

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^+ , Ca^{2+} , Sr^{2+} , and Ba^{2+}). 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 Ba^{2+} 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 insight 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 Ba^{2+} 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 Ba^{2+} complex with Trp,^{3a} in contrast with the CS structure of alkali metal ion complexes with Trp.⁴ 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 Ba^{2+} on account of the strong solvation capacity of the side-chain amide $C=O$.⁸ The present work extends this comparison by contrasting Ba^{2+} with Ca^{2+} , 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

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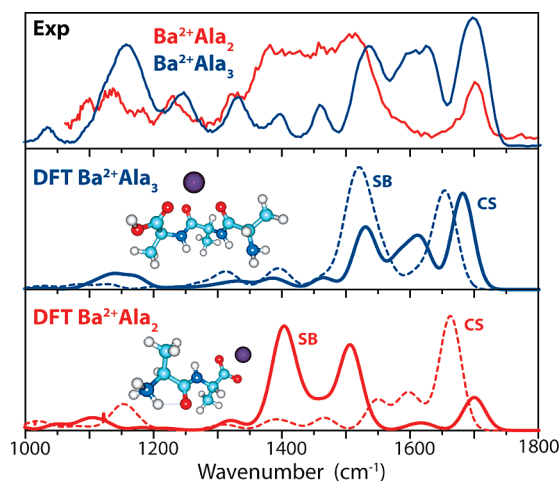


Figure 1. Switch from SB to CS upon going from two to three residues. The IRMPD spectrum for $\text{Ba}^{2+}\text{AlaAlaAla}$ and the simulations are shown in blue; corresponding plots for $\text{Ba}^{2+}\text{AlaAla}$ and the simulations are in red.

76 for optimization were guided by the results of the careful
77 searches of conformation space for sodium complexes described
78 by Balaj et al.^{7a} Trial geometries for the AlaPhe and PheAla
79 complexes were based on the structures surveyed for alkali ion
80 complexes by Polfer et al.⁸

81 Chain Length

82 Figure 1 contrasts the IRMPD spectra of the Ba^{2+} complexes
83 with AlaAla and AlaAlaAla, along with computed spectra of
84 the lowest-energy CS and SB isomers for both complexes. The
85 experimental spectra of the two complexes are different,
86 indicating different structural motifs. The AlaAla complex
87 spectrum is typical for SB, while AlaAlaAla suggests a CS
88 structure. CS conformations are often distinguished by the C=O
89 stretch at around 1600–1700 cm^{-1} (in doubly charged com-
90 plexes) and the OH bend at 1150 cm^{-1} . SB conformations have
91 been characterized by the intense NH_3^+ bend near 1400 cm^{-1} ,
92 as in the present AlaAla case. However, in the computed
93 spectrum of SB AlaAlaAla, spectral features related to the NH_3^+
94 group are blue-shifted into a busy spectral region above 1500
95 cm^{-1} by strong H-bonding to two oxygens, so that it is not useful
96 to look for a diagnostic peak near 1400 cm^{-1} . Here, differentia-
97 tion between CS and SB relies on the clear differences between
98 CS and SB at around 1600 and 1150 cm^{-1} . The AlaAla spectrum
99 shows all major peaks expected for SB, and little or no intensity
100 at the peak positions expected for CS. There is some congestion
101 in the 1450 cm^{-1} region, which, although it is unexplained, is
102 certainly not indicative of a CS contribution. On the other hand,
103 the AlaAlaAla spectrum gives a satisfactory fit to the expecta-
104 tions for a CS complex and is marked as having little SB
105 contribution, particularly by the low intensity at 1650 cm^{-1} .

106 The switch from SB (AlaAla) to CS (AlaAlaAla) can be
107 attributed to the increasing stability of the CS conformation.
108 Coordination with carbonyl groups is the most favorable of the
109 solvating interactions available to the metal ion,⁸ and in the
110 AlaAlaAla case, it is sterically feasible for three carbonyls
111 to chelate the ion, as shown in Figure 1 (middle panel). In the
112 AlaAla case, only two carbonyls are available for chelation,
113 making the CS conformation relatively less attractive.

