Experimental and Theoretical Investigations of Infrared Multiple Photon Dissociation Spectra of Asparagine Complexes with Zn$^{2+}$ and Cd$^{2+}$ and Their Deamidation Processes

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*Supporting Information

ABSTRACT: Complexes of asparagine (Asn) cationized with Zn$^{2+}$ and Cd$^{2+}$ were examined by infrared multiple photon dissociation (IRMPD) action spectroscopy using light generated from a free electron laser. Electrospray ionization yielded complexes of deprotonated Asn with Zn$^{2+}$, [Zn(Asn$^{-}$H)$^+$], and intact Asn with CdCl$^+$, CdCl$^+(\text{Asn})$. Series of low energy conformers for each complex were found using quantum chemical calculations in order to identify the structures formed experimentally. The experimentally obtained spectra were compared to those calculated from optimized structures at the B3LYP/6-311+G(d,p) level for [Zn(Asn$^{-}$H)]$^+$ and the B3LYP/def2-TZVP level with an SDD effective core potential on cadmium for the CdCl$^+(\text{Asn})$ system. The main binding motif observed for the CdCl$^+$ complex is a charge solvated, tridentate [N, CO, COs] structure where the metal binds to the backbone amino group and carbonyl oxygens of the carboxylic acid and side-chain amide groups. The Zn$^{2+}$ system deprotonates at the backbone carboxylic acid and prefers a [N, CO$^-$, C Os] binding motif, where binding was observed at the carboxylate site along with the backbone amino group and side-chain carbonyl groups. In both cases, the theoretically determined lowest-energy conformers explain the experimental [Zn(Asn$^{-}$H)$^+$] and CdCl$^+(\text{Asn})$ spectra well. Additionally, complete mechanistic pathways were found for each of the major dissociation reactions of [Zn(Asn$^{-}$H)$^+$] (primary loss of CO$_2$, followed by the sequential loss of NH$_3$) and CdCl$^+(\text{Asn})$ (concomitant loss of NH$_3$ + CO).

INTRODUCTION

Deamidation at asparagine (Asn) residues is a post translational modification that occurs in proteins and plays an important role in degenerative aging diseases, such as Parkinson’s and Alzheimer’s.1-4 Thus, asparagine containing systems have been studied extensively. In the gas phase, our group has studied complexes of asparagine cationized with Li$^+$, Na$^+$, K$^+$, Rb$^+$, Cs$^+$, and Ba$^{2+}$ by infrared multiple photon dissociation (IRMPD)5 spectroscopy as well as asparagine cationized with H$^+$, Na$^+$, and K$^+$ by threshold collision-induced dissociation (TCID) in order to study the structural and energetic characteristics of these complexes.6-8 In the present study, IRMPD spectroscopy was utilized to study asparagine cationized with Zn$^{2+}$ and Cd$^{2+}$, as these two metals play an important role in binding to amino acids under biological conditions, particularly in zinc finger domains.9,10 Zn$^{2+}$ binds preferentially to cysteine (Cys) and histidine (His) amino acid sites within proteins, although it is not obvious why those amino acids are preferred. The replacement of Zn$^{2+}$ centers with Cd$^{2+}$ and additional cations has been documented; however, the nature of the metal dependence of these systems is not yet completely understood.11,12 Previous IRMPD studies have evaluated the binding of Zn$^{2+}$ and Cd$^{2+}$ with His,13 Cys and CysOMe,14 Gln,15 and Ser,16 thereby elucidating important structural information regarding the metal dependence of these systems. In the current work, we continue this evaluation of amino acid binding to Zn$^{2+}$ and Cd$^{2+}$, with an additional interest in evaluating the deamidation processes of these Asn complexes.

In order to definitively determine conformations of the [Zn(Asn$^{-}$H)$^+$] and CdCl$^+(\text{Asn})$ complexes formed experimentally, IRMPD action spectra for each complex were measured. Experimental spectra are then compared to spectra calculated for a series of low-energy conformers with optimized structures and vibrational frequencies determined at the B3LYP/6-311+G(dp) or B3LYP/def2-TZVP level of theory,
where an SDD effective core potential was used for cadmium. Comparison of the calculated and experimental spectra of each system allows for clear identification of the populated conformation of each complex. Theory also provides mechanistic details of the deamidation processes observed. Results presented here provide a great deal of information regarding metal dependence effects with respect to binding affinity, structural characteristics, and deamidation processes. Combined with results of our previous studies, analysis of these complexes allows for a more complete understanding of the metal dependence of other biologically relevant amino acid systems, e.g., the preferential binding to Cys and His.

Experimental and Computational Section

Mass Spectrometry and Photodissociation. Experiments were performed at the free electron lasers for infrared experiments (FELIX) facility of Radboud University in the Netherlands. A 4.7 T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, described elsewhere, was used to measure the IRMPD spectra. Ions were generated using an electrospray ionization (ESI) source and then accumulated in a hexapole trap for about 5 s before being pulse extracted through a quadrupole bender and injected into the ICR cell via a radiofrequency (rf) octopole ion guide. Electrostatic switching of the dc bias of the octopole was used to avoid collisional heating of the ions. Once trapped in the ICR cell, the ion of interest (assumed to be roughly at room temperature) was mass selected using a stored waveform inverse Fourier transform (SWIFT) excitation pulse. These ions were irradiated with FELIX for 2–3 s at a 10 Hz macropulse repetition rate (energy up to 45 mJ per macropulse and a bandwidth of 0.5% of the central frequency). The IRMPD spectra were generated by plotting the photo-fragmentation yield, \( Y = \frac{\sum I_p}{\sum I_p + \sum I_f} \), where \( I_p \) and \( I_f \) are the integrated intensities of the parent and fragment ion mass peaks (where the sum includes all isotopes), respectively, as a function of the frequency of IR radiation. The yield is linearly corrected for frequency dependent variation in the laser pulse energy. 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 because of the incoherent rather than coherent nature of the multiple photon excitation process. IRMPD fragmentation of the \([\text{Zn}(\text{Asn}−\text{H})]^+\) complex appeared to be saturated at wavenumbers corresponding to the highest intensity bands such that a spectrum with 5 dB attenuation of the laser power was also recorded below 1720 cm\(^{-1}\). Comparable levels of attenuation have been used in studies of similar systems.

Metalated Asn complexes were prepared from solutions of 1.0 mM Asn and 1.0 mM Zn(NO\(_3\))\(_2\) or CdCl\(_2\) in 60:40 MeOH/H\(_2\)O solvent using a Micromass Z-Spray ESI source. Flow rates of 6 \(\mu\)L/min were used, and the electrospray needle was held at a voltage of 2.4 kV. In the case of the Zn\(^{2+}\) complex, the ESI source generated a \([\text{Zn}(\text{Asn}−\text{H})\text{ACN}]^+\) complex, in which the asparagine is deprotonated and ACN = acetonitrile, CH\(_2\)CN. (The ACN was present adventitiously from previous experiments.) In this case, a CO\(_2\) laser was used to irradiate the sample for 0.3 s to remove the ACN ligand, leaving the \([\text{Zn}(\text{Asn}−\text{H})]^+\) complex. The resulting ions were mass isolated and allowed to cool radiatively for 0.4 s. Electrospray of the Cd\(^{2+}\) solution generated CdCl\(_2\)(Asn), which did not dissociate upon CO\(_2\) laser irradiation.

Computational Details. In order to determine low-lying conformers of the cationized Asn complexes, a series of 10–15 unique conformers of Asn were first optimized at the B3LYP/6-311+G(d,p) level, using the Gaussian 09 suite of programs. For the \([\text{Zn}(\text{Asn}−\text{H})]^+\) system, each of these structures were deprotonated (all possible sites were explored) and a Zn\(^{2+}\) cation was introduced to the system (all reasonable binding sites were explored). For the CdCl\(_2\)(Asn) system, CdCl\(_2\) was added to Asn exploring all possible binding sites. Initial optimizations of the metalated complexes were then done using the “loose” keyword to utilize a large step size of 0.01 au and a rms force constant of 0.0017 to facilitate convergence. Unique structures were then further optimized at the B3LYP/6-311+G(d,p) level of theory for Zn\(^{2+}\) complexes and at the B3LYP/def2-TZVP level, where def2-TZVP is a size-consistent basis set for all atoms and includes triple-\(\zeta\) + polarization functions with a small core (8-electron) effective core potential (ECP) on Cd. The def2-TZVP basis set and corresponding ECP were obtained from the EMSL basis set exchange. These combinations of level of theory, basis set, and ECP have previously proven to provide accurate structural information with complexes of similar size and composition. Geometry optimizations of metalated structures were also conducted including corrections for empirical dispersion at the B3LYP-GD3BJ level.

