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Librational fluctuations in protein glasses

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ABSTRACT

Librational motions in the region of the protein "glass" (or dynamic) transition are analysed for spin-labelled haemoglobin, serum albumin and β-lactoglobulin by EPR spectroscopy. A discontinuity in the temperature dependence of the mean-square librational amplitude, $\langle\alpha^2\rangle$, occurs in the region of 200 K as found for the mean-square atomic displacement, $\langle r^2\rangle$, at the protein dynamic transition by Mössbauer spectroscopy and neutron scattering. The discontinuity in $\langle\alpha^2\rangle$ vs. T can be described by the Vogel–Tammann–Fulcher equation, implying a finite glass transition temperature. Above the dynamic transition, $\langle\alpha^2\rangle$ vs. $1/T$ can be approximated by the Arrhenius law with activation energies similar to those usually found for $\langle r^2\rangle$, and relaxation processes in glass-forming media and the hydration shells of proteins. Similar results are found for librational fluctuations of membranous Na,K-ATPase spin-labelled either on superficial –SH groups or on those essential to activity.

1. Introduction

Several aspects of the conformational behaviour of solvated proteins strongly resemble the properties of glass-forming liquids [1–4]. At a characteristic temperature, in the region of 200 K, the hydrated protein becomes frozen into a non-equilibrium distribution of almost isoenergetic conformational substates [5,6]. This recalls the heterogeneous metastable distribution of local environments that is found in the glassy state [7]. Below 200 K, the protein motions are restricted to harmonic vibrations and librations, as in a solid and characteristic also of the vitreous state. In this regime, the mean-square atomic displacements, $\langle r^2\rangle$, of the protein increase linearly with temperature. Above 200 K, a dynamic transition [8] takes place beyond which thermal fluctuations establish equilibrium between the different conformational substates. The atomic displacements of the hydrated protein then increase much more rapidly with increasing temperature [3,8], which characterises the onset of stochastic (i.e., diffusive) motions. This situation corresponds to the onset of rapid translational diffusion that takes place on melting of vitreous systems at the glass transition. The position of the dynamic protein transition depends on the rate of heating and on the timescale of motions to which the experimental method for detecting the transition is sensitive [3,9], just as for the glass transition.

The dynamic transition of the glass-like protein state was characterised originally by the discontinuity in temperature dependence of translational displacements, from measurements of $\langle r^2\rangle$ either by Mössbauer spectroscopy [10,11] or quasi-elastic neutron scattering [8,12–14]. The purpose of this communication is to show that the dynamic protein transition is accompanied also by the onset of Brownian torsional fluctuations, as recorded by the electron paramagnetic resonance (EPR) spectra of spin-labelled proteins. In particular, it is found that the temperature dependence of the librational motion above the transition is very similar to that of the atomic displacements, which are recorded by the $\langle r^2\rangle$ parameter. Previously, we have demonstrated the multi-state nature of the energy landscape of spin-labelled proteins in the glassy state, both by analysis of conventional EPR line shapes and by time-resolved EPR [6,15].

2. Method of analysis

The mean-square amplitude of librational motion, $\langle\alpha^2\rangle$, can be derived from the motionally averaged hyperfine splittings, $2\langle A_{zz}\rangle$, in the EPR spectra of the spin-labelled proteins, according to Ref. [16]:

$$\left\langle \alpha^2 \right\rangle = \frac{A_{zz} - \langle A_{zz}\rangle}{A_{zz} - A_{xx}} \tag{1}$$

where the angular brackets indicate a motionally averaged hyperfine tensor (of principal elements A_{xx}, A_{yy} and A_{zz}) — see also Ref. [17]. Eq. (1) is limited to small librational amplitudes. In the stochastic regime, the mean-square diffusive displacement at short times t' is given by an Einstein-type relation:

$$\langle \alpha^2 \rangle = 2D_\alpha t' \tag{2}$$

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where D_α is the effective rotational diffusion coefficient for libration within the torsional potential well. The temperature dependence of the effective diffusion coefficient is determined by an activation barrier that is associated with the rotational friction coefficient, f_α, where $D_\alpha = k_BT / f_\alpha$ [18].

