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Hydrated complexes of tryptophan: ion dip infrared spectroscopy in the 'molecular fingerprint' region, 100–2000 cm^{-1}

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The infrared spectra of hydrated complexes of tryptophan have been recorded in the gas phase over the range 160–800 cm^{-1} using double resonance IR-UV ion dip spectroscopy. Despite the problems arising from severe UV spectral overlap of unresolved resonances, the IR measurements, combined with new DFT and *ab initio* calculations have allowed a re-assessment of the spectral assignments proposed in an earlier combined near-infrared/quantum computational investigation [L. C. Snoek, R. T. Kroemer and J. P. Simons, *Phys. Chem. Chem. Phys.* 2002, **4**, 2130]. It has reinforced the conclusion that hydration leads to conformational restructuring and also served to focus attention on the information that can be provided by spectroscopic measurements obtained in the mid and far IR.

1. Introduction

In a recent IR-UV ion dip spectroscopic study of the amino acid tryptophan, isolated in the gas phase,[1] it was possible to record the IR spectra in the N–H and O–H stretch region of its six lowest lying molecular conformers. This success depended upon the availability and separate resolution of distinct, non-overlapping UV spectral features that could be associated with one conformer at a time. Prompted by this and by earlier successful spectroscopic and structural investigations of the hydrated complexes of 3-propionic acid,[2] and of 2-indole acetic and 3-indole propionic acids,[3] the same strategy was subsequently applied to an investigation of the hydrated complexes of tryptophan.[4] Unfortunately, severe spectral overlap in their congested resonant two-photon ionisation (R2PI) spectra prevented the separate detection of individual species. Assignment of the IR ion-dip spectrum (recorded between 2900 cm^{-1} and 3800 cm^{-1} in the mass channel of the singly hydrated tryptophan ion) was further complicated by the broad and very diffuse features appearing towards the lower wavenumbers which could reflect spectral overlap or the broadening of spectrally shifted bands perturbed by hydrogen-bonded interactions.

The possibility of problems arising from spectral congestion was not unexpected.[4,5] The tryptophan molecule accommodates a flexible side chain which adopts at least six energetically low-lying conformations:[1,5] water molecules might bind to any of these at a number of alternative sites. Bound water molecules might select particularly favourable conformers but they might also change the preferred molecular conformation(s), to include perhaps, conformers that are not populated in the bare amino acid. Attachment of a water molecule to phenylalanine stabilises its second-most stable conformer of the monomer, which presents a favourable *syn* carboxylic acid structure; its minimum energy conformation presents an unfavourable *anti* structure and a 'closed' hydrogen-bonded network.[6] Understanding the influence of hydration on molecular conformation[7,8] and also on the electronic charge distribution in biological molecules,[9] particularly in the amino acids where multiple hydration must eventually lead to zwitterion formation,[4,10] is a key issue.

One way of addressing these issues is through appeal to quantum chemical computation, essential for interpreting and assigning the IR spectra of large and complex molecular assemblies (see for example, articles included in refs. 11 and 12). The earlier near-IR investigation of hydrated tryptophan[4] included a series of calculated structures and the corresponding O–H and N–H vibrational spectra of its lowest lying singly, doubly and triply hydrated clusters, computed using density functional theory (DFT), as well as their relative energies, computed *ab initio* and including electron correlation. Comparisons between the experimental data and the computed spectra, taking into account the relative energies of the computed structures, led to the tentative conclusion that the principal components of the observed UV and IR spectra were likely to be associated with triply hydrated complexes of a newly populated extended conformer of tryptophan, rather than singly or multiply hydrated complexes of the most favoured conformer of bare tryptophan.

Since completion of this study improved quantum computational codes[13] for calculations involving molecular complexes[14] have become available. Additionally, the successful application of a free-electron laser source (FELIX),[15,16] generating pulsed infrared radiation from the mid-IR down to the far IR region, to IR ion-dip studies of nucleic acid bases[17] and lactose[18] as well as to tryptophan itself[19] has encouraged a reinvestigation of the hydrated clusters of tryptophan.

The IR ion dip spectrum of hydrated tryptophan, measured over a broad spectral range from 160 cm^{-1} to 1800 cm^{-1}, has been recorded and analysed in the light of new *ab initio*

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calculations. An attempt has been made to disentangle the contributions to the IR spectrum from different tryptophan-water complexes. The study throws new light on the earlier tentative assignments that were based upon an analysis of the spectra in the N–H and O–H stretch region. The new data also serve to focus attention on the information that could be provided by extending spectroscopic measurements into the far IR and THz regions.