Vibrational frequencies were calculated at these levels of theory and scaled by 0.975 for comparison to the IRMPD spectra. This scaling factor has been shown to account for known inaccuracies in the calculated frequencies, and therefore gives good agreement with well-resolved peaks in other IRMPD spectra. The calculated frequencies were broadened using a 30 cm\(^{-1}\) full width at half-maximum Gaussian line shape when used for comparison to the experimentally determined spectra. This broadening accounts for the finite laser bandwidth, unresolved rotational structure of the ions (which are near room temperature), and anharmonicity of the vibrational mode in combination with broadening as a result of the multiple photon absorption process.

Relative energies were determined for B3LYP geometries using single point energies calculated at the B3LYP, B3P86, and MP2(full) levels using 6-311+G(2d,2p) (Zn\(^{2+}\) complexes) and def2-TZVP (Cd\(^{2+}\) complexes) basis sets. Relative B3LYP-GD3BJ single point energies using these larger basis sets and the B3LYP-GD3BJ geometries were also computed. Zero point energy (ZPE) corrections were applied to single point energies in order to provide 0 K relative enthalpies. Thermal corrections to obtain 298 K Gibbs free energies were calculated as outlined previously from 0 K relative enthalpies by using the rigid rotor/harmonic oscillator approximation with the calculated rotational constants and vibrational frequencies. Vibrational frequencies were scaled by 0.989 when used for zero point energy (ZPE) and thermal corrections.

Results and Discussion

IRMPD Action Spectroscopy. IRMPD action spectra for the \([\text{Zn}(\text{Asn}−\text{H})]^+\) and CdCl\(_2\)(Asn) complexes are shown in Figure 1, where the main product fragments in each case were used to determine the yield spectrum. Linear corrections of the laser power were applied and result in higher relative intensities of the high frequency bands and minor changes in the low frequency region. In each case, the spectral region was scanned from 5.5 to 10 \(\mu\)m (1820 to 1000 cm\(^{-1}\)), as there are no unique
Photodissociation of $[\text{Zn(Asn−H)}]^+$ and CdCl$^+(\text{Asn})$ complexes both result in fragments corresponding to deamidation. However, these processes proceed via pathways different from each other and different from those observed in the related glutamine (Gln) systems, as discussed in more detail below. IRMPD of the parent $[\text{Zn(Asn−H)}]^+$ complex resulted in the fragmentation pattern shown in reactions 1 and 2, where the numbers in brackets indicate the mass to charge ratio of the ion. Because $^{66}\text{Zn}$ is the most abundant isotope (49.17% natural abundance), the complex of this isotope is designated here as the parent ion. Channels corresponding to $^{66}\text{Zn}$ and $^{68}\text{Zn}$ (27.73 and 18.45% natural abundance, respectively) were also monitored and included in yield calculations shown in Figure 1.

$$\begin{align*}
[Zn(Asn-H)]^+ & \rightarrow \text{[Zn(C}_3\text{H}_2\text{O}_2\text{N}_2])^{151} + \text{CO}_2 \\
[Zn(C}_3\text{H}_2\text{O}_2\text{N}_2)]^{151} & \rightarrow \text{[Zn(C}_3\text{H}_4\text{O}_2\text{N}]^{134] + \text{NH}_3)
\end{align*}$$

The major product appears at $m/z$ 151 and results from the loss of CO$_2$, reaction 1. As the Asn ligand is deprotonated at the carboxylic acid site, the loss of CO$_2$ is clearly a reasonable fragment. As shown by reaction 2, deamidation occurs via a secondary loss from $m/z$ 151; however, the $m/z$ 134 product had an intensity not significantly above the noise level (consistent with its identification as a secondary product) and therefore was not included in the measurement of the spectrum. For comparison, $[\text{Zn(Gln−H)}]^+$ dissociation also resulted in CO$_2$ loss but was accompanied by Zn loss as well. Then, sequential deamidation occurred, and the deamidation product channel exhibited a much higher intensity.

The IRMPD of CdCl$^+(\text{Asn})$ resulted in one major fragment, as shown in reaction 3. Here, masses are chosen to correspond to the most abundant isotopes of Cd and Cl, $^{114}\text{Cd}$ and $^{35}\text{Cl}$, such that $m/z$ 281 is designated as the parent ion and $m/z$ = 236 as the fragment. The complexes associated with $m/z$ values corresponding to $^{113}\text{Cd}$, $^{114}\text{Cd}$, $^{115}\text{Cd}$, $^{37}\text{Cl}$ were also monitored and included in yield calculations shown in Figure 1.

$$\text{CdCl}^+(\text{Asn})[281] + nhv \rightarrow \text{CdCl}^+(\text{C}_2\text{H}_2\text{O}_2\text{N})[236] + \text{NH}_3 + \text{CO}$$

The $m/z$ 236 product likely corresponds to the concomitant loss of (NH$_3$ + CO) from the Asn ligand, but neither product associated with the possible primary losses was observed, as illustrated in Figure S2. Although the sequence of losses is experimentally indistinguishable, deamidation occurring as the primary loss, followed by sequential dissociation of CO, would be consistent with reaction pathways found for the decomposition of H$^+(\text{Asn})$ by CID$^3$ and CdCl$^+(\text{Gln})$ by IRMPD.

This result will be explored in more detail theoretically below. Theoretical Results: Relative Energies. Tables S1 and S2 (Supporting Information) give relative single point energies and free energies for conformers of $[\text{Zn(Asn−H)}]^+$ and CdCl$^+(\text{Asn})$, respectively, calculated at the B3LYP, B3LYP-GD3BJ, B3P86, and MP2(full) levels of theory (relative to the lowest-energy isomer at each level of theory). Complexes are named using nomenclature outlined previously. In short, the complexes are designated by their metal binding site in brackets, with the deprotonation site (if present) indicated by a negative sign. In most systems, both the backbone and side-chain carboxyls are binding sites with a subscript s (for side chain) designating the latter. The designation of the metal binding site is then followed by the amino acid orientation, which is represented by the characterisation of dihedral angles as cis (c, for angles between 0 and 45°), gauche (g, 45−135°), or trans (t, 135−180°). In a few cases, signs of the gauche angles (+ or −) are also needed in order to distinguish otherwise identical names. Dihedral angles were measured starting from the carboxylic acid hydrogen (unless this site is deprotonated) or the analogous proton on NH$_2$ in zwitterionic structures and going to the terminal side-chain nitrogen. In cases where deprotonation does not occur at any of the metal binding sites, the deprotonation site is added in parentheses. When deprotonation occurs at a carbon along the backbone, standard nomenclature is used ($\alpha, \beta, \gamma, \ldots$).

At all levels of theory, the predicted lowest energy $[\text{Zn(Asn−H)}]^+$ complex adopts a tridentate [N, CO$\gamma^−$, CO$_\alpha^−$] binding motif (Figure 2), where the metal binds to the backbone amino group, the carboxyl oxygen of the deprotonated carboxylic acid group, and the carboxyl oxygen of the amide side-chain group. Only one conformation having this binding motif was found, although all other conformers up to 82 kJ/mol across all levels of theory are also characterized as tridentate. Two [N$^−$, CO$_\alpha$, CO$_\gamma$] conformers (Figure 2) are the next lowest in energy, having relative 0 K energies 39−53 kJ/mol above the ground conformer. The more favorable energetics of the $\text{tg}_{g,t}$ conformer appear to be the result of less constrained backbone dihedral angles such that the sum of the $\angle$NMO angles of the $\text{tg}_{g,t}$ conformer is slightly greater than that of the $\text{tg}_{g,t}$ conformer. Two tridentate conformers, [CO$_\gamma^−$, CO$_\alpha^−$, CO$_\gamma^−$] (Figure 2) and [CO$_\gamma^−$, N$_\gamma$, CO$_\gamma^−$] (not shown), were found where binding takes place at both oxygens of the deprotonated carboxylate group. Here, the possible binding sites at the amide side-chain group have a significant effect on the relative energetics, as the [CO$_\gamma^−$, N$_\gamma$] conformer (76−85 kJ/mol relative to the ground conformer) is 56−65 kJ/mol lower in energy than the [CO$_\gamma^−$, N$_\gamma$] conformer. A similar effect

