J|A|C|S ARTICLES

Published on Web 02/25/2009

Solvation Dynamics of Model Peptides Probed by Terahertz Spectroscopy. Observation of the Onset of Collective Network Motions

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Abstract: We have studied the solvation of model peptides at low hydration levels by terahertz absorption spectroscopy. We have recorded the concentration-dependent terahertz absorption coefficients of *N*-acetyl-glycine-amide (NAGA), *N*-acetyl-glycine-methylamide (NAGMA), *N*-acetyl-leucine-amide (NALA), *N*-acetyl-leucine-methylamide (NALMA), and *N*-acetyl-tryptophan-amide (NATA) in aqueous solution. We find a dramatic decrease in the THz absorption, if the number of water molecules per solute is less than 18−20. This change is taken as a signature for the breakdown of peptide−water network motions, which supports the hypothesis that a minimum number of hydration waters is required to activate these motions. This is well below a monolayer coverage of the model peptides. It is interesting to note that the required hydration level corresponds to the number of water molecules which are required for biological functionality.

Introduction

Solvent motions affect a broad range of protein functions from conformational fluctuations to ligand binding and catalysis.[1−3] Although solvent dynamics occur on the picosecond time scale, it activates slower protein dynamics, for example relevant in folding processes, via liquid-state fluctuations.[2] In return, the protein also changes the structure and dynamics of the solvent.[4] To elucidate the mechanisms of protein−water dynamical coupling, solvent dynamics must be studied over a wide range of time scales from fast processes in the sub-picosecond region to diffusive motions on the picosecond-to-nanosecond time scale.

Above all, there is interest in processes on the sub-picosecond-to-picosecond time scales, which is the typical regime of hydrogen-bond dynamics within the water network. We have recently shown that terahertz (THz) absorption spectroscopy is able to probe directly the change of the fast water network motions of hydrated biomolecular solutes within a wide (>10 Å) solvation shell.[5−9]

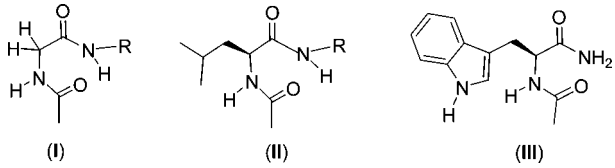
THz spectroscopy probes the sub-picosecond−picosecond intermolecular dynamics of water very sensitively. Solutes such as carbohydrates[5−9] as well as the model peptides considered here have far fewer low-frequency modes than those of water and can be considered as almost transparent solutes. A detailed study of the THz spectra of tripeptides and deformation modes for crystalline dipipetide nanotubes has been carried out by Plusquellic and co-workers.[10,11] Replacing water by the solutes in the binary mixtures therefore generates holes in the spectral density of the low-frequency modes. Thus, one naively expects only a linear decrease of the THz absorption coefficient with increasing solute concentration. However, in real systems this simple two-component model does not hold; we observed a change of the absorption coefficient of water by the solute, resulting in an onset of nonlinearity at specific concentrations. A three-component model comprising distinct absorption coefficients of the solute, shell water, and bulk water has rationalized the terahertz absorption of carbohydrate solutions quite well.[8,9] As observed in all cases studied so far, the curvature is convex with regard to the abscissa. This is a direct consequence of the fact that the terahertz absorption coefficient of water in the hydration shell is higher than that of bulk water (called "THz excess"). As an exciting feature, the "dynamical hydration shell" in which water is affected by the solute substantially exceeds the range of the static hydration shell, as the static hydration shell, which probes the influence on the water molecules, is probed by scattering experiments.[12]

Here, we address specifically two questions: One concerns the ongoing discussion of whether the hydration dynamics are determined exclusively by the topography or whether we can find systematic differences with chemical composition (e.g.,

