Peptide Length, Steric Effects, and Ion Solvation Govern Zwitterion Stabilization in Barium-Chelated Di- and Tripeptides

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Infrared multiple-photon dissociation (IRMPD) spectroscopy has given infrared spectra of complexes of di- and tripeptides (AlaAla, AlaAlaAla, AlaPhe, PheAla) with singly and doubly charged metal ions (K+, Ca2+, Se2+, and Ba2+). The switch between charge-solvated (CS) and salt-bridged zwitterion (SB) conformations is displayed through highly diagnostic features in the mid-infrared. Systematic trends are found correlating with the length of the peptide chain (tripeptides favoring CS conformations), metal ion size (larger metals favoring SB conformations), metal ion charge (doubly charged ions favoring SB conformations), and sterically available Lewis-basic side-chain interactions with the metal ion (for example a cation—π interaction with Ba2+ stabilizes CS for PheAla but not for AlaPhe). The principle is that CS conformations are favored for small metal ions with high charge density and extensive microsolvation of the charge by Lewis-basic groups, especially amide carbonyls; SB conformations are favored by metal ions of high charge but low charge density, which are better stabilized by salt-bridge Coulomb interactions.

Introduction

Much recent attention has been devoted to conformational variations in metal-ion-complexed amino acids1 in the gas phase, using infrared action spectroscopy2–4 and other approaches.5,6 However, as compared with amino acids, introducing one or more amide groups as one progresses to peptide ligands induces a fundamental change in the available modes of chelation of the metal ion. In addition, new energetic and steric considerations affect the conformational preferences. Hence, to obtain insights into metal ion interactions with peptide chains, one needs to go beyond simple amino acid–metal ion complexes, so that amide groups are available for metal ion interaction. Some work on such complexes has been reported, but this landscape is still much less explored.6–9

Structures of the complexes are characterized by whether the peptide adopts a zwitterionic (salt-bridged, SB) conformation or a canonical (charge-solvating, CS) conformation. Within these main structural motifs, there are further refinements of which Lewis-basic sites of the peptide participate in chelation of the metal ion. Important features influencing the fine balance between different binding geometries include the length of the peptide chain, the presence and sequence of side chains, and the size, electronic properties, and charge of the metal ion. The present work investigates several model complexes by infrared multiple-photon dissociation (IRMPD) spectroscopy, showing how relatively small changes in these features can translate into large-scale alterations of the conformation of the complex.

The divalent alkaline-earth Ba2+ ion is particularly interesting in comparison with the heavily studied alkali ions because its juxtaposition of high ionic charge with weak solvation interactions makes possible a crossover from CS conformations (typical of alkali ions) to SB conformations. A striking example is the SB structure of the Ba2+ complex with Trp,3a in contrast with the CS structure of alkali metal ion complexes with Trp.3b Further work has suggested that this contrast holds for nearly all monomeric amino acids. An interesting exception is the asparagine amino acid,2f which forms a CS structure upon complexation with Ba2+, which has the same charge but stronger solvating interactions with basic ligands.

Experimental infrared spectra of the gas-phase complexes were obtained by IRMPD. A Fourier-transform ion cyclotron resonance (FTICR) mass spectrometer interfaced to the FELIX free-electron laser light source was used, as has been described elsewhere.4 Complexes were formed by electrospray ionization (ESI) using methanol/water as the solvent and the peptide and the metal chloride or nitrate salt at around 1 mM concentrations. The complexes under study were mass-isolated in the FTICR and subsequently irradiated with FELIX for typically 3 s. An IR spectrum was reconstructed by plotting the fragment yield as a function of the photon energy of the radiation. Computations were performed at the B3LYP/6-31+G(d,p) (SDD ECP on Ba) level for vibrational spectra (frequencies scaled by 0.975) and at the B3LYP/6-311+G(d,p) (SDD on Ba) level for energies. For AlaAla and AlaAlaAla complexes, candidate geometries were obtained by IRMPD spectroscopy, showing
Chain Length

