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The Dynamics of Water−Protein Interaction Studied by Ultrafast Optical Kerr-Effect Spectroscopy

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Abstract: Changes in the ultrafast dynamics and terahertz Raman spectrum accompanying a helix-to-coil transition of a homo-polypeptide have been observed for the first time. Formation of the α-helix is associated with a shift to lower frequency of a broad Raman band attributable to solvent-peptide intermolecular hydrogen bonding. This band facilitates direct spectroscopic observation of so-called hydration water near a peptide and yields the first quantitative estimate of the time scale of the ultrafast dynamics in the solvation shell, which range from 0.18 to 0.33 ps (185−100 cm^{-1}) depending on the secondary structure of the peptide. Such fast motions of solvent molecules have been referred to as the "lubricant of life" and are thought to play key roles in determining structure and activity of proteins.

Introduction

Protein structure plays a vital role in determining their biological function and a thorough understanding of the factors that either determine the secondary structure or control changes between conformational states is of widespread interest. Consequently, the helix to coil transition (HCT) has been the subject of a great many studies.[1−3] To date, however, the focus of this attention has been on the changes in structure and dynamics pertaining solely to the polypeptide backbone. Latterly, it has been realized that solvent water molecules also have a significant part to play, but their exact role is not well understood.

The main problem is that the solvent dynamics apparently occur on many timescales, meaning that a range of techniques is required to obtain a complete picture.[4−12] Molecular dynamics simulations,[4−8] supported by fluorescence spectroscopy,[13,14] NMR,[15] and inelastic neutron scattering experiments[11] report rotational dynamics ranging from a few picoseconds for bulk water[6] up to hundreds of picoseconds. The latter have been attributed to suppressed dynamics of so-called hydration or bound water[4−6] held near the protein surface by hydrogen bonds. The properties of this bound water have attracted a lot of interest because of the discovery of such water molecules deep within the structures of proteins (e.g., in enzyme binding pockets) leading to suggestions that these are crucial to the function of the biomolecules. This is supported by observations that a minimum level of hydration is required for an enzyme to function.[8] It has also been predicted by simulations that the presence of a hydration (or hydrogen-bonding) site on a protein causes dramatic changes in the local solvent density.[4−6]

Despite this, little is known about the *mechanism* through which water interacts with the protein, particularly with respect to controlling secondary structure. A decade ago it was suggested that residues in unfolded proteins and in disordered loop regions of molten-globule-like states (such as might occur in the active site of enzymes) "flicker" between α-helical, β-sheet, and PPII-helix structures with a rate of about 10^{12} s^{-1} at room temperature.[9] It was suggested that these flickering motions are associated with picosecond rearrangements of the hydrogen-bond network of water, both in the bulk and near the peptide backbone.[9] These motions are thought to enable protein folding and structural rearrangements, hence the phrase "lubricant of life". To date, there has been no direct observation of this effect and no quantitative estimate of the timescales involved. Here, we report changes in the terahertz Raman spectrum of a homo-polypeptide accompanying a secondary structure (α-helix to coil) transition, changes that can be attributed to variations in solvent−peptide hydrogen bonding.

The technique used is optically heterodyne-detected optical Kerr-effect (OHD-OKE) spectroscopy.[16−26] This method is

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widely used for measuring the time-dependent third-order polarizability anisotropy of liquids.[27] In addition to the access to ultrafast molecular dynamics, OHD-OKE spectroscopy provides, via Fourier transformation, an excellent method for recovering terahertz frequency Raman scattering data at frequencies that are often obscured by the Rayleigh line in spontaneous Raman spectroscopic methods. OHD-OKE has been used extensively to study the dynamics of pure liquids and binary mixtures[27−29] and is increasingly being employed in investigations of more complex liquid-phase systems. Examples of the latter include microemulsions,[30−33] sol−gel confined liquids,[34−37] and of direct relevance to this study, liquid crystals,[38−41] polymers,[42−44] biopolymers, and proteins.[45,46] In particular, Giraud et al.[46] studied a range of samples including di-L-alanine, poly-L-alanine, and four globular proteins to determine the effect of local structure on the low-frequency vibrational modes of these molecules. More recently, we have applied the technique to study the dynamics of hydrogen-bonded liquid systems.[47,48]

