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IRMPD spectroscopic investigation of gas-phase complexes of deprotonated penicillin G with Ba^{2+}, Zn^{2+} and Cd^{2+}

Robert C. Dunbar[a,*], Jos Oomens[b,c], Galina Orlova[d], Diethard K. Bohme[e]

[a] Chemistry Department, Case Western Reserve University, Cleveland, OH 44106, United States
[b] FOM—Institute for Plasma Physics Rijnhuizen, Edisonbaan 14, NL-3439 MN Nieuwegein, The Netherlands
[c] University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands
[d] Department of Chemistry, St. Francis Xavier University, Antigonish, Nova Scotia, Canada B2G 2W5
[e] Department of Chemistry and Centre for Research in Mass Spectrometry, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3

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ABSTRACT

The bare deprotonated penicillin G, and its complexes with the Ba^{2+}, Zn^{2+} and Cd^{2+} ions, have been formed by electrospray ionization and studied by IRMPD (infrared multiple photon dissociation) spectroscopy using FT-ICR (Fourier-transform ion cyclotron resonance) mass spectrometry with the FELIX free electron laser infrared light source. The spectra in the 900–2000 cm^{-1} range have been interpreted by comparison with DFT-computed spectra. The spectrum of deprotonated penicillin G is in good agreement with calculations for a mixture of the two most favorable conformations, and is in good accord with the condensed-phase spectrum, except that the symmetric and antisymmetric carboxylate peaks are split much farther apart in the gas phase. The Ba(Penicillin-H)$^+$ ion is assigned as the simple complex with barium chelated by all three carbonyl oxygens, along with a cation-π interaction with the phenyl ring. The Zn(Penicillin-H)$^+$ spectrum is consistent with simple complexation in a similar tridentate conformation, probably without cation-π interaction, but the agreement with the calculated spectrum is not excellent, and rearrangement is not ruled out. The Cd(Penicillin-H)$^+$ complex probably has the simple tridentate conformation with strong cation-π interaction when sprayed from methanol solution, but rearranges when sprayed from acetonitrile to a new spectroscopically unique conformation, which was not identified with confidence. For this latter complex, the most stable calculated structure found has a deprotonated amide nitrogen and a cadmium–nitrogen covalent bond.

1. Introduction

The binding of metal ions to β-lactam antibiotics, notably including penicillin, has been of considerable interest, since the antimicrobial activity of these drugs and the mechanism of bacterial resistance are intimately tied to their interactions with metal ions. Anacona and Figueroa [1] in their extensive exploration of the penam family of metal-ion complexes note the longstanding [2–4] appreciation of the activity of these drugs in the protein-synthesis machinery, involving metal ion binding to the nucleotide building blocks, to the enzymes, and to the drugs themselves. The high reactivity of the penam molecules in transition-metal environments, for instance with nickel [5] or zinc [6], is considered to be involved in bacterial resistance to penicillins, and invites exploration of the details of possible reaction pathways.

Emerging spectroscopic and other techniques for detailed structural analysis of metal-ion complexes in the gas phase, as well as rapidly expanding computational approaches, present new opportunities to deepen the knowledge of such interactions and reactivity (for a few illustrations, see Refs. [7–19]). Complexes of the barium dication with model amino-acid and amide-based systems of biological relevance have been the subject of several gas-phase spectroscopic and computational investigations [20–23], showing that this cation can serve as a model for the complexation behavior of large metal ions having relatively low interaction strength. Zinc and cadmium dications, in contrast, are strongly interacting transition metal ions that can provide insight into the biologically interesting family of highly reactive transition metals that also includes Ni^{2+}, Co^{2+}, Cu^{2+} and Pd^{2+} [1,24,25].

Although not yet a subject of gas-phase study to our knowledge, the interaction of divalent metals with penicillin, as well as related antibiotics with an amide-linked side chain, has been the subject of numerous condensed-phase studies having a focus on the spectroscopy of the complexes. Anacona's group has presented a series of a dozen or more studies whose common theme is interaction

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[*] Corresponding author. Tel.: +1 216 368 3712; fax: +1 216 368 3006.
E-mail addresses: rcd@po.cwru.edu (R.C. Dunbar), j.oomens@rijnhuizen.nl (J. Oomens).

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of divalent (Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Cd(II)) and trivalent transition metals (Fe(III), La(III)), with a variety of β-lactam ligands (for example [1,26]). They emphasize the commonality of the IR spectroscopy, tracing the small frequency variations of a set of metal-sensitive mid-IR normal modes across the mid-IR wavelength range, as discussed below for the IR spectra of penicillin complexes [1]. Chohan and Supuran [27,28] have reported IR-spectroscopic data for divalent transition metal complexes of two antibiotics in this class [27,28] (not including penicillin). Mukherjee and Ghosh, as part of a series of publications on divalent transition metal ions interacting with ampicillin (an amino-substituted variant of penicillin G), have considered the possible binding modes as a function of pH [25].

The degradation of penicillin G (and other β-lactams) in methanolic solution is strongly catalyzed by divalent transition metal ions including zinc(II) [6] and cadmium(II) [29], with Cd(II) being particularly effective. The catalysis is postulated to involve initial formation of simple 1:1 or 1:2 metal–ligand complexes, followed by degradation reactions. The present study was aimed at characterizing the 1:1 complexes under the solvent free conditions of the gas phase. Since the ions trapped and characterized spectroscopically in these experiments have the mass of the un-degraded 1:1 complex, it is known that they represent complexes which have not gained or lost mass, and can thus be taken to reflect the initial stages of the penicillin degradation sequence. However, the ions emerging from the electrospray sampling process may or may not have undergone internal rearrangement. The goal here was to use the incisive characterization provided by the emerging technique of gas-phase infrared multiple photon dissociation (IRMPD) spectroscopy [30] with these ions to determine whether they retain the structure of a simple metal/ligand addition complex, or whether rearrangement takes place in the initial complex-formation stage of the reaction processes. Accordingly, IRMPD study was undertaken of the barium ion complex, where the low reactivity of the metal may not be conducive to rearrangement, and the zinc and cadmium ion complexes, which are known to have particularly high propensity for reaction. It was also interesting to characterize the spectroscopy of the bare gas-phase penicillin G anion, and to compare and correlate the spectroscopy of the gas-phase penicillin anion and its (cationic) metal ion complexes with corresponding ions in the condensed phase.

