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Conformations and vibrational spectroscopy of metal-ion/polylalanine complexes

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ABSTRACT

The thermochemistry and structures of complexes of dialanine and trialanine with a series of singly and doubly charged metal ions have been examined by spectroscopic and computational approaches. Complexes with Li^+, K^+, Cs^+, Ca^{2+}, Sr^{2+} and Ba^{2+} were formed by electrospray ionization, and studied by infrared multiple photon dissociation spectroscopy (IRMPD) in a Fourier-transform ion cyclotron resonance (FTICR) mass spectrometer using irradiation in the 1000–1900 cm^{-1} infrared (IR) region from the FELIX free electron laser. Spectra were correlated with computed thermochemistry and simulated IR spectra from density functional (DFT) calculations of likely candidate conformations to make conformational assignments and characterize the variation of characteristic normal mode frequencies as a function of metal-ion identity. All trialanine complexes were found to have the charge-solvated (CS) form of the ligand with all three carbonyl oxygens chelating the metal. Dialanine was more varied: the Ba complex had largely the salt-bridge (SB) form, the Sr and Ca complexes showed a mixture of CS and SB, the Cs complex was CS with (OO) chelation, and the K, Na and Li complexes were CS in an undetermined mixture of (OO) and (NOO) chelation. Binding of the metal ion to the carboxyl carbonyl and to the amide carbonyl(s) (Amide I band) gave a red shift of the C=O stretches, and a blue shift to the Amide II band. The frequency shifts were greater for more strongly interacting metal ions (smaller size, larger charge), and were found to be linearly proportional to the metal–ligand binding energy.

1. Introduction

The binding patterns of metal ions to amino acids have received much attention, thanks in large measure to recent developments in spectroscopic [1–17], thermochemical [18–20], ion-mobility [21] and computational tools for structure determination of the gas-phase complexes. Large advances have been achieved in understanding the choices between zwitterion and canonical conformations, as well as other conformational variations. The role of metal-side-chain interactions in governing these choices has been clarified in numerous cases. Spectroscopic approaches have played an important part in these recent advances and are of particular interest to the present work; the reviewing of developments in this area is reasonably current [13,22–25].

Towards the overall goal of carrying gas-phase understanding into the broader domain of metal-ion interactions with peptides, proteins, enzymes and related biomolecules, it is equally important to look at model systems containing the peptide linkage, namely dipeptides and longer peptide chains, especially since the metal-binding strength of the amide carbonyl group is suggested by simple modeling [26] to dominate over other commonly available Lewis-basic ligation sites in metal/ion peptide complexes. A baseline of understanding of such complexes is starting to develop [20,26–40], but there is still no extensive information about even the simplest systems. The present work expands the understanding of metal-ion complexation to polyalanines, representing some of the simplest models of the peptide chain.

A basis of knowledge about the sodium ion complexes of dialanine and trialanine was laid in computational studies [20,29,32,37,38,41]. Spectroscopic confirmation of the structures emerging from electrospray production of the sodium complexes was achieved by the Ohanessian group [28] working with the CLIO laser, who reported fine agreement between the observed IRMPD spectra of the dialanine and trialanine complexes with predicted IR spectra for the lowest-enthalpy conformations. The present authors looked at IRMPD spectra of these same ligands with some other metal ions [7], having in mind the possibility of conformational variations with metal ions of larger size or higher charge, and reported that there is in fact a tendency to switch from the canonical, charge-solvated (CS) structural pattern of the

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alkali metal ions to the zwitterionic, salt-bridge (SB) pattern for the alkaline earth ions, particularly barium. The present study expands and deepens the consideration given in this previous work to the effects of peptide chain length and metal-ion characteristics on the structures and the IR spectroscopy of these $M^{z+}(Ala_n)$ complexes.

A premise of the present work is that there are characteristic bands in the IRMPD spectra of peptide complexes that can be traced across variations in metal ion and chain length, and reflect the character of the interactions of the metal ion with the Lewis-basic sites of the peptide chain. A useful background of understanding is provided by the spectroscopy of the mono-amino acids, which has been reported extensively (as for instance in many of the papers and reviews noted above.) A principal theme of much of this work is differentiating the metal-complexed amino acids in the CS versus the SB forms. This effort has been favored by a particularly happy match between the IR wavelength range of free electron laser light sources and the existence of strong and well differentiated vibrational absorptions which characterize CS and SB ligand binding patterns. The CS form, having –COOH and $–NH_2$ terminal groups, characteristically gives a strong metal-complexed C═O stretching peak standing out on the blue side of the spectrum around 1700 cm^{-1}. In one CS variation (small metal ions with high charge density), the NH_2 group may chelate the metal ion, which is signalled by a prominent free hydroxyl COH bending peak near 1150 cm^{-1}. In another CS variation (larger metal ions) the acidic OH hydrogen bonds to the NH_2 group, giving rise to a prominent H-bonded hydroxyl bending mode near 1400 cm^{-1}. The SB conformation, having $–COO^-$ and $–NH_3^+$ terminal groups, typically shows the carboxylate OCO stretching mode shifted to longer wavelength as compared to the carboxylic acid C═O stretch, along with disappearance of the free COH peak and appearance of strong features near 1400 cm^{-1} due to ammonium N–H bending motions. This set of typical IR characteristics serves as a baseline for interpreting the cationized amino acid spectra, filled out and made more precise by detailed quantum-chemical calculations of the actual conformations possible within these main motifs. A principal focus of the present work on the polyalanines is to sort out the additions and modifications to this picture introduced by the presence of the amide linkage(s) and the enhanced conformational flexibility of the di- and trialanine ligands.

In the 1400–1800 cm^{-1} range the strong, characteristic bands singled out here for discussion include modes of the terminal groups (COOH and NH_2, or COO^- and NH_3^+) already well known from amino acid studies, plus the additional amide-linkage-based modes Amide I (C═O stretch) and Amide II (NH bend plus CN stretch). In addition, the free hydroxyl bending mode near 1150 cm^{-1} continues to be a useful marker. Accordingly, in the spectra discussed in detail below, we will look for terminal CO or COO^- stretching modes, NH_2 or NH_3^+ bending modes, Amide I and Amide II, and a terminal hydroxyl bending mode. The simplifying advantage of focusing on the polyalanine model ligands in this study, as opposed to residues with functionalized side chains, is that the spectroscopic situation is not complicated by strong overlapping side-chain modes.