Experimental

The experimental OHD-OKE method has been described in detail elsewhere.[47,49,50] The ultrafast light source is a Kerr-lens mode-locked titanium-sapphire laser operating at 800 nm with a bandwidth of 35 nm and repetition rate of 80 MHz. The pulse duration, measured by second order autocorrelation at the sample position was <30 fs.

Poly-L-lysine (PLL) of molecular weight 10−40 000 was purchased as the hydrobromide (HBr) salt from Sigma-Aldrich along with all other chemicals used in this study; all were used without further purification. PLL-containing samples were of concentration 100 mg/mL of solvent. All samples were filtered using 0.2 μm filters (Millipore) to remove dust and allowed to equilibrate for at least 24 h at the laboratory temperature prior to use. Mid-infrared spectra were recorded using a FTIR spectrometer (Bruker Vertex 70).

A detailed description of the Fourier transform deconvolution procedure used to recover the Raman spectral densities from the OHD-OKE data can be found elsewhere.[27,30] Put simply, the Raman spectral density, undistorted by the finite instrument response time, is obtained from the imaginary part of the ratio of the Fourier transform of the OHD-OKE data to that of the second-order autocorrelation of the laser pulse.

Results and Discussion

Fourier-transform infrared (FTIR) spectroscopy of the amide-I absorption band was used to determine the secondary structure of PLL as a function of methanol concentration. The frequency of the amide-I band is well-known to be sensitive to peptide secondary structure.[1,2] The results are shown in Figure 1a and b. As the methanol concentration was increased, the amide-I band gradually blue-shifted until a concentration of 80% was reached, whereupon a sudden decrease in the amide-I peak frequency occurred.

When PLL is dissolved in water at neutral pH, it assumes the random-coil conformation.[10] Increasing the methanol concentration decreased the strength of the hydrogen bonding between the peptide backbone and the solvent. This caused a steady increase in the amide-I frequency, which continues until the α-helical conformation (having intramolecular hydrogen bonds) becomes more stable than the random coil. The lowered amide-I frequency in the α-helix-state is due to the strong intramolecular hydrogen bonds, which significantly weaken the amide bond in comparison to those found in the random coil configuration.

The effect of temperature on the methanol-dependence of the PLL HCT was also determined using FTIR. Using a methanol-water concentration of 78% (corresponding to the random coil state but close to the observed transition point) a reduction in temperature was shown to result in a transition to the α-helical state. This process was observed to be reversible. Similar behavior was observed when a methanol concentration of 83% (corresponding to the α-helix at room temperature) was used. Once again, higher temperatures favored the random coil configuration. The results for these samples are compared in Figure 1c, and arrows in Figure 1b summarize the effect of temperature on the HCT. Thus, the HCT could be studied with solvents of constant methanol content by changing the temperature of a mixture near the transition point.

The OHD-OKE responses of PLL in solutions of 30, 50, 70 (random coil), and 95% (α-helix) methanol in water were obtained. Figure 1d shows the time domain data; the sharp spike at time zero corresponds to the electronic hyperpolarizability of the sample and closely follows the instrument response function, which is shown for comparison. The small shoulder between 0 and 300 fs is due to nuclear dynamics. The magnitude of this portion of the OHD-OKE response is determined by the molecular third-order polarizability tensor, and it can be seen that this is weak in this case. In spite of this, however, it is clear that the OHD-OKE method yields data with a high signal-to-noise ratio, hence the preference for this approach over linear