2. *Ab initio* calculations

The first computational investigation of the conformational landscape of singly and multiply hydrated complexes of tryptophan[4] was conducted using revision 7 of the Gaussian 98 package (G98.R7).[20] Since then some algorithmic errors in the treatment of hydrated clusters in that particular version of Gaussian have come to light and as discussed in ref. 14, it cannot be regarded as reliable; in consequence all of the hydrated tryptophan structures computed previously have been re-optimised using the Gaussian 03 (G03) package.[13] The calculations were conducted initially at the B3LYP/6-31+G(d) level of theory, which was also employed for computation of the harmonic vibrational frequencies. Dynamic electron correlation was accommodated by performing single point calculations at the second-order Møller-Plesset (MP2) level of theory on these structures, using a 6-311++G(d,p) basis set (MP2/6-311++G(d,p)//B3LYP/6-31+G(d)).

As anticipated, the optimised structures of hydrated tryptophan complexes and their relative energies calculated using G03 differed in some cases from those computed previously using G98.R7. Additionally, some of the local minima on the G98.R7 energy hypersurface disappeared and in several cases pairs of different starting structures resulted in a single structure on the G03 hypersurface. As a consequence all structures considered in the previous study[4] have been recalculated with G03. The resulting relative energies are given in Table 1, where the structural notation follows that employed in ref. 4. An extreme illustration of the different structures that can be obtained using the two versions of Gaussian is given in Fig. 1, which shows the superimposed minimum energy structures of a triply hydrated complex (conformer **X_b3**[4]) located at a relative energy 15 kJ mol^{-1} above that of the global minimum structure using G98.R7 but located only 3.51 kJ mol^{-1} above the global minimum using G03.

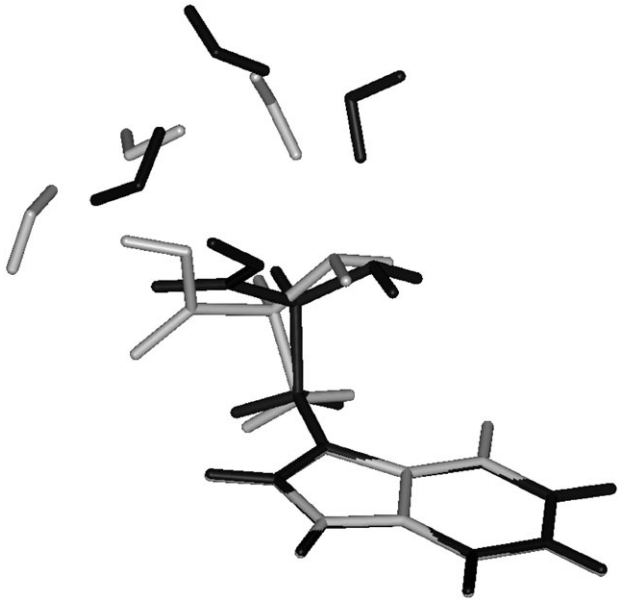

Fig. 1 Comparison of the optimised minimum energy structures (B3LYP/6-31+G(d)) of the triply hydrated complex, trp·W$_3$(**X_b3**) obtained using the Gaussian packages, G03 (dark gray) and G98.R7 (light gray). The most easily recognisable difference between both structures is the dihedral angle about the bond connecting the aliphatic chain and the indole group, but other, more subtle, differences, *e.g.* the arrangement of the water chain and the lack of planarity of the COOH group in the G98 structure, are also expected to have a large effect on the energy of the structures.

Table 1 Relative energies (E_{rel}/kJ mol^{-1}) calculated at the B3LYP/6-31+G* and MP2/6-311++G**//B3LYP/6-31+G* levels of theory for the most stable conformers of the trp·W$_n$ (n = 1, 2 and 3) clusters. The energies are corrected for the B3LYP/6-31+G* zero point energy. The tryptophan conformer labels follow those used earlier[4]

