

The First Step in Glycine Solvation: The Glycine–Water Complex

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The jet-cooled spontaneous Raman spectrum of a glycine–water complex (Gly + H₂O), the first step in amino acid hydration, is reported. The low-frequency vibrational spectrum (below 500 cm^{−1}) of the solvated molecule is recorded and assigned using quantum chemical data calculated from *ab initio* (MP2) and DFT (B3LYP, BLYP, PBE0 = PBE1PBE). Anharmonic corrections to Raman and infrared (IR) active vibrations are calculated using second-order perturbation theory at the MP2/6-31+G(d) level. The acquired spectra at medium resolution (hwhm of ~4 cm^{−1}) allow different conformers of the glycine–water heterodimer to be distinguished. Three different dimer conformations are observed and identified; selective collision-induced relaxation processes are used to estimate their relative stability. The results are compared with recent theoretical predictions and microwave (MW) spectroscopy data. The premise that the acidic character of the OH group of the carboxylic acid dominates the interaction between water and glycine is confirmed. The addition of a water molecule is found to greatly change the potential energy surface and conformational preferences of H₂NCH₂COOH. Water stabilizes conformations in which formation of a closed-ring, H-bonded structure is possible. Simultaneous participation by the carboxyl oxygen of the amino acid in two hydrogen bonds is found to be unfavorable. It may be expected that the addition of extra water molecules could ultimately lead to the stabilization of the Gly zwitterion.

1. Introduction

Solvation is known to play a major role in the assembly of protein tertiary and quaternary structures, as well as in protein dynamics.¹ Water molecules interact with proteins on many length and time scales. Therefore, understanding the sophisticated process of biomolecule solvation is not possible without a complete understanding of the solvation processes relevant to its building blocks. Because of this fact, the formation of hydration shells around the simplest biomolecules has been extensively experimentally and theoretically investigated.¹

Despite their importance and the considerable effort spent on their study, biomolecule–solvent interactions are inadequately understood because of the considerable difficulty associated with these experiments.^{1c} Detailed hydration mechanisms are not yet clear, even for the simplest biomolecules (e.g., amino acids).

Characterization of the isolated clusters formed by an amino acid (AA) and water molecules offers considerable support for such studies, since the effects of intermolecular interactions are completely eliminated in the gas phase.^{1b}

In 2006, Alonso and co-workers^{1b} observed the hydration of glycine in a supersonic jet by using laser ablation in combination with Fourier transform microwave spectroscopy. The microwave (rotational) spectrum has revealed that water binds to glycine through two hydrogen bonds, which bridge the carboxylic acid group in a closed planar structure (image D1, Figure 1). It has been concluded that in the complex with a single water molecule, glycine retains the most stable conformer of the parent unhydrated molecule (Figure 1).

In 2010, the first observation of a gas-phase spontaneous Raman spectrum of an amino acid, glycine (Gly, H₂NCH₂COOH), was reported by Balabin.^{2c} *Ab initio* (MP2) and density functional theory (DFT) calculations with the large basis set

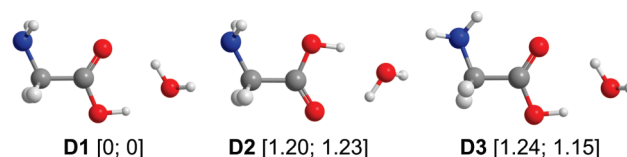


Figure 1. The three lowest-energy glycine–water complexes.^{1c,d,2c} The relative energy differences (in kcal mol^{−1}), calculated at the theoretical B3LYP and MP2 levels,¹¹ are shown in square brackets.

were used to support vibrational assignments. Jet-cooling of glycine molecules was observed by varying the laser–nozzle distance. The acquired medium resolution spectra have allowed the different glycine conformations to be distinguished. The structures of the two most stable glycine conformers, previously observed by electron diffraction and microwave spectroscopy, have been confirmed. Evidence has been reported for a third conformation (gauche–trans–trans), which has not been previously experimentally reported.^{2c}

In 2008, computations exploring the microsolvation of neutral and zwitterionic glycine were reported by Bachrach using the PBE1PBE density functional.^{1d} A broad configuration search was performed to identify the lowest-energy clusters of glycine with one to seven water molecules. The structures of the clusters are analyzed on the basis of the hydrogen-bonding network established among the water molecules and between water and glycine.

In this paper, we present the first vibrational spectroscopy study of a cluster formed by a nonaromatic AA and one water molecule. Glycine (Gly, H₂NCH₂COOH), the simplest amino acid, has been used. Two questions are addressed: (i) how a water molecule binds to an amino acid and (ii) what conformational changes are induced in Gly by this cluster formation.

