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Effects of solvent concentration and composition on protein dynamics: ^{13}C MAS NMR studies of elastin in glycerol–water mixtures

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ABSTRACT

We use ^{13}C CP MAS NMR to investigate the dependence of elastin dynamics on the concentration and composition of the solvent at various temperatures. For elastin in pure glycerol, line-shape analysis shows that larger-scale fluctuations of the protein backbone require a minimum glycerol concentration of ~0.6 g/g at ambient temperature, while smaller-scale fluctuations are activated at lower solvation levels of ~0.2 g/g. Immersing elastin in various glycerol–water mixtures, we observe at room temperature that the protein mobility is higher for lower glycerol fractions in the solvent and, thus, lower solvent viscosity. When decreasing the temperature, the elastin spectra approach the line shape for the rigid protein at 245 K for all studied samples, indicating that the protein ceases to be mobile on the experimental time scale of ~10^{-5} s. Our findings yield evidence for a strong coupling between elastin fluctuations and solvent dynamics and, hence, such interaction is not restricted to the case of protein–water mixtures. Spectral resolution of different carbon species reveals that the protein-solvent couplings can, however, be different for side chain and backbone units. We discuss these results against the background of the slaving model for protein dynamics.

1. Introduction

It is nowadays widely accepted that the environment of a protein is a crucial factor for its function [1]. While there is consensus that the presence of a solvent is essential, the underlying interaction between solvent and protein is still a matter of debate. One popular view on this topic is the slaving concept [2,3], which proposes that solvent fluctuations in the immediate vicinity of the protein surface control small-scale protein dynamics, while those at sufficient distance govern large-scale protein motions. However, a recent study argued that the slaving concept needs to be reconsidered taking into account a mutual influence of solvent and protein dynamics [4]. Also, it was conjectured that the solvent modulates the energy barriers to protein relaxations and, hence, it does not slave, but rather plasticize the protein [5]. Thus, the exact action of the solvent is not yet fully understood.

Important insights into environmental influences on protein motions can be gained from variation of the temperature and the solvent. The former approach was taken in studies on the dynamical transition i.e., a freezing of protein relaxations upon cooling, providing clear evidence for the importance of a coupling of protein and solvent dynamics [6–10]. While interactions between proteins and solvents were mostly studied for the example of water, the case of glycerol is also of high relevance since this molecule plays important roles as a cryoprotective agent and in life sciences [11,12]. The dynamical behavior of various protein–glycerol mixtures was investigated by means of molecular dynamics simulations [13,14] as well as neutron scattering [15] and dielectric spectroscopy [16,17] experiments. It was reported that motions of the protein become slower when using glycerol rather than water as the solvent. For the dynamics of glycerol, two studies found a slowdown in the mixtures with respect to the bulk [16], at least in the immediate vicinity of the protein surface [13], while other works did not observe such effect [17].

Unlike in many scientific approaches, proteins in cells are not surrounded by a pure solvent, but are embedded in heterogeneous environments. Therefore, use of binary solvent mixtures is the next step to understand protein dynamics under biological conditions. Experimental and computational studies on proteins in binary solvents reported that the dynamics of the protein slow down with increasing viscosity of the solvent [18–22]. For proteins in glycerol–water mixtures, it was found that this change of the dynamical behavior is accompanied by preferential hydration, i.e., by the formation of a solvation shell with increased water concentration relative to the nominal solvent composition [23–27].

Elastin, one of the main components of connective tissue, differs structurally and dynamically from often used model proteins like lysozyme or myoglobin due to the higher molecular weight, the lack of a defined secondary structure, and the higher fraction of hydrophobic amino acids. A number of studies analyzed the origin of the characteristic elastic properties of this protein [28–32]. Dielectric measurements focusing on the hydration dependence showed an onset of protein dynamics at a hydration level of ~0.25 g/g, corresponding to ~1.2

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water molecules per elastin residue [33]. Moreover, use of this technique revealed that water and glycerol exhibit broad distributions of mobilities at the elastin surface [17,34].

Nuclear magnetic resonance (NMR) has proven to be an excellent tool to ascertain the interplay of elastin with solvents. On the one hand, ^{13}C NMR yields valuable insights into the protein dynamics. In particular, combination of the cross polarization (CP) and magic angle spinning (MAS) techniques enables spectral resolution of signals from structurally different carbons in the solid state, whereby the intensity and width of the various resonances yield information about molecular dynamics [35]. So far, ^{13}C CP MAS NMR line-shape analysis was used to show that elastin dynamics sets in above a hydration level of 0.2 g/g [36,37], consistent with the abovementioned dielectric studies [33]. Also, it was employed to investigate the temperature-dependent mobility for dry and hydrated elastin [38,39]. On the other hand, ^{2}H NMR provides straightforward access to the solvent dynamics. We exploited this capability to determine the rates and mechanisms of water and glycerol motions in elastin matrices over broad time and temperature ranges [39–43].

Here, we apply ^{13}C CP MAS NMR to determine the effects of solvent concentration and composition on elastin dynamics for moderate solvation levels. To study the concentration dependence, glycerol is used as the solvent in our approach, while water was employed in previous works [36,37]. Hence, a comparison of present and previous data can provide further insights into protein–solvent couplings for the example of elastin, which has a high biological value and, at the same time, a molecular structure different from that of frequently studied model proteins. To investigate the composition dependence, glycerol–water mixtures are used so that the solvent viscosity can be varied by changing the temperature or the fractions of the solvent components. Finally, we exploit that our findings for the elastin dynamics can be discussed against the background of a large body of literature results for solvent motion near this protein.

