Zn$^{2+}$ and Cd$^{2+}$ cationized serine complexes: infrared multiple photon dissociation spectroscopy and density functional theory investigations†

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The gas-phase structures of zinc and cadmium dications bound to serine (Ser) are investigated by infrared multiple photon dissociation (IRMPD) action spectroscopy using the free electron laser FELIX, in combination with ab initio calculations. To identify the structures of the experimentally observed species, [Zn(Ser-H)CH$_3$CN]$^+$ and CdCl$^+$ (Ser), the measured action spectra are compared to linear absorption spectra calculated at the B3LYP/6-311+G(d,p) level for Zn$^{2+}$ containing complexes and B3LYP/def2-TZVP levels for Cd$^{2+}$ containing complexes. Good agreement between the observed IRMPD spectra and the predicted spectra allows identification of the isomers present. The intact amino acid interacting with cadmium chloride adopts a tridentate chelation involving the amino acid backbone amine and carbonyl groups as well as the hydroxyl group of the side-chain, [N,CO,OH]. The presence of two low-energy conformers is observed for the deprotonated serine–zinc complex, with the same tridentate coordination as for the cadmium complex but proton loss occurs at both the hydroxyl side-chain, [N,CO,OH], and the carboxylic acid of the amino acid backbone, [N,CO$^-$,OH]. These results are profitably compared with the analogous results previously obtained for comparable complexes with cysteine.

Introduction

Recently, the interactions of cysteine (Cys)$^1$ and histidine (His)$^2$ with transition metal dications, Zn$^{2+}$ and Cd$^{2+}$, have been studied using infrared multiple photon dissociation (IRMPD) action spectroscopy. These studies were motivated by the biological relevance of zinc ions coordinating to these specific amino acids, as is observed in metalloproteins, and by the susceptibility of such zinc binding sites to toxic metal (Cd) replacement.$^3,4$ A large number of these metal-dependent proteins, such as zinc fingers, have been found to contain cysteine-rich sequence motifs capable of coordinating Zn$^{2+}$ ions, utilizing the metal ion for structural integrity.$^5$ These zinc finger proteins are characterized by divalent Zn$^{2+}$ centers directly coordinated to four amino acid residues, most commonly binding Cys and His.$^5$ Our work is designed to help interpret why Zn$^{2+}$ preferentially binds to Cys and His, and how replacing these residues with other amino acids might influence the protein. We recently examined such interactions for glutamine (Gln),$^6$ and in the current work, we expand our metalated amino acid examinations to include serine (Ser), which differs from Cys only by the replacement of oxygen for the sulfur in the side-chain. Preferential binding of Zn$^{2+}$ to Cys over Ser has been observed in metalloproteins, as is the case in the prokaryotic zinc finger domain of the Ros protein.$^7$ In that study, Baglivo et al. found that when Cys is replaced by Ser in the coordination sphere of the zinc finger domain, Cys$_2$His$_2$ to Ser$_2$His$_2$, the protein can still fold into a functional protein, but the zinc ion is lost. Moreover, they found that even single amino acid mutations of Cys to Ser in the first coordinating position of the zinc finger would transform the domain from zinc-binding to non-zinc-binding. Thus, the presence of Cys in this domain is essential for binding the zinc ion to the protein.$^7$ A pairwise examination at the fundamental level of metal binding with the amino acid Ser compared to Cys could provide valuable information about this strong metal-dependent binding of proteins.

In addition to the IRMPD action spectroscopy studies of the transition metal dications Zn$^{2+}$ and Cd$^{2+}$ with Cys,$^1$ His,$^2$ and Gln,$^6$ complexes of alkali-metal cations, Li$^+$, Na$^+$, K$^+$, Rb$^+$, and Cs$^+$ with single amino acid Ser$^8$, Cys$^9$, His$_{10}$ and Asn$_{11}$ have been examined. The alkali-metal ions interacted to form M$^+$ (Ser) and M$^+$ (Cys) directly from the electrospray ionization (ESI) source; however, for group 12 metals, the ESI source does...
Experimental and theoretical methods

Mass spectrometry and IRMPD spectroscopy

Experiments were performed at the FELIX Laboratory, Radboud University, The Netherlands, using the Free Electron Laser for Infrared Experiments (FELIX) beam line. Experiments have been described previously, but a brief description follows. A 4.7 T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer was used to record the IRMPD spectra. The metal–serine complex ions were generated using a Micromass Z-spray electrospray ionization source. Solutions of 1.0 mM Ser with 1.0 mM zinc nitrate or cadmium chloride in a 50:50 methanol:H₂O solution were used with a flow rate of 3–10 μL min⁻¹. Ions generated by the ESI source were accumulated in a hexapole trap for 5–7 s before being pulse extracted through a quadrupole bender and injected into the ICR cell via a radiofrequency (rf) octopole ion guide. Ion capturing was affected by the electrostatic switching of the dc bias of the octopole to avoid collisional heating of the ions. Once trapped in the ICR cell, the ions were allowed to cool radiatively to room temperature for up to 0.4 s. The ion of interest was mass isolated using a stored waveform inverse Fourier transform (SWIFT) excitation pulse and irradiated with FELIX for 3–5 s at a 5 or 10 Hz macropulse repetition rate (energy up to 45 mJ per pulse and the bandwidth is 0.5% of the central frequency).

At each wavelength, the parent and fragment ion intensities were used to determine the fractional yield as \( \sum I_f / (I_p + \sum I_f) \), where \( I_p \) and \( I_f \) are the integrated intensities of the parent and fragment ion mass peaks (including all isotopes as detailed below), respectively. The IRMPD spectra were generated by plotting the yield as a function of the wavenumber of IR radiation after correcting for laser power. Infrared spectra obtained using IRMPD methods are comparable to those recorded using linear absorption techniques, in part because the spectra result from incoherent, rather than coherent, multiple photon excitation. Previous modeling studies have demonstrated the near-linear absorption character of IRMPD studies. For some bands (here the high frequency range for CdCl⁻(Ser)), the laser energy drops quickly, which results in a yield higher than one after correcting for the laser power. The application of a linear laser power correction is well described in the literature, and is appropriate because the power dependence is basically linear until saturation begins, again because of the incoherent rather than the coherent nature of the multiple photon excitation process. A yield greater than unity is an indication that the band would have been saturated if the laser power was higher.