Conformer	E_{rel} (B3LYP)	E_{rel} (MP2)
Trp·W$_1$		
2b2	0.04	0
6a	3.09	1.28
2a1	0	2.14
5b	3.03	2.92
2b1	1.84	3.21
1a_COO	3.99	3.44
2a2	1.09	3.72
5a	3.86	7.89
7a	1.71	10.23
6b	2.61	11.55
7b	4.73	13.01
Trp·W$_2$		
2b	0	0
2a	1.19	10.74
5b	4.32	11.45
5a	4.23	15.21
7a	5.74	20.64
7b	8.63	23.38
Trp·W$_3$		
7a	0	0
7b	1.91	1.90
Y	4.11	3.24

Fig. 2 displays the structures of the lowest-lying singly, doubly and triply hydrated clusters, together with those of the three lowest energy conformers of bare tryptophan.[1] Single point energy calculations conducted at the MP2 level of theory lead to changes in their relative energies compared with those determined using DFT. This is exemplified when comparing the relative energies of the pair of doubly hydrated clusters trp·W$_2$ (**2b**), and (**2a**). For trp·W$_2$ (**2b**) a relatively strong stabilization of the complex is observed at the MP2 level of theory. Unlike trp·W$_2$ (**2a**), the structure of trp·W$_2$ (**2b**) facilitates an interaction between one of the bound water molecules and the π-electron cloud of the indole ring. Since this interaction is largely dispersive it is underestimated by the B3LYP functional, or DFT calculations in general. A recent study of the benzene.W$_1$ complex, which compares MP2 and B3LYP calculations (using a 6-311G(2d,2p) basis set) for water oriented in the 'down' geometry,[21] reports an energy contribution due to dispersive interaction of ~7 kJ mol^{-1}, a value very similar to the relative energy difference between the DFT and MP2 calculations in this study for trp·W$_2$ (**2b**). A less dramatic manifestation of such effects is revealed in the relative energies of the trp·W$_1$ (**2b2**) and (**2a1**) complexes, which are quasi degenerate at the DFT level: the MP2 calculation shows that the conformation (**2b2**) is more stable by *ca.* 2 kJ mol^{-1}, resulting from an H$_2$O–π interaction. The absence of this interaction in the lowest-lying triply hydrated complexes, trp·W$_3$ (**7a_b3**) and (**7b_b3**), explains why the relative energies computed for these structures at the MP2 level are virtually identical to those computed using DFT.

