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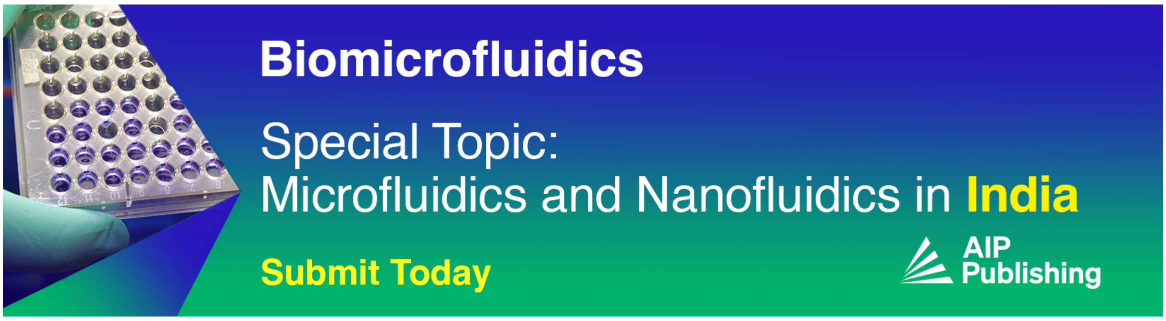

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Fourier transform infrared spectroscopic and theoretical study of water interactions with glycine and its *N*-methylated derivatives

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In this study we attempt to explain the molecular aspects of amino acids' hydration. Glycine and its *N*-methylated derivatives: *N*-methylglycine, *N*,*N*-dimethylglycine, and *N*,*N*,*N*-trimethylglycine were used as model solutes in aqueous solution, applying FT-IR spectroscopy as the experimental method. The quantitative version of the difference spectra method enabled us to obtain the solute-affected HDO spectra as probes of influenced water. The spectral results were confronted with density functional theory calculated structures of small hydration complexes of the solutes using the polarizable continuum model. It appears that the hydration of amino acids in the zwitterionic form can be understood allowing a synchronized fluctuation of hydrogen bonding between the solute and the water molecules. This effect is caused by a noncooperative interaction of water molecules with electrophilic groups of amino acid and by intramolecular hydrogen bond, allowing proton transfer from the carboxylic to the amine group, accomplishing by the chain of two to four water molecules. As a result, an instantaneous water-induced asymmetry of the carboxylate and the amino group of amino acid molecule is observed and recorded as HDO band splitting. Water molecules interacting with the carboxylate group give component bands at 2543 ± 11 and 2467 ± 15 cm^{-1}, whereas water molecules interacting with protons of the amine group give rise to the bands at 2611 ± 15 and 2413 ± 12 cm^{-1}. These hydration effects have not been recognized before and there are reasons to expect their validity for other amino acids. © *2011 American Institute of Physics*. [doi:10.1063/1.3567202]

INTRODUCTION

Amino acids are the basic building blocks of all proteins. Glycine is the simplest amino acid and its interactions with surrounding water molecules are of particular biological interest. Glycine and its *N*-methyl derivatives belong to a class of small organic compounds (osmolytes) produced in living cells under unfavorable environmental conditions.[1–3] It is believed that osmolytes stabilize the native state of globular proteins rather by modification of solvent properties than direct interactions with them.[4–10] In spite of many studies, the detailed mechanism of this stabilization is not completely understood.

It is known that the glycine molecule exists in the neutral form in the gas phase, whereas in aqueous solution and in the solid state, the zwitterionic conformer is the predominant form.[11–17] The zwitterionic form of glycine is stable in solution thanks to favorable interactions with the solvent. Many theoretical works on the stability of glycine zwitterion were carried out.[18–35] Some calculations indicate that the stability of the zwitterionic form in solution arises from direct intramolecular proton transfer from the carboxylic to the amino groups.[19,21,31,32] Another computational results support a mechanism where water molecules mediate this transfer.[18,20,22–24,26–29,33–35] These studies suggest that one water molecule is not enough to stabilize the zwitterionic structure of glycine.[18,26,29] However, two water molecules are able to stabilize the glycine zwitterion.[18,23] Calculations by Kassab *et al.*[20] and Chaudhari *et al.*[28] show that three water molecules are needed to stabilize the zwitterion conformer of glycine.

Glycine and its *N*-methyl derivatives were extensively studied experimentally by means of IR,[11,35–38] Raman[36–40] and dielectric relaxation[41] spectroscopy, neutron diffraction,[42,43] by calorimetry[12] and theoretically by *ab initio* calculations with various basis sets[4,11,18,20,23,24,26,28,29,33–35,37,38,44–47] and by simulations with both Monte Carlo[4,48] and molecular dynamics (MD) (Refs. 25, 27, 31, and 49–52) methods, including also *ab initio* molecular dynamics.[27,53] These studies present different views of the interactions between these amino acids and surrounding water.

We specialize in gaining subtle information about the energetic and structural state of water molecules hydrating different solutes on the basis of vibrational spectra of water in solution.[54] In the case of amino acids we expected specific solvent-induced effects of symmetry breaking of amino acid hydrophilic groups by the analogy to the result recently obtained for carboxylate anions.[55] Spectral studies exclusively on amino acid as a solute could not be sufficient, however, because common and nonadditive influence of the carboxylate and the amino group on water hydration shell of the amino acid molecule could be expected. Therefore, a series of *N*-methylated derivatives of glycine have been studied in this work, as solutes in aqueous solutions, to help understand hydration of glycine and thus other amino acids.