Computational details

Possible geometries for Zn²⁺(Ser) complexes were taken from the previously examined geometries of Li⁺(Ser) complexes, which were determined using a simulated annealing procedure that combines annealing cycles and ab initio calculations. Structures were optimized using density functional theory (DFT) and the Gaussian09 suite of programs at the B3LYP/6-31G(d) level of theory, using the “loose” keyword to utilize a large step size of 0.01 au and a rms force constant of 0.0017 to ensure a rapid geometry convergence. These structures were refined by geometry optimization at the B3LYP/6-31+G(d,p) level. From these optimized structures, the Ser residue was deprotonated at the likely sites (OH, COOH, or NH₂) yielding [Zn(Ser-H)]⁺ structures that were refined again in the loose optimization step. The converged structures were then chosen for further refinement with the acetonitrile (ACN) ligand added, optimized at the loose level. Unique [Zn(Ser-H)ACN]⁺ conformers underwent final geometry optimization and vibrational frequency calculations at the B3LYP/6-311+G(d,p) level of theory. Geometry optimizations utilizing empirical dispersion corrections were also determined at the B3LYP-GD3BJ/6-311+G(d,p) level. The CdCl⁻(Ser) complexes went through the same geometry optimization procedure except the def₂-TZVP basis set was used, where def₂-TZVP is a size-consistent basis set for all atoms and includes triple-ξ + polarization functions with a small core (28 electron) effective
core potential (ECP) on Cd.\textsuperscript{28,29} The def2-TZVP basis set and affiliated ECP were obtained from the EMSL basis set exchange.\textsuperscript{30}

To determine the accurate relative energies of the isomers of the Zn (Cd) complexes at both 0 and 298 K, single-point energy calculations were carried out at the B3LYP, B3LYP-GD3BJ,\textsuperscript{27} B3P86,\textsuperscript{31} and MP2(full)\textsuperscript{12} (where full indicates correlation of all electrons) levels using the 6-311+G(2d,2p) (def2-TZVPP) basis sets. For all species, zero point energy (ZPE) corrections and thermal corrections to free energies at 298 K utilized vibrational frequencies scaled by 0.989. The transition states (TSs) of the [Zn(Ser-H)ACN]\textsuperscript{+} photofragmentation processes were found through a series of relaxed potential energy scans along the likely reaction coordinates at the B3LYP/6-31G(d) level.\textsuperscript{25,33,34} Geometry optimizations and frequency calculations of the TSs and intermediates (INTs) were performed at the B3LYP/6-311+G(d,p) level. Here the vibrational frequencies of the TSs were found to have only one imaginary frequency, and all INTs were determined to be vibrationally stable.

For comparison to IRMPD experimental spectra, calculated frequencies were scaled by 0.975, as this factor leads to good agreement between the calculated and experimentally well-resolved peaks and is consistent with previous IRMPD studies of metal–amino acid complexes.\textsuperscript{2,10,15–38} The calculated vibrational frequencies are broadened using a 30 cm\textsuperscript{-1} full width at half maximum Gaussian line shape for comparison with experimental spectra. This broadening accounts for the finite laser bandwidth, unresolved rotational structure of the ions (which are near room temperature), anharmonicity of the vibrational mode, and broadening as a result of the multiple photon absorption process.\textsuperscript{19}

### Results

**IRMPD action spectroscopy**

The IRMPD action spectra of [Zn(Ser-H)ACN]\textsuperscript{+} and CdCl\textsuperscript{+}(Ser) were examined from 550 to 1800 cm\textsuperscript{-1} (Fig. 1). The IRMPD action spectra shown here correspond to the fractional yield of the product ions as a function of free electron laser wavenumber. Dominant dissociation pathways for the photodissociation of CdCl\textsuperscript{+}(Ser) resulted in the loss of H\textsubscript{2}O and (H\textsubscript{2}O + CO); whereas, for [Zn(Ser-H)ACN]\textsuperscript{+}, the photodissociation fragmentation corresponded only to the loss of CH\textsubscript{2}O, agreeing with the IRMPD fragmentation channel previously observed for the deprotonated serine anion (Ser-H)\textsuperscript{−}.\textsuperscript{39} Notably, ACN loss was not observed and these fragmentation pathways differ from those observed for the IRMPD\textsuperscript{8} and collision-induced dissociation (CID)\textsuperscript{40} studies of M\textsuperscript{+}(Ser) where M\textsuperscript{+} = Na\textsuperscript{+}, K\textsuperscript{+}, Rb\textsuperscript{+}, and Cs\textsuperscript{+}, in which dissociation resulted in the loss of the intact ligand to form the atomic metal cation. Fragment mass channels of 167 and 151 m/z were also observed during the photodissociation of [Zn(Ser-H)ACN]\textsuperscript{+}; however, they were not included in the fractional yield spectrum in Fig. 1. These fragmentation mass channels are such that they cannot be from the parent complex as there are no Zn isotopes for either mass channel and their spectra are very different from that for the loss of formaldehyde from [Zn(Ser-H)ACN]\textsuperscript{+}. These fragments are most likely contaminants or formed from species that were above the selected SWIFT mass range.

Comparison of the spectra in Fig. 1 shows that the features observed for the intact amino acid and deprotonated system are similar in several respects. Particularly, [Zn(Ser-H)ACN]\textsuperscript{+} and CdCl\textsuperscript{+}(Ser) have parallel spectra for the low to middle wavenumber range, less than 1600 cm\textsuperscript{-1}, but obvious spectral differences appear in the higher frequency range associated with C=O stretching. Key differences between the Zn spectrum and that for Cd include: the intensity growth of the 1230 cm\textsuperscript{-1} band for Zn, the weakness of the 1430 cm\textsuperscript{-1} band for Zn, and most obviously, the two spectral features observed in the high frequency range from 1650–1760 cm\textsuperscript{-1} for Zn compared to only one for Cd. As will be documented below, this is indicative of multiple isomers present for the Zn complex.

**Overview of theoretical results**

As described above, the low-energy structures of [Zn(Ser-H)ACN]\textsuperscript{+} and CdCl\textsuperscript{+}(Ser) complexes were optimized at the B3LYP/6-311+G(d,p) and B3LYP/def2-TZVP levels of theory, respectively, with single point energies calculated using slightly larger basis sets and several levels of theory. Details of the calculated distinct low-lying conformers of [Zn(Ser-H)ACN]\textsuperscript{+} and CdCl\textsuperscript{+}(Ser) complexes are provided in the ESI\textsuperscript{†} along with their corresponding optimized geometry structures, Fig. S1 and S2, respectively. The nomenclature used to identify the different structural isomers is based on that described in previous studies of metal–amino acid complexes.\textsuperscript{1,6,9,11,40,41} Briefly, conformations are identified by their metal binding site in brackets, where backbone binding sites always precede side-chain binding. In cases where the binding site is ambiguous, “s” is used to specify a side-chain group. The binding site designation is followed by a description of the amino acid orientation by a series of dihedral angles.
The angles are denoted as “c” (cis) for angles < 45°, “g” (gauche) for angles between 45° and 135°, and “t” (trans) for angles > 135°. For charge-solvated (CS) structures, these dihedral angles start with the carboxylic acid hydrogen atom and proceed along the molecule to the terminal hydrogen of the amino acid side-chain, _HOCC, _OCCC, _CCCO, and _COOH. The former or latter angles are missing if the carboxylic acid or hydroxyl has been deprotonated, respectively. The deprotonation site is designated by a negative ion symbol on the appropriate atom. In the case of salt-bridge (SB) structures, the proton originally on either the carboxylic acid or hydroxyl terminus is attached instead to the nitrogen. For SB complexes deprotonated at the carboxylic acid, the first dihedral angle starts at this bridging proton, moves along the H-bond toward the deprotonated site, and then includes the same three dihedral angles as above.