For a spin-label EPR experiment of this type, the characteristic timescale of observation, t', is determined by the inverse intrinsic line width $\approx T_2^*$ and the effective diffusion coefficient (or correlation time, τ_c). Resolution of a measurable shift in hyperfine splitting requires that the numerator in Eq. (1) should be at least ~10^{-2} of the line width. In the temperature range above 200 K, where the current measurements are made, this corresponds to an experimental uncertainty in $\langle\alpha^2\rangle$ that is ~5×10^{-3} rad^2 [6]. From Eqs. (1) and (2), the corresponding minimum value of t' is:

$$t'_{\min} = \frac{1}{2D_\alpha}\frac{10^{-2}/T_2^*}{\gamma_e(A_{zz}-A_{xx})} \approx 0.005\tau_c \tag{3}$$

where γ_e is the gyromagnetic ratio of the electron. Of course, the effective diffusion coefficient and τ_c are temperature dependent, but for $\tau_c \approx 2 \times 10^{-9}$ s (see Ref. [6]) Eq. (3) predicts that $t'_{\min} \approx 10^{-11}$ s. This corresponds to the temperature below which $\langle\alpha^2\rangle$ becomes too small to be measured by conventional CW EPR. Note that this limit on resolution is not instrumental but determined by the spectroscopic properties of the spin-labelled system, because EPR spectra are recorded with field-modulation amplitudes that are much less than the intrinsic line widths. This therefore differs from the situation that obtains with neutron scattering (see, e.g., Ref. [19]). In the temperature range over which the librational amplitudes are measurable by EPR, inhomogeneous line broadening is small and therefore $T_2^* \approx T_2$, the true transverse relaxation time that determines the Lorentzian line width [6].

3. Soluble proteins

Fig. 1 shows the temperature dependence of the mean-square librational amplitude, $\langle\alpha^2\rangle$, for spin-labelled human haemoglobin (Hb), human serum albumin (HSA) and bovine β-lactoglobulin (β-LG). Hb is spin-labelled on Cys-β93 of the β-subunits with 4-maleimido-2,2,6,6-tetramethylpiperidine-1-oxyl (6-MSL); HSA is spin-labelled on Cys-34 with 3-maleimido-tetramethylpyrrolidine-1-oxyl (5-MSL); and β-LG is spin labelled on Cys-121 with 5-MSL. Both Hb and HSA are α-helical proteins, whereas β-LG consists of a β-barrel. Data are given in Fig. 1 for the proteins in water (i.e., buffer) and in a glass-forming 60% v/v glycerol–water mixture. Librational motions of appreciable amplitude set in at temperatures of 180−200 K, for the proteins in water and slightly above this for the protein in glycerol–water mixtures. The diffusive librations that occur above 200 K are a property of the hydrated protein, because they are virtually absent for the lyophilised spin-labelled proteins. Time-resolved (spin echo-detected) EPR spectra show that they take place on a sub-nanosecond time scale [6,20].

Considerable variation is found between the librational amplitudes, $\langle\alpha^2\rangle$, of the different systems in Fig. 1. This arises from heterogeneity between the different single sites of labelling, which would become averaged if many residues were labelled on a particular protein. Mean square displacements, $\langle r^2\rangle$, averaged over all non-exchangeable protons by neutron scattering, are found to be rather similar for different α-helical proteins, for example, myoglobin and lysozyme [19]. Recently, however, it was found that corresponding values of $\langle r^2\rangle$ for a β-barrel protein are of much smaller amplitude [21]. This correlates with the smaller values of $\langle\alpha^2\rangle$ obtained for β-LG than for Hb in Fig. 1.

