

VIBRATIONAL SPECTROSCOPY OF BARE AND SOLVATED IONIC COMPLEXES OF BIOLOGICAL RELEVANCE

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Received 12 June 2008; received (revised) 15 August 2008; accepted 15 August 2008

Published online 24 February 2009 in Wiley InterScience (www.interscience.wiley.com) DOI 10.1002/mas.20215

The low density of ions in mass spectrometers generally precludes direct infrared (IR) absorption measurements. The IR spectrum of an ion can nonetheless be obtained by inducing photodissociation of the ion using a high-intensity tunable laser. The emergence of free electron lasers (FELs) and recent breakthroughs in bench-top lasers based on nonlinear optics have now made it possible to routinely record IR spectra of gas-phase ions. As the energy of one IR photon is insufficient to cause dissociation of molecules and strongly bound complexes, two main experimental strategies have been developed to effect photodissociation. In infrared multiple-photon dissociation (IR-MPD) many photons are absorbed resonantly and their energy is stored in the bath of vibrational modes, leading to dissociation. In the "messenger" technique a weakly bound van der Waals atom is detached upon absorption of a single photon. Fundamental, historical, and practical aspects of these methods will be presented. Both of these approaches make use of very different methods of ion preparation and manipulation. While in IR-MPD ions are irradiated in trapping mass spectrometers, the "messenger" technique is generally carried out in molecular beam instruments. The main focus of this review is the application of IR spectroscopy to biologically relevant molecular systems (amino acids, peptides, proteins). Particular issues that will be addressed here include gas-phase zwitterions, the (chemical) structures of peptides and their collision-induced dissociation (CID) products, IR spectra of gas-phase proteins, and the chelation of metal–ligand complexes. Another growing area of research is IR spectroscopy on solvated clusters, which offer a bridge between the gas-phase and solution environments. The development of state-of-the-art computational approaches has gone hand-in-hand with advances in experimental techniques. The main advantage of gas-phase cluster research, as opposed to condensed-phase experiments, is that the systems of interest can be understood in detail and structural effects can be studied in isolation. It will be shown that IR spectroscopy of mass-selected (bio)molecular systems is now well-placed to address specific questions on the individual effect of charge carriers (protons and metal ions), as well as solvent molecules on the overall structure. © 2009 Wiley Periodicals, Inc., *Mass Spec Rev* 28:468–494, 2009

Keywords: infrared photodissociation; spectroscopy; ions; biomolecules; solvation; zwitterion

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I. INTRODUCTION

Despite the major advances mass spectrometry (MS) has brought to (bio)analytical sciences, there are surprisingly few techniques available to directly characterize the structures of ions in mass spectrometers. Such techniques include electron diffraction (Kruckeberg et al., 2000; Schooss et al., 2005), ultraviolet (Dunbar, 2000) and infrared (IR) spectroscopy (Duncan, 2000; Oomens et al., 2006; Asmis & Sauer, 2007), fluorescence measurements (Iavarone et al., 2007) and ion mobility (Clemmer & Jarrold, 1997; Wyttenbach & Bowers, 2003). Of these, only ion mobility can be considered to have evolved beyond a merely "academic" level of interest, with the recent development of a commercially available ion mobility mass spectrometer (Giles et al., 2004). While ion mobility mass spectrometry is highly effective at yielding an overall structure of the ion by virtue of the collision cross-section, the technique is far less useful in deriving detailed chemical information, with the exception of small complexes such as carbon clusters (von Helden et al., 1991; Bowers et al., 1993; Clemmer et al., 1994) and atomic metal ion spin states (Kemper & Bowers, 1990).

Infrared (IR) spectroscopy of mass-selected molecular ions, on the other hand, is well-suited to give chemically relevant information on the ion. The vibrational frequencies of intense IR absorptions, such as modes involving CO, NH, and OH groups, are generally very sensitive to hydrogen bonding, which make them excellent probes to investigate the detailed aspects of the structures of biologically relevant molecules. Particularly in combination with modern electronic structure calculations, such as density functional theory (DFT), IR spectroscopy has become a powerful structural tool in MS research that is now being applied in a growing number of labs around the world.

Historically, ion spectra were first obtained as emission spectra from electrical discharges (Herzberg, 1950, 1966, 1971). Such spectra can be difficult to interpret, given the many quantum states that are populated and the presence of impurities. Some of the first direct IR absorption spectra of ions include the pioneering work by Schwarz (1977) and Oka (1980). Saykally and co-workers developed a method to measure the IR spectra of ions where the contribution of neutral species could be eliminated, by employing velocity modulation techniques for discharges (Gudemann et al., 1983). They were also the first to observe direct absorption spectra of ions in fast ion beams (Coe et al., 1989), which is much more challenging, given the reduced ion density (10^4 ions/cm³) compared to electrical discharges (10^{12} ions/cm³). In general, the low density of ions only allows their direct absorption to be observed in cases where the

absorption cross-section is high, the density can be maintained at a high enough level, the rovibrational partition function is relatively low, and/or the absorption path length is long. These requirements are optimally fulfilled in a slit jet discharge expansion, which has become the most popular application of direct absorption spectroscopy on molecular ions (Anderson et al., 1996; Linnartz, Verdes, & Speck, 2000).

To overcome the problem of low ion densities impeding the measurement of direct absorption spectra of ions, one can make use of changes in a characteristic property of the ion, such as the quantum state, the charge or the mass of the ion, induced by the absorption of one or more photons. Such an approach is generally referred to as an “action” spectroscopy method. One of the first applications of action spectroscopy to ionic species was reported for the HD^+ molecular ion in 1976, where the change in collision cross section upon IR absorption was used (Wing et al., 1976). Since low ion densities are no longer critical, action spectroscopy can be applied to mass-selected ions, which greatly enhanced the applicability of, and the interest in, IR ion spectroscopy.

In the mid-1980s YT Lee and co-workers developed the vibrational pre-dissociation spectroscopy technique (Okumura, Yeh, & Lee, 1985), which spawned the new field of IR spectroscopy of clusters. In Lee’s set-up, cold (~ 130 K) cluster ions were generated in an expansion and ionized by electron impact; his tandem mass spectrometer allowed the precursor ion to be mass selected, trapped in an rf trap to be irradiated with a tunable IR laser and the photodissociation products to be detected as a function of wavelength. The profound influence of Lee’s work on gas-phase spectroscopy of clusters has been reviewed recently (Lisy, 2006).

In the case of photodissociation of weakly bound systems, such as a van der Waals rare gas complex, where the sole purpose of the rare gas atom is to signal photon absorption by the ion of interest, the approach is referred to as the “messenger” technique (Okumura et al., 1986). The binding strength of the rare gas is very low so that the complex can photodissociate upon absorption of a single photon, resulting in a spectrum that closely resembles a linear absorption spectrum. Moreover, the low binding energy ensures that the structure of the target ion is virtually unaffected, compared to more strongly bound systems, such as YT Lee’s spectra of $\text{H}_3\text{O}_2^+\text{H}_2$ complexes, which show red-shifting and splitting of vibrational bands (Yeh et al., 1989; Okumura et al., 1990). Hence, minimizing the binding energy of the messenger atom by going from Ar to Ne for instance, can be of importance (Hammer et al., 2005). Nonetheless, perturbation of the symmetry of the system can influence the spectroscopic selection rules and thus change the spectrum, regardless of the nature of the messenger atom (Bakker et al., 2002; Bopp, Roscioli, & Johnson, 2007). For small weakly bound clusters it is even possible to obtain rotationally resolved spectra using IR photodissociation approaches in tandem mass spectrometers; an excellent review on high-resolution spectroscopy of cluster ions is available (Bieske & Dopfer, 2000).

An ion trap mass spectrometer has the advantage that all three steps in a photodissociation experiment (i.e., mass isolation, trapping and mass detection) can be carried out in the trap. For room-temperature ions weakly bound complexes cannot be formed and to dissociate more strongly bound species using IR

radiation, one must be able to induce multiple photon absorption. Although the trap increases the interaction time, more powerful IR laser sources are generally required. Beauchamp and co-workers first demonstrated IR multiple photon dissociation (IR-MPD) spectroscopy of mass-selected ions using a tunable CO_2 laser in a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Woodin, Bomse, & Beauchamp, 1978). Using these methods, Eyler showed that IR spectra of covalently bound ions could be recorded (Baykut et al., 1985) and Beauchamp applied it to negative ions (Shin & Beauchamp, 1990). In addition to CO_2 lasers (Atrill & Stace, 2000), Stace and co-workers used CO lasers to access different parts of the IR spectrum (Odeneye & Stace, 2005). Unfortunately, gas-discharge lasers, such as CO_2 and CO lasers have a limited and discontinuous tuning range, which constrains their spectroscopic usefulness. In 2000, we showed that it was possible to use the high-intensity, widely tunable IR radiation produced by a free electron laser (FEL) to obtain IR-MPD spectra of mass-selected gas-phase ions (Oomens et al., 2000). Since this first publication, there has been a renewed interest in the IR-MPD spectroscopy of ions and successful user programs have been developed at the FEL facilities FELIX in the Netherlands and CLIO in France. Figure 1 shows the upward trend in the number of publications on FEL-based IR spectroscopy of ions from 2000 to 2008.

In this special edition on *photodissociation*, this review aims to give an overview of vibrational spectroscopy of ions, with particular emphasis on the application of IR spectroscopy of biomolecular ions and their solvation. Other areas of spectroscopy of ions in this edition are discussed by Jennifer Brodbelt on IR-MPD in quadrupole ion traps (Brodbelt, 2009), by John Eyler on IR-MPD in Penning traps (Eyler, 2009) and by James Reilly on UV photofragmentation of biomolecular ions (Reilly, 2009). This review also surveys recent developments to complement a range of reviews that have appeared on IR spectroscopy of ions over the years (Bieske & Dopfer, 2000; Duncan, 2000, 2003; Oomens et al., 2003, 2006; Dopfer, 2005; Lisy, 2006; Asmis & Sauer, 2007; MacAleese & Maitre, 2007; Polfer & Oomens, 2007).

II. EXPERIMENTAL TECHNIQUES

A. Ion Preparation and Mass Spectrometry Techniques

Roughly speaking, two experimental approaches to IR gas-phase ion spectroscopy are most popular nowadays: methods based on supersonic expansions and methods based on ion trapping. A combination of these methods is also possible. Ionization of the species in the beam can be effected in various ways: a plasma discharge in the nozzle (Anderson et al., 1996; Linnartz, Verdes, & Speck, 2000), electron impact ionization (Okumura, Yeh, & Lee, 1985; Bieske et al., 1995; Olkhov, Nizkorodov, & Dopfer, 1998; Hammer et al., 2005), and UV photoionization (Piest, von Helden, & Meijer, 1999; Pino, Boudin, & Brechignac, 1999). Metal (oxide) clusters as well as metal–organic complexes are commonly generated by laser vaporization producing both charged and neutral species; hence, no post-ionization is required

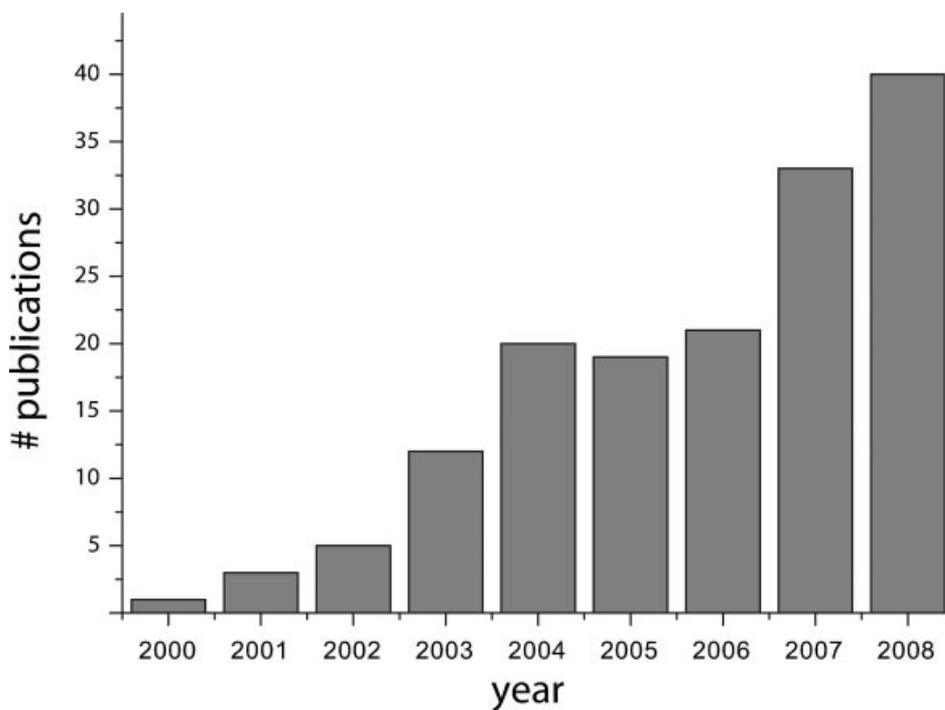


FIGURE 1. Summary of peer-reviewed publications on IR spectra of gas-phase ions from 2000 to 2008 using free electron lasers.

to record ion spectra (Duncan, 2003; Fielicke et al., 2004a, 2005; Walters et al., 2005a; Fielicke, Rabin, & Meijer, 2006; Velasquez et al., 2006). The Simons group in Oxford has used a novel photochemical proton transfer approach to generate protonated (bio)molecules and measure their IR-MPD spectra in supersonic expansions (MacLeod & Simons, 2004, 2006). Expansion cooling in a seeded molecular beam produces molecules with internal energies in the range of 10 K, such that weakly bound complexes can be generated. Hence, IR spectra are often recorded by applying the messenger technique, where the absorption of an IR photon induces detachment of the tag atom or molecule, which is detected in a mass spectrometer (Okumura et al., 1986). Molecular beam spectroscopy of involatile molecular ions can be carried out using a laser desorption source (Meijer et al., 1990).

