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Chirality-Induced Conformational Preferences in Peptide−Metal Ion Binding Revealed by IR Spectroscopy

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ABSTRACT: Chirality reversal of a residue in a peptide can change its mode of binding to a metal ion, as shown here experimentally by gas-phase IR spectroscopy of peptide−metal ion complexes. The binding conformations of Li^+, Na^+, and H^+ with the LL and DL stereoisomers of PhePhe were compared through IR ion spectroscopy using the FELIX free-electron laser. For the DL isomer, both Li^+ and Na^+ exclusively coordinate to the amide O atom, the carboxyl O atom, and one of the aromatic rings (the OOR conformation), while for the LL isomer, a mixture of the OOR and NOR conformations was found. The stereochemically induced change in conformation is shown to reflect the strength of an NH···π interaction remote from the metal ion site. Protonated PhePhe shows no stereochemically induced variation in binding geometry.

As a peptide folds into its stable conformation, subtle intramolecular interactions govern the geometry choices. Similarly, the aggregation of biomolecule building blocks into clusters or aggregates can be influenced by small variations in intermolecular interactions. In addition, peptide conformational preferences can be steered by complexation, chelation, or sequestration of metal ions in cages formed by specific peptide folding, which has widespread importance for host−guest recognition, ion-channel transmission, enzyme active-site binding, and so on. The interactions and consequent conformational preferences in the structures built by such molecular assemblies can be influenced and even switched by subtle alterations in the sequence and chirality of the amino acid chain. Details of such processes can be exposed using gas-phase model studies, which avoid the complicating effects of solvent interactions as well as unwieldy large-molecule substrates but still give insight into analogous aggregation, recognition, and complexation in large solution-phase systems.

A wide range of work has been reported in which a diastereomeric differential affecting the binding of optically active reactants forms the basis for chiral recognition. Cooks' group reported the marked preference for homochiral aggregation of serine in the formation of the "magic-number" octamer complex (as well as smaller aggregates),[1] which has also been studied recently with IR multiple-photon dissociation (IRMPD) spectroscopy.[2] The noncovalent host−guest interactions of peptide ions with caging host partners were explored by Lebrilla's group, who found strong chiral variations.[3] Two- or threefold binding of small peptides to Cu(II) depends on the chirality of the ligands, forming the basis for a widely appreciated method developed by Cooks' group for determination of the chirality and enantiomeric excess of chiral samples.[4,5] Variations of this approach have explored chirally dependent multiphoton ionization, fragmentation, and spectroscopy[6] of gas-phase diastereomeric dimer species, including both metal-bridged[7] and proton-bridged[8] systems.

Recent IRMPD spectroscopy studies performed at the CLIO free-electron laser showed that chiral variations in a polypeptide chain can engender robust, readily observable differences in their IRMPD spectra, although these were not interpreted in terms of different conformational preferences.[9] In a similar vein, the present work exploits the sensitivity of the IRMPD spectrum to ion structural variations, allowing us for the first time to compare and analyze gas-phase geometries for binding of a metal ion to a dipeptide in its LL (natural) and DL (unnatural) enantiomeric forms. We deliberately chose a dipeptide with bulky, metal-ion-interacting side chains in order to maximize the steric cross-talk between the two chiral residues in the complex. Aromatic amino acids are known for their strong chiral selectivity effects.[5] PhePhe provides the possibility of strong cation−π interactions between the metal ion and one or both of the side chains as well as other sterically inhomogeneous phenyl interactions. Here we present a spectroscopic investigation of PhePhe complexation with alkali metal ions that provides a graphic example of biomolecular conformations determined by ligand chiral properties, mediated in this case by the varying availability of a proton−π interaction of the aromatic side chain with the amide group.

M^+−PhePhe complexes ($M^+ = Li^+$, Na^+, H^+) were formed by electrospray ionization. Following isolation of the desired complex ion in a Fourier-transform ion cyclotron resonance (FT-ICR) ion trap, irradiation with the tunable output of the FELIX IR free-electron laser[10] was used to induce wavelength-selective dissociation. Recording the fragmentation efficiency as a function of laser frequency then yielded an IR spectrum, as described previously.[11]

Figure 1 compares the IRMPD spectra of three cations bound alternatively to the LL and DL forms of PhePhe. (In the achiral environment experienced by the ions, the behavior of the DD and LD complexes is necessarily the same as that of the LL and DL complexes, so observation of the other forms would be superfluous). On the basis of previous interpretations of IR spectra of

