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Dimeric Complexes of Tryptophan with M^{2+} Metal Ions

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IRMPD spectroscopy using the FELIX free electron laser and a Fourier transform ICR mass spectrometer was used to characterize the structures of electrosprayed dimer complexes $M^{2+}Trp_2$ of tryptophan with a series of eight doubly charged metal ions, including alkaline earths Ca, Sr, and Ba, and transition metals Zn, Cd, Mn, Co, and Ni. With the support of DFT thermochemical calculations, at least three different structural motifs were distinguished spectroscopically, depending critically on the nature of the metal ion. The spectral signatures of a ligand in the charge-solvated (CS) configuration, namely peaks near 1730 and 1150 cm^{-1}, were prominent in all the spectra, and it was clear that all the dimer complexes contain at least one CS ligand. The spectra indicated that the second ligand is zwitterionic (ZW) for all complexes except the Ni case, with the second ligand having an extended binding geometry with smaller metals but showing some admixture of a compact chelated geometry with larger alkaline earths. It was concluded that these dimer complexes have a mixed configuration of ligands, denoted CS/ZW. The $Ni^{2+}Trp_2$ complex is exceptional, with the spectroscopy and the thermochemical calculation both indicating a CS/CS configuration of ligands. This geometry appears to correlate with the exceptionally small size and high binding strength of the Ni^{2+} cation. The complex $CdClTrp^{1+}$ was also obtained and gave a clear spectrum showing a CS ligand configuration. The presence of a CS ligand in all the dimeric complexes of the 2+ metals is an interesting contrast with the monomer complex $Ba^{2+}Trp$, in which the ligand is ZW.

Introduction

Tryptophan's packing around a metal cation is a situation of interesting complexity.[1] With three Lewis-basic heteroatoms along with the cation-friendly aromatic system,[2] the choices of binding pattern and conformation give rise to numerous possibilities. Added to this flexibility is the possibility for the amino acid to bind in either the canonical ("charge-solvated", or CS) form, or the zwitterionic (ZW) form which gives rise to a salt bridge (SB) form when complexed to a metal cation.

Interaction of a metal cation with the negative end of the large indole dipole seems to play an important role in the function of some ion channels (of which the most heavily studied model is the gramicidin-A channel).[3] For instance, the organization of the Trp residues and their indole side chains and the interaction of the indoles with the cation at the entrance to the ion channel of gramicidin S has been of recent interest.[4,5] As another example, gating of the Ca^{2+} channel in *N*-methyl-D-aspartate receptors is known to involve participation of a Trp residue.[6]

Except for basic amino acids (proline, arginine, lysine) the binding of an amino acid to a singly charged metal ion in the gas phase favors the ligand in the CS configuration.[1,7−25] The recent observation of electrosprayed Trp/barium complexes in the +2 charge state,[26] using infrared spectroscopy to characterize the structure, was the first experimental demonstration that the higher metal-ion charge can bring about a switch to the ZW configuration. This work was soon followed by the demonstration of similar ZW ligand character in a number of other amino acid/Ba^{2+} complexes.[27]

The steric crowding around a cation has possible effects on the preferred conformation when the cation interacts with more than one ligand. Given that a single Trp on Ba^{2+} is zwitterionic, it is interesting to ask about the conformation adopted by the second ligand in forming the $M^{2+}Trp_2$ "dimer" complex. It has proven possible, not only with Ba^{2+} but also with a number of other doubly charged metal ions, to electrospray such dimer complexes in the mass spectrometer coupled to the FELIX free electron laser, opening the opportunity to make structure determinations and comparisons with the previous work with monomeric complexes. Moreover, since it has not yet been possible to obtain spectroscopic information about monomeric M^{2+} amino acid complexes with metals other than barium, the study of the dimer complexes gives the first insights via this approach into binding patterns of Trp with a variety of doubly charged metal ions.

There has been study of the structures of proton-bound dimers of several amino acids. The Fridgen group, as well as the McMahon group, have studied the IR spectroscopy of proton-bound dimers of aliphatic amino acids, notably glycine, using similar techniques and a similar wavelength range to those used here.[28,29] They assigned the nonprotonated amino acid ligand to have a nonzwitterionic structure with the carbonyl bound to the bridging proton, but it was also suggested that for the proton-bridged dimer systems the IR spectrum in the 800−2000 cm^{-1} region was not a very incisive tool for isomeric structure assignments. A study by the McLafferty group of a series of proton-bound dimers in the hydrogen-stretching region (using OPO laser excitation, 3000−3800 cm^{-1})[30] also looked at the glycine proton-bound dimer, but its spectrum was interpreted

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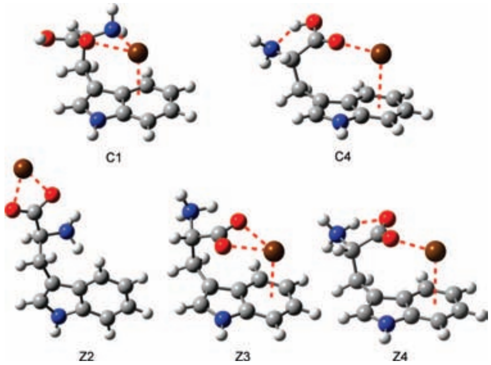
SCHEME 1



in terms of a different structure having the proton bridging between the two nitrogens, with a nonzwitterionic glycine. Computationally and spectroscopically it appears to be difficult to make definitive structure assignments of the proton-bound systems. Dimer ions of amino acids bound by other metal ions rather than protons do not seem to have been the subjects of previous structure determination efforts.