Commonly, molecular beam IR ion spectroscopy makes use of either time-of-flight MS or tandem mass spectrometers, with or without an ion trap (see Fig. 2). In TOF instruments, interaction with the IR laser takes place in, or slightly upstream from the extraction zone of the TOF-MS. Using a reflectron-TOF, Duncan and co-workers showed that introducing the IR laser in the turning point of the reflectron has the advantage that the first leg can be used to mass-select ions prior to IR interrogation (Duncan, 2003; Jaeger, Pillai, & Duncan, 2004). The advantage of instruments based on tandem mass spectrometers is that mass-selection prior to IR irradiation is a standard feature. The middle section, where interaction with the IR laser takes place, can be either a multipole ion guide or a linear ion trap.

A mass-selective ion trapping device, such as a quadrupole rf ion trap or an ICR trap, allows ions to be trapped for extended

periods of time, during which they can be mass isolated, reacted, and irradiated with a laser for variable periods of time. Novel ionization methods can be conveniently coupled to quadrupole and ICR ion traps. The development of soft ionization techniques such as matrix-assisted laser desorption/ionization (MALDI) (Hillenkamp et al., 1991) and electrospray ionization (ESI) (Fenn et al., 1989) have made it possible to bring intact large molecules into the gas phase, thus laying the foundation for the important role that mass spectrometry now plays in the biological sciences.

A recent development by the group of Rizzo has been the use of a closed-cycle He refrigerated 22-pole ion trap (after the design of Gerlich) incorporated into a tandem mass spectrometer, which opens up a wealth of new possibilities for gas-phase spectroscopy of biologically relevant ions (Stearns et al., 2007b). In addition, it has been shown that in liquid He cooled multipole traps, van der Waals clusters with He can be formed so that the messenger technique can be applied even without a supersonic expansion (Brummer et al., 2003).

A comparative study by Simon et al. (2007) on IR-MPD spectra obtained in an FT-ICR and an rf quadrupole trap, indicated that the quadrupole trap allows much more efficient photodissociation, as would be expected from the enhanced overlap between the ion cloud and the laser beam, and that the spectral bands are narrower. This is rationalized by the buffer gas cooling in the quadrupole trap, which thermalizes the ions more effectively prior to IR-MPD. It should also be noted that different ionization methods were used in both experiments: MALDI for the FT-ICR experiment versus ESI for the ion trap. On the other hand, FT-ICR instruments have considerable advantages over quadrupole ion traps, such as higher mass resolution, more

accurate ion manipulation, including mass isolations, and lower pressure, thus limiting collisional damping in the IR-MPD process.

B. Tunable Laser Systems

Historically, gas discharge lasers and in particular CO₂ and CO lasers (Patel, 1965; Urban, 1995; Bijnen et al., 1996) have been used for IR-MPD ion spectroscopy in the 9–11 and 5–7.5 μm wavelength regions, respectively (Baykut et al., 1985; Shin & Beauchamp, 1990; Odeneye & Stace, 2005). These laser sources feature high output powers, which make them useful for multiple photon excitation, but the limited and discontinuous tuning ranges put severe constraints on the spectroscopic information that can be obtained.

Over the past decade, a revolution has taken place in the performance of tunable IR bench-top laser systems. Laser systems based on nonlinear frequency down-conversion, such as optical parametric oscillation and amplification (OPO/OPA) and difference frequency generation (DFG) have matured considerably and are now commercially available. These developments have been made possible by the enhanced performances of novel birefringent materials and of solid-state laser sources, used as pump source in nearly all OPO and DFG applications over the past decade.

A popular way of generating IR radiation in the 3 μm wavelength range is by mixing the fundamental of a Nd:YAG laser with the output of a tunable dye laser, which is pumped by the second harmonic of the same Nd:YAG laser (Gerhards, 2004). Using an additional OPA amplification stage, such systems produce several tens of millijoules per pulse nowadays. In addition, the signal (1.75–1.85 μm) and the idler (2.5–2.75 μm) output from the OPA stage can be used as input for another difference frequency mixing process in newly developed AgGaSe₂ crystals. It has thus become possible to extend the wavelength range down to the biologically important 6–10 μm range (Gerhards, Unterberg, & Gerlach, 2002).

Another approach, that is based solely on solid-state laser technology, has been to use a pulsed Nd:YAG laser pumped OPO to generate IR radiation in the 3 μm range, which can be amplified in an OPA stage. Using AgGaSe₂ crystals, signal and idler output can again be mixed generating light down to approximately 600 cm⁻¹ (see e.g., Diken et al., 2005; Slipchenko et al., 2006; Douberly et al., 2008b). High-resolution studies may be performed using a cw single-frequency Nd:YAG laser as pump source and a periodically poled birefringent crystal (Myers et al., 1995). Such a combination has been shown to produce narrow band cw IR radiation in the 3–5 μm range with cw output powers exceeding 1 W (Bosenberg et al., 1996; van Herpen, Bisson, & Harren, 2003).

Various other routes to access the mid-IR spectral range exist, but they have not been widely used to obtain gas-phase ion spectra. A solid para-hydrogen Raman shifter at 4 K in combination with a tunable near-IR OPO has been reported to cover a wavelength range of 4.4–8 μm with pulse energies of 0.1–1 mJ (Kuyanov, Momose, & Vilesov, 2004). Quantum-cascade lasers have evolved to become popular semi-conductor lasers for the mid-IR wavelength range (Faist et al., 1994). They

are available at many (even custom-made) wavelengths, but have a fairly narrow tuning range, which makes them particularly useful in, for example, trace gas detection or high-resolution spectroscopy.

While bench-top laser systems are suited to carry out experiments in the near IR (2500–4000 cm⁻¹) and are pushing ever further into the mid-IR (800–2000 cm⁻¹), the far-IR (<500 cm⁻¹) so far remains the exclusive domain of FELs. As FELs are based on free electrons, unbound to an atomic nucleus, their output wavelength is not hindered by self-absorption of the lasing medium. Hence, the laser can in principle be tuned to any wavelength in the electromagnetic spectrum. Ground-breaking developments in FEL science are taking place in the X-ray region of the spectrum (DESY Hamburg, LCLS Stanford). In terms of pulse energies, FELs also considerably surpass their bench-top “competitors.” This is essential in experiments where multiple photons need to be absorbed to induce photodissociation (see following section).

A free electron laser is based on the radiation (Bremsstrahlung) generated by accelerated relativistic electrons (see e.g., Colson et al., 2002). Very briefly, relativistic electron bunches of several tens of MeV are created, commonly using rf linear accelerators. The electron bunches are injected into a periodic magnetic field structure, called an undulator, where they undergo a wiggling motion induced by the Lorentz force. This angular acceleration generates the Bremsstrahlung upon which the free electron laser is based. The wavelength of the light is determined by the energy of the electrons and the magnetic field strength of the undulator. There are various IR free electron laser facilities around the world, though only few are applied for gas-phase spectroscopy. These FELs, which include CLIO in Orsay, France, FELIX in Nieuwegein, The Netherlands, and FEL-SUT in Tokyo, Japan, have in common that the wavelength can be tuned conveniently. This is achieved by varying the magnetic field strength of the undulator by changing the gap between the two arrays of magnets. At FELIX, this results, for instance, in a tuning range of about a factor of three in wavelength for a given setting of the electron beam energy. Other IR free electron lasers for spectroscopic experiments are currently being conceptualized, designed, and constructed (e.g., in Daresbury, UK, Nijmegen, The Netherlands, Berlin, Germany, and at the NHMFL, Tallahassee, FL, USA).

III. MECHANISM OF INFRARED MULTIPLE-PHOTON DISSOCIATION (IR-MPD)

With the emergence of FELs, IR-MPD spectroscopy of gaseous ions has recently seen rapid growth, though it has been successfully applied with line tunable gas-discharge lasers for several decades. Although IR-MPD spectra are often directly compared to spectra generated by quantum-chemical computations, one should keep in mind that the multiple photon excitation mechanism may influence the appearance of the spectrum, which could therefore differ from the calculated linear absorption spectrum. Various molecular parameters apart from frequency and intensity may affect the actual bands observed in an IR-MPD spectrum, such as the dissociation threshold, the anharmonicities of the vibrational potentials, and the kinetic shift (Lifshitz, 2002).

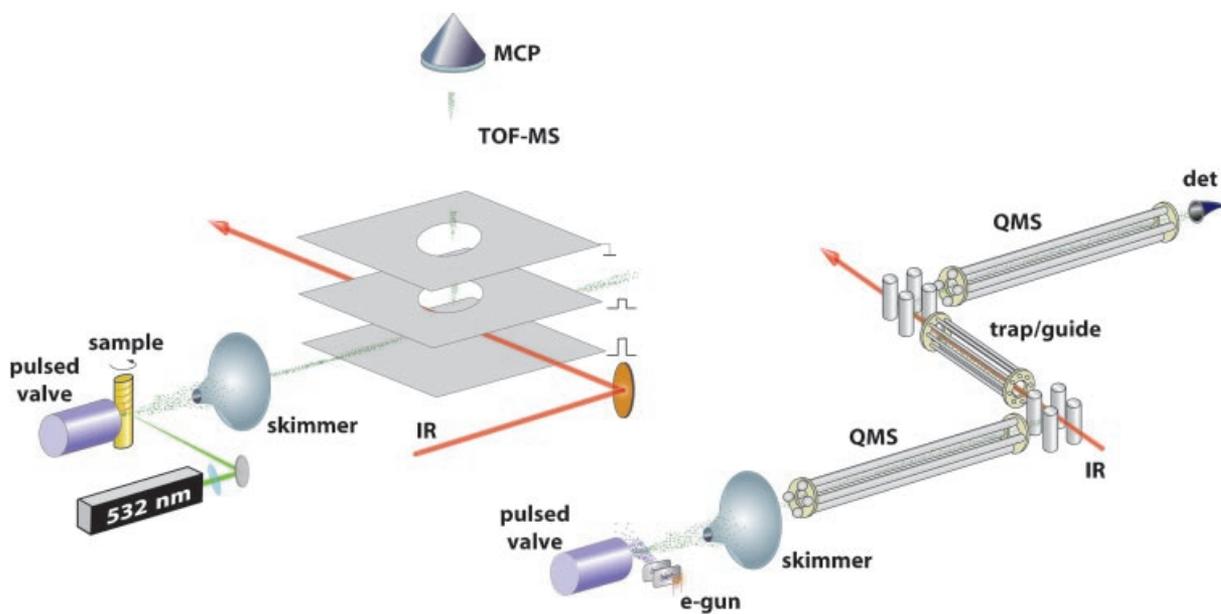


FIGURE 2

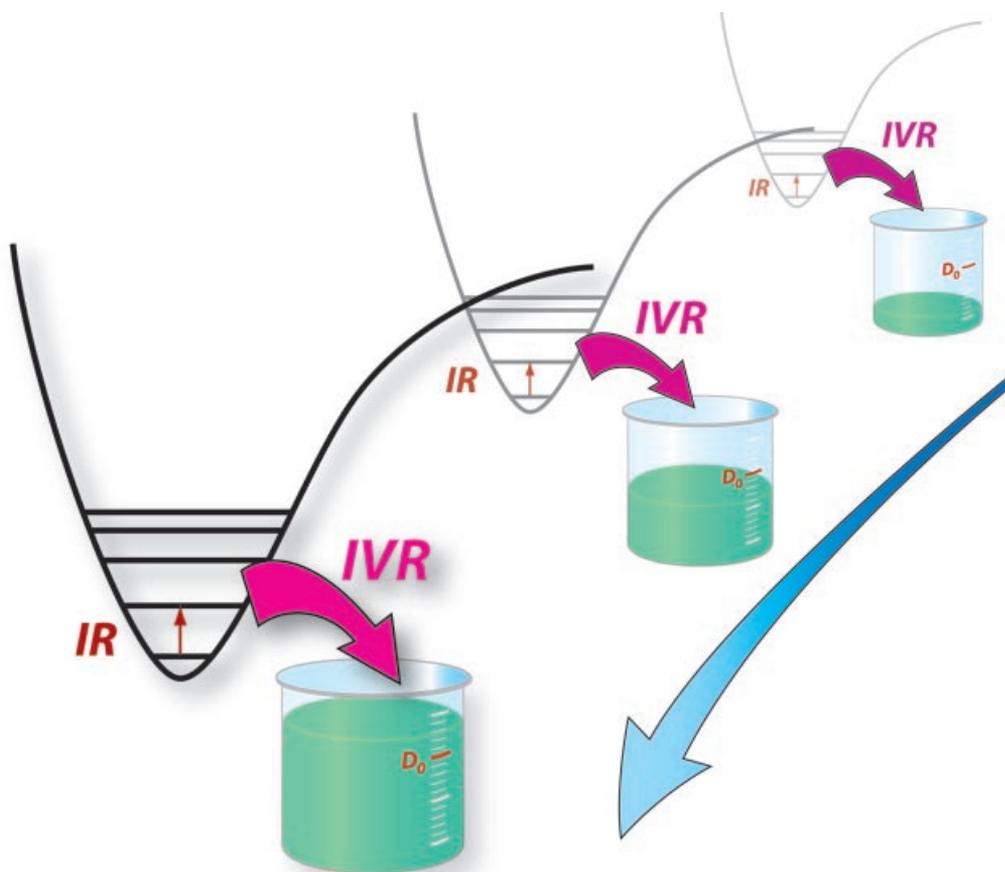


FIGURE 3

FIGURE 2. Two typical implementations of molecular beam ion spectroscopy. The set-up on the left combines a laser vaporization source with a TOF mass spectrometer. Ionic metal (oxide) clusters or metal–organic complexes can thus be generated. The species, often as rare-gas van der Waals complexes, undergo dissociation upon resonant IR irradiation in the extraction zone of the TOF, which is monitored by pulsed-field extraction into the TOF-MS. The set-up on the right is based on a tandem mass spectrometer, equipped with a supersonic expansion source. The precursor is seeded in a carrier gas and ionized by electron impact just downstream from the nozzle. The ions are then mass selected, irradiated with an IR laser source, and either the undissociated parent or the fragment yield is monitored with a second QMS.

FIGURE 3. Impression of the IR-MPD mechanism in polyatomic molecules, showing how the energy pumped into a specific vibrational mode is quickly redistributed over the bath of background states by virtue of IVR. The molecule can thus sequentially absorb many photons on the same transition, while the energy is stored in the bath. Once the internal energy reaches the dissociation threshold (red mark), the molecule can undergo unimolecular dissociation.