**Theoretical results for [Zn(Ser-H)ACN]⁺**

The optimized structures of select and distinctive low-lying conformations of [Zn(Ser-H)ACN]⁺ are shown in Fig. 2a–d with their corresponding linear IR spectra. Energies at 0 and 298 K of the calculated conformations of these complexes relative to the ground structure (GS) calculated at four different levels of theory are given in Table 1. In all [Zn(Ser-H)ACN]⁺ complexes, the acetonitrile ligand binds in a near linear configuration on the opposite side of the zinc cation away from the amino acid. All levels of theory predict that the lowest energy structure has deprotonated Ser binding in a tridentate fashion to Zn; however, the preferred site of deprotonation differs with the level of theory (Table 1). DFT levels of theory, including B3LYP with empirical dispersion corrections, find that the lowest energy structure at 0 and 298 K is [N,CO,O⁻]tgg (Fig. 2a), Zn bound to the amino nitrogen and carbonyl of the Ser backbone with added complexation to the deprotonated side-chain hydroxyl. In contrast, the MP2(full) level of theory predicts that deprotonation occurs at the carboxylic acid terminus, [N,CO,OH]ggt (Fig. 2b), although this conformer is lower in free energy at 298 K by only 0.2 kJ mol⁻¹. Comparison of bond lengths in Table 2 reveals that the [N,CO,OH]ggt conformer has shorter M²⁺–OC and M²⁺–N bond distances than the [N,CO,O⁻]tgg conformer by 0.29 and 0.07 Å, respectively. Conversely, the tridentate deprotonated side-chain hydroxyl, [N,CO,O⁻]tgg, conformer binds more closely at the M²⁺–O site by 0.29 Å. This shift in coordination distances between the two conformers can be clearly seen in the optimized structures provided in Fig. 2a and b.

The next lowest energy conformer of [Zn(Ser-H)ACN]⁺ is the deprotonated tridentate [N,CO,O⁻]cgg conformer (Fig. 2c). In the tgg GS, the carboxylic hydrogen forms an intramolecular hydrogen bond (H-bond) with the carbonyl oxygen. The gg conformer...
breaks this intramolecular bond, leading to a 19–21 kJ mol⁻¹ increase in the free energy at 298 K relative to the tgg GS. The lowest energy bidentate species is the metal bound to the amine and deprotonated side-chain hydroxyl. Additional higher energy structures located are shown in Fig. S1 (ESI) and include [N,CO,O]tgg, [N,CO]tgg, [N,CO,O]tgg, [CO₂]tc, and various [CO₂] binding motifs.

Although the [Zn(Ser-H)]⁺ species were not observed experimentally, it is valuable to compare the theoretical results for this complex with those for the species contaminated by ACN. Tables 2 and 3 provide direct comparison of the geometries and relative enthalpies, respectively, of low-lying ACN-free and ACN-contaminated deprotonated conformers. The [Zn(Ser-H)]⁺ [N,CO,OH]tgg conformer is calculated to be 12–20 kJ mol⁻¹ higher in free energy than the [N,CO,O]⁺ GS, meaning it could only be found at most population 0.8% of an ion population equilibrated at 298 K. In contrast, as seen in Table 1, the presence of the ACN ligand stabilizes the [N,CO,OH]tgg conformer such that it would populate between 8 and 52% of ions at 298 K. Zn²⁺ prefers to adopt a 4-coordinate geometry such that the addition of the ACN ligand to the metal stabilizes the complexes (as demonstrated experimentally by the inability to remove the ACN ligand when irradiated with a high power CO₂ laser).

### Table 1 Relative enthalpies at 0 K and free energies at 298 K (kJ mol⁻¹) of [Zn(Ser-H)ACN]⁺ conformers

<table>
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<tr>
<th>Structure</th>
<th>B3LYP</th>
<th>B3LYP-GD3BJ</th>
<th>B3P86</th>
<th>MP2(full)</th>
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<td>[N,CO,O]tg</td>
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<td>0.0</td>
<td>0.0</td>
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<td>[N,CO,OH]tgg</td>
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<td>0.0 (0.0)</td>
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<td>[N,CO,O]tg</td>
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<td>18.6 (18.8)</td>
<td>19.0 (19.2)</td>
<td>21.2 (20.5)</td>
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<td>115.8 (118.5)</td>
<td>116.8 (118.5)</td>
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* Free energies at 298 K are in parentheses. Calculations performed at the stated level of theory using a 6-311+G(2d,2p) basis set. Geometries and vibrational frequencies calculated at the B3LYP/6-311+G(d,p) level. Energies include ZPE corrections scaled by 0.998. s = side-chain. Salt-bridge between NH₃⁺ and COO⁻ groups.

