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Does hydrated glycine act as solidification nucleus at multi-kilobar conditions?

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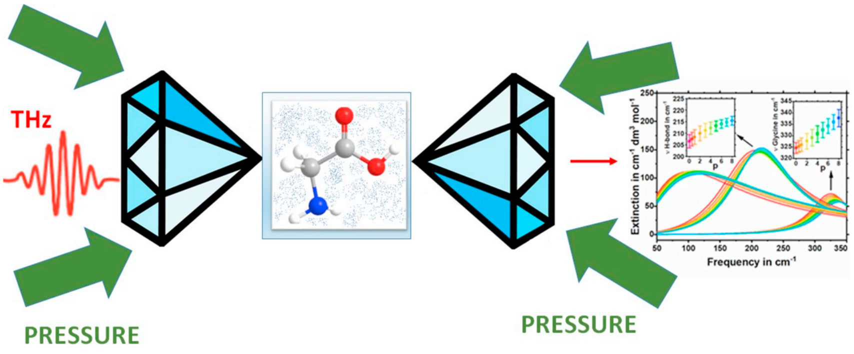
HIGHLIGHTS

- THz study of 1.5 M glycine from 1 bar to 8 kbar.
- Linear compression of glycine from linear blue shift.
- Non-linear compression of water from non linear blue shift.
- Microscopic origin of the macroscopic changes, as the liquid-solid phase transition.

GRAPHICAL ABSTRACT



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ABSTRACT

The investigation of aqueous solutions containing biomolecules as a function of thermodynamic parameters, such as the pressure, is crucial for understanding biological processes. Here we report the first low frequency spectra of 1.5 M aqueous glycine from ambient pressure up to 8 kbar, i.e. in the pressure range which is crucial for understanding biological processes under extreme conditions.

We observe a linear pressure dependent blue shift of the specific N-C-C-O open/close mode at ~320 cm^{-1} indicating an increasing compression of the solvated glycine. In contrast, the characteristic peak of the hydrogen bond hydration water network centered, at ambient conditions, at ~184 cm^{-1} non-linearly blue shifts with increasing pressure, as well, but with a slower rate than the intramolecular peak.

This indicates that the macroscopic liquid-solid phase transition observed above 8 kbar pressure is driven by hydrated glycine as solidification nucleus.

1. Introduction

Solvation of biomolecules in water is a central topic in molecular sciences [1–7]. Nowadays it is largely recognized that the hydration water (i.e. water close to solutes) behaves differently from the bulk liquid [8]. Since hydrogen bonds reorganize on sub-picosecond time scales [9], THz spectroscopy offers an excellent means by which to study intermolecular hydrogen bond network dynamics and its coupling with the solute. In previous publications, we investigated the solvation of small ions, amino acids, and proteins in water at ambient

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conditions [10–12]. Among the 20 proteinogenic standard amino acids glycine is the simplest one and has served as a prototype for many hydration studies of zwitterions. Under physiological conditions glycine is a zwitterion ($NH_3^+CH_2COO^-$) with a negatively charged carboxyl group and a positively charged amino group, whereas it is neutral in the gas phase [13]. Since it plays an important role for protein stability, aggregation and misfolding [14,15], glycine has been investigated under several different conditions in the IR regime, including measurements of the first gas phase spectra of glycine [16] as well as ab initio calculations of aqueous glycine solutions under acidic and basic conditions [17]. A recent joint THz spectroscopy and ab initio molecular dynamics (AIMD) simulations study of glycine is reported in ref. [18]. Here the authors were able to decipher the low frequency spectrum of this amino acid in water. The spectrum could be decomposed into contributions from the intermolecular modes of the super-solvation complex and the intramolecular modes of the solute. The N-C-C-O open/close mode of glycine at 320 cm^{-1} has been found to be the most pronounced mode in the THz spectrum.

Nevertheless, little is known at the molecular level about the solvation and the solvation dynamics of biomolecules at high hydrostatic pressure (HHP) conditions despite the fact that these conditions are of utmost importance in understanding life under extreme conditions. Pressure can indeed modulate solvation properties, alter non-covalent interactions, affect molecular distances and change supermolecular structures. It is worth to point out that under ambient conditions even simple alterations can cause the unfolding of proteins. In the deep sea some marine species survive in extreme pressure conditions (even above 0.8 kbar) without evident modification in the protein structure. Therefore, the investigation of the thermodynamic evolution of the solute-solvent ensemble can shed light on specific, unclear biomolecular mechanisms such as the one allowing piezophilic species to survive at high pressures [19].