2. Experimental and computational

2.1. Computational

Calculations were performed using Gaussian 03 [31] at the B3LYP/6–31 + g(d,p) level unless otherwise noted, fully geometry optimized. No imaginary frequencies were encountered for structures where vibrations were calculated. For the Cd^{2+} and Ba^{2+} complexes, the sdd relativistic effective core potential was used on the metal ion. Energies given are simple, uncorrected electronic energies at the local minimum-energy optimized structures. In cases where the vibrational frequencies were calculated, an additional value is reported, namely the (relative) free energy (rigid-rotor harmonic oscillators at 298 K), corrected for thermal enthalpy at 298 K and for vibrational zero-point energies. The computed frequencies were scaled by 0.975 as has been found appropriate in previous analyses of IRMPD spectra of cationized amino acids and small peptides in this wavelength range. Computed infrared spectra were convoluted with a Gaussian lineshape with a FWHM of 20 cm^{-1} (occasionally 30 cm^{-1}) for visual comparison to experimental spectra.

The conformation space of the ions in this study is large, and global systematic searching for conformations was not feasible. Within the limits of retention of the bicyclic β-lactam core of the molecule, extensive searches were made of the conformations that seemed likely to have low energy, both those involving simple additive complexation of the metal ion, and those involving migration of the amide proton. The present interpretations are based on the range of possible structures covered in this way. Some of the present results suggest the possibility of even more extensive rearrangements, offering scope in future work for exploration of more disruptive reaction processes.

3. Experimental

Infrared spectra of the ionized gas-phase complexes were measured as IRMPD (infrared multiple-photon dissociation) spectra, based on the concept of action spectroscopy, whereby the dissociation as a function of laser wavelength is considered as a proxy for the inaccessible linear direct absorption spectrum [7,8,19,32]. The FTICR mass spectrometer interfaced to the FELIX free-electron laser light source at the FOM Institute (Rijnhuizen) was used, as has been described in detail [7,30,32,33]. Ions were produced by electrospray ionization (ESI) from a solution of penicillin G and the metal salt (metal chloride or nitrate) usually at a 1 mM concentration of each. The penicillin anions, the barium complex, and the first set of cadmium complexes were sprayed from methanol/water (~70:30), while the zinc complex and the second set of cadmium complexes were sprayed from acetonitrile/water (~80:20) as discussed below. Ions were accumulated and stored for collisional and radiative cooling during 4 s in a hexapole linear trap, followed by mass isolation in the FTICR ion trap and irradiation by FELIX for typically 3 s. The IR spectrum was reconstructed by summing all major fragment ions and plotting the total fragment yield as a function of the photon energy. A linear correction corresponding to the measured laser intensity as a function of wavelength was applied as has become common in such experiments, and the laser wavelength was calibrated two or three times a day, or following a change in laser parameters.

Many of the peaks in this set of spectra seem relatively broad compared with the typical results of IRMPD studies of small metal-ion complexes. We attribute this in part to power broadening effects due to high laser intensity, and in part to the complexity and flexibility of the penicillin framework which allows greater conformational heterogeneity than is often possible in small, tightly bound ionic complexes. As an indication of the degree of laser power broadening, a partial spectrum of the bare anion similar to that shown below was taken at three times lower laser intensity. This spectrum showed noticeable narrowing of the peaks, with its narrowest peaks near 30 cm^{-1} in width (in the 1200–1800 cm^{-1} region), which is of the same order as the laser linewidth.

No consistent pattern was found in the identities of the photodissociation fragment ions. Each of the four parent ions gave more than one fragment mass, and no two fragments had the same mass, or the same neutral-molecule loss mass. Consideration of these fragmentation patterns was not thought to be useful, and was not pursued.

4. Results and discussion

A baseline for spectroscopic interpretation of the complexes is provided by the bare deprotonated penicillin G. Computationally only two closely related structures of low energy were located (see Fig. 1 for the more stable structure, Anion I. The other conformation Anion II, is less stable by 1 kJ mol^{-1}, differing only by a rotation of the phenyl side chain by 120°, and gives a practically identical calculated IR spectrum). The IRMPD spectrum of the deprotonated