The present work is the first characterization of the structures of doubly charged two-ligand complexes of an aromatic amino acid with a metal ion. A previous study with some relevance[15] looked at the dimer complex of Phe with Zn(II) in which one of the amino acids was deprotonated, giving the singly charged complex Zn(Phe)(Phe-H)$^+$. Based on the infrared spectrum, and supported by computational confirmation of the most stable structure, this work concluded that the Phe ligand was in the CS configuration, and that the two ligands wrap around the zinc in a compact chelating conformation involving metal ion interaction with basic nitrogen, oxygen, and ring π binding sites on both ligands.

Several binding motifs were found to be energetically favorable for singly charged monomer complexes,[1] and our thinking about the possible configurations of the dimer complexes was based on combinations of these motifs for two ligands around the metal ion. The monomer motifs considered most reasonable were the five shown in Scheme 1, which are labeled in agreement with the labeling scheme in the previous publication.[1] The first two (C1 and C4) are CS forms, while the last three (Z2, Z3, and Z4) are ZW forms. Various combinations of these monomer binding motifs, with various possible orientations of the two ligands, were tested with lower-level B3LYP calculations (typically 3-21 g) to develop the set of promising candidate structures for high-level optimization for each metal ion.

For the singly charged cations previously surveyed[1] the most stable monomer configurations were CS, with the 3-fold chelated form C1 being best for smaller, more tightly binding metal ions, and the 2-fold form C4 becoming competitive in energy upon going to larger, more weakly bound metal ions. For the monomer complex of the doubly charged Ba^{2+} cation, the compact zwitterion Z3 was the most stable configuration.[26]

Calculations reported in ref 26 for the doubly charged monomer complexes indicated that the CS configuration C1 was more stable than the best ZW configuration (Z3) for all the metals considered, with the exception of the larger alkaline earths Ca^{2+}, Sr^{2+}, and Ba^{2+}. Further calculations have indicated that the "extended" zwitterion conformation Z2 is disfavored compared with compact chelated conformations by substantial margins of 50 kJ/mol or more. Interestingly, the 2-fold chelated configuration C4 that is relatively favorable for singly charged complexes was found, in the case of the doubly charged complexes that we examined, to rearrange exothermically by proton transfer (spontaneously or with low barrier) to the related zwitterion Z4. This suggests that the transferred proton in this situation might actually be considered as a shared proton, with a highly anharmonic potential energy well. However, both the C4 and Z4 monomer chelation motifs were found to be substantially less stable than other configurations in all the doubly charged cases, and the Z4 binding motif was also found to be an unfavorable ligand conformation in the doubly charged dimer complexes. Thus, the C4 and Z4 configuration, with the possible shared proton, appear to have no direct relevance in the present consideration of the spectroscopy of these complexes.

Methods

Calculations. The calculations were done with DFT/B3LYP using the Gaussian 03 package.[31] A basis of 6-31+g(d,p) was used for all atoms, except that the sdd basis with a relativistic effective core potential was used for the metal atom in the complexes of Sr, Ba, and Cd. These basis set choices have been found satisfactory for calculating vibrational frequencies and intensities in other chelated metal-ion complexes.

The assumption that the closed-shell metal complexes (alkaline earths, Zn, and Cd) are most stable in singlet spin states is not in doubt. For Ni^{2+}, either triplet or singlet spin states are possible, but it is not likely that there are large enough differences in ligand effects to reverse the inherent greater stability of the triplet (168 kJ/mol more stable than the singlet for the bare ion). One check of this was made for the C1/C1 isomer of NiTrp$_2^{+2}$, for which the triplet state was calculated to be more stable than the singlet state by 41 kJ/mol (both states structure-optimized). For other isomers of this complex, only triplets were considered. The computed vibrational frequencies were scaled by 0.975, which is in accord with the scale factors found appropriate in other studies of comparable systems.

IR Spectroscopy. Solutions of 1 mM tryptophan with the nitrate or chloride salt of Ca, Sr, Ba, Mn, Co, Ni, Zn, and Cd were made up in 80% MeOH and 20% H$_2$O. The metal cation−tryptophan complexes were generated by electrospray ionization (ESI) and accumulated in a hexapole, prior to ion transfer to the Penning trap of a laboratory-built Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer.[32] Ion capturing was effected using electrostatic pulsing of the ion transfer octopole, as described in a recent publication.[15] The ion of interest was mass isolated and then irradiated with 10 macropulses from the FELIX laser to induce IR photodissociation. Given the low density of gas-phase ions, direct absorption spectroscopy is not feasible, and thus the ion spectroscopic information was derived through the common 'action' spectroscopy approach.[33,34] In IRMPD, resonant absorption of light causes photodissociation, the yield of which is recorded as a function of wavelength to obtain the IRMPD spectrum. FELIX is capable of delivering high-energy macropulses (typically 60 mJ) over a very wide wavelength region (3−250 μm), thus enabling many structurally relevant vibrational regions to be explored. While the IRMPD spectrum is not identical to a linear absorption spectrum, the assumption that the IRMPD yield is linearly proportional to the IR absorption intensity has been considered to be a useful approximation,[35] making it possible to view the IRMPD spectrum as a reflection of the true IR absorption spectrum. Nevertheless, there are some caveats when interpreting IRMPD spectra by comparison with calculated linear absorption spectra, such as a general red-shifting and broadening of bands, as well as changes in the intensities of bands due to the presence of nearby bands.[35]

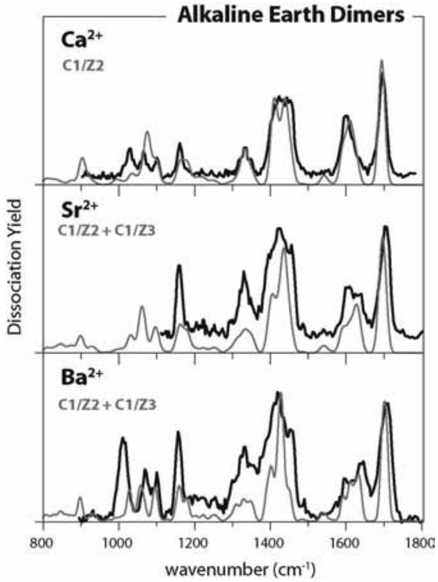


Figure 1. Spectra of the three alkaline earth complexes (black), with computed spectra (gray) for the indicated conformer or mixture of conformers.