2. Experimental and computational methods

2.1. Computational considerations

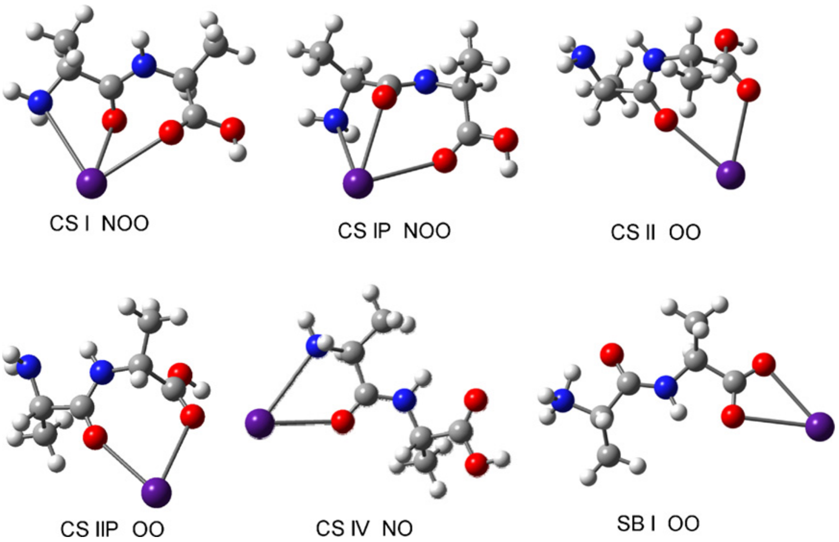
Calculations were performed using Gaussian 03, normally at the B3LYP/6-311 + g(d,p) level, fully geometry optimized. For atoms heavier than Kr (namely Sr, Cs, and Ba) the sdd relativistic effective core potential included in the Gaussian program suite was used. No imaginary frequencies were encountered. For economy, some of the structures found to lie far above the ground state energy were calculated at the cheaper double-zeta level, B3LYP/6-31 + g(d,p). Energies were corrected for zero-point energy effects and for thermal energy at 298 K. The computed spectra were scaled by 0.975 (6-31 + g(d,p)) or 0.98 (6-311 + g(d,p)) as has been found appropriate in previous analyses of IRMPD spectra of cationized amino acids and small peptides in this wavelength range. They were convoluted with a Gaussian lineshape with a FWHM of 30 or 40 cm^{-1}. Free energy values were based on an assumption of rigid-rotor/harmonic-oscillator contributions to the entropies. The aim of this study was not to obtain accurate binding thermochemistry of these systems, so no basis set superposition corrections were applied.

The construction of Fig. 8 in Section 3.3 defines the "binding energy" of the metal ion with trialanine. This quantity is the binding energy of the ion to a hypothetical conformation of trialanine, displayed as Fig. S14, which has no intramolecular hydrogen bonds to the oxygen or nitrogen atoms, but still occupies a local minimum on the potential energy surface so as to allow an energy optimization and normal mode frequency calculations. This neutral conformation is employed here to provide a valid reference point for the C═O vibrational modes unperturbed by extraneous hydrogen bonds, and allows the plotting in Fig. 8 of frequency points at "zero" binding energy for which the normal modes are unperturbed by metal-ion interactions. The true metal-ion binding energies would be obtained by subtracting a constant "rearrangement energy" from all the values given below. Using the neutral ground state conformation assigned by Wang et al. [20] this rearrangement energy is 9 kJ/mol. Note that the correlations with binding energy discussed in Section 3.3 remain linear with the same slope regardless of the conformation assumed for the reference neutral ligand, except that the frequencies plotted at zero binding energy are dependent on this choice. In order to estimate the actual absolute binding energies of the complexes (given in Table 2), the literature ground state structures of the neutrals [20,42] were optimized at the same computational level as the rest of this work.

2.2. Comparison of basis sets

It was interesting to test whether less expensive double-zeta calculations (6-31 + g(d,p)) would be useful, and a number of comparisons were made. It was found that energies and spectra were very similar except for Ca^{2+} complexes, for which the peak positions in the carboxyl/amide region (1500–1800 cm^{-1}) changed by as much as 25 cm^{-1} and relative intensities changed by as much as factors of 2 (as displayed in the Supplementary Material, Figs. S13–14). One especially prominent discrepancy was $Ca^{2+}(Ala_2)$ SB-I, where the splitting between the NH_3^+ peak near 1400 and the COO^- peak near 1500 was found to decrease by a factor of 2 upon going to the larger basis (Fig. S13). In the case of $Ca^{2+}(Ala_3)$ CS-II (Fig. S14), where the conformation assignment is quite secure, the triple-zeta result agrees substantially better with the IRMPD spectrum than the double-zeta basis. The further-expanded 6-311 + g(2d,2p) basis gave no significant changes. The general conclusion was that spectra (though not necessarily the energies) calculated using the double-zeta basis were reliable except for the strongly interacting Ca^{2+} complexes.

At the triple-zeta level, it has been suggested that the thermochemistry of complexes of heavy elements is better treated using the Def2TZVP basis set, which includes its own effective core potential [9]. This larger basis was not used for the complete set of the present structure-survey calculations, but was used for several of the critical Ca, Sr and Ba dialanine comparisons. The thermochemistry from these calculations was indeed significantly more consistent with our results than the 6-311 + g(x,x) results (more favorable to the SB structure), and these appar-



Scheme 1. Low-Energy M^{z+}AlaAla conformations.

ently improved relative energies are accepted in the discussion below. On the other hand, comparison of the calculated spectra using Def2TZVP in the Ca cases showed insignificant differences in peak positions and intensities (using the identical scaling factor).

2.3. Experimental

Infrared spectra of the gas-phase complexes were obtained in the action spectroscopy mode 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 in previous work [12,26]. Electrospray ionization (ESI) using methanol/water as the solvent with peptide and salt (metal chloride or nitrate) at around 1 mM concentrations was applied as the ion production method. The complexes under study were accumulated and stored for collisional and radiative cooling during 1–2 s in a hexapole linear trap, followed by mass isolation in the FTICR ion trap and irradiation by FELIX for typically 3 s. The IR spectrum was reconstructed by summing and plotting the yields of all major fragment ions as a function of the photon energy of the radiation. A power correction was applied as a function of wavelength using the approximation of linear dependence of the dissociation yield on the laser intensity. The reasonably wavelength-independent nature of the FEL output (dropping off only at the extremes of the tuning range by not more than a factor of 2) ensured that this approximation to the true intensity dependence of the yield did not result in severe distortions of the spectra.