It is possible to find in literature several studies of crystalline glycine under extreme conditions, but only a few on the hydrated glycine under HHP conditions exist. Glycine in its crystalline has form three different polymorphous expressions (α -in which glycine crystallizes at ambient conditions-, β and γ). In all of them glycine exists as a zwitterion. Murli et al. [20] performed thermodynamic studies via Raman measurements on α-glycine up to 23 GPa (230 kbar), revealing a lack of a structural phase transition even though they recognized the presence of changes in the N—H · · · O hydrogen bond interaction upon compression. In contrast, Goryainov found by polarized optical microscopy experiments a reversible pressure-induced phase transition in β-glycine at 0.76 GPa (~8 kbar) [21]. The phase transition was also confirmed by Raman spectroscopy. The transition is associated to the rearrangement of the hydrogen bond network as well as rotations and twisting of the zwitterions.

Organic osmolytes such as amino acids, sugars and trimethylamine-N-oxide (TMAO) have been found to be crucial under pressure and thermal stress conditions [22]. In fact, although the influence of hydrostatic pressure on bio-chemical reactions has been known for some time, a lack of knowledge from the molecular point of view exists about (bio)chemical reactions occurring at elevated pressure conditions. It is well known that life can withstand extreme conditions regarding pressure and temperature. Only few spectroscopic studies focusing on aqueous glycine under high hydrostatic pressure are reported [23].

In order to fill this gap of experimental investigations, we report in the following the THz absorption study of 1.5 M aqueous glycine solution in the frequency range from 50 cm^{-1} to 350 cm^{-1} as a function of external applied pressure using a diamond anvil cell.

2. Materials and methods

2.1. Equipment

We recorded THz-Far infrared (THz/FIR) spectra of 1.5 M aqueous solutions of glycine (Carl Roth GmbH, purity ≥99%) in the frequency range 50–350 cm^{-1} as a function of an applied external pressure. In order to record the small pressure-induced changes in the THz absorption of solvated amino acids, we recorded spectra at two distinct set-ups. This permitted us to check the consistency of the data. We used a Fourier transform infrared spectrometer (FTIR, Vertex 80 V, Bruker) with an external He-cooled bolometer (Infrared Laboratories, Inc., Tucson, USA) for light detection and as a source of radiation both a commercial mercury lamp (for the in-house setup, Bochum) and the synchrotron light (at the beamline AILES, Soleil). The pressure was varied between ambient condition and 8 kbar with steps of 1 kbar. Above 8 kbar, we found a reproducibly liquid-solid transition phase of the sample, leading to a cell leakage.

For the in-house measurements, we used a commercially available Diamond Anvil Cell, DAC, (VivoDAC, Almax Easylab, Diksmuide, Belgium), consisting of a diamond anvil and a flat diamond window. The sample is placed in the center of a brass ring without any pre-indentation with an outer diameter of 3.95 mm, an inner diameter of 0.5 mm and a thickness of approximately 40 μm, as manufactured by LMB Automation (Iserlohn, Germany). The light was collimated using a 6× beam condenser from Pike Technologies (Madison, USA) and the pressure was applied via a gas membrane connected to an automatic pressurizing system (Pace 5000, by GE Measurements, Billerica, USA). A more detailed description of the setup is provided in a previous publication [24]. The pressure inside the cell was determined with an accuracy of about 300 bar, employing the pressure-dependent shift of an absorption band of crystalline quartz. The changes in the thickness of the sample cell as a function of the gas membrane pressure were measured ex-situ using two confocal distance sensors (Micro-Epsilon IFS 2403, Micro-Epsilon, Ortenburg). Further details are reported in the E.S.I.

The set-up at AILES beamline enables to use the ultra-bright synchrotron radiation source and pressures even above 8 kbar. It was also used to compare our pressure calibration with the in-cell pressure calibration.