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Vibrational spectroscopy is an ideally suited method for investigation of solute hydration. The time scale of the response of vibrational spectroscopy, ~10^{-14} s in the range of water stretching vibrations, allows registering even relatively short-lived species formed by water molecules influenced by a solute. Vibrations of semiheavy water (HDO), isotopically diluted in H_2O, have been used as a sensitive probe of local molecular surroundings and thus of solute hydration. HDO spectra are free of the interpretative and experimental problems connected with spectra of ordinary water. To receive information about the structure and interactions inside the hydration sphere, the contribution of bulk water to the spectrum should be removed as to obtain the solute-affected water spectrum. This has been done applying the quantitative version of the difference spectra method developed in our laboratory. More details on this and related topics can be found in the recent review.[54]

In this paper, we present results of the FT-IR spectroscopic study on the hydration properties of glycine (Gly) and its *N*-methylated derivatives: *N*-methylglycine (NMG, sarcosine), *N*,*N*-dimethylgycine (DMG), and *N*,*N*,*N*-trimethylglycine (TMG, betaine) in aqueous solution. The interaction between water molecules and the studied solutes, and the structure of water around them, were investigated on the basis of the band shapes of solute-affected HDO spectra. The obtained experimental results were confronted with DFT calculated structures of solute aqueous clusters, using the polarizable continuum model of solvation.

Direct observation of the solvent dynamics for aqueous glycine solutions was rarely attempted before. It can be indirectly inferred from the experimentally observed rates of vibrational relaxation of glycine CH stretching fundamental.[56,57] In these investigations the H_2O (Ref. 56) or D_2O (Ref. 57) stretching fundamental was used as a "molecular thermometer," as the vibrational excitation of the solute molecule is eventually released to the surrounding bath. In D_2O, the thermometer response time constant was 7.2 ps, larger than the 1.8 ps time constant of pure solvent.[57] This hints at a slower water dynamics in the glycine hydration sphere. This notion is further supported by the measurements of anisotropy decay of femtosecond midinfrared spectra of aqueous solutions of some hydrophobic solutes, including the amino acid proline.[58] The reorientation of water (HDO) molecules could be represented by a fast (~2 ps) component as in pure water and a slow (>10 ps) component associated with solvation shell water molecules. The presence of the latter indicates a large degree of water "immobilization" in the vicinity of hydrophobic groups (in glycine, the single methylene group is expected to effectively immobilize ~4 OH groups).[58]

The results cited above validate, to a large extent, our methodology of comparison of the static vibrational (difference) spectra with the static *ab initio* calculations. The clusters that we believe to represent the situation around the solute molecule may in fact correspond to fairly stable hydration complexes, albeit engaged in a dynamic equilibrium, rather than being just transient forms. The time scale of the phenomena studied with dynamic experiments indicates that they might influence the static linear IR experiments as well.

EXPERIMENTAL PROCEDURES

Chemicals and solutions

Glycine (99%, Sigma), sarcosine (*N*-methylglycine, ≥99%, Fluka), *N*,*N*-dimethylglycine (>98%, Alfa Aesar), and anhydrous betaine (*N*,*N*,*N*-trimethylglycine, ≥99%, Fluka) were used without further purification. D_2O was purchased from Aldrich (99.96% deuterium).

Stock solutions were prepared by dissolving weighted amounts of solutes in deionized water. Sample solutions for the HDO spectra were made by adding 4% (by weight) of D_2O relative to H_2O ($H_2O + D_2O = 2HDO$, $K \approx 4$) and the reference solutions (without D_2O) by adding the same molar amounts of H_2O. All solution densities were measured using an Anton Paar DMA 5000 densitometer at 25.000 ± 0.001 °C.

The series of glycine and NMG solutions were in the range of molality (m) between 0 and 1 mol kg^{-1} at an approximate step of 0.2 mol kg^{-1}. For TMG and DMG solutions molalities were in the range between 0 and 2 mol kg^{-1}.

IR measurements

FT-IR spectra of aqueous solutions of glycine and its *N*-methylated derivatives were recorded on Nicolet 8700 spectrometer (Thermo Electron Co.) with a resolution of 4 cm^{-1} in the range of 500–5000 cm^{-1}. One hundred twenty-eight scans for each spectrum were made. The spectrometer was purged with dry nitrogen. A cell with CaF_2 windows was used and the path length of the cell was 0.0289 mm, as determined interferometrically. The temperature was monitored by a thermocouple inside the cell and was kept at 25.0 ± 0.1 °C by circulating thermostated water through the mounting plates of the cell.

Computational details

The optimized geometries and energies of the nonhydrated and hydrated structures of glycine and its *N*-methyl derivatives were calculated using density functional theory (DFT) with Becke's three parameter (B3) exchange functional, along with the Lee–Yang–Parr (LYP) nonlocal correlation functional (B3LYP).[59,60] Calculations were performed with the use of a standard Pople's 6-311++G(d,p) basis set.[61,62] The polarizable continuum model (PCM) formalism of self-consistent reaction field theory (SCRF) was used to study systems in the liquid phase.[63–65] All calculations have been carried out using the GAUSIAN 03 program package.[66] The program HyperChem 8 (Hypercube, Gainesville, FL) was applied to the preparation of entrance data and for visualization of computed results.