Note that the lowest-energy conformer of glycine water complex (image D1 in Figure 1) has already been identified by

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TABLE 1: Calculated Harmonic Vibrations of the First Six Lowest-Energy Conformers of the Glycine–Water Complex (D1–D6) at the MP2/aug-cc-pVTZ Theory Level^a

	D1		D2		D3	
ν_{33}	61	(4)	28	(1)	69	(2)
ν_{32}	74	(1)	76	(2)	101	(4)
ν_{31}	142	(11)	152	(24)	144	(13)
ν_{30}	196	(6)	188	(0)	194	(50)
ν_{29}	215	(61)	248	(76)	198	(35)
ν_{28}	262	(81)	269	(80)	258	(100)
ν_{27}	291	(110)	303	(52)	313	(47)
ν_{26}	369	(59)	379	(62)	366	(61)
ν_{25}	493	(17)	515	(30)	491	(12)

	D4		D5		D6	
ν_{33}	31	(3)	32	(4)	58	(5)
ν_{32}	79	(2)	46	(29)	92	(19)
ν_{31}	114	(110)	119	(44)	124	(34)
ν_{30}	124	(9)	155	(520)	154	(15)
ν_{29}	163	(7)	215	(9)	169	(67)
ν_{28}	254	(28)	239	(30)	235	(83)
ν_{27}	293	(26)	291	(5)	330	(31)
ν_{26}	384	(122)	455	(73)	377	(54)
ν_{25}	472	(30)	469	(32)	381	(116)

^a Calculated Raman activities ($\text{Å}^4 \text{ a.m.u.}^{-1}$) are shown in parentheses.

microwave (MW) spectroscopy; however, this fact does not make extra studies by vibrational spectroscopy unnecessary. The MW and Raman techniques provide very different information about the molecules under study: rotational constants and geometrical parameters (MW) and vibrational frequencies (IR/Raman). Together, these values (the coordinates of the minimal points and the second derivatives) result in better experimental description of the molecular potential energy surface (PES).

2. Experimental Section

2.1. Raman Spectroscopy. A high-sensitivity Raman spectrometer containing a high-power 457 nm CW DPSS laser (19.8 W) and a retroreflecting multipass (67-pass) cell was used for the experimental Raman spectral characterization.^{2a,b} The spectrometer was modified to maximize signal intensity and stability at the expense of a lower resolution and a reduced frequency range. The setup was equipped with a jet-cooled source containing a heatable valve ($\leq 250^\circ\text{C}$, $\pm 0.05^\circ\text{C}$) and housed a stainless steel nozzle ($625 \times 250 \mu\text{m}$; $D_{\text{eff}} = 446 \mu\text{m}$).

A glycine sample (>99.9% purity) supplied by the Novosibirsk Institute of Organic Chemistry (Novosibirsk, Russia) was used throughout the study. A He/Ne/Ar gas mixture (ratio 85:14:1) was used as a carrier gas. Gaseous glycine with a partial pressure of ~ 10 Pa was expanded from a gas reservoir (183°C) through the nozzle into a chamber. The smallest possible vapor pressure of water was used to exclude the possibility of formation of other clusters. Spectra were collected for 24 h, and 3–14 spectra were averaged.

The spectrum of water, recorded under the same experimental conditions, was subtracted to eliminate water dimer/trimer influence.^{3,4} A signal-to-noise ratio (SNR; before filtering) that exceeded 5.2 was achieved.

2.2. Computational. Quantum chemistry ab initio (MP2) and DFT calculations with the aug-cc-pVTZ basis set were used to interpret the spectroscopic results.^{2–4} Structures of 10 glycine–water complexes (see Figure 1 for the three lowest-energy ones) were optimized at B3LYP/aug-cc-pVTZ, BLYP/aug-cc-pVTZ, PBE0/aug-cc-pVTZ, and MP2/aug-cc-pVTZ, and vibrational

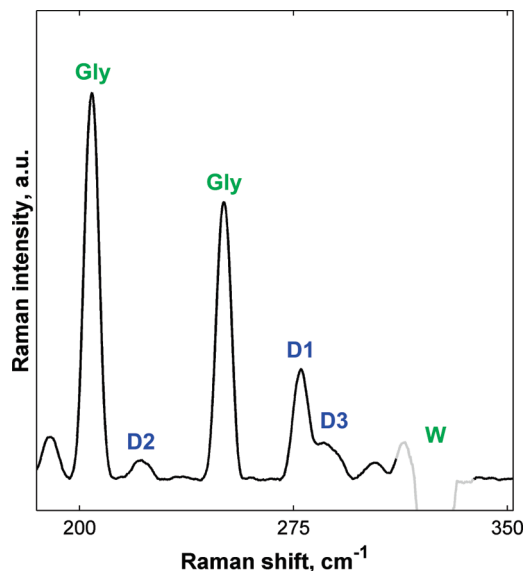


Figure 2. Experimental jet-cooled Raman spectra of glycine–water complexes ($x/D_{\text{eff}} = 3.6$) after water spectrum subtraction and Savitzky–Golay filtering. The region containing water dimer vibrations (W, $310\text{--}340 \text{ cm}^{-1}$) is shown in gray. Spectrum was collected for 24 h; 12 spectra were used for averaging. See Figure 3 here and Figure 2 in ref 2c for an approximate noise level in the spectral data.