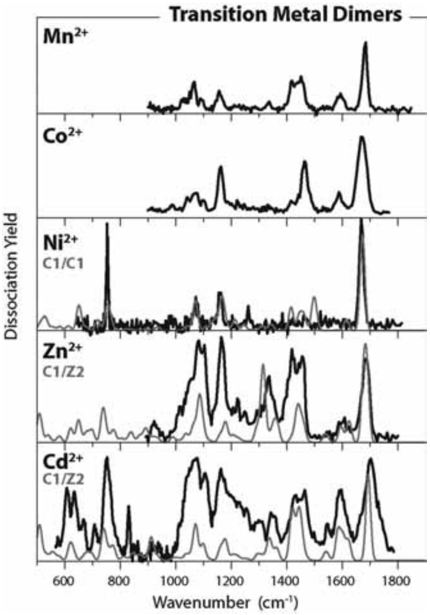


Figure 2. IRMPD spectra of the transition metal $M^{2+}Trp_2$ complexes (black), along with calculated spectra of the most stable isomers that were located in the calculations (gray). Note that the calculated spectrum for the Ni complex is the CS/CS configuration, while the others are CS/ZW configurations.

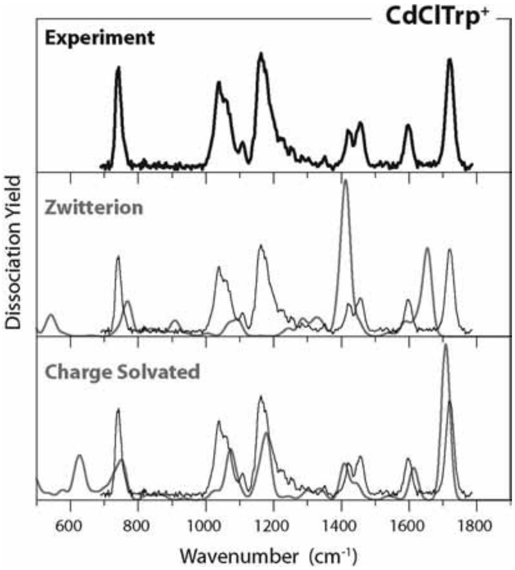


Figure 3. IRMPD spectrum of $CdClTrp^{1+}$, along with calculated spectra for the ZW (Z3 conformation) and CS (C1 conformation) structures of the complex.

Such effects can be ascribed at least in part to anharmonicities of the vibrational modes due to the large number of photons (i.e., typically tens to hundreds) that are absorbed by each ion in the IRMPD process.

Photodissociation of the dimer ions usually yielded two observed product channels. One was the loss of mass 17 (presumably NH_3) to give $(P\text{-}17)^{2+}$ (where P is the parent mass). The other was the charge-splitting dissociation giving $(MTrp - H)^+$ and $(Trp + H)^+$ (where M is the metal mass). For $CdClTrp^{1+}$, products were seen for loss of mass 17 (presumably NH_3) and mass 45 (presumably COOH), and appearance of a peak at *m*/*z* 132 (largest isotope component) thought to be $Cd(H_2O)^+$. The photodissociation spectra are plotted as the sum of all product ions, which represents the disappearance spectrum of the parent ion, and is expected to reflect the photon absorption spectrum of the parent ion without any complications arising from secondary dissociations of fragment ions.

Results and Discussion

Figure 1 and Figure 2 show the FELIX spectra of the eight complexes, along with calculated linear IR absorption spectra predicted for the lowest-energy configurations of the ligands. For Ca, Zn, and Cd, the calculated spectra are for the C1/Z2 isomer; those for Sr and Ba are an equal mixture of C1/Z2 and C1/Z3 while for Ni, the calculated spectrum is C1/C1. Spectra were not calculated for Mn and Co. Finally, the spectrum of $CdTrpCl^{+1}$ is shown in Figure 3, along with calculated spectra for both the C1 and the Z3 configurations of the Trp ligand.

The results of the thermochemical calculations are shown in Table 1. Zero-Kelvin binding energies are shown for all the species calculated, and (in parentheses) binding free energies at 298 K that correct for thermal effects and for vibrational and rotational entropies. The binding energy values represent the energy for removal of all Trp ligands from the complex.

Table 2 shows some bond length results from the calculations. Only results for a CS ligand are shown, but the M−O, M−N, and M−ring distances for a ZW ligand were all quite similar to those for the CS ligand.

Structures and Spectroscopic Characteristics. As typical examples, Figure 4 displays calculated spectra for a number of the most stable isomers of the barium complex. In the Supporting Information are shown some additional isomer spectra for the Ba case, as well as the spectra that were calculated for complexes of all the other metals in this work. The 0 K energy of each isomer relative to the most stable form is noted on the plots.