### Table 2 Bond distances (Å) and bond angles (°) for select low-energy conformers of metalated serine

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<th>Complex</th>
<th>Structure</th>
<th>r(M–N)</th>
<th>r(M–OC)</th>
<th>r(M–OH₃)</th>
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<th>∠NMOₘ</th>
<th>∠OMOₘ</th>
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<td>78.1</td>
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<td>2.15</td>
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<td>75.9</td>
<td>84.8</td>
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<td>1.84</td>
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<td>[Zn(Ser-H)⁺]</td>
<td>[N,CO,O]tgg</td>
<td>1.88</td>
<td>1.84</td>
<td>109.8</td>
<td>32.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Zn(Ser-H)⁺]</td>
<td>[CO₂]tc</td>
<td>1.91</td>
<td>2.14</td>
<td>2.13</td>
<td>89.1</td>
<td>84.9</td>
<td>84.8</td>
<td>35.2</td>
</tr>
<tr>
<td>[Zn(Ser-H)⁺]</td>
<td>[CO₂]tc</td>
<td>2.04/2.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Geometries calculated at the B3LYP/6-311+G(d,p) level for Zn-containing complexes. s = side-chain. Salt-bridge between NH₃⁺ and COO⁻ groups. Hydroxyl of backbone carboxylic acid. "∠OMO on backbone carboxylate oxygens."
Interestingly, comparison of [Zn(Ser-H)ACN]+ and [Zn(Ser-H)+] relative free energies (Tables 1 and 3) shows that the ACN containing conformers all have a lower free energy relative to the ground conformer than the corresponding ACN-free structures, although [N,CO,O]/C0tggt and [N/C0,C0,CO,OH]tgggt are stabilized comparably to the GS. This phenomenon can be explained by examining the geometric effects of adding ACN to the [Zn(Ser-H)+] complexes, as seen in Table 2. For most complexes, the addition of ACN increases the metal–ligand bond lengths, a natural consequence of electron donation from acetonitrile to the metal center. In contrast, the Zn–N bond of both [N,CO,O]/C0tggt and [N/C0,C0,CO,OH]tgggt decreases upon ACN addition as does the Zn–OC bonds of [CO2/C0]ggg, such that these structures benefit less from coordination of the fourth coordination site. With the exception of the [CO2/C0]ggg conformer, all of the complexes see a decrease in ligand binding site bond angles upon ACN attachment, again indicating that the metal generally moves away from the (Ser-H)+ ligand. Overall, the addition of ACN increases the metal–ligand bond lengths, a natural consequence of electron donation from acetonitrile to the metal center.

Table 3  Relative enthalpies at 0 K and free energies at 298 K (kJ mol−1) of low-lying conformers of [Zn(Ser-H)]+ #

<table>
<thead>
<tr>
<th>Structure</th>
<th>B3LYP</th>
<th>B3LYP-GD3BJ</th>
<th>B3P86</th>
<th>MP2(full)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[N,CO/O ]ggg</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>[N,CO,OH]tggt</td>
<td>16.1 (17.1)</td>
<td>14.3 (15.5)</td>
<td>18.9 (19.9)</td>
<td>11.1 (12.0)</td>
</tr>
<tr>
<td>[N,CO/O ]ggg</td>
<td>21.8 (22.0)</td>
<td>21.3 (21.5)</td>
<td>21.3 (21.4)</td>
<td>22.0 (22.2)</td>
</tr>
<tr>
<td>[N,CO/O ]tggt</td>
<td>49.5 (50.5)</td>
<td>52.4 (53.4)</td>
<td>57.3 (58.4)</td>
<td>58.2 (59.2)</td>
</tr>
<tr>
<td>[N,CO/O ]tggt</td>
<td>63.5 (65.2)</td>
<td>66.1 (67.8)</td>
<td>71.6 (73.2)</td>
<td>68.4 (70.0)</td>
</tr>
<tr>
<td>[N,CO/O,OH]tggt</td>
<td>46.5 (47.1)</td>
<td>46.6 (45.2)</td>
<td>50.8 (51.4)</td>
<td>42.7 (43.3)</td>
</tr>
<tr>
<td>[CO2/O ]ctc</td>
<td>50.3 (51.3)</td>
<td>52.1 (53.0)</td>
<td>56.9 (57.8)</td>
<td>56.1 (57.1)</td>
</tr>
<tr>
<td>[CO2/O ]ctc</td>
<td>70.4 (71.3)</td>
<td>74.4 (75.3)</td>
<td>75.1 (76.0)</td>
<td>88.2 (89.1)</td>
</tr>
<tr>
<td>[N,CO,OH]tggt</td>
<td>52.1 (53.3)</td>
<td>53.3 (54.5)</td>
<td>55.4 (56.6)</td>
<td>53.5 (54.6)</td>
</tr>
<tr>
<td>[CO2 ]ggg</td>
<td>102.8 (106.7)</td>
<td>114.4 (118.3)</td>
<td>120.9 (124.8)</td>
<td>156.0 (159.9)</td>
</tr>
</tbody>
</table>

# Free energies at 298 K are in parentheses. Calculations performed at the stated level of theory using a 6-311+G(2d,2p) basis set. Geometries and vibrational frequencies calculated at the B3LYP/6-311+G(d,p) level. Energies include ZPE corrections scaled by 0.989. s = side-chain. 5 Salt-bridge between NΗ3+ and COO− groups.

Theoretical results for CdCl+(Ser)
The optimized structures of select and distinctive low-lying conformations of CdCl+(Ser) with their corresponding linear IR spectra are shown in Fig. 3a–c with relative energies in Table 4. Calculations at all four levels of theory find that the charge-solvated tridentate [N,CO,OH]tggt structure is the ground structure (GS) for CdCl+(Ser). As seen in Fig. 3a, in this conformer, Cd is bound to the amino nitrogen, r(Cd–N) = 2.33 Å, carbonyl oxygen, r(Cd–O) = 2.39 Å, and side-chain hydroxyl oxygen, r(Cd–O) = 2.51 Å, and the carboxylic hydrogen forms an intramolecular H-bond with the carbonyl oxygen. Similar to the deprotonated species, when this intramolecular bond is broken, as in the [N,CO,OH]tggt conformer, the relative free energy at 298 K is found to be 28–30 kJ mol−1 higher than the tggt conformer.

If the metal ion interaction with the hydroxyl side-chain is lost and only the backbone amino acid interaction is maintained, bidentate [N,CO] conformers are formed with tcgt, tgtt, and tgtg orientations. The lowest of these bidentate conformers is

Fig. 3 Comparison of the experimental IRMPD spectrum of CdCl+(Ser) with IR spectra and structures of select low-lying conformers calculated at the B3LYP/def2-TZVP level. Relative 298 K free energies in kJ mol−1 from Table 4 given in parentheses are calculated at the B3LYP, B3LYP-GD3BJ, B3P86, and MP2(full) levels with the def2-TZVPP basis set. For structures: grey = C, red = O, blue = N, white = H, green = Cl and yellow = Cd. Dashed lines indicate hydrogen bonds.
Table 4 | Relative enthalpies at 0 K and free energies at 298 K (kJ mol$^{-1}$) of CdCl$^+$ (Ser) conformers$^a$

