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Low frequency dynamics in the enzyme superoxide dismutase revealed by inelastic neutron scattering

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Abstract

The atomic dynamics of the enzyme Cu/Zn superoxide dismutase hydrated to 0.34 g D_2O/g protein has been investigated by means of inelastic neutron scattering at different temperatures between 50 and 300 K. At all temperatures the inelastic part of the spectrum is well separated from the quasi-elastic region. The vibrational frequency distribution function $G(\omega)$ shows, for all temperatures, a Debye-like behaviour up to about 5 meV.

Keywords: Protein; Glass transition; Phonon density of states

Results from different spectroscopic techniques and from molecular dynamics simulations have demonstrated that structural and dynamical properties at the molecular level are both relevant in determining the functional behaviour of proteins. Mössbauer and neutron inelastic (INS) and quasi-elastic scattering (QENS) studies evidenced some features common to most hydrated globular proteins. Below ~180 K the atomic dynamics is mostly harmonic, while above this temperature more complex motions involving transitions between different conformational substates of the polypeptide scaffolding become apparent [1, 2].

We have initiated a systematic study of the dynamics of an entirely β-sheet protein: Cu, Zn superoxide dismutase (SOD) which is well characterized from the crystallographic point of view and with a 3-D structure which has been described in detail for different species. Each subunit is formed by eight antiparallel β-strands joined by three external loops. The enzyme active site is constituted by a metal cluster with a copper and a zinc atom coupled together by a bridging imidazolate side chain. In recent QENS experiments performed at the ISIS we have investigated the temperature dependence of the elastic and quasi-elastic scattering from moderately hydrated SOD [3, 4]. These measurements have shown the existence of a dynamic transition similar to that observed in other globular proteins although with a much less intense quasi-elastic component. This difference is probably related to the high structural rigidity of this entire β-sheet protein. In this paper we report preliminary results of INS studies on this protein.

The protein was purified from bovine erythrocytes. After purification it was dialysed against water to remove any salt and was then fully D_2O exchanged. The measurements were performed on a sample hydrated at 34.6% (g D_2O/g protein) in a temperature range between 50 and 300 K. The measurements were performed on the time-of-flight spectrometer MIBEMOL at the Orphèe reactor, CEA, Saclay. The spectrometer operated with a incident energy of 2.27 meV, covering an energy transfer range $\hbar\omega$ from 0 to 20 meV, with an elastic energy resolution of 0.098 meV, and with an elastic momentum transfer, Q, ranging from 0.29 to 1.94 $Å^{-1}$. Time-of-flight raw

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spectra were corrected and normalized according to standard procedures [5].

Experimental data were used to obtain the dynamical structure factor at each temperature according to the one-phonon quasi-harmonic approximation:

$$S(Q,\omega) = e^{-Q^2\langle u^2\rangle}[A(Q)\delta(\omega) + S_{qel}(Q,\omega) + S_{vib}(Q,\omega)], \quad (1)$$

where $A(Q)$ is the EISF, $S_{qel}(Q,\omega)$ and $S_{vib}(Q,\omega)$ are the quasi-elastic and inelastic scattering contributions, respectively.

From the latter function a proton weighted frequency distribution function $G(\omega)$ can be derived as

$$G(\omega) = \lim_{Q\to 0} \frac{S(Q,\omega)}{Q^2\hbar}\omega\left(1 - \exp\left(-\frac{\hbar\omega}{K_B T}\right)\right). \quad (2)$$

In Eq. (1), the Debye–Waller factor $\exp(-Q^2\langle u^2\rangle)$ was estimated from previous QENS data on the same protein [3]. In Eqs. (1) and (2) we have assumed that the $G(\omega)$ function is temperature independent, being the temperature dependence of the vibrational spectrum only included in the Debye–Waller and Bose factor, as for an harmonic system.

Looking to the ratios $S(Q,\omega;T)/S(Q,\omega;T = 50\text{ K})$ we observe that above $\sim$2 meV these ratios follow with reasonable accuracy the ones given by a Bose population factor; in addition, from the single $S(Q,\omega;T)$ functions, we find that the quasi-elastic contribution to the scattering appears always well separated from the inelastic one. For this protein we can then conclude that:

(i) even at room temperature the inelastic scattering contribution is well separated from the quasi-elastic one, the latter being limited to energies below $\sim$1.5 meV;

(ii) the inelastic scattering is, to a good approximation, described by a quasi-harmonic temperature scaling.

In Fig. 1 the $G(\omega)$ function derived for the different temperatures, according to Eq. (2) are plotted in the 0–11 meV energy range. It can be seen that below

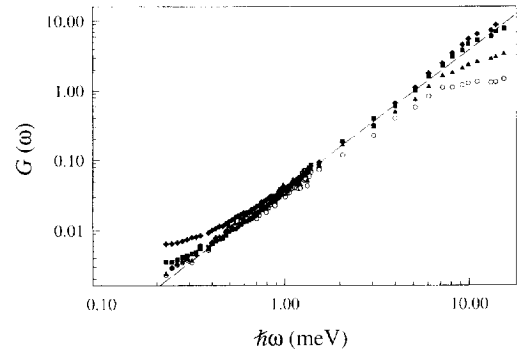

Fig. 1. Log–Log plot of the proton weighted vibrational frequency distribution at 50 K (○), 180 K (▲), 230 K (■), and 300 K (♦).

$\sim$5 meV $G(\omega)$ follows a Debye behaviour ($G(\omega) \propto \omega^2$). At higher energies the $G(\omega)$ functions remain always lower than the plotted straight line (Debye-like behaviour) for temperatures $\leqslant 180\ K$. Above this temperature an extra intensity appears in the 6–11 meV region leading the curves slightly above the Debye one. This increase of scattering intensity can be correlated to the progressive activation of conformational degrees of freedom which are also responsible for the fall-off of the elastic scattering intensity (Debye–Waller factor) in the same temperature range.

If one assumes a sound velocity for this protein of the order of 3000 m/s, the Debye frequency $\omega \simeq$ 5 meV for the cross-over frequency of Fig. 1 gives a length scale of about 70–100Å. This result agrees reasonably well with the SOD dimension of the order of 60Å.

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