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Role of Hydration in the Phase Transition of Polypeptides Investigated by NMR and Raman Spectroscopy

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Summary: NMR, Raman spectroscopy and *ab initio* quantum-chemical calculations have been employed to investigate the role of the hydration water in the inverse temperature transition of elastin-derived biopolymers represented by poly(Gly-Val-Gly-Val-Pro) and poly(Ala-Val-Gly-Val-Pro). Temperature and concentration dependences of the Raman spectra measured for water solutions of polymers and of a low-molecular-weight model have been correlated with the vibrational frequencies calculated at the DFT (B3LYP) and MP2 levels for the peptide segment surrounded by a growing number of water molecules. The results indicate strong hydration before the transition that, in addition to water hydrogen-bonded to amide groups, includes hydrophobic hydration of non-polar groups by a dynamic cluster of several water molecules. According to ^{1}H longitudinal and transverse relaxation of HOD signals in D_2O solutions, the number of water molecules motionally correlated with the polymer is about 4 per one amino acid residue.

Keywords: NMR; quantum chemistry; Raman spectroscopy; transitions; water-soluble polymers

Introduction

Hydration and hydrophobic interactions are important factors in the stability of the folded structure of water-soluble polypeptides and proteins. In this study we have employed NMR, Raman spectroscopy and *ab initio* quantum-chemical calculations to investigate the role of the hydration water in the inverse temperature transition of elastin-derived biopolymers represented by poly(Gly-Val-Gly-Val-Pro) (*1*, R=H) and poly(Ala-Val-Gly-Val-Pro) (*1*, R=CH_3).

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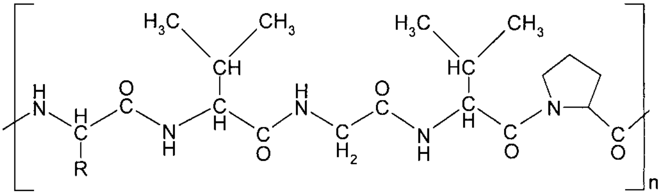

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At low temperatures, they are soluble in aqueous media. Under gradual heating, a critical temperature can be found, at which the polypeptide molecules undergo a reversible change of state leading to their strong conformational change, aggregation and eventually sedimentation.[1-3] According to theoretical predictions, the pre-aggregation changes include entropy-driven changes in specific molecular hydration, chain conformation and internal hydrogen bonding. Analysis of the spectral changes observed in the temperature and concentration dependences has shown that polypentapeptides exhibit vibrations sensitive to both changes in the conformational structure of the polymer (C-H bending) and to local changes in the surrounding medium of the polymer chain caused by hydrophobic or hydrophilic interactions (C-H stretching).[4,5] The aim of this study is to correlate data provided by NMR and Raman spectroscopy with the results of *ab initio* calculations for a model structure of amide polymers surrounded by a growing number of water molecules, from one water molecule to a clathrate-like structure.

Results and discussion

The entropy-driven change in the polymer hydration and competition for hydration between non-polar and polar groups are key postulates in the Urry's explanation of the thermoreversible behavior of elastin-like polypeptides[1,7] and thus the polymer related dynamic states of water are of primary interest. The hydration degree of poly(Gly-Val-Gly-Val-Pro) at temperatures below transition has already been studied. Urry used microwave dielectric relaxation data to estimate the amount of water of hydrophobic hydration and found about 20 water molecules per amino acid residue.[7] The calorimetric study of polymer solutions in water-excess and water-deficit conditions indicate that complete saturation of poly(Gly-Val-Gly-Val-Pro) by water of hydration corresponds to 170 water molecules per pentamer as an upper limit.[8] The hydrophobically perturbed water experimentally delineated from bulk water thus extends the surface layer and the waters of hydrophobic hydration have to be understood as a more or less diffuse sheath of structured water molecules variously correlated with the polymer.[7,9] Molecular dynamics simulations of polypentapeptides with explicit water[10,11] support this view but indicate a rather strong change in water-polymer correlation at the transition temperature.