To dissociate an ion using IR radiation, typically tens to hundreds of photons need to be absorbed to achieve an observable IR-MPD yield. Already in the 1970s, evidence was found that this process occurs through non-coherent multiple photon absorption (Yablonovitch, Bloembergen, & Mukamel, 1977; Grant et al., 1978; Bagratashvili et al., 1985), mediated by intramolecular vibrational redistribution, IVR (Lehmann, Scoles, & Pate, 1994). A coherent “ladder-climbing” mechanism in a single vibrational potential well would be impeded by the “anharmonicity bottleneck,” which causes the laser to gradually run out of resonance as the molecule is excited. Instead, photon absorption is followed by statistical energy redistribution over all vibrational degrees of freedom, facilitated by vibrational couplings in polyatomic molecules. A subsequent photon can then be absorbed on the same (fundamental) transition and its energy will eventually be stored in the bath of vibrational background states (see Fig. 3). This process repeats itself many times. The internal energy distribution attained after the laser pulse then determines whether or not the molecule undergoes (unimolecular) dissociation, and whether the dissociation is branched over multiple dissociation channels.

Along with avoiding the anharmonicity bottleneck, IVR also wipes out any recollection of the initially excited vibrational mode and is thus assumed to prevent mode-selective dissociation. Moreover, in the limit that the randomization is complete, that is, that all phase space is probed by IVR, different conformers are expected to show the same fragmentation behavior, if the barrier to isomerization is lower than the dissociation thresholds (Bush et al., 2007b). If, on the other hand, the thermodynamic or kinetic barriers are sufficiently high, such as often the case for structural isomers, different structures may be observed to show differences in fragmentation (Jockusch, Price, & Williams, 1999; Solca & Dopfer, 2004).

Different thresholds for different dissociation channels may cause small differences in the spectral responses found in each of the channels, which sample different parts of the population of excited molecules. A clear example was found for the para-amino benzoyl cation, where the C≡O stretching mode was observed at slightly different frequencies depending on the dissociation channel monitored. In the higher energy dissociation channel the band is slightly red-shifted compared to that in a lower energy channel (Oomens et al., 2004a). This can be qualitatively understood in the sense that to reach the higher

energy threshold, the laser needs to be tuned to slightly longer wavelengths because of the anharmonic red-shift increasing with increasing internal energy. In addition, we observed that changing the laser pulse energy changes the branching in the two channels to the point where the higher energy channel becomes dominant. This can be rationalized in terms of unimolecular dissociation theory, where the dissociation rate into higher energy channels eventually becomes dominant at higher internal energies because of the higher pre-exponential factor (i.e., “looser” transition states) in the expression for the dissociation rate. These findings sketch a picture where at each wavelength, a particular excited state distribution is created depending on the details of the vibrational potentials and the laser radiation parameters, which determines the branching into the different exit channels. In most cases, these effects are very marginal so that the spectra in the various channels are very similar. Their contributions are then summed to give the final IR-MPD spectrum. An example where the relative yields in competing dissociation channels was found to be strikingly dependent on wavelength were a series of Li⁺-cationized glucose–glucose disaccharide isomers (Polfer et al., 2006b). In fact, each of these linkage- and anomeric isomers displayed distinct wavelength-dependent photodissociation patterns that can be considered as spectral fingerprints. This approach appears to have potential as a method to distinguish isomers by mass spectrometry.

IV. IR SPECTROSCOPY ON BARE BIOLOGICAL IONS

While it is not completely clear yet what role mass spectrometry will play in the higher-order structural characterization of biomolecules, many techniques have been applied to this task, including electron capture dissociation (ECD) (McLafferty et al., 1998; Kelleher et al., 1999), black-body infrared radiative dissociation (BIRD) (Price, Schnier, & Williams, 1996), H/D exchange (Campbell et al., 1995; Konermann & Simmons, 2003), ion mobility (Clemmer, Hudgins, & Jarrold, 1995; Valentine & Clemmer, 1997; Bernstein et al., 2005); IR spectroscopy is a relative newcomer to this field. Nonetheless, IR spectroscopy is well-suited to answer some specific questions about biomolecular ions, including the location of the charge, the

presence of chemical moieties and the secondary structure of peptides and proteins, for which other gas-phase techniques are arguably less direct probes of the structure.

A. Zwitterions in the Gas Phase?

The question of identifying zwitterionic (ZW) structures of amino acids or peptides in the gas phase, which result in salt bridge (SB) complexes upon binding with a metal cation, has been the subject of much debate (Wytenbach, Witt, & Bowers, 1999, 2000; Moision & Armentrout, 2002; Lemoff & Williams, 2004; Kamariotis et al., 2006), yielding at times contradictory or inconclusive results. Amino acids are generally not thought to exist as zwitterions in the gas phase, due to the absence of the polar solvent. However, for instance, metal cations can stabilize the deprotonated carboxylate group, thus lowering the energy of the ZW form, often to a degree where it becomes competitive with the charge solvated (CS) form. A delicate balance between CS and ZW structures thus exists, and subtle effects such as the proton affinity of the amino acid, the presence of aromatic rings, the nature of the (alkali) ion, the charge of the metal ion as well as steric effects can change the balance. Mainly by virtue of the difference in stretching frequency between the carboxylate (COO⁻) and carboxylic acid (C=O) groups, IR spectroscopy has been very successful in identifying CS versus ZW structures.

Ohanessian and co-workers investigated the sodium-tagged glycine and proline amino acids by IR spectroscopy (Kapota et al., 2004). Proline displays a band at ~1696 cm⁻¹ assigned to the antisymmetric CO stretch of the carboxylate group, which is considerably red-shifted compared to the CO stretch of the carboxylic acid group for glycine at 1740 cm⁻¹. This difference can be attributed to the presence of the SB structure for proline and mainly the non-zwitterionic CS structure for glycine. This result is consistent with calculations, as proline displays a higher basicity secondary amine group as opposed to the lower basicity primary amine in glycine. A second diagnostic mode for zwitterionic proline in the mid-IR region of the spectrum is the symmetric CO stretch of the carboxylate group at 1400 cm⁻¹.

The Williams group has studied a wide range of potential gas-phase zwitterionic structures using mainly BIRD and computational approaches; see for instance (Jockusch, Lemoff, & Williams, 2001; Lemoff, Bush, & Williams, 2003). These studies had indicated that in the case of arginine, the most basic amino acid, the size of the alkali metal cation determines whether the zwitterionic form is adopted or not: whereas Li⁺ appears to be too small as a cation to interact favorably with zwitterionic arginine, the larger alkali metals chelate the carboxylate group much more effectively, thus promoting the zwitterionic form (Jockusch, Price, & Williams, 1999). Note that this trend is in complete disagreement with what Wytenbach and Bowers suggested for the alkali metal series of glycine and alanine (Wytenbach, Witt, & Bowers, 2000). IR measurements by Williams and co-workers in the NH and OH stretch region have validated their previous hypothesis, showing that the transition between non-zwitterion and ZW in Arg occurs in between lithium and sodium (Bush et al., 2007b). Figure 4 shows the IR spectrum for ArgK⁺, where the computed spectrum for the ZW structure E gives the most convincing agreement. This study has recently been followed up by a mid-IR investigation by Jockusch

and co-workers of the complete alkali metal series for arginine, confirming the same trends (Forbes et al., 2007). For the next most basic amino acid, lysine, the cross-over from charge solvated to salt-bridge structures occurs at K⁺, although both structures coexist up to the largest alkali ion, Cs⁺ (Bush et al., 2007a).

The π -cloud of aromatic amino acids presents a good binding site for alkali metals thus increasing the capability of these amino acids to solvate the metal ion. Moreover, these molecules lack a highly basic site. It is hence not surprising that the ZW structure is not observed for tryptophan regardless of the alkali metal ion complexed, as determined by IR spectroscopy (Polfer, Oomens, & Dunbar, 2006). Nonetheless, two CS structures are present in this case, a tridentate N/O/ring or bidentate O/ring structure, based on the diagnostic vibrations at 1150 cm⁻¹ (N/O/ring) and 1400 cm⁻¹ (O/ring). The more compact N/O/ring structure is favored for the smaller cations Li⁺ and Na⁺, where the amino acid is able to “wrap” around the metal ion better. The twofold chelating O/ring structure becomes progressively more favored for the larger cations. The cross-over in energetics is predicted to occur around K⁺ and this is mirrored by the relative intensities of the diagnostic bands for each structure. A similar trend is observed for the alkali metal ion series complexed to glutamine (Gln). Threefold chelation involving the C- and N-terminus as well as the side chain carbonyl is observed for the smaller alkali metal ions, whereas the decreasing polarizing effect of the larger alkali ions favors a twofold chelation with the amide and carbonyl C=O groups, combined with an H-bond between the C- and N-termini (Bush et al., 2008b). A zwitterionic structure is not observed for any of the alkali ions.

Infrared (IR) spectra have also been recorded for the full alkali metal ion series complexed with the alcoholic side chain amino acids serine (Armentrout et al., 2008) and threonine (Rodgers et al., 2008). Despite their similar side chains, there are subtle differences in their behavior upon alkali metal ion binding. Most notably, the presence of a ZW structure is experimentally observed for Ser complexed with Cs, whereas for Thr exclusively charge solvated structures are found.

To probe the reactivity of these species, K⁺Trp was subjected to gas-phase H/D exchange with CH₃OD. The resulting singly deuterated product ion displayed a weak IR band at 1680 cm⁻¹ which was not present in the spectrum of the non-deuterated precursor (Polfer, Dunbar, & Oomens, 2007). This band is consistent with the appearance of the ZW structure, as this band matches the predicted position of the antisymmetric CO stretch of the carboxylate group. Based on the relative band intensities, the abundance of the ZW structure was approximated at 20%, which means that the majority of the ion population in fact adopted the lowest energy CS structure. Zwitterionic intermediates of HDX reactions had been hypothesized by others (Wytenbach et al., 2003; Rožman et al., 2006), but this was the first direct evidence that the bare zwitterionic isomer could be isolated in the gas phase. While H/D exchange is clearly not as direct a technique in probing structures as IR spectroscopy, these results even suggest that H/D exchange can be an invasive technique, in fact changing the structure of the analyte.

When tryptophan is chelated to a divalent alkaline-earth metal barium cation, it was found to exclusively form ZW

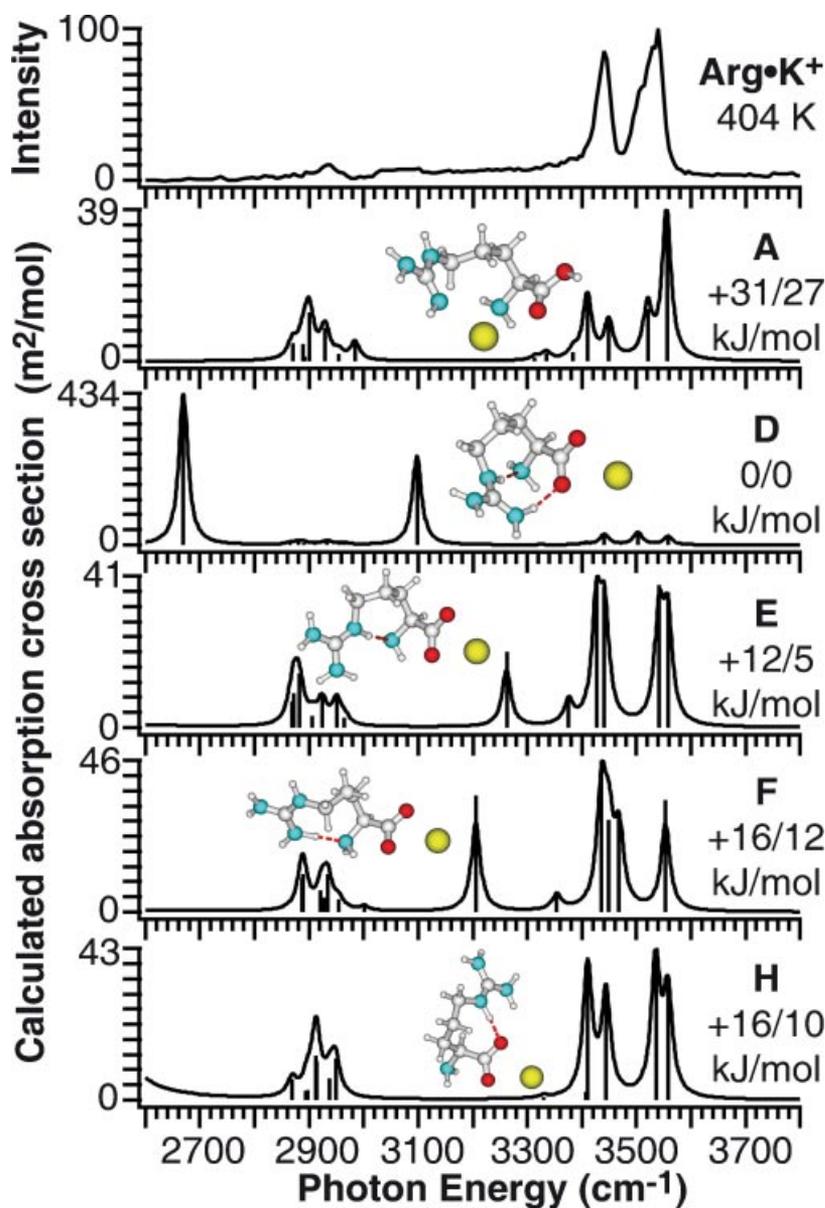


FIGURE 4. IR photodissociation spectrum of ArgK⁺ recorded at a temperature of 404 K compared to calculated spectra of putative structures: non-zwitterionic structure A and zwitterionic structures D, E, F, and H. The slightly higher-energy ZW structure E appears to give the best agreement with the experimental spectrum. The free energies are given for each structure at 0 K and at 404 K. Reprinted with permission from Bush et al. (2007b). Copyright (2007) American Chemical Society. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

structures (Dunbar, Polfer, & Oomens, 2007). This is consistent with previous theoretical studies on amino acid complexes with divalent metals (Strittmatter, Lemoff, & Williams, 2000; Hoyau et al., 2001; Ai, Bu, & Han, 2003). It appears that the higher charge on the metal cation can no longer be adequately solvated by the Lewis basic groups of the amino acid, thus forcing the amino acid to adopt a zwitterionic structure so that the carboxylate group can coordinate to the 2+ charge forming a ZW structure. The diagnostic bands of the ZW structure are the antisymmetric stretch of the carboxylate group at ~ 1600 cm⁻¹

and the NH₃⁺ umbrella mode at ~ 1450 cm⁻¹, as shown in Figure 5. Further studies by Williams and co-workers suggest that most amino acids adopt a ZW structure upon complexation to Ba²⁺ (Bush et al., 2008a).