The most stable binding motifs for the dimers that we located (see Table 1) were C1/Z2, C1/C1, and C1/Z3. These are illustrated (for the Ca case) in Scheme 2. These three geometries did not change very much in going across the series of metals, aside from the variation in metal−ligand bond lengths for metals of different sizes (Table 2).

There are obviously many possible conformational combinations of the two ligands in the dimer complex, and careful

TABLE 1: Calculated Energies for Dissociation of Both Ligands (Dimers) or One Ligand (Monomers) Off of the 2+ Metal Ions[a]

	Ca	Sr	Ba	Zn	Cd	CdCl	Ni
				Dimers			
C1/C1	949 (812)	871 (743)	756 (629)	1506 (1365)	1290 (1152)		1576 (1427)[b]
C1/Z2	953 (829)	878 (758)	765 (648)	1518 (1379)	1302 (1175)		1572 (1428)[c]
C1/Z3	952 (819)	878 (742)	763 (636)	1507 (1359)	1276 (1144)		1517
Z3/Z3	928 (792)		742 (619)				
Z3/Z2			757 (630)				
Z2/Z2			748 (630)				
Z4/Z4	920 (797)			1475 (1334)			
				Monomers			
C1	678	581	508	1154	955	410	1198
Z3	688	590	517	1112	932	369	1148
C5	668	564	493			369	
Z2	602		460			378	
Z5			501				

[a] Energies shown are the 0 K dissociation energies, and the 298 K dissociation free energies (values in parentheses). More positive values indicate higher stability of the complex. All values in kJ/mol. [b] MPW1PW91 value for C1/C1 is 1571 (1423) kJ/mol. [c] MPW1PW91 value for C1/Z2 is 1561 (1418) kJ/mol.

TABLE 2: Metal−Ligand Bond Lengths (Å)[a]

	Ca	Sr	Ba	Zn	Cd	CdCl	Ni
				Dimers			
M−O	2.41	2.53	2.71	2.11	2.37	2.44	2.09
M−N	2.61	2.74	2.91	2.11	2.40	2.34	2.09
M−ring	2.86	2.92	3.10	2.26	2.45	2.70	2.01
				Monomers			
M−O	2.29		2.62	2.01	2.25		2.01
M−N	2.53		2.91	2.08	2.31		2.03
M−ring	2.47		2.93	2.16	2.25		2.01

[a] For the monomers, the conformation is C1. For the dimers, the conformation is C1/Z2, and the bond lengths refer to the C1 ligand.

consideration of the evidence is needed to distinguish them. It is useful to make initial assignments of the ion structures based on clear-cut signature peaks expected for the different likely ligand configurations. Then further confirmation and refinement can be made via individual calculations of the most likely configurations of the specific $M^{2+}(Trp)_2$ complex in question.

The spectroscopic signatures of the CS and the ZW configurations of Trp ligands can be assigned based on surveying the calculated spectra of both monomer and dimer complexes to pick out normal modes and IR features that are consistently identifiable with one or the other form. However, there is additional insight to be gained by drawing on experience from a number of 1+ complexes, both with Trp and also with other amino acids,[15−17,22,26−28,36,37] and additionally by comparing the spectra of an authentic ZW monomer (Ba) with that of an authentic CS monomer. We do not yet have an authentic monomer 2+ metal ion with a single CS ligand, but the $CdClTrp^{1+}$ complex gives us a single-Trp complex on a metal ion having an electrostatic environment approximating a true 2+ complex, and is quite assuredly in the CS configuration. Figure 4 provides good illustrations of the characteristic peaks for different structures, and the spectra displayed in the Supporting Information give many further illustrations.

The outstanding signature spectral features of CS Trp ligands are considered to be[1,15−17,27,28] the carboxyl C═O stretch at 1700−1750 cm^{-1} and the COH bend at 1150−1170 cm^{-1}. These are both strong consistent peaks, and both appear in regions having no expected ZW features for likely ZW conformations.

The ZW situation is more difficult, because all the expected ZW signatures coincide more or less with weak or moderate features also expected for possible CS configurations. Thus, the identification of a ZW ligand has to be based on the signature peaks being stronger than would be expected for CS, and assignments of ZW ligands depend heavily on the calculated spectra for the specific complex in question. The useful signature peaks are taken to be the NH_3^+ umbrella mode at 1400−1450 cm^{-1}, and the C_α−H bend at 1300−1350 cm^{-1}. The mixed mode (NH_3^+ bend plus COO^- antisymmetric stretch) near 1600 cm^{-1} can be very characteristic in particular cases, but in general it suffers from unpredictable confusion with the CS mode (NH_2 scissors) also near 1600 cm^{-1}.