Spectral data analysis

The difference spectra method has been applied to isolate the solute-affected HDO spectrum, on the basis of analysis of spectra series measured for different concentrations of aqueous solutions and the bulk HDO spectrum.[67,68] The method is based on the assumption that it is possible to divide water in solution into two additive contributions: the bulk water and the affected water. Its validity has been independently proven by molecular dynamics simulations[69,70] as well as by the chemometric analysis of solvent infrared spectra in solutions.[71] The mentioned contributions correspond to the spectrum of bulk water (identical to the pure HDO spectrum), ε_b, and to the solute-affected water spectrum, ε_a, respectively. Accordingly, the total concentration of water in solution (c, mol dm^{-3}) is equal to $c_a + c_b$, then the mean molar absorptivity of water in solution (ε) is equal to $(\varepsilon_{a}c_{a} + \varepsilon_{b}c_{b})/c$. Hence,

$$\varepsilon_a = \frac{c}{c_a}(\varepsilon - \varepsilon_b) + \varepsilon_b, \qquad (1)$$

or in molalities,

$$\varepsilon_a = \frac{1}{NM}\frac{(\varepsilon - \varepsilon_b)}{m} + \varepsilon_b, \qquad (2)$$

where N parameter is the "affected number", equal to the number of moles of water affected by 1 mole of solute, M is the mean (4% D_2O in H_2O in the case of HDO spectra) molar mass of water (kg mol^{-1}), and m is the molality of the solution (mol kg^{-1}).

The fraction $(\varepsilon - \varepsilon_b)/m$ can be referred to as the difference spectrum, though in reality the spectral difference $\varepsilon - \varepsilon_b$ is additionally scaled by molality of the solution. When $m \to 0$, Eq. (2) reduces to

$$\varepsilon_a = \frac{1}{NM}\left(\frac{\partial\varepsilon}{\partial m}\right)_{m=0} + \varepsilon_b. \qquad (3)$$

Equation (3) gives the basis for the extraction of the solute-affected water spectrum, extrapolated to infinite dilution.

The derivative $\partial\varepsilon/\partial m$, in the infinite dilution limit, is obtained by an approximation of the molar absorption coefficient value as a function of molality at each wavenumber. The use of linear regression for glycine and quadratic regression for the other solutes ($R^2 > 0.9997$) was justified.

According to Eq. (3), it is possible to assign the solute-affected water spectrum to the known affected number N. The proper value of N can be found by analyzing the whole series of trial affected spectra for different values of N. These spectra are fitted using a linear baseline, a minimal number of analytical bands (which give adequate fit), and the bulk water spectrum. The product of Gaussian and Lorentzian peak functions is used as the starting analytical band shape. All parameters (including Gaussian to Lorentzian ratio) are unconstrained during the fit, with the exception of the bulk solvent spectrum, for which only intensity is allowed to vary. The maximum value of N, for which the contribution of the bulk water spectrum in the solute-affected water spectrum is negligible (the practical threshold value is set at 0.5% of the total integrated intensity of the ε_a spectrum), is treated as the "true" value of N, and the corresponding spectrum as the true affected water spectrum. The detailed procedure of spectral data analysis toward extraction of the solute-affected water spectrum is described in Refs. 67 and 68. This method is used extensively in our laboratory to investigate solute–solvent interactions.[55,69,70,72–75] It can be added that the recently developed method of chemometric analysis of HDO spectra[71] provided essentially the same results that the method applied in this work.

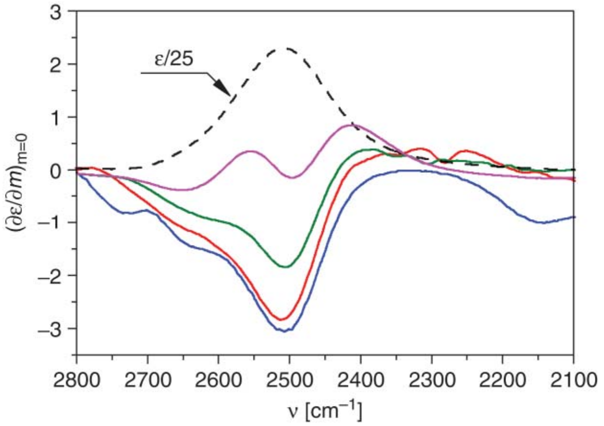

FIG. 1. Derivatives $(\partial\varepsilon/\partial m)_{m=0}$ in the OD stretching region for aqueous solutions of Gly (blue), NMG (red), DMG (green), and TMG (pink). The bulk HDO spectrum (black, dashed) has been shown for comparison purposes (in molar absorptivity scale divided by 25).

All spectra have been handled and analyzed using commercial PC softwares: OMNIC (Thermo Electron Corporation), GRAMS/32 (Galactic Industries Corporation, Salem, USA), and RAZOR (Spectrum Square Associates, Inc., Ithaca, USA) run under GRAMS/32.

RESULTS AND DISCUSSION

Spectral results

Figure 1 shows the derivatives $(\partial\varepsilon/\partial m)_{m=0}$ for ν(OD) vibration band of HDO in aqueous solutions of Gly, NMG, DMG, and TMG, which determine the corresponding solute-affected HDO spectra according to Eq. (3) and the band-fitting procedure summarized above. All solute-affected spectra have been collected in Fig. 2, together with the bulk HDO spectrum. They have been obtained for the following affected numbers: $N = 5.8$ for Gly, $N = 5.0$ for NMG, $N = 4.8$ for DMG, and $N = 4.0$ for TMG. They can be considered as "affected spectra" in a diluted solution, theoretically infinitely diluted, due to the extrapolation procedure applied.

Except for the TMG-affected HDO spectrum, the other spectra in Fig. 2 include not only the absorption of HDO but also absorption contribution of isotopically substituted amine groups (N–D stretching vibrations).[76] At the same time, affected HDO spectra for Gly, NMG, and DMG solutions have to show additional types (relative to TMG) of interacting water molecules due to the presence of the amine groups. In the low-frequency slope of affected spectra for NMG and DMG a "jagged structure" is visible, which is most probably due to the overtone and combination bands of deformation vibrations of deuterium substituted amine group of NMG and DMG. These bands cannot be compensated for in the subtraction procedure.