<table>
<thead>
<tr>
<th>Structure</th>
<th>B3LYP</th>
<th>B3LYP-GD3BJ</th>
<th>B3P86</th>
<th>MP2(full)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[N,CO,OH]$^a_{cgg}$</td>
<td>2.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>[N,CO]$^a_{cgg}$</td>
<td>14.6 (14.9)</td>
<td>20.7 (20.8)</td>
<td>16.5 (16.6)</td>
<td>25.9 (26.0)</td>
</tr>
<tr>
<td>CO$_2^+$</td>
<td>17.1 (17.8)</td>
<td>28.9 (29.6)</td>
<td>20.2 (20.9)</td>
<td>30.2 (30.9)</td>
</tr>
<tr>
<td>N$<em>2$O$^b</em>{cgg}$</td>
<td>19.0 (20.2)</td>
<td>25.0 (25.2)</td>
<td>21.6 (21.8)</td>
<td>31.0 (31.3)</td>
</tr>
<tr>
<td>[N,CO]$^b_{cgg}$</td>
<td>19.5 (20.6)</td>
<td>31.9 (33.0)</td>
<td>23.6 (24.7)</td>
<td>34.4 (35.6)</td>
</tr>
<tr>
<td>[N,CO,OH]$^b_{cgg}$</td>
<td>21.9 (22.5)</td>
<td>26.6 (27.2)</td>
<td>23.5 (24.1)</td>
<td>34.1 (34.7)</td>
</tr>
<tr>
<td>[N,OH]$^b_{cgg}$</td>
<td>27.3 (27.7)</td>
<td>31.6 (31.9)</td>
<td>30.3 (30.6)</td>
<td>34.6 (34.9)</td>
</tr>
<tr>
<td>[N,CO,OH]$^b_{cgg}$</td>
<td>28.5 (28.8)</td>
<td>28.1 (28.4)</td>
<td>28.2 (28.5)</td>
<td>30.0 (30.3)</td>
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<td>[N,OH,OH]$^b_{cgg}$</td>
<td>30.3 (30.7)</td>
<td>28.9 (29.2)</td>
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<td>27.3 (27.5)</td>
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<td>[N,OH]$^b_{ttt}$</td>
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<td>42.0 (42.6)</td>
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<td>[N,OH]$^b_{cgg}$</td>
<td>43.9 (44.5)</td>
<td>50.4 (51.0)</td>
<td>46.4 (46.9)</td>
<td>62.2 (62.7)</td>
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<tr>
<td>[CO2]$^b_{cgg}$</td>
<td>44.8 (45.6)</td>
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<td>47.4 (48.2)</td>
<td>61.7 (62.4)</td>
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<tr>
<td>[CO2]$^b_{tcgg}$</td>
<td>49.3 (49.8)</td>
<td>62.2 (62.7)</td>
<td>51.6 (52.2)</td>
<td>69.8 (70.3)</td>
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<tr>
<td>[CO2]$^b_{gtg}$</td>
<td>49.4 (50.6)</td>
<td>61.0 (62.1)</td>
<td>53.1 (54.3)</td>
<td>66.6 (67.8)</td>
</tr>
<tr>
<td>[CO2]$^b_{s}$</td>
<td>56.4 (56.8)</td>
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<tr>
<td>[CO2]$^b_{s}$</td>
<td>60.4 (60.9)</td>
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<td>61.7 (62.2)</td>
<td>73.9 (74.4)</td>
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<td>60.0 (61.2)</td>
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<td>66.7 (67.9)</td>
<td>80.8 (82.0)</td>
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<td>65.6 (66.3)</td>
<td>65.4 (66.2)</td>
<td>68.0 (68.7)</td>
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<td>[CO2]$^b_{s}$</td>
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<td>77.5 (77.7)</td>
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<td>60.0 (61.7)</td>
<td>67.3 (69.0)</td>
<td>66.3 (67.9)</td>
<td>80.4 (82.1)</td>
</tr>
<tr>
<td>[CO2]$^b_{s}$</td>
<td>62.9 (63.2)</td>
<td>66.3 (66.6)</td>
<td>65.3 (65.6)</td>
<td>71.0 (71.4)</td>
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<td>[CO2]$^b_{s}$</td>
<td>64.1 (63.8)</td>
<td>75.7 (75.5)</td>
<td>67.6 (67.3)</td>
<td>78.8 (78.5)</td>
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<tr>
<td>[CO2]$^b_{s}$</td>
<td>103.1 (103.6)</td>
<td>115.3 (115.8)</td>
<td>107.9 (108.4)</td>
<td>128.5 (129.0)</td>
</tr>
<tr>
<td>[CO2]$^b_{s}$</td>
<td>118.5 (120.9)</td>
<td>123.8 (126.1)</td>
<td>128.5 (130.8)</td>
<td>133.4 (135.7)</td>
</tr>
<tr>
<td>[CO2]$^b_{s}$</td>
<td>136.4 (137.8)</td>
<td>148.6 (150.0)</td>
<td>146.4 (147.8)</td>
<td>159.7 (161.1)</td>
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</table>

$^a$ Free energies at 298 K are in parentheses. Calculations performed at the B3LYP level using a def2-TZVP basis set. Geometries and vibrational frequencies calculated at the B3LYP/def2-TZVP level. Energies include ZPE corrections scaled by 0.989. $s$ = side-chain. $^b$ Salt-bridge between NH$_3^+$ and COO$^-$ groups. $^c$ Side-bridge between NH$_3^+$ and CO$_2^-$ groups.

In making these comparisons, it should be taken into consideration that the calculated IR intensities are based on single photon absorption, which sometimes do not correspond directly to the multiphoton process of the action spectra.

The highest frequency band observed at 1762 cm$^{-1}$ corresponds to the carbonyl stretch, which explains its large intensity. Interaction with the zinc dication results in red shift compared to that calculated for free Ser at 1791 cm$^{-1}$. The [N,CO$_2^+$,OH]$_{cgg}$ conformer predicts this band lies at 1756 cm$^{-1}$, in good agreement with the action spectrum. In addition, the peaks observed at 1227 and 1047 cm$^{-1}$ are consistent with the next most intense bands in the calculated IR spectrum of this conformer. Clearly, this conformer does not reproduce many of the other peaks observed experimentally. The peak observed at 1667 cm$^{-1}$ suggests the presence of the [N,CO$_2^+$,O]$_{cgg}$ conformer, which theory predicts has a C–O stretch at 1665 cm$^{-1}$ for the deprotonated carboxylic acid. The presence of this conformer is confirmed by reproduction of the peaks observed at 1147 and 992 cm$^{-1}$. Both low-energy conformers have vibrations in good agreement with the bands observed at 639 and 712 cm$^{-1}$ associated with COOH wagging. The band observed at 1577 cm$^{-1}$ associated with the NH$_3^+$ bend motion is predicted to be at 1606 and 1604 cm$^{-1}$ for the [N,CO$_2^+$,O]$_{cgg}$ and [N,CO$_2^+$,OH]$_{cgg}$ conformers, respectively. This vibrational mode is known to have especially strong anharmonic character in metallated amino acids accounting for the significant shift in the action spectra, as observed consistently in IRMPD amino acid studies. For instance, the 28 cm$^{-1}$ shift in this mode observed here is comparable to those seen for [M(Cys-H)]$^+$, between 26 and 30 cm$^{-1}$, and actually less than those observed for M$^+$ (Ser) where M$^+$ = K$^+$, Rb$^+$, and Cs$^+$, which exhibit shifts up to 40 cm$^{-1}$. Overall, we conclude that the experimental spectrum can be explained nicely by a combination of the two lowest energy conformers. The appearance of multiple conformers is consistent with the relative enthalpies and free energies calculated for this system, Table 1. The relative intensities of the two C–O stretch bands suggest that [N,CO$_2^+$,OH]$_{cgg}$ has a higher abundance than the [N,CO$_2^+$,O]$_{cgg}$ conformer. This result is consistent with the relative free energies calculated at the MP2(full) level of theory, which indicates that [N,CO$_2^+$,OH]$_{cgg}$ can account for 52% of an equilibrated ion population at 298 K.