Figure 1. Infrared multiple photon dissociation (IRMPD) action spectra of Zn$^{2+}$ and Cd$^{2+}$ complexes of Asn. Spectra taken with attenuated laser power (in decibels) are indicated in red.
regarding the specificity of binding at the amide group is observed for the \([N, CO^-, NO]^-\) conformers, which lie 71–87 kJ/mol higher than the \([N, CO^-, CO]^-\) ground conformer. Thus, there is much more stability associated with binding at the oxygen of the carbonyl group compared with the amide nitrogen, as also confirmed by the shorter M–O distance in the \([N, CO^-]CO_2^-\) conformer (1.96 Å) compared with the M–N distances in the \([N, CO^-, NO]^-\] and \([N, CO^-, NO]^-\] conformers (2.09 and 2.06 Å, respectively). Notably, one tridentate conformer was found where binding takes place at a deprotonated carbon of the backbone chain, \([N, CO, C^-]CO_2^-\), which has relative energies 104–111 kJ/mol higher than the ground conformer. Three conformers where deprotonation occurs at the amide nitrogen, \([N, CO, CO_2^-]NO]^-\), were found, where the lowest energy conformer of this type, \([N, CO, CO_2^-]NO]^-\), is shown in Figure 2.

Several bidentate binding motifs were also found for \([Zn(Asn−H)]^+\) with relative energies 83–251 kJ/mol higher in energy than the ground conformer, clearly indicating an increased stability with the tridentate conformers. The two lowest energy bidentate conformers have similar relative energies (83–95 kJ/mol higher than the ground conformer) and involve binding at the carbonyl of the amide side chain coupled with either the deprotonated backbone carbonyl or amine sites. The highest energy \([Zn(Asn−H)]^+\) conformer, \([CO_2^-]ctg\) (175–251 kJ/mol above the ground conformer), is also characterized by a bidentate binding motif where binding only takes place at both oxygens of the carboxylate group.

Similar to the \([Zn(Asn−H)]^+\) conformers, the lowest energy CdCl\(^{−}\)(Asn) conformer is characterized by a \([N, CO, CO_2^-]ctg\) binding motif (Figure 3). This is similar to what is observed in other amino acid systems. The next four lowest energy conformers lie within ~40 kJ/mol of the ground conformer, but only one of those conformers, \([N,OH,CO]^-\)ctgt, is characterized by a tridentate binding motif. One zwitterionic form of CdCl\(^{−}\)(Asn), \([CO_2^-]ctgt\), was found to be only 20–32 kJ/mol higher than the ground conformer, and is stabilized by NH–OC and NH–OC hydrogen bonds (Figure 3).

This zwitterionic binding motif was also found to be a low-energy conformer of Asn cationized with Li\(^+\), Na\(^+\), K\(^+\), Rb\(^+\), Cs\(^+\), and Ba\(^2+\), as well as Glu complexes cationized with Zn\(^2+\) and Cd\(^2+\), and Li\(^+\), Na\(^+\), K\(^+\), and Cs\(^+\). Similar to the \([Zn(Asn−H)]^+\) conformers, CdCl\(^{−}\)(Asn) conformers exhibit a strong specificity for binding to the carbonyl of the amide side chain. Namely, the \([N, CO, NO]^-\) conformer is 50–60 kJ/mol higher in energy than the \([N, CO, CO_2^-]\) ground conformer, nearly equivalent to the analogous increase in energy observed with the \([Zn(Asn−H)]^+\) conformers.

**Theoretical Results: [Zn(Asn−H)]\(^{+}\) Structures.** A full description of the key geometric parameters of each major identified binding motif for the \([Zn(Asn−H)]^+\) conformers is given in Table S3 (Supporting Information), where our structural analysis provides a great deal of information on the binding affinity and conformational effects of these complexes.

As stated above, only one conformer of the \([N, CO^-, CO]^-\) binding motif was located for the \([Zn(Asn−H)]^+\) complex, the \([N, CO^-, CO]^-\)ctg ground conformer. Here, deprotonation occurs at the carboxylic acid site, as is also found for \([N, CO_2^-]NO]^-\), \([CO_2^-]ctg\), and \([N, CO^-]NO]^-\). All of these conformers are characterized by relatively short M–O distances, ranging from 1.81 to 1.89 Å, where the shortest distance is observed in the bidentate \([CO_2^-]CO_2^-\). Likewise, conformers where deprotonation occurs at the amino nitrogen \([N^+, CO, CO_2^-]NO]^-\) exhibit the shortest M–N distances, 1.85–1.90 Å. The longest M–N distances are observed in conformers where deprotonation occurs at the amide group, \([N, CO, CO_2^-]NO]^-\), and in one instance, at a carbon of the backbone, \([N, CO, C^-]CO_2^-\). Other notable comparisons arise from examining the M–O distances in \([N^+, CO, CO_2^-]NO]^-\) conformers (2.14–2.18 Å), which are much shorter than the M–O distance in the analogous \([N^+, OH, CO_2^-]NO]^-\) conformer (2.37 Å). This stronger interaction leads to favorable energetics of the \([N^+, CO, CO_2^-]NO]^-\) conformers, 33–41 kJ/mol lower in energy than \([N^+, OH, CO_2^-]ctg\). Likewise, the M–N and M–O distances are very similar for the \([N, CO^-, CO_2^-]ctg\) and \([N, CO_2^-]NO]^-\) conformers (the lowest energy conformers of each type), although the M–Y\(^-\) distance increases by about 0.1 Å when the carboxyl binding site is replaced with the amide nitrogen.

A few trends were also observed in the \(\angle XMO, \angle XMY_1\), and \(\angle OMY_2\) angles. Of the tridentate \([Zn(Asn−H)]^+\) complexes,
the [N, CO, COs]-tgtt conformer exhibits the smallest ∠NMO, 78.6°. The next smallest angles were observed with those involving deprotonation at the amide site (78.7°–81.5°). Here, the more constrained ∠NMO angles, in addition to other factors, clearly have effects on the energetics of these conformers (100–126 kJ/mol above the ground conformer). The bidentate conformers, specifically [N+, COs]-tttt, [N+, COs]-cgtt, and [N, NOS]-tgtt, have the largest ∠NMO angles (111, 114, and 116°, respectively). Although these conformers are clearly less constrained with respect to these angles and have relatively short M–N and M–O distances (indicative of tight binding), the presence of the additional binding site in the tridentate conformations is clearly important, as these bidentate conformers are 84–122 kJ/mol higher in energy than the tridentate ground conformer.

**Theoretical Results: CdCl'(Asn) Structures.** Table S4 (Supporting Information) lists the key geometric parameters of major binding motifs for the CdCl'(Asn) conformers. All CdCl'(Asn) complexes exhibit longer M–O and M–O distances than the [Zn(Asn–H)]+ complex characterized by a similar binding motif. This reflects the smaller ionic radius of Zn2+ (0.60 Å) as compared to that of Cd2+ (0.78 Å), as well as tighter binding in the [Zn(Asn–H)]+ complexes as a result of stronger electrostatic interactions between the metal center and deprotonated anion ligand as compared with the intact amino acid in the Cd2+ complexes. Here, M–N distances are ~0.3 Å longer than those in comparable [Zn(Asn–H)]+ complexes, where this effect is mainly a result of the ionic radius, coupled with tighter binding of the anionic ligand as described above. Similar to the [Zn(Asn–H)]+ system, there was only one conformer having the [N, CO, COs] binding motif for the CdCl'(Asn) conformers, [N, CO, COs]-tgtt. Along with [N, OH, COs]-tgtt and [N, CO, NOS]-tggtt, these tridentate conformers exhibit the longest M–N distances (2.37, 2.32, and 2.34 Å, respectively). Understandably, the shortest M–N distances of the CdCl'(Asn) conformers are observed in the bidentate conformations, with the [N, OH]-tgtt conformer having the shortest distance, 2.18 Å. The longest M–O distances result from binding at the hydroxyl oxygen ([N, OH, COs], [N, OH], and [OH, COs]) as compared with the alternative carbonyl oxygen as observed in the ground conformer. Clearly, there are stronger electrostatic interactions when binding the carbonyl to the metal center.

