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Metal ion binding to peptides: Oxygen or nitrogen sites?

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ABSTRACT

Infrared multiple-photon dissociation (IRMPD) spectroscopy was used to probe the conformations of gas-phase metal-ion complexes between a series of five metal ions and six small peptide ligands. This report is presented in recognition and tribute for the Armentrout group's long and hugely productive interest in metal-ion binding to gas-phase ligands. The metal ions (K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}) span a range of ligand binding strengths, and the ligands include several dipeptides and tripeptides, and one tetrapeptide. The weaker metal ions generally form charge-solvated (CS) complexes binding amide carbonyl oxygen, while the strongest metal ion, nickel, deprotonates the amide nitrogens, probably through iminol tautomerization, and binds to the amide nitrogens. The Amide II vibrational mode (1500–1550 cm^{-1}) is found to be an excellent marker for the presence or absence of protons on amide nitrogens in a complex. The magnesium ion marks a boundary between these two structural motifs, forming iminol complexes with the dipeptides and switching to CS complexes for the tripeptides FGG and FGGF. Compared with solution-phase behavior, the iminol binding mode shown by Mg^{2+} for the smallest peptides is surprising, since this ion is considered as generally binding in a CS mode in solution. The present results for the larger peptides reconcile this surprising difference, showing that larger peptide ligands revert to the expected CS binding pattern for gas-phase Mg^{2+}.

1. Introduction

The conformational space of the ligands surrounding metal ions bound by, or embedded in, peptides is of great importance in understanding biological roles of metal ions. Amino acid side chains often play a role in the binding, but since each peptide residue in its own right offers both an oxygen and a nitrogen as potential backbone binding sites, it is conceivable that some, if not all, of the enclosing ligand sites around the metal ion are backbone amide sites. Small model peptides allow a systematic characterization of the strengths and conformational preferences of such chelating interactions using small model peptides in the gas phase.

Consideration of backbone binding preferences using model peptides that lack strongly basic side chain ligation sites is a natural starting point for a program of wider study of the details of metal-ion/peptide complexation using tools of gas-phase spectroscopy and mass spectrometry. Fundamental to considering binding to the backbone amide-linkage sites is the choice the system makes between ligating the metal ion through microsolvation by proximity to peptide carbonyl oxygen, or instead through deprotonation of amide nitrogen and metal-nitrogen bond formation. Structures **1** and **2** illustrate these two contrasting motifs for a compact conformation of $Ba^{2+}(Ala)_5$ characterized by extensive metal-oxygen chelation [1], and for a typical conformation of $Ni^{2+}(Ala)_4$

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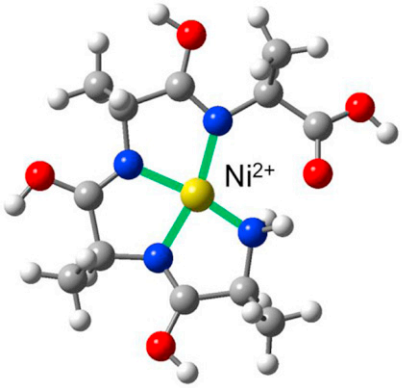
exhibiting metal-nitrogen chelation [2].



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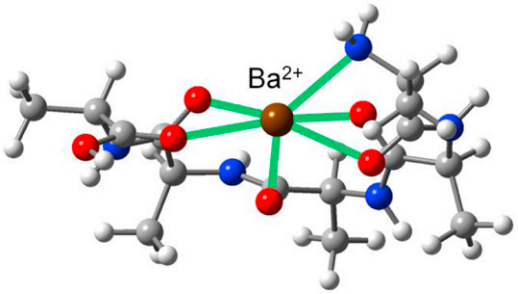

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Ni(II) is representative of strong-binding metal ions, typically transition metals, which form bonds to deprotonated backbone amide nitrogen atoms. The normal Ni^{2+} binding pattern is a nitrogen planar square, which may be capped by a more weakly-bound fifth ligand in a square pyramidal conformation [2,3]. For example, the common metal-carrying protein human serum albumin has a well characterized binding pocket for transition metal ions (including Ni^{2+}) at the N-terminal sequence motif (NH_2-Asp-Ala-His-). Its square-planar nitrogen framework is composed of the deprotonated amide nitrogens of the Asp and Ala residues, the terminal amine nitrogen, a side chain nitrogen on His, with a carboxylate oxygen from Asp at the capping site [4,5]. Another example is the binding site of oxytocin modeled by structure (**1**) for +2 transition metals at the N-terminus (**1**) formed from three deprotonated amide nitrogens and the N-terminal amine [6]. In their review of the solution chemistry of peptide complexes of metal ions, Sigel and Martin [7] highlight the role of side-chain anchoring groups in stabilizing the deprotonated-amide complexes of strong-binding transition metal ions including nickel. They do not specifically discuss the cation–π interaction with favorably situated aromatic side chains as an anchoring chelation site, although previous results from our laboratory [8,9], as well as work by Hu et al. [10] have shown the anchoring ability of such cation–π interactions in the gas phase.