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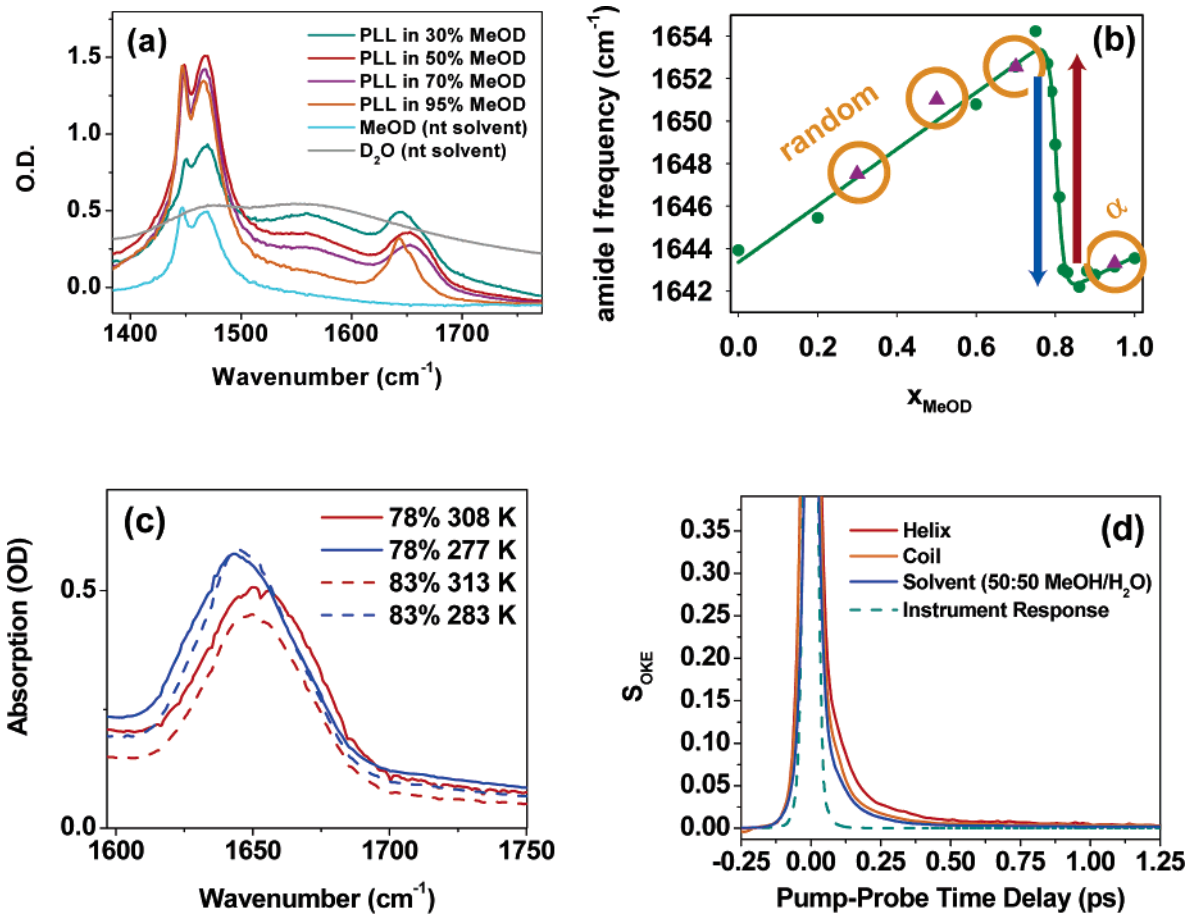

Figure 1. (a) FTIR spectra in the amide-I (~1650 cm^{-1}) region of poly-L-lysine (PLL) dissolved in aqueous solutions containing 30, 50, 70, and 95% MeOD by volume (deuterated solvents were used in order to prevent the amide-I band from being obscured). FTIR spectra of the neat solvents are also shown. (b) Plot of amide-I peak frequency of PLL as a function of methanol concentration (X_{MeOD}). Orange circles indicate the samples used in OHD-OKE studies, whereas the red and blue arrows demonstrate the effect of temperature on the helix-to-coil transition (HCT) (see text). (c) Effect of temperature on the amide-I transition frequency of PLL in 78% (solid lines) and 83% (dashed lines) MeOD. Cooling the sample induces a coil to helix transition and a red-shift of the absorption. (d) Example OHD-OKE responses of PLL in helical and random coil conformations. The OHD-OKE response of a typical solvent mixture is also shown along with the instrument response function for the purpose of comparison.

