Peptide Conformations

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Peptide Bond Tautomerization Induced by Divalent Metal Ions: Characterization of the Iminol Configuration**

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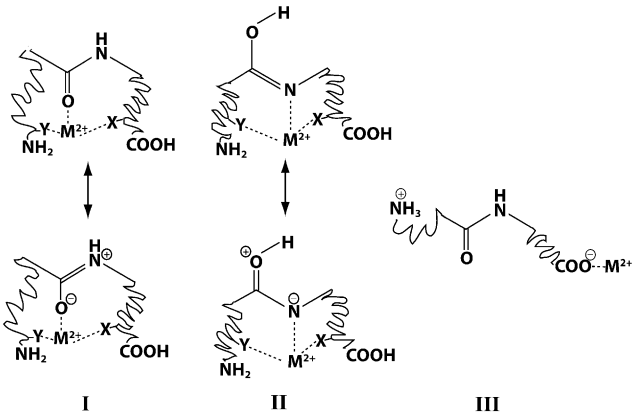
A metal ion that attaches to a peptide backbone has the choice of binding to an amide carbonyl oxygen atom or to an amide nitrogen atom by replacement of the amide proton.[1–5] Coordination to the nitrogen atom is normally accompanied by elimination of the proton, thereby resulting in deprotonation of the ligand. An alternative possibility, not involving deprotonation, is the unusual amide/iminol tautomerization.[6] In the gas phase, outright elimination of the proton is energetically costly, so that metal complexation without deprotonation is frequently observed and could well be accompanied by tautomerization. The present results show that coordination of active metal ions to amide nitrogen atoms through iminol tautomerism (analogous to the well-known keto/enol tautomerism of ketones) can indeed be a favorable gas-phase binding pattern.

Such a chemical change is readily identified by infrared multiple-photon dissociation (IRMPD) spectroscopy, which is an emerging tool for structural investigation of gas-phase complexes of selected masses and which uses powerful new IR laser sources.[7–11] We apply this tool to investigate the possible conformations of gas-phase complexes that are composed of model dipeptides with active divalent metal ions generated by electrospray ionization. The complexes characterized herein bridge the conceptual division between simple complexation and complexation with deprotonation. In fact the complexes are shown to contain the metal/amide-nitrogen bond, but without concurrent deprotonation.

We have previously investigated complexation of alkali and alkaline-earth ions by the dipeptide PhePhe, which provides a good model for interaction of ions with the peptide amide linkage and gives insight into stabilization and structure determination through cation–π interactions.[12] This dipeptide readily forms +2 complexes with divalent metal ions.

The binding patterns of interest are shown in Scheme 1. Charge-solvated (CS) binding, pattern I, is normal for alkali-metal ions and tends to maximize the number of coordinated amide carbonyl groups. In the iminol binding pattern II the metal ion coordinates a negatively charged deprotonated amide nitrogen atom. Both patterns I and II are best described by two principal resonance forms. Also possible are variants of the zwitterion binding mode III. However, previous work with the dipeptide PhePhe,[12] as well as calculations in the present study, have indicated that gas-phase zwitterion motifs of type III are much higher in energy than motifs of type I and II for the metal ion partners of interest here (Mg^{2+}, Ni^{2+}, Co^{2+}) and are thus unlikely. Figure 1 shows representative computed geometries.



Scheme 1. Binding motifs of metal ions complexed to peptides. Sites X and Y are other backbone, side-chain, or terminal-group Lewis-basic chelation points.