Interestingly, the M–N, M–O, and ∠NMO geometric parameters do not differ significantly for the [N, OH, COs]-tgtt and [N, COs]-tgtt conformers, where the specified distances are within 0.04 Å and the angles agree within 3°. These structural similarities also result in very comparable energetics, as the bidentate conformer is only 0.1–12 kJ/mol higher in energy than the [N, OH, COs] complex, a much smaller energetic penalty than observed in the [Zn(Asn–H)]+ system where bidentate complexes systematically had a much higher relative energy than the ground conformer. A much more noticeable difference between comparable bidentate and tridentate structures for the CdCl'(Asn) system is observed between the [N, CO, COs]-tgtt and [N, COs]-tgtt conformers. Here, the differences between M–N and M–O distances and ∠NMOs were larger, 0.05 Å, 0.09 Å, and 7°, respectively. The energetic cost associated with removing one of the binding sites is also more obvious, as the [N, COs] conformer is 25–34 kJ/mol higher in energy than the [N, CO, COs] ground conformer.

**Comparison of Experimental and Theoretical IR Spectra: [Zn(Asn–H)]+.** In comparing the experimental and theoretical IR spectra, it should be remembered that the experimental IRMPD intensities are not always reproduced by the calculated linear absorption spectrum; however, infrared spectra obtained using IRMPD methods are generally

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**Figure 3.** Structures of low-energy CdCl'(Asn) conformers calculated at the B3LYP/def2-TZVP level of theory. Relative single point enthalpies (0 K) in kJ/mol are given at the B3LYP, B3LYP-GD3BJ, B3P86, and MP2(full) levels, respectively. Short dashed lines indicate hydrogen bonds (up to 2.5 Å). Metal–ligand interactions are shown by long dashed lines.
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.30,35

As shown in Figure 4, the most intense spectral features in the computed spectra of the lowest energy [N, CO−, C Os]-ggt conformer correlate fairly well with those observed in the experimental [Zn(Asn−H)]+ spectrum. Here, we have combined the 0 and 5 dB experimental spectra for ease of comparison to the calculated spectra, where clearly the composite spectrum is dominated by the 0 dB contributions at high and low frequencies, with bands from 1300−1500 cm−1 indicative of the 5 dB contribution to the spectrum. The bands observed at 1552 (amide CO stretch), 1647 (amide NH2 bend), and 1738 cm−1 (stretch of uncoordinated CO in carboxylate) are consistent with those calculated for the [N, CO−, C Os]-ggt conformer. Here, the most intense band (1647 cm−1) is reproduced very well in frequency and intensity by the calculated spectrum, although the band corresponding to the CO stretch of the carboxylate is blue-shifted about 20 cm−1 compared with the experimental spectrum. However, the [N, CO−, C Os]-ggt species is the only low-lying conformer that exhibits a carboxylate CO stretch in this region. The weak band at 1600 cm−1 in the calculated spectrum for the [N, CO−, C Os]-ggt conformer was not explicitly resolved experimentally, although there is a small shoulder present at 0 dB. This band correlates to the NH2 bend of the backbone amino group and characteristically exhibits a red shift of about 25 cm−1 as a result of the strong anharmonic nature of this vibrational mode.15,36−41 A similar shift would produce a peak at 1575 cm−1 that would overlap with the band at 1552 cm−1, thus accounting for the difference in experimental and theoretical intensities.

There is also reasonable agreement between the experimental and [N, CO−, C Os]-ggt calculated spectra in the lower frequency region. For the experimental/calculated spectra, bands were located at (1053/1053 cm−1, amino NH2 wagging), (1131/1130 cm−1, amino NH2 twisting, with minor backbone motions), (1185/1196 cm−1, primarily backbone CH2 twisting), (1238/1230 cm−1, backbone CCH bending with minor contributions from coordinated CO stretching), (1292/1296 cm−1, amino NH2 twisting), and (1449/1434 and 1414 cm−1, amide NOC bending with backbone CH2 wagging). Of these bands, the peaks located at 1131, 1238, and 1292 cm−1 all have experimental intensities much higher than predicted theoretically. Likewise, the bands at 1053, 1185, and 1449 cm−1 were all calculated to be either comparable or higher in intensity than is observed experimentally. The only spectral feature of the experimental [Zn(Asn−H)]+ spectrum that is not reproduced in the calculated spectra is the band at 1490 cm−1, a discrepancy for which we have no ready explanation, although a band shifted by anharmonic effects is one possibility.

The calculated spectra for four additional conformers are also given in Figure 4. The [N+, CO3, CO2]+ conformers were found to have two intense spectral features centered about the band at 1647 cm−1 of the experimental spectrum but did not reproduce the 1738 cm−1 band associated with CO stretching of the
deprotonated carboxylic acid (because deprotonation is occurring at the amino nitrogen). Interestingly, \([\text{CO}_2^-\text{C} \text{ Os}]\text{-gcg}\) was the only conformer that exhibits a band in the region where the 1490 cm\(^{-1}\) peak is observed in the experimental spectrum. Again, this conformer does not reproduce the CO stretching band observed in the experimental spectrum.

\([\text{N}^-\text{OH}, \text{CO}_3^-]\text{-tggt}\), the only additional conformer shown that exhibits a CO stretch in the high frequency region, is characterized by a blue-shift by \(\sim 80\) cm\(^{-1}\) with respect to the experimental band at 1738 cm\(^{-1}\), Figure 4.

Therefore, the experimental spectrum appears to be most consistent with the calculated spectrum for \([\text{N}, \text{CO}^-\text{C} \text{ Os}]\text{-ggt}\), the predicted lowest energy \([\text{Zn(Asn}^-\text{H})]^+\) conformer. In addition to a spectral comparison, the probability that \([\text{N}, \text{CO}^-\text{C} \text{ Os}]\text{-ggt}\) was the conformer formed experimentally was also assessed on the basis of an equilibrium distribution of conformers. According to the calculated free energies at all levels of theory, the \([\text{N}, \text{CO}^-\text{C} \text{ Os}]\text{-ggt}\) conformer would have a population of \(\geq 99.99\%\) at 298 K.

**Comparison of Experimental and Theoretical IR Spectra: CdCl\(^+\)(Asn).** Similar to the Zn\(^{2+}\) system, the experimental IRMPD action spectrum of CdCl\(^+\)(Asn) is predicted very well by the lowest-energy conformer, \([\text{N},\text{CO}_2\text{O}]\text{-ggt}\); see Figure 5. The main spectral features of the CdCl\(^+\)(Asn) spectrum are observed at 1040, 1149, 1417, 1586, 1644, and 1720 cm\(^{-1}\). Computed bands at 1721 (coordinated CO stretch of the carboxylic acid) and 1656 cm\(^{-1}\) (CO stretch of the amide group, with contributions from amide NH\(_2\) bending) reproduce the experimental spectrum extremely well in frequency (shifts within \(\sim 10\) cm\(^{-1}\)) and intensity, and are probably the most diagnostic. The remaining bands computed at 1412 (backbone CH\(_2\) bending with minor contributions from COH bending and CNH bending), 1161 (primarily COH bending with a small contribution from NH\(_2\) rocking), and 1038 cm\(^{-1}\) (amino NH\(_2\) wagging) all exhibit comparable behavior to the experimental spectrum, with the only significant deviation in frequency being the band at 1161 cm\(^{-1}\), which is blue-shifted by \(\sim 10\) cm\(^{-1}\).

Additionally, for the \([\text{N}, \text{CO}, \text{CO}_3^-]\text{-tggt}\) conformer, there is a predicted band near 1610 cm\(^{-1}\) corresponding to the NH\(_2\) band of the backbone amino group that is not clearly observed in the experimental spectrum at that frequency. As mentioned above, this effect has been seen in similar systems where it was concluded that strong anharmonic effects red shift the NH\(_2\) band in all cases. A red shift of the same magnitude shown in previous studies would produce a peak at \(\sim 1585\) cm\(^{-1}\) in the \([\text{N}, \text{CO}, \text{CO}_3^-]\text{-tggt}\) spectrum, thus overlapping the predicted band at 1594 cm\(^{-1}\). A combination of both bands in this frequency range would explain why the peak at 1586 cm\(^{-1}\) in the experimental spectrum exhibits a higher relative intensity compared with the predicted spectrum.