3. Results and discussion

3.1. Thermochemistry

3.1.1. Structures and nomenclature

The substantial amount of computational and experimental work reported on the lithium, sodium and potassium complexes of polyglycine and polyalanine complexes gives a good picture of the available conformations. However, the relative energetics of the alanine systems are not always the same as the glycine systems, so that the polyalanine structure surveys of Refs. [20,28] are the most relevant to the present work.

3.1.2. Dialanine

Scheme 1 shows the binding patterns that we considered as possible candidates for dialanine complex ground states. The numbering follows that of Balaj et al. [28] with respect to the structures that are displayed in their paper (their Fig. 2). Note that CS-I and CS IP are a diastereomer pair, with our structure CS-I being that displayed as their structure AA-Na^+ I. Our CS-II and CS-IIP (their structure AA-Na^+ II) are two conformations behaving consistently as distinct potential energy minima, differing only in the conformations of the 7-membered ring. Our structure CS-IV is similar to their AA-Na^+ IV. Our structure SB-I is the zwitterionic structure which we found to be clearly the most stable zwitterion for all metal ions. An alternative zwitterion not pictured (SB-II), with all three oxygens coordinated to the metal ion, was the next most stable zwitterion, but was substantially less stable than SB-I.

Table 1 shows the energetics calculated for the dialanine complexes. There is a sharp contrast between the singly and doubly charged metal ions. For the former, the bidentate (OO) structures CS-II or CS-IIP are definitely preferred (although which of the two diastereomers is favored seems to vary depending on the metal ion.) The alternative CS-I (NOO) and CS-IV (NO) binding motifs are less favorable, although not completely unreasonable. In an interesting crossover, the most favorable CS conformation for the alkaline earth complexes is the tridentate (NOO) pattern. For the doubly charged metal ions, the added stabilization gained by coordinating the terminal nitrogen evidently overcomes any steric and entropic disadvantage of the tridentate conformations, and structures CS-I or CS-IP are preferred.

For the alkalis, no zwitterion (SB) structure was found with a stability that would make it a likely competitor to the CS structures. The SB-I structure noted in Table 1 was the best. For the alkaline earths, however, the SB-I zwitterion becomes competitive with CS structures. As we progress from Ca to Sr to Ba, the zwitterion becomes progressively more favorable, and may be the ground state for Sr and Ba. In accordance with the spectral evidence below, we consider that the Ca and Sr complex populations are a mixture of SB and CS conformations, and the Ba complex is almost completely SB.

The zwitterion structure SB-II with all three oxygens coordinating the metal ion was found for Ca and Sr, but this structure was inferior to SB-I (Table 1) because of the disruption of the salt-bridge, as well as its lower entropy. Although it is energetically

Table 1
Metal-ion AlaAla thermochemical calculations. DFT energies relative to the lowest energy conformation for each metal (corrected for zpe and thermal effects at 300 K). Binding energies (last row) are not corrected for basis set superposition error, which is usually minor at this computational level, nor for zpe or thermal effects. These are absolute binding energies relative to the neutral ground state. Free energies in parentheses are relative to the lowest free energy conformation (kJ/mol).

	Li	Na	K	Cs	Ca	Sr	Ba
CS-IIP (OO)	0 (0)	0 (0)	0 (0)	8 (9)	33 (27)	24 (23)	16 (21)
CS-II (OO)	6 (4)	9 (9)	1 (1)	0 (0)	41 (32)	32 (30)	17 (22)
CS-IP (NOO)[a]	11 (14)	9 (13)	13 (13)	15 (19)	**0 (0)**	**0 (0)**	**3 (11)**
					0 (0)	0 (0)	0 (5)
CS-I (NOO)[a]	13 (16)	10 (12)	16 (16)	21 (24)	**4 (3)**	**4 (4)**	**7 (14)**
					4 (3)	4 (4)	4 (8)
CS-IV (NO)	16 (15)	19 (20)	23 (20)	24 (24)	44 (43)		55
SB-I (OO)[a]			56 (52)	65 (63)	**12 (1)**	**5 (1)**	**0 (0)**
					18 (7)	12 (8)	3 (0)
SB-II (OOO)					28 (26)	28 (26)	31 (29)
Binding	308	218	160	114	656	554	477

[a] Boldface numbers use the Def2TZVP basis. Plainface numbers are 6-311 + g(d,p).

Table 2
Thermochemistry calculated for some metal-ion AlaAlaAla complexes as in Table 1.

	K	Cs	Ca	Sr	Ba
CS-II (OOO)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CS-IId (OOO)	16 (16)	18 (19)	12 (11)	15 (14)	18 (17)
CS-III (OOO)	17 (31)	15 (29)	37 (49)	32 (38)	29 (36)
CS-I (NOOO)	37 (40)	37 (44)	14 (20)	18 (24)	20 (25)
CS-IV (NOO)	16 (19)	31	56		
SB-III (OOO)	[a]	[a]	30 (42)	26 (40)	25 (36)
Binding	200	144	790	679	592

[a] Not a stable potential energy minimum.

unfavorable, the spectrum suggests that it cannot be ruled out as a possible contributor, and it is noted briefly below in discussing the spectroscopy.