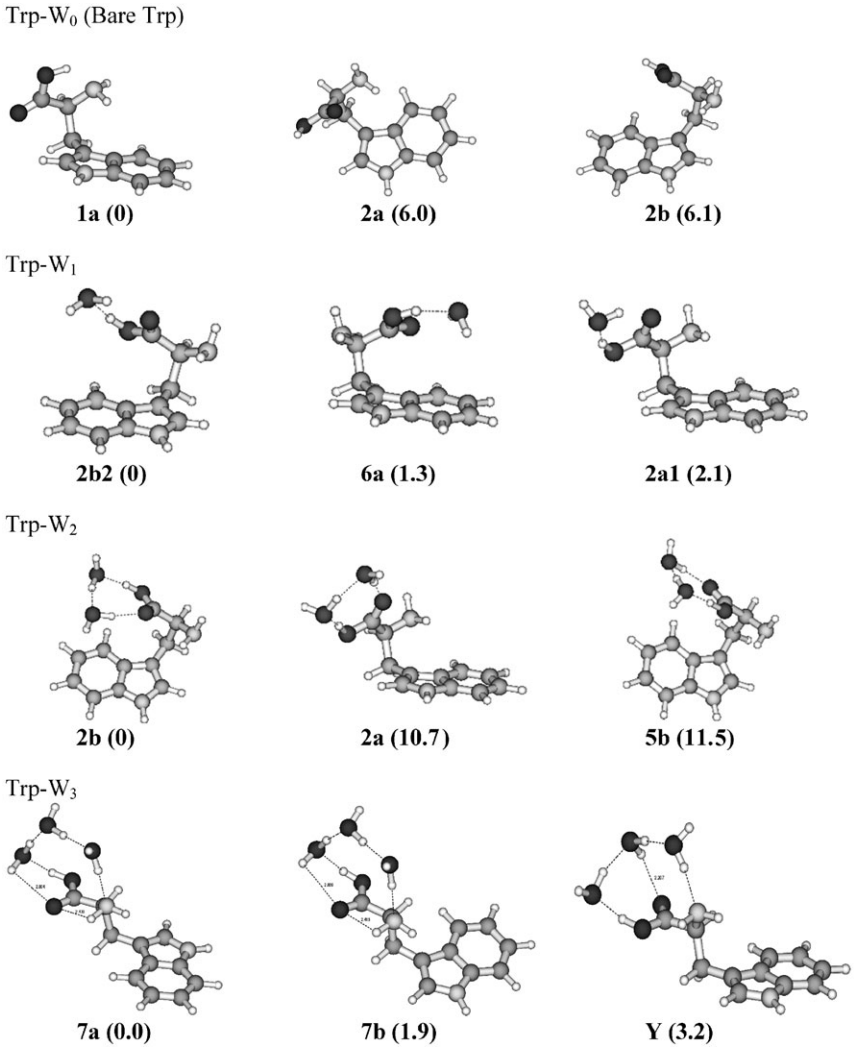

Fig. 2 Structures and relative energies of the lowest-lying conformers of hydrated tryptophan. Energies are given in kJ mol^{-1} at the B3LYP/6-31+G*//MP2/6-311++G** with B3LYP/6-31+G* zero point energy correction. Data and structures for bare tryptophan are taken from ref. 1.

3. Spectroscopy experiments

Tryptophan samples were mixed with graphite powder and applied to the surface of a graphite bar located directly below the orifice of a pulsed valve (R. M. Jordan, 0.5 mm diameter, 10 Hz pulse rate) where they were vaporised into an expanding pulsed Ar jet using a focused Nd:YAG laser (Thales Diva II, 1064 nm, <1 mJ per pulse, 10 ns pulse duration). The argon (mixed with water vapour prior to the expansion, mixing ratio $\approx 0.25\%$) had a backing pressure of 4 bar. Complexes of tryptophan and water were formed *via* many-body collisions in the collision zone of the supersonic expansion. The beam was skimmed and intersected by the IR and UV laser beams. Molecular ions created by R2PI were mass separated and detected using a linear time-of-flight mass spectrometer (Jordan). In the IR ion-dip experiments, the UV laser was tuned to the desired resonant two photon ionisation (R2PI) wavelengths. When the IR laser induced a transition in the molecular complexes, to transfer population out of the ground vibrational state, it led to a reduction in the detected ion signal.[8] The IR radiation was provided by the free electron laser FELIX,[15,16] which produces a train of ps laser pulses over a few μs, tuneable between 40 and 2000 cm^{-1} and with a bandwidth of approximately 1% of the central frequency (fwhm).

Strong ion signals were observed only in the singly hydrated mass channel, trp · $W_{n=1}^{+}$. The signals associated with higher complexes were either weak ($n = 2$) or undetectable ($n > 2$). Fig. 3 shows the R2PI spectrum monitored in the trp · W_1^{+} mass channel (222 u). The associated IR ion dip spectra were recorded using the same mass channel.

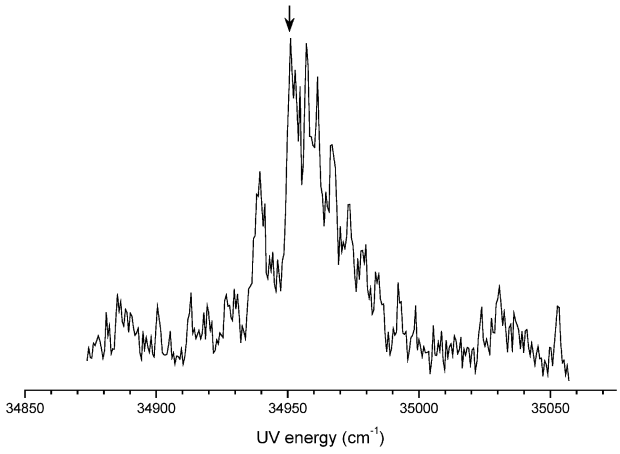

Fig. 3 R2PI spectrum of the hydrated complexes of tryptophan monitored in the trp · W_1^{+} ion mass channel. The arrow indicates the UV wavenumber (corresponding to the peak at 34950 cm^{-1}, labelled 'P1' in ref. 4) used to monitor the ion signal in the IR-UV ion dip experiments. Experiments using alternative UV features in the central region of the R2PI spectrum generated essentially identical IR ion dip spectra.