For the measurements performed at AILES, the DAC cell consists of two anvil diamonds. The sample is placed in the hole of a gasket with 50 μm diameter. The ring is an indented stainless-steel disk, with a thickness of 30–40 μm. Here, we loaded in the cell a few ruby crystals together with the sample, to record the ruby fluorescence lines. The frequency shift of the peak centered at 695 nm provides a direct measurement of the in-situ pressure. Further details can be found in another publication [25] and in the E.S.I.

Each spectrum has been acquired after achieving hydrostatic equilibrium. The temperature was kept constant at 20 °C.

2.2. Data analysis

For the analysis of the THz spectra, we apply the Lambert-Beer law, see eq. (1):

$$I(\nu, P) = I_0 e^{-\alpha(\nu,P)} d_P \, I(\nu, P) = I_0 e^{-\alpha_{sol}(\nu,P) dP} \tag{1}$$

where I(ν,P) is the transmitted intensity of the sample solution at the pressure P, I_0 the intensity of the sample solution at ambient pressure, $\alpha_{sol}(\nu, P)$ is the pressure dependent absorption coefficient at frequency ν and d_P is the cell thickness as a function of the pressure. Each spectrum is obtained as an average of 128 spectra at the same temperature and pressure conditions. Fig. S13 in the SI provides a comparison between measurements at AILES and in the in-house laboratory. In the following, we discuss only the results for measurements at the laboratories in Bochum.

We assume that the total absorption α_{sol} of our sample solution can be described as the sum of the absorption contributions of the solute, the bulk water and the hydration water, namely $\alpha_{sol} = \alpha_s + \alpha_{bulk} + \alpha_{hydr}$. Apart from α_{sol}, we can directly measure only the contribution of the bulk (pure) water. In order to extract the behavior of the glycine in water and to obtain its effective absorption, α_s^{eff} we subtract the expected absorption of the bulk water in the sample as follows. Starting from the density of the 1.5 M glycine at a certain pressure *P,* we calculate the volume occupied by the bulk water in the sample at the pressure *P*. From the total absorption spectrum, the bulk absorption spectrum is subtracted after scaling it by the fraction occupied by water in the solution. In this sense, the effective absorption has to be considered as the result of a volume correction [26]:

$$\alpha_{gly}^{eff}(\nu, P) = \alpha_{sol} - \frac{c_w^{sol}(P)}{c_w^{bulk}(P)}\alpha_{bulk}(\nu, P) \tag{2}$$

where $c_w{}^{sol}(P)$ and $c_w{}^{bulk}(P)$ are the water concentrations in the solution and in pure water at pressure *P*, respectively, as determined by density measurements and the Tait equation (see SI for details) and $\alpha_{\mathrm{bulk}}(\nu, P)$ is the absorption of the bulk water at pressure *P* measured with the same procedure as for the 1.5 M glycine solution.

For an ideal solution $\alpha_{gly}{}^{eff}(\nu, P)$ is proportional to the concentration and thus the effective extinction $\varepsilon_{gly}^{eff}(\nu, P) = \frac{\alpha_{gly}^{eff}(\nu, P)}{c_s(P)}$ is independent of the solute concentration $c_s(P)$. Note that in the present case, also the solute concentration varies with the internal applied pressure P.

As in previous publications [27], we deconvolute each spectrum into a superposition of damped harmonic oscillators, eq. (3), functions of the form:

$$f\ (\nu, \nu_D, A_0, \Delta) = \frac{A_0 \nu \Delta^2}{4\pi^3\left(\left({\nu_D}^2 + \left(\frac{\Delta}{2\pi}\right)^2 - \nu^2\right)^2 + 4\left(\frac{\Delta}{2\pi}\right)^2 \nu^2\right)} \tag{3}$$

with ν_{D} as the effective center frequency of the band, A_0 the amplitude and Δ as the width of the band, respectively. Due to damping, ν_D is shifted compared to the center frequency of the corresponding unperturbed harmonic oscillator ν_0, eq. (4):

$$\nu_0 = \sqrt{{\nu_D}^2 + \left(\frac{\Delta}{2\pi}\right)^2} \tag{4}$$

In the following, when we present and discuss the center frequencies we refer to the center frequency ν_0 of the unperturbed harmonic oscillator. Results from the fitting procedure are within the 2σ.