Raman or IR methods. In such a situation, it is more instructive to consider the data in the frequency regime and Figure 2a, b, and c show the terahertz Raman spectra of the solutions, solvents, and difference spectra. The difference spectra show a band at ~100 cm^{-1} (3 THz) for the α-helical conformation and ~185 cm^{-1} (5.5 THz) for the random-coil. Data from the three random-coil samples show that this band is insensitive to solvent composition and dependent only upon the PLL conformation. This is shown in Figure 2a by a comparison of the difference spectra recovered from the 30 and 50% methanol samples. The amplitude of the band is slightly lower in the 30% sample but the peak shows no significant shift. The random-coil spectra in Figure 2a also exhibit a very low-frequency peak corresponding to a 3.5 ps decay in the time-domain. This indicates a slow process such as rotational diffusion that apparently occurs only in the random-coil conformation. It is interesting to note that the data from the α-helical sample is somewhat noisier than that of the random coil. This is possibly due to the increased inhomogeneity in the α-helical sample as a result of decreased solvent−solute interactions and manifests itself in the frequency domain as irreproducible oscillations.

Previous studies of peptides and proteins in solution[45] have employed fits to a series of Brownian oscillators in an attempt to shed more light on the origins of the observed bands. In this case, however, the fact that the signals recovered from the PLL samples show little structure means that it is more instructive to compare the raw data.

The broad band at 100 or 185 cm^{-1} corresponds to time domain dynamics on timescales of 0.33 and 0.18 ps. It should be noted that, although the same information is contained in both the time and frequency-domain data, the weak signals from PLL mean that it is significantly easier to determine these timescales in this case following solvent subtraction in the frequency domain. This band has several possible origins. Previous studies[51,32] have shown that intramolecular backbone-torsional motions are responsible for bands observed near 100 cm^{-1} in polymer systems and in simulations of the spectra of α-helices. However, such an explanation is inconsistent with the spectral change observed; stiffening of the backbone caused by increased intramolecular hydrogen bonding in the helix implies an expected *increase* in the frequency of such a mode.

Lysine side-chain librations could be responsible for these low-frequency bands. In the random-coil conformation, solvent-peptide hydrogen bonds are formed.[10] This would cause the first solvent shell to be more structured and stiffen the potential for side-chain libration. This would lead to the observed frequency

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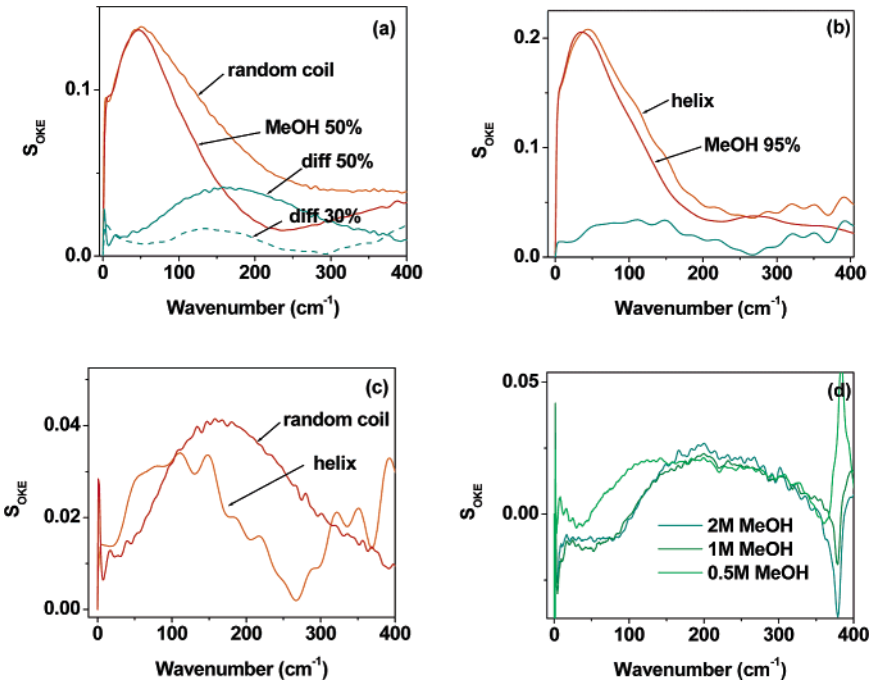