The temperature dependence of the librational motion that is recorded by EPR strongly resembles that of the diffusive displacements measured by Mössbauer spectroscopy and quasi-elastic neutron scattering [8,11]. In particular, it can be described by the Vogel–Tammann–Fulcher equation that is appropriate to glass-forming solvents [3]:

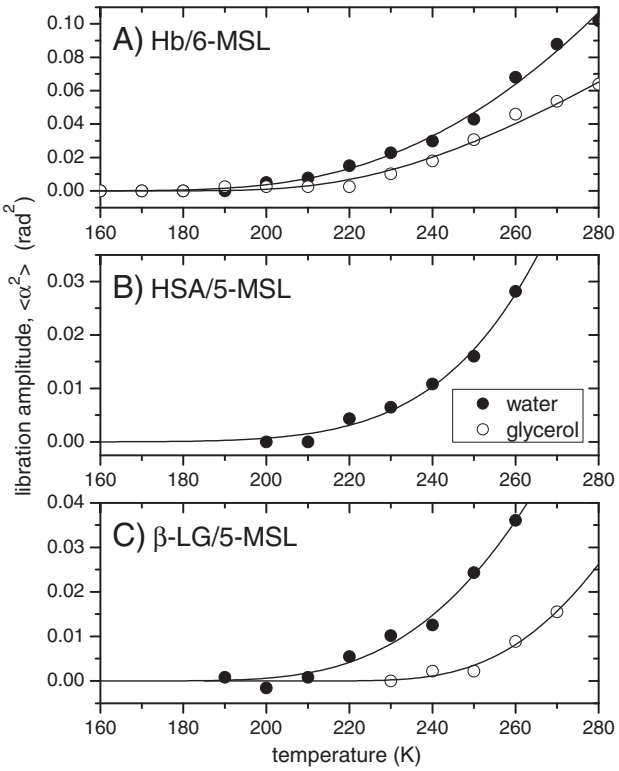

Fig. 1. Temperature dependence of the mean-square amplitude of librational motion, $\langle\alpha^2\rangle$, for: A. 6-MSL-labelled haemoglobin in water (solid circles), and in 60% v/v glycerol–water (open circles); B. 5-MSL-labelled human serum albumin in water (solid circles); C. 5-MSL-labelled β-lactoglobulin in water (solid circles), and in 60% v/v glycerol–water (open circles). Solid lines are fits of Eq. (4) that were performed on a log scale vs. 1/T.

$$\left\langle\alpha^2\right\rangle = A\exp(-BT_o/(T-T_o)) \tag{4}$$

where T_o the temperature at which $\langle\alpha^2\rangle \to 0$, and A and B are fitting constants. An equation of this form is also the basis for the free-volume model of translational diffusion [22,23]. Qualitative fits of Eq. (4) to the spin-label EPR data are shown by the solid lines in Fig. 1. Note that the fitting parameters can be determined only with limited precision, because the fractional errors in the data become rather large as $\langle\alpha^2\rangle \to 0$ (see the discussion surrounding Eq. (3) above). Nonetheless, the fits are adequate to establish that T_o increases when 60% glycerol is added to the medium. Although the form of the temperature dependence of the librational amplitudes is rather similar in 60% aqueous glycerol to that in water, reflecting only the difference in effective glass transition temperatures, the absolute values of $\langle\alpha^2\rangle$ are lower in 60% glycerol. This effect of solvent viscosity is expected for the diffusion coefficient (i.e., $D_\alpha = k_BT / f_\alpha$), or more generally from Kramers theory for the coupling of dynamic processes to the environment [24].

Most significantly, the temperature dependence of the spin-labelled proteins reported here strongly resembles the librational motions found for small spin labels in glass-forming solvents by Paschenko et al. [25] and in our own measurements with 5-MSL alone and the TEMPONE (2,2,6,6-tetramethylpiperidine-4-oxo-1-oxyl) spin label in 60% aqueous glycerol (data not shown). It is also interesting to note that qualitatively similar data are found for the librational motions of spin-labelled lipid chains in membranes [26]. The latter results are