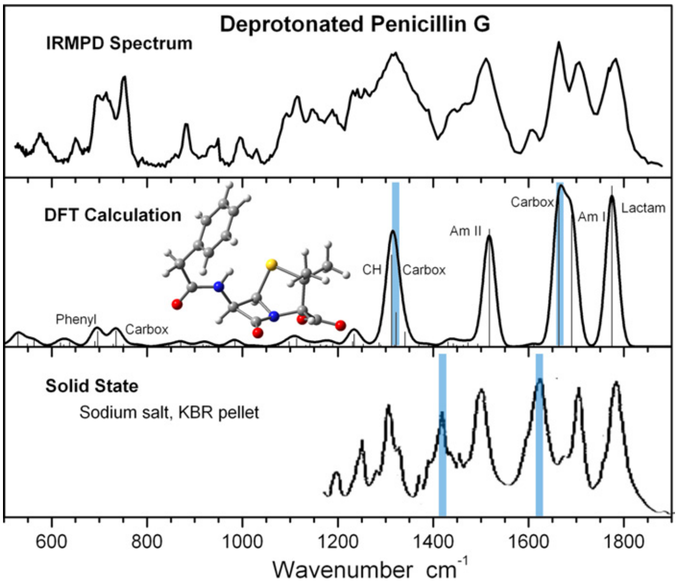

Fig. 1. Spectroscopy of the deprotonated penicillin G. The gas-phase IRMPD (top panel) and DFT-calculated spectrum of the Anion I conformation (middle panel) are the present results, while the bottom panel is abstracted from Ref. [1]. The peaks marked in blue in the calculated spectrum and in the solid state spectrum are the pair of normal modes, asymmetric (high energy) and symmetric (low energy) COO^- stretching vibrations, of the carboxylate moiety, which are known to be perturbed by crystal or counterion effects so as to be less widely split apart in the solid phase than in the unperturbed gas phase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

penicillin G is displayed in Fig. 1. Along with this spectrum the computed IR spectrum of the Anion I is shown, as well as the solid-state IR spectrum of the sodium salt reported in Ref. [1].

Although the IRMPD features are broadened compared with the (arbitrary) width of 30 cm^{-1} chosen for the calculated spectrum, the prominent features of the predicted and observed spectra are in excellent accord. As marked in the figure, the expected peaks of high intensity are listed in Table 1. The major peaks correspond very well in position and, as well as can be expected, in intensity (given the well-known deficiencies in IRMPD spectra in reproducing accurate relative peak intensities corresponding to the actual linear IR absorption intensities [30]).

The extensive condensed phase literature of the metal penicillinates has emphasized the characteristic antisymmetric and symmetric stretching vibrations of the COO^- group [1,24,34]. These two carboxylate frequencies are well established as being highly characteristic of the environment of the carboxylate group, both for free anions and also for complexes bound to strongly complexing metal-ions [24,34]. The condensed-phase antisymmetric stretch is typically in the 1550–1600 cm^{-1} range, and the symmetric stretch lies quite close to 1400 cm^{-1}, with variations depending on metal counter ion, environment, effects of groups neighboring the carboxylate, and so on. The splitting between these two peaks is usually in the 100–200 cm^{-1} range. It is of interest to compare the gas-phase and computational spectra of the free deprotonated penicillin G and the metal-complexed compounds with results from literature. In a paper of interest in the present context, Anacona and Figueroa [1] show the solid-state IR spectra of the free deprotonated penicillin G anion (from the sodium penicillinate spectrum) and the Zn and La complexes (actually dimer complexes) over the range of about 2100–1150 cm^{-1}. Fig. 1 shows the comparison of their solid-state spectrum of deprotonated penicillin G sodium salt with the present gas-phase spectrum. Close correspondence in frequencies is observed between IRMPD and solid-state band positions of the lactam, Amide I, Amide II and CH modes. However, the two carboxylate frequencies assigned here for the gas-phase free deprotonated penicillin G (1663 and ~1315 cm^{-1}) are substantially different from the condensed-phase peaks assigned by Anacona to the two free carboxylate modes (1621 and 1418 cm^{-1}) (highlighted in blue in Fig. 1). The sharp difference between gas-phase and condensed-phase frequencies of these modes is likely a consequence of the differences in environment. Recently gas-phase IRMPD spectroscopy of some simpler solvent-free carboxylate anions, supported by computations, produced frequencies for the gas-phase free carboxylate that correspond quite well to the results presented here [35]. Several benzoates were reported to have antisymmetric stretches at 1626–1650 cm^{-1}, and symmetric stretches at 1300–1311 cm^{-1}, depending on the ring substituents [35], closely corresponding with our assignments of 1663 and ~1315 cm^{-1}. In this report Oomens et al. also noted larger splitting between the carboxylate peaks in the gas phase compared with solid state spectra of the same benzoate anions.

Table 1
The expected peaks of high intensity in the gas-phase IRMPD spectra of deprotonated penicillin G ("observed" frequencies are our proposed assignment of the IRMPD spectrum).

Feature	Frequency (cm^{-1})	
	DFT	Observed
C=O of the lactam group	1774	1778
"Amide I" mode (C=O stretch of the amide group)	1691	1703
Antisymmetric COO^- stretch	1663	1663
"Amide II" mode (N–H bend of the amide group)	1516	1507
Symmetric COO^- stretch	1323	[a]
Mixed mode composed of a number of C–H bends	1311	[a]
Symmetric COO^- bend	735	752
Out-of-plane ring C–H bends	696	697–717

[a] Unresolved multiplet near 1315 cm^{-1}.

As we consider the metal-bound anions in the following discussion, we might take several lessons from the results for the free deprotonated penicillin G: (1) The carboxylate peaks in the gas-phase, while expected to be substantial in intensity, may not provide standout markers of predominant intensity as is characteristic of condensed phases. (2) The positions of these two peaks may be very different from condensed phase, and the splitting between them may be much larger (by a factor of 2 or more) than is typical in condensed phases. (3) While there is not enough experience from gas phase to draw experimental generalizations about carboxylate anion modes in varying molecular contexts, DFT computational predictions appear to offer a reliable guide to their likely gas-phase positions.

4.1. *Metal ion complexes*

The metal complexes having the carboxylate moiety bound to the metal ion can either be bidentate (both COO^- oxygens symmetrically bound head-on) or monodentate (one oxygen bound side-on) [34]. In the analysis of the solution-phase complexes of divalent transition-metal complexes, Anacona and Figueroa consider the complexation to be monodentate [1]. Similarly, the computations of possible structures for the present gas-phase complexes show that the structures which chelate the metal ion with a bidentate carboxylate (e.g., structure COO in Fig. 2) are much less stable than various monodentate possibilities. Thus we can limit our consideration to structures with monodentate carboxylate binding.