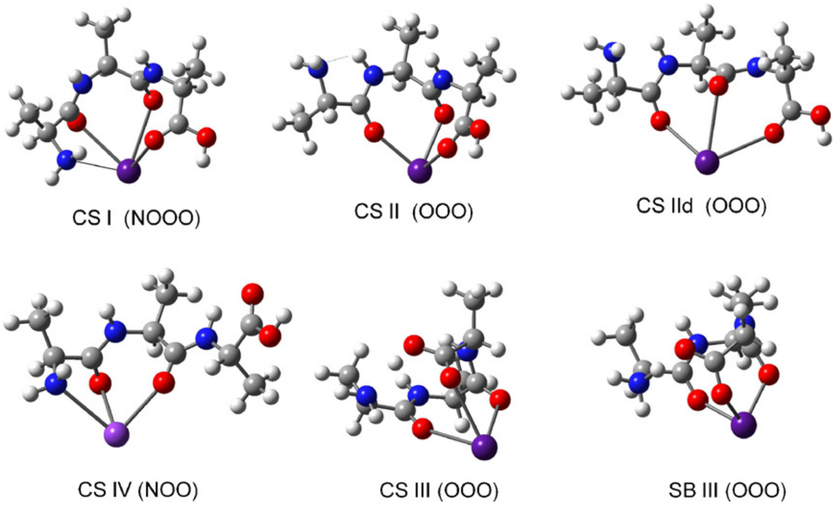
3.1.3. *Trialanine*

No exhaustive conformational search was attempted for the trialanine complexes, which offer a large number of possibilities, and for which the sodium complexes have already been extensively surveyed [20,28]. However, calculations were done on a substantial number of candidate structures considered as interesting possibilities, in order to get a sense of the variety of spectroscopic behavior and metal-ion trends that would correspond to different conformational themes. The lowest energy examples of these conformations are shown in Scheme 2. The CS conformers designated CS-I and CS-II are equivalent to those designated AAA-Na$^+$ 1-diast and AAA-Na$^+$ 2-diast by Balaj et al. [28], and CS-IId is a diastereomer variant of CS-II which is equivalent to their AAA-Na$^+$ 2. Our CS-IV is similar to (but not the same as) their AAA-Na$^+$ 4. Our CS-III and SB-III do not correspond to structures drawn in their paper.

The twin structures CS-III and SB-III are interconverted by the transfer of a proton, which may be considered as a shared proton occupying an anharmonic double potential energy well, as has been discussed for other comparable situations [1,14,43–47]. For the alkaline earth complexes, the two positions of the proton both give potential energy minima at the DFT level, while for the alkali complexes only the CS conformation was found as a minimum. However, the present systems give no chance to explore the interesting spectroscopy of this situation, since this class of conformations is not energetically competitive with the CS-II ground states.

The thermochemistry of these conformations is shown in Table 2. Continuing the pattern of the sodium case [28], the CS-II (OOO) conformation with the metal coordinated to all three $C{=}O$ groups was invariably the best of the tested structures by a convincing margin. No reason emerged to expect that the spectra would show other significant contributing conformations. As seen below, the spectra of the two diasteriomeric versions of CS-II are quite similar, but the thermochemistry of Table 2 indicates that the specific conformer designated CS-II is substantially more favorable than CS-IId.

The experience of other smaller systems had suggested to us that some zwitterionic conformation of the Ba complex would likely approach or surpass the CS conformations in stability, but surprisingly, even for Ba we did not find any zwitterion whose stability



Scheme 2. Representative conformations of M$^+$AlaAlaAla.

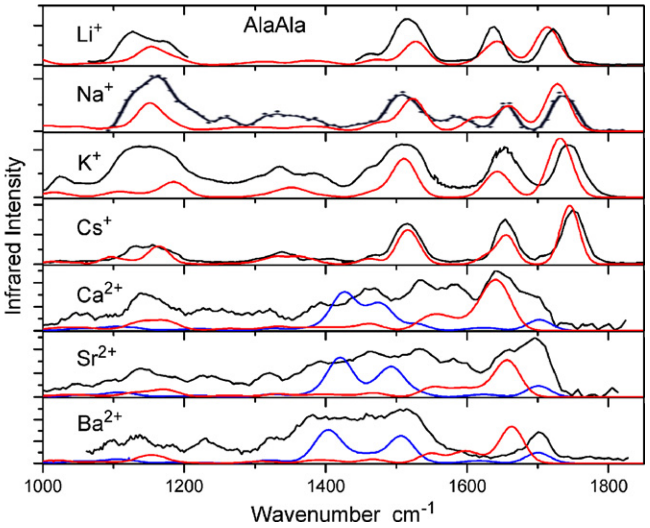


Fig. 1. IRMPD spectra of the dialanine complexes (black traces), along with the calculated CS (red) and SB (blue) conformations considered most likely to dominate the ground state populations. (The IRMPD plot for the Na^+ complex is extracted from Ref. [28].)

approached being competitive with the CS-II conformation. For the alkaline earths, the SB-III conformer is slightly more favorable than the proton-shifted CS-III, and, while it is much less favorable than CS-II, it is still a conformation with decent stability. For the alkali complexes, SB-III is not stable with respect to proton transfer to CS-III, and no other zwitterion structure was uncovered that was within 75 kJ/mol of the best charge-solvated structures.

3.2. Assignments of spectral peaks and ion conformations

3.2.1. Dialanine spectra

Fig. 1 displays the IRMPD spectra of the seven dialanine complexes. Also shown (in red) is the calculated spectrum of the CS conformation in Table 1 that we consider likely to be the dominant CS contributor to the observed spectrum. For the alkaline earth complexes, the calculated spectra of the most stable SB complex are also displayed (in blue).

Experimental band widths in IRMPD spectra typically range upward from about 25 cm^{-1}, governed by thermal broadening (room temperature or above, depending on the effectiveness of the collisional and radiative thermalization in the hexapole accumulation trap), anharmonicity effects in the IRMPD process [48,49], and conformational inhomogeneity. The FEL bandwidth (about 0.6%, or 5–10 cm^{-1}) [49] contributes only marginally to the overall linewidths. The FWHM peak widths in the present work in the 1000–1800 cm^{-1} region range upward from about 30 cm^{-1} for the narrowest well defined non-overlapped peaks (for instance, the peak near 1650 cm^{-1}).

3.2.2. Conformational implications

First the structure implications of the spectra can be considered. In drawing structure conclusions, the calculated spectra of the likely candidate conformations are compared with the observed spectra. As examples of the appearance of these different calculated spectral patterns, Figs. 2 and 3 show sets of calculated spectra for the Cs and Ca complexes. The Cs case is a straightforward situation of a single charge-solvated (OO) binding pattern (with two diastereomeric conformations) giving the lowest energy conformations and also giving an excellent fit to the observed spectrum. On the other hand, the Ca case is obviously a complicated story with a mixture of SB and CS binding patterns, for which a definitive assignment will not be achieved here. The regions most amenable to interpretation in this spectrum are the carbonyl/Amide I region around 1600–1750 cm^{-1}, and the COH bending region at 1150 cm^{-1}. Comparison with the computed CS1 and SB1 spectra in Figs. 1 and 3 shows that elements of both the CS and the SB conformations are present in the spectrum, indicating a mixture of conformers.