4. Results and spectral analysis

4.1. Mid-IR spectrum (700–2000 cm^{-1})

The mid-IR-UV ion-dip spectrum of hydrated tryptophan is shown in Fig. 4. Although the experimental spectrum is recorded in the trp · W_1^+ mass channel, it does not exclude contributions from larger clusters, such as trp · W_2 and trp · W_3, which are known to fragment in the UV detection scheme.[4] Consequently, spectra calculated for the most stable multiply hydrated conformational structures trp · W_2 (**2b**) and trp · W_3 (**7a**), as well as for trp · W_1(**2b2**), are also displayed, together with a composite spectrum for the two lowest-lying conformers of trp · W_3 (**7a**) and (**7b**) (tentatively assigned to the near IR spectra observed previously[4]). As the density of IR active modes in the mid-IR region is quite large the calculated spectra are presented as convolutions of the stick spectra with a Gaussian line shape function of the same width as the bandwidth of FELIX, ~1% of the central wavelength. From inspection it appears that none of the individual calculated spectra or the composite spectrum shown in Fig. 4 match the experimental spectrum.

The observed spectrum displays many more features than calculated for the most stable singly hydrated structure, trp · W_1 (**2b2**), consistent with contributions from complexes containing more water molecules and/or multiple conformers of singly hydrated tryptophan – though no plausible combination of spectra including only trp · W_1 conformers could be found to reproduce the experimental spectrum and in the previous study in the near infrared O–H and N–H stretch regions, no such combination could be found either.[4]

Fig. 5 compares the experimental IR spectrum with a calculated *composite* spectrum which includes, as an alternative trial, equal contributions from each of the most stable structures, trp · W_1 (**2b2**), trp · W_2 (**2b**) and trp · W_3 (**7a**). With some exceptions, identified in Fig. 5 by light and dark grey rectangles, its structure presents a much improved correlation with the observed spectrum encouraging a more detailed analysis; it begins with a review of the IR spectra of bare tryptophan conformers.[1,19]

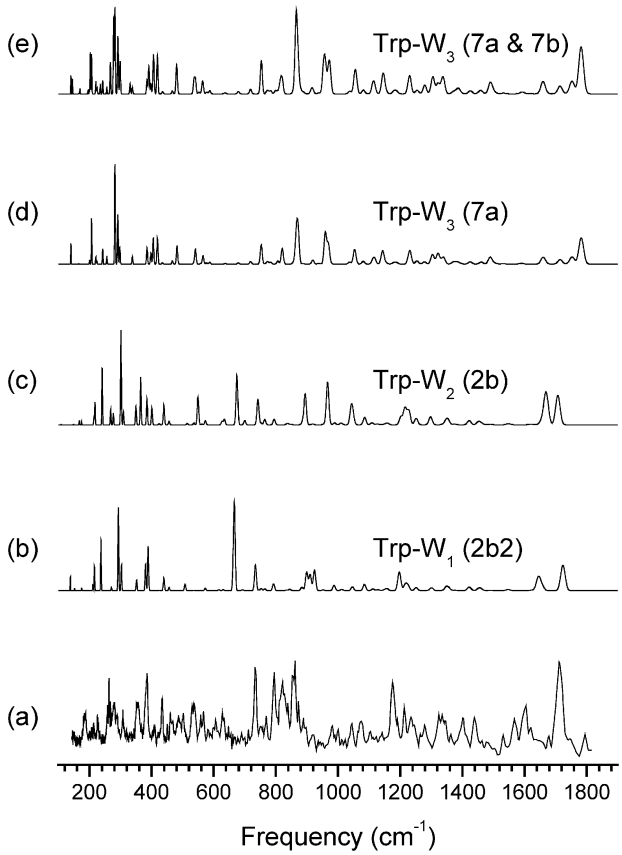

Fig. 4 Comparison of the experimental IR spectrum (a) of hydrated tryptophan complexes with the spectra calculated for the most stable singly, doubly and triply hydrated structures: (b) trp · W_1 (**2b2**), (c) trp · W_2 (**2b**), (d) trp · W_3 (**7a**) and (e) the calculated superposition spectrum of the structures, trp · W_3 (**7a**,**7b**).

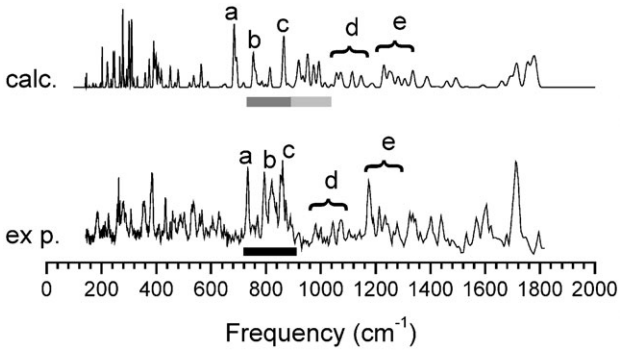

Fig. 5 Comparison of the experimental IR ion dip spectrum with a calculated superposition spectrum including equally weighted contributions from the most stable conformers trp · W_1(**2b2**), trp · W_2(**2b**) and trp · W_3(**7a**). The lines and groups of lines labelled a, b, c, d and e are discussed later in the text.