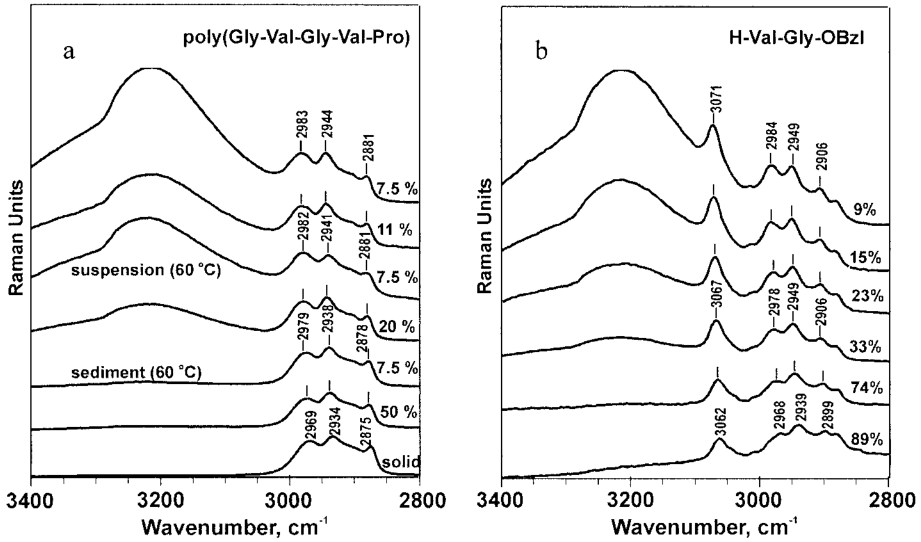

Figure 1. Raman spectra of poly(Gly-Val-Gly-Val-Pro): 7.5, 11, 20, and 50 wt % in H_2O measured at room temperature, a suspension and a sediment in H_2O at 60°C, and the original solid sample at room temperature (a). Raman spectra of H-Val-Gly-OBzl solutions in H_2O at indicated concentrations (b).

Spectral changes observed in the temperature and concentration dependences show (Figure 1a) that elastin-like polypentapeptides exhibit vibrations (C-H stretch modes) sensitive to the local changes in the surrounding medium of the polymer chain caused by hydrophilic and hydrophobic interactions. Compared to 7.5 wt % water solution the C-H stretch bands are shifted to lower wavenumbers by ~ 6 - 9 cm^{-1} in 50 wt % water solution and the shifts reach the value of 14 cm^{-1} for dry solid samples. Analogous concentration changes of the interactions with solvent molecules and corresponding frequency shifts of the C-H stretch bands are observed also for a low-molecular-weight model, H-Val-Gly-OBzl (Figure 1b). The notion that frequency shifts are related to the changes in the intermolecular arrangement of water molecules in the vicinity of hydrophobic and hydrophilic groups of amino acid residues[4] is confirmed by *ab initio* quantum chemical calculations. We have studied interactions of a peptide segment corresponding to the amino acid residue (alanin) surrounded by hydrogen bonded water molecules arranged in strings or clusters (Figure 2). The calculations were carried out at the density functional theory (DFT, B3LYP functional)[12] and Møller-Plesset (MP2) levels of theory with the 6-31G(d) being the basis set employing the Gaussian 98 program package.[13]

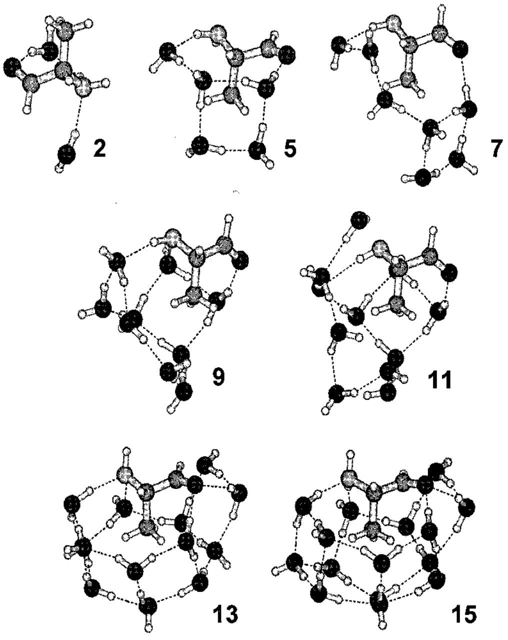

Figure 2. B3LYP/6-31G(d) optimized structures of the model $NH_2CH(CH_3)CHO$ with increasing number of H_2O molecules.

From the analysis of the experimental frequency shifts and its relation to the data obtained in model calculations follows that changes observed in the Raman spectra are predominantly caused by blue-shifting hydrogen bonding C-H...OH_2. The spectral manifestation and the nature of improper, blue-shifting hydrogen-bonded complexes X-H...Y are entirely different from those of standard hydrogen-bonded complexes.[14] While the standard complexes are characterized by an elongation of the X-H bond and a corresponding red shift of the respective stretch frequency, blue-shift complexes exhibit a contraction of the X-H bond and a blue shift of the X-H stretch frequency. Both types of complexes are characterized by electron-density transfer from the proton acceptor to the proton donor.[14]