Fig. 2 shows the calculated structures and thermochemistry for a number of the conformations explored for which the metal ion is chelated by an essentially native deprotonated penicillin G ligand. It can be seen that the low-energy structures of this type are all characterized by chelation of the metal ion with all three

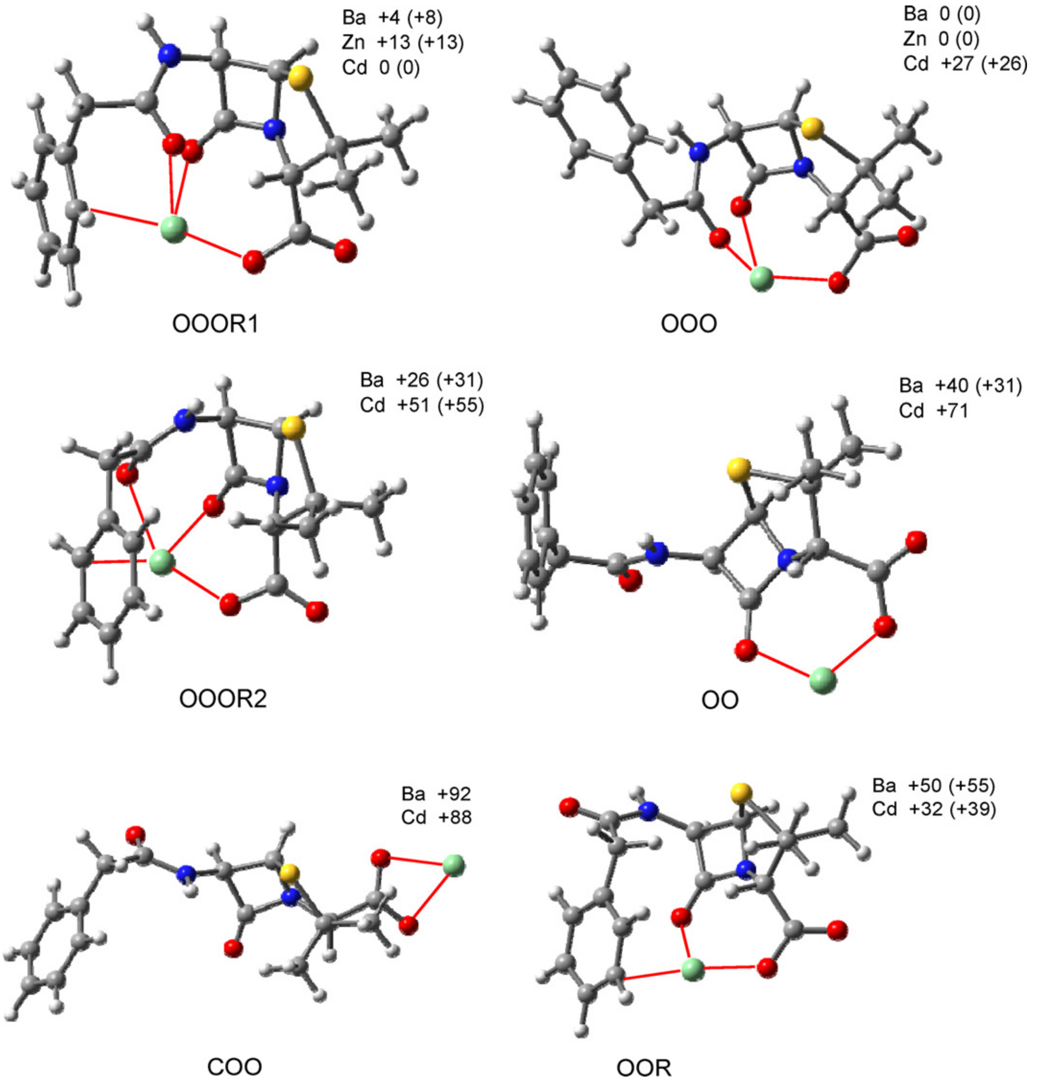

Fig. 2. Structures and thermochemistry of penicillin G complexes (cadmium complexes Cd(Penicillin-H)$^+$ are shown for illustration). Energies (kJ mol^{-1}) are shown relative to the most stable of the complexes of each metal shown in this figure. Values in parentheses are relative free energies at 298 K.

of the carbonyl oxygens of penicillin. Added stabilization may be provided by chelation to the aromatic ring in a cation-π configuration [36]. The cation-π effect is strongest for cadmium, where the ring-chelated form OOOR1 is the most stable complex. For zinc, the intrinsic strength of the cation-π interaction is probably strong as well, but the smaller size of the zinc ion appears to lead to excessive strain in the OOOR1 structure, and the most stable complex is the non-π-chelated structure OOO. For barium, the metal-ion ring interaction is intrinsically weaker, making OOOR1 and OOO very close in stability.

Another possibility is deprotonation of the amide nitrogen by proton transfer to the carboxylate, replacing the amide proton with a covalently N-bound metal ion. Two types of complexes of this latter type are shown in Fig. 3. In one case the metal ion is chelated by the lactam carbonyl and in the other case by the sulfur atom. These deprotonated-amide structures are surprisingly stable, particularly for zinc. With the transition metals zinc and cadmium the ground state is calculated to be the deprotonated structure NSR3. With barium the unrearranged complex OOO remains the ground state even when the covalent deprotonated-amide possibilities are taken into account. Mukherjee and Ghosh [25] suggest that metal ion attachment with amide deprotonation is also thermodynamically accessible in solution (with ampicillin, in their study), but only when driven by very high pH. Given the high stability of the gas-phase deprotonated amide structures, a primary interest of the present study is thus whether the unrearranged complex conformations favored for the solution complexes are retained upon ionization and transfer to the gas phase, or whether the complexes of the transition metal ions find their way to more stable deprotonated-amide ground state conformations.