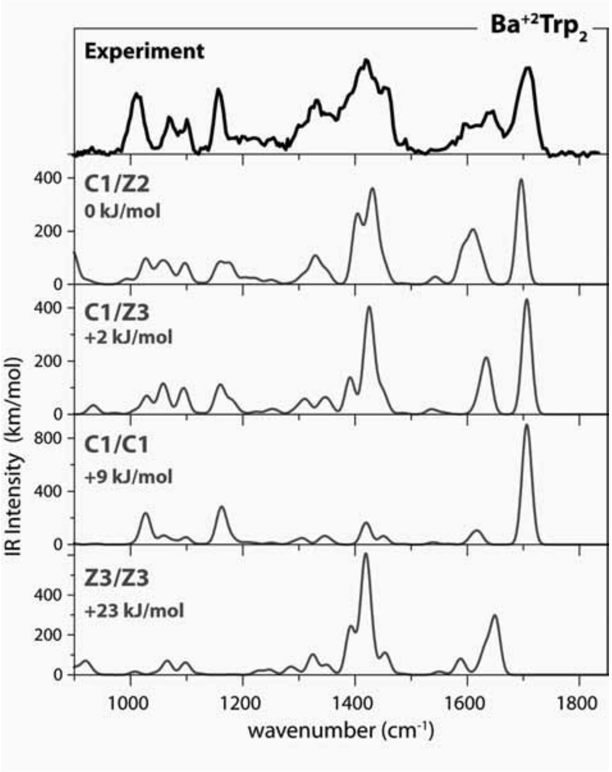
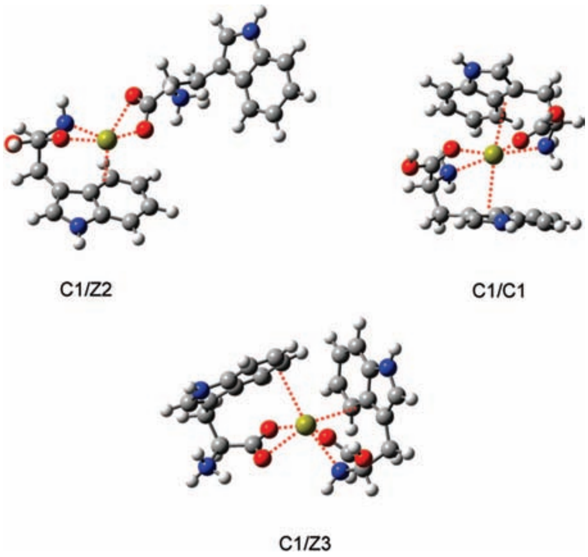


Figure 4. Computed spectra of some of the most thermochemically favored isomers of the Ba dimer complex. (See also Figures S3 and S4, Supporting Information). The designations of the two ligand configurations correspond to the labels on the structural motifs displayed in Scheme 1.

SCHEME 2



The conformation of the ZW ligand in the alkaline earth dimer complexes is also interesting. As seen in Table 1, the extended (C1/Z2) and compact (C1/Z3) binding configurations of the ligand have almost equal calculated energies for Ca, Sr, and Ba. The calculated free energies appear to favor the looser extended conformation slightly. (The MPW1PW91 functional, which is thought to give a slightly better representation of cation−π interactions than B3LYP,[38] actually gives greater stability by a margin of 3 kJ/mol for the compact structure vs the extended structure in the Ba case.) We conclude that the thermochemical difference between these structures of the mixed-ligand dimer is unclear within the uncertainties in the DFT calculations.

The spectroscopic evidence is clearer. As seen in Figure 4 and in Figures S1−S6, Supporting Information, the two conformations are expected to have very similar spectra except that the strong feature in the 1600 cm^{-1} region (mixed NH_3^+ bend and antisymmetric COO^- stretch) shows a distinct shift of about 30 cm^{-1} between the two structures. The Ca^{2+} spectrum shows an excellent fit to the C1/Z2 prediction (Figure 1), while the Sr^{2+} and Ba^{2+} spectra are much better fitted by a mixture of C1/Z3 and C1/Z2 forms (Figure 1, Figure 4, Figures S2 and S3, Supporting Information). There is evidently a delicate balance between the compact and extended binding conformations of this ligand on the alkaline earth metal ions, with the smaller metal ion (Ca) favoring the extended form, and the larger Sr and Ba ions giving more more nearly equal stability to the compact, chelated form.

Alkaline Earth Metals. As shown in Table 1, a direct analogy with the monomer complexes would lead us to expect all three of the alkaline earth complexes studied here to have ZW ligands, although the energetic advantage over the CS form for the monomer complexes is not large. Furthermore, the 3-fold chelated Z3 conformation is strongly favored over the extended Z2 conformation for the monomers.

The situation is definitely different when there are two ligands, so that the spectra of the dimer complexes in Figure 1 all show prominently the characteristic features of a CS ligand, notably the strong C=O stretch around 1700 cm^{-1} and the COH bend near 1150 cm^{-1}. At least one of the two ligands is clearly CS. The evidence is strong that the other ligand has a ZW configuration, most notably because of the strong feature at 1400−1450 cm^{-1}. Whether the ZW ligand favors the extended (Z2) or chelated (Z3) binding conformation depends on the metal ion.

***Ca^{2+}*.** The calcium dimer ion is apparently the most straightforward. The spectrum shows the signature peaks expected for both a CS and a ZW ligand, and the spectrum gives an excellent fit to the predicted spectrum for the C1/Z2 configuration. As noted above, the C1/Z3 variant is thermochemically competitive with this but gives a distinctly worse spectroscopic fit. Thus, the C1/Z2 configuration is assigned confidently as the predominant one.

***Ba^{2+}*.** The two strongest features at 1700 and 1430 cm^{-1} suggest that there are comparable populations of CS ligands and ZW ligands, and it seems firmly established that this is a CS/ZW dimer ion. This is in accord with the thermochemical calculations (Table 1), which found no structures of either CS/CS or ZW/ZW that were as stable as the mixed-ligand structures. The spectrum between 1600 and 1640 cm^{-1} does not fit well to either the C1/Z3 or C1/Z2 predictions (which are shown in Figure S3, Supporting Information) but fits quite well to a 50/50 mixture of these two structures, as shown by the gray curve of Figure 1.