(1) Rupley, J. A.; Careri, G. *Adv. Protein Chem.* **1991**, *41*, 37.
(2) Fenimore, P. W.; Frauenfelder, H.; McMahon, B. H.; Parak, F. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16047.
(3) Pal, S. K.; Zewail, A. H. *Chem. Rev.* **2004**, *104*, 2099.
(4) Despa, F.; Fernandez, A.; Berry, R. S. *Phys. Rev. Lett.* **2004**, *93*, 228104.
(5) Ebbinghaus, S.; Kim, S. J.; Heyden, M.; Yu, X.; Heugen, U.; Gruebele, M.; Leitner, D. M.; Havenith, M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 20749.
(6) Ebbinghaus, S.; Kim, S. J.; Heyden, M.; Yu, X.; Gruebele, M.; Leitner, D. M.; Havenith, M. *J. Am. Chem. Soc.* **2008**, *130*, 2374.
(7) Born, B.; Kim, S. J.; Ebbinghaus, S.; Gruebele, M.; Havenith, M. *Faraday Discuss.* **2009**, *141*, 161.
(8) Heugen, U.; Schwaab, G.; Bründermann, E.; Heyden, M.; Yu, X.; Leitner, D. M.; Havenith, M. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12301.
(9) Heyden, M.; Bründermann, E.; Heugen, U.; Niehues, G.; Leitner, D. M.; Havenith, M. *J. Am. Chem. Soc.* **2008**, *130*, 5772.
(10) Zhang, H.; Siegrist, K.; Plusquellic, D. F.; Gregurick, S. K. *J. Am. Chem. Soc.* **2008**, *130*, 17846.
(11) Siegrist, K.; Bucher, C. R.; Mandelbaum, I.; Hight Walker, A. R.; Radhakrishnan, B.; Gregurick, S. K.; Plusquellic, D. F. *J. Am. Chem. Soc.* **2006**, *128*, 5764.
(12) Head-Gordon, T.; Sorenson, J. M.; Pertsemidis, A.; Glaeser, R. M. *Biophys. J.* **1997**, *73*, 2106.

10.1021/ja808997y CCC: $40.75

Scheme 1. (**Ia**) *N*-Acetyl-glycine-amide (NAGA, R = H); (**Ib**) *N*-acetyl-glycine-methylamide (NAGMA, R = CH_3); (**IIa**) *N*-acetyl-leucine -amide (NALA, R = H); (**IIb**) *N*-acetyl-glycine-methylamide (NALMA, R = CH_3); (**III**) *N*-acetyl-tryptophan-amide (NATA)



hydrophilic versus hydrophobic). The second concerns the question of what is happening at low hydration levels for the collective water network motions as probed by THz spectroscopy. Among others, such confined states are present in the crowded environment of living cells, where macromolecules may occupy up to 40% of the volume of the cytoplasm and are typically separated by only 1−2 nm.[13] This is a regime of high interest because in order to achieve their normal functionality, proteins have to overcome a hydration threshold. Studies of wet protein powders have shown that the onset of enzyme function typically requires about 0.4 g water per gram protein,[1] which is well below monolayer coverage. Experimental studies of such highly confined states of water near proteins are not trivial because at the concentrations of interest, proteins may aggregate and precipitate. However, they allow us to probe how many water molecules are required to establish the strong absorbing collective water network modes which are probed around 3 THz (where the solute by itself is mostly transparent for small peptides). By contrast, at the protein concentrations used in our previous experiments, we have mostly probed the hydration water far away from the surface (up to 20 Å).

Experimental Section

NAGA (**Ia**) was obtained from Alfa Aesar (Karlsruhe, Germany); all other model peptides, **Ib**, **IIa**, **IIb**, and **III**, were obtained from Bachem (Weil am Rhein, Germany). The powders were dissolved in distilled water, and the solutions were adjusted to pH 4 by adding traces of aqueous HCl. For optimum stirring, the samples were mixed with a vortexer, and small amounts of the aggregated or nondissolved powder were removed by centrifugation at 10 000 rpm for 10 min before each measurement. The solubility was found to depend strongly on the pH of the solutions. For example, solutions of NALA in distilled water (pH 7) became turbid at a molar concentration of about 1.25 M in agreement with reports in the literature.[14] By acidification to pH 4 we could, however, achieve supersaturated solutions up to 3 M, which could be kept stable over the duration of the experiments. The maximum concentrations achieved at pH 4 for the other model peptides were also markedly higher than reported in the literature.