Experiments were performed using a Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer with ESI source, coupled to the free-electron laser for infrared experiments (FELIX) as has been detailed elsewhere.[10,12] Metal-ion/peptide complexes were generated by standard electrospray ionization from a solution containing peptide and metal salt. Target ions were trapped and mass-selected in

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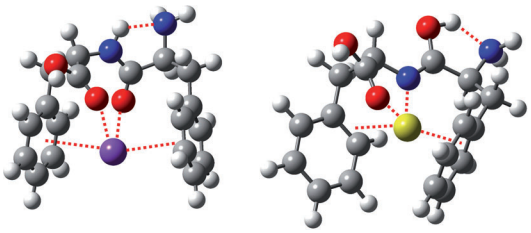

Figure 1. Favored PhePhe complexes of K^+ (type I) and Ni^{2+} (type II). On the left is shown the K^+PhePhe CS complex and on the right the Ni^{2+}PhePhe iminol complex. In the iminol motif, the amide proton relocates to the amide carbonyl oxygen atom and forms a hydrogen bond to the terminal amino nitrogen atom. The complexes of both types are stabilized by cation–π interactions with the phenyl rings. C gray, H white, O red, N blue, K^+ purple, Ni^{2+} yellow.

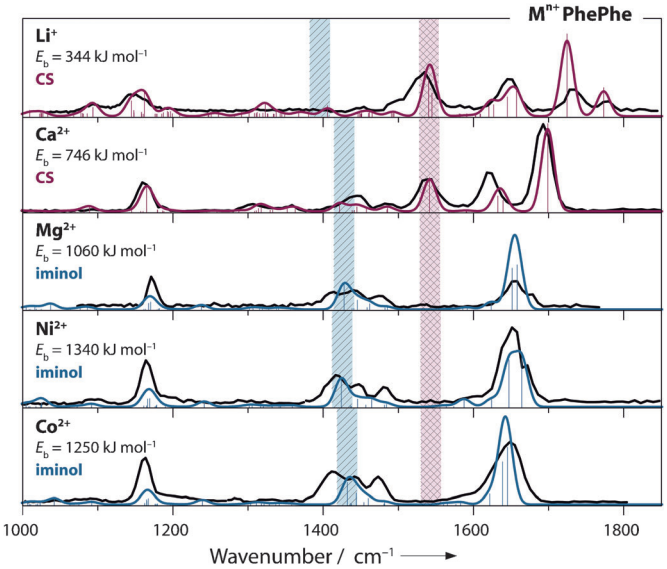

Figure 2. IRMPD spectra of metal-ion/PhePhe complexes (black). Spectra and calculations for Li^+ and Ca^{2+} complexes from Ref. [12]. Red and blue traces are the calculated spectra of the lowest-energy structures found in each case (CS for Li^+ and Ca^{2+} complexes, and iminol for Mg^{2+}, Ni^{2+}, and Co^{2+} complexes), except that the Li^+ complex assumes an OOR/NOR mixture (see Ref. [12]). E_b values are calculated binding energies for detaching the metal ion from the most stable complex and suggest that the iminol structure is favored for strongly binding metal ions. The blue bars show the positions of the diagnostic iminol OH bend/C=N stretch; the purple bars indicate the amide II band (N–H bend) that is diagnostic for an intact amide linkage.

the FT-ICR cell and were irradiated with the wavelength-tunable infrared light from the laser. By plotting the sum of all dissociation channels normalized to the total ion count as a function of laser frequency, an infrared action spectrum was generated and interpreted as a surrogate IR spectrum of the complex. DFT-computed linear IR spectra of candidate ion structures were compared with the observed spectra, where the calculated relative energeties provided additional guidance, to assign conformational and tautomeric structures.

Figure 2 displays mid-IR IRMPD spectra, revealing the abrupt shift in binding motif for more-strongly interacting metal ions. The Li^+ and Ca^{2+} spectra are typical of the CS binding motif (Scheme 1 (I) and Figure 1). The CS pattern is, however, inconsistent with the spectra observed for the Mg^{2+}, Ni^{2+}, and Co^{2+} complexes. Most obvious is the absence of the strong amide II feature (amide N–H bending) near 1550 cm^{-1} (marked in purple). Moreover a group of peaks between 1400 and 1500 cm^{-1} emerges that is expected for the iminol structure (marked in blue). The iminol binding motif is the only structure found for which calculated spectra give good agreement with the IRMPD spectra of the Mg^{2+}, Ni^{2+}, and Co^{2+} complexes.