Figure 5 shows spectra for an additional four low-lying structures (20–41 kJ/mol higher than the lowest-energy conformer), but the spectral features exhibited in those cases do not match the observed CdCl\(^+\)(Asn) spectrum to a degree that would suggest any of these conformers were formed experimentally. Specifically, the main spectral features in the zwitterionic \([\text{CO}_2^-\text{C} \text{ Os}]\text{-ctgt}\) conformer (20–32 kJ/mol higher than the lowest-energy conformer) are intense bands at 1409 and 1436 cm\(^{-1}\) for which there is only a very weak band in the experimental spectrum. All other spectral features do not correspond to any bands present in the CdCl\(^+\)(Asn) spectrum. The only additional tridentate conformer shown, \([\text{N}, \text{OH}, \text{CO}_3^-]\text{-tggt}\), only reproduces two of the major high frequency bands (those at 1586 and 1644 cm\(^{-1}\)). The absence of the predicted band at 1810 cm\(^{-1}\) and the failure to reproduce
any lower frequency features clearly suggest this conformer is not being formed experimentally. Of the two bidentate conformers shown, [N, CO, COs]-tgtt reproduces all bands present in the experimental spectrum with the exception of the coordinated CO stretch of the carboxylic acid, as no binding at that site occurs in this conformer. Instead, a blue shift of ~60 cm<sup>−1</sup> is observed for the CO stretching band in this spectrum, which is clearly not observed experimentally.

Here, the spectral analysis indicates that solely [N, CO, COs]-tgtt is present experimentally, consistent with theoretical calculations at all levels of theory. This conclusion also agrees with an equilibrium distribution of conformers at 298 K, which suggests the [N, CO, COs]-tggt conformer would have a population ≥99.99%.

**Overall Comparison.** Here we find it useful to give a more comprehensive analysis of the spectral features exhibited in both the [Zn(Asn−H)]<sup>+</sup> and CdCl'<sup>+</sup>(Asn) IRMPD action spectra. As shown in Figures 1, 4, and 5, the main spectral features in the [Zn(Asn−H)]<sup>+</sup> complex are observed at 1053 (0 dB), 1131 (0 dB), 1185, 1292, 1449, 1490, 1552, 1647, and 1720 cm<sup>−1</sup>, whereas those in the CdCl'<sup>+</sup>(Asn) spectrum appear at 1040, 1149, 1422, 1586, 1644, and 1720 cm<sup>−1</sup>. Of the three most intense and diagnostic peaks of the [Zn(Asn−H)]<sup>+</sup> and CdCl'<sup>+</sup>(Asn) spectra, the amide NH<sub>2</sub> bending mode at 1647 and 1644 cm<sup>−1</sup>, respectively, shifts very little. This is a reasonable observation given the same side-chain amide functionality and the fact that metal dependent effects should be minimal, as there is no complexation at this site. In contrast, the CO stretch of the amide experiences a large shift (from 1552 cm<sup>−1</sup> for the Zn<sup>2+</sup> complex to 1586 cm<sup>−1</sup> for the Cd<sup>2+</sup> complex). Here, the larger shift likely results from more significant perturbations caused by differences in the metal cation binding strength. This can be directly related to the size of the metal center, as smaller metals allow shorter M−O and M−X distances, which translates into stronger electrostatic interactions between the metal and amino acid ligand. Interestingly, this blue shifting of the Cd<sup>2+</sup> complex (relative to the Zn<sup>2+</sup> complex) is consistent with previous studies of alkali metal cation complexes with Asn<sup>+</sup>, although a much larger shift is observed in the current study. This is likely a result of the much stronger M−L interactions in the Zn<sup>2+</sup> and Cd<sup>2+</sup> Asn complexes as compared to those for the singly charged alkali metal complexes. A smaller shift of 18 cm<sup>−1</sup> in the opposite direction is observed for the CO stretch of the carboxylic acid, from 1738 to 1720 cm<sup>−1</sup>. The CO band from the stretching of the carboxylate group in the Zn<sup>2+</sup> spectrum lies above that in the Cd<sup>2+</sup> spectrum (opposite of what was observed in our previous Asn IRMPD study of alkali metal complexes), an effect that can be attributed primarily to deprotonation at the carboxylic site in [Zn(Asn−H)]<sup>+</sup>. The analogous effect was previously observed in a study of His and Gln complexes of Zn<sup>2+</sup> and Cd<sup>2+</sup> where the shift is about 40 cm<sup>−1</sup>.13,15

**Spectral Comparison to Zn<sup>2+</sup>/Cd<sup>2+</sup> Gln.** The IRMPD spectra for [Zn(Gln−H)]<sup>+</sup> and CdCl'<sup>+</sup>(Gln) have been measured previously.13 Figure 6 shows the comparison between those spectra and the spectra measured for [Zn(Asn−H)]<sup>+</sup> and CdCl'<sup>+</sup>(Asn) in the current study. It is clear that the spectra for each metal agree closely with one another, as expected given the same side-chain functionality. For the [Zn(Gln−H)]<sup>+</sup> and [Zn(Asn−H)]<sup>+</sup> complexes, two of the three major diagnostic peaks are nearly identical in frequency (1557 and 1552 cm<sup>−1</sup>, amide CO stretch) and (1647 and 1647 cm<sup>−1</sup>, amide NH<sub>2</sub> bend), and only the CO stretch of the carboxylate group experiences a larger shift (~20 cm<sup>−1</sup>). The opposite correlation is observed for the Cd<sup>2+</sup> systems, where the frequency shifts are more evident at the amide CO stretching (1597 and 1586 cm<sup>−1</sup>) and NH<sub>2</sub> bending (1662 and 1644 cm<sup>−1</sup>) bands in the CdCl'<sup>+</sup>(Gln) and CdCl'<sup>+</sup>(Asn) spectra, respectively. Interestingly, all other bands that are represented in both Asn and Gln systems are red-shifted in the Asn spectra by 10−42 cm<sup>−1</sup> (with the only exception being the band at 1053 cm<sup>−1</sup> in both the [Zn(Gln−H)]<sup>+</sup> and [Zn(Asn−H)]<sup>+</sup> spectra). This red shifting is consistent with previous studies, which have concluded that Asn absorbs ~10 cm<sup>−1</sup> lower than Gln as a result of the slightly different environments in which intramolecular interactions (hydrogen bonding) occur.22 Specifically, previous theoretical and experimental studies revealed that, as hydrogen bonds weaken (and lengthen), there is typically an associated red shift.13 On the basis of these conclusions, it appears that the additional carbon present in the Gln backbone affects the hydrogen bonding environment sufficiently that corresponding bands in the Asn and Gln spectra (other than the high-

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**Figure 6.** Infrared multiple photon dissociation (IRMPD) action spectra of Zn<sup>2+</sup> and Cd<sup>2+</sup> complexes with Asn and Gln.15

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frequency region discussed above) of Figure 6 are red-shifted in the [Zn(Asn−H)]⁺ and CdCl⁺(Asn) spectra compared to spectra for the analogous Gln complexes.

