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Dynamical structural distributions in proteins

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Abstract

A moment analysis of the self-intermediate scattering function is used to reconstruct the distribution of structural displacements in the small protein myoglobin as a function of time, temperature and environment. Model-independent information is obtained on mean squared displacements and the Gauss-deviation. Two types of molecular motions were identified: rotational jumps of side chains and small-scale translational displacements induced by interfacial water. The displacements of water on the protein surface increase sublinearly with time and the fourth moment deviates from a Gaussian value.

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Keywords: Protein dynamical transition; Myoglobin; Neutron scattering line

1. Introduction

Globular proteins are close-packed structures with densities approaching those of molecular crystals. However, biological function is closely connected with conformational changes and considerable structural plasticity is required to enable the exchange of small ligands between a dense protein interior and the solvent. The general plasticizer is liquid water interacting with the protein surface. To characterize the spatial and time properties of conformational distributions using dynamic neutron scattering is thus of considerable interest [1–4]. In the following, it is shown how the displacement distribution can be reconstructed from experimental scattering functions based on a moment expansion. The neutron scattering cross-section of D_2O-hydrated proteins is dominated (95%) by the non-exchangeable hydrogen atoms and thus incoherent scattering. The corresponding self-intermediate scattering function, $I_{s,i}(\mathbf{Q},t)$ records displacements of individual hydrogen atoms (i) [5,6,8]:

$$I_{s,i}(\mathbf{Q},t) = \langle \exp(i\mathbf{Q}\mathbf{r}_i(0)) \cdot \exp(-i\mathbf{Q}\mathbf{r}_i(t)) \rangle. \quad (1)$$

The scattering vector $\mathbf{Q}$ is the instrumental parameter to modify the spatial scale probed by the scattering process.

From $I_{s,i}(\mathbf{Q},t)$ one derives by a Fourier transform the displacement distribution function $G_{s,i}(\mathbf{r},t)$:

$$G_{s,i}(\mathbf{r},t) = \int \frac{d^3Q}{(2\pi)^3} \exp(-i\mathbf{Q}\mathbf{r}) \cdot I_{s,i}(\mathbf{Q},t). \quad (2)$$

It denotes the probability density, that atom (i) which is initially at $\mathbf{r}_0$ moves to a position $\mathbf{r}$ within a time interval t: for a classical system, averaged over all atoms i, this is equivalent to:

$$G_s(\mathbf{r},t) = \int d^3r_0\, p(\mathbf{r}_0+\mathbf{r},\mathbf{r}_0,t) \cdot p_0(\mathbf{r}_0) \quad (3)$$

with the equilibrium distribution

$$p_0(\mathbf{r}) = p(\mathbf{r},\mathbf{r}_0,t=\infty). \quad (4)$$

Due to limitations of the experimental Q-range, a direct transform according to Eq. (2) is rarely possible. Alternatively, one can reconstruct the displacement distribution from an expansion of $I_s(\mathbf{Q},t)$ in powers of Q^2:

$$I_s(\mathbf{Q},t) \approx 1 - \tfrac{1}{2} \cdot Q^2 \cdot \langle (\hat{Q}\mathbf{r})^2 \rangle(t) + \tfrac{1}{24} \cdot Q^4 \cdot \langle (\hat{Q}\mathbf{r})^4 \rangle. \quad (5)$$

Thus, a polynomial fit of the intermediate scattering function at fixed time $I(Q,t_{fix})$ versus Q^2 yields the appropriate information on the moments of $G(r,t)$.

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2. Results: protein dynamics

In the following, we focus on experiments performed with myoglobin, a small helical protein, which stores oxygen in muscle tissue. The protein was embedded in three different environments: (a) water: fully hydrated (0.35 g D_2O/g protein); (b) vacuum (lyophilized to less than 0.05 g/g) and (c) a glassy perdeuterated glucose matrix, $T_g = 325$ K. The backscattering spectrometer IN13 (ILL, Grenoble) provides an unusually large Q-range up to 5 Å^{-1}. Fig. 1 shows representative scattering data approximating the intermediate scattering function $I(Q, t = 50\,\text{ps})$ at fixed time at various temperatures in the three environments.

Dehydrated and glassy myoglobin display similar scattering functions, while hydration leads to an additional decrease in the scattering function at high Q. A minimum of two Gaussian distributions is required to fit the data, which deviate from a straight line above 160 K.