4.2. Structure of the gas-phase Ba(Penicillin-H)$^+$ complex

The spectrum of the Ba^{2+} complex is shown in Fig. 4. It differs substantially from the bare deprotonated penicillin G, showing that metal-ion interaction has major effects on the structure and spectroscopy. The two conformations OOOR and OOO that chelate the ion with three oxygen atoms are by far the most stable of all possibilities investigated. Comparing these two conformations, it appears that the cation-π interaction that can stabilize the ring-coordinated OOOR structure is counterbalanced by the extra strain involved in swinging the ring into that position, so these two conformations differ only by 4 kJ mol^{-1} in energy at this level of computation. When the greater conformational freedom of the OOO structure is taken into account through the relative free energies, the OOO structure appears to be slightly favored (by

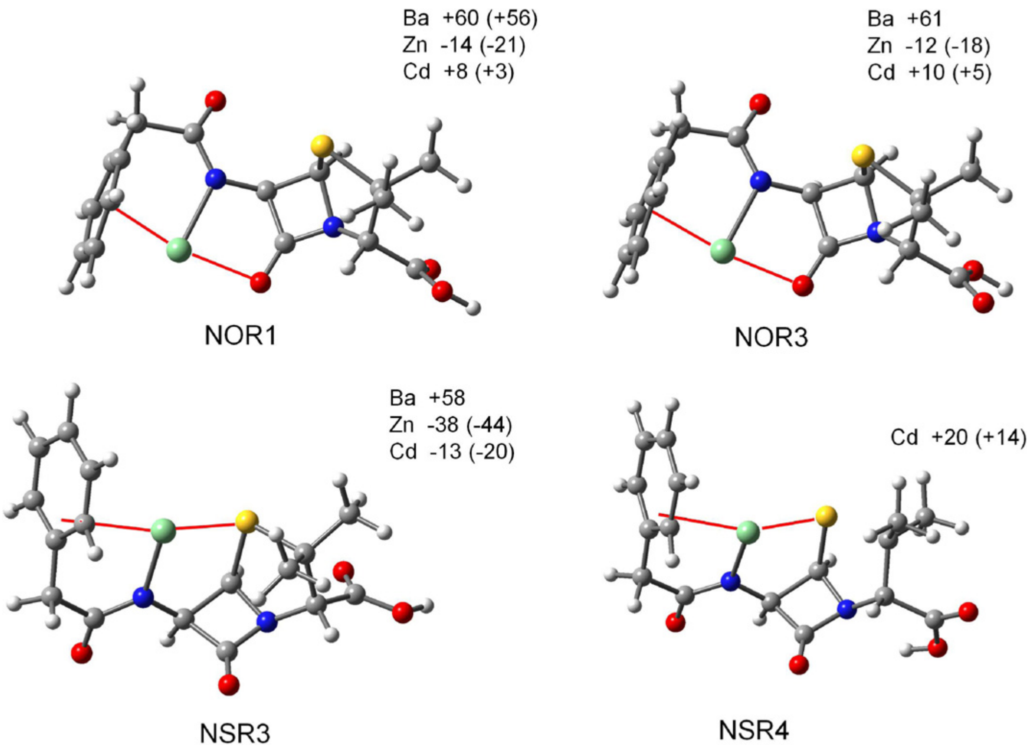

Fig. 3. Structures involving amide deprotonation and covalent metal-ion binding. Energies and free energies are given relative to the most stable unrearranged complex (Fig. 2), even when that reference structure is not the global ground state structure.

8 kJ mol^{-1}) at room temperature. However, these two structures are so close in energy that a mixture with substantial contributions from both is well within computational uncertainty. (It has been suggested that B3LYP often slightly underestimates cation-π interactions [37–41]. Using the MPW1PW91 functional, the OOOR1 structure is calculated to be several kJ mol^{-1} more favorable than with B3LYP, making these two conformations truly equal within computational uncertainty.) The alternative arrangement of the three oxygens represented by structure OOOR2 is feasible, but less favorable by about 25–30 kJ mol^{-1}.

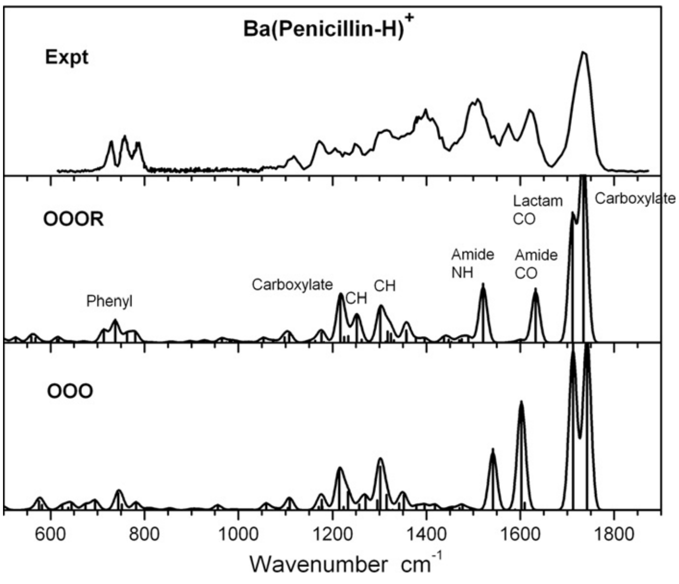

Fig. 4. IRMPD spectrum, and calculations for the two most favorable complex conformations of the Ba-Penicillin complex.