Interestingly, the band at ∼1300 cm⁻¹ in the [Zn(Asn−H)]⁺ spectrum is not observed in the Gln system with any significant intensity, although the lowest energy conformers of both systems are predicted to have a band in this region, which is due to backbone CH₂ and amino NH₂ motions. There are indeed two weak bands in the predicted [Zn(Asn−H)]⁺ [N, CO−, CO−, Os]-ggt spectrum (CH₂ wagging and CH₂ rocking), see Figure 4, which likely explain the observed intensity. The Gln system is only predicted to have one band (a combination of CH₂ wagging and rocking) near this frequency. Interestingly, this band also appears to be metal dependent, as it is not observed in the CdCl⁺(Asn) spectrum. Furthermore, this band was not observed in Asn or Gln complexes with Li⁺, was observed with only moderate intensity with Na⁺, and was observed with the highest intensity with Cs⁺.5,32

Deamidation Pathways for [Zn(Asn−H)]⁺. Under biological conditions, the mechanism for deamidation of Asn residues is believed to occur via formation of a five-membered cyclic succinimide intermediate.4 Because the [Zn(Asn−H)]⁺ complex has been deprotonated at the carboxylic acid, deamidation of this complex cannot occur facilely via the same pathway and likely requires more intramolecular rearrangement. It has also been shown experimentally and theoretically that conformational and steric effects play obvious roles in controlling the rate of ring closure, which requires the transfer of a hydrogen to the amide NH₂ to form the NH₃ leaving group. Interestingly, both Asn complexes studied here exhibited dissociation patterns that suggest deamidation is occurring while the metal center still participates in binding, whereas different behavior was observed with Gln complexes with Zn²⁺.15 Thus, the structural and spectral analysis of these complexes is an important factor in evaluating the deamidation properties of these and other biologically relevant systems, specifically in comparison with the Gln complexes of Zn²⁺.15

The complete mechanistic pathways for the major dissociation reactions 1−3 were evaluated, with a particular interest in the deamidation processes. Here, the distinction between ammonia loss via the amino or amide group is particularly important, because the deamidation process has been thoroughly documented as being detrimental to protein health.4 Thus, we have evaluated the complete mechanistic pathways for each of the possible dissociation processes leading to m/z 134, where the reaction coordinates for deamidation (amide NH₃ loss) and deamination (amino NH₃ loss) from [Zn(C₃H₇ON₂)]⁺ are given in Figures 7 and 8, respectively. Optimized B3LYP/6-311+G(d,p) structures of the major dissociation species are given, and relative single point energies given at the B3LYP, B3LYP-GD3BJ, B3P86, and MP2(full) levels of theory are given in Table 1. The starting structure for each of these pathways, [Zn(C₃H₇ON₂)]⁺, was found via simple C−C bond cleavage, resulting in the loss of the carboxylate group (CO₂) from the [N, CO−, CO−, Os]-ggt lowest energy conformer. Additional higher energy conformers of [Zn(C₃H₇ON₂)]⁺ are given in the Supporting Information.

The lowest energy pathway for ammonia loss (Figure 7) from [Zn(C₃H₇ON₂)]⁺ was found to occur from the amide group. From [Zn(C₃H₇ON₂)]⁺, proton transfer from the N to N site occurs, passing through TS₆. This transfer results in the formation of the NH₃ leaving group, as shown in INT₆.
than those with the dication counterpart. The relatively high

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Figure 8. Reaction coordinate for ammonia loss via the backbone amino group from \([\text{Zn(C}_{3}\text{H}_{4}\text{ON})]^+\) calculated at the MP2(full)/6-311+G(2d,2p) level of theory, using B3LYP/6-311+G(d,p) geometry optimizations. Structures for intermediates lie below the reaction coordinate, and those for TSs lie above. Dashed lines (gray) indicate M–L bonds. Dotted lines (black) indicate bonds being broken or formed for transition states.

Table 1. Relative Energies (0 K) and Free Energies (298 K) of Major Reaction Species Forming m/z 134 from [Zn(Asn−H)]^+\)

<table>
<thead>
<tr>
<th>reaction</th>
<th>structure</th>
<th>B3LYP/GD3BJ(^{a})</th>
<th>B3LYP6</th>
<th>MP2(full)</th>
</tr>
</thead>
<tbody>
<tr>
<td>deamidation</td>
<td>[N, CO^−, CO_2(^−)]^+</td>
<td>0.0 (0.0)/0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>[Zn(C(_2)H(_4)ON(^+))+ CO_2</td>
<td>33.2 (35.6)/57.6 (60.0)</td>
<td>68.8 (71.2)</td>
<td>86.5 (88.9)</td>
</tr>
<tr>
<td></td>
<td>TS(_{2A}) [N−N(_2)]</td>
<td>102.8 (109.8)/132.2 (139.1)</td>
<td>157.1 (164.0)</td>
<td>164.6 (171.6)</td>
</tr>
<tr>
<td></td>
<td>INT(_{1D})</td>
<td>108.0 (112.1)/134.3 (138.5)</td>
<td>165.3 (169.6)</td>
<td>169.6 (173.9)</td>
</tr>
<tr>
<td></td>
<td>TS(<em>{2D}) [N−C(</em>\alpha)]</td>
<td>202.8 (212.6)/240.9 (250.7)</td>
<td>281.9 (291.7)</td>
<td>254.1 (263.9)</td>
</tr>
<tr>
<td></td>
<td>INT(_{2D})</td>
<td>192.0 (202.4)/229.3 (238.9)</td>
<td>267.2 (277.7)</td>
<td>251.1 (261.6)</td>
</tr>
<tr>
<td></td>
<td>[Zn(C(_2)H(_4)ON(^+))+ CO_2 + NH_3</td>
<td>250.4 (255.6)/294.0 (296.9)</td>
<td>331.2 (306.4)</td>
<td>307.4 (282.7)</td>
</tr>
<tr>
<td></td>
<td>[N, CO^−, CO_2(^−)]^+</td>
<td>0.0 (0.0)/0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>amino loss</td>
<td>[Zn(C(_2)H(_4)ON(^+))+ CO_2</td>
<td>33.2 (35.6)/57.6 (60.0)</td>
<td>68.8 (71.2)</td>
<td>86.5 (88.9)</td>
</tr>
<tr>
<td></td>
<td>TS(_{2A})</td>
<td>273.3 (271.5)/290.2 (288.5)</td>
<td>287.4 (285.7)</td>
<td>292.2 (290.5)</td>
</tr>
<tr>
<td></td>
<td>INT(_{1A})</td>
<td>230.2 (231.2)/254.1 (252.2)</td>
<td>254.8 (255.9)</td>
<td>248.9 (250.0)</td>
</tr>
<tr>
<td></td>
<td>TS(_{2A})</td>
<td>348.1 (352.0)/368.2 (372.1)</td>
<td>387.1 (391.1)</td>
<td>416.5 (420.4)</td>
</tr>
<tr>
<td></td>
<td>INT(_{2A})</td>
<td>3.5 (15.6)/39.7 (51.8)</td>
<td>65.1 (77.2)</td>
<td>46.5 (58.6)</td>
</tr>
<tr>
<td></td>
<td>[Zn(C(_2)H(_4)ON(^+))+ CO_2 + NH_3</td>
<td>145.0 (123.4)/193.1 (171.5)</td>
<td>216.1 (194.5)</td>
<td>203.3 (181.8)</td>
</tr>
</tbody>
</table>

\(^{a}\)Relative single point energies and free energies in parentheses calculated at the level of theory indicated using a 6-311+G(2d,2p) basis set.

\(^{b}\)Empirical dispersion corrected B3LYP-GD3BJ values are given in bold.

Deamidation Pathways for CdCl\(^+\)(Asn). For the CdCl\(^+\)(Asn) complex, the most plausible deamidation pathway would likely involve hydrogen transfer from the carboxylic acid group to the amide NH\(_2\), similar to the mechanism detailed for deamination of protonated and sodiated Asn.\(^{43,44}\) These studies concluded that deamination resulted in the formation of 3-amino succinic anhydride (aSA), paralleling the mechanism proposed in biological systems where the deamination process is believed to proceed through a succinimide intermediate. Deviations from this pathway arise when the additional CO loss is considered, an important aspect of the dissociation pathways exhibited in the current study, where only concomitant loss of NH\(_3\) + CO is observed. For H\(^+\)(Asn), secondary CO loss was observed at considerably higher energy than deamination,\(^{37}\) and

elongating the C–N\(_{\alpha}\) amide bond by ~0.2 Å. Further elongation of this bond results in rupture via TS\(_{2D}\). The complex formed, INT\(_{2D}\), is stabilized by the ammonia remaining loosely bound to the system via hydrogen bonding. Simple dissociation of this complex can lead to [Zn-(C\(_2\)H\(_4\)ON\(^+)\)+ + NH\(_3\), which requires 250–331 kJ/mol relative to [Zn(Asn−H)]. An interesting observation made here is that the proton transfer of TS\(_{2D}\) results in the stabilization of INT\(_{1D}\) in such a way (NH\(_3\)^+ formation) that the charge is now located on the ligand, rather than residing on zinc. Thus, along the PES, Zn behaves as a neutral rather than its dication counterpart, as is evident from the longer M–L interactions and (although this is difficult to ascertain from the orientation of the structures shown) the fact that Zn lies out of the plane of the ligand. Zn–L bond lengths are given in Figure 7, where it can be seen that interactions involving neutral zinc are 0.3–1.2 Å longer than those with the dication counterpart. The relatively high
for Na+(Asn), no CO loss was observed via TCID, even though energies up to 480 kJ/mol (5.0 eV) were monitored. Here, we explore the pathways for concomitant loss as well as the possibility of primary ammonia loss (Figure 9), followed by the sequential loss of CO (see the Supporting Information). The possibility that CH$_3$NO is lost as formamide was also explored, although no reasonable pathway could be found. Instead, any pathway that could conceivably lead to loss of formamide led to preferential formation of H$_2$N–CO–NH$_2$.