Fig. 2 also includes the predicted spectra for two conformers that are next lowest in energy, Table 1. In both cases, all levels of theory place these structures sufficiently high in energy that they should not be populated if equilibrated at 298 K. The [N,CO$_2^+$,O]$_{cgg}$ spectrum has similarities to the [N,CO$_2^+$,OH]$_{cgg}$ spectrum, particularly for the C–O stretch (1688 cm$^{-1}$), NH$_2$ bend (1606 cm$^{-1}$), and NH$_3^+$ wag (996 cm$^{-1}$) vibrational modes. However, it also predicts intense peaks at 1289 and 1396 cm$^{-1}$ that are clearly absent from the experimental spectrum. The former band is unique to this [N,CO$_2^+$,O]$_{cgg}$ conformer as it is from HOCC bending motion centered at the carboxylic acid carbon, when there is no intramolecular bonding within the carboxylic acid. The bidentate [N,OH$_2^+$]$_{cgg}$ spectrum also has a band at 1759 cm$^{-1}$, corresponding to the C–O stretch of an intact carboxylic acid but no longer coordinated to the metal cation.
The rest of the spectrum matches the experiment reasonably well in the wavenumber position, but not in relative intensity. Furthermore, the band near 630 cm\(^{-1}\) is much too broad compared to the experiment and peaks predicted between 1300 and 1450 cm\(^{-1}\) that are not observed experimentally.

**Comparison of experimental and theoretical spectra: CdCl\(^{2+}\)(Ser)**

Compared with the experimental spectrum of [Zn(Ser-H)ACN]\(^{+}\) (Fig. 1), the CdCl\(^{2+}\)(Ser) spectrum retains most of the same characteristics but there is only one C–O stretch band and more intensity in the 1350–1500 cm\(^{-1}\) region. Fig. 3 shows the experimental IRMPD action spectrum of CdCl\(^{2+}\)(Ser) compared with theoretical predictions for the three lowest energy conformations, Table 4. It is clear that the bands predicted for the consensus GS, [N,CO,OH]tggt, correspond well to the observed spectrum. All major bands are present with comparable theoretical and experimental frequencies. The largest difference can be found for the band at 1586 cm\(^{-1}\) where there is a 26 cm\(^{-1}\) shift to the blue in the predicted spectrum, a common anharmonic shift for the band at 1586 cm\(^{-1}\). Compared with the experimental spectrum of [Zn(Ser-H)ACN]\(^{+}\), a common anharmonic shift for the band at 1586 cm\(^{-1}\) shows that that interaction of intact Ser with Cd\(^{2+}\) results in an even larger red shift from the free Ser C–O band compared to the interaction with Zn\(^{2+}\). The [N,CO,OH]tggt conformer predicts this band at 1704 cm\(^{-1}\), in excellent agreement with the observed band. The next lowest energy conformer, [N,CO]tggt, predicts a red-shifted carbonyl stretch at 1687 cm\(^{-1}\). The carboxylate C–O stretch band for the salt-bridge conformer, [CO\(_2\)]cgg, is shifted even further to 1637 cm\(^{-1}\). The position of this band and other spectral discrepancies with the action spectrum confirm that the higher energy salt-bridge conformer is not present experimentally.

Except for the carbonyl stretch, the predicted spectrum of the higher energy bidentate conformer, [N,CO]tggt, agrees reasonably with the observed action spectrum, although there are additional disagreements in diagnostic bands that unequivocally assign the CdCl\(^{2+}\)(Ser) experimental spectrum as tridentate [N,CO,OH]tggt. Specifically, the 1013 and 1052 cm\(^{-1}\) bands, which are associated with the COH stretch and NH\(_2\) wag, respectively, match the GS spectrum well, whereas the [N,CO]tggt spectrum does not predict the COH stretch band and calculates that the NH\(_2\) wag vibrational mode is shifted to the blue by 20 cm\(^{-1}\) compared to the experiment. Another diagnostic region is apparent in the sequence of three bands corresponding to carboxylic acid hydrogen wagging observed at 624, 691, and 764 cm\(^{-1}\). The relative intensities as well as their positions are nicely predicted by the GS, whereas these three bands for the [N,CO]tggt conformer are all red shifted and the relative intensity ratio is different from the experiment. Therefore, we assign the [N,CO,OH]tggt ground conformer as the only structure present experimentally. Although the possibility that small contributions from the [N,CO]tggt conformer cannot be completely eliminated, its calculated relative energy suggests that its equilibrium 298 K population is less than 0.3%.

Notably, the CdCl\(^{2+}\)(Ser) action spectrum has a band at \(~1430\) cm\(^{-1}\) band, which was not observed for [Zn(Ser-H)ACN]\(^{+}\), Fig. 1. This vibrational band can be assigned to the intact carboxylic acid HOC bend. Because the experimental ion population of the Zn complex appears to be dominated by the deprotonated carboxylic acid conformer, which naturally lacks this vibrational mode, this comparison helps validate the conclusions regarding the relative populations discerned above.

**Photofragmentation pathways**

Reaction coordinate calculations were performed to elucidate the photodissociation pathways of [Zn(Ser-H)ACN]\(^{+}\), as two sterically different parent ion conformers, [N,CO,O,OH]tggt and [N,CO,OH]tggt, were present experimentally yet only one distinct fragment ion was observed. Interestingly, the only fragment ion observed corresponds to the loss of neutral formaldehyde, which can be imagined to be lost easily in a charge remote process from the [N,CO,O,OH]tggt parent ion conformer (Fig. 2). However, as determined above, the [N,CO,OH]tggt conformer probably has a higher population. Therefore it needs to be investigated how the [N,CO,OH]tggt conformer loses formaldehyde and why other potential pathways, such as the loss of neutral carbon dioxide or the ACN ligand, are not observed.