The present study focuses on model peptides which enable experiments at very low hydration levels with systematically varied chemical properties. In these model peptides, defined in Scheme 1, side chains of various hydrophobicities are attached to a blocked polypeptide backbone. Specifically, we consider hydrophilic *N*-acetyl-glycine-amide (NAGA, (**Ia**)) and the homologue *N*-acetyl-leucine-amide (NALA, (**IIa**)) which involves a hydrophobic alkyl chain. The profitable use of this class of solutes goes back to the work of Head-Gordon and co-workers,[12,14−16] who have studied

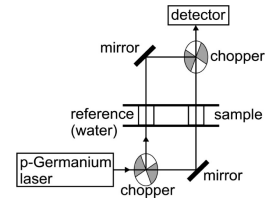


Figure 1. p-Ge difference spectrometer. Displayed is the path of the THz radiation. The mirror chopper splits the THz radiation emitted from a p-Ge laser into two distinct beams, probing the reference (water) and the sample solution, respectively. Both are focused onto the detector.

hydration dynamics on longer time scales of *N*-acetyl-glycine-methylamide (NAGMA, (**Ib**)) and *N*-acetyl-leucine-methylamide (NALMA, (**IIb**)) by dielectric spectroscopy, neutron scattering, and molecular dynamics simulation. These two solutes differ from NAGA and NALA by a methyl residue at the terminal amide group.

We have investigated NAGMA and NALMA as well; however, the highly interesting regime of very low hydration levels could only be reached with NAGA and NALA. Finally, we performed some experiments with *N*-acetyl-tryptophan-amide (NATA, (**III**)), which is used as a label in fluorescence spectroscopy and is typical for tryptophan-containing segments in proteins.[17] For NATA, the accessible concentrations were more than 2 orders of magnitude lower than those of the other model peptides.

We have used two THz spectrometer setups to measure the THz absorption of model peptides: One spectrometer is a commercial FTIR spectrometer (Vertex 80v, Bruker); the second is our p-Ge spectrometer (see Figure 1).[18]

The THz absorption coefficients (α) of bulk water and the solvated biomolecule are both large (on the order of 400 cm^{-1}). The solute-induced change is relatively small ($\Delta\alpha$ is on the order of 10−20 cm^{-1}) but carries the essential information. Using the p-Ge spectrometer, we have measured the integrated absorption coefficient in the spectral range from 2.1 to 2.8 THz. The transmitted intensities of the laser pulse were measured at a fixed layer thickness using a standard Bruker liquid sample cell with Teflon spacers and z-cut quartz windows. The layer thickness of the aqueous sample was determined to be (52.6 ± 0.3) μm using FTIR spectroscopy. The temperature of the sample was kept at (20 ± 0.1) °C by using a Peltier element. The measured humidity near the purged sample cell was below 8%. In order to determine the difference in absorption between protein solution and buffer blanks with a high precision, we used the p-Ge difference spectrometer. The setup is designed to minimize systematic errors due to temperature drifts or changes in the air humidity as would be present in the case of subsequent measurements. Specifically, we determine:

$$\alpha(c) = \alpha_{\mathrm{sample}}(c) - \alpha_{\mathrm{buffer}}(c),$$

with $\alpha_{\mathrm{sample}}(c)$ and $\alpha_{\mathrm{buffer}}(c)$ being the integrated absorption coefficients (2.1 to 2.8 THz) of the sample and buffer at a given concentration c. The analysis of our p-Ge data is explained in detail in a previous publication.[9]

The remaining errors are caused by manual refilling of the sample cell, which leads to slight sample-to-sample, cell positioning, or path length variations. By data evaluation with Beer's law, the absorption coefficient of the sample and the reference in the two separate channels were determined.