***Sr^{2+}*.** The strontium case is quite similar to barium. The ion definitely has a CS/ZW structure, based both on the thermochemical expectations and on the major features of the spectrum. The shape of the spectrum in the 1600−1640 cm^{-1} region suggests a mixture of extended C1/Z2 and compact C1/Z3 conformations. The predicted spectrum in Figure 1 takes a 50/50 mixture of these structures, giving a fairly good fit, although a somewhat greater proportion of the C1/Z2 variant might give a still better fit and would be more consistent with the thermochemistry of Table 1. Thus the two largest ions, Sr^{2+} and Ba^{2+}, are the only ones showing clear spectroscopic signatures for the compact C1/Z3 structure in which both rings have cation−π interactions with the metal ion. Apparently steric crowding in the smaller ions, i.e. Ca^{2+} and the transition metal ions (vide infra), favors opening up the binding of the ZW ligand so that its ring system is remote from the metal ion.

Trp Binding to a Metal Chloride Complex. Except for the Ba complex, it has not yet proven possible to obtain the spectrum of a monomer complex of a 2+ metal ion with Trp, and we have no authentic example of the IRMPD spectrum of a 2+ Trp complex with the ligand in the CS configuration. However, we were able to produce the $CdClTrp^{1+}$ complex by electrospray and to establish its conformation by IRMPD spectroscopy, as shown in Figure 3. The calculated CS spectrum is clearly an excellent fit to the observed spectrum. While this is not a 2+ ion, nevertheless the environment of the ligand attached to the Cd^{2+} center should approach more closely to such an ion than the previously studied 1+ strongly bound complexes such as the Ag^+ complex.[1] It thus gives some indication of the spectroscopy expected for strongly binding 2+ Trp complexes, and provides a striking contrast with the more weakly binding Ba^{2+} complex which has its ligand in the ZW configuration. As shown in Table 1, the CS conformation of this complex is fully in accord with the thermochemical expectation, since the CS configuration is favored by about 32 kJ/mol over the best ZW configuration.

A correlation between Trp binding energy and the (calculated) preference for CS versus ZW binding was proposed for monatomic metal ions in ref 26. The correlation was dependent on the metal ion charge, showing different approximately linear proportionalities for 1+ and 2+ metal ions. The calculated binding energy of 410 kJ/mol for Trp to $CdCl^+$ places this ion quite close to typical 1+ ions in terms of this empirical correlation, if the ion is viewed as equivalent to a monatomic ion. This might lead to an expectation of a CS conformation for the Trp ligand, similar to the alkali metal ions.[1] On the other hand, ref 39 took the point of view that the benzene ligand sees the $M^{2+}Cl^-$ ion as having more of the character of a 2+ metal ion, with a spectator Cl^- ion. In this latter point of view, the higher effective charge of the Cd^{2+} ion might be expected to favor the ZW conformation for the Trp ligand. The fact that the CS ligand conformation actually is strongly favored for $CdClTrp^{1+}$ suggests that the large, chelating Trp ligand, in contrast to benzene,[39] sees $CdCl^+$ as a singly charged entity, rather than having the character of a Cd^{2+} ion with an appended Cl^- ligand. The structure of the CS complex is shown in the Supporting Information.

Transition Metals. The closed shell transition metals Zn^{2+} and Cd^{2+} give very similar spectra which are also quite similar to those of the alkaline earth complexes. As shown in Figure 2, the fits to the calculated C1/Z2 spectrum are good, and it is reasonable to assign this as the actual structure in both cases. Compared with the alkaline earths, the peaks in the lower energy region 1000−1200 cm^{-1} seem to be strongly enhanced. The calculated spectra predict somewhat greater intensities of peaks in this region, but the enhancement seems larger than expected and is not understood.

Two of the transition metal spectra were taken to wavelengths longer than that of the alkaline earths. We can note the strong peak around 750 cm^{-1}, which is a universally observed feature of the tryptophan side chain. We have not found any useful structural information to be available from this peak or from others in the low-frequency region.

Dimer complexes of the two open-shell transition metals Mn^{2+} and Co^{2+} show spectra that are very similar to those of the closed-shell metal ions. Apparently these complexes are similarly mixed CS/ZW conformations. Given the fact that multiple spin states must be considered, and the likely presence of multiple open-shell electrons in these two complexes, calculations were not further attempted in this study.

The Ni^{2+} complex is the most interesting of the open-shell complexes. This is the most tightly binding complex of the systems for which energy calculations were performed here. Consequently, the Ni^{2+} complex is most likely to favor CS binding. The complex was difficult to photodissociate, and only the rather noisy spectrum shown could be obtained, but it is sufficient to indicate that the characteristic ZW peak near 1450 cm^{-1} is absent or nearly so. The comparison with calculated spectra in Figure S7 suggests that this spectrum gives a much better match to the CS/CS conformation than to the CS/ZW conformation. It appears that this complex is unique among those studied here in having both ligands in the CS conformation.

This suggestion is supported by the energy computations. As shown in Table 1, the B3LYP result for free energies gives virtually equal stabilities for C1/C1 and C1/Z2. However, in line with our experience that the cation−π interaction with transition metal ions is somewhat better treated by the MPW1PW91 functional,[38] we repeated these two calculations with the latter functional, and as indicated in the table footnotes, we found that the C1/C1 conformation is favored by the small, but suggestive, increment of 5 kJ/mol. This level of calculation is certainly not definitive in view of the smallness of the free-energy difference, but it indicates that our suggestion of a thermochemical advantage for the CS/CS configuration, and of unique character for the Ni^{2+} complex, is not unreasonable.