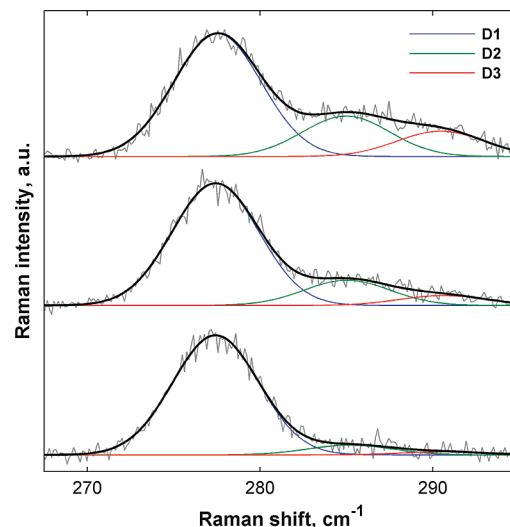


Figure 3. Deconvolution of the $272\text{--}294 \text{ cm}^{-1}$ band system. Laser–nozzle distance (x/D_{eff}) is 3.6 (top), 5.4 (middle), and 7.2 (bottom). Note that formation of higher molecular weight clusters (e.g., Gly + 2W) is not expected at these laser–nozzle distances.²

analyses were conducted on these structures (Table 1). Note that $\text{PBE0} = \text{PBE1PBE}$.

Anharmonic (PT2) calculations at the MP2/6-31+G* theory level were also performed.⁵ The MP2 level of theory was found to be much less dependent on the basis set size than DFT-B3LYP,^{2d} so it was chosen for anharmonic correction calculation. Standard Gaussian 03 parameters were used for anharmonic correction calculations.⁵

3. Results and Discussion

Figure 2 depicts the experimental Raman spectrum of the glycine–water mixture observed in the jet stream. Free glycine peaks that did not disappear when the water stream was turned off were observed at 204 , 250 , and 303 cm^{-1} .

The first two originated from the *ttt* (**Ip**) glycine conformation, and the third originated from the *ccc* (**IIn**) conformer.^{2c} For the

TABLE 2: Comparison of Experimental and Calculated Parameters of Raman Active Vibrations of Glycine–Water Complexes (D1–D3)

		frequency, cm ⁻¹		depolarization ratio (×10 ²) ^a	
		calcd (anharm.)		exptl	calcd
		exptl	MP2 corr		
D1	ν_{29}	190.0 ± 2.0	201	75 ± 5	74
	ν_{27}	277.5 ± 1.2	277	20 ± 8	22
D2	ν_{29}	217.2 ± 2.0	195 (203) ^b	78 ± 12	74
	ν_{28}	223.8 ± 1.9	262 (230) ^b	70 ± 12	74
	ν_{27}	285.0 ± 2.5	292	25 ± 12	22
D3	ν_{27}	290.4 ± 3.1	302	66 ± 11	73

^a Ref 10. ^b With anharmonic correction from ν_{29} of **D1**. See text for details.

experimental parameters used, no signal from the *gtt* (**IVn**) conformation was observed; this seemed to selectively relax to a global minimum structure (*ttt*).

Six Raman peaks of glycine–water complexes can be seen in Figure 2. Three of these peaks overlap and produce one broad band in the 272–294 cm⁻¹ region. Two well-separated peaks were identified to originate from the **D1** (190 cm⁻¹) and **D2** (~220 cm⁻¹) glycine–water heterodimers. The latter may have been produced by two close vibrations that, after deconvolution, appear to be at 217 and 224 cm⁻¹ (see Table 2).

Table 2 depicts the comparison between experimental and theoretical vibrational frequencies of glycine–water complexes. It can be seen that anharmonic calculation (even at the relatively low MP2/6-31+G* level of theory) was required to correctly predict frequency ranges. This was especially true when water was participating in the molecular vibrations. Harmonic calculations were inadequate, even at relatively high theory levels, resulting in mean absolute deviations of 40–50 cm⁻¹ or 13–17%. The MP2/6-31+G* theoretical level seemed to incorrectly describe the Fermi resonance between the ν_{28} and ν_{29} modes of **D2**. When anharmonic correction from the ν_{29} mode of **D1** was applied, much better agreement between the theoretical and experimental values was achieved. It is important to note that depolarization and intensity ratios were necessary to unambiguously assign conformer vibrations.