Figure 3 shows the spectra from Fig. 2 decomposed into physically meaningful bands. Base lines for the presented spectra have been leveled and offset to zero. The component bands correspond to three categories: ν(OD) of HDO, N–D stretching vibration bands, and the already mentioned overtones connected with the isotopic effect. The origin of the first two band groups will be discussed and justified with the help of DFT calculations.

DFT calculations

All DFT calculations presented were performed using the PCM model. It appears from our experience that PCM model better reproduces the geometry of hydrated complexes, giving results much more compatible with infrared spectra relative to those obtained in the gas phase.[70] In most cases the hydrated complexes were initially optimized in the gas phase and next reoptimized within the PCM model: we have verified that the PCM algorithm occasionally stabilized the initially assumed geometry of a complex. Energetic data obtained in the PCM model influence the calculation of the hydrogen bond energy, as the difference between the energy of a cluster in the water continuum and the energies of separated molecules in the water continuum. The result obtained in this way corresponds rather to the covalent contribution to the hydrogen bond energy as discussed in more detail previously.[70]

Calculation of ν(ND) frequency

To interpret component bands in the solute-affected HDO spectra in Fig. 3, the bands corresponding to ν(ND) vibrations of deuterium substituted amine groups of Gly, NMG, and DMG have to be identified first. DFT calculations were performed in the continuum liquid water phase. In aqueous solutions, glycines may form different hydrated structures, therefore ND band positions were calculated for various hydrated complexes.

All calculated wavenumbers of ν(ND) vibrations for the optimized clusters have been collected in Table I, together with the experimental values obtained from independent decompositions of all affected spectra from Fig. 3 into analytical component bands. The calculated structures have been specified in Table I and referred to the figure showing the corresponding clusters [Fig. 5(a)]. Decomposition procedure has been performed without fixing any parameters of analytical

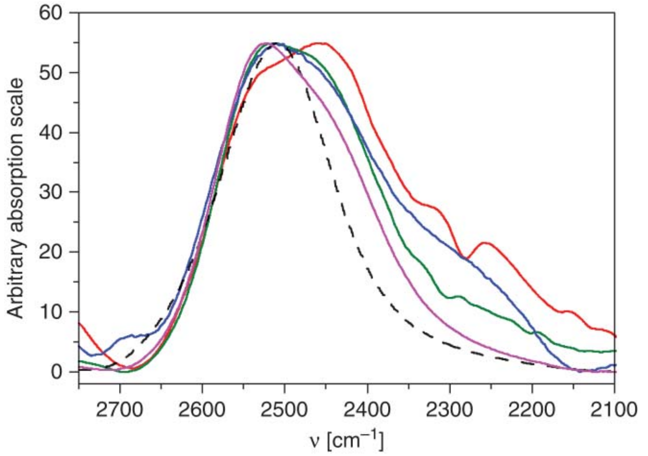

FIG. 2. Solute-affected HDO spectra in the OD stretching region for Gly (blue), NMG (red), DMG (green), and TMG (pink) together with the bulk HDO spectrum (black, dashed). The spectra have been scaled to the same maximum absorption value for better comparison.