3. Results and discussion

In order to understand the behavior of proteins under HHP conditions and their coupling with the solvent, we measured THz spectra of 1.5 M glycine in water from ambient pressure up to 8 kbar at constant room temperature. The results for the extinction coefficient of the solute are reported in Fig. 1.

For the low and the high frequency region of the spectra, we observe a decrease of the overall intensity with increasing pressure, while the range between 170 cm^{-1} and 270 cm^{-1} shows an increasing intensity with increasing pressure.

In the following analysis, each spectrum is deconvoluted into three modes (see inset of Fig. 1). The total fit is the sum of three damped harmonic oscillators, whose center frequencies are at ~ 80 cm^{-1}, ~184 cm^{-1} and ~320 cm^{-1}. From ab initio MD simulations, we can assign the band at 184 cm^{-1} to the solute−solvent hydrogen bond stretching mode in the solvation shells of the charged $-\mathrm{NH}_3{}^+$ and $-\mathrm{COO}^-$ groups [18,28] and the band at ~320 cm^{-1} to the N-C-C-O open/close backbone mode of glycine [29], respectively. We restrict our analysis to the modes above 100 cm^{-1}, since the low frequency band cannot be assigned unambiguously to a single specific mode. In fact, the low frequency part of these spectra is the superposition of the cage mode of glycine [28] and the Debye relaxation term [8]:

In Fig. 2, we plot the deconvolved features of the extinction spectra as a function of the pressure. The left and the right insets show the pressure-dependent shift of the oscillator unperturbed center frequency of the hydrogen bond intermolecular network and the internal backbone mode of glycine, respectively. For the intramolecular mode of glycine, we observe a linear blue shift with pressure. Remarkably, we observe a different response in the spectroscopic fingerprint of the collective mode at ~184 cm^{-1}, which describes the coupling of the glycine to the water network. In fact, even if the center frequency of the H-bond intermolecular stretching mode blue shifts with pressure, it levels off for pressure higher than 4–5 kbar.

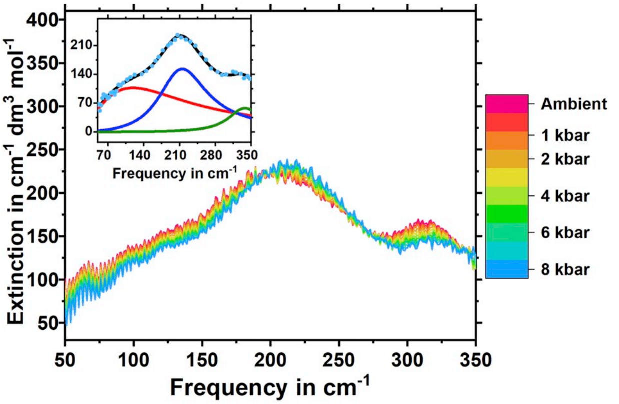

Fig. 1. Effective THz/FIR extinction spectra of glycine as a function of the applied pressure. All measurements were conducted at constant room temperature. Inset: example of the dissection of the glycine aqueous spectrum at 8 kbar. The light blue dotted curve is the acquired spectrum, the black solid line is its fit, and the solid red line is the fit of the peak associated with the cage mode, the solid blue line the fit of the H-bond stretching mode, the solid green the fit of the N-C-C-O open/close glycine specific. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

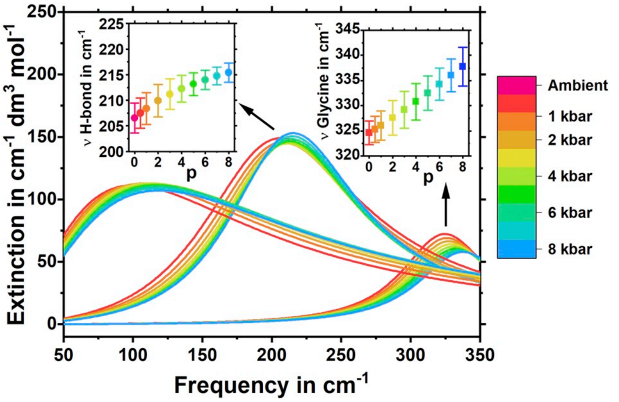

Fig. 2. Plot of the deconvolved features of the extinction spectra with the three damped harmonic oscillators. Left inset: peak center frequency of the H-bond intermolecular stretching mode as a function of the pressure. Right inset: peak center frequency of the NCCO open/close mode of glycine as a function of the pressure.