Figure 2. OHD-OKE spectra of PLL solutions (orange) in (a) random coil and (b) α-helical conformations along with the solvent responses (red) and difference spectra (green). (c) Comparison of the two difference spectra; (d) difference spectra for a solution of 1 M hydroquinone (1,4-dihydroxybenzene) in acetonitrile with stoichiometrically small quantities of methanol added.[47]

shift. However, this explanation is inconsistent with inelastic neutron-scattering (INS) studies of proteins in which a peak at about 25 cm^{-1}, the Boson peak, is typically observed and has been assigned to side-chain libration.[52] It should be noted that this peak is Raman-active and will contribute to the observed spectra at low frequencies. That it is not specifically observed is due to the broad nature of the low-frequency response and to the fact that the INS technique is very sensitive to hydrogen atom motions which Raman spectroscopy, where the signal arises from the molecular polarizability tensor, is not.

This leaves solvent-peptide intermolecular hydrogen bonding. Recent studies of a model system for poly-L-tyrosine (which contains a phenolic side chain), featuring phenol derivatives dissolved in methanol and acetonitrile revealed a broad band located at around 200 cm^{-1} when methanol was present (Figure 2d).[47] FTIR data showed that the proportion of hydrogen bonding in the methanol-containing samples was significantly higher than in acetonitrile.[47] Subsequently, this band was assigned to the liquid-phase analogue of transitions reported in gas-phase fluorescence spectra of phenol-$(methanol)_n$ clusters[53] arising from modes involving intermolecular hydrogen bonds. The similarity to the band observed here supports the assignment of these bands to solvent-peptide hydrogen-bond motions. It is also persuasive to note that the reduced solvent-peptide interactions upon formation of the helix[10] would yield an expected red-shift. It should be noted that the peaks occurring at around 400 cm^{-1} are due to intramolecular vibrational modes of acetonitrile. These are extremely strong and the solvent subtraction process has resulted in the observed artifacts.[47]

A variety of studies[4−7] have concluded that so-called bound water exists near hydrogen-bonding sites on the surface of proteins. This bound water is reported[6] to have a significantly longer residence time near the protein than bulk water, along with suppressed rotational dynamics. Results also suggest that this localized water exhibits an increased density relative to that of the bulk, to the extent that it approaches a glassy state.[6,8] PLL, in the random coil conformation, possesses many of these hydration sites because of the lack of intramolecular hydrogen bonding. This increased solvent-peptide interaction in this conformation will lead to a higher observed Raman frequency for intermolecular modes.

Despite the ability of OHD-OKE spectroscopy to observe rotational diffusive dynamics,[27] suppressed rotational relaxation of solvent near the peptide[5,6] was not observed. However, the timescales reported are on the order of hundreds of picoseconds.[6] These lie beyond the scope of our spectrometer as a result of the weak signal obtained from the PLL samples. Although it is possible to cover such timescales with the spectrometer, the OHD-OKE signal obtained has disappeared within pump−probe delay times of 5−10 ps.

It is necessary to determine that the shift in the Raman band is not simply due to changes in the strength of interaction or mass arising from the different solvents. The 70 cm^{-1} shift is too large to arise simply from the change in mass of the solvent however and the fact that the position of the band is insensitive to solvent composition in the random-coil state also negates this argument.