114 Metal Ion Size and Charge

115 Figure 2 displays the progression of spectra for the dipeptide
116 complexes of four metal ions having different charge states and

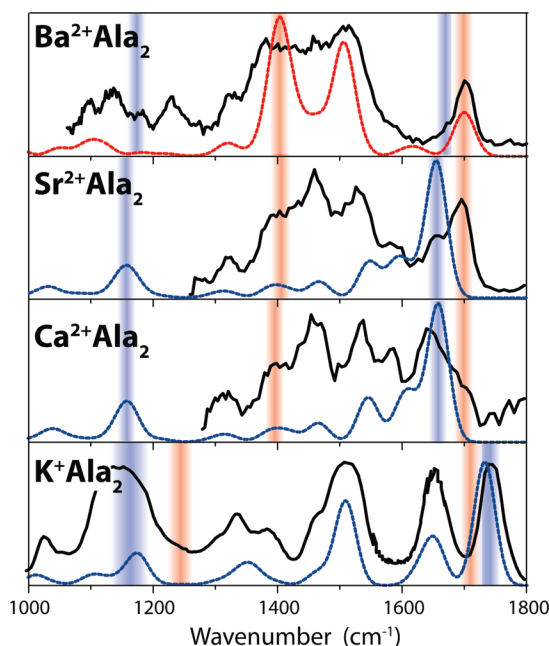


Figure 2. Series of AlaAla metal ion complex spectra showing the relative prominence of SB versus CS signature features. Calculated positions of the characteristic SB peaks are indicated in orange, and CS peaks are in blue. Traces of calculated spectra show the most stable SB conformation in the Ba complex (red) and the most stable CS conformations for the Sr, Ca, and K complexes (blue).

117 ion sizes. Figure 2 overlays the spectra with the calculated
118 positions of the CS signature peaks (carboxyl C=O stretch and
119 OH bend) in blue and the SB signature peaks (amide C=O
120 stretch and NH_3^+ bend) in orange. Comparison of the observed
121 spectra with the calculated predictions shows that Ba^{2+} is
122 exclusively SB. Sr^{2+} gives a complex which appears largely
123 SB but with a shoulder near 1660 cm^{-1} and other features that
124 clearly indicate an admixture of some CS ions in the population.
125 Ca^{2+} also displays a mixed population, but with a higher fraction
126 of CS than that for Sr^{2+} . Finally, K^+ purely exhibits a CS
127 population. These assignments are illustrated on the figure by
128 plots of the most stable SB conformation for the Ba complex
129 (red) and the most stable CS conformations for the other
130 complexes (blue). (Not surprisingly, the K^+AlaAla spectrum is
131 very similar to the Na^+AlaAla spectrum reported and assigned
132 as CS by Balaj et al.^{7a}) Higher charge is known to favor a SB
133 conformation,³ giving the transition from Ba^{2+} (SB) to K^+ (CS).
134 Large ion size favors a SB conformation, giving the progression
135 from Ba^{2+} (SB) to Ca^{2+} (substantially CS). (Pauling ionic radii
136 of Ba^{2+} , Sr^{2+} , Ca^{2+} , K^+ , and Na^+ , are respectively, 1.35, 1.13,
137 0.99, 0.95, and 1.33 Å.)

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

145 Side Chain and Sequence

146 The spectrum of $\text{Ba}^{2+}\text{AlaPhe}$ (Figure 3) is quite similar to
147 that of $\text{Ba}^{2+}\text{AlaAla}$ (Figure 2), indicating a SB conformation,
148 which implies that no available geometry allows sufficient side-
149 chain chelation to stabilize the solvated (CS) conformation.
150 However, in the sequence-reversed complex $\text{Ba}^{2+}\text{PheAla}$, the