The simplest interpretation of the observed spectroscopic changes is that the pressure leads to an increased compression of the glycine. As a result, the effective force constant acting on the specific glycine N-C-C-O open/close mode is increased and the band at ~320 cm^{-1} is blue-shifted. The pressure simultaneously compresses the hydrogen-bond-network structure of the water, as well, but with a less intense response than for the intermolecular glycine mode. In fact, the frequency of the glycine backbone mode blue shifts in overall 13 cm^{-1} while the frequency associated to the hydrogen-bond network blue shifts 9 cm^{-1} at the outmost. Since the peak at ~184 cm^{-1} is dominated by the cross-correlation term between the glycine and the water dipole moments up to the second hydration shell, the less pronounced blue shift indicates that the hydration water withstands longer the pressurization than the glycine. For low regime pressures (below 4 kbar) the glycine and the hydration water experiment the same compression, while at higher pressure a structural mismatch in the compression leads to an abrupt change of phase. In fact, no spectra are available for pressures higher than 8 kbar since the sample immediately experiences a phase transition from the liquid to the solid phase above this pressure. To support our assignment, we compared the water band in the 1.5 M glycine at 8 kbar and in the amorphous ice. Interestingly we have a good agreement between the center frequency of the amorphous ice band [30] and the center frequency of the hydrogen bond intermolecular mode at 8 kbar. The center frequency of the amorphous ice band (see ESI for details on the fitting procedure) has been fitted at [218.2 ± 2.0] cm^{-1} while the hydrogen bond band at 8 kbar is centered at [215.4 ± 2.1] cm^{-1}. The agreement within the error bars suggests that the structural mismatch between glycine and its hydration water facilitates the solidification of water under HHP, while this occurs at higher pressure in the pure water. Thus, hydrated glycine acts as nucleation site for solidification. A second hint of the liquid-solid phase transition comes from a recent theoretical work by Gupta and coworkers in which they simulated the THz absorption spectrum of the partially frozen aqueous glycine [31]. The center frequency of the NCCO open/close mode of the free glycine in fixed water is found at around 336 cm^{-1}, in accordance with our findings in the spectrum at 8 kbar [337.7 ± 3.8] cm^{-1}. Thus, we conclude that the increase of the high hydrostatic pressure is responsible for two different modifications in the solvent-solute interaction: one is associated with a pure volume inclusion effect on the glycine in water and the other is the limited change in the localized hydration water complex structure and dynamics. The former takes place continuously from ambient pressure to a maximum of 8 kbar while the latter has a less pronounced effect because of the different underlying molecular mechanisms.

4. Conclusions

We recorded THz spectra of 1.5 M glycine solution from ambient pressure to 8 kbar in a diamond anvil cell in the spectral range between 50 cm^{-1} and 350 cm^{-1}.

The dissection of each spectrum into its characteristic peaks suggested that the pressure acts simultaneously on both the glycine and the hydrogen bond water complex. We observe a clear linear blue shift of the glycine backbone mode (N-C-C-O open/close at ~320 cm^{-1}) with pressure. We found a similar effect on the localized solvation complex band (at ~184 cm^{-1}). This also experiences a blue shift as a function of the pressure reaching a plateau for pressures higher than 4–5 kbar. At 8 kbar, the center frequency of the H-bond network mode is interestingly similar to that of amorphous ice. We speculate that this similarity might foster the transition from the liquid to the solid state and the glycine dissolved in water is responsible for the early liquid-solid phase transition of the solution at pressures higher than 8 kbar. We propose that the different blue shifts are an evidence of a growing structural mismatch between glycine and its hydration water, which leads to a hydration water structure and dynamics close to that of amorphous ice when pressures reach 8 kbar. At this pressure, hydrated glycine likely acts as nucleation site leading to the early liquid-solid phase transition compared to bulk water at the same HHP conditions. We speculate that this might be the reason why the content of glycine in deep-sea marine species (anemones) decreases with increasing water depth -and consequently increasing pressure [32]. This first work on the simplest amino acid paves the road for further studies for understanding how more complex samples -peptides and later proteins- can maintain their structure in critical conditions such as the HHP.

Conflict of interests

There are no conflicts to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bpc.2019.106215.

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