The more weakly binding alkali and alkaline earth ions attach to oxygen (usually carbonyl) sites [11–13]. Structure **2** shows a recently calculated example of the gas-phase structure of a charge solvated (CS) complex (with pentaalanine) of an alkaline earth ion (Ba^{2+}) [1]. The tendency to retain some hydration is pronounced with alkaline earths, especially Mg^{2+}. For instance, the fourfold oxygen-bound Mg^{2+} ions characterized in the magnesium transport protein MgtE are at least partially, and perhaps fully, hydrated [14]. One environment involving direct contact of desolvated alkali and alkaline earth ions with backbone carbonyls is the interior of ion channel proteins and ion transporters. For example, potassium [15,16] and calcium transport proteins apparently strip off hydration water of the ion passing through the cavity; these binding sites are apparently always oxygens. Similarly, Lunin et al. [17] consider that the Mg^{2+} ion is at least partially stripped of solvent waters during passage through the selective pore of the CorA protein, and the X-ray results of Eshagi et al. [18] also support this picture. An example of bound calcium apparently stripped of water in a static complex using oxygen binding sites is the calcium binding protein calmodulin [19,20] where it is sevenfold coordinated, with just one water ligand.

One way to build up understanding of metal-ion binding preferences in this context is through the structural characterization of isolated gas-phase complexes of metal ions with peptides. Rodgers and Armentrout [21] have explored the application of thermochemical approaches to this task. The group of Lisy have used infrared photodissociation spectroscopy in the 3 μm wavelength region in numerous studies characterizing the interactions of metal ions (usually Na^+ and K^+) with model ligands involving for example amide-carbonyl oxygen [22], aromatic basic sites like phenol [23], or bidentate chelation structures [24]. The recently developed infrared-spectroscopic tool of infrared multiple photon dissociation spectroscopy (IRMPD), based on the combination of powerful tunable infrared light source with a mass spectrometer, has the power to characterize the in situ structures of mass selected gas-phase complexes [8,25–39]. Application of this approach in the mid-infrared wavelength region has been found to be particularly good at distinguishing the two different binding modes of interest here, and the free electron laser (FEL) laser light source coupled to an FTICR mass spectrometer at the FELIX facility has been brought to bear on study of gas-phase metal-ion peptide complexes. The comparable facility at the CLIO FEL has engaged in similar studies. Much has been learned about binding patterns in dipeptides [9,26,28,33,34,40], as well as larger peptides [1,8,26,31,35,37,41–44].

Here we report work extending up to a tetrapeptide ligand, and present a more extensive and systematic account of trends in binding as a function of peptide size and metal identity than previously. By systematic comparison across both a series of metal ions and a series of peptide ligands, new insight emerges on how the metal ion environment is affected by the increasing number of residues in the chain. The magnesium ion turns out to be pivotal in the progression of increasingly strongly bound metal ions, showing a key switch from small-peptide to large-peptide characteristics.

The amide-linkage binding sites of alkali ions have consistently been found to be the carbonyl oxygens in the so-called charge solvated (CS) binding mode. (The N-terminal amine nitrogen is sometimes also implicated, as well as Lewis-basic side-chain binding sites when they are available.) In general the alkaline earth divalent metal ions Ca^{2+}, Sr^{2+} and Ba^{2+} have also preferred oxygen binding. For dipeptides, where only a single backbone carbonyl is available for oxygen chelation, binding of alkaline earth ions to the C-terminal carboxyl end group in a zwitterion binding mode is also possible if an additional anchor site is available to stabilize the zwitterion [41]. Ba^{2+} often prefers the zwitterion binding mode, while Sr^{2+} and Ca^{2+} have shown evidence of a mixture of zwitterion and CS complexes [41]. More recently, it has been discovered that strongly binding divalent metal ions, notably Mg^{2+}, Ni^{2+} and Co^{2+}, displace the amide proton and bind to the amide nitrogen in dipeptides, with the ligand undergoing an iminol tautomerization [9,45]. A point of interest [9] was that this iminol binding mode to the amide nitrogen was preferred for the magnesium

ion, in contrast to condensed-phase experience indicating a strong preference for amide oxygen binding of this metal ion in larger peptides. A goal of the present work was to determine whether this difference in behavior relates to the difference between gas phase and condensed phases, or whether it is instead a trend intrinsic to the number of residues in the chain. Furthermore, Ca^{2+}, while showing a preference for oxygen binding to dialanine, also showed evidence of a component of nitrogen-bound iminol ions in a population of dipeptide complexes [9], and another goal of the present work was to further investigate the occurrence and conditions for amide nitrogen binding of Ca^{2+}.