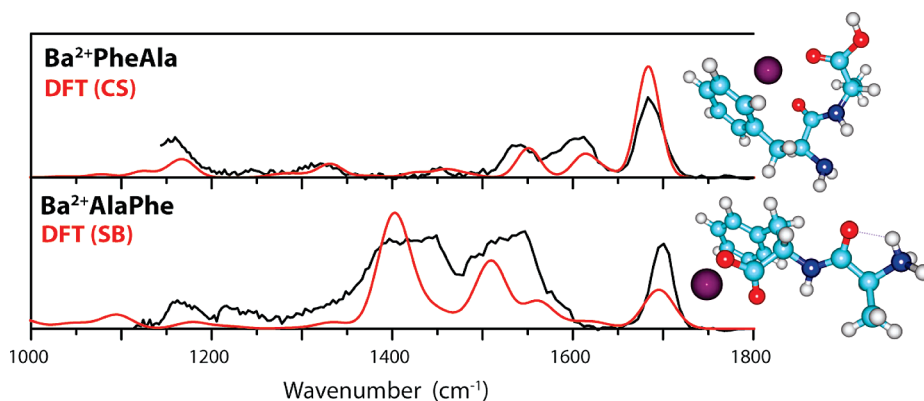


Figure 3. Contrasting spectra (black) of Ba^{2+} complexes of AlaPhe and PheAla. Red traces are simulated spectra of the indicated conformations.

151 phenyl group is able to chelate the metal ion in a cation- π
 152 binding configuration, along with both carbonyl groups, which
 153 stabilizes the CS conformation and results in an entirely different
 154 spectrum matching the CS simulation. In contrast, sequence
 155 independence was observed for alkali metal complexes of
 156 AlaPhe and PheAla, which are always CS.⁸ On the other hand,
 157 a high basicity side chain can induce a comparable sequence-
 158 dependent variation of conformational preference even with
 159 alkali metal cations, as was recently reported for Li^+ and Na^+
 160 complexed to ArgGly and GlyArg.⁹

161 Discussion

162 Increasing relative favorability of the CS conformation
 163 correlates with increasing charge density of the cation, which
 164 promotes solvation of the metal ion by the carbonyls and other
 165 Lewis-basic ligands, and with increasing numbers of sterically
 166 available chelating points. Of all of the metal cations studied
 167 to date, Ba^{2+} is the most favorable to stabilizing a SB structure
 168 for a peptide. The present results show that SB is favored for
 169 AlaAla, but SB is not observed even with Ba^{2+} for the tripeptide
 170 AlaAlaAla (Figure 1). This suggests that the chelating potential
 171 of AlaAlaAla is sufficient to stabilize CS regardless of metal
 172 ion characteristics. Whether this is true for triply (or higher)
 173 charged metal ions is an intriguing open question.

174 The CS/SB choices illustrated here for dipeptide complexes
 175 are governed by the strength of the electrostatic microsolvation
 176 of the cation. Stronger and/or more extensive solvation is
 177 promoted, often leading to favorable CS, when several amide
 178 CO groups are accessible (Figure 1), or the charge density of
 179 the cation is high (Figure 2), or a Lewis-basic side chain is
 180 accessible for additional chelation (Figure 3). It will be
 181 interesting to study peptides larger than three residues, where
 182 more than two amide carbonyls are potentially available for
 183 chelation.

184 Of the functional groups available in typical peptides, amide
 185 carbonyl groups are most strongly bound to metal cations,⁸ and
 186 complexes of peptides up to tripeptides, whose structures have
 187 been assigned, have shown all available amide carbonyls (as
 188 well as the carboxyl carbonyl in most cases) chelating the metal
 189 in the most stable CS conformation. On the other hand, the
 190 situation can be different for complexes with SB ground states
 191 (as in Figure 3, lower panel), where maximizing the electrostatic
 192 salt-bridged interactions of charged groups is more important
 193 than maximizing microsolvation of the metal ion. This line of
 194 reasoning rationalizes the observation that doubly charged metal
 195 ions are inherently more likely to favor SB than singly charged
 196 ones, as illustrated by the present examples.

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