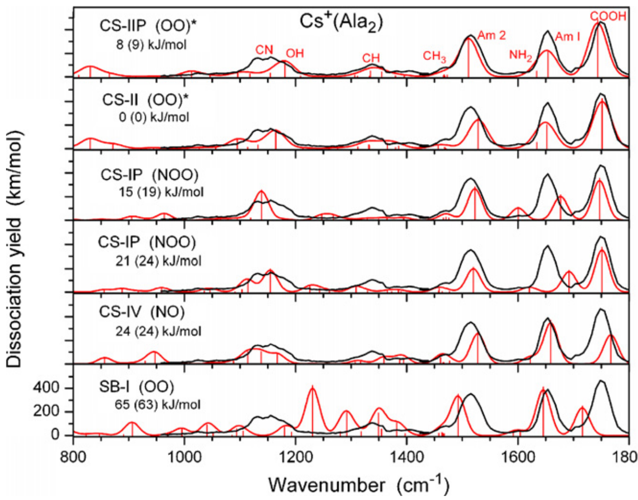


Fig. 2. Calculated spectra of Cs^+(AlaAla). Red traces are calculated spectra, black traces are IRMPD experimental results. Calculated intensities (km/mol) use a uniform scale as labelled for the bottom panel. Experimental intensities are dissociation yields (arbitrary units). Plots marked with an asterisk (*) are B3LYP/6-311 + g(d,p). Those without asterisk are B3LYP/6-31 + g(d,p). Energies (free energies) as in Table 1. (For interpretation of the references to color in the figure caption, the reader is referred to the web version of the article.)

The more straightforward case of Ba^{2+}, as shown in Fig. 1 (as well as Ref. [7]) represents the largely complete crossover to a purely SB structure as reflected in the spectroscopy (that is, the blue trace for SB1 in Fig. 1 gives quite a reasonable match to the IRMPD spectrum). Calculated spectra for various conformations of the dialanine complexes of all the metals are shown for reference in Supplementary Materials.

Detailed inspection of the calculated alkali/dialanine spectra in Figs. S1–S7 shows that one may or may not be able to differenti-

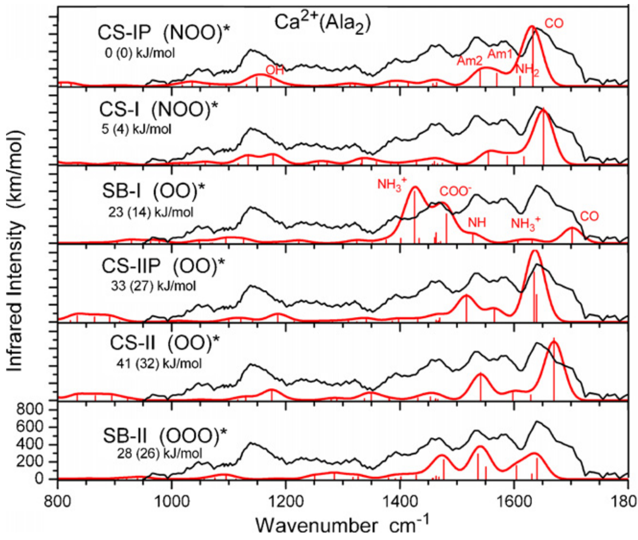


Fig. 3. Calculated spectra of Ca^{2+}(AlaAla). Red traces are calculated, black traces are IRMPD experiment. Calculated intensities (km/mol) use a uniform scale as labelled for the bottom panel. Experimental intensities are dissociation yields (arbitrary units). All calculations are B3LYP/6-311 + g(d,p), indicated by *. Energies (free energies) as in Table 1. (For interpretation of the references to color in the figure caption, the reader is referred to the web version of the article.)

ate the (OO) from the (NOO) conformations. For the lighter alkalis Na^+ and K^+ the spectra of CS-I, CS-IP, CS-II and CS-IIP are nearly all possible fits to the observed spectra. Only the CS-IV (NO) conformation is convincingly ruled out in all cases. This agrees with the conclusions of Balaj et al. for the Na^+ case. One might argue that the more highly-chelated CS-IP (NOO) conformation for Li^+/dialanine gives the best spectroscopic fit by a significant margin, in spite of the apparent energy advantage calculated for the (OO) conformations, but we would not take this as very conclusive evidence, and consider the Li conformation also to be undecided. On the other hand, for the heavy alkali Cs^+, the (OO) conformations give much better spectroscopic fits, as well as significant energy advantages over the (NOO) conformation, and we make the definite (OO) conformational assignment for this complex. Such lowered chelation of the metal ion for large metals is reminiscent, for instance, of the parallel situation for serine [1] or tryptophan [12].

In Fig. 2 (marked on the CS-IIP plot) and Fig. 3 (marked on the CS-IP and SB-I plots) we have shown the vibrational mode assignments of some of the prominent modes as they appear in the most likely conformations. The four principal modes that are further analyzed in Section 3.3 are shown, as well as some modes that are expected to be less intense. Note that the NH_2 scissor mode of the terminal amino group (CS conformers) is consistently located in the vicinity of the Amide I peak, but it is not a generally useful marker of a free NH_2 group because its intensity is generally low and it is often covered by the wing of the Amide I peak.

The most interesting feature of the alkaline earth complexes is the observed mixture of CS and SB conformations, as noted in Ref. [7]. Apparently for these metal ions the factors favoring CS (namely, the strong coordination or microsolvation of the metal ion by the two carbonyl oxygens, and the intrinsically greater stability in gas-phase of the canonical form of the terminal NH_2 and COOH groups compared with the charge-separated zwitterion NH_3^+ and COO^- groups) is accurately balanced by the factors favoring the zwitterion (namely, the large salt-bridge stabilization for the doubly charged ion, and the higher entropy of the extended SB structure). As noted above, the most stable CS configurations are the CS-IP configurations. For Ca and Sr, the spectrum in the 1600–1750 region is most reasonably interpreted as showing a mixture of SB-I and CS-I; there is both a characteristic CS peak near 1650 cm^{-1} and also a characteristic SB peak near 1700 cm^{-1} (although these two peaks are not fully resolved.) The Ba case shows little sign of the CS-I characteristic peak at 1650 cm^{-1}, and we consider this population to consist predominantly of SB-I. It is consistent with the thermochemistry of Table 2 that we see decreasing fractions of CS-I as we progress from Ca to Sr to Ba. Further supporting the assignment of partial CS-I population in the Ca and Sr cases, but not in the Ba case, is the significant intensity for the free-OH bending mode near 1150 for the Ca case, but little such intensity for the Ba case. (The Sr case has too poor a signal/noise ratio to make even a speculative judgement of this feature.)