From a computational point of view, Gly alone is a much simpler system than Gly + 1W, especially in a vibrational low-frequency range. First of all, intermolecular complex is in need of a larger basis set and higher-level electron correlation treatment for accurate energies and vibrational analysis. Second, the RRHO approximation is much better fulfilled for a single molecule than for its complex.^{2c}

Figure 3 shows a deconvolution of the large 3-band system in the 272–294 cm⁻¹ spectral region. All three bands were clearly visible at small distances (*x*) between the laser and nozzle. The intensity (*I*) ratios of the peaks seemed to significantly change along the jet stream. By comparing these ratios, conclusions about the relative stability of different dimers could be drawn. I_{D2}/I_{D1} changed from 0.31 ± 0.02 to 0.21 ± 0.02 and, finally, to 0.08 ± 0.02 for an x/D_{eff} of 3.6, 5.4, and 7.2, respectively. The corresponding intensity ratios for the **D3/D2** pair (I_{D3}/I_{D2}) were 0.63 ± 0.10, 0.40 ± 0.10, and 0.31 ± 0.23. Thus, the experiment suggests that the relative stabilities of the observed glycine–water complexes satisfied the relation **D1** > **D2** > **D3**. This result (**D2** > **D3**) confirmed the PBE1PBE and B3LYP data, but it contradicted the HF and MP2 results.^{1c,d}

Note that neither zero-point energy (ZPE) nor basis set superposition error (BSSE) corrections were performed in the

reported calculations.⁶ The effect of ZPE or BSSE correction can significantly change the relative energy (enthalpy, free energy) values, predicted by quantum chemistry.

Since the predicted energy difference between **D2** and **D3** was very small (<0.1 kcal mol⁻¹), extra theoretical work is needed to calculate more precise energy values. After such calculations, a more certain conclusion can be drawn about the consistency between experimental and theoretical results. Note that the need for extra theoretical work is even more evident, considering that the mathematical manipulation (deconvolution) of the vibrational bands in the 272–294 cm⁻¹ region of the experimental Raman spectra is required.^{7,8}

Recently, Alonso et al.^{1b} experimentally studied water binding to Gly using molecular-beam Fourier transform microwave spectroscopy (LA-MB-FTMW). The structural parameters obtained by microwave spectroscopy showed that the acidic character of the OH group of the carboxylic acid dominated the interaction between water and *ttt* glycine (**D1**).^{1b} Alonso et al.^{1b} were able to observe the **D1** structure due to only either the small dipole moment components of other dimers or their relaxation upon collision. Our results confirm the possibility of the selective relaxation of Gly–H₂O conformation under jet-cooled conditions.

Good spatial resolution (ability to scan the molecular beam at different distances to the nozzle) greatly increased the probability of finding conformers, other than the one with the lowest energy, using Raman spectroscopy. It allowed these conformational changes, induced by hydration, to be experimentally observed. Raman spectroscopy data showed that the addition of just one water molecule greatly changed the conformational preferences of the amino acid. In the presence of water, the *ttt*-type conformation still had the least energy, as was expected, since extra hydrogen bonds do not compete with the intramolecular hydrogen bonds in *ttt* glycine; however, **D2** became the second-lowest-energy structure in the presence of water, whereas it had only the fourth (*tct*, **IIIp**)-lowest energy in free glycine. The possibility of forming a closed-ring structure (in which the water bridge benefits from an enhanced cooperative effect^{1b,7}) decreased the relative energy of this conformer by 0.50 kcal mol⁻¹ (MP2). Note that CCSD(T) or higher-order results can be different. In contrast, the participation of carbonyl oxygen in two H bonds simultaneously made the *gtt* structure less stable by ~0.10 kcal mol⁻¹.

4. Conclusions

In conclusion, the formation of three different glycine–water clusters was observed using Raman spectroscopy. One can claim that the addition of a water molecule changed the glycine potential energy surface and its conformational preferences.^{3,4,7} The shift of vibrational frequencies by 10–25% is observed.

Water stabilized conformations in which the formation of a closed-ring H-bonded structure was possible.⁸ Participation of Gly hydroxyl oxygen in two hydrogen bonds simultaneously was unfavorable (note that absence of *ccc*-type structure). However, one might ask whether the absence of the *ccc*-based structure is due to the inability of the hydroxyl group to form two hydrogen bonds (one as donor and one as acceptor) or of the inability of the water molecule to form two hydrogens bonds (acting as a donor for each bond). Such an alternative (“water-based”) point of view is, of course, possible.¹²

It may be expected that the addition of extra water molecules could lead to other changes in the glycine PES, ultimately leading to the stabilization of the Gly zwitterion (⁺H₃NCH₂–COO⁻).⁹

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