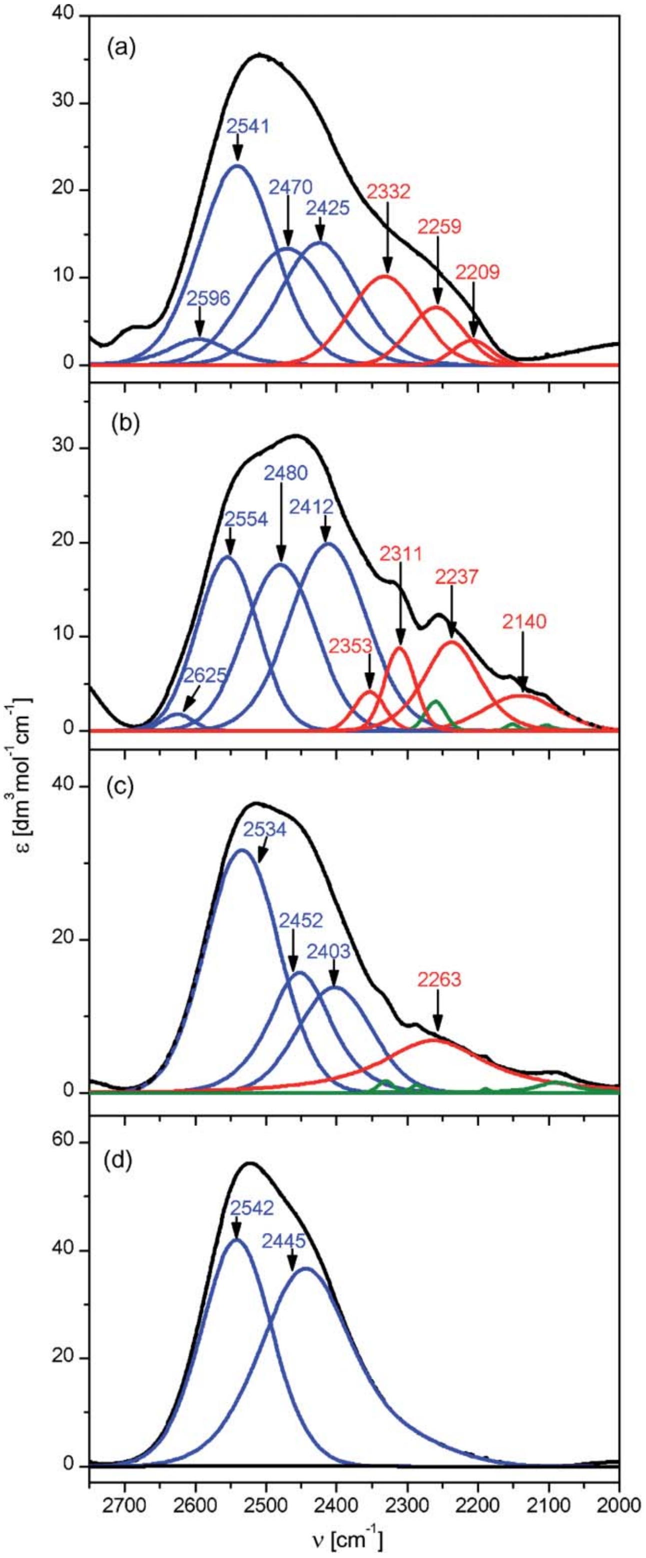

FIG. 3. Decomposition of affected spectra in the OD stretching region for (a) Gly, (b) NMG, (c) DMG, and (d) TMG, into component bands. Solid black line: original affected spectrum; dotted black line: sum of the component bands (covered by the solid line of the original spectrum); blue line: OD component bands; red line: ND component bands; green line: overtone bands connected with the isotopic effect (see text).

TABLE I. Experimental and theoretical values of ν(ND) band positions

	ν_{ND}[a]	ν_{ND}[b]
	2332	2344[c]
Gly	2259	2281[c]
	2209	2255[c]
	2311	2310[c]
NMG	2237	2239[c]
	2353	2374[d]
	2140	2153[d]
		2210[e]
DMG	2263	2180–2265[f]

[a] Experimental values of ν(ND) band position (cm^{-1}) (see Fig. 3).

[b] Calculated (DFT method, PCM model) values of ν(ND) band position (cm^{-1}), for the following aqueous clusters shown in Fig. 5(a).

[c] Structure of the **K** type for Gly and **F** type for NMG with additional water molecule interacting with hydrogen atom of the amine group, not engaged with hydrogen bonding with other molecules within the hydration complex.

[d] Structure of the **H** type.

[e] Structure of the **F** type.

[f] Different modifications of the structure **H** [hydrogen atoms of the amine group in plane in Fig. 5(a) are substituted by methyl groups for DMG molecule].

bands of the general shape of Gaussian–Lorentzian product peak functions.

Several band positions corresponding to the ν(ND) vibrations are possible. For Gly, three component bands obtained from DFT calculations (Table I) are recognized in Gly-affected HDO spectrum in Fig. 3(a). In this case one structure indicated in Table I corresponds to the affected spectrum in Fig. 3(a). It can be added that other considered structures give qualitatively similar results. For NMG, ν(ND) vibrations spread over a range of 2153–2374 cm^{-1}, which may be identified as four N–D component bands visible in the NMG-affected HDO spectrum in Fig. 3(b). In the case of DMG, the calculated frequencies correspond to the wavenumber range of 2180–2265 cm^{-1}, visible in the DMG-affected HDO spectrum in Fig. 3(c). As it can be seen, there is a good agreement between the calculated and the experimental band positions. The important and interesting observation is that protons of GLY and NMG amine groups are strongly nonequivalent with respect to the interaction with the hydrating water molecules.

H-bond energy calculation

For better understanding of simultaneous interactions of water molecules with different electrophilic centers of Gly at first calculations for small complexes were performed (shown in Fig. 4). The total energy, covalent contributions to the hydrogen bond energy, and cooperativity energy data are shown in Table II. It is evident that water molecule forms stronger H-bond (in a sense already explained) with the amine group than with the carboxylic group of glycine. These results are in good agreement with other theoretical studies on the glycine zwitterion.[37,46] Simultaneous interactions of water molecules with different centers of the same group of glycine are noncooperative. This is indicated by positive values of ΔE in Table II, which appear for interactions with the carboxylic group (structures **C** and **D** in Fig. 4) and with the amine group (structures **F** and **G** in Fig. 4). The especially strong effect takes place for water molecules interacting simultaneously with both oxygen atoms of carboxylic group (structure **D** in Fig. 4). This is the main reason why carboxylate anions display nonequivalent interaction with hydrating water via both oxygen atoms, appearing as the recently observed splitting of the hydrated HDO infrared band.[55] Nonequivalent interactions of water molecules with amine protons of GLY and NMG, appearing as the splitting of the absorption band attributed to ν(ND) vibrations (Fig. 3), can now be justified by a noncooperative effect observed for structures **F** and **G** in Fig. 4. It can also be noticed that the polarization effect

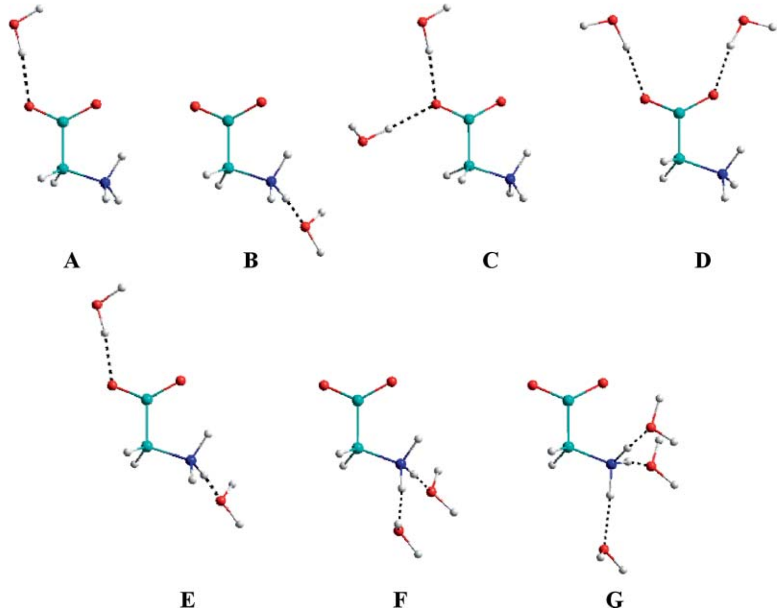

FIG. 4. Hydrogen bonds around hydrophilic groups of glycine with water molecules. Red spheres denote oxygen atoms; dark blue spheres denote nitrogen atoms; gray spheres denote hydrogen atoms. The hydrogen bonds are indicated by black dashed lines.