Bare tryptophan. The most stable conformational structure of bare tryptophan, (**1a**) (shown in Fig. 2), is not retained in the hydrated clusters, presumably because the bridging water molecule(s) favour a configuration in which the carboxylic acid group is twisted away from the *anti* conformation favoured by the *intra*-molecular OH → NH_2 hydrogen bond, into the *syn* conformation favoured by *inter*-molecular hydrogen bonding. Conformers (**2b**), (**2a**) and (**7a,b**), in Fig. 2, all display *syn*-conformations. It should be noted that while conformers (**2a**) and (**2b**) have been observed for the bare molecule,[1,5,19] conformers (**7a**) and (**7b**) have not.

A direct comparison of the mid-far IR spectrum of 'bare' trp (**2b**) with that of its hydrated complexes is not possible, since this conformer has not been observed yet in this spectral region, but fortunately that of the similar structure trp (**2a**) has been reported.[19] In Fig. 6 the observed mid-IR spectrum of trp (**2a**) is compared with the calculated spectra of trp (**2a**) and (**2b**), which both reproduce the experimental mid-IR spectrum well. There is some discrepancy around the spectral region labelled "2", which is dominated by the inversion motion of the amino group; the harmonic calculations predict this to lie some 60 cm^{-1} higher than observed.[19] On the other hand, there is very good agreement in the regions labelled "1" (*ca.* 750 cm^{-1}) and "3" (*ca.* 1100–1150 cm^{-1}), where the vibrational modes are associated with the indole CH umbrella modes and with the deformation modes of the amino acid group (mostly dominated by the HOC bending of the carboxylic acid group), respectively. Since these modes are well reproduced in the harmonic approximation for the monomer, they can be used as reference points for assigning the experimental spectrum of the *hydrated* amino acid. Given the calculated structures of the most stable hydrated complexes (Fig. 2), it is possible to predict qualitatively, the influence of bound water molecules on these two reference modes.

Hydrated tryptophan. In hydrated tryptophan the indole CH umbrella mode should not be greatly perturbed. This is confirmed by the calculations, which predict virtually no change upon single, double or triple hydration, allowing its assignment to the feature labelled "b" in Fig. 5. Fig. 7 shows the individual components of the composite spectrum shown in Fig. 5 as stick spectra. The intensity of the indole CH umbrella mode "b" is enhanced by the closely overlapping contributions from all

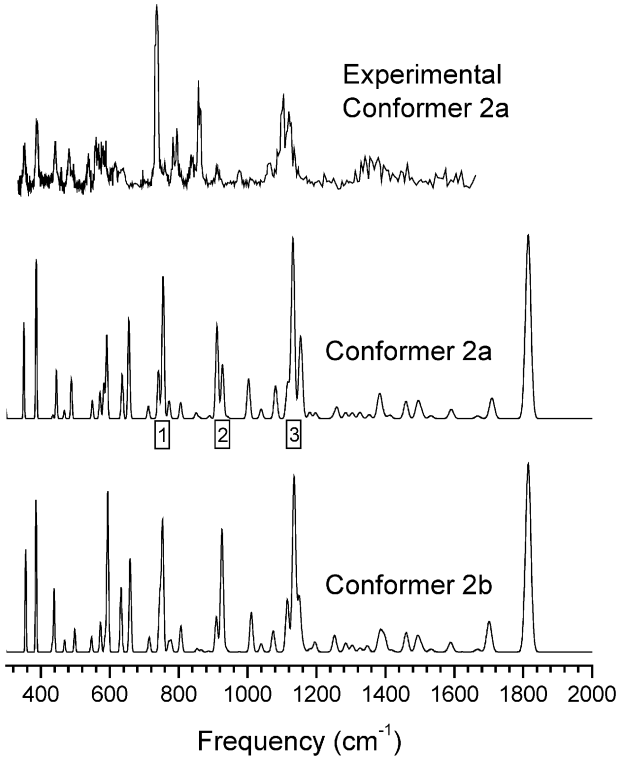

Fig. 6 Comparison of the calculated mid-far IR spectra of trp (**2a**) and (**2b**) and the experimental spectrum of trp (**2a**) observed by Bakker *et al.*[19] Note the similarity between the predicted IR spectra (**2a**) and (**2b**), and the good agreement with the experimental data, suggesting their usefulness for 'reference points' to help assign the hydrated tryptophan spectrum.