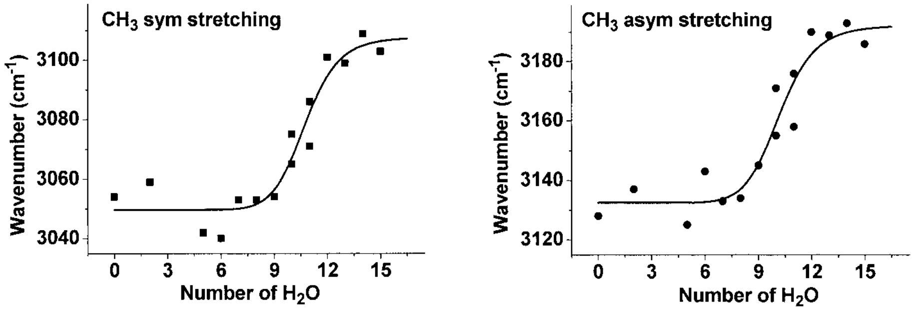

Figure 3. Calculated (B3LYP/6-31G(d)) frequencies for symmetric and asymmetric CH_3 stretch vibrations of $NH_2CH(CH_3)CHO$ in a cluster with n molecules of H_2O.

It is seen in Figure 3 that *ab initio* calculated frequencies of the C-H stretch vibrational modes exhibit strong dependence on the number of hydrated water molecules. In order to exclude the effect of the method, some of the points in Figure 3 have been recalculated at the MP2 level and the results show analogous frequency differences. For the structures with cage-like clusters of high number of water molecules (n = 12 – 15) around the methyl group the frequency shifts reach the value of ~ 60 cm^{-1} relative to the typical values obtained for the isolated model or the model with hydrogen bonded water molecules. However, upper limit for the experimentally determined frequency shifts due to hydration lies in the range 6 – 16 cm^{-1} both in polypentapeptides and in H-Val-Gly-OBzl (Figure 1) and it suggests that only several water molecules (approximately up to 10) can be strongly correlated with the peptide segment corresponding to one amino acid residue.

As a sensitive probe into water motional states and interactions can serve relaxation rates of protons in water. Under no polymer-water interactions, longitudinal and transverse relaxation times T_1 and T_2 should be equal at given temperature due to extreme narrowing and increase with increasing temperature. Experimental results shown in Figure 4 are quite different from this expected course (due to radiation damping in H_2O, we used the ^{1}HOD signal in D_2O solutions of the polymers). Large difference between T_1 and T_2 determined for both polymer solutions originates from various sources, the most important being chemical exchange and motional hindering due to transient interactions with the polymer. The chemical exchange is formed by contributions of HOD protons exchange either with excess deuterons in predominant D_2O or with deuterons in ND-CO groups of the polymer. It was shown[6] that all contributions of the chemical exchange can be estimated by using feasible approximations and from the difference between T_1 and T_2 we can determine steady state mole fraction φ of HOD molecules in direct interaction with polymer chains.

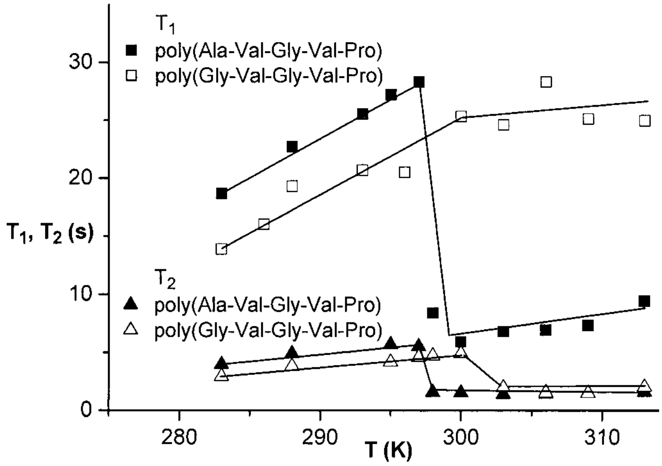

Figure 4. T_1 and T_2 relaxation times of HOD in 5% w solutions of poly(Gly-Val-Gly-Val-Pro) and poly(Ala-Val-Gly-Val-Pro) in D_2O.

Obtained value of $\varphi \approx 0.04$ for the 5 w% solution of polypentapeptides with an average of 105

D_2O molecules per one amino acid residue indicates that about four water molecules per one residue are statististically correlated with polymer chains. Due to the uncertainties in the estimation, the number is considered as a lower limit. This number of correlated water molecules is substantially lower than values obtained in dielectric relaxation[7] and DSC[8] studies but it corresponds to strong hydration water, which includes hydrophobic hydration besides hydrogen-bonded water. On the other hand, relatively small number of hydrophobically hydrated water molecules following from NMR relaxation data is in good agreement with the results from Raman spectroscopy and *ab initio* calculations given above.

Conclusion

The results question the widespread notion of hydration water as ordered clathrate structures surrounding non-polar groups. For such ordered structures the *ab initio* calculations predict significantly larger changes of the vibrational frequencies in comparison with the experimentally determined values.

^1H longitudinal and transverse relaxation of HOD signals in D_2O solutions indicate that the number of water molecules motionally correlated with the polymer is about 4 per one amino acid residue at 300 K, in accordance with the finding mentioned above.

Acknowledgment

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