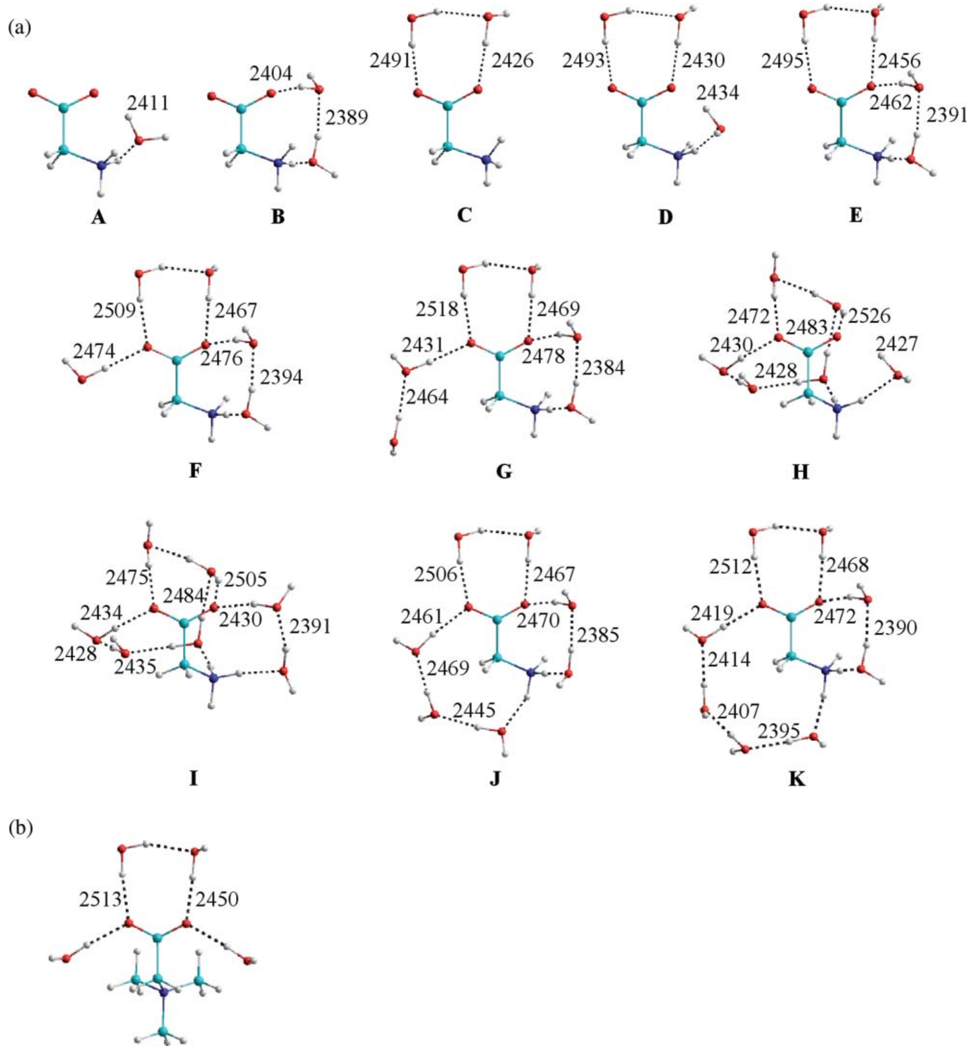

FIG. 5. Different aqueous clusters of zwitterionic Gly (a) and TMG (b) calculated in the PCM model and the corresponding vibrational frequencies (ν_{OD}) obtained from transformation of interatomic oxygen–oxygen distances (R_{OO}) to the OD band position of HDO (ν_{OD}) with the aid of the empirical relation from Ref. 76 (see text). The hydrogen bonds are indicated by black dashed lines.

within amino acid molecule caused by simultaneous interaction of water molecules with the amino and carboxylic groups is negligible, as evidenced by a slightly cooperative effect (Table II) calculated for the structure **E** in Fig. 4.

Calculated structures of hydration complexes and interpretation of spectral results for affected HDO

In order to identify the component bands of HDO affected by glycine and its *N*-methyl derivatives, shown in Fig. 3, we have to know OD vibration frequencies of HDO placed in the positions of water molecules in the calculated hydration complexes. We did not use analytically calculated vibrational frequencies for the comparison with HDO spectra; however, as we verified previously[77] that they poorly correlated with the spectra, even when scaled. This is because stretching vibrations of water are strongly anharmonic and this anharmonicity is significantly dependent on water hydrogen bond strength (and thus stretching frequencies).[78] Instead, we use interatomic oxygen–oxygen distances (R_{OO})

TABLE II. Total energies, covalent contributions to the hydrogen-bond energies, and cooperativity energies for the clusters of zwitterionic glycine and water molecules calculated at the B3LYP/6-31++G(d,p) level of theory in a simulated aqueous environment (PCM model).

		E[a]	$E_{\text{H-bond}}$[b]	ΔE[c]
	H_2O	−76.4523460		
	Gly	−284.471558		
A	Gly + H_2O	−360.928086	−10.98	
B	Gly + H_2O	−360.929417	−14.47	
C	Gly + $2H_2O$	−437.381074	−12.67	+9.29
D	Gly + $2H_2O$	−437.377327	−2.83	+18.53
E	Gly + $2H_2O$	−437.385952	−25.47	−0.02
F	Gly + $2H_2O$	−437.385450	−24.15	+4.79
G	Gly + $3H_2O$	−513.840305	−30.74	+12.68

[a] Total energy (hartree).