Figure 9. Reaction coordinate for aSA-[N,CO] formation from CdCl⁺(Asn) calculated at the MP2(full)/def2-TZVPP level of theory from B3LYP/def2-TZVP geometry optimizations. Structures for intermediates lie below the reaction coordinate, and those for TSs lie above. Dashed lines (gray) indicate hydrogen bonds (up to 2.5 Å). Dotted lines (black) indicate bonds being broken or formed for transition states.

Figure 10. Reaction coordinates for the concomitant NH$_3$ and CO loss from CdCl⁺(Asn) calculated at the MP2(full)/def2-TZVPP//B3LYP/def2-TZVP level of theory. Structures for intermediates lie below the reaction coordinate, and those for TSs lie above. Dashed lines (gray) indicate hydrogen bonds (up to 2.5 Å). Dotted lines (black) indicate bonds being broken or formed for transition states.
of CdCl\官兵(NH₃ + CO) is the concomitant loss of (NH₃ + CO), as shown in Figure 10.

The PES showing the loss of ammonia to form CdCl\官兵(aSA) is given in Figure 9, with energies of all reaction species given in Table 2. The first elementary step involves rotation of the \( \angle \text{CCO} \) dihedral angle such that the hydrogen bond of the carboxylic acid is broken, thus allowing a more facile proton transfer to Nₛ to form the NH₃ leaving group. To induce this proton transfer, a series of \( \angle \text{CCCN} \) dihedral angle rotations result in the decreased distance between Nₛ and the hydrogen of the carboxylic acid in INT₃N. Proton transfer occurs as the complex passes through TS₃N characterized by concerted motions of NH₃ formation, C–NH₃ bond rupture, and ring closure. (Here, IRC calculations confirm the identity of TS₃N to ensure that there was not another TS leading to the formation of the NH₃ leaving group.) This TS leads to formation of CdCl\官兵(aSA)[N, CO\官兵(NH₃NH₃)] (168–235 kJ/mol higher in energy than the ground conformer), in which the subscript indicates the ammonia is hydrogen bound to the NH group. This sequence of steps parallels those found previously for deamidation of Asn cationized by Na⁺ and H⁺.⁶

Thus, we observe concomitant (NH₃ + CO) loss such that deamidation alone is not observed. Thus, dissociation of CdCl\官兵(Asn) would appear to follow a pathway in which deamidation occurs at sufficient temperature (and high-energy single collisions of TCID are more likely to produce entropically favored products, whereas the multiple low-energy collisional processes used to induce deamidation in the spectroscopic study should yield enthalpically favored products. Thus, the different experimental conditions may allow for alternate pathways for deamidation than the pathways paralleling the TCID deamidations of H⁺(Asn) and Na⁺(Asn).

Our exploration of the PES for (NH₃ + CO) loss is shown in Figure 10, with relative energies given in Table 3. Here, the first three elementary steps are equivalent to the pathway given in Table 2. Relative single point energies and free energies in parentheses calculated at the level of theory indicated using a def2-TZVPP basis set and SDD ECP for Cd. Empirical dispersion corrected B3LYP-GD3BJ values are given in bold.

### Table 2. Relative Energies (0 K) and Free Energies (298 K) of Major Reaction Species Forming the aSA Intermediate from CdCl\官兵(Asn)⁶

<table>
<thead>
<tr>
<th>structure</th>
<th>B3LYP/GD3BJ°</th>
<th>B3P86</th>
<th>MP2(full)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[N, CO, CO\官兵]·tggt</td>
<td>0.0 (0.0)/0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>TS₅N</td>
<td>62.6 (59.9)/62.0 (59.3)</td>
<td>63.6 (60.9)</td>
<td>65.1 (62.4)</td>
</tr>
<tr>
<td>INT₅N</td>
<td>63.5 (61.3)/66.1 (63.9)</td>
<td>63.7 (61.6)</td>
<td>70.9 (68.7)</td>
</tr>
<tr>
<td>TS₆N</td>
<td>63.8 (59.7)/65.8 (61.6)</td>
<td>64.6 (60.4)</td>
<td>70.2 (66.1)</td>
</tr>
<tr>
<td>INT₆N</td>
<td>32.5 (29.1)/38.6 (35.2)</td>
<td>29.7 (26.3)</td>
<td>48.4 (45.0)</td>
</tr>
<tr>
<td>TS₇N</td>
<td>121.8 (117.2)/126.8 (122.1)</td>
<td>120.5 (115.8)</td>
<td>131.5 (126.9)</td>
</tr>
<tr>
<td>INT₇N</td>
<td>100.2 (97.3)/103.0 (100.0)</td>
<td>100.5 (97.6)</td>
<td>108.4 (105.5)</td>
</tr>
<tr>
<td>TS₈N</td>
<td>159.2 (157.1)/163.8 (161.6)</td>
<td>161.6 (159.5)</td>
<td>164.4 (162.4)</td>
</tr>
<tr>
<td>INT₈N</td>
<td>93.9 (94.4)/105.7 (106.2)</td>
<td>98.2 (98.6)</td>
<td>105.6 (106.1)</td>
</tr>
<tr>
<td>aSA[N, CO] + NH₃</td>
<td>167.7 (159.0)/188.4 (153.6)</td>
<td>177.4 (142.6)</td>
<td>235.1 (200.3)</td>
</tr>
</tbody>
</table>

Relative single point energies and free energies in parentheses calculated at the level of theory indicated using a def2-TZVPP basis set and SDD ECP for Cd. Empirical dispersion corrected B3LYP-GD3BJ values are given in bold.

### Table 3. Relative Energies (0 K) and Free Energies (298 K) of Major Reaction Species Forming m/z 236 via (NH₃ + CO) Loss from CdCl\官兵(Asn)⁶

<table>
<thead>
<tr>
<th>structure</th>
<th>B3LYP/GD3BJ°</th>
<th>B3P86</th>
<th>MP2(full)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[N, CO, CO\官兵]·tggt</td>
<td>0.0 (0.0)/0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>TS₁N</td>
<td>62.6 (59.9)/62.0 (59.3)</td>
<td>63.6 (60.9)</td>
<td>65.1 (62.4)</td>
</tr>
<tr>
<td>INT₁N</td>
<td>63.5 (61.3)/66.1 (63.9)</td>
<td>63.7 (61.6)</td>
<td>70.9 (68.7)</td>
</tr>
<tr>
<td>TS₂N</td>
<td>63.8 (59.7)/65.8 (61.6)</td>
<td>64.6 (60.4)</td>
<td>70.2 (66.1)</td>
</tr>
<tr>
<td>INT₂N</td>
<td>32.5 (29.1)/38.6 (35.2)</td>
<td>29.7 (26.3)</td>
<td>48.4 (45.0)</td>
</tr>
<tr>
<td>TS₃N</td>
<td>121.8 (117.2)/126.8 (122.1)</td>
<td>120.5 (115.8)</td>
<td>131.5 (126.9)</td>
</tr>
<tr>
<td>INT₃N</td>
<td>100.2 (97.3)/103.0 (100.0)</td>
<td>100.5 (97.6)</td>
<td>108.4 (105.5)</td>
</tr>
<tr>
<td>TS₄N</td>
<td>159.2 (157.1)/163.8 (161.6)</td>
<td>161.6 (159.5)</td>
<td>164.4 (162.4)</td>
</tr>
<tr>
<td>INT₄N</td>
<td>93.9 (94.4)/105.7 (106.2)</td>
<td>98.2 (98.6)</td>
<td>105.6 (106.1)</td>
</tr>
<tr>
<td>aSA[N, CO] + NH₃</td>
<td>167.7 (159.0)/188.4 (153.6)</td>
<td>177.4 (142.6)</td>
<td>235.1 (200.3)</td>
</tr>
</tbody>
</table>