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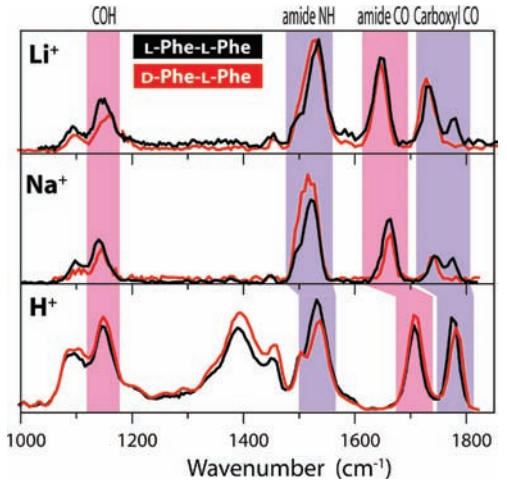

Figure 1. Experimental IRMPD spectra of (top, middle) alkali metal ion complexes with the LL and DL stereoisomers of the dipeptide PhePhe and (bottom) the protonated LL and DL dipeptides. The splitting of the carboxyl CO stretching peak into two components in the LL complexes (black traces), which is evident for Na^+ and Li^+, reflects the nearly equal stabilities of two spectrally distinct conformers. For the DL dipeptide (red traces), only one of the conformeric motifs is formed with Na^+ and Li^+, as indicated by the complete absence of one of the carboxyl CO stretching bands.

alkali ion (di)peptide complexes,[12] it is known that the present complexes exclusively form charge-solvated (as opposed to zwitterionic) structures. Also on the basis of previous experience, all main bands in the current spectra could readily be assigned to the various vibrational modes of the peptides, including carboxyl (C═O) stretching, amide carbonyl stretching (Amide I), amide N−H bending (Amide II), carboxyl C−O−H bending, and aromatic out-of-plane C−H bending. The protonated molecule also shows a strong characteristic NH_3^+ feature near 1400 cm^{-1}.

Having seen that these spectra show the expected general features, we can compare the chirally distinguished pairs more closely for information about the influence of the stereochemistry on the metal-ion binding patterns. Nothing in the H^+PhePhe spectra suggests a conformeric difference between the stereoisomers. It is the Na^+ and Li^+ complexes that show the striking spectral differences between the DL and LL stereoisomers that give these results their interest.

Most noteworthy are the differences in the carbonyl stretching region (1650−1800 cm^{-1}). Specifically, the LL forms of the Li^+ and Na^+ complexes show a distinct and highly diagnostic band on the high-frequency side that is absent for the analogous complexes involving the DL stereoisomer. A peak at this position (1780 cm^{-1}) can correspond only to the C═O stretch of a free carboxyl group unperturbed by a significant interaction with the metal ion. The C═O stretches at lower frequencies, 1730 (Na^+) and 1740 (Li^+) cm^{-1}, are typical for metal-bound carboxyl groups. The spectra thus unmistakably show the simultaneous presence of two conformers for the LL complexes, whereas only one structure is present for the DL complexes. We assign the conformer common to both isomers as one in which the metal ion is coordinated to the amide oxygen, the carboxyl carbonyl oxygen, and one of the phenyl rings (the OOR conformation). In the conformer observed only for the LL isomer, the metal ion is coordinated to the N-terminal nitrogen, the amide oxygen, and one of the phenyl rings (the NOR conformation).

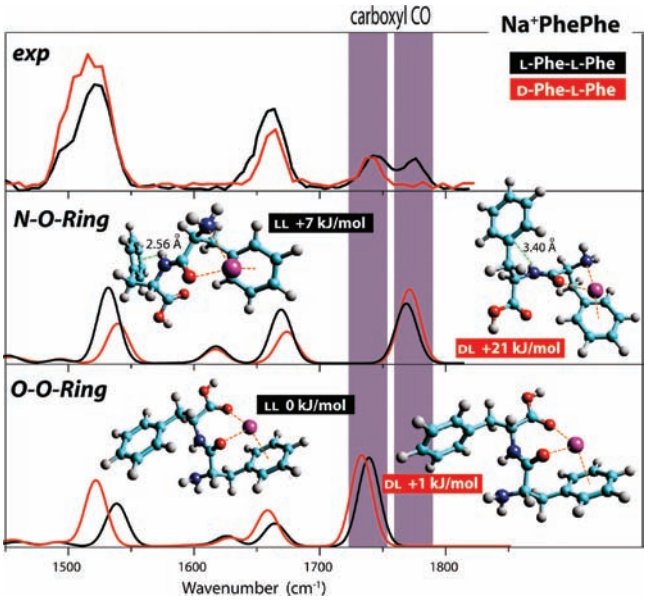

Figure 2. Comparison of (top) and (middle, bottom) calculated IR spectra for the LL and DL stereoisomers of Na^+PhePhe in the lowest-energy (middle) NOR and (bottom) OOR conformations. The calculated spectra of these two conformers are observably different only in the carboxyl C═O stretching region, which is highlighted in purple. For a given metal-binding conformation, the calculated LL and DL spectra are similar within the computational uncertainty. Thus, the strong difference observed in the IRMPD spectra indicates the presence of two conformers for the LL complex but only one for the DL complex. The noted energy values are the enthalpies at 0 K relative to the value for the OOR conformer of the LL complex.