Figure 1 contrasts the IRMPD spectra of the Ba\(^{2+}\) complexes with AlaAla and AlaAlaAla, along with computed spectra of the lowest-energy CS and SB isomers for both complexes. The experimental spectra of the two complexes are different, indicating different structural motifs. The AlaAla complex spectrum is typical for SB, while AlaAlaAla suggests a CS structure. CS conformations are often distinguished by the C=O stretch at around 1600–1700 cm\(^{-1}\) (in doubly charged complexes) and the OH bend at 1150 cm\(^{-1}\). SB conformations have been characterized by the intense NH\(_3\)O stretch and NH\(_3\) bend near 1400 cm\(^{-1}\), as in the present AlaAla case. However, in the computed spectrum of SB AlaAlaAla, spectral features related to the NH\(_3\)O group are blue-shifted into a busy spectral region above 1500 cm\(^{-1}\) by strong H-bonding to two oxygens, so that it is not useful to look for a diagnostic peak near 1400 cm\(^{-1}\). Here, differentiation between CS and SB relies on the clear differences between CS and SB at around 1600 and 1150 cm\(^{-1}\). The AlaAla spectrum shows all major peaks expected for SB, and little or no intensity at the peak positions expected for CS. There is some congestion in the 1450 cm\(^{-1}\) region, which, although it is unexplained, is certainly not indicative of a CS contribution. On the other hand, the AlaAlaAla spectrum gives a satisfactory fit to the expectations for a CS complex and is marked as having little SB contribution, particularly by the low intensity at 1650 cm\(^{-1}\).

The switch from SB (AlaAla) to CS (AlaAlaAla) can be attributed to the increasing stability of the CS conformation. Coordination with carbonyl groups is the most favorable of the solvating interactions available to the metal ion,\(^6\) and in the AlaAlaAla case, it is sterically feasible for three carbonyls to chelate the ion, as shown in Figure 1 (middle panel). In the AlaAla case, only two carbonyls are available for chelation, making the CS conformation relatively less attractive.

Metal Ion Size and Charge

Figure 2 displays the progression of spectra for the dipeptide complexes of four metal ions having different charge states and ion sizes. Figure 2 overlays the spectra with the calculated predictions shows that Ba\(^{2+}\) is exclusively SB, Sr\(^{2+}\) gives a complex which appears largely SB but with a shoulder near 1660 cm\(^{-1}\) and other features that clearly indicate an admixture of some CS ions in the population. Ca\(^{2+}\) also displays a mixed population, but with a higher fraction of CS than that for Sr\(^{2+}\). Finally, K\(^{+}\) purely exhibits a CS population. These assignments are illustrated on the figure by plots of the most stable SB conformation for the Ba complex (red) and the most stable CS conformations for the Sr, Ca, and K complexes (blue). (Not surprisingly, the K\(^{+}\)AlaAla spectrum is very similar to the Na\(^{+}\)AlaAla spectrum reported and assigned as CS by Balaj et al.\(^7\)) Higher charge is known to favor a SB conformation,\(^3\) giving the transition from Ba\(^{2+}\) (SB) to K\(^{+}\) (CS). Large ion size favors a SB conformation, giving the progression from Ba\(^{2+}\) (SB) to Ca\(^{2+}\) (substantially CS). (Pauling ionic radii of Ba\(^{2+}\), Sr\(^{2+}\), Ca\(^{2+}\), K\(^{+}\), and Na\(^{+}\), are respectively, 1.35, 1.13, 0.99, 0.95, and 1.33 Å.)

The progression toward CS in Figure 2 along this sequence of examples from Ba\(^{2+}\) to K\(^{+}\) is supported by free-energy calculations. For Ba\(^{2+}\), SB is favored over CS by 5 kJ/mol, and for Sr\(^{2+}\), it is favored by 2 kJ/mol, while for Ca\(^{2+}\) (–2 kJ/mol) and K\(^{+}\) (–56 kJ/mol), CS is favored. The overall picture is that increasing the charge density favors CS (Ba\(^{2+}\) to Ca\(^{2+}\)), whereas increasing the charge favors SB (K\(^{+}\) to Ba\(^{2+}\)).

Side Chain and Sequence

The spectrum of Ba\(^{2+}\)AlaPhe (Figure 3) is quite similar to that of Ba\(^{2+}\)AlaAla (Figure 2), indicating a SB conformation, which implies that no available geometry allows sufficient side-chain chelation to stabilize the solvated (CS) conformation. However, in the sequence-reversed complex Ba\(^{2+}\)PheAla, the
ions are inherently more likely to favor SB than singly charged reasoning rationalizes the observation that doubly charged metal complexes of AlaPhe and PheAla, which are always CS.8 On the other hand, a high basicity side chain can induce a comparable sequence-dependent variation of conformational preference even with alkalai metal cations, as was recently reported for Li+ and Na+ complexed to ArgGly and GlyArg.9

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References and Notes


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