To exclude solvent-composition effects definitively, experiments were carried out on samples with methanol-water mixtures near the HCT transition, with temperature used to induce the conformational change. Deuterated solvents allowed verification of the PLL conformation using FTIR prior to the OHD-OKE experiments (Figure 1c). Spectra were obtained for

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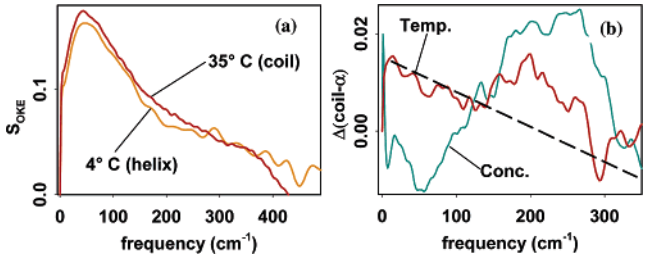

Figure 3. (a) OHD-OKE spectra of 100 mg/mL PLL dissolved in 78% methanol at 308 and 277 K (35 and 4 °C). (b) (red) Difference spectrum from (a) and (cyan) double difference spectrum from data in Figure 2, which is directly comparable to the red curve, see text.

two samples containing 78 and 83% methanol. The former was studied at 308 (coil) and 277 K (α-helix) and the latter at 313 (coil) and 283 K (α-helix).

The results of the experiments are shown in Figure 3a, which shows the terahertz Raman spectra obtained for the 78% methanol solution. The results for both samples were consistent and no solvent subtraction was used because the solvents are identical. Figure 3b shows the response of α-helical PLL subtracted from that of the random coil. A broad band appears at around 200 cm^{-1} in the random coil state and an increase in intensity below 100 cm^{-1} is observed. The appearance of the band near 200 cm^{-1} is consistent with the observations from the concentration dependent experiments above. The corresponding double difference (coil−α-helix) spectrum from those experiments is shown in Figure 3b for comparison.

The increased intensity below 100 cm^{-1} in the random coil relative to that of the α-helix in the temperature-dependent experiments is not observed in the concentration-dependent data, which suggest a greater intensity in the α-helical conformation. This Boson peak region[6,8] is attributable to librational modes of the peptide side chains and to the glassy nature of the hydration shell near the peptide backbone. Studies of the Boson peak[8,12] report temperature-related increases in the low-frequency density of states, which may account for the increased spectral density observed here. To confirm this, experiments were performed to determine the effect of increased temperature on the spectral density of PLL dissolved in 30% methanol solution and of the solvent alone. Temperature-induced changes in the spectral density of the solvent proved to be very slight, in line with previous OHD-OKE studies,[27] whereas heating the PLL-containing sample caused increased Raman scattering intensity below 100 cm^{-1} identical to that shown in Figure 3b. We conclude that the temperature-induced baseline for this experiment is approximately as shown by the dashed line in Figure 3b.

Thus, the agreement between the concentration and temperature-controlled experiments is very good, the appearance of a band due to the random coil conformation is clearly observed in both cases. Furthermore, the peak of this band is slightly lower in the temperature-dependent experiment, as would be anticipated given the use of deuterated solvents.

Previous OHD-OKE studies of peptides[45,46] have observed increased Raman-scattering intensity at low-frequency due to cooperative effects within the α-helix resulting in an enhanced dipole moment. The difference spectrum from the concentration experiment (Figure 3b) shows a negative contribution in the region below 100 cm^{-1}, indicating increased amplitude in the α-helical state, but definitive assignment to cooperative effects is impossible due to the range of possible contributions to this region.

It is concluded that the broad Raman band observed in these PLL-containing samples is attributable to solvent-peptide hydrogen bonding and shows that important dynamics are taking place in these systems on timescales of 0.18−0.33 ps, which are dependent upon the conformational state of the peptide. Such observations are consistent with the dynamics predicted to constitute the "lubricant of life".[9]

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