Results

We have measured an overview spectrum of NALA in acidic (pH 4) aqueous solution for concentrations from 0 to 3 M using

(13) Ball, P. *Chem. Rev.* **2008**, *108*, 74.
(14) Russo, D.; Hura, G.; Head-Gordon, T. *Biophys. J.* **2004**, *86*, 1852.
(15) Russo, D.; Murarka, R. K.; Hura, G.; Vershell, E.; Copley, J. R. D.; Head-Gordon, T. *J. Phys. Chem. B* **2004**, *108*, 19885.
(16) Russo, D.; Murarka, R. K.; Copley, J. R. D.; Head-Gordon, T. *J. Phys. Chem. B* **2005**, *109*, 12966.
(17) Pal, S. K.; Peon, J.; Zewail, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 1763.
(18) Bergner, A.; Heugen, U.; Bründermann, E.; Schwaab, G.; Havenith, M.; Chamberlin, D. R.; Haller, E. E. *Rev. Sci. Instrum.* **2005**, *76*, 063110.

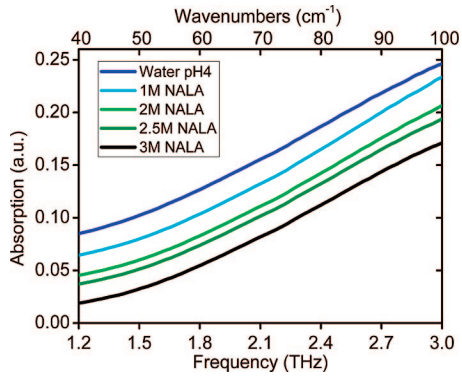


Figure 2. FTIR spectrum of NALA dissolved in aqueous solution at pH 4 and 20 °C. The frequency-dependent absorption in the THz region decreases due to an increasing solute concentration.

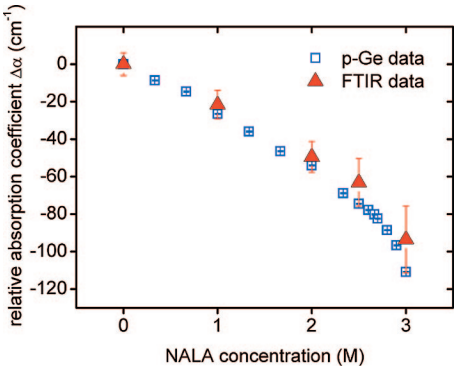


Figure 3. Comparison of the concentration-dependent relative THz absorption coefficient $\Delta\alpha$ measured at 20 °C with a p-Ge spectrometer and an FTIR spectrometer of aqueous solutions of NALA at pH 4 (α_{sample}) relative to α_{buffer} at pH 4. Concentrations are given in molar units. Error bars demonstrate the statistical error.

our FTIR spectrometer in the THz region under vacuum conditions. The result is shown in Figure 2. We find a continuous increase in THz absorption with frequency for all NALA concentrations from 1.2 to 3 THz. With increasing solute concentration, the terahertz absorption is found to be decreased, in accordance with the expected "THz defect".

Water shows a two times stronger absorption than 3 M NALA at 2.4 THz (80 cm^{-1}) whereas the absorption curves of the more dilute NALA solutions are between these two absorption curves, the more dilute in concentration the more water-like in absorption. Because this decrease is frequency independent, in the following we will focus on the decrease of absorbance with increasing solute concentration. Concentration-dependent measurements of NALA as measured at both set-ups, the Bruker FTIR spectrometer and our p-Ge laser spectrometer, are compared in Figure 3 and show agreement within their experimental error.

Due to the higher radiation power, the p-Ge spectrometer measurements have a higher experimental accuracy (less than 1%) and thereby allow a more precise determination of the influence of hydration dynamics by the solute.

Figure 4 shows experimental results for $\Delta\alpha$ as a function of the peptide concentration expressed in molar units. Up to about 1.5 to 2 M, we find a similar behavior as for the disaccharides: $\Delta\alpha$ decreases with increasing solute concentration, with a slight deviation from linearity. For higher concentrations, we find a clear change in curvature. For small concentrations, the linear approximated slope is negative (-7.2 cm^{-1} M^{-1} for NAGA and