To investigate the loss of neutral CO\(_2\) from [N,CO,OH]tggt, a complete reaction coordinate path for this dissociation was investigated theoretically. Fig. 4 shows the potential energy surface generated for the loss of neutral CO\(_2\) by a charge remote process with relative energies at multiple levels of theory listed in Table 5. In this mechanism, the rate-limiting barrier TS\(_C\) corresponds to breaking the amino acid backbone C–C bond, 159–173 kJ mol\(^{-1}\) higher in energy than the [N,CO,O,OH]tggt conformer. The loss of the M–OC interaction does not occur yet as the CO\(_2\) ligand moves to stay coordinated with the metal ion in INT\(_C\). The M–N coordination is lost in the process and now the metal coordinates to the newly formed anionic carboxylic acid, such that Zn remains 4-coordinate, [C\(^{−}\),OH][CO][ACN]. Complete loss of CO\(_2\) from the metal ion from INT\(_C\) requires an additional 12–23 kJ mol\(^{-1}\) energy.

The loss of neutral CH\(_2\)O from the (Ser-H)\(^−\) parent ion has been observed in IRMPD\(^{39}\) and CID\(^{42}\) studies and investigated by molecular modeling.\(^{43}\) It has been suggested that the loss of CH\(_2\)O from (Ser-H)\(^−\) may occur by a charge-direct process.\(^{44}\) In that study, the deprotonation site was the carboxylic acid and fragmentation was initiated by endothermic proton transfer from the side-chain hydroxyl to the carboxylate, resulting in a facile and exothermic anion-induced elimination of CH\(_2\)O. A similar process was found to be the lowest energy pathway in this study, where Fig. 4 shows the reaction coordinate for the loss of CH\(_2\)O from [Zn(Ser-H)ACN]\(^{+}\) [N,CO,OH]tggt. (It can also be noted that we also located a higher energy charge-direct process for the loss of CH\(_2\)O. In this mechanism, the first step is proton transfer from the side-chain hydroxyl to the carboxylate \(\text{via} \ T3\), which requires 84–91 kJ mol\(^{-1}\). Facile rotation of the COOH group forms INT\(_{1f}\), [N,CO,O,OH]tggt, which rearranges to the other populated conformer, INT\(_{2f}\), by rotation of the hydroxyl group over TS\(_{2f}\) (51–54 kJ mol\(^{-1}\) above the GS). In [N,CO,OH]tggt, the side-chain is positioned to allow the cleavage of the carbon–carbon bond to free CH\(_2\)O \(\text{via} \ T3\), which requires 74–99 kJ mol\(^{-1}\) energy. The CH\(_2\)O moves to stay...
coordinated to the metal ion in INT3f, [N,CO\(^{-}\)][CH\(_2\)O][ACN], where here c indicates a cis COOH dihedral angle. Elimination of formaldehyde requires an additional 83–101 kJ mol\(^{-1}\) of energy after INT3f. Note that this CH\(_2\)O loss mechanism forms the [N,CO][ACN] product, rather than the lowest energy product conformer, [N,CO\(^{-}\)][ACN] (Table 5), which requires proton transfer from COOH to the alpha-carbon radical. Mechanisms for this proton transfer were located but all contained a transition state lying > 245 kJ mol\(^{-1}\) above reactants (see, for example, Fig. S3 of the ESI†). Overall, the rate-limiting transition state for the loss of formaldehyde is the final products, a loose phase space limit (PSL) TS, which lie 148–192 kJ mol\(^{-1}\) above the GS. Theory predicts that this TS is below the rate-limiting TS\(_c\) for CO\(_2\) loss by 11 kJ mol\(^{-1}\) at the B3LYP level, but 2–28 kJ mol\(^{-1}\) above at the other levels examined, Table 5. To examine how these relative energetics would influence the competition between these two competitive channels, we performed RRKM analysis for H\(_2\)CO and CO\(_2\) loss using relative energy differences stipulated by all levels of theory. In all cases, the loose PSL TS for the loss of CH\(_2\)O dominated the tight TS for CO\(_2\) loss (TSC) by an order of magnitude or more at time scales relevant to the ICR detection (< 100 ms). For CO\(_2\) loss to be observed, we calculate that TSC would need to be lower in relative energy by > 34 kJ mol\(^{-1}\), well over the difference predicted by any level of theory. Thus, proton transfer between the deprotonated side-chain hydroxyl and carboxylic acid sites over TS1G requires only 84–91 kJ mol\(^{-1}\), well below either dissociation channel. Thus proton transfer is facile and permits the loss of formaldehyde from both [N,CO\(^{-}\)][ACN] and [N,CO\(^{-}\)][OH]\(_{gt}\) conformers. The failure to remove the ACN ligand can also be understood as this requires much more energy (221–235 kJ mol\(^{-1}\)) than the decomposition routes shown in Fig. 4.

### Discussion

#### Comparison with M\(^2+\)(Cys)

The amino acids cysteine and serine differ only by the substitution of sulfur for the oxygen in the side-chain and have
proven to exchange easily in proteins.\textsuperscript{7,45} Comparison of the present work on Ser with the corresponding study of Cys\textsuperscript{1} demonstrates that such equivalent amino acid exchange is not as facile upon interaction with metal dications. In the previous study of Cys and its methyl ester, CysOMe, the ESI source formed [M(Cys-H)ACN]\textsuperscript{+}, [M(CysOMe-H)ACN]\textsuperscript{+} and CdCl\textsuperscript{+}(CysOMe) for M = Zn and Cd. The ACN contamination ligand was easily removed from [M(Cys-H)ACN]\textsuperscript{+} and [M(CysOMe-H)ACN]\textsuperscript{+} by CO\textsubscript{2} laser irradiation, thereby forming stable 3-coordinate [M(Cys-H)]\textsuperscript{+} and [M(CysOMe-H)]\textsuperscript{+} complexes.\textsuperscript{1} Comparably for histidine, both 3- and 4-coordinate systems, [M(His-H)]\textsuperscript{+}, CdCl\textsuperscript{+}(His), and M\textsuperscript{2+}(His)\textsubscript{2} could be formed. Conversely, the equivalent [M(Ser-H)]\textsuperscript{+} complexes could not be isolated either in the source or by additional laser irradiation. Instead the acetonitrile ligand (for Zn\textsuperscript{2+}) or spectator ion (Cl\textsuperscript{-} for Cd\textsuperscript{2+}) needs to be present, and even after FELIX light irradiation, the [M(Ser-H)]\textsuperscript{+} deprotonated species were never observed because fragmentation leads to the decomposition of the amino acid instead of the loss of the ACN ligand. The need to bind these non-amino acid ligands to the metal and the inability to remove these ligands by irradiation indicates that Ser binds less effectively to Zn\textsuperscript{2+} and Cd\textsuperscript{2+} than Cys and His.