three clusters.† In contrast, the deformation modes in the hydrated amino acid group should be significantly perturbed by the attached water molecules; the directionality of the hydrogen bonding to the 'bridging' water molecule(s) will 'stiffen' the HCO angle and shift its deformation frequency from *ca.* 1100 cm^{-1} towards higher wavenumbers. The groups of lines labelled "e" in the calculated spectra in Figs. 5 and 7, located at *ca.* 1200–1300 cm^{-1}, can be assigned to the perturbed deformational modes of the amino acid group.

The features labelled "a" and "c" correspond to *inter*-molecular rocking modes of the bound water molecules. Their calculated frequencies and intensities are in good agreement with the experimental spectrum. There is also a good correspondence between calculation and experiment for the group of features labelled "d" in Figs. 5 and 7, which are associated predominantly with a coupled C–OH torsion and water rocking motions and result mostly from the trp · W_2 (**2b**) and trp · W_3 (**7a**) clusters.

The only striking disagreement between the experimental and theoretical spectra shown in Fig. 5 appears in the zones marked with the dark and light grey rectangles. The vibrational structure predicted by the calculation between 900 and 1000 cm^{-1} (light grey rectangle) is due to NH_2 inversion modes. Previous mid-IR studies of bare tryptophan[19] and of aniline[22] show this particular motion to be very poorly reproduced in the harmonic approximation; the calculated frequencies have to be shifted downwards by several tens to hundreds of wavenumbers to match the observed spectra. If the structure marked with the light grey rectangle were also shifted to lower wavenumbers, by *ca.* 100 cm^{-1}, it would lie in the region marked by the dark grey area where it would provide a better match with the group of experimental features located between 700 and 900 cm^{-1} (black area).

† A similar enhancement was shown for the indole NH stretch mode observed in the near IR.[4]

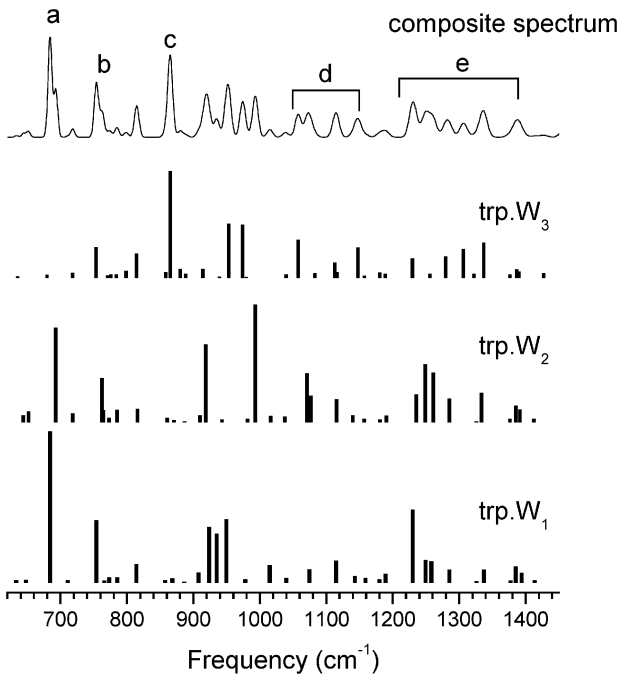

Fig. 7 Contributions made by singly, doubly and triply hydrated complexes to the convolution of the calculated superposition spectrum. The harmonic frequencies calculated for each component are represented as stick spectra. The labelling of the individual lines or groups of lines discussed in the text corresponds to those shown in Fig. 6.

The region between 1350 cm^{-1} and 1600 cm^{-1} is composed of a superposition of many weak absorption bands, dominated by ring breathing modes and by deformation modes of the carboxylic acid tail; it includes contributions originating from both singly and multiply hydrated clusters. The three strongest lines between 1650 and 1730 cm^{-1} can be assigned to H_2O bending modes, associated with the triply, singly and doubly hydrated clusters, (W_3, W_1 and W_2, running from low to high wavenumbers) and the remaining lines (1750–1800 cm^{-1}) can be assigned to CO stretching and coupled H_2O bending modes, associated with trp · W_2, W_1 and W_3 (again running from low to high wavenumbers).