The apparent poorly resolved peaks in the congested spectra of the alkaline earths between 1600 and 1350 cm^{-1} could be imaginatively assigned to various features of the candidate conformations. The most convincing possibility might be to match the three CS-I modes at about 1550, 1460 and 1400 cm^{-1} with the apparent bumps in the Ca and Sr spectra. However, this exercise is rather speculative and unproductive, and will not be pursued, keeping alive the hope for better-resolved spectra in the future.

For the K^+ and Cs^+ complexes, assignment of any important contribution of the SB-I conformation is ruled out by the failure to match the SB amide peak calculated at 1705 cm^{-1} and the NH_3^+ peak calculated at 1490 cm^{-1}, as well as the fact that the SB structure is energetically very unfavorable. Balaj et al. [28] also saw no indication of such a structure in their assignment of the Na^+ complex.

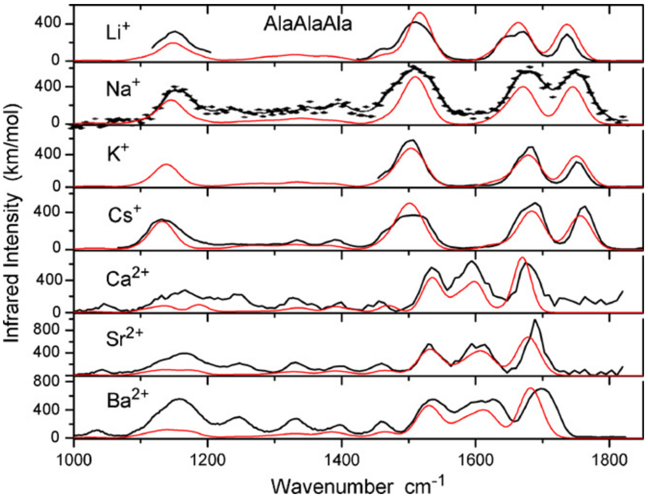


Fig. 4. IRMPD spectra of the trialanine complexes (black traces), along with the calculated CS (red) conformations considered most likely to dominate the ground state populations. (The IRMPD plot for the Na^+ complex is extracted from Ref. [28].) (For interpretation of the references to color in the figure caption, the reader is referred to the web version of the article.)

The diastereomer partners CS-I and CS-IP have quite similar calculated spectra in the cases where we calculated both. This is also true for the closely similar conformation pair CS-II and CS-IIP. (See the spectral plots in Figs. S1–S7 in the Supplementary Information.) Distinguishing conformations within these pairs spectroscopically is in general not feasible, nor do the energetics give a convincing basis for distinguishing them.

3.2.3. *Trialanine spectra*

Fig. 4 shows the spectra for the metal complexes with trialanine. Also shown in this figure are the computed spectra for the CS-II (OOO) structure, which is computed to be the lowest energy structure (in agreement with Balaj et al. [28] for the Na^+ case.) The agreement is very satisfactory in all cases, and this assignment seems very reasonable. Calculated spectra for the various metals and conformations are shown in Figs. S8–S12 (except the Li and Na cases, for which only the obvious CS-II conformation was calculated as shown in Fig. 4, making further plotting superfluous). The CS-IId conformation (the diastereomer of CS-II) gives spectra that are practically the same as CS-II, but have consistently higher energy (Table 2), so it is not considered likely to be a major contributor to the populations. Two other possibilities can be dismissed quickly. The lowest SB conformation is SB-III. This is in all cases higher in energy by more than 10 kJ/mol than CS-II, and has calculated spectra in strong disagreement with those observed. The CS-I conformer (NOOO) is a highly-chelated structure giving maximal solvation of the cation. For the alkaline earths, the CS-I energies are calculated to be not much higher than CS-II, and the spectra provide possible matches to the IRMPD results (see Supplementary Figs. S10–12), but there seems no reason to consider it seriously as a likely significant competitor to CS-II. Thus for the alkaline earths as well, CS-II is the obvious assignment, based both on energetics and on spectral comparisons. For illustration, Fig. 5 shows calculated spectra for the low-energy conformations of the Cs^+ complex.

3.3. *Frequency trends*

There are some regular trends in the peak positions as the metal ion changes. It is valuable to systematize the frequencies of some of the characteristic metal ion/peptide vibrational modes, as reflected in these small polyalanine examples, as a future guide to recognizing similar complexation patterns in metal-ion complexes of

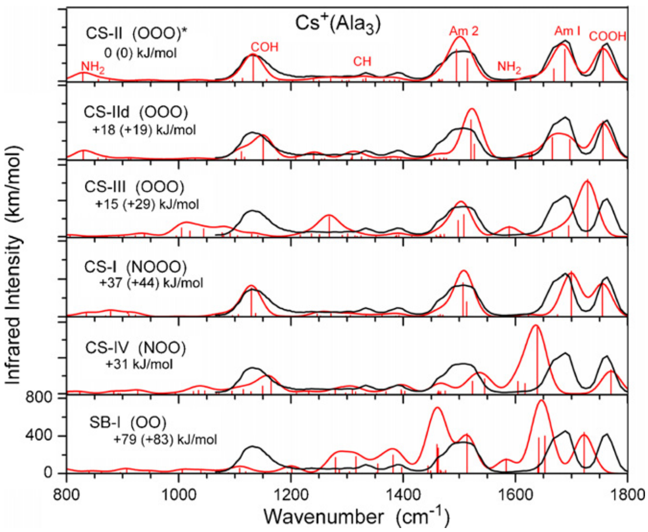


Fig. 5. Calculated spectra of Cs$^+$(AlaAla). Red traces are calculated, black traces are IRMPD experiment. Calculated intensities (km/mol) use a uniform scale as labelled for the bottom panel. Experimental intensities are dissociation yields (arbitrary units). * designates 6-311 + g(d,p) basis. Energies and free energies as in Table 2. (For interpretation of the references to color in the figure caption, the reader is referred to the web version of the article.)

other, perhaps larger, peptides. Our guiding hypothesis here is that there are regular trends as a function of the strength of the metal-ion/ligand interaction, spanning the series of metals from the strongly interacting Ca^{2+} ion to the weakly interacting Cs$^+$ ion. Figs. 6 and 7 trace the shifting positions of the characteristic IR peaks in the spectra of the complexes studied here.