The predicted spectra of the two favored conformations are also displayed in Fig. 4. There is reasonable correspondence of the observed spectrum with the computations for either of these structures or more likely a mixture. The lactam C═O and the carboxylate antisymmetric stretch lie close in frequency, and are suggested to overlap in the single feature near 1730 cm^{-1}. The amide NH bend (Amide II) forms the strong feature at 1500 cm^{-1}. The amide C═O (Amide I) is predicted to lie at distinctly different positions in the two conformations, so that the two peaks in the 1550–1650 cm^{-1} region can be ascribed to non-overlapping contributions of this mode from a mixture of the two conformations. The carboxylate symmetric stretch of the metal-coordinated group is predicted to be at even lower frequency (1210 cm^{-1}) than in the bare deprotonated penicillin G, mixing quite strongly with several aliphatic C–H bends, and not very intense. Assigning it as a weak peak, not standing out in the poorly resolved cluster in this region, seems possible. Several mixed modes containing combinations of aliphatic C–H bends are predicted between 1150 and 1350 cm^{-1}; associating these specifically with the several moderate peaks observed in this region cannot be done with confidence, but appears plausible.

Thus nearly all features in the spectrum can be matched with calculations for a mixture of the two conformations that feature tridentate binding to the three accessible carbonyl-type oxygens of penicillin G. An unresolved question is the nature of the strong, broad peak observed around 1400 cm^{-1}, which is not predicted in any of the structures for which we have calculated spectra. This feature could arise from a contribution to the population of conformers not identified in the computations, or it could represent a failure of this computational approach to reproduce the position or the intensity of a mode at this position in the known conformations. Perhaps the most interesting spectroscopic point of these observations is that the symmetric stretching mode of the metal-bound carboxylate group, which is so characteristic in the condensed phase, is either suppressed to relatively low intensity at its computed frequency near 1200 cm^{-1} (as the computations suggest), or

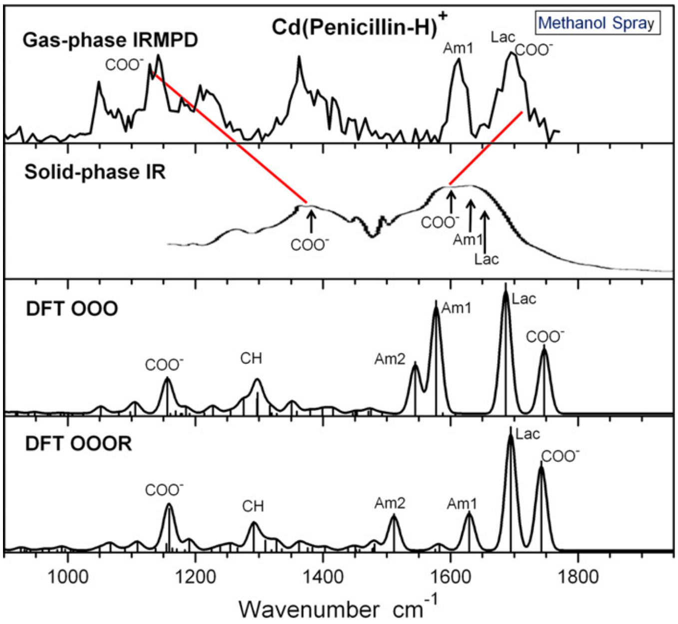

Fig. 5. Top panel, IRMPD spectrum of the cadmium complex sprayed from methanol solution. A tentative assignment is labeled using the designations "Lac", lactam C=O; "COO−", carboxylate CO stretch; "CH", hydrogen bend; "Am1", amide CO stretch mixed with C–N stretch; "Am2", amide N–H bend. Second panel is the solid phase spectrum of $ZnPen_2Cl{\cdot}3H_2O$ abstracted from Ref. [1], with peak assignments as given in the reference indicated with arrows. The red lines indicate the suggested shift of the carboxylate modes between solid state and gas phase. Third and fourth panels are calculated spectra of two conformations of the gas-phase complex. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

its frequency is calculated extremely erroneously by this level of theory. (It could be attractive to postulate that the peak observed at 1400 cm^{-1} actually corresponds to the fugitive carboxylate COO^- symmetric stretching mode, but it is hard to accept that these computations could give such a grossly inaccurate prediction while getting the rest of the spectrum right. No such difficulty was evident in the calculations of Ref. [35], nor in the present calculations of the bare deprotonated penicillin G, all of which apparently gave reasonable predictions for the symmetric carboxylate stretch.)

4.3. Structure of the gas-phase Cd(Penicillin-H)$^+$ complex from methanol solution

Fig. 5 displays the spectrum of the gas-phase Cd(Penicillin-H)$^+$ complex obtained from methanol solution. The spectrum seems roughly consistent with the prediction for the most stable complex conformation, OOOR1. The match with the non-ring-complexed conformation OOO is also roughly consistent, although the energetic penalty of about 26 kJ mol^{-1} for this structure weighs against such an assignment. Similar to the barium complex, the strong peak observed near 1400 cm^{-1} is not well accounted for by either of these predicted spectra, although the cluster of calculated hydrogen bending modes extending broadly over the 1200–1400 cm^{-1} region can be taken as a possible correspondence. It can be concluded that there are spectral features in this spectrum that can be associated with the predicted lactam, carboxylate and Amide 1 peaks, but the correspondences are less than excellent, and we are not confident of the assignment of this structure.