2. Experimental

The apparatus and general experimental procedures have been described in detail previously [46]. Ions were generated by electrospray ionization (ESI) from acetonitrile/water solutions of the nitrates or chlorides of the metals, usually at 1 mM concentration of both salt and ligand. Ions were thermalized for about 5 s in a linear hexapole trap, before introduction into the FT-ICR cell where they were mass-isolated and subsequently irradiated for about 2–6 s by the infrared beam from FELIX. 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 linear correction corresponding to the measured laser intensity as a function of wavelength was applied, and the laser wavelength was calibrated using a grating spectrometer two or three times a day, or following a change in laser parameters.

Several of the target ions did not emerge with useful abundance from the ESI source except solvated by one or two acetonitrile molecules. It was often possible to desolvate these by a 0.1–1.5 s pulse of irradiation from a 35-W continuous-wave CO_2 laser, followed by isolation of the desired species from the laser-dissociation fragments. Among those for which desolvation was successful were Ca^{2+}FG, Ca^{2+}FGGF, Mg^{2+}FG, Mg^{2+}FF, Mg^{2+}FGGF, Co^{2+}FF, Ni^{2+}FF, and Ni^{2+}AAA. Some interesting species, among them Cu^{2+}FA and Ni^{2+}FG could not be successfully desolvated without decomposition of the desired parent ion.

3. Results

The panel to the right in Fig. 1 displays the series of ligand variations for each of the five metal ions surveyed. Some of the spectra have been analyzed and published previously, providing a solid interpretive basis for correlating spectroscopic characteristics with the different structure types of the complexes. A large number of new spectra are also reported, notably including all of the ones displayed for the FGG and FGGF ligands. A regrouping of the spectra by ligand instead of by metal is also helpful in visualizing the trends, and such a regrouped set of spectra is displayed as the left column in Fig. 1.

The principal point of the present report is that three different binding modes can usually be distinguished based on characteristic spectral features. Before discussing in detail the choice of spectroscopic markers and their application to this set of ions, it may be helpful to summarize our final structure assignments of the spectra of Fig. 1. The complexes of K^+ are all assigned as CS structures, as are the Ba^{2+} and Ca^{2+} complexes of AAA, FA, FG, FF, FGG and FGGF. Also CS are the Mg^{2+} complexes of FGG and FGGF. The Ba^{2+} complex of AA is assigned as ZW, while the cluttered spectrum of the Ca^{2+} complex of AA suggests a mixture of ZW and CS. The iminol assignments are the Mg^{2+} complexes of FF, FA and FG, and the Ni^{2+} complexes of AAA, FA, FF, FGG and FGGF. Note that the Ca^{2+} complexes of FA and FG seem inconclusive, likely mixtures of structures with at least some CS. In the following, we detail the spectroscopic indicators (and some computed thermochemical stabilities) forming the basis for these assignments.

3.1. Charge solvated (CS)

The five K^+ spectra are typical examples. The pattern of three major peaks at 1750 cm^{-1} (metal-bound terminal carboxyl carbonyl stretch), Amide I at 1650 cm^{-1} and Amide II at 1520 cm^{-1} is reproduced in many examples both here and in the literature. The carboxyl stretch and the Amide II peak are subject to important wavelength shifts as a function of metal ion identity, as has been well characterized [8]. The Amide I region is not always a simple sharp peak, apparently in part because of the inconsistent appearance of the NH_2 scissors mode in this same region. However, the Amide II peak is highly predictable, strong and predictable for all the complexes containing amide NH groups, and we take this feature as a useful marker of the presence of amide linkages in their conventional tautomerization motif.

The CS pattern can be recognized in the spectra for most of the complexes. The Ba^{2+} complexes except for dialanine appear as CS structures, as is also the case for the Ca^{2+} complexes other than dialanine. The Mg^{2+} complexes with FGG and FGGF give spectra conforming very nicely to the CS pattern. Only the Ni^{2+} complexes show no obvious examples of CS binding.

3.2. Zwitterion

For dipeptides, with only a single amide carbonyl to chelate the metal ion, a zwitterion conformation of the ligand has been found to be competitive with CS binding giving (for the AA ligand) partial (Ca^{2+}) or complete (Ba^{2+}) complexation in this mode [41]. Indicating the presence of ZW binding, the Amide I peak around 1650 cm^{-1} signaling metal-coordinated amide carbonyl groups weakens or disappears, as in the Ba^{2+}AA spectrum in Fig. 1, and the carboxylate asymmetric COO^- stretch is calculated and observed near 1700 cm^{-1}.