Previously, comparison of experimental and theoretical IR spectra allowed us to conclude that Zn\textsuperscript{2+} and Cd\textsuperscript{2+} bind to deprotonated Cys in a tridentate [N,CO,S\textsuperscript{-}]ggg configuration, with deprotonation occurring at the sulfur side-chain.\textsuperscript{1} For CdCl\textsuperscript{+}(CysOMe), a similar tridentate binding motif is found, [N,CO,S], and likewise CdCl\textsuperscript{+} binds to intact Ser as [N,CO,OH]. These binding motifs are also found for alkali metal cations binding to Ser\textsuperscript{40} and Cys.\textsuperscript{8} The side-chain hydroxyl is polar and partially negative and thus interacts strongly with the metal dications, even in the presence of spectator ions and ligands as found in the present study. The [Zn(Ser-H)ACN]\textsuperscript{+} complex is also found to have a tridentate ligand binding geometry, analogous to the deprotonated Cys systems. However, unlike the unambiguous assignment of deprotonation at the thiol for Cys, the site of deprotonation for Ser is mixed. Deprotonation at the carboxylic acid becomes competitive with the side-chain deprotonation for the lowest energy conformer, as demonstrated experimentally by the IR action spectrum. DFT levels of theory calculate that the [N,CO,OH]ggg conformer is more favorable than [N,CO,S\textsuperscript{-}]ggg, being ~2 J mol\textsuperscript{-1} less favorable at 298 K. Thus, both metal dications (Zn and Cd) bind to Ser in a tridentate motif coordinated to the amine and carbonyl groups of the amino acid backbone and the hydroxyl group of the side-chain. Our study finds that the CdCl\textsuperscript{+}(Ser) complex can be assigned as the predicted ground conformer, [N,CO,OH]ggg; whereas both [N,CO,OH]ggg and [N,CO,S\textsuperscript{-}]ggg, the two lowest energy conformers, were observed for the [Zn(Ser-H)ACN]\textsuperscript{+} complex. Relative intensities of the diagnostic C=O stretch bands in the IRMPD action spectrum suggest that the latter level of theory is the most accurate for this study.

As such, one might expect the carboxylic acid to be the preferred site of deprotonation for both of these amino acids. However, as we observe in this study, interaction with Zn\textsuperscript{2+} changes the relative probabilities for deprotonation as deprotonation of SH in Cys is highly favored and deprotonations of OH and COOH in Ser are competitive when binding to Zn\textsuperscript{2+}. One plausible rationale is that Zn\textsuperscript{2+} prefers bonding with more localized anion sites, O\textsuperscript{-} and S\textsuperscript{-}, over the delocalized CO\textsubscript{2}\textsuperscript{-}, although the latter coordination site remains competitive because its deprotonation is enthalpically favored.

Theory can also be used to examine the relative energies of low-lying conformers of [Zn(Ser-H)]\textsuperscript{+} (Table 3), which allows for a direct comparison with the previously studied [Zn(Cys-H)]\textsuperscript{+} system.\textsuperscript{1} Deprotonation at the side-chain is predicted to be the ground conformer for both Cys and Ser interacting with zinc, finding each species to have a tridentate [N,CO,X\textsuperscript{-}]ggg ground conformer, where X = S or O. For Cys, all levels of theory find that this structure is lower in energy than all other structures by >25 kJ mol\textsuperscript{-1} with deprotonation at the carboxylic acid, [N,CO,S\textsuperscript{-}]ggg, being ~42 kJ mol\textsuperscript{-1} less favorable at 298 K. In contrast, for Ser, Table 3 shows that this gap is 12–20 kJ mol\textsuperscript{-1}, with decarboxylation at the carboxylic acid, [N,CO\textsuperscript{-},OH]ggg, lying closest in energy. Furthermore, this energy difference is dramatically reduced upon coordination by a fourth ligand, as in the experimentally observed [Zn(Ser-H)ACN]\textsuperscript{+} system.

**Overview**

The IRMPD action spectra of cationized serine have been obtained in the region of 550–1800 cm\textsuperscript{-1} for complexes with Zn\textsuperscript{2+} and Cd\textsuperscript{2+}. Experimental spectra compared with IR spectra calculated at the B3LYP/6-311+G(d,p) level for Zn\textsuperscript{2+} and at the B3LYP/def2-TZVP level for Cd\textsuperscript{2+} containing complexes allow the identification of conformations present in the experiment. Action spectra were collected for complexes of Zn\textsuperscript{2+} with deprotonated serine and a metal bound acetonitrile spectator ligand and Cd\textsuperscript{2+} with a chlorinate spectator anion interacting with intact serine. Thus, in both cases, only 4-coordinate systems are formed for the Ser ligand. Comparison of experimental and theoretical IR spectra show that both metal dications (Zn and Cd) bind to Ser in a tridentate motif coordinated to the amine and carbonyl groups of the amino acid backbone and the hydroxyl group of the side-chain. Relative intensities of the diagnostic C=O stretch bands in the [Zn(Ser-H)ACN]\textsuperscript{+} action spectra suggest deprotonation at the carboxylic acid conformer is the more populated conformer, in agreement with the results at the MP2(full) level of theory. Deprotonation at this site differs from the previously studied deprotonated cysteine complexes, where the site of deprotonation for both Zn\textsuperscript{2+} and Cd\textsuperscript{2+} was assigned unambiguously to be the thiol side-chain.\textsuperscript{1} Although not experimentally observed, \textit{ab initio} calculations of the [Zn(Ser-H)]\textsuperscript{+} complex show that the conformer with deprotonation of the carboxylic acid is stabilized by the addition of the acetonitrile ligand yielding a desirable 4-coordinate complex.
The ACN ligand pulls electron density away from the metal, thereby relaxing the coordination of the amino acid ligand.

The types of complexes for amino acids interacting with metal dication, Zn$^{2+}$ and Cd$^{2+}$, can be experimentally generated change from 3-coordinate for Cys and His to 4-coordinate complexes for Ser, indicating that stabilization of the Ser complex requires the extra ligand. As noted above, it is energetically more favorable for the side-chain thiol of Cys to undergo deprotonation to bind with the metal dication, than to deprotonate the hydroxyl side-chain of Ser. To compensate for this energetic cost, deprotonated Ser binds to the metal dication, rather than to deprotonate the hydroxyl side-chain of Ser. This result parallels the observation that the metal–amino acid complex interaction is lost when Ser replaces Cys in zinc-finger protein studies. The results presented here provide valuable information for expanding our understanding of the fundamental aspects of metal-amino acid preferential binding phenomena in metal-dependent proteins.

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