Fig. 2 displays the resulting displacement distribution functions at various temperatures referring to a fixed instrumental time window. A change in temperature shifts the effective time scale of molecular motions with respect to the instrumental window. At low temperature only vibrational motions are resolved, which implies a Gaussian distribution of displacements. The corresponding maximum broadens slightly with increase in temperature. The distinct shift and broadening of the maximum above 200 K reveals additional small scale diffusive displacements. This feature is absent in the dry and vitrified sample (Fig. 2) and thus represents the liquid aspect of protein motions. In contrast, displacements on a scale of 1.5 Å are observed in all three environments above 200 K. On such a scale only hydrogen motions due to rotational jumps are plausible. Fits to a scattering function describing three-fold jumps

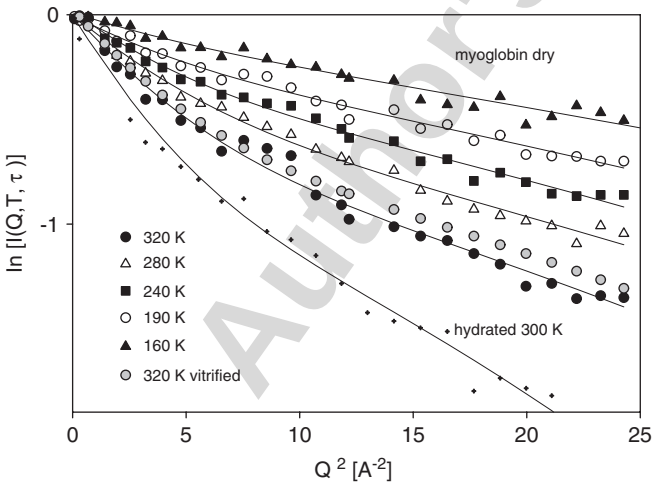

Fig. 1. Approximate $I(Q, \tau = 50\,\text{ps})$ of myoglobin in three different environments, vacuum (dehydrated), vitrified (perdeuterated glucose glass) and D_2O-hydrated (0.35 g/g) using the thermal backscattering spectrometer IN13 at the ILL in Grenoble. The data are corrected for multiple scattering.

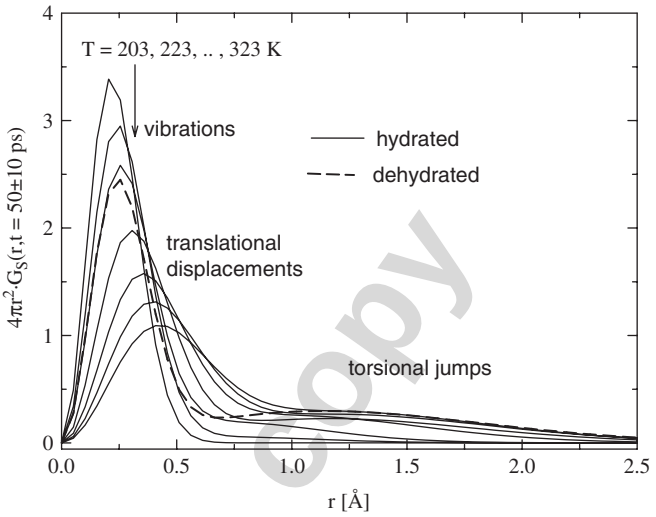

Fig. 2. Displacement distribution, $4\pi r^2 G(r, t = 50\,\text{ps})$, of hydrated (and dehydrated) myoglobin at fixed time with increasing temperature in steps of 20 degrees, derived from the fits in Fig. 1, dashed line: dehydrated myoglobin at 300 K.

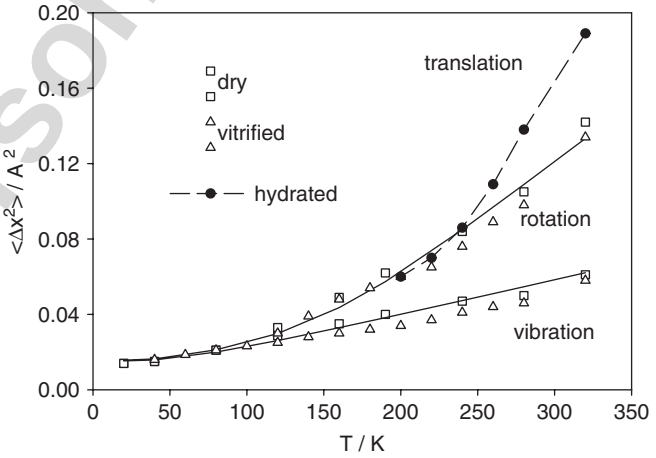

Fig. 3. Second moment of the displacement distribution at fixed time in three different environments, vacuum (dehydrated, open squares), vitrified (perdeuterated glucose glass, open triangles) and D_2O-hydrated (0.35 g/g, full circles) using the thermal backscattering spectrometer IN13 at the ILL in Grenoble. The data are corrected for multiple scattering.

can fit the data quite well as shown in Fig. 1. This result suggests that rotational transitions of side chains, in particular of methyl groups, in the protein interior are not strongly coupled to the properties of the environment [8,9]. Torsional motions of methyls occur also in molecular crystals. This component thus represents the solid aspect of structural fluctuations. Fig. 3 shows the temperature evolution of the second moment of the distribution with respect to the three environments.