3.3. Iminol (enol tautomerized peptide bond)

In the iminol binding motif, the metal ion coordinates to the deprotonated amide nitrogen, as described in Ref. [9]. The outstanding signal of binding of the metal to the amide nitrogens is the disappearance of the Amide II peak between 1500 and 1550 cm^{-1}, the observation of which is strongly indicative of the presence of a proton bound to the amide nitrogen. The present survey does not focus on computational comparisons, but it is important to stress that a substantial body of computational results (many not yet published) support the proposition that this Amide II feature signaling the proton-bearing amide nitrogen is consistent across a wide range of complexes, not being strongly affected by the nature of the metal, length of the peptide chain, or variations in hydrogen bonding motif. It would be useful in addition to identify a reliable positive spectroscopic marker of the iminol conformation, but such an effort is not very successful. The closest thing to such a marker is the cluster of peaks at 1400–1450 cm^{-1}, which calculations suggest is due in part to a hydrogen bending motion of the iminol OH proton, along with other hydrogen bending motions. This cluster is observed with varying intensity in most of the spectra identified as likely iminol conformations (strongly for Mg^{+2}FA/Mg^{2+}FG, Mg^{2+}FF, Ni^{2+}FA and Ni^{2+}FGGF, weakly for Ni^{2+}FGG and Ca^{2+}FF) and is weak or absent in the CS cases, but its intensity, position and shape are too erratic for this to serve as an unambiguous conformational marker (probably because of variations in the hydrogen bonding nature of the hydroxyl hydrogen of the iminol tautomer).

Most of the complexes of K^+, Ba^{2+} and Ca^{2+} are clearly CS conformations. Exceptionally, the dipeptide AA does not fit this