A useful point of comparison for the present results on transition-metal complexes is the solid-phase spectroscopy of similar complexes of various lactam antibiotics described by Anacona and Figueroa in their extensive series of publications, among them Ref. [1] which reports results for a number of transition-metal ion complexes of penicillin G. (The spectrum reproduced here from Anacona et al. is the zinc complex, which they report to be very similar to the cadmium spectrum.) As indicated in the figure, the corresponding peaks assigned to the carboxylate symmetric and antisymmetric stretches in the condensed phase spectrum are much closer together than in the gas-phase spectrum. They are assigned by Anacona at 1585 and 1370 cm^{-1} for the cadmium dimer complex of penicillin G.

We have no experimental precedent for the positions of the gas-phase carboxylate peaks for a monodentate carboxylate bound to Cd^{2+}. Bush et al. [20] calculated the carboxylate peaks in the monodentate carboxylate conformation of the deprotonated Ba^{2+} valine complex at 1724 and 1308 cm^{-1}. It appears reasonable that for the more strongly interacting cadmium ion the splitting would be even larger, making our present assignment of these peaks in the cadmium complex at ∼1720 and 1140 cm^{-1} appear reasonable.

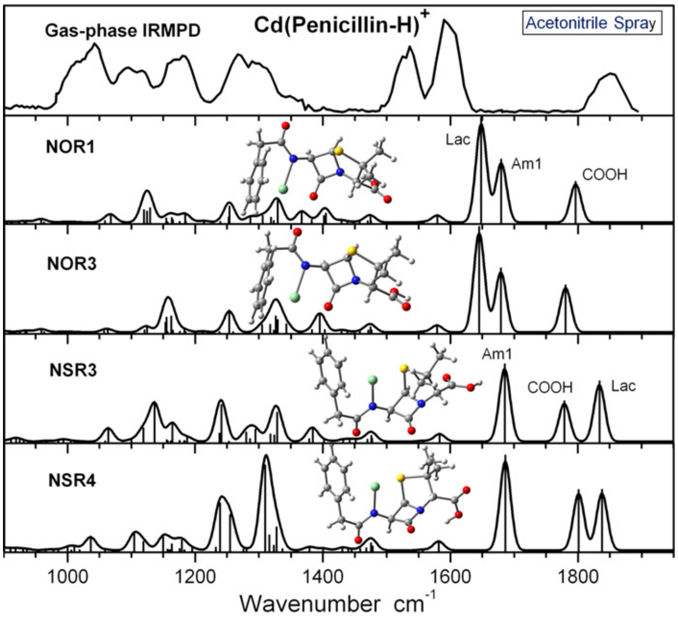

Fig. 6. IRMPD spectrum and possible calculated deprotonated-amide structures for the Cd(Penicillin-H)$^+$ complex sprayed from acetonitrile/water. The pair of NOR structures, and the pair of NSR structures, differ in the orientation of the hydroxyl on COOH.

4.4. Structure of the gas-phase Cd(Penicillin-H)$^+$ complex from acetonitrile solution

We have found it much easier to obtain useful ion abundances of doubly charged metal-ion complexes by electrospraying from acetonitrile (with ∼30% water) than from aqueous methanol solutions. This was found to be true for the difficult cadmium complex, which gave good parent ion abundances easily from acetonitrile, but unexpectedly the IRMPD spectrum of the resulting ion showed it to be drastically different from that of the ion described above, sprayed from methanol. The spectrum is shown in Fig. 6, and comparison with Fig. 5 immediately shows its different character.

It is a challenge to assign a structure to this spectrum, and no fully satisfactory match was found. Particularly for conjugated anions it is known that ESI from methanol versus acetonitrile solutions can generate different gas-phase isomers [42,43], resulting from the higher solvation energy of charge-localized structures in protic versus aprotic solvents, whereas such a preferential stabilization does not occur for charge-delocalized structures, which are preferred in the gas phase [44]. The absence of peaks between 1600 and 1800 cm^{-1} indicates that no carbonyl oxygen (amide, lactam or

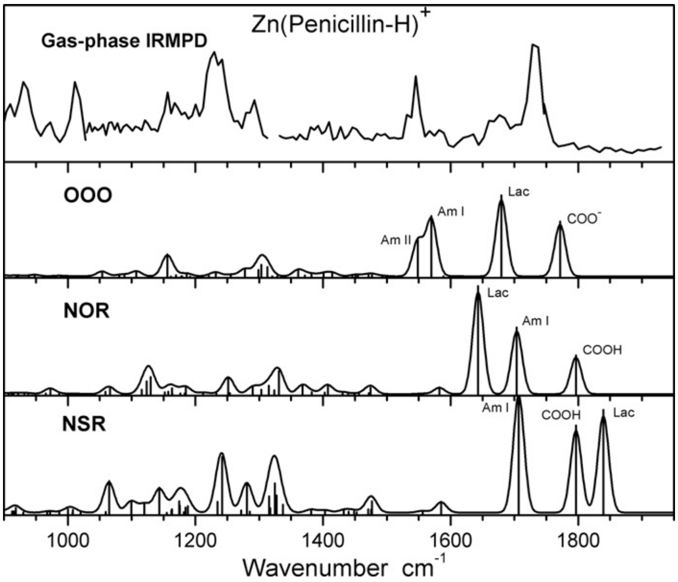

Fig. 7. IRMPD spectrum of Zn(Penicillin-H)$^+$ sprayed from acetonitrile/water solution. Calculated spectra are shown for representative structures of three possible types of gas-phase complexes.