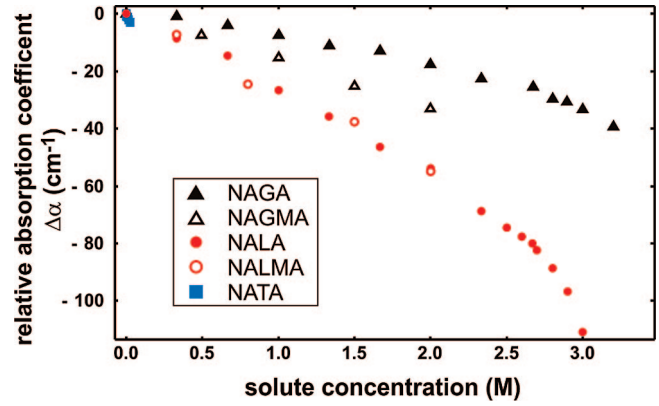


Figure 4. Concentration dependence of the relative THz absorption coefficient α between 2.1 to 2.8 THz measured with a p-Ge spectrometer of aqueous solutions of NAGA, NAGMA, NALA, NALMA, and NATA at pH 4, relative to α of the aqueous reference at pH 4. Concentrations are given in molar units. The statistical error bars are smaller than the symbols. The temperature is (20 ± 0.1) °C.

-26.5 cm^{-1} M^{-1} for NALA). The methyl substitution of one of the amide hydrogen atoms in the hydrophobic NALA/NALMA pair has practically no effect on $\Delta\alpha$. For the hydrophilic NAGA/NAGMA pair, the effect is larger, and the initial slope of -15.2 cm^{-1} M^{-1} for NAGMA is intermediate between those of NALA and NAGA. Finally, the decrease of $\Delta\alpha$ observed for highly hydrophobic NATA with concentration exceeds that of the other peptides by far; the approximate initial slope amounts to -(120 ± 10) cm^{-1} M^{-1}.

Discussion

In total, the sequence of the curves correlates systematically with an increasing polarizability associated with the surface charge density and increasing hydrophobicity. This supports our previous speculation that while the collective modes which are influenced by hydrophilic parts of the solute give rise to an increase in absorption at 3 THz, hydrophobic parts lead to a net decrease of the absorption coefficient.[7] We thereby support the theory that the chemical composition, especially the interface of hydrophobic and hydrophilic domains, and not exclusively the topography determines the solvation dynamics: This supports the results of simulations which predict quite distinct dielectric spectra for NAGMA and NALMA.[19] It is also in line with a recent THz study of Markelz and co-workers which proves the tertiary structure formation is not important for the onset of the dynamical transition.[20]

Whereas for NAGMA and NALMA the solubility restricts the concentration regime to below 2 M, for the new studied model peptides NAGA and NALA, we can go beyond that. Above 2 M, the concentration dependence of $\Delta\alpha$ changes dramatically. These high concentrations now probe the range where we observe the transition from local low frequency modes of the peptides to collective peptide water network oscillations.

For this purpose it is highly instructive to characterize the solutions in terms of the water-to-solute molecular ratio. As a rough measure, we note that a monolayer of water around NALMA should comprise about 25 waters.[17] The first hydration shell of NALA should contain roughly the same number of water molecules, while the hydration numbers of NAGA (and

(19) Murarka, R. K.; Head-Gordon, T. *J. Phys. Chem. B* **2008**, *112*, 179.
(20) He, Y.; Ku, P. I.; Knab, J. R.; Chen, J. Y.; Markelz, A. G. *Phys. Rev. Lett.* **2008**, *101*, 178103.

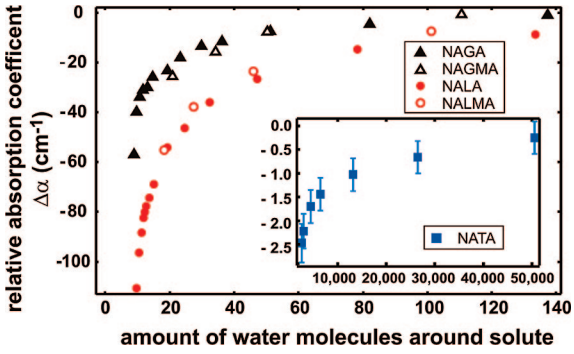


Figure 5. THz absorption coefficient α between 2.1−2.8 THz of the model peptide samples NAGA, NAGMA, NALA, and NALMA dissolved in water at pH 4, relative to α of the aqueous reference at pH 4 as a function of the hydration level characterized by the water-to-solute molecular ratio. The inset shows a THz study of NATA.