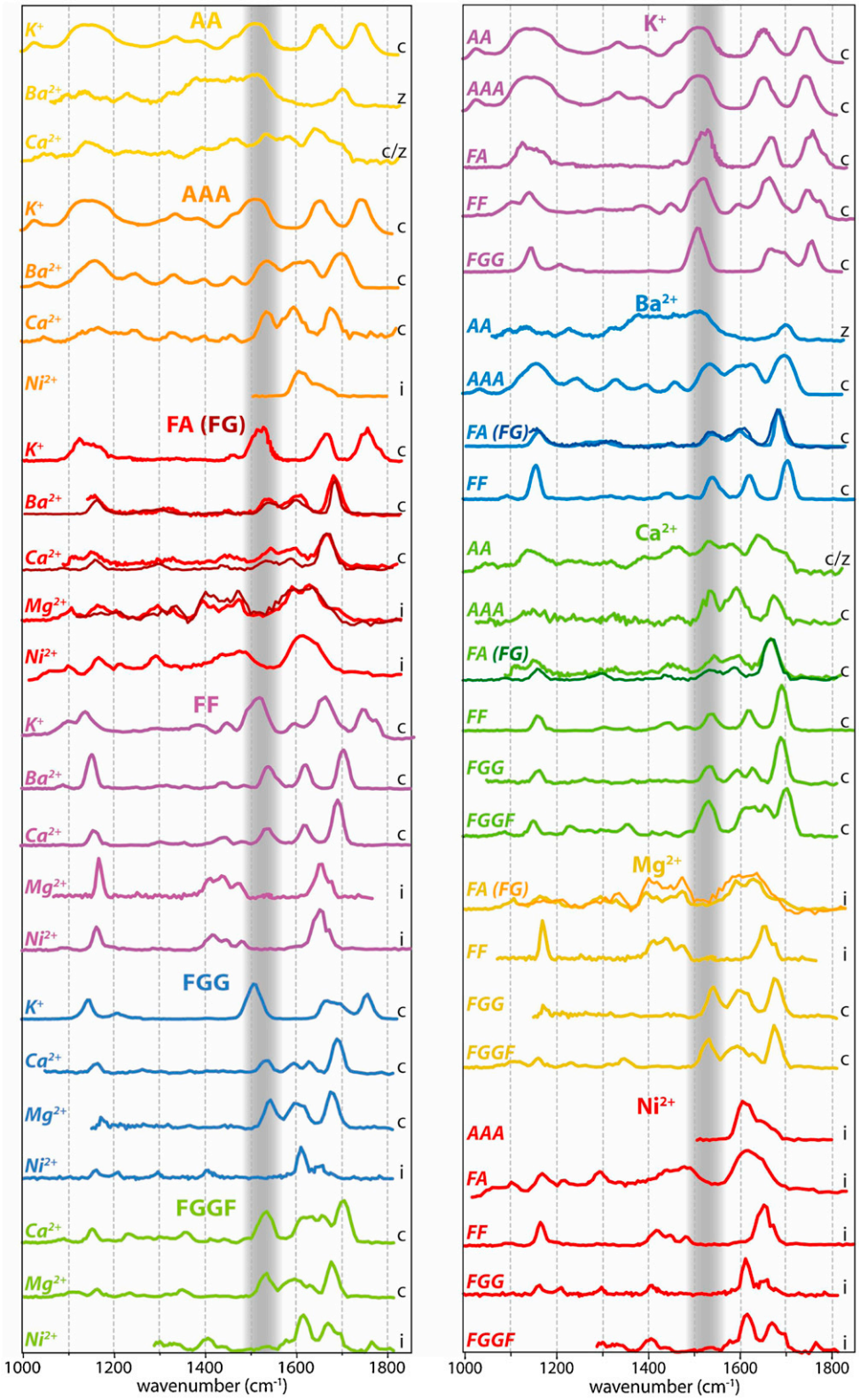

Fig. 1. IRMPD spectra of five metal ions complexed to various peptide ligands. To the right, the spectra are grouped by metal ion, while to the left, the same spectra are grouped by ligand. The gray shading indicates the approximate position of the amide NH bending mode ("Amide II mode"). The presence of this band indicates that the ligand is in the 'normal' amide configuration, while absence of the band suggests a tautomerization to the iminol motif, in which the proton has moved from the amide nitrogen to the amide oxygen atom. The *c,z,i*-letter code to the right of each spectrum indicates our assignment as charge-solvated, zwitterion or iminol, respectively. Note that the spectra for the FG (PheGly) ligand have been superimposed on the FA spectra in a slightly darker color, and that the spectra of this pair of ligands are consistently very similar.

spectroscopic pattern well for either Ba^{2+} or Ca^{2+}. The Ba^{2+}AA complex was previously assigned as a ZW conformation [41]. A partial contribution from an iminol-type conformation (which was not considered as a possibility in Ref. [41]) would also be consistent with the spectroscopic picture, accounting for the high intensity near 1400 cm^{-1}, but this structure was calculated to be thermochemically disfavored by a very large amount (at least 53 kJ mol^{-1}). The Ca^{2+}AA spectrum was previously assigned as a mixture of ZW and CS structures [41], and may include other structures as well. No compelling new spectroscopic or thermochemical arguments have emerged to require reevaluation of these two species, and there seems no reason to change these previous conformational assignments for these two ions.

The Ni^{2+} complexes are all clearly iminol structures. (The AA complex would be of interest here as well, but could unfortunately not be produced for Ni^{2+} or Mg^{2+} in our instrument.) The most interesting metal is Mg^{2+}, whose FA, FG and FF complexes clearly lack an Amide II band, but whose complexes with the FGG and FGGF ligands clearly show a strong Amide II band as part of clearly CS-pattern spectra. Thus the Mg^{2+} complexation shows a transition from deprotonated amide nitrogen coordination (presumably in the iminol configuration) for the dipeptides to oxygen coordination for the tri- and tetrapeptides. Furthermore, the CS spectra of the two larger complexes show no sign of a peak in the 1400–1450 cm^{-1} region, which we take as a further indication that all of the two (or three) amide groups are oxygen coordinated, and none of the possible amide nitrogens are metal-coordinated. On the other hand, in the Ni^{2+} case, the FGG and FGGF complexes show no sign of an Amide II peak, which suggests that all of the (two or three) amide linkages have the metal ion bound to the deprotonated nitrogen.

4. Discussion

4.1. Transition from oxygen to amide nitrogen coordination

The behavior observed for this series of complexes strengthens the hope that useful parallels can be found between gas-phase and condensed-phase binding of metal ions to peptide frameworks. The previous observation [9] that magnesium ions prefer iminol binding to at least one dipeptide in the gas phase was an interesting and surprising contrast to the general expectation from condensed-phase experience of consistent oxygen binding for this metal ion in large peptides. The present results, showing that magnesium ions revert to oxygen binding in the gas-phase complexes with a tripeptide and a tetrapeptide, reconcile these previous findings. The larger peptide ligands are obviously more comparable to larger polypeptides that are of primary concern in condensed-phase binding, so it is useful to find that the binding propensity in gas phase becomes similar to experience with larger peptides in condensed phase even for a ligand as small as a tripeptide.