Relative single point energies and free energies in parentheses calculated at the level of theory indicated using a def2-TZVPP basis set and SDD ECP for Cd. Empirical dispersion corrected B3LYP-GD3BJ values are given in bold.
Figure 9 for CdCl⁺(aSA)[N, CO] formation. From INT₆₅₈ rotation of the ≤COOC dihedral angle from trans to cis (passing through gauche TS₆₅₈) results in the formation of INT₆₅₆, where heavy metal binding occurs at the oxygen sites of the carboxylic acid, [COOH]. From here, proton transfer to the amide NH₃ group can occur facilely, yielding the zwiterionic INT₆₅₆, [CO₂⁻, O], in which the NH₃ leaving group is formed. Elongation of the C−NH₃ bond over TS₆₅₈ results in the concerted motions of bond rupture and aSA formation, where stabilization of these motions results in INT₆₅₆, CdCl⁺(aSA)[CO, O(NH₃H₄H₄)], where the ammonia is hydrogen bonded to the amine group. Next, CdCl⁺ migrates to break the [CO, O] interaction of the carboxylic acid oxygen and reform the analogous interaction with the carbonyl oxygen of the side chain. Although INT₆₆₄ is 5−6 kJ/mol lower in energy than INT₆₅₈, TS₆₅₈ is sufficiently high in energy (198−220 kJ/mol) that this is the rate-limiting TS at the B3LYP level of theory. We also explored the possibility of forming INT₆₅₈ by CdCl⁺ migration to the side-chain carbonyl prior to aSA formation, but no such pathway could be isolated. From INT₆₅₈ decarbonylation can occur from the backbone carbonyl adjacent to the nitrogen site by elongation of the NC−CO bond leading to TS₆₅₈. This motion yields INT₆₅₈, where the NH₃ and CO ligands remain hydrogen bound to the complex at the terminal NH₃ site. Again, this complex is zwiterionic with positive charge on the nitrogen and delocalized negative charge on the carboxylate group, a species that parallels that found in the H⁺(Asn) study. (Notably, decarbonylation from INT₆₅₈ is much higher in energy than the pathway shown because the final product formed is much less stable.) From this intermediate, CO can be lost most easily, as it requires only 6−77 kJ/mol (100−209 kJ/mol above the reactants) and NH₃ loss requires about 30 kJ/mol more (140−233 kJ/mol above reactants). Loss of both ligands yields CdCl⁺(C₃H₅O₂N) + NH₃ + CO, 186−347 kJ/mol relative to the ground reactant conformer. Importantly, the energy of TS₆₅₈ (173−213 kJ/mol) is the lowest energy TS for decarbonylation that we have found, suggesting that the complex is most likely to lose CO via this pathway. Alternate pathways (where metal binding is taking place at [N, CO] or [CO, O] of the backbone) were located, although each of these TSs were significantly higher in energy. Select pathways exploring these TSs are given in the Supporting Information.

From an energetic point of view, primary deamidation via the loose, phase-space limit TS of Figure 9 (168−188 kJ/mol) should occur at lower energies relative to the concomitant pathway via TS₆₅₈ detailed in Figure 10 (198−216 kJ/mol) according to the DFT level of theory. However, MP2(full) theory indicates that TS₆₅₈ (220 kJ/mol) is lower than the deamidation products of Figure 9 (235 kJ/mol), such that these pathways could be competitive. Once over TS₆₅₈ the system should preferentially lose CO first, and require additional energy to lose NH₃ as well. Significantly, although the pathways shown in Figures 9 and 10 may be competitive, neither pathway clearly explains why the concomitant loss of (NH₃ + CO) is observed rather than observing loss of just NH₃ (Figure 9) or CO (Figure 10).

Select higher energy conformers of CdCl⁺(C₃H₅O₂N) are given in the Supporting Information (Figure S2). Here, several additional pathways were found for the loss of (NH₃ + CO) from CdCl⁺(Asn), but all lie at higher energy than that shown in Figure 10, as detailed in the Supporting Information (Figures S3−S6).

From a structural perspective, comparison of the geometric analysis of both Zn²⁺/Cd²⁺ Asn and Zn²⁺/Cd²⁺ Gln systems is particularly helpful in analyzing the deamidation patterns exhibited by each of the complexes. Here, the most interesting observation is that Zn + CO₂ dissociation from [Zn(Asn−H)⁺], loss of CO₂ occurs before ammonia loss but the Zn²⁺ metal center is still bound. On the basis of M−N, M−O, and M−CO distances, very similar lengths were found for the deprotonated Asn (2.06, 1.89, and 1.96 Å, respectively) and Gln (2.06, 1.88, and 1.93 Å, respectively) complexes with Zn²⁺. The most noticeable difference in the geometric parameters is the significantly smaller OOMO angle in the current Asn study (110.7° for Asn, compared with 125.6° for Gln). This effect is attributed to the extra carbon in the backbone of Gln, which increases this angle. Although the additional carbon allows for increased flexibility along the backbone, the more constrained OOMO angle in the Asn complex appears to help maintain a compact conformation that is more suitable for cyclization (where the ability to form the cyclic succinimide structure is particularly important for the deamidation process). A similar trend in smaller OXYM and OOMY angles is observed in the Cd²⁺ system as well, although the differences are not as pronounced. Such small differences are consistent with the dissociation patterns observed, where Cd²⁺ remains more loosely bound (compared with Zn²⁺) to the system in both Asn and Gln studies. Here, the distinct difference in deamidation pathways for both systems arises from the fact that deamidation was observed as the only primary loss of the Cd²⁺ Gln system, whereas the simultaneous loss of NH₃ and CO was detected in the current study as the only dissociation product of CdCl⁺(Asn).

CONCLUSIONS

IRMPD action spectra for complexes of asparagine cationized with Zn²⁺ and Cd²⁺ were measured in the region of 1000−1850 cm⁻¹ and were compared to calculated IR spectra at the B3LYP/6-31+G(d,p) and B3LYP/def2-TZVP levels of theory, respectively. For both [Zn(Asn−H)⁺] and CdCl⁺(Asn), the theoretically determined ground conformer is found to reproduce the experimental spectra well. The [N, CO⁺, CO₂]−ggt conformer of the [Zn(Asn−H)⁺] complex is confirmed to be the complex present experimentally, with no major contributions from higher lying conformers. For the CdCl⁺(Asn) complex, the experimental IRMPD spectrum was predicted best by the [N, CO, CO₂]−ggtt conformer.

The analysis regarding the changes in vibrational modes and geometric parameters also provides valuable information about the binding strength of the metal center within the complex, a characterization instrumental in determining the metal dependence of these and other biologically relevant systems. The shorter M−O and M−X distances of the [Zn(Asn−H)⁺] complex should characteristically lead to stronger metal−amino acid binding compared to binding observed in CdCl⁺(Asn). This weaker binding along with the less constrained dihedral angles in the CdCl⁺(Asn) complex appears to facilitate the formation of a cyclic succinic anhydride structure that is necessary for deamidation to occur. We have previously correlated these findings (regarding the ability for movement within the complex as well as metal chelation throughout the dissociation process) to the observation of...
differing pathways for deamidation, as the Zn\(^{2+}\) center dissociates prior to deamidation in the Zn\(^{2+}\) Gln system. In the current study, however, deamidation was observed with the Zn\(^{2+}\) center intact (although long M–L interactions were observed and, here, Zn acts as a neutral rather than a dication after CO\(_2\) loss), although no cyclization was determined to occur.

The characteristic vibrational modes in the \([\text{Zn(Asn–H)}]^+\) and CdCl\(^+\)(Asn) spectra also provide valuable information with respect to the metal dependence of the system (as given by the band shifts observed in our spectral results), although some contributions to those shifts arise from the inherent difference in binding between Zn\(^{2+}\) and anionic (Asn–H) versus CdCl\(^+\) and neutral Asn. Overall, the results presented here provide valuable fundamental information for the continuation of our metal-amino acid studies, specifically regarding the metal dependence of biologically important systems. Specifically, the different deamidation patterns observed between the Asn and Gln systems are an important aspect to evaluate in other larger, more biologically relevant systems.

**REFERENCES**