For larger molecular systems, such as peptides, the number of vibrational modes becomes very large, leading to increased spectral congestion. The largest single molecule that has so far been identified as a zwitterion in the gas phase based on IR spectroscopy is the pentapeptide bradykinin fragment 1–5 (Arg-Pro-Pro-Gly-Phe) by Polfer and co-workers (Polfer et al., 2005a).

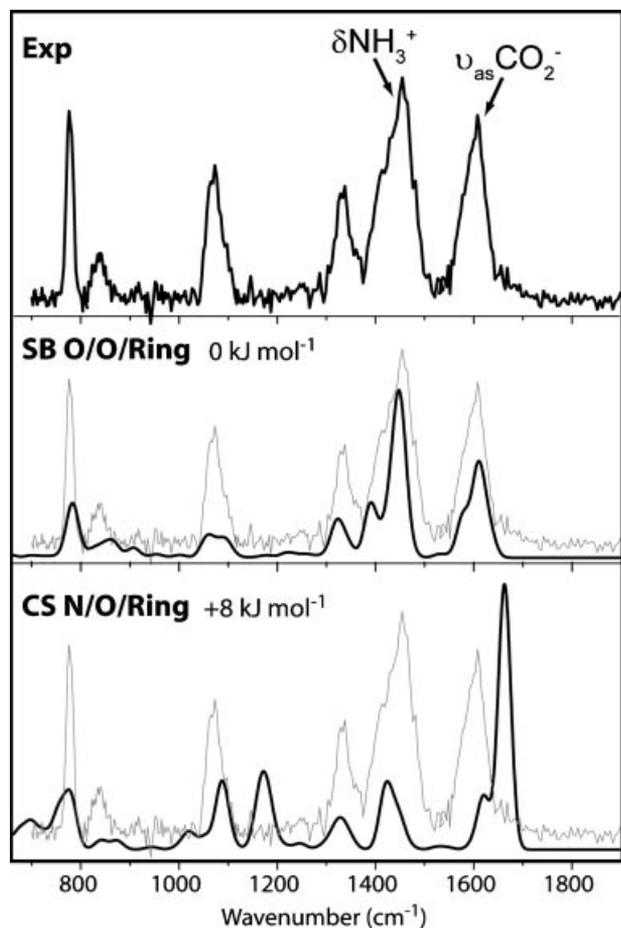


FIGURE 5. IR-MPD spectrum of Ba^{2+}Trp compared to the calculated spectra of the salt bridge (SB) and charge solvation (CS) binding motifs. The diagnostic antisymmetric CO_2^- stretch (1600 cm^{-1}) and NH_3^+ umbrella (1450 cm^{-1}) bands are labeled and appear like a “fingerprint” for the exclusive presence of SB. Data taken from Dunbar, Polfer, and Oomens (2007b).

As the size of the molecular system increases further, the increase in the number of vibrational modes also leads to a relative “diluting” of the absorption cross-section of the carboxylic acid mode. In other words, in a large peptide or protein the intensity of the C-terminal carboxylate or carboxylic acid CO stretch is too low to be measurable compared to other strong vibrational modes, such as the amide I or amide II modes (Oomens et al., 2005).

Another interesting approach is to study dimers or higher multimers of amino acids which are merely non-covalently bound. Studies by McLafferty on glycine and lysine proton-bound dimers suggested the presence of zwitterions for the lysine-containing systems (Oh et al., 2005), whereas studies by McMahan and co-workers (Wu & McMahan, 2007a) on dimers of proton-bound glycine and proline showed that only proline forms zwitterionic structures. This once again confirms that basic amino acids tend to form zwitterionic structures, whereas “normal” amino acids typically do not. It is still a matter of debate how much detailed information IR spectroscopy can yield on room-temperature ions. For these proton-bound complexes

many conformations are possible and multiple structures may be populated at room temperature. Rajabi and Fridgen recently showed that the IR spectra of proton-bound glycine and alanine look almost identical (Rajabi & Fridgen, 2008). Nonetheless, they were able to distinguish which conformation is favored, based on differences in the asymmetric stretch mode of the shared proton. Note that they also predicted a different structure for the proton-bound glycine dimer compared to McLafferty and co-workers. Both studies also made use of different wavelength regions, $700\text{--}2000\text{ cm}^{-1}$ for Fridgen et al. compared to $3050\text{--}3800\text{ cm}^{-1}$ for McLafferty and co-workers, which underlines the importance of considering wider wavelength regions for structural elucidation.

Chang and co-workers presented the IR spectra of a series of serine multimers (Kong et al., 2006), going from the dimer to the “magical” octamer (Cooks et al., 2001; Takats et al., 2003). They found that the intensity of the carboxylic acid OH stretch at 3565 cm^{-1} progressively decreases as a function of the serine cluster size, eventually disappearing at the octamer, whereas a band appears at 3270 cm^{-1} , which they ascribed to the NH stretch of the NH_3^+ group involved in intramolecular N–H...O hydrogen bonding. Given the difficulty in modeling such structures, they were only able to compare their experimental results to calculated spectra of the dimer, however, the spectra give compelling evidence that the octamer is made up of zwitterionic serine amino acids.

The increasing body of experimental results also allows a classification of the trends that assist zwitterion stabilization. Table 1 shows the spectroscopically confirmed presence for either charge solvation or SB structures as a function of the alkali metal cation size and the gas-phase proton affinity. The results clearly show that an increasing metal cation size favors zwitterion stabilization, as previously suggested by Williams and co-workers (Jockusch, Price, & Williams, 1999). The proton affinity scale has some merit in predicting the stabilization of ZW structures, as the occurrence of zwitterionic arginine and lysine clearly show. Nonetheless, a distinct cut-off for ZW based on PA is more difficult to establish. For instance, proline forms a ZW structure with Na^+ , whereas tryptophan does not, despite both having very similar proton affinities. The ability to make predictions based on the PA is also complicated by the fact that the PA scale itself has come under review a number of times in recent years (Harrison, 1997; Hunter & Lias, 1998; Maksic & Kovacevic, 1999; Mezzache et al., 2005; Bleiholder, Suhai, & Paizs, 2006). Amino acids differ in their side-chains, and thus differ in the types of chelation motifs that can be formed. The binding of the metal cation to the carboxylic acid or carboxylate groups of the amino acid also constrains binding to other groups. For instance, the competition between metal ion binding and intramolecular hydrogen bonding is probably an important structure determining factor. Smaller alkali ions are known to be more polarizing. The size of the alkali metal cation would therefore be expected to affect the number of chelating molecular sites. All of these effects subtly combine to a fine balance between the relative stabilities of CS and ZW structures, which often makes it difficult to predict a priori the most stable conformation.

For alkaline-earth M^{2+} metals a similar behavior has been proposed as for alkali metals, in that the larger cations are thought

TABLE 1. Zwitterionic (ZW) versus non-zwitterionic (NZ) forms of amino acid alkali metal ion complexes as identified by IR spectroscopy

AA	PA ^a (kcal/mol)	Li ⁺	Na ⁺	K ⁺	Rb ⁺	Cs ⁺
Gly	211.4		NZ ^b			
Cys	214.4					
Ala	215.0					
Ser	217.3	NZ ^c	NZ ^c	NZ ^c	NZ ^c	NZ/ZW ^c
Val	217.7					
Asp	218.0					
Leu	218.2					
Ile	218.7					
Thr	218.7	NZ ^d	NZ ^d	NZ ^d	NZ ^d	NZ/ZW ^d
Phe	220.6			NZ ^e		
Tyr	221.2			NZ ^e		
Trp	223.8	NZ ^f	NZ ^f	NZ ^f	NZ ^f	NZ ^f
Met	224.0					
Pro	224.0		ZW ^b			
Asn	224.2					
Glu	225.8					
Gln	231.9	NZ ^g	NZ ^g	NZ ^g	NZ ^g	NZ ^g
His	233.7					
Lys	237.3	NZ ^h	NZ ^h	NZ/ZW ^h		NZ/ZW ⁱ
Arg	253.3	NZ ^j	ZW/NZ ^j	ZW ^j	ZW ^j	ZW ^j

^aBleiholder, Suhai, and Paizs (2006).^bKapota et al. (2004).^cArmentrout et al. (2008).^dRodgers et al. (2008).^ePolfer et al. (2005a).^fPolfer, Oomens, and Dunbar (2006).^gBush et al. (2008b).^hBush et al. (2007a).ⁱBush, Oomens, and Williams (2009).^jBush et al. (2007a,b).

to favor the ZW structure whereas smaller metals do not (Strittmatter, Lemoff, & Williams, 2000). For tryptophan-bound alkaline earth metals this was found to give a poor correlation. Instead, a plot of the relative ZW stability versus complex binding energy gave a surprisingly good correlation (see Fig. 6). This suggests that weakly binding divalent metal cations favor the ZW structure over the CS form. Although it is not clear yet if this correlation is true for all amino acids, recent experiments have shown purely ZW structures for complexes of Ba²⁺ with Arg, Val, Pro, Gln, and Ser (Bush et al., 2008a).

Given the number of IR measurements on zwitterionic/SB structures in the gas phase, the question arises as to whether there is a true “fingerprint IR spectrum of zwitterions” in the gas phase. In basic amino acids a side-chain group is the proton acceptor site. This is also reflected in the vibrational spectrum,

yielding a different IR spectrum for each individual species that is studied. Only a few spectra on non-basic amino acid zwitterionic structures have so far been presented: the serine octamer (Kong et al., 2006) spectrum in the H-stretching region and a series of Ba²⁺-adducted amino acids (Bush et al., 2008a; Dunbar, Polfer, & Oomens, 2007) in the mid-IR region. These spectra come closest to a general fingerprint for gas-phase zwitterionic amino acids, where the proton is transferred from the acid to the amino group. Nonetheless, the choice of the charge carrier and the overall size of the system also affect the positions of the diagnostic bands, as seen in the red-shifted antisymmetric CO₂⁻ mode for the Ba²⁺-bound Trp. Indeed, the position of the asymmetric carboxylate stretching mode was found to be very sensitive to the chelation structure (Bush et al., 2008a) as is also well known from carboxylate salts in condensed-phase

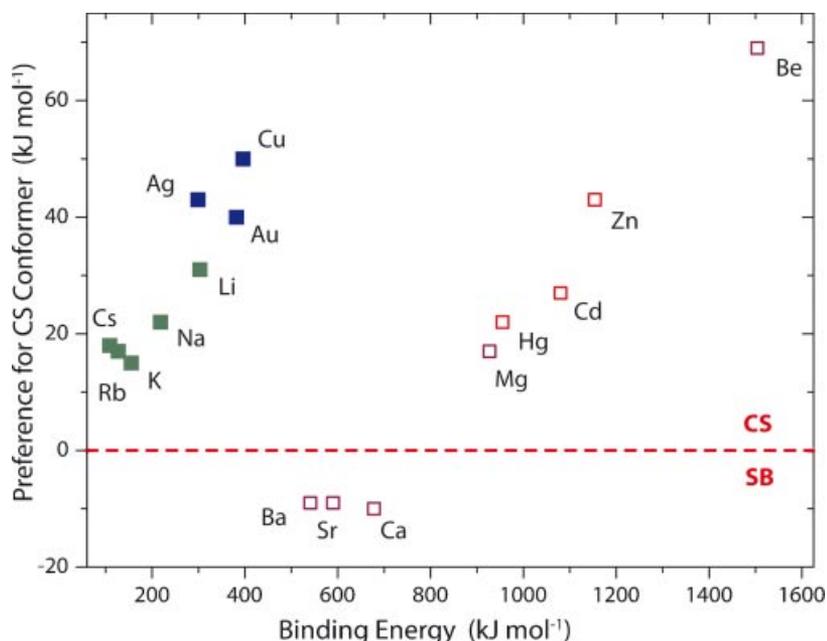


FIGURE 6. Relative preference for the charge solvation (CS) conformer as a function of the binding energy of the complex for Trp. Reprinted with permission from Dunbar, Polfer, and Oomens (2007). Copyright (2007) American Chemical Society. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

spectroscopy (see e.g., Spinner, 1967). Oomens and co-workers addressed this point by studying the inherent symmetric and antisymmetric carboxylate CO_2^- stretch modes of the benzoate anion and substituted variants in the gas phase (Oomens & Steill, 2008).

While IR spectroscopy is the most direct method to identify gas-phase ZW complexes, sometimes caveats remain in the interpretation. The first IR measurements of a putative zwitterionic structure in the gas phase were carried out by Saykally and co-workers on neutral arginine using cavity ring-down spectroscopy (Chapo et al., 1998). Their results suggested that the major constituent of the population was non-zwitterionic, but that some zwitterionic conformers could not be excluded, based on a weak band at 1600 cm^{-1} . Later calculations by Gutowski and co-workers suggested a different interpretation of the vibrational bands, in fact rendering the assignment of the conformers inconclusive (Rak et al., 2001).

B. Site of Proton Attachment on Peptides and Structures of Fragment Ions

In spite of an extended body of experimental and theoretical evidence on relative proton affinities and gas-phase basicities for amino acids (Harrison, 1997; Hunter & Lias, 1998; Bleiholder, Suhai, & Paizs, 2006), the site of proton attachment in a peptide is not always unambiguous, and hence direct structural methods are required. IR spectroscopy is a particularly useful candidate as a structural tool, as the presence of the charge will generally result in a vibration that is characteristic of a particular site of proton attachment and this vibration will generally be intense by virtue

of the charge. All of the published studies on proton-attached peptides have employed ESI or MALDI to generate the ion of interest, with the exception of Vaden et al., who employed a chemical proton transfer technique (MacLeod & Simons, 2004) on a number of peptide-based systems in the $3\text{ }\mu\text{m}$ region (Vaden et al., 2007, 2008a,b).