The evidence from the present results for FGG and FGGF complexes is that either all of the amide linkages bind the metal to their carbonyl oxygens, or all of them deprotonate at the nitrogens and bind the metal there. Transitional cases with some but not all of the amide nitrogens bound to metal would be a possibility for magnesium or nickel, but were not encountered. Ni^{2+} is well known for the favorable formation of planar complexation structures with an array of bound nitrogens (including nitrogens from deprotonated amide linkages as well as available nitrogens from the terminal amino group and from side chains) surrounding the metal ion (Structure **1**), most favorably in a square planar arrangement of the four nitrogen atoms. Solution results provide support for the "all or nothing" nature of the amide deprotonation observed in the present cases. In solution, the successive association constants for deprotonation and attachment of the metal ion increase at successive amide nitrogens in triglycine and tetraglycine [7], which suggests that if conditions favor metal ion deprotonation and attachment at one amide nitrogen, the second (and third) nitrogens will be even more favorable for attachment. It is thus not surprising to see the Ni^{2+} ion in the present complexes binding all of the amide linkages with metal-nitrogen coordination. It will be interesting with further detailed study to assess whether the Ni^{2+}FGGF complex is truly planar and tetradentate with all four nitrogens, similar to the well known behavior of this ion with tetrapeptides (like GGGG) in solution [2,47].

The alkaline-earth complexes with the dipeptide AA appear to be mixed populations [41], and do not fall easily into the patterns of the other ligands. However, the anchoring effect of the N-terminal phenyl group seems to stabilize the complexes of FA and FF into more settled CS conformations for these relatively weak-binding metal ions. The additional stability provided by this phenyl side chain seemed necessary in our instrument for dipeptide complex formation with the metal ions Mg^{2+} and Ni^{2+} (since no such complexes were obtained using AA). AAA complex formation with Ni^{2+} was also difficult to observe, but the Ni^{2+}AAA complex was briefly obtained in sufficient abundance to give us the partial spectrum shown in Fig. 1, which shows clearly the similarity of this complex to the other Ni^{2+} complexes, and similarly shows the absence of an Amide II peak between 1500 and 1550 cm^{-1}. Thus for Ni^{2+} the propensity for iminol complexation is sufficiently strong that the anchoring effect of an N-terminal phenyl side chain is not necessary for gas-phase formation of the tripeptide complex with AAA having both amide nitrogens cooperatively deprotonated and metal-chelated. Martin [2] notes that this is the normal binding mode of tripeptides to nickel ions under basic conditions in solution, whereas dipeptides (at least those without effective side-chain anchors) form only hexacoordinate bis complexes. It is thus not surprising that we were unable to form monomeric Ni^{2+} complexes with AA in the gas phase.

Over the present set of complexes, comparisons with calculated thermochemistry (both published results as well as some to be described in detail in subsequent publications) show that the observed predominant structure resulting from electrospray as inferred from the present spectroscopic results are in accord with the lowest energy calculated structure (or one of the lowest structures when there are multiple structures within a few kJ mol^{-1} of the lowest one). It is not safe to rely on such agreement in general, since cases are already known where a higher energy structure is kinetically trapped as the predominant species extracted to gas phase by electrospray. (As an example, see the p-hydroxybenzoic acid case recently worked out in an elegant study by the group of the late Schröder [48].) Thus, for instance, the thermochemical justification above for discounting the possible presence of an Iminol component in the population of Ba^{2+}AA is a weak argument, and it would be rash to take this as a firm conclusion in the absence of further evidence from another source.

4.2. Size and binding strengths

The binding behavior of the different metal ions shows wide differences. Two properties of the metal ion have been thought about as possibly correlating with the binding patterns, namely the metal-ion size and the binding strength. Size can be characterized by the familiar ionic radii (r_{ion}), derived largely from solid-state crystals (see Table 1). Perhaps more to the present point, one can also characterize their chelation sizes (r_{ch}) using their typical bond distances from Lewis-basic chelating atoms (carbonyl oxygen is most relevant here) which can be accurately calculated with modern computations. A set of r_{ch} values is shown in Table 1 taken from our recent calculations of the metal ion distance to the amide carbonyl oxygen in the FF complexes [28], which provide a

Table 1
Radii and binding strengths of ions for the FF ligand. The r_{ion} values are standard ionic radii. The r_{ch} values are derived from the calculated metal–oxygen bonds in the FF complexes. Although the ground state of the nickel complex is an iminol complex with no oxygen ligand, for comparability with the other metal ions the values given here are for the computationally accessible CS binding mode.

	K^+	Ba^{2+}	Ca^{2+}	Mg^{2+}	Ni^{2+}
r_{ion} (Å)	1.33	1.34	0.99	0.66	0.69
r_{ch}[a] (Å)	1.91	1.84	1.61	1.28	1.27
D_{FF} (kJ mol^{-1})[b]	183	597	746	1062	1314

[a] DFT value of metal/amide carbonyl distance in M^{n+}FF after subtraction of an oxygen radius of 0.66 Å. (Geometries from Ref. [28] and present calculations.)
[b] DFT values from Ref. [28].

convenient series of chelated complexes whose conformations are quite confidently known.