Metal-ion binding to the amide-linked backbone of peptides has had longstanding interest in condensed-phase metal-ion chemistry and was well-characterized and reviewed[1–4] by the 1980s. A more recent review with biological focus was given by Sovago and Osz.[5] Carbonyl binding is universal for alkali-metal ions and has also been considered normal for alkaline-earth ions in condensed phase. On the other hand, divalent and trivalent transition-metal ions are well-known to bind deprotonated amide nitrogen atoms in condensed phases. A notable recent example in the protein literature is Cu^{II} binding by the prion protein.[13]

In gas phase, numerous studies of simple complexation without proton loss by various techniques (e.g. Refs. [12, 14–23]) have generally shown that alkali and alkaline-earth metal ions bind peptide chains in the CS configuration (motif I). The present study shows that this generalization does not extend to complexes with active transition-metal ions.

On the other hand, overtly deprotonated gas-phase complexes of alkaline-earth and transition-metal ions commonly exhibit binding of the metal ion to the amide nitrogen atom.[24–29] There is an interesting contrast of these earlier gas-phase studies of deprotonated complexes with the present results: For the nondeprotonated complexes studied herein, a clear distinction is seen between the amide-nitrogen attachment of magnesium ions versus the oxygen attachment of calcium ions. This distinction between calcium and magnesium is reinforced by the computational results, as shown in Figure 3. Such a difference in binding propensities is also characteristic in condensed phases.[2,3]

Binding of metal ions to the amide nitrogen atom is always anchored by further chelation of the metal ion. The literature of organometallic and biological systems has emphasized anchoring by backbone carbonyl groups or the terminal NH_2 group. Hu et al.[26] suggested cation–π interactions with aromatic side chains as alternative anchors in gas-phase deprotonated complexes, thereby presaging the present observation of the ring π site of the Phe side chain as an effective anchor in systems that are not deprotonated. In the present iminol complexes, the terminal NH_2 group, which might be expected to provide a metal-binding anchor point, is tied up in stabilizing the shared-proton site that is occupied by the original amide proton (Figure 1), and instead the C-terminal carbonyl oxygen atom anchors the metal ion.

In conclusion, three distinctive aspects of this newly observed binding configuration emerge here: 1) Deprotonation does not occur, but instead the proton migrates across the amide group by amide/iminol tautomerization. 2) An "anchor" chelating group that stabilizes the iminol binding

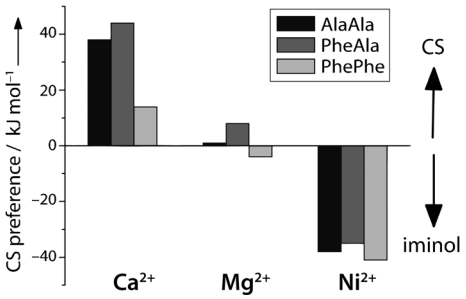

Figure 3. Coordination preferences of complexes as a function of metal and ligand identities. It is of interest whether stabilization of the structure by the double cation–π interaction in PhePhe is important to the stability of the iminol conformation. The Figure shows a computational test of this hypothesis through comparison with PheAla and AlaAla, and shows that the strong preference for iminol binding for Ni^{2+} ions does not depend on the presence of either phenyl group. (See structures of the nickel complexes in Figure S1 in the Supporting Information.) Iminol binding is increasingly favored in the order $Ca^{2+} < Mg^{2+} < Ni^{2+}$. Computations used the Gaussian 03 program suite with the B3LYP functional and the 6-31 + G(d,p) basis. Vibrational frequencies were scaled by 0.975, and vibrational zero point energy corrections (but not basis set superposition error corrections) were applied.

mode is the N-terminal phenyl group of Phe, if such a side chain is present. 3) In addition to the active transition metals, the iminol binding mode is favorable for magnesium(II), whereas this metal seldom or never engages in binding to deprotonated amide nitrogen atoms in the condensed phase.

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