Below 20 K only zero point vibrations contribute to the displacements (0.014 ± 0.003 Å^2), the vibrational component follows a coth-function with temperature. The displacements of the vitrified sample are slightly lower than those of the hydrated or dry sample. Anharmonic

enhancements become significant above 120 K with all three samples, reflecting rotational motions of side chains. The onset is gradual and consistent with an Arrhenius temperature dependence of rotational jump rates. The rotational rates have been measured with dehydrated myoglobin and the side chain of crystalline alanine dipeptide as displayed in Fig. 4. The solid lines in Fig. 3, in the case of dehydrated myoglobin, were obtained based on the structure factor of methyl group rotation, a calculated partial cross section of 25% and the jump rates of Fig. 4. Water induces additional translational motions, which become resolved above 240 K. The onset of water-assisted dynamics is abrupt due to unfreezing of hydration water. Glass transitions are generally associated with a super-Arrhenius temperature dependence of the respective relaxation rates [8].

3. Dynamics of protein-interfacial water

The scattering functions of hydration water are calculated by subtracting the spectra of H_2O-hydrated from those of D_2O-hydrated proteins taking into account the exchangeable protons [7]. Myoglobin contains about 1200 non-exchangeable hydrogen atoms, while about 400 water molecules (and thus 800 protons) or 0.35 g water/g protein are required for full hydration.

Fig. 5 shows the resulting intermediate scattering function of interfacial water after a numerical Fourier transform of the corrected difference spectrum. Two dynamical components related to a fast vibration (translational band at 48 cm^{-1}) and slow diffusive relaxation can be identified. A polynomial fit of second order in Q^2 at fixed time yields the respective moments of the displacement distribution:

$$I(Q,t) \approx A(t) + B(t)\cdot Q^2 + C(t)\cdot Q^4 + \cdots \quad (6)$$

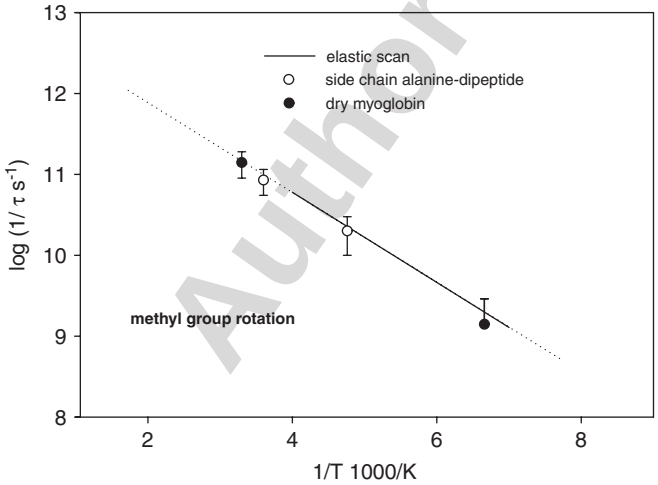

Fig. 4. Arrhenius plot of methyl group rotation rates, derived from quasielastic spectra (IN5, Grenoble) with dehydrated myoglobin and alanine dipeptide crystals [10], the full line was derived from elastic scan data (IN13, Grenoble) on alanine dipeptide crystals.

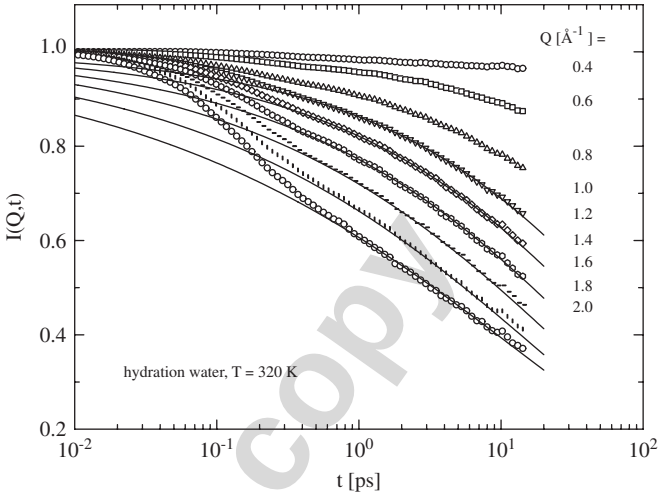

Fig. 5. Intermediate scattering function of protein hydration water based on spectral data (IN6, ILL, Grenoble) of H_2O-hydrated myoglobin (0.35 g/g) at various values of Q [7].