2. Methodical and experimental aspects

2.1. Methodical aspects

In our case of moderate solvation levels, proteins, including elastin, are expected to exhibit anisotropic internal dynamics [37–39,44–46]. ^{13}C CP MAS NMR spectra provide valuable information about such fluctuations [35,47]. Specifically, when molecular dynamics is absent or slow, i.e., when the correlation time is longer than 10^{-4} s, ^{13}C CP MAS NMR spectra feature well resolved lines. Anisotropic motions with a characteristic time scale in the range 10^{-4}–10^{-6} s lead to a line broadening and a loss of signal intensity due to a reduced CP efficiency [48] and an interference of the stochastic time dependence of molecular dynamics with the coherent averaging associated with the applied dipolar decoupling and sample spinning techniques [49–51]. Finally, highly restricted reorientations with correlation times shorter than 10^{-6} s result in narrow lines as motional averaging is completed on the experimental time scales, while less restricted reorientations on this time scale can cause a merging or disappearance of lines [35]. Altogether, ^{13}C CP MAS NMR spectra probe restricted protein dynamics on the experimental time scale ~10^{-5} s via a line broadening and reduced signal intensity.

2.2. Experimental aspects

We study elastin from bovine neck ligament, which was purchased from Sigma-Aldrich and used without further purification. To investigate the dependence of protein dynamics on the solvent concentration, elastin and glycerol were carefully mixed to obtain samples with various solvation levels s. In the following, we specify the values of s in $g_{solvent}/g_{elastin}$, where we drop the indices for convenience. Six samples with solvation levels s between 0.0 and 0.8 g/g are studied. The influence of the solvent composition is analyzed for elastin in glycerol–water mixtures. We prepared samples with a fixed solvation level of $s = 0.3$ g/g and glycerol:water weight ratios of 0:100 (pure water), 25:75, 50:50, 75:25, 94:6, and 100:0 (pure glycerol), corresponding to molar ratios of 0:100, 6:94, 16:84, 37:63, 75:25, and 100:0. Prior to the MAS experiments, the samples were stored in MAS rotors at room temperature for several days to ensure an appropriate solvent distribution. No significant differences were observed when repeating some of the measurements after several weeks. We excluded a loss of solvent during the storage times by weighing.

^{13}C CP MAS NMR experiments were performed on a TecMag Apollo spectrometer working at 8.57 T, corresponding to a 1H frequency of 354.8 MHz and a ^{13}C frequency of 89.2 MHz. A 4 mm double resonance MAS probe was used. For CP, a 1H 90° pulse of 2.7 μs length was followed by a contact time of 0.8 ms. Field strengths for high-power cw decoupling during acquisition were in the range 70–80 kHz. The spinning frequencies were 5.5 and 6.0 kHz during the measurements of elastin–glycerol samples and elastin–glycerol–water mixtures, respectively. The temperature was calibrated using the ^{207}Pb chemical shift of lead nitrate $Pb(NO)_3$ [52].

The spectra were obtained from free induction decays of 4096 data points with a dwell time of 4.0 μs. Prior to Fourier transformation, we applied a baseline correction, we doubled the number of data points by zero filling, and we multiplied the time signals with a Gaussian corresponding to a line broadening of $\sigma = 60$ Hz. The ^{13}C chemical shifts were referenced with respect to tetramethylsilane using adamantane as external standard. To account for different sample masses and temperature effects, the spectra are scaled by the elastin masses and, for elastin in the binary solvent, multiplied with the temperature to correct for the Curie factor.

3. Results

3.1. Dependence on the glycerol concentration

Fig. 1 shows the ^{13}C CP MAS NMR spectrum of dry elastin at ambient temperature together with an assignment of the carbon resonances to amino acid residues according to Ref. [53]. Three spectral regions are distinguishable. A broad resonance at ~173 ppm can be attributed to backbone carbons in carbonyl groups. In the region between 70 and 40 ppm, we see four peaks from other carbons in the protein backbone, mostly aliphatic C_α. The third region between 30 and 15 ppm consists of three or four resolved peaks. The most upfield peak can be assigned to the methyl groups of valine and alanine, the others are C_β and C_γ side chain carbons of the amino acids.

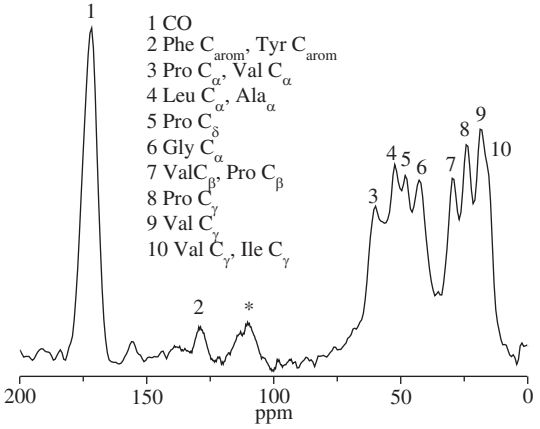

Fig. 1. ^{13}C CP MAS NMR spectrum of dry elastin at ambient temperature together with an assignment of the resonances according to Ref. [53]. The asterisk marks a spinning sideband of the carbonyl signal.