Desfrancois and co-workers have looked at small peptide systems such as Ala-Ala (see Fig. 7) and Ala-His to show that the site of proton attachment is on the N-terminus for Ala-Ala (Lucas et al., 2004), whereas the imidazole side-chain nitrogen is the preferential site of attachment for Ala-His (Lucas et al., 2005). Moreover, they were able to establish that the neighboring amide NHCO was preferentially in the trans configuration.

Proton attachment to a series of methyl esters of amino acids has been reported by Simon et al. (2007), confirming the amino group as the site of protonation and hydrogen bonding by this group to the ester CO. Their study also includes a useful comparison of MALDI- and ESI-generated ions, though this was in fact carried out in two different mass spectrometers, an FT-ICR and quadrupole ion trap mass spectrometer. It is apparent from their results that the ions generated by MALDI have higher internal energies, which is manifested by the broader and slightly red-shifted bands. Wu and McMahon have also recently investigated the site of proton attachment in GlyGlyGly and AlaAlaAla by mid-IR spectroscopy (Wu & McMahon, 2007b), showing that a substantial proportion of the ions carry the proton on the amide carbonyl. This is contrary to the expectation that solely the N-terminus carries the proton for room-temperature ions.

In the pentapeptide Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) the N-terminus was identified as the main site of proton

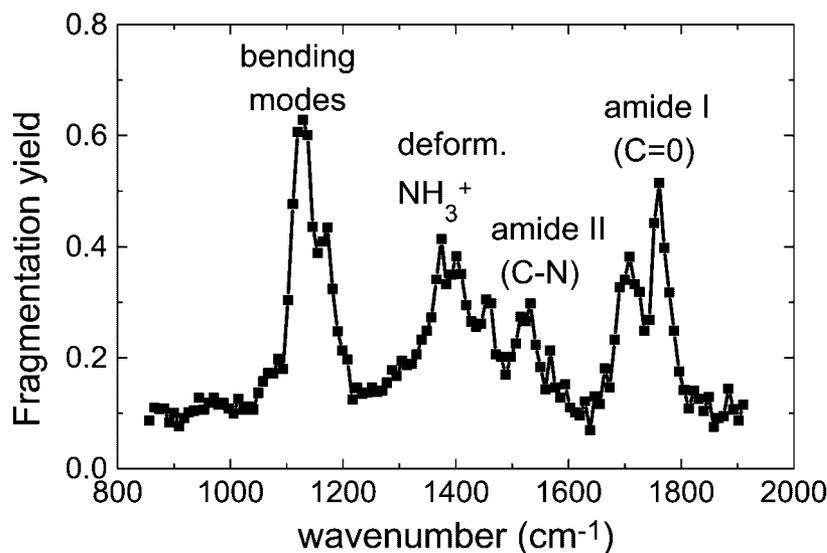


FIGURE 7. IR photodissociation spectrum of protonated dialanine, showing the chemically diagnostic bands. The NH_3^+ deformation mode establishes the site of proton attachment as the *N*-terminal amino group. The position of the amide I $\text{C}=\text{O}$ stretch mode is in accord with a *trans* conformation of the neighboring amide group. Reproduced from Lucas et al. (2004), by permission of the PCCP Owner Societies, copyright 2007 (“dx.doi.org/10.1039/b316053j” or “www.rsc.org/pccp/Publishing/Journals/CP/article.asp?doi=b316053j”).

attachment by Polfer and co-workers (Polfer et al., 2007b), though a small proportion of proton attachment to the carbonyl oxygens could not be excluded. Activation of this ion by collisions yields abundant b_4 and a_4 product ions. An IR study of these CID product ions gave insights into the chemistry of the dissociation mechanism (Polfer et al., 2005b, 2007b). In the case of the b_4 ions, there is compelling evidence that oxazolone ring structures were formed, based on the diagnostic CO stretch bands of the oxazolone ring. These bands fall into a region of the spectrum higher in frequency than the conventional amide I (CO stretch) at 1700 cm^{-1} , where generally no other bands are encountered, and their identification is therefore unambiguous. The exact position of this band depends on the site of proton attachment and the secondary structure of the fragment and multiple structures were observed to coexist. The IR spectrum also indicates the presence of a fully cyclic structure, which displays a diagnostic band at 1440 cm^{-1} , that is not predicted for the oxazolone-type structures. However, as this region of the spectrum is congested, the assignment is less secure than for the oxazolone-type structures.

For the a_4 fragment, the presence of a fully cyclic structure is more convincing, as there are two diagnostic bands that are not predicted by the linear imine structure: the out-of-plane bending mode at 1400 cm^{-1} and the in-plane bending mode at 1600 cm^{-1} of the NH_2^+ group (cyc labeled bands in Fig. 8). Overall, the spectrum suggests a mixture of linear imine and cyclic structures for a_4 . This has recently been supported by ion mobility results by Polfer et al., where a distribution of structures was observed (Polfer et al., 2008). Cyclic fragment structures can give rise to non-direct sequence ions that do not reflect the original amino acid sequence (Harrison et al., 2006). This raises a concern for using tandem mass spectrometry in the identification of proteins and may explain the high abundance of unidentified peaks in

proteomics CID mass spectra of tryptic digest peptides (Simpson et al., 2000). Chamot-Rooke and co-workers have recently employed IR spectroscopy to identify the chemical structures of peptide ECD “c” fragments, showing that an amide structure is formed (Frison et al., 2008), as opposed to the enol imine structure originally proposed by McLafferty and co-workers (Zubarev, Kelleher, & McLafferty, 1998).

C. Vibrationally Resolved Electronic Spectra

In UV photodissociation spectroscopy (UV-PD) of an aromatic ion, absorption of a UV photon causes excitation to a vibronic level of the $S_1\ \pi\pi^*$ electronic state. In amino acids, it has been shown that this can be followed by intersystem crossing to the dissociative $\pi\sigma^*$ state, resulting in fragmentation of the ion (Kang et al., 2004, 2005). Alternatively, internal conversion to the ground electronic state can take place, resulting in vibrationally excited ground-state ions that dissociate in a number of pathways (El Aribi et al., 2004). The UV-PD spectrum is also obtained by monitoring the photodissociation yield as a function of wavelength, but generally absorption of a single photon is sufficient to induce dissociation. For some small organic radical compounds, it had been shown that vibrationally resolved UV-PD spectra could be obtained, even for 300 K ions, as reviewed by Dunbar (2000). For floppy biomolecules, no such vibrationally resolved UV-PD spectra have been reported for room-temperature ions. Attempts by Weinkauff and co-workers in cooling TrpH^+ in a Paul trap to liquid nitrogen temperature also gave rise to broad electronic spectra (Nolting, Marian, & Weinkauff, 2004). However, Rizzo and co-workers have recently shown that cooling TyrH^+ close to liquid He temperature (estimated $T \sim 6\text{ K}$) in a 22-pole rf ion trap based on the design by

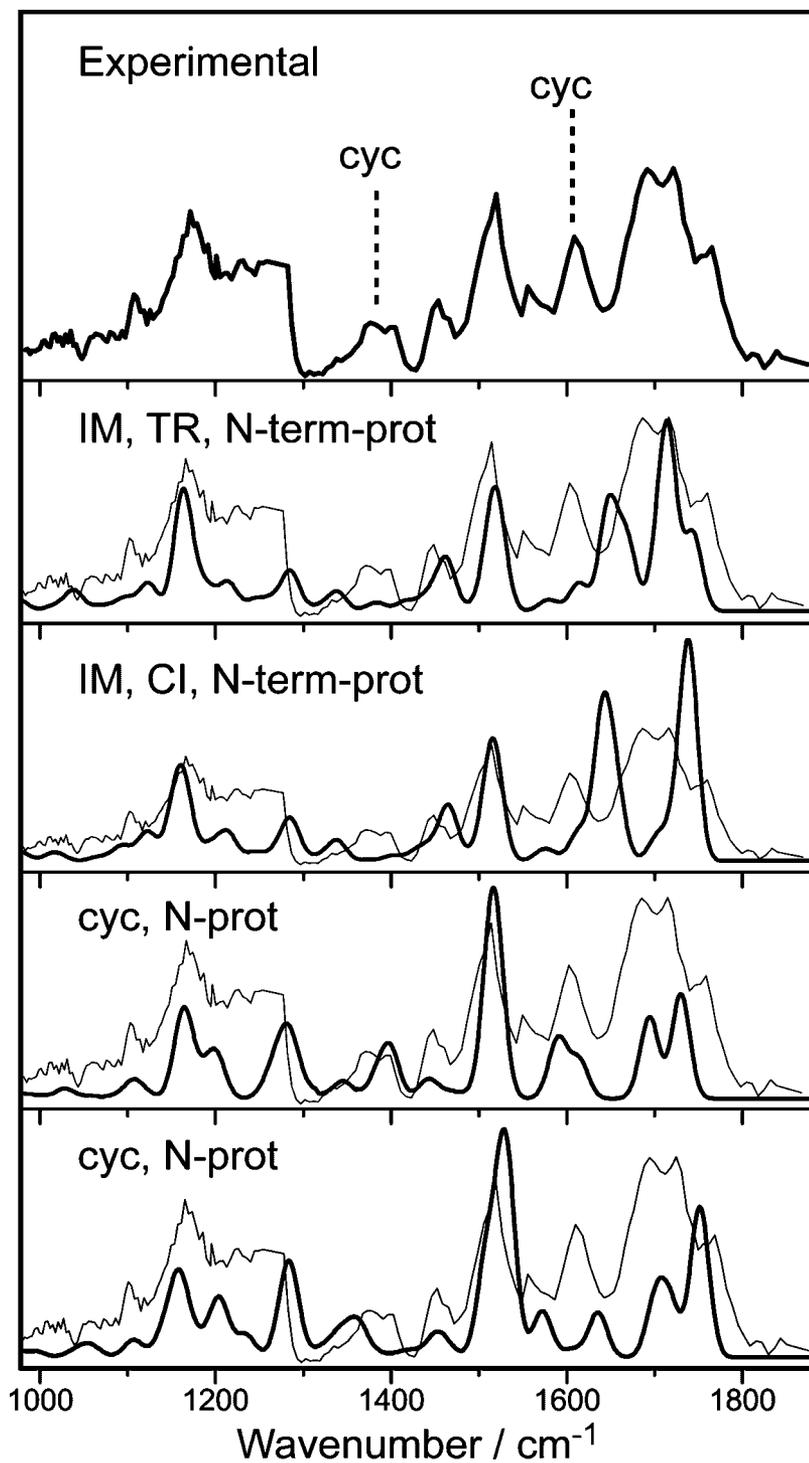


FIGURE 8. IR-MPD spectrum of the a_4 CID fragment ion of Leu-enkephalin compared to calculated spectra for linear imine (IM) structures and cyclic (cyc) structures. The diagnostic modes for the cyclic structure at 1400 (out-of-plane NH_2^+ bend) and 1600 cm^{-1} (in-plane NH_2^+ bend) are labeled (cyc). (Data taken from Polfer et al., 2007b.)

Gerlich and co-workers (Gerlich, 1993; Wang et al., 2003) yields a UV-PD spectrum with extremely narrow bands (FWHM $\sim 2.7 \text{ cm}^{-1}$) (Boyarkin et al., 2006).

Surprisingly, UV-PD of liquid He cooled TrpH^+ still resulted in unresolved, broad spectral features. This suggests fast, non-radiative decay of the excited state for TrpH^+ (Boyarkin et al., 2006). Later solvation experiments on TrpH^+ confirmed that attachment of two water molecules extends the life-time of the electronic state, thus resulting in beautifully vibrationally resolved UV-PD spectra for $\text{TrpH}^+(\text{H}_2\text{O})_2$ (Mercier et al., 2006).

Rizzo and co-workers have recently employed UV-PD in combination with IR spectroscopy (Stearns et al., 2007b), in analogy to IR ion-dip spectroscopy for neutrals (Snoek et al., 2000; Nir et al., 2001; Zwier, 2001; Bakker et al., 2003; Chin et al., 2005). In this IR–UV double resonance depletion spectroscopy technique an IR laser is crossed with the ion cloud prior to a fixed UV laser wavelength irradiation: resonant IR absorption causes depopulation of the vibrational ground state for a fraction of the ions, thus resulting in a dip of the UV-induced photodissociation signal. In essence, the UV laser allows selection of a particular conformer; the conformer-selective IR spectrum can be recorded by monitoring the dip in the UV dissociation as a function of IR wavelength. They have recently applied this approach to study larger peptide-based systems to investigate α -helix formation in alanine-rich peptides with C-terminal lysine, as originally proposed by Jarrold and co-workers (Hudgins, Ratner, & Jarrold, 1998; Hudgins & Jarrold, 1999). Figure 9 presents the many N–H stretch bands that can be resolved for different UV-selected conformers (Stearns, Boyarkin, & Rizzo, 2007). Their results are consistent with gas-phase α -helical structures for these compounds, as indicated by the N–H...O=C hydrogen bonding interactions in the stick structure. Although the N–H stretch is effectively a local oscillator, the extent of hydrogen bonding strongly affects its frequency. This effectively restrains the types of conformations that are possible.

A drawback of UV-PD as opposed to direct IR spectroscopy is related to life-time issues of the electronic state, which can give rise to broadening. UV-PD also requires the presence of a UV chromophore, thus restricting the study to aromatic-containing systems (e.g., tryptophan, tyrosine, or phenylalanine). Nonetheless, UV-PD spectroscopy offers considerable advantages over “conventional” IR-MPD spectroscopy in that absorption of a single photon is sufficient to induce a hole burning signal. This eliminates the problem of anharmonic broadening and gives rise to relatively high-resolution IR spectra. Moreover, particular conformers can be selected by fixing the UV laser wavelength, thus allowing conformer-specific IR spectra to be obtained.

D. Metal–Ligand Interactions

1. Ion-Molecule Complexes and Metal Clusters

Determining the geometric and electronic structure of (transition) metal ion ligand complexes has been one of the most successful applications of IR photodissociation spectroscopy in recent years. Such complexes are not only interesting from a

biological viewpoint, as discussed further below, but also in catalysis and cluster sciences. Standard spectroscopic methods have been unable to answer a number of basic structural questions because the coordination environment is often not well defined in condensed phase samples. Performing IR spectroscopy on mass-selected species on the other hand often provides more unambiguous answers. From an experimental point of view, typical binding energies are on the order of one or a few eV, which makes the complexes stable in an ion trap mass spectrometer and at the same time amenable to IR multiple photon dissociation.