[b] Covalent contribution to the hydrogen-bond energy calculated as the difference between the energy of a cluster in the water continuum and the sum of the energies of separated molecules in the water continuum (kJ mol^{-1}).

[c] Hydrogen-bond energy differences between polyhydrated clusters and sum of hydrogen-bond energies of monohydrated glycine (kJ mol^{-1}). The complexes are referred to by their symbols as shown in Fig. 4.

TABLE III. Total energies and covalent contributions to the hydrogen-bond energies per one water molecule of glycine–water clusters calculated at the B3LYP/6-311++G(d,p) level of theory in a simulated aqueous environment (PCM model).

		E^a	$E_{\mathrm{H-bond}/n\mathrm{H_2O}}{}^b$
	H_2O	−76.4523460	
	Gly	−284.471558	
A	Gly + $1H_2O$	−360.925622	−4.51
B	Gly + $2H_2O$	−437.383098	−8.99
C	Gly + $2H_2O$	−437.380400	−5.45
D	Gly + $3H_2O$	−513.838554	−8.72
E	Gly + $4H_2O$	−590.294764	−9.07
F	Gly + $5H_2O$	−666.747958	−7.70
G	Gly + $6H_2O$	−743.198027	−5.42
H	Gly + $6H_2O$	−743.195362	−4.26
I	Gly + $7H_2O$	−819.650706	−4.77
J	Gly + $7H_2O$	−819.652656	−5.50
K	Gly + $8H_2O$	−896.112198	−7.18

[a] Total energy (hartree).

[b] Covalent contribution to the hydrogen-bond energy per one water molecule calculated as the difference between the energy of a cluster in the water continuum and the sum of the energies of separated molecules in the water continuum and divided by the number of water molecules (kJ mol^{-1}). The complexes are referred to by their symbols as shown in Fig. 5(a).

in hydration complexes and calculate respective band position (ν^o_{OD}), with the aid of the correlation curve ν^o_{OD} versus R_{OO} of Berglund *et al.*[79] Generally, shorter R_{OO} distances correspond to lower wavenumbers and also to stronger hydrogen bonds.[80]

Figure 5 shows the calculated hydration complexes for Gly and TMG. The respective complexes for NMG and DMG (not shown) do not demonstrate any additional new types of interactions with water molecules than Gly and TMG, which delimit the studied series of glycine *N*-methyl derivatives. Hydration complexes for Gly (of the Gly · nH_2O type) selected in Fig. 5(a) do not exhaust all capabilities but are the most stable from the considered and calculated, taking into account the covalent contribution to the hydrogen-bond energy per one water molecule (data collected in Table III). Only these structures of hydration complexes for $n \geq 6$ are included in Fig. 5(a) that correspond well to frequencies of HDO spectra in Fig. 3(a). Respective oxygen–oxygen distances in complexes have been recalculated to HDO band positions and shown in Fig. 5 for easier comparison with the frequencies marked on solute-affected HDO spectra in Fig. 3.

Gradual growth of hydration complexes of glycine shown in Fig. 5(a) assists in observing noncooperative hydrogen bonding of water molecules with the carboxylic group. On the other hand, we can observe cooperative interactions between water H-bonds: water molecule H-bonded to one oxygen atom of the carboxylic group and simultaneously to another water molecule H-bonded to the second oxygen atom of this group, strengthens this H-bond with the second oxygen atom [see the complex **C** in Fig. 5(a) as well as the complex in Fig. 5(b) for TMG]. The second important observation confirms stronger H-bonds of water molecules to the amine group than to the carboxylic group of glycine in the hydration complex.

It can be noticed that one water molecule cannot form effectively two hydrogen bonds with amine hydrogen atom and carboxyl oxygen atom of glycine simultaneously because of steric restrictions [complex **A** in Fig. 5(a)]. The hydration complex of the structure **B** shows high stabilization, measured by hydrogen bond energy per one water molecule (Table III). It illustrates that two water molecules can serve probably as the smallest proton transferring system from carboxylate to amine group of amino acid molecule. This complex is too small, however, to explain the infrared spectra. The complex **E** shows especially high stabilization (Table III) and can qualitatively explain spectra for glycine. Further complexes with the chain of following water molecules attached to the oxygen atom of glycine still improved quantitative description of the spectra, in the order of structures **G**, **J**, and **K** in Fig. 5(a). The closed chain formed by four water molecules, like in the structure **K**, seems to be even more effective in proton transfer than the chain of two molecules. This can be inferred from the fact that for the structure **K**, the N–H bond for the group forming hydrogen bond with the four-molecule chain is longer than for the group forming hydrogen bond with the two-molecule chain.

Among the Gly · $6H_2O$ type complexes in Fig. 5(a), apart from the more stable structure **G** the structure **H** also corresponds well to the infrared spectra. Likewise among complexes of the Gly · $7H_2O$ type, apart from the more stable structure **J** the structure **I** also corresponds well to the spectra. Perhaps both these less energetically preferable complexes (**H** and **I**) are more stabilized by entropy, having a less symmetric and less ordered structure. It seems that the three-water molecule closed chain, characteristic for these structures, can also serve in transferring proton within the hydration complex of glycine.