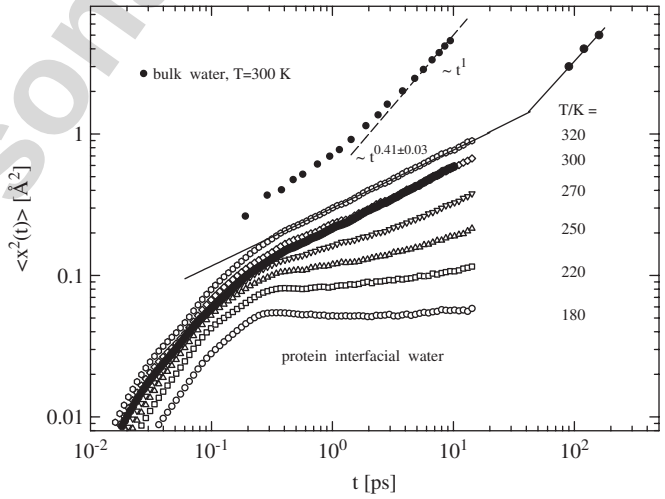

Fig. 6. Time-resolved displacements of bulk and of interfacial water (myoglobin) at various temperatures (IN6 and IN16 (full circles) ILL, Grenoble).

$A(t)$ reflects the Q-independent background of multiple scattering [7], $B(t) = \frac{1}{6}\langle r^2(t)\rangle$ and $C(t) = \frac{1}{120}\langle r^4(t)\rangle$.

Fig. 6 compares the time dependent displacements of bulk water [7] to those of protein interfacial water at various temperatures. The initial rise at short times is due to vibrational motions, above 1 ps translational diffusion takes over. Bulk water reaches the Stoke–Einstein regime after 2 ps on a spatial scale of the intermolecular distance of 2.8 Å. The cage effect due to nearest neighbour interactions dominates up to 1 ps. In contrast in the protein-water interface the cage effect leads to an extended region of sub-linear diffusion. The back-scattering data (IN16) displayed in Fig. 6 suggest that the linear range is reached at times exceeding 100 ps. Interfacial water does not crystallize. It is thus an excellent system to study the glass transition of thin

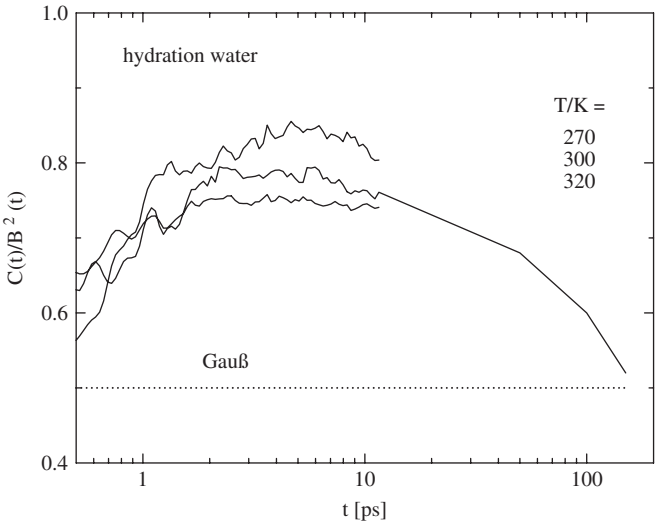

Fig. 7. Time-resolved fourth moment of the displacement distribution of protein hydration water.

liquids. Below 180 K the cage effect induces structural arrest on large scale. Neutron scattering experiments have shown that the protein structural fluctuations exhibit a dynamic anomaly near 180 K coupled to the glass transition of water [1,8]. The fourth moment provides additional information on the displacement distribution. Fig. 7 shows the so-called non-Gauss parameter C/B^2 versus time, which is 0.5 for a Gaussian distribution. At short time, this value is assumed reflecting vibrational motions. At intermediate times the distribution becomes highly non-Gaussian due to a combination of localized and displaced water molecules. At long times, the Gaussian diffusion limit is reached.

4. Conclusions

A moment analysis of the intermediate scattering function provides model-independent information about molecular displacement distributions and their time evolution. Two types of protein-internal motions were detected: rotational jumps and continuous diffusive displacements of side chains on a local scale. From a dynamical point of view proteins and liquids are radically different. Liquids exhibit short-range order and long-range translational diffusion. Molecular displacements are continuous and isotropic. Proteins in contrast are long-range ordered, but molecular diffusion is of short range. Internal displacements are discontinuous and anisotropic. Rotational transitions persist even without water and with a rigid external environment. The interaction with water introduces liquid aspects to otherwise solid-like protein molecules: The small-scale diffusive displacements of side-chains induced by water open up transient vacancies enabling small ligands to penetrate the compact protein structure [11].

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