Figure 2 compares the experimental IR spectra for the Na^+ complexes to spectra calculated using density functional theory (DFT) at the B3LYP/6-31+g(d,p)/6-311+g(d,p) level (frequencies scaled by 0.98). It is seen that the calculation for the OOR conformation accurately matches the observed position of the metal-bound C═O stretch at 1740 cm^{-1}, while the calculation for the NOR conformation matches the 1770 cm^{-1} peak observed for the DL isomer.

For both alkali ions, the ground state is the OOR conformation, which gives roughly equal energies for the LL and DL complexes. Figure 3 displays the calculated free energies of the NOR conformations relative to these ground states for the DL and LL isomers. For Na^+, for example, the NOR conformation is only 8 kJ/mol higher in free energy for LL, but it is 24 kJ/mol higher for DL. As a first approach to interpreting the spectra of room-temperature populations that show mixtures of conformations, it is common to consider that conformations having free energies within a few multiples of k_BT above the ground state (3 kJ mol^{-1} near room temperature) are likely to make substantial contributions to the population mixture. (The observed fractions of the NOR conformations in these two systems are quantitatively somewhat greater than those predicted by such a thermal model, but given the likelihood of incomplete cooling of the ions in the cell to thermal equilibrium as well as the computational uncertainties in the computed free energies, we consider that this point of view still gives an informative perspective.) Thermochemistry therefore rationalizes why the LL complex shows a significant fraction of NOR conformers while DL shows none. The somewhat higher relative energy of the Li^+ NOR conformation can also account for its somewhat smaller relative peak height

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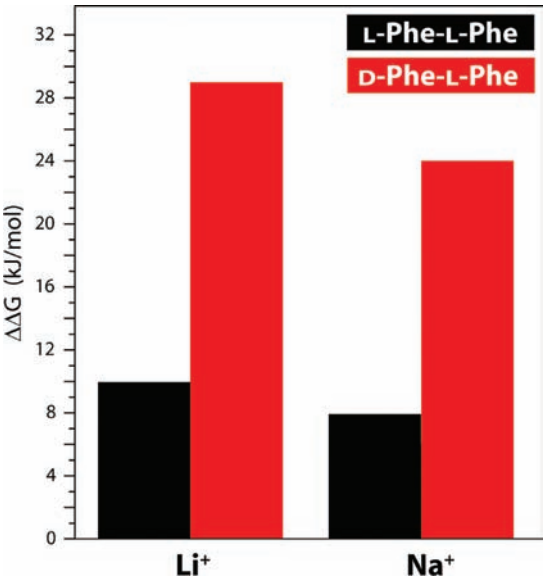

Figure 3. Calculated stabilities of the NOR conformation relative to the OOR conformation. Values are given as free energies at 298 K relative to the OOR ground state of each complex.

(Figure 1) for the LL complex, although this last argument pushes the limits of confidence of both the experimental and computational results.

The chirality-induced differences in metal-ion binding for the LL and DL complexes are not large energetically, and the difference would not be observable by many mass-spectrometric approaches. However, spectroscopic distinction is possible through a sensitive thermodynamic amplification: in order for comparable bands of the OOR and NOR conformations to appear together in the spectrum, the conformations must have free energies within a few kJ/mol of each other (assuming equilibrium conformer populations in the ICR cell). For the LL complexes, the NOR conformation is slightly less stable than the OOR conformation but still present as a significant fraction. For the DL complexes, the NOR conformation is less favorable by more than 20 kJ/mol, suppressing its population below observability.

The chiral discrimination is not enforced by differences in the metal-binding pocket created by the chelating ligand. Comparison of the DL and LL structures of the NOR conformer (Figure 2) shows that the binding geometries around the metal ions are very similar. The chirally distinct energetics of the two complexes originate from interactions *remote from the metal-binding site*, namely, the intramolecular amide NH$\cdots\pi$ interactions with the remote phenyl ring. Gloaguen et al.[13] studied the folding of neutral acetyl-capped PhePhe and found that an NH$\cdots\pi$ interaction of this type is a determinant of the conformation in that case. They calculated H$\cdots$ring distances of between 2.44 and 2.54 Å, and we have calculated a very similar distance of 2.56 Å for the LL complex. In contrast, the H$\cdots$ring distance in the DL case is 3.40 Å (see Figure 2), indicating a much weaker stabilizing interaction.

The observation of a strong chirally governed difference in metal binding reported here is remarkable in that it originates from an intramolecular interaction remote from the metal-binding site. The dependence of this interaction on subtle details of the conformations illustrates that predicting such effects in general is unique to each individual situation but amenable to modern DFT analysis.

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