In Fig. 6 for the trialanine complexes, there is no question about the CS nature of the conformations, and furthermore there is excellent agreement of the observed spectra with the CS-II calculations. Thus, the black trend lines connecting corresponding peaks down the series can be taken to approximately represent both the experimental and the calculated trends. (The solid black dots and the solid black trend lines plot direct experimental data, while the dashed red trend lines rely in part on theoretical values.) The top trace designated “neutral” is a computed reference spectrum of a model conformer of neutral trialanine which is a potential energy minimum, but has no hydrogen bonds to the carbonyl oxygens (see Supplementary Fig. S16). It is thus not a representation of the actual (hydrogen-bonded) ground state of trialanine, but rather is a reference structure comparable to the CS-II conformation of the metal-ion complexes, which also has no intramolecular hydrogen bonds to carbonyl oxygens in the peptide chain.

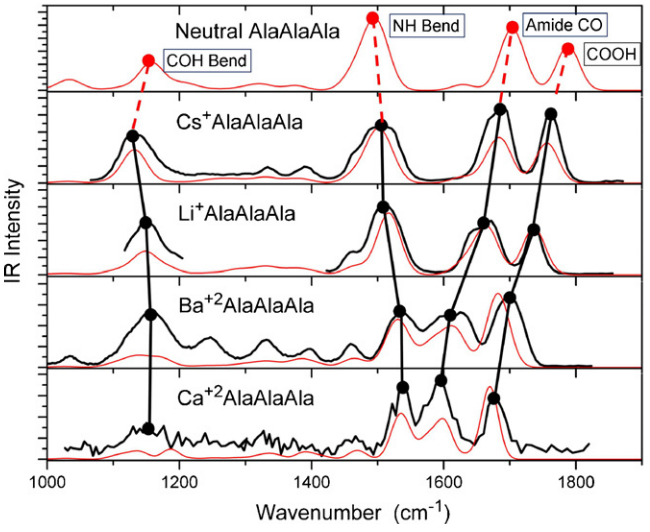


Fig. 6. Trends of the four characteristic modes of trialanine complexes. The dashed trend lines are correlations relying partly on computational results, while the solid trend lines are based on the assigned peak positions in the experimental spectra. Red traces are computed spectra, and black traces are experimental IRMPD results. (For interpretation of the references to color in the figure caption, the reader is referred to the web version of the article.)

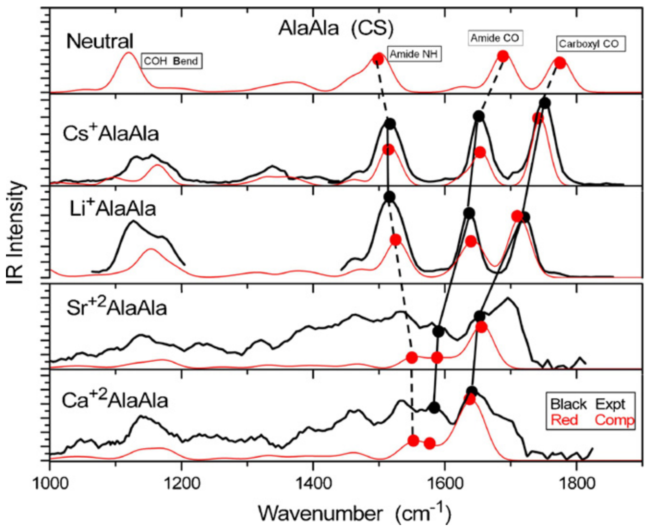


Fig. 7. Trends of the four characteristic modes of dialanine CS complexes. The dashed trend lines are correlations relying on computational results, while the solid trend lines are based on the assigned peak positions in the experimental spectra. Red traces are computed spectra and black traces are experimental IRMPD results. (For interpretation of the references to color in the figure caption, the reader is referred to the web version of the article.)

A similar trend plot for the dialanine complexes is shown in Fig. 7. As with the trialanine case, the “neutral” reference spectrum is a calculated conformation having no hydrogen bonding to the carbonyl groups (see Fig. S15). This plot is more problematic than the trialanine case, because the mixture of SB ions in the populations for the alkaline earth cases makes the assignment of the CS peak positions uncertain at best, and for some points requires complete reliance on computed spectra (as indicated by the dashed trend lines connecting points which are not fully experimentally derived). Nevertheless, it is clear from the plot that the dipeptides behave in a similar way to the tripeptides. This gives support to our hope that the systematic trends described here relating metal-ion binding to the perturbations of the characteristic IR peaks will retain some utility in extending to larger and more complicated peptide complexes.

Since we have little data for zwitterion conformations, we have not tried to systematize the peaks in the dialanine SB structures, and have limited the analysis to the CS conformations. We can note in passing that the one zwitterion normal mode for which we can make any confident conclusions, namely the amide carbonyl stretch near 1700 cm^{-1} (see Fig. 1), seems to be essentially unchanged in position from Ca to Sr to Ba. This is natural, since the metal ion bound to the carboxylate in these SB-I structures is remote, both in space and also along the chain, from the amide C=O bond.

Two prominent modes reflect the metal-coordinated COOH group (peptide C-terminus). One, the COH bend, appears near 1150 cm^{-1}. This mode seems to be a consistent marker for the presence of a free terminal COOH lacking a hydrogen-bonding interaction with neighboring groups. However, assigning a position to this mode is problematic, because it is usually encroached on the long-wavelength side by a cluster of CH bending modes between

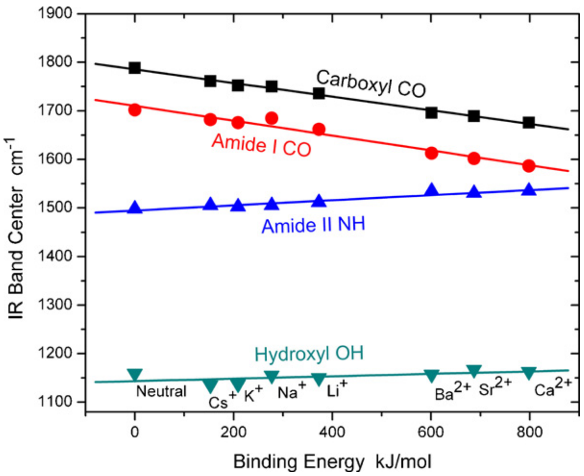


Fig. 8. Plots of the four characteristic modes of the trialanine complexes vs. binding energy of the metal ion. Band center frequencies are IRMPD experimental values except for the neutral molecule, for which the values are calculated using the non-interacting geometry described in the text. Lines are linear regression fits to the points.