Comparing One Trp Ligand Versus Two. Figure 5 displays the spectroscopic appearance of complexes with either one or two Trp ligands. (The Ba monomer spectrum is from ref 26, and the other three spectra are reproduced from Figures 1 and 2). The Ba and Cd cases are both fairly large ions which are interesting to compare, because Ba is a weakly binding ion favoring the ZW conformation for the first Trp ligand while Cd is a strongly binding ion favoring the CS conformation for one Trp ligand. The Ba comparison is straightforward: the monomer complex is a clear-cut ZW ligand, and the second Trp adds as a CS ligand to give a dimer of CS/ZW mixed character. On the other hand, the Cd "monomer" complex (represented by the Trp/chloride complex) has a CS ligand and adds its second Trp ligand as a ZW ligand to end up also as a dimer CS/ZW configuration.

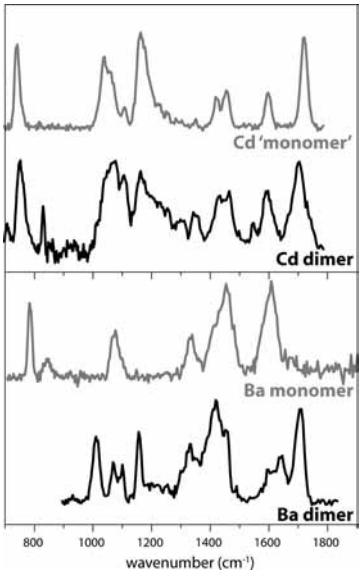


Figure 5. Comparison of spectra of M^{2+} ions with one versus two Trp ligands. The "Cd 'monomer' " spectrum is the spectrum of $CdClTrp^{1+}$.

It is noteworthy that the Cd dimer spectrum, in this direct comparison with the "monomer" spectrum, has little in it that points overtly to the presence of the ZW ligand. The modest peak near 1350 cm^{-1}, and the somewhat greater intensity of the 1600 cm^{-1} peak compared with the "monomer" spectrum, are the only features that can be considered as signals of the ZW ligand. This illustrates the point made above that the identification of a ZW ligand in the presence of a CS ligand in these dimer complexes is not straightforward and rests on detailed matching with the computed spectrum, combined with thermochemical calculations. Note that the fit of this Cd dimer spectrum to the C1/C1 computed spectra, shown in the Supporting Information, is very poor, so the presence of the ZW ligand is actually strongly indicated by the spectroscopy as well as by the thermochemistry.

Conclusions

IRMPD spectroscopy in concert with DFT calculations was applied to assign the structures of the $M^{2+}Trp_2$ dimer complexes in the gas phase. The IRMPD spectra of all of these dimer complexes show unequivocally that at least one ligand is present in the CS configuration. This is clear from the strong feature (C═O stretch) at about 1730 cm^{-1} in all the spectra and is reinforced by the obvious presence of the COH bending mode at 1150 cm^{-1}. Calculated energies and spectra indicate the N/O/ring 3-fold chelated conformation of the CS ligand as the most likely. The fact that at least one ligand in the dimers is CS contrasts with the situation with the monomer complex of Ba^{2+}, which was shown to be ZW, and with the calculated most-stable structures of the Sr^{2+} and Ca^{2+} monomers, which are also ZW.

Except for the Ni case, the second ligand in all of the dimers studied has a ZW configuration, as indicated by the strong features at 1400−1450 cm^{-1} (NH_3^+ umbrella mode). We conclude that these populations consist predominantly of ions with mixed-configuration CS/ZW structures. Energy calculations indicate the extended Z2 binding conformation of the ZW ligand as most favorable for the transition metal cases, but for the alkaline earths the alternative compact, chelated Z3 conformation is so close to Z2 in stability that we do not trust the calculations to predict the conformation for these cases. The spectrum in the Ca^{2+} case gives an excellent fit to the calculated spectrum for C1/Z2, but the two largest metal ions in this study, Sr^{2+} and Ba^{2+}, show spectroscopic evidence of a mixture of Z2 and Z3 configurations of the ZW ligand, showing IR profiles in the region of the zwitterionic mode around 1600−1640 cm^{-1} (COO^- stretch/NH_3 bend), indicating both C1/Z2 and C1/Z3 conformations. We conclude that the compact and extended modes of chelation of the ZW ligand are quite closely balanced in stability, and the steric crowding around the smaller metals acts to push the binding toward the extended-ligand pattern.

The Ni dimer spectrum, while of low quality, is sufficiently clear to indicate that this ion, uniquely among those examined, has no ZW ligand and most likely has the C1/C1 compact structure. This ion is also unique among those calculated in having the C1/C1 structure as the most stable of the structures examined computationally. This case seems to support the principle that strong metal−ligand binding is accompanied by a preference for the CS ligand binding configuration.

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Supporting Information Available: Additional computed spectra of dimer complexes of relevant metal ions and conformations; structure of $CdClTrp^{1+}$ (C1 conformation). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Polfer, N. C.; Oomens, J.; Dunbar, R. C. *Phys. Chem. Chem. Phys.* **2006**, *8*, 2744.

(2) Dougherty, D. A. *Science* **1996**, *271*, 163.

(3) Durrant, J. D.; Caywood, D.; David, D., D D *Biophys. J.* **2006**, *91*, 3230.

(4) Hu, W.; Cross, T. A. *Biochemistry* **1995**, *34*, 14147.

(5) Cotten, M.; Tian, C.; Busath, D. D.; Shirts, R. B.; Cross, T. A. *Biochemistry* **1999**, *38*, 9185.

(6) Buck, D. P.; Howitt, S. M.; Clements, J. D. *Biophys. J.* **2000**, *79*, 2454.

(7) Stepanian, S. G.; Reva, I. D.; Radchenko, E. D.; Adamowicz, L. *J. Phys. Chem.* **1998**, *102*, 4623.

(8) Stepanian, S. G.; Reva, I. D.; Radchenko, E. D.; Adamowicz, L. *J. Phys. Chem. A* **1999**, *103*, 4404.