4.2. Far infrared spectrum (100–700 cm^{-1})

The qualitative agreement between the calculated 'composite' mid-far IR spectrum and the observed spectrum is not maintained at wavenumbers below 700 cm^{-1}. Similar behaviour has been observed in the mid-far IR spectrum of the disaccharide *O*-benzyl-lactoside:[18] although there the experimental spectrum was in excellent correspondence with spectra computed *ab initio* throughout the spectral region 800–3800 cm^{-1}, the agreement below 800 cm^{-1} was qualitative at best.

The vibrational bands in the far IR are associated with large amplitude motions including torsional modes and delocalized vibrations involving global molecular motions. These can be strongly coupled and highly anharmonic and since the calculated frequencies are obtained by using the harmonic approximation, it is not surprising that the theoretical predictions fail in this region. The lack of agreement could also be explained by the poor description of dispersive interaction by DFT, though it is worth mentioning that for smaller systems, for example the principal conformer of bare tryptophan,[19] the agreement between experiment and calculation at the lowest energy end of the spectrum is much more satisfying.

5. Concluding remarks

Despite the severe spectral congestion, in both the UV and IR regions, which prevents the spectral isolation of the

individually resolved hydrated tryptophan clusters, it has been possible to analyse their composite IR spectrum on the basis of *ab initio* calculations and also by reference to earlier experimental studies of hydrated clusters and other 'isolated' molecules.[1–7,18,19,21–23] The analysis modifies and extends the conclusion reached in a previous study,[4] which identified the major contribution to the near-IR spectrum as resulting from the pair of triply hydrated tryptophan clusters, trp · W_3 (**7a,b**). The mid-IR data suggest important contributions are also made by its most stable singly and doubly hydrated complexes. This study also addresses a number of other important issues.

5.1. Conformational preferences

The computed conformations of the amino acid group adopted in the most stable hydrated complexes of tryptophan do not correspond to those that have been identified in the bare molecule but, not surprisingly, to those that offer the best binding *motifs* for water. This selectivity can be seen as a minimalist picture of conformational molecular recognition which plays such an important role in many biological processes. It is not clear whether the selection process is achieved in a passive manner or more interestingly, through active conformational change promoted by the non-covalent interaction with the approaching water molecule(s). Active dynamical conformational adaptation will depend on the intermolecular interaction strength and the potential energy barriers to conformational change. The vibrational motions associated with conformational change lie in the low energy region of the IR spectrum: a good reason to call for the implementation of theories able to describe correctly these motions.

5.2. Zwitterions: a correction

In the earlier investigation[4] an ion signal was apparently observed in the trp · W_3^+ mass channel when the tryptophan was vaporised by laser ablation but not when it was vaporised in an oven. The *ab initio* calculations indicated the possibility of zwitterion formation in the triply hydrated clusters. Following the new experiments reported here, the original measurements were reviewed. A careful recalibration of the TOF spectrometer identified the signal attributed earlier to trp · W_3^+ (mass 258 u) as a dimer formed by the indole fragment of tryptophan (dimer mass 260 u), which is produced more efficiently by intense laser ablation than by oven evaporation. Its IR-UV ion dip spectrum recorded between 3500 and 3800 cm^{-1}, presented a strong absorption band at 3525 cm^{-1}, associated with the indole NH stretch, but OH bands, which would have been expected for a hydrated zwitterion at higher wave numbers, could not be detected.

5.3. Low frequency modes

Spectroscopic interrogation of the highest energy vibrational modes, the OH or NH stretches, provides key structural information since their proton donor or acceptor roles support the hydrogen bonded networks that rigidify biomolecular conformational structures. In contrast, spectroscopic interrogation of the lowest energy modes can in principle, provide key dynamical information, for example changes in conformational shape evolving along torsional and bending coordinates. The ambient temperatures at which biological processes occur correspond to energies *ca.* 2.5 kJ mol^{-1} (200 cm^{-1}). Their spectroscopic interrogation using free electron[17–19] or THz[24] laser sources, stimulated Raman ion dip techniques[23] or stimulated emission population spectroscopy[25] is just beginning, but their analysis and interpretation depends upon the creation and development of new models for their theoretical description.

Acknowledgements

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