The mixture of hydration complexes from **E** to **G**, and **J** and **K** as well as complexes **H** and **I** in Fig. 5(a) can be considered as the main individual corresponding to the glycine-affected HDO spectrum. It has been obtained for the affected number $N = 5.8$, close to the average composition of the considered group of complexes. Therefore, the above-indicated hydrates (perhaps not excluding also ones with more water molecules reported previously[38]) are representative of the complex dynamic environment for the central solute. We have also checked that the corresponding complexes calculated for alanine adopt the same structures as already mentioned for glycine. These hydration patterns may therefore be general for amino acids.

The structure of hydration complex TMG · $4H_2O$, shown in Fig. 5(b), corresponds to the spectrum of TMG-affected HDO in Fig. 3(d), obtained for $N = 4$. Additional water molecules in this complex surround its alkyl groups in a clathratelike fashion (not shown). It is well known that water molecules in such a state are very slightly perturbed relative to the bulk water.[63]

It seems to be clear that the splitting of TMG-affected HDO (component bands at 2542 and 2445 cm^{-1}) results from the water-induced symmetry breaking of the carboxylic group, observed previously for carboxylate anions.[55] The same effect takes place for Gly, NMG, and DMG, and the band is split to 2543 ± 11 and 2467 ± 15 cm^{-1} components. The second band is blueshifted relative to the respective band of TMG. This is caused by a strong hydrogen bond (2420 ± 5 cm^{-1}) with the same oxygen atom of the chain of water molecules linking the carboxylic with the amine group (in the case of Gly, NMG, and DMG).

The somewhat contradictory results obtained previously for aqueous triflouorocarboxylate anion should be mentioned here as well. These authors found the solvent reorganization time around the COO^- group to be just 0.82 ps.[81] However, we found previously that the CF_3COO^- anion is different from the simple alkyl carboxylates (more representative of the glycine carboxylic group), since it displays a single affected HDO band at ~2550 cm^{-1},[82] while the latter clearly show the presence of two component bands,[55] as discussed above. This indicates that instantaneous water configurations leading to charge localization on COO^- seem to be long-lived enough to register this fact in the linear IR spectra.

High-wavenumbers component bands present in Gly- and NMG-affected HDO spectra in Fig. 3, at 2596 and 2625 cm^{-1}, respectively, are small but very important for understanding of hydration of amino acids in the zwitterionic form. It should be noted that the urea-affected HDO spectrum shows similar small component band at 2631 cm^{-1}, which is due to a water molecule weakly interacting with the hydrogen atom of the amine group.[67] In the case of urea, hydrogen bonding via oxygen atom of carbonyl group is much stronger than via hydrogen atom of uncharged amino group. The last interaction is also weaker than the one between water molecules.

It appears that in the case of NMG one hydrogen atom of the charged amine group, carrying almost entire positive charge of this group, forms a strong H-bond with the water molecule which through the successive two or even four water molecules links to the oxygen atom of the carboxylic group, which in turn carries almost entire negative charge of this group. Such asymmetry of charge distribution, caused by water interactions, ensures direct intramolecular proton transfer from the carboxylic to the amino groups. This effect is rooted in noncooperative interactions already discussed. It can be added that one water molecule is enough to stabilize the zwitterionic structures of NMG and DMG when hydration complex is calculated in the gas phase, while at least two water molecules, linking the carboxylic and amine groups, are needed to stabilize the zwitterionic form of Gly. The band at 2413 ± 12 cm^{-1} is attributed to these chains of water molecules in the case of hydration complexes of the mentioned solutes. Water molecule weakly H-bonded with the second almost uncharged hydrogen atom of the amine group shows the band position at 2625 cm^{-1} in the case of NMG. For Gly, the averaged respective band with the maximum at 2596 cm^{-1} corresponds to water molecules interacting with two weakly charged hydrogen atoms. In the case of DMG, only one strongly charged hydrogen atom is present, which causes the band at the low-wavenumbers position (at 2403 cm^{-1}) and there is no band at the high-wavenumbers position.

CONCLUSIONS

Solutes selected for studies in aqueous solutions within this work are important osmolytes, stabilizing the native state of proteins, but they also enable us to depict hydration patterns characteristic for amino acids, taking glycine as a simple representative.

Hydration of glycine in the zwitterionic form, and of amino acids in general, can be understood allowing a fluctuating synchronization of hydrogen bonding of water molecules with their interaction centers. This is caused by a lack of cooperativity of water interactions with the electrophilic groups of amino acid and by intramolecular hydrogen bond chain, allowing proton transfer from the carboxylic to the amino group, consisting of two, three, or even four water molecules. The instantaneous water-induced asymmetry of the solute is observed by the fast responding infrared spectroscopy. The band splitting of HDO molecules involved in interaction with the carboxylic group gives component bands at 2543 ± 11 and 2467 ± 15 cm^{-1}. Even stronger is the effect caused by interaction with the amine group: HDO molecules involved in proton transfer within the amino acid molecule give rise to the band at 2413 ± 12 cm^{-1}, whereas HDO weakly interacting with the hydrogen atom of amine group, which is almost devoid of positive charge, shows the band at 2611 ± 15 cm^{-1}.

It should also be mentioned that very recent *ab initio* molecular dynamics simulations[53] confirm nonequivalent water interaction with oxygen atoms of the carboxylate group of glycine. Also, radial distribution function presented in this work for ammonium hydrogen atoms and oxygen atoms of hydrating water molecules shows for one H_N–O_N correlation pair slight, but distinct, difference in the first maximum, relative to the two other correlation pairs. In spite of this and other results presented, authors of that paper claim that rotational dynamics averages the state of protons in the $–NH_3^+$ group, so they also negate water intramolecular hydrogen bond connecting the ammonium and the carboxylate groups. These last two conclusions are opposite to our spectral observations and findings.

Properties of the studied solutes as osmolytes will be discussed in a successive paper in the context of their influence on a model protein in aqueous solution.

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