1100 and 1150 cm^{-1}. Thus we have not tried to trace the trend of this mode in Fig. 7, and the trend line plotted for it in Fig. 6 must be taken with caution. Roughly speaking, however, this mode does not seem to change position very strongly as a function of metal-ion interaction. The other characteristic mode of the terminal carboxyl is the C═O stretch of the C-terminal carbonyl, which in all the present cases is coordinated with the metal ion. This peak progresses strongly to higher frequency with decreasing metal-ion coordination strength. Thus for Ca^{2+}(AlaAla) it is near 1650, while for Cs^{+}(AlaAla) it has moved all the way to 1750 cm^{-1}. Interestingly, this peak is systematically higher in frequency for the AlaAlaAla complexes than the AlaAla complexes, with the difference being greatest for Ca and Sr. We can speculate that the greater extent of chelation of the metal by the two amide oxygens of AlaAlaAla weakens the metal-ion interaction with the C-terminal carbonyl oxygen compared with AlaAla.

It has been generally accepted that the mode frequencies of the metal-interacting modes shift their positions to an extent that depends in a qualitative way with the strength of the metal-ion interaction with the ligand. Plotting the shifts as a function of the binding energy of the metal ion, as shown in Fig. 8 for the tripeptide complexes, puts this fuzzy expectation on a surprisingly quantitative footing. All four of the characteristic modes focused on here show strikingly linear correlation of their frequency shifts with binding energy. The two carbonyl stretches, whose oxygens are directly bound to the metal, show strong perturbation to lower frequency with increasing bond energy. On the other hand, the two hydrogen bending modes, which are only affected indirectly by the electron-withdrawing effect of the metal ion through three intervening atoms, show a weaker, but also linear, perturbation toward higher frequency with increasing bond strength, although the 1150 cm^{-1} mode shifts only a small amount. It is notable that these frequency trends correlate with the bond energy in particular, and not in a direct way with other characteristics of the metal such as size (ionic radius), charge, hardness/softness, electronegativity, or mass.

The two amide groups of trialanine give rise to two C═O stretching vibrations which comprise Amide I (see Fig. 2). These modes are coupled, giving symmetric and antisymmetric combinations with somewhat different frequencies (calculated splitting of 25–30 cm^{-1}). The experimental Amide I features show this splitting more or less clearly. In the trend plots of Figs. 6 and 8, we have estimated an average of the two apparent peak positions of the split Amide I cluster.

Velasquez et al. [50] described a similar metal-ion-induced red shift of the carbonyl stretch for acetone complexes of Mg^{+}, Al^{+} and Ca^{+} and gave an MO-oriented rationalization of the effect which posited a depletion of bonding electron density from the C═O bonding region of the high-lying MO's. Their open-shell magnesium and calcium cases are not strictly comparable to the present complexes, but the closed-shell aluminum complex is a good model of the metal-ion/carbonyl interaction, differing chiefly from the present complexes in that the acetone complexes have linear C═O–M^{+} bonding while the present complexes have bent C═O–M^{+} geometry. The red shift effect does not depend on significant transfer of electron density onto the metal ion for these weakly interacting closed-shell metal ions. Illustrating this principle, we found that the band shifts for the Na^{+}/acetone complex are reproduced quite closely if the sodium ion is replaced by a bare +1 charge at the same position.

The frequency shifts displayed in the plots of Figs. 6 and 7 serve as a graphic and quantitative probe of the perturbing interaction of the charge site with the carboxyl carbonyl and the amide carbonyl(s), as well as the indirect perturbation of the amide NH group. For a particular structure (in this case CS2), the strength of interaction between the metal ion and its solvating carbonyl ligands depends on (1) the metal-ion charge, (2) the size of the metal ion, and (3) the number of chelating carbonyl ligands. The plots of frequency shifts in Fig. 8 quantify these trends. The metal charge has the largest influence, as seen by the fact that the frequency shifts for all the doubly charged ions are greater than those for any of the singly charged ions. Within a single charge state, the interaction is stronger, and the frequency shifts are larger, for the smaller metal ions.

4. Conclusions

It was expected that the survey of the characteristic normal modes of the terminal groups and the amide linkage would show shifts of the mode frequencies reflecting the desire of the metal ion to find stabilizing microsolvation interactions with the peptide ligands, and this was indeed found to be the case. What was striking and unexpected in this study was finding that these shifts are actually accurately correlated in linear fashion with the binding energy of the metal ion to the ligand. Why these correlations should be linear between these particular two markers of solvating perturbations needs more profound theoretical consideration that we can offer. But the fact of this linearity should be valuable in future study of metal-ion/peptide complexes through the medium of infrared spectroscopy.

It will be of interest to see whether similar correlations exist for even more strongly bound metal ions (transition metals, or metal ions of higher charge). Analogous correlations will also be interesting to look for in side-chain-based vibrational modes of amino acids with functionalized side chains, with the possibility that vibrational perturbations can be established as quantitative measures of the strength of metal-ion/side-chain interaction.

Trend plots like Figs. 6 and 7 seem valuable in solidifying confidence in conformational assignments of the complexes with different metal ions, and in tracking the identity of the normal modes corresponding to prominent peaks. For instance, having accurate knowledge of the peak shifts of Amide I and Amide II, one can make confident comparisons of these important vibrational features between peptides bound to very disparate metal ions. As an illustration in the present work, the conclusion that the 1750 cm^{-1} peak in the alkaline earth complexes actually belongs to an SB component of the population would be very uncertain in the absence of systematic understanding of the positions expected for carboxyl and amide C=O groups in SB vs. CS conformations.

The observation of the mixed CS/SB populations in the Ca and Sr complexes of dialanine is among a still small number of cases where these two classes of conformations are in such close balance of stability. (At equilibrium at 298 K, a free energy difference not exceeding about 3 kJ/mole is required to keep the relative populations within a factor of 3 of each other.) A further route to understanding of this thermochemical balance can lie in studying progressive hydration of these systems, which is in general expected to push the systems toward SB conformations but has not yet received much attention over a range of metal-ion systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2010.07.001.

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