carboxylate) is present and coordinated to the metal ion, while the strong peak at 1850 cm^{-1} indicates the presence of a free lactam carbonyl or a free COOH carbonyl. No reasonably stable complexes of native deprotonated penicillin G ligand could be found without stabilization of the system by metal chelation to both the amide and lactam carbonyls. However, two very favorable classes of complex were found in which a proton shifts to the carboxylate group from either the amide nitrogen or the ring sulfur, and is replaced by a covalently bound metal ion. Structures of these two types are shown in Fig. 3. Figs. 2 and 3 show that structures of these types are actually more stable than complexes of the native anion ligand for Zn^{2+} and, in some cases, for Cd^{2+}, although not for Ba^{2+}. No structure of this rearranged type was found that closely reproduces the observed spectrum of the Cd^{2+} complex. However, the representative calculated spectra shown in Fig. 6 are not hopelessly far off, showing that a free lactam carbonyl or a free COOH can account for a short wavelength peak in the vicinity of the peak observed at 1850 cm^{-1}. The observed spectral pattern might be matched more closely by a rearranged structure involving disruption of the β-lactam core not explored here.

4.5. Structure of the gas-phase Zn(Penicillin-H)$^+$ complex

It was found possible to form the Zn(Penicillin-H)$^+$ complex spraying from acetonitrile, and the spectrum was obtained as shown in Fig. 7. The assignment of the structure or structures giving rise to this spectrum is not clear. The most stable complex not involving major rearrangement is the OOO structure, for which the match to the high-frequency region (1400–1950 cm^{-1}) is unconvincing, but not impossible. Referring to the comparison with the calculated OOO spectrum in Fig. 7, the intense peak at 1730 cm^{-1} corresponds to the COO$^-$ asymmetric stretch, which appears lower than predicted by 30–40 cm^{-1}. The lactam C=O stretch is matched at 1675 cm^{-1}, although with lower intensity than predicted. The split peak at 1530–1540 cm^{-1} would correspond well to the prediction for overlapping Amide 1 and Amide 2 bands. The observed spectrum at frequencies lower than 1400 cm^{-1} bears no clear relation to the predicted peaks, although we can again note that this region is rich with hydrogen bending modes that do not appear to be well calculated for these systems, and lack of agreement in this wavelength region might not be surprising.

In contrast to the spectrum of the cadmium complex sprayed from acetonitrile, the zinc complex has no peak in the high-frequency region above 1750 cm^{-1}, which would correspond to the presence of a carbonyl group (lactam, amide, or carboxyl) not coordinated to the metal ion. The poor match in the high-frequency region of the spectrum to either of the representative deprotonated-amide structures shown in Fig. 7 illustrates this absence of unchelated carbonyl groups. Thus, while a rearrangement upon complexation to zinc is quite strongly suggested by the spectrum, such a rearrangement definitely does not have the same character as that induced by cadmium complexation.

5. Conclusions

The prominent features of the predicted and observed deprotonated penicillin G anion spectra are in good accord, and the structure assignment and the accuracy of the calculations seem well assured. Most of the features in the spectrum of the barium(II) penicillin G complex can be matched with calculated bands for a mixture of the two conformations that feature tridentate binding to the three accessible carbonyl-type oxygens of penicillin G. The most interesting spectroscopic point of the spectroscopic results for this ion is that the symmetric stretching mode of the metal-bound carboxylate group, which is so characteristic in the condensed phase, is either suppressed to relatively low intensity at its computed frequency near 1200 cm^{-1}, or its wavelength is calculated extremely erroneously by this level of theory.

It was unexpected that the cadmium(II) complex would exhibit completely different structures depending on the nature of the electrospray solvent. Obviously, one of the structures is a non-ground-state structure in which the complex is kinetically trapped in the gas phase. Based on the existence of at least one deprotonated-amide structure (NSR3) calculated to be more stable than any simple-complex structure, and the resemblance of the spectrum in Fig. 6 to spectra anticipated for various deprotonated-amide structures, we can suggest that the cadmium(II) complex structure from the acetonitrile electrospray is the gas-phase thermodynamic ground state. In this interpretation, the structure obtained from a methanol ESI solution could be the solution ground state (probably OOO), which is a simple addition complex unable to rearrange to a more stable covalently bound metal structure upon transfer to the gas phase. Alternatively the complex formed in methanol solution could be a kinetically trapped metastable conformation which is unable to rearrange in methanol to a more stable thermodynamic ground state. At this point it is not clear whether the postulated deprotonated-amide structure emerging from the acetonitrile spray is the stable form in acetonitrile solution, or whether acetonitrile assists the conversion of the complex into such a structure in the course of electrospray ionization.

Given this interpretation of the cadmium-complex spectroscopy, it is curious that the zinc complex, which is computed to have even more stable deprotonated-amide structures than the cadmium complex, shows no sign of such a structure in the acetonitrile-sprayed ions. On the other hand, it is known that the solution-phase penicillin degradation process initiating with metal ion/penicillin complexation is much slower for zinc ions than for cadmium ions [6], which is consistent with the present assignment of rearrangement of the cadmium complex but not the zinc complex (when both are sprayed from acetonitrile).

The speculative match of the Zn^{2+} complex spectrum with the OOO-structure is the best we have found, but we do not consider the structure of this species to be definitively settled. What seems well established is that the zinc and cadmium complexes characterized in Figs. 6 and 7 have different structures. These spectra are expected to be very similar if the structures are similar, whereas actually the observed spectra are quite different. The inference is that at least one of these structures involves a rearrangement away from the simple oxygen-chelated (OOO) structure, but this situation must be left for future clarification. The strongest conclusion from the spectrum of the zinc complex is that if a rearrangement takes place, it does not leave a lactam carbonyl oxygen unchelated, or form an unchelated COOH group, since the distinctive spectral signatures of these features are definitely absent.

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