Coordination of Fe^+ and other transition metal (TM) ions to unsaturated hydrocarbons has been investigated at the CLIO FEL by Maître and co-workers (Simon et al., 2004; Bakker et al., 2007). Another early study involving the CLIO FEL focused on complexes of Nb^+ with acetonitrile (Reinhard et al., 2004). Depending on the cluster size, spectroscopic evidence for a reductive nitrile coupling reaction was found. Reactants and products on a ketone decarbonylation reaction path induced by CO^+ for cobalt were spectroscopically characterized by Dunbar, Moore, and Oomens (2007).

Heterogeneous catalysts often consist of small TM clusters deposited on a metal oxide support. IR action spectroscopy can be used to obtain fundamental insights into the catalytic properties of these systems by studying the systems in complete isolation in the gas phase. Fielicke and co-workers employed the far IR radiation of FELIX to study the structure of cationic pure metal clusters (Fielicke et al., 2004a). Individual cationic vanadium clusters in the size range from 6 to 23 were shown to possess very distinctive far-IR spectra, indicating that the clusters each have very different structures and symmetries (Ratsch et al., 2005). The binding of small reactant molecules, in particular CO, is often used to probe cluster characteristics by recording shifts in the CO stretch vibration. Clear trends with charge and size have been observed for cationic, neutral as well as anionic Rh clusters, in particular whether CO coordinates to 1, 2, or 3 metal atoms, for individual metal cluster sizes ($n = 4–15$) (Fielicke et al., 2004b). Vanadium oxide based catalysts are used in the production of various bulk chemicals, which has motivated several studies into the fundamental structures of vanadium oxide clusters ranging in size from VO_2 to V_8O_{20} , as recently reviewed by Asmis and Sauer (2007). Most gas-phase IR studies on bare vanadium oxide clusters (cationic and anionic) have been performed by the group of Asmis (Brummer et al., 2003; Asmis & Sauer, 2007). Fielicke and co-workers investigated the complexation of metal oxide cluster cations with small hydrocarbon molecules in view of their use as oxidation catalysts (Fielicke et al., 2003).

Cation– π interaction between aromatic molecules and TM ions has been intensively studied and gas-phase IR spectroscopy provides an ideal tool to study the geometric as well as electronic structures of these systems. The spin state on the metal has a profound influence on the structure of the complex and hence on its vibrational frequencies, so that spin promotion induced by the ligand field can actually be probed by IR spectroscopy. Duncan and co-workers studied complexes of the first-row TMs with one and two benzene ligands (Jaeger et al., 2004b; Duncan, 2008). They have also investigated metal cation– π binding to acetylene (Walters et al., 2005b) and binding to carbonyls (Velasquez et al., 2006). Competition between ring binding and heteroatom binding of functionalized aromatics to TM ions has been

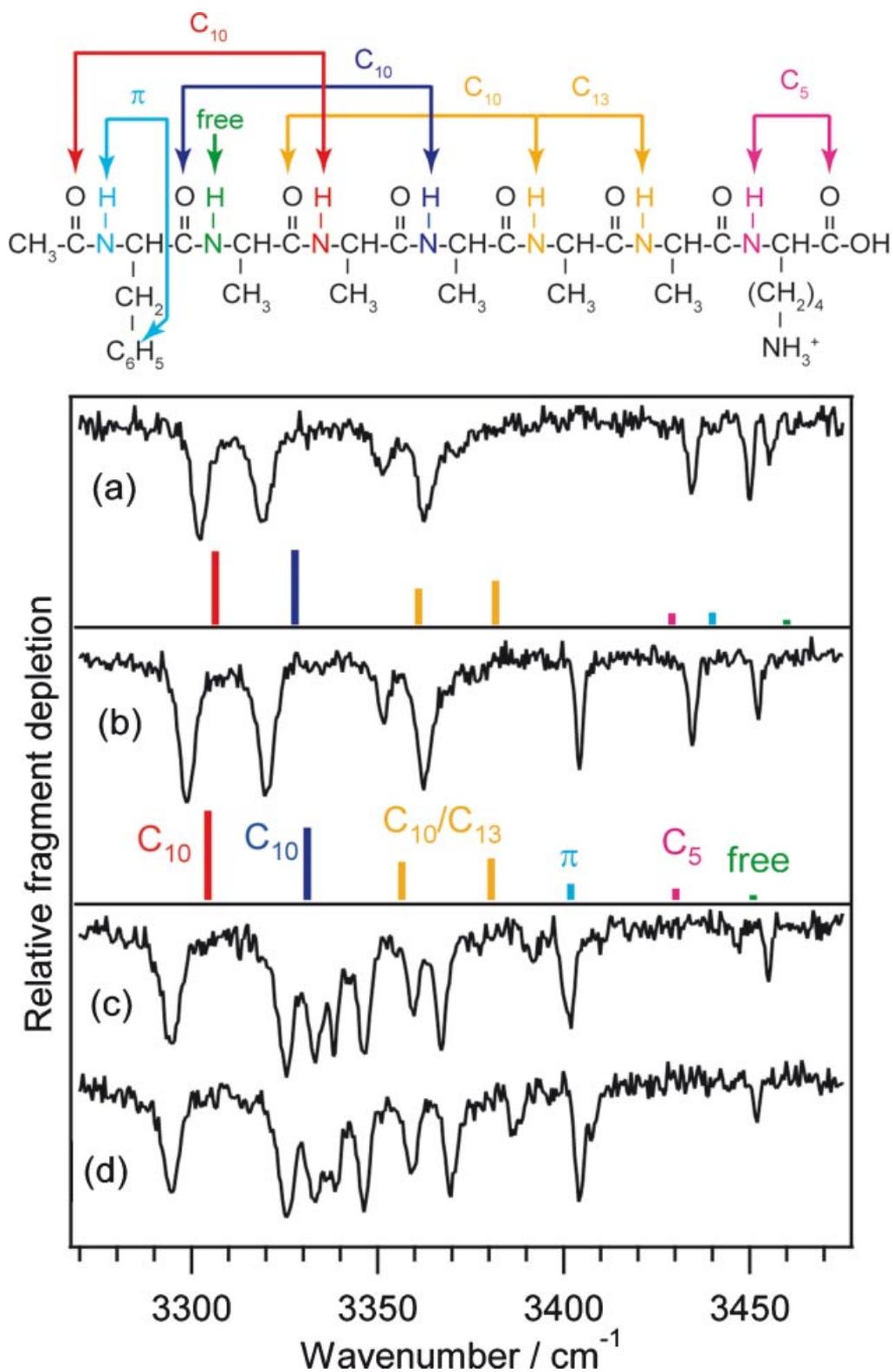


FIGURE 9. IR-UV double resonance depletion spectra for UV-selected conformers of Ac-Phe-(Ala)₅-Lys-H⁺ (a and b) and Ac-Phe-(Ala)₁₀-Lys-H⁺ (c and d). The stick spectra in the panels a and b were calculated using B3LYP/6-31G** with a scaling factor of 0.952. The peaks are labeled by the type of interaction and correspond to the color-coded oscillators and hydrogen-bonding patterns shown schematically above. Reprinted with permission from Stearns, Boyarkin, and Rizzo (2007). Copyright (2007) American Chemical Society. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

addressed by Dunbar and co-workers (Oomens et al., 2004b; Moore et al., 2005). IR spectra of complexes of polyaromatic molecules with Fe^+ were recorded to evaluate their astrophysical relevance (Simon et al., 2008; Szczepanski et al., 2006).

Coordination around *f*-block metals is of importance to understand for instance the proliferation of these toxic metals in ground water, but presents a true challenge to current quantum chemical methods. Determining the structure of a variety of such complexes via IR ion spectroscopy has been carried out by Groenewold and co-workers in collaboration with several computational experts including Visscher and others (Groenewold et al., 2006, 2008).

2. Metal–Ligand Interactions of Biological Relevance

The importance of metal ions in biology can hardly be overestimated, whether as catalytic sites in enzymes or for structural selectivity (Finney & O'Halloran, 2003). Metal–ligand complexes are inherently ionic and can be conveniently produced by ESI. Smaller gas-phase complexes lend themselves very well as model systems to state-of-the-art DFT calculations, where effects can be studied in isolation. In particular *biomimetic* complexes are well suited for this approach, as they display biological activity, while also being of a sufficiently small size to be considered by gas-phase IR spectroscopy.

Dunbar and co-workers investigated the zinc amino acid dimer complex $[\text{Zn} + \text{Phe}_2\text{-H}]^+$, which is de-protonated on one of the Phe carboxylic acids (Polfer et al., 2006a). This model system serves as a simple analogue to the competing binding sites available to a metal in a protein. In spite of the small nature of the complex, a large number of binding motifs can be envisaged, involving chelation to the acid O's, amino N's and the π -cloud of the aromatic side-chain. The theoretical structures predict significant differences in the calculated IR spectra, and merely the lowest-energy conformation, which is in a tetrahedral-type chelation, is consistent with all of the vibrational modes in the spectrum. This is also consistent with the general preference of Zn^{2+} to form tetrahedral binding sites in proteins (Berg & Shi, 1996).

E. IR Spectra of Proteins

McLafferty and co-workers (Oh et al., 2002) and von Helden and co-workers (Oomens et al., 2005) have tried to push the barriers of how large a molecule can be to still be able to measure its IR spectrum in the gas phase. McLafferty's group carried out IR photodissociation experiments on bovine ubiquitin ions. Photodissociating such a large molecule is not trivial and hence they actually carried out these experiments on protein ions that had captured an electron, but had not dissociated, the so-called odd-electron “reduced” $[\text{M} + n\text{H}]^{(n-1)+}$ ions. They observed a relatively broad band centered at $\sim 3350 \text{ cm}^{-1}$, but the assignment of this band is difficult for lack of a suitable comparison.

Von Helden and co-workers measured the charge-state resolved mid-IR spectra ($1400\text{--}1800 \text{ cm}^{-1}$) of bovine cytochrome *c* (104 aa residues, 12.5 kDa) using K^+ tagging and were able to resolve three bands over this region (see Fig. 10). Of these, the amide I band (CO stretch) is routinely used in solution-phase

studies to determine the secondary structure of a protein. The central position of the amide I band in the gas phase at 1660 cm^{-1} is close to where it appears in solution for an alpha-helical structure ($1648\text{--}1658 \text{ cm}^{-1}$). For the higher charge states (15^+ and 16^+) a blue-shift of this band is observed, which is consistent with reduced hydrogen bonding. While the amide II band (NH bend) at 1535 cm^{-1} does not display an interesting behavior as a function of charge state, the third band at 1483 cm^{-1} is more intriguing. This band shows a striking increase in intensity upon going to higher charge states. This band is still awaiting assignment, for lack of a solution-phase equivalent.

Despite the current inability to interpret these spectra in detail, these proof-of-principle experiments have shown that IR spectra of even such large molecules can be recorded. Moreover, only macromolecules such as proteins could realistically conserve solution-phase elements upon their transfer in the gas phase, as the barriers of structural interconversion are high. As merely the condensed-phase structure is really relevant to biologists, this is an important aspect in terms of defining the relevance of these measurements for the wider biological scientific community.

V. IR SPECTROSCOPY ON SOLVATED COMPLEXES

Microsolvation experiments, where clusters with a known number of solvent molecules are structurally characterized, are a promising avenue to bridge the gap between gas-phase experiments and the actual biological environment. These experiments may thus shed more light on the imperative question of the relevance of intrinsically gas-phase MS methods for true *in vivo* biomolecular systems. Here, a number of solvated molecular complexes are reviewed, with relevance to zwitterion stabilization, as well as metal ion and proton solvation.

A. Zwitterion Stabilization

The transition from an isolated non-zwitterionic amino acid in the gas phase to a zwitterion in (aqueous) solution can be conveniently investigated in microsolvation experiments. Simons and co-workers presented IR–UV hole-burning spectra on neutral singly hydrated tryptophan in the $100\text{--}1800 \text{ cm}^{-1}$ (Carcabal et al., 2004), as well as the $2800\text{--}2900 \text{ cm}^{-1}$ spectral ranges (Snoek, Kroemer, & Simons, 2002). Brutschy and co-workers extended this to singly and doubly hydrated phenylalanine (Ebata et al., 2006). In these smaller clusters there was little evidence for the presence of zwitterionic structures. Oomens and co-workers studied a complete series of tryptophan–water and tryptophan–methanol clusters with up to nine solvent molecules by IR ion dip spectroscopy in the mid-IR range (Blom et al., 2007). These results suggest that 5–6 water molecules are required for the zwitterionic structure to become competitive in energy with the non-zwitterionic form. Although the behavior of Trp appears to be similar in water and in methanol, the results for methanol are more convincing, showing the appearance of a small, yet distinct, band at 1650 cm^{-1} starting at five solvent molecules. This band is consistent with the