(9) Stepanian, S. G.; Reva, I. D.; Radchenko, E. D.; Rosado, M. T. S.; Duarte, M.; Fausto, R.; Adamowicz, L. *J. Phys. Chem. A* **1998**, *102*, 1041.

(10) Blanco, S.; Lesarri, A.; Lopez, J. C.; Alonso, J. L. *J. Am. Chem. Soc.* **2004**, *126*, 11675.

(11) Lesarri, A.; Cocinero, E. J.; Lopez, J. C.; Alonso, J. L. *Angew. Chem., Int. Ed.* **2004**, *45*, 605.

(12) Rak, J.; Skurski, P.; Simons, J.; Gutowski, M. *J. Am. Chem. Soc.* **2001**, *123*, 11695.

(13) Talley, J. M.; Cerda, B. A.; Ohanessian, G.; Wesdemiotis, C. *Chem. Eur. J.* **2002**, *8*, 1377.

(14) Cerda, B. A.; Wesdemiotis, C. *Int. J. Mass Spectrom.* **1999**, *185−187*, 107.

(15) Polfer, N. C.; Oomens, J.; Moore, D. T.; von Helden, G.; Meijer, G.; Dunbar, R. C. *J. Am. Chem. Soc.* **2006**, *128*, 517.

(16) Forbes, M. W.; Bush, M. F.; Polfer, N. C.; Oomens, J.; Dunbar, R. C.; Williams, E. R.; Jockusch, R. A. *J. Phys. Chem. A* **2007**, *111*, 11759.

(17) Polfer, N. C.; Paizs, B.; Snoek, L. C.; Compagnon, I.; Suhai, S.; Meijer, G.; von Helden, G.; Oomens, J. *J. Am. Chem. Soc.* **2005**, *127*, 8571.

(18) Jockusch, R. A.; Price, W. D.; Williams, E. R. *J. Phys. Chem. A* **1999**, *103*, 9266.

(19) Kish, M. M.; Ohanessian, G.; Wesdemiotis, C. *Int. J. Mass Spectrom.* **2003**, *227*, 509.

(20) Marino, T.; Russo, N.; Toscano, M. *J. Phys. Chem. B* **2003**, *107*, 2588.

(21) Shoeib, T.; Siu, K. W. M.; Hopkinson, A. C. *J. Phys. Chem. A* **2002**, *106*, 6121.

(22) Kapota, C.; Lemaire, J.; Maitre, P.; Ohanessian, G. *J. Am. Chem. Soc.* **2004**, *126*, 1836.

(23) Lemoff, A. S.; Bush, M. F.; Williams, E. R. *J. Am. Chem. Soc.* **2003**, *125*, 13576.

(24) Lemoff, A. S.; Bush, M. F.; Williams, E. R. *J. Phys. Chem. A* **2005**, *109*, 1903.

(25) Lemoff, A. S.; Bush, M. F.; Wu, C. C.; Williams, E. R. *J. Am. Chem. Soc.* **2005**, *127*, 10276.

(26) Dunbar, R. C.; Polfer, N. C.; Oomens, J. *J. Am. Chem. Soc.* **2007**, *129*, 14562.

(27) Bush, M. F.; Oomens, J.; Saykally, R. J.; Williams, E. R. *J. Am. Chem. Soc.* **2008**, *130*, 6463. *J. Phys. Chem. A* **2008**, *112*, 8578.

(28) Rajabi, K.; Fridgen, T. D. *J. Phys. Chem. A* **2008**, *112*, 23.

(29) Wu, R.; McMahon, T. B. *J. Am. Chem. Soc.* **2007**, *129*, 4864.

(30) Oh, H.-B.; Lin, C.; Hwang, H. Y.; Zhai, H.; Breuker, K.; Zabrouskov, V.; Carpenter, B. K.; McLafferty, F. W. *J. Am. Chem. Soc.* **2005**, *127*, 4076.

(31) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R. ; Montgomery J.A. Jr.; Vreven, T.; Kudin, K. N.; Burant, J.C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al.Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian, Inc., Wallingford, CT, 2004.

(32) Valle, J.; Eyler, J. R.; Oomens, J.; Moore, D. T.; van der Meer, A. F. G.; von Helden, G.; Meijer, G.; Hendrickson, C. L.; Marshall, A. G.; Blakney, G. T. *Rev. Sci. Instrum.* **2005**, *76*, 023103.

(33) Dunbar, R. C. *Int. J. Mass Spectrom.* **2000**, *200*, 571.

(34) Duncan, M. A. *Int. J. Mass Spectrom.* **2000**, *200*, 545.

(35) Oomens, J.; Tielens, A. G. G. M.; Sartakov, B.; von Helden, G.; Meijer, G *Astrophys. J.* **2003**, *591*, 968.

(36) Lucas, B.; Gregoire, G.; Lemaire, J.; Maître, P.; Ortega, J.-M.; Rupenyan, A.; Reimann, B.; Schermann, J. P.; Desfrançois, C. *Phys. Chem. Chem. Phys.* **2004**, *6*, 2659.

(37) Balaj, O. P.; Kapota, C.; Lemaire, J.; Ohanessian, G. *Int. J. Mass Spectrom.* **2008**, *269*, 196.

(38) Oomens, J.; Moore, D. T.; von Helden, G.; Meijer, G.; Dunbar, R. C. *J. Am. Chem. Soc.* **2004**, *126*, 724.

(39) Gapeev, A.; Dunbar, R. C. *J. Phys. Chem. A* **2000**, *104*, 4084.

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