The chelation binding strength of a metal ion obviously varies depending on the ligand, but a useful comparative set of values can be given for the different metals referenced to the same ligand. We give in Table 1 the binding strength values D_{FF} calculated for the present series of metal ions bound to FF.

Using these data, we can ask whether it is more useful to look at the size or the binding energy in predicting whether two metal ions will show similar or different binding patterns. One possible comparison within this data set is K^+ and Ba^{2+}, which have nearly the same size but very different binding energies. They show very different binding patterns for the AA complex (see Fig. 1), but for the other complexes with more extensively chelating ligands the binding appears to be CS for both metals. However, it is not very meaningful to compare their binding behavior, since the difference in charge is obviously a major confounding factor. Further complicating this particular comparison is the fact that Ba^{2+}, uniquely among the metal ions in this set, may have a propensity for salt-bridge formation with dipeptides in a zwitterion binding pattern [8,41]. Mg^{2+} and Ni^{2+} are the same size, and have the same charge, so a comparison is more meaningful. Their binding behavior to FGG and FGGF is quite different. This contrast is easily reconciled if we consider the binding strength to be a more informative predictive variable than size, since Ni is a much more strongly binding metal than Mg.

5. Conclusions

The present spectroscopic survey of a range of ligands and metal ions gives a more comprehensive overall view than previous more focused studies of the possible binding modes and propensities for metal ions with small peptides. It is not surprising to find, parallel to solution phase, that the more weakly binding metal ions favor binding to the amide carbonyls, while the much stronger-binding nickel ion is able to deprotonate the amide nitrogens (via the iminol tautomerism) and bind in metal-nitrogen-coordinated conformations. An interesting feature is the transitional behavior found for the magnesium ion, which switches from its (surprising) gas-phase behavior as an iminol-binding metal for dipeptides, to its oxygen-binding behavior, as expected by analogy to its binding behavior with peptides in solution.

The Amide II feature was found, as in previous work, to be a clear and unambiguous marker of the presence of one or more amide N–H moieties, showing small and predictable wavelength shifts upon complexation, and is thus an exceptionally useful probe for metal-ion binding to the amide nitrogen atoms.