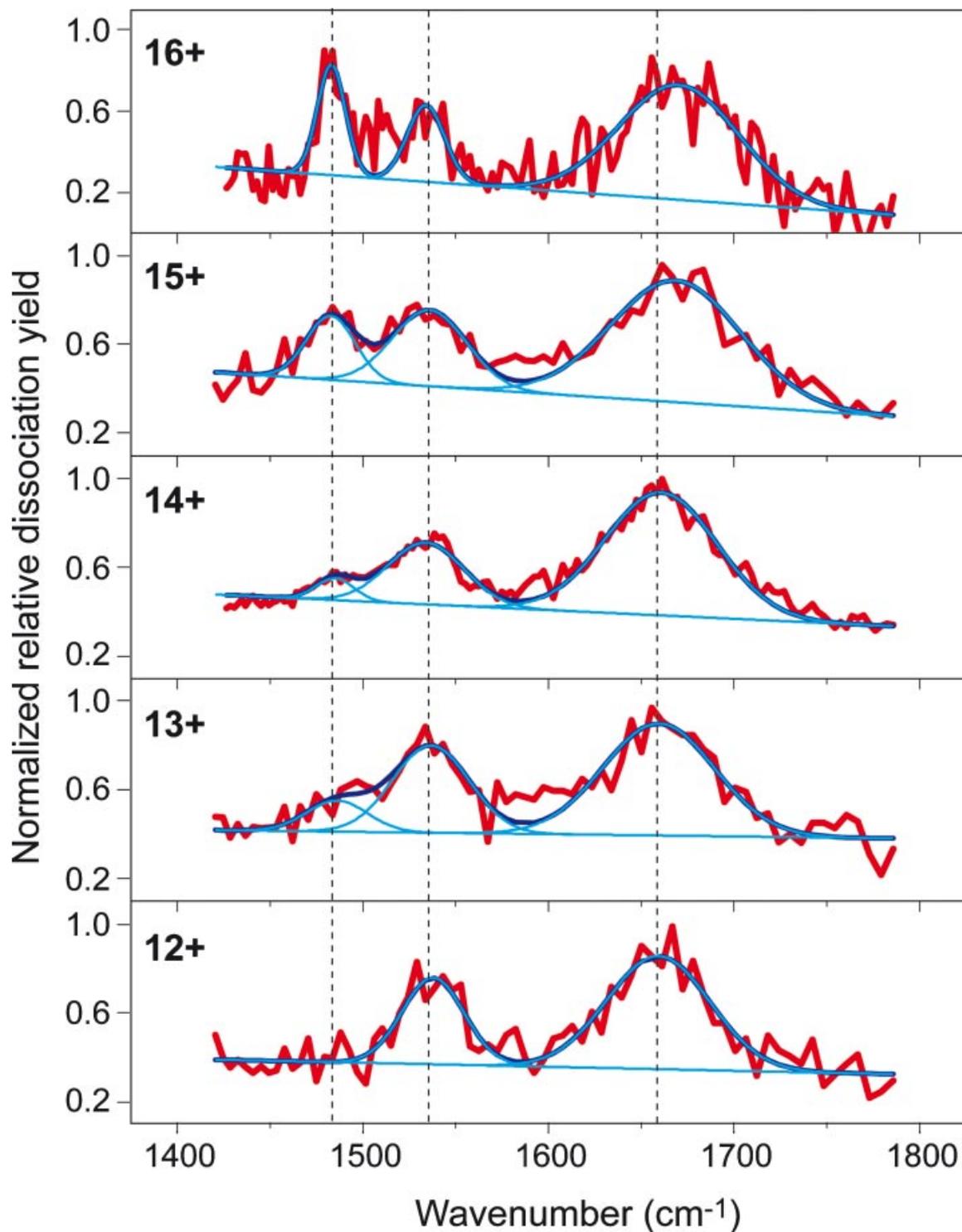


FIGURE 10. IR-MPD spectra of a series of charge states of bovine cytochrome *c* from 12+ to 16+, showing the labeled vibrations amide I (1660 cm^{-1}), amide II (1535 cm^{-1}) and unassigned mode (1480 cm^{-1}). Reproduced from Oomens et al., 2005, by permission of the PCCP Owner Societies, copyright 2005. (“[dx.doi.org/10.1039/b502322j](https://doi.org/10.1039/b502322j)” or “www.rsc.org/pccp/Publishing/Journals/CP/article.asp?doi=b502322j”) [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

antisymmetric carboxylate stretch band in the FTIR spectrum of Trp in solution. Nonetheless, the non-zwitterionic form, identified by the carboxylic acid CO stretch band at 1690 cm^{-1} , is dominant at five solvent molecules, and remains important even at nine methanol molecules. This suggests a very gradual transition from the non-zwitterionic form to the zwitterion. By comparison to the previous section, it appears that a single metal di-cation (e.g., Ba^{2+}) is much more potent at stabilizing the zwitterionic form of tryptophan than nine polar solvent molecules.

Rizzo and co-workers carried out an IR study on lithiated valine–water clusters (Kamariotis et al., 2006). Even four water molecules did not result in the appearance of zwitterions, in fact contradicting earlier findings based on BIRD (Lemoff & Williams, 2004). The BIRD study had suggested that zwitterions were formed upon the addition of a third water molecule, based on the computed binding energies. The IR spectrum is a more direct probe of the structure than BIRD measurements. Nonetheless, the discrepancy could also be due to differences in the internal energy of the ions, the time-scale of the experiment and, importantly, differences in the ion formation; it is unclear whether the complexes are formed via evaporation of larger droplets generated in the nanospray or by condensation onto the bare ion (Rodriguez-Cruz, Klassen, & Williams, 1999).

B. Solvated Metal Cations

Lisy and co-workers investigated size-selected clusters containing benzene and water complexed to the alkali metals sodium and potassium (Cabarcos, Weinheimer, & Lisy, 1999). These clusters serve as model systems for the selectivity of potassium over

sodium for K^+ ion channels in living cells. Since Na^+ has a smaller cation size than K^+ it is unexpected that the ion channel selectively lets the larger K^+ pass through. These channels are rich in aromatic amino acids, suggesting that cation– π interactions are important in explaining the selectivity (Dougherty, 1996). Gas-phase IR measurements on $\text{M}^+(\text{C}_6\text{H}_6)_n(\text{H}_2\text{O})_m$ complexes, where $M = \text{Na}$ or K , are shown in Figure 11. Whereas the Na^+ complexes display narrow bands, the K^+ complex IR bands are broad. This is consistent with extensive water–water hydrogen bonding in the case of K^+ and shows that the cation– π interaction of K^+ with benzene is more important than K^+ interactions with water. In other words, K^+ is dehydrated to form cation– π interactions. In contrast, the cation– π interactions of Na^+ with benzene are weaker, leading to an intact $\text{Na}^+(\text{H}_2\text{O})_4$ inner solvation shell. In terms of the potassium ion channel, while K^+ is de-solvated in the vicinity of the ion channel and can pass through, the solvated $\text{Na}^+(\text{H}_2\text{O})_4$ remains intact and is too large to pass through the channel. This demonstrates that small changes in the binding affinity to competing ligands affect the selectivity of molecular machines, such as ion channels.

Lisy and co-workers have considered other mixed complexes containing an alkali metal chelated by water molecules and a competitive ligand, such as hexafluorobenzene (Patwari & Lisy, 2003), para-difluorobenzene (Miller & Lisy, 2006a), indole (Miller & Lisy, 2006b) and *n*-methylacetamide (Miller & Lisy, 2007). Such systematic studies can yield detailed information on the preferred chelation and solvation motifs that are formed.

The groups of Lisy and Duncan have studied other solvated alkali and alkaline earth metal complexes using laser evaporation and beam expansion set-ups (Lisy, 1997; Vaden, Forinash, & Lisy, 2002; Walker et al., 2003). Williams and co-workers have generated Ca^{2+} -water clusters with up to 10 solvent

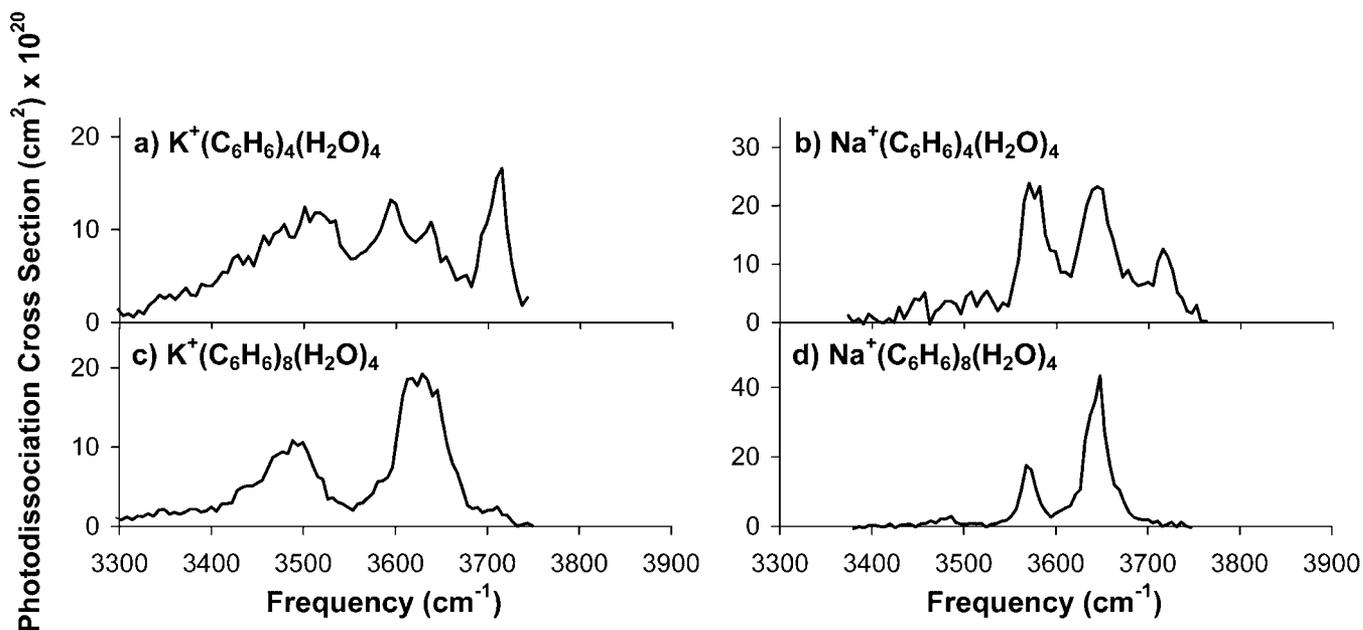


FIGURE 11. IR photodissociation spectra of (a) $\text{K}^+(\text{C}_6\text{H}_6)_4(\text{H}_2\text{O})_4$, (b) $\text{Na}^+(\text{C}_6\text{H}_6)_4(\text{H}_2\text{O})_4$, (c) $\text{K}^+(\text{C}_6\text{H}_6)_8(\text{H}_2\text{O})_4$, and (d) $\text{Na}^+(\text{C}_6\text{H}_6)_8(\text{H}_2\text{O})_4$. Reused with permission from Orlando M. Cabarcos, Corey J. Weinheimer, and James M. Lisy, *Journal of Chemical Physics*, 110, 8429 (1999). Copyright 1999, American Institute of Physics.

molecules by ESI (Bush, Saykally, & Williams, 2007). IR spectroscopy of these mass-selected clusters yields information on the primary and secondary solvation shells around the cations. Solvation of TM ions by water and various other small molecules has been investigated in a molecular beam in the group of Duncan (Walters, Pillai & Duncan, 2005; Walters et al., 2005a; Velasquez et al., 2006; Pillai, Jaeger, & Duncan, 2007). As TM-solvent binding energies are much higher than in their alkali metal counterparts, photodissociation becomes more difficult to achieve on intact complexes. The experimental strategy for these systems therefore involves rare gas tagging, such as argon, to apply the “messenger” technique. On the computational front, a large number of structures have to be considered for solvated metal complexes. Nonetheless, at least qualitative agreement between experiment and theory is now achievable (Kolaski et al., 2007).

C. Hydrated Proton Complexes

Protons play a central catalytic role at the cellular level, for instance in proton-mediated enzymatic reactions (Gerlt et al., 1997). It is necessary for cells to regulate their internal pH using proton-pumps and thus maintain a membrane potential. Many of the mechanistic aspects of these pumps are only partially understood (Oesterhelt et al., 2000; Kuhlbrandt, Zeelen, & Dietrich, 2002). The fact that nature can control proton transfer is all the more impressive since protons display anomalously high mobilities in aqueous solutions that are thought not to be limited by diffusion processes. Although the exact proton transfer mechanism is still being debated, protons are thought to migrate by concerted proton “hopping” or “tunneling” (Huckel, 1928; Bernal & Fowler, 1936; Stearn & Eyring, 1937), based on the original hypothesis by Grotthuss more than 200 years ago (Grotthuss, 1806). Hence, there is much interest in understanding the hydrogen bonding structures that protons form with surrounding water molecules or other ligands. The elemental structures in these hydrogen-bonded networks are believed to be “*Eigen*” (hydronium H_3O^+ , the *Eigen* core) (Eigen & Demeyer, 1958; Eigen, 1964) or “*Zundel*” structures ($\text{H}_2\text{O} \cdots \text{H}^+ \cdots \text{OH}_2$) (Zundel, 1976). A large number of studies have addressed the structures of the solvated proton present in bulk water, and it is beyond the scope of this review to discuss those here.

In the gas phase small solvated proton clusters can be formed and mass selected so that they can be studied individually by IR spectroscopy. These results can then be compared to high-level theoretical spectra for a structural assignment. The first gas-phase IR spectra of water clusters were recorded by Schwarz more than 30 years ago (Schwarz, 1977). In those experiments the relative contributions of individual water clusters were approximately controlled by adjusting the vapor pressure. The superimposed absorption spectra of different clusters and the relatively low resolution room-temperature spectra only allowed the general trends towards higher clusters to be observed.

The first IR spectra of mass-selected hydrated hydronium clusters were obtained 20 years ago by Lee and co-workers (Okumura et al., 1986; Yeh et al., 1989). Although these clusters were cold, the IR spectra in the OH stretching range did not allow

identifying either the *Eigen* or *Zundel* structural motifs. Even a recent full-dimensional quantum DMC calculation by McCoy and co-workers (McCoy et al., 2005) based on a new *ab initio* potential energy surface (Huang, Braams, & Bowman, 2005) did not yield a satisfactory match with the experimental spectrum.

The much wider tuning range of the FEL made it possible to extend the measured IR spectra in the mid-IR range. Asmis et al. measured the IR-MPD spectrum of the protonated water dimer *Zundel* ion in the range from 620 to 1900 cm^{-1} (Asmis et al., 2003), where structurally important modes, such as the proton asymmetric stretch, are present. Based on 4D quantum-chemical calculations by Sauer and a deuteration experiment they assigned the asymmetric stretch to the peak at 1317 cm^{-1} . In this C_{2v} structure the proton is shared equally between the two water molecules. The very flat potential makes it very challenging to calculate the vibrational modes accurately, especially in the case of the asymmetric proton-bound mode: the low mass of the proton results in large-amplitude motions, which are difficult to calculate accurately and which tend to couple easily with other local modes. A further complication concerns the spectrum of H_5O_2^+ recorded 1 year later at the CLIO FEL facility (Fridgen et al., 2004), which curiously differs from the spectrum by Asmis et al. Among others, the low-frequency bands are blue-shifted by 70–120 cm^{-1} , relative to the spectrum measured by Asmis et al. These discrepancies could be due to differences in the ion generation and possibly the ion temperature, which was room temperature in the latter experiments. The IR spectrum of Fridgen et al. showed a reasonable agreement with the harmonic DFT-calculated spectrum, suggesting that the lower-frequency 990 cm^{-1} band can be assigned to the asymmetric proton stretch vibration.