NAGMA) should be somewhat lower; on the basis of molecular models, one estimates 18 to 20 waters per solute.

Figure 5 shows the change in absorption (measured as $\Delta\alpha$) expressed in terms of the water-to-solvent molecular ratio. The conversion from molar units is based on measured mass densities of the solutions. The inset of Figure 5 shows results for NATA, for which the solubility limits the hydration levels to values by 2 orders of magnitude above those achieved with the more hydrophilic peptides. The curve observed for NALMA is practically indistinguishable from that for NALA. In addition, the conversion of the composition variable also removes the difference between NAGMA and NAGA.

Interestingly, the solute-induced change of the solvation dynamics, as probed by THz spectroscopy, is now independent of size, however clearly dependent on the insertion of hydrophilic or hydrophobic parts, as suggested by other studies.[7,19]

Figure 5 reveals several interesting features. Based on the hydration numbers quoted above, the experiments probe the coupled peptide water network motions up to three to four hydration shells around the peptide, to highly confined states, where the peptide−water network motion is measured when on the average only single water molecules or small water clusters are shared by several peptide molecules. At high hydration levels, the curves show a slightly bended decreasing curvature similar as for the carbohydrates.[8,9] However, as soon as the concentration is so high that only the hydration water in the first shell is probed, we find a dramatic change: the absorption coefficient begins to break down with a rapid drop-down of the absorption by more than 25% compared to bulk water.

In the absence of any THz absorption for the model peptide ($\alpha_{peptide} = 0$), $\Delta\alpha$ should approach a value of about -420 cm^{-1} However, even in the complete absence of water molecules, we expect absorption attributed to the low frequency intermolecular modes of the peptides.[10,11,21] For carbohydrates, these contributions were found to be of the order of $\alpha_{solute} = 20$ to 50 cm^{-1}. Thus, a relative absorption coefficient $\Delta\alpha$ of -370 to -400 cm^{-1} would imply the absence of any water network motions in the investigated frequency range.

Among the peptides under testing, the absorption curves recorded for NAGA and NALA solutions cover a sufficiently large segment of the concentration dependence to allow an accurate extrapolation. As an exciting feature, the intermolecular water network motions cannot be extrapolated to zero water concentration, which stresses the failure of a simple two-component model. Rather the curves show a nonlinear behavior with a divergent absorption drop at water-to-solute ratios of (9 ± 0.5) for NALA and (8.5 ± 0.5) for NAGA.

Our results indicate that it needs a minimum number of hydration waters to activate the collective water−peptide network motions as probed by THz spectroscopy. This number is well below a monolayer coverage of the model peptides. Estimates based on the hydration numbers quoted above imply a coverage of 35% for hydrophobic NALA and 45% for hydrophilic NAGA relative to complete hydration. It appears that the higher minimum coverage of hydrophilic NAGA is beyond the uncertainty of these estimates.

The results show a striking resemblance to hydration effects upon the functionality of proteins. In the latter case, there seems to be a hydration threshold for the onset of enzyme activities well below monolayer coverage at hydration levels similar to those observed here.[1] The present results are along with others[22] suggesting that the activation of intermolecular network motions of water will be a key step in the activation of protein functionality.

Acknowledgment. We acknowledge Dr. P. Sasisanker for peptide preparation. B.B. thanks the Ruhr-University Research School for financial support. M.H. gratefully acknowledges the Human Frontier Science Program for financial support. The FTIR spectrometer was funded by BMBF grant 05S7PC2.

JA808997Y

(21) Kutteruf, M. R.; Brown, C. M.; Iwaki, L. K.; Campbell, M. B.; Korter, T. M.; Heilweil, E. J. *Chem. Phys. Lett.* **2003**, *375*, 337.
(22) Special issue *Chem. Phys. Chem.* **2008**, 9 (18).