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References

[1] R.C. Dunbar, J.D. Steill, N.C. Polfer, J. Oomens, Journal of Physical Chemistry A (2012), http://dx.doi.org/10.1016/j.ijms.2012.10.006.
[2] R.B. Martin, in: A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems: Nickel and Its Role in Biology, Marcel Dekker, New York, 1988, p. 123.
[3] R.K. Andrews, R.L. Blakely, B. Zerner, in: A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems: Nickel and Its Role in Biology, Marcel Dekker, New York, 1988, p. 165.
[4] J.D. Glennon, B. Sarkar, Biochemical Journal 203 (1982) 15.
[5] J.P. Laussac, B. Sarkar, Biochemistry 23 (1984) 2832.
[6] T. Wyttenbach, D. Liu, M.T. Bowers, Journal of the American Chemical Society 130 (2008) 5993.
[7] H. Sigel, R.B. Martin, Chemical Reviews 82 (1982) 385.
[8] R.C. Dunbar, J.D. Steill, J. Oomens, International Journal of Mass Spectrometry 297 (2010) 107.
[9] R.C. Dunbar, J.D. Steill, N.C. Polfer, J. Oomens, Angewandte Chemie International Edition 51 (2012) 4591.
[10] P. Hu, C. Sorensen, M.L. Gross, Journal of the American Society for Mass Spectrometry 6 (1995) 1079.
[11] R.B. Martin, in: A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems: Probing of Proteins by Metal Ions and Their Low-Molecular-Weight Complexes, Marcel Dekker, New York, 1984, p. 1.
[12] R.B. Martin, in: A. Sigel, H. Sigel (Eds.), Magnesium and Its Role in Biology, Nutrition and Physiology, Marcel Dekker, New York, 1990, p. 1.
[13] C.B. Black, H.W. Huang, J.A. Cowan, Coordination Chemistry Reviews 135/136 (1994) 165.
[14] M. Hattori, Y. Tanaka, S. Fukai, R. Ishitani, O. Nureki, Nature 448 (2007) 1072.
[15] D.A. Doyle, J.M. Cabral, R.A. Pfuetzner, A. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, R. MacKinnon, Science (Washington D. C.) 280 (1998) 69.
[16] Y. Jiang, A. Lee, J. Chen, V. Ruta, M. Cadene, B.T. Chait, R. MacKinnon, Nature (London U. K.) 423 (2003) 33.
[17] V.V. Lunin, E. Dobrovetsky, G. Khutoreskaya, R. Zhang, A. Joachimiak, D.A. Doyle, A. Bochkarev, M.E. Maguire, A.M. Edwards, C.M. Koth, Nature (London U. K.) 440 (2006) 833.
[18] S. Eshaghi, D. Niegowski, A. Kohl, D.M. Molina, S.A. Lesley, P. Nordlund, Science 313 (2006) 354.
[19] R. Chattopadhyaya, W.E. Meador, A.R. Means, F.A. Quiocho, Journal of Molecular Biology 228 (1992) 1177.
[20] Y.S. Babu, C.E. Bugg, W.J. Cook, Journal of Molecular Biology 204 (1988) 191.
[21] M.T. Rodgers, P.B. Armentrout, Accounts of Chemical Research 37 (2004) 989.
[22] D.J. Miller, J.M. Lisy, Journal of Physical Chemistry A 111 (2007) 12409.
[23] T.D. Vaden, J.M. Lisy, Journal of Chemical Physics 120 (2004) 721.
[24] A.L. Nicely, D.J. Miller, J.M. Lisy, Journal of the American Chemical Society 131 (2009) 6314.
[25] J. Oomens, B.G. Sartakov, G. Meijer, G. von Helden, International Journal of Mass Spectrometry 254 (2006) 1.
[26] O.P. Balaj, C. Kapota, J. Lemaire, G. Ohanessian, International Journal of Mass Spectrometry 269 (2008) 196.
[27] M. Benzakour, M. McHarfi, A. Cartier, A. Daoudi, Journal of Molecular Structure-Theochem 710 (2004) 169.
[28] R.C. Dunbar, J.D. Steill, J. Oomens, Journal of the American Chemical Society 133 (2011) 9376.
[29] J.R. Eyler, Mass Spectrometry Reviews 28 (2009) 448.
[30] T.D. Fridgen, Mass Spectrometry Reviews 28 (2009) 586.
[31] J. Oomens, N. Polfer, D.T. Moore, L. van der Meer, A.G. Marshall, J.R. Eyler, G. Meijer, G. von Helden, Physical Chemistry Chemical Physics 7 (2005) 1345.
[32] N.C. Polfer, J. Oomens, Mass Spectrometry Reviews 28 (2009) 468.
[33] N.C. Polfer, J. Oomens, R.C. Dunbar, ChemPhysChem 9 (2008) 579.
[34] J.S. Prell, M. Demireva, J. Oomens, E.R. Williams, Journal of the American Chemical Society 131 (2009) 1232.
[35] J.S. Prell, T.G. Flick, J. Oomens, G. Berden, E.R. Williams, Journal of Physical Chemistry A 114 (2010) 854.
[36] T.R. Rizzo, J.A. Stearns, O.V. Boyarkin, International Reviews in Physical Chemistry 28 (2009) 481.
[37] D. Semrouni, O.P. Balaj, F. Calvo, C.F. Correia, C. Clavaguéra, G. Ohanessian, Journal of the American Society for Mass Spectrometry 21 (2010) 728.
[38] M. Citir, C.S. Hinton, J. Oomens, J.D. Steill, P.B. Armentrout, Journal of Physical Chemistry A 116 (2012) 1532.
[39] A.L. Heaton, V.N. Bowman, J. Oomens, J.D. Steill, P.B. Armentrout, Journal of Physical Chemistry A 113 (2009) 5519.
[40] R.C. Dunbar, J.D. Steill, J. Oomens, J. Am. Chem. Soc 133 (2011) 1212.
[41] R.C. Dunbar, J. Steill, N.C. Polfer, J. Oomens, Journal of Physical Chemistry B 113 (2009) 10552.
[42] N.C. Polfer, B. Paizs, L.C. Snoek, I. Compagnon, S. Suhai, G. Meijer, G. von Helden, J. Oomens, Journal of the American Chemical Society 127 (2005) 8571.
[43] D. Semrouni, C. Clavaguera, J.-P. Dognon, G. Ohanessian, International Journal of Mass Spectrometry 297 (2010) 152.

[44] O.P. Balaj, D. Semrouni, V. Steinmetz, E. Nicol, C. Clavaguera, G. Ohanessian, Chemistry – A European Journal 18 (2012) 4583.
[45] D.P. Fairlie, T.C. Woon, W.A. Wickramasinghe, A.C. Willis, Inorganic Chemistry 33 (1994) 6425.
[46] J. Valle, J.R. Eyler, J. Oomens, D.T. Moore, A.F.G. van der Meer, G. von Helden, G. Meijer, C.L. Hendrickson, A.G. Marshall, G.T. Blakney, Review of Scientific Instruments 76 (2005) 023103.
[47] R.B. Martin, in: A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems: Probing of Proteins by Metal Ions and their Low-Molecular-Weight Complexes, 2001, p. 1.
[48] D. Schröder, M. Buděšinský, J. Roithová, Journal of the American Chemical Society (2012), http://dx.doi.org/10.1021/ja3060589, Articles ASAP.