More recently, the group of Johnson and co-workers managed to record the IR spectrum of rare-gas tagged H_5O_2^+ (Hammer et al., 2005), using frequency mixing in AgGaSe_2 crystals, as originally pioneered by Gerhards, Unterberg, and Gerlach (2002). They confirmed the low-frequency bands at 928 and 1047 cm^{-1} , in analogy to the results of Asmis et al.

While the messenger technique used by Johnson and co-workers has the advantage that merely one photon is required to induce photodissociation, the presence of the rare gas atom disturbs the symmetry of the structure. This effect is clearly observed for Ar, and is amplified when multiple Ar atoms are added (Headrick, Bopp, & Johnson, 2004; Park, Shin, & Kim, 2007). Though Ne also changes the symmetry of the system, the binding energy and the inherent influence on the structure is less than for Ar. Moreover, the calculations suggest that the rare gas atom binds to the non-bonded water OH groups, thus not interfering with the proton itself. A full-dimensional diffusion Monte-Carlo and multimode/vibrational configuration interaction (MM/VCI) on bare H_5O_2^+ yielded a convincing agreement for the asymmetric high-intensity proton-bound mode around 1000 cm^{-1} and the lower-intensity water binding modes around 1800 cm^{-1} (McCoy et al., 2005). Nonetheless, the doublet feature around 1000 cm^{-1} , which has so far not been reproduced successfully by the calculations, remains an open question.

Johnson and co-workers also recorded a continuous series of solvated proton clusters in the range from $\text{H}^+(\text{H}_2\text{O})_{2-11}$ (Headrick et al., 2005) (see Fig. 12). Remarkable differences

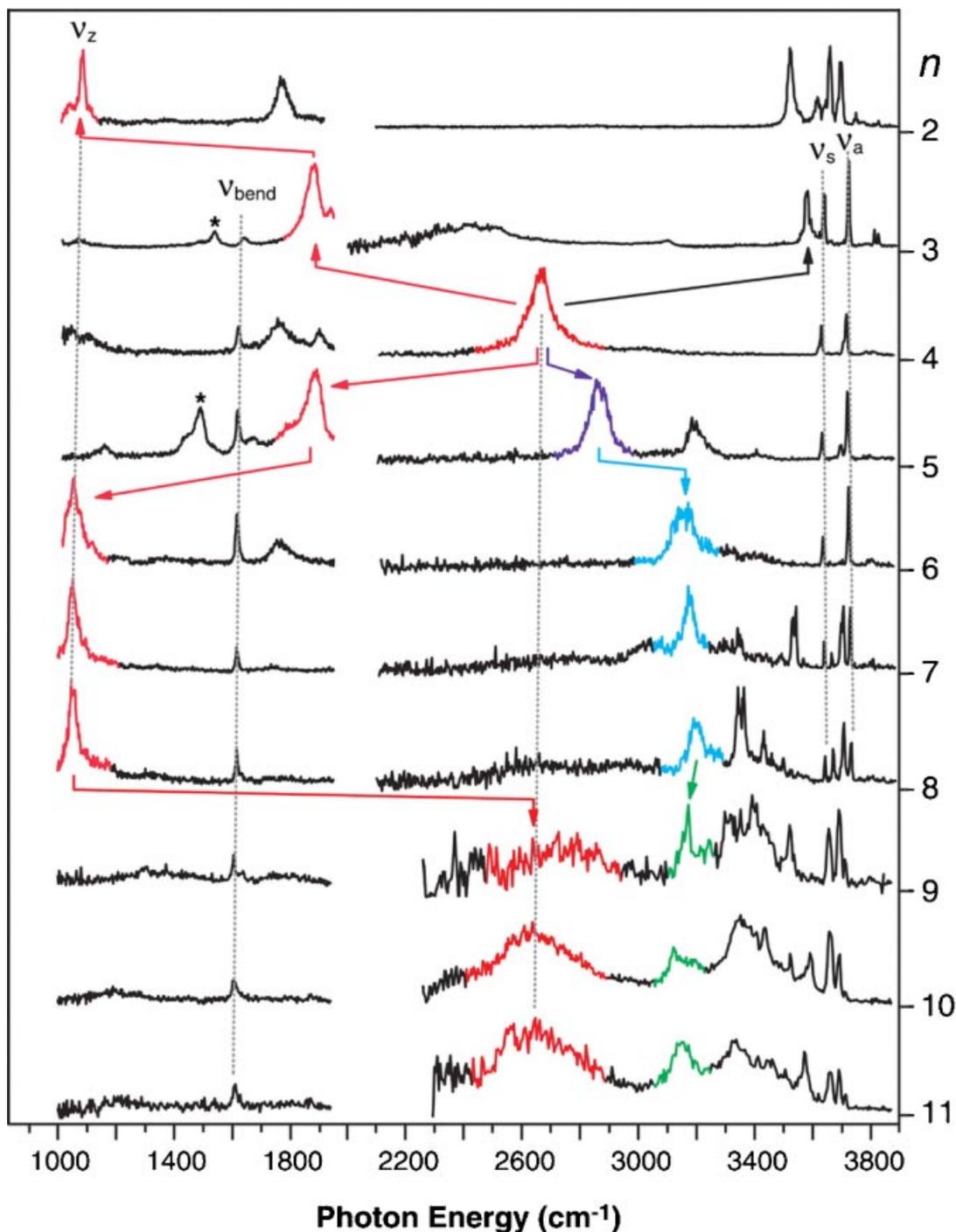


FIGURE 12. Argon pre-dissociation spectra of $\text{H}^+(\text{H}_2\text{O})_n$, $n = 2-11$. The bands most closely associated with the motions of H atoms bearing excess charge are highlighted in red. Bands derived from the OH stretches bridging the *Zundel* ion to the first hydration shell are highlighted in blue, whereas the analogous bands involving the *Eigen* ion are indicated in green. The purple band for $n = 5$ is assigned to the OH stretches of the H_3O^+ ion bound to two single acceptor water molecules. The spectral interpretation predicts the *Eigen* motif for $n = 3-5$ and $9-11$, whereas the *Zundel* motif is observed for $n = 2$ and $6-8$. From Headrick et al. (2005), reprinted with permission from AAAS, copyright 2005. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

between the spectra in the series can be seen, showing that the *Zundel* and *Eigen* structural motifs are recognizable based on their vibrational signatures. For $\text{H}^+(\text{H}_2\text{O})_4$ the *Eigen* core structure is adopted, based on the strong band at 2665 cm^{-1} , which was assigned to the asymmetric OH stretching mode. While the 3–5 H_2O clusters adopt *Eigen* core structures, the 2 and 6–8 water complexes are present as *Zundel* structures. However, for the larger 9–11 clusters again the *Eigen* structure is preferred. Based on these latter results, it turns out that the *Eigen* structural motif was correctly identified in the original experiments by Schwarz, even if the interpretation was complicated by the lack of mass selection.

D. Other Solvated Proton Complexes

Moore et al. recorded the first gas-phase IR-MPD spectra of proton-bound dimers other than water, in particular a series of proton-bound ether dimers. In these spectra, similar bands were observed as in the case of the *Zundel* ion (Asmis et al., 2003), leading Moore et al. to suggest that there is a “vibrational signature” for the $\text{O}\cdots\text{H}^+\cdots\text{O}$ bridge (Moore et al., 2004). Again, subsequent studies by Fridgen and co-workers showed that the appearance of these spectra is very sensitive to experimental conditions, observing shifts of several tens of cm^{-1} , particularly for modes involving the shared proton (Fridgen et al., 2005). The anharmonic nature of the potential makes these modes very susceptible to shifts depending on the internal energy of the system. Fridgen and co-workers later extended their spectroscopic studies on proton-bound systems to include alcohols, ketones and even amino acids (Fridgen et al., 2006).

The room-temperature IR-MPD studies mentioned above suffer from extensive spectral broadening, complicating the interpretation of the spectra. Later studies by Johnson and co-workers on cold proton-bound dimer complexes generated in a supersonic expansion showed that these messenger-technique pre-dissociation spectra are much better resolved than the corresponding IR-MPD spectra (Roscioli, McCunn, & Johnson, 2007). This suggests that the IR spectra of such complexes depend strongly on internal energy and/or that broadening due to the multiple photon excitation in IR-MPD is extensive for these “floppy” systems. In addition, these systematic studies by Johnson as well as studies by Fridgen and co-workers on a series of proton-bound homo- and heterodimers showed a good correlation between the frequency of the proton-bound mode and the difference in proton affinity (ΔPA) between both ligands (Fridgen et al., 2005). The homodimers displayed the proton-bound mode $<1000\text{ cm}^{-1}$, whereas this mode was shifted to $>3000\text{ cm}^{-1}$ in the heterodimers with the largest difference in proton affinity (e.g., CH_3OH and Ar, $\Delta\text{PA} = 385\text{ kJ mol}^{-1}$). This equates to a frequency shift of more than 2000 cm^{-1} , which can be rationalized by very different potential energy surfaces for this mode, which had also been predicted by Fridgen based on calculations (Fridgen, 2006). In the case of the homodimer, the potential energy surface is very flat, resulting in a large-amplitude motion at a low frequency. In heterodimers, this potential energy surface becomes asymmetric and steeper as the difference in proton affinity increases. The resulting proton-

bound mode progressively shifts to higher frequency, while the amplitude of the vibration decreases.

Iyengar and co-workers have recently applied *ab initio* molecular dynamics (AIMD) simulations to the proton-bound dimethyl ether dimer (Li, Moore, & Iyengar, 2008). They were able to qualitatively reproduce both the argon-tagged spectra from Johnson and co-workers (Roscioli, McCunn, & Johnson, 2007) and the IR-MPD spectra from Moore et al. (2004). In particular, they could explain the doublet feature around 1000 cm^{-1} in the argon-tagged spectra as coupling between the shared proton stretch and ligand vibrations, such as the backbone COC stretch, the oxygen–oxygen stretch and the methyl wag modes. The main conclusion from this work is that spectral broadening occurs as a result of anharmonic coupling between harmonic vibrational modes and intensity borrowing by dark states. This results in IR spectra that are intimately dependent on the initial internal energy of the probed complexes.

Duncan and co-workers considered protonated acetone (Douberly et al., 2008a) and carbon dioxide (Douberly et al., 2008b) complexes, where they estimated the effect of Ar attachment on the OH^+ stretch frequency; this effect was found to be much more pronounced for $\text{H}^+(\text{CO}_2)\text{Ar}$ than for acetone. This shows once again that while Ar attachment certainly results in high-quality spectra, some caution needs to be exercised in the interpretation of such spectra, due to the perturbing effect of the van der Waals atom on the symmetry of the complex. Published experimental IR spectra of solvated proton complexes display striking differences, which may be due to differences in ion formation, the temperatures of the clusters and the method of photodissociation. While current theoretical approaches still struggle to adequately reproduce the experimental data, recent developments (Li, Moore, & Iyengar, 2008) are encouraging that these challenges can be overcome in the near future.

VI. CONCLUSIONS AND SUMMARY

Infrared (IR) spectroscopy of mass-selected ions has seen a steady increase over the past years, mainly due to recent developments in tunable IR light sources, such as FELs and lasers based on nonlinear frequency down-conversion. IR-MPD and the “messenger” technique remain the most popular approaches to carry out IR spectroscopy experiments. The IR–UV double resonance photofragmentation technique on cold ions pioneered by Rizzo and co-workers has the advantage that IR spectra of conformer-selected ions can be recorded. This promising method has boosted the use of liquid He cooled multipole rf traps in recent years, particularly for spectroscopic studies.

This review has attempted to give a summary of recent work in IR photodissociation spectroscopy with a particular emphasis on bare and solvated biomolecular complexes. Vibrational spectroscopy is particularly useful in determining the *chemical structure* of ions, thus being valuable for instance in distinguishing between zwitterionic and non-zwitterionic forms of gas-phase amino acids. The vibrational bands in these complexes are susceptible to sites being protonated and the extent of electrostatic interaction with metal cations. Secondary structural motifs are often determined by hydrogen bonding interactions, which

leave a clear imprint on the IR spectrum. Vibrational spectroscopy is also highly suited in the structural elucidation of peptides and their CID products. The site of proton attachment and the chemical moieties formed are identifiable by IR spectroscopy. In these cases IR spectroscopy seems to yield the most direct information on the chemical structure, which would be difficult to obtain with other gas-phase structural methods on ions, such as ion mobility, CID or H/D exchange. In an attempt to determine what the limits of molecular size are for IR photodissociation spectroscopy of ions, whole proteins (i.e., bovine ubiquitin and bovine cytochrome *c*) have been successfully studied. In principle, the positions of the main bands (i.e., amide A, I and II) carry information about the secondary structures of these gas-phase proteins. While such large molecules yield partly resolvable bands, an interpretation of the results is nonetheless complicated by an inability to calculate the vibrational spectra. Moreover, it remains to be seen how relevant these gas-phase measurements are with respect to biological molecules in their native environment. IR spectroscopy on microsolvated clusters holds much promise to bridge this gap. Much fewer experiments have been carried out to date on solvated complexes, due to more challenging experimental conditions, where the solvated complexes must be cooled to be stable over the length of the measurement. Among the scientific questions that have been addressed in some detail are the solvation shells formed around cations and the proton, as well as zwitterion stabilization. Many more interesting experiments with relevance to biology are likely to be carried out over the coming decade, including the study of discrete aggregation states of proteins and sequential hydration of peptides and *biomimetic* systems. The IR study of mass-selected clusters remains very attractive, as discrete complexes in mixtures can be separated and the complexity of these molecular systems is often compatible with high-level calculations, thus allowing a detailed understanding of the structural driving forces.

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