Dynamics of Hydrogen Atoms in Superoxide Dismutase by Quasielastic Neutron Scattering

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ABSTRACT The low energy dynamics of the enzyme Cu,Zn superoxide dismutase have been investigated by means of quasielastic neutron scattering in the temperature range 4–320 K. Below 200 K the scattering is purely elastic, while above this temperature a pronounced decrease in the elastic intensity is observed, together with the onset of a small quasielastic component. This behavior is similar to that previously observed in other more flexible globular proteins, and can be attributed to transitions between slightly different conformational substates of the protein tertiary structure. The presence of only a small quasielastic component, whose intensity is ≤25% of the total spectrum, is related to the high structural rigidity of this protein.

INTRODUCTION

Pioneering studies of the molecular dynamics of hydrated biopolymers by neutron inelastic scattering date back to the early seventies (Dahlborg and Rupprecht, 1971). It was then realized that scattering techniques employing cold neutrons could contribute significantly to the understanding of hydration processes in biological materials owing to the unique possibility provided by neutrons of resolving both structural and dynamical details at the molecular level.

More recently the advent of new high-resolution inelastic spectrometers together with the increase of source fluxes made it possible to perform quantitative studies of the dynamics of complex biomolecular assemblies as proteins and nucleic acids (Randall et al., 1978; Grimm et al., 1987). quasielastic scattering (QENS), which covers the energy domain from the meV to the neV region (i.e., times $\sim 10^{-12}$ to 10^{-8} s) is particularly relevant for such studies, because it allows researchers to investigate a broad spectrum of excitations ranging from local motions of small molecular subunits to slower processes involving cooperative motions of massive parts of the macromolecule (Bée, 1988; Deriu, 1993). The effectiveness of this technique for biological studies is also due to the incoherent neutron-scattering cross section of protons, which is much larger than that of deuterium and of most other elements present in biological matter. In proteins hydrogen atoms are uniformly distributed over the macromolecule, and this technique therefore provides information on the average dynamics of the polypeptide chains.

In recent years detailed QENS studies have been performed on the dynamics of the whole α-helical protein myoglobin from sperm whale (Doster et al., 1989, 1990). These studies demonstrated that the proton dynamics of hydrated myoglobin up to ~180 K is mostly vibrational with a quasiharmonic behavior. Above this temperature a drastic decrease of the elastic scattering intensity was observed, together with the appearance of a broad quasielastic component. This was attributed to the onset of a dynamic transition between conformational substates of the protein tertiary structure of slightly different energy. Similar results in agreement with this model have also been obtained, both on myoglobin and on other globular proteins, with different spectroscopic techniques as Mössbauer (Parak et al., 1981) and optical spectroscopy (Di Pace, 1992). Molecular dynamics computer simulations, which can now be performed on systems of such structural complexity, have also contributed to a deeper understanding of the nature of internal protein motions (Smith, 1991; Furois-Corbin, 1993).

With the aim of extending the present knowledge of the microscopic dynamics of globular proteins, we have undertaken a systematic study of the dynamics of an entirely β-sheet protein: Cu,Zn superoxide dismutase (SOD). SOD is a well characterized protein from a structural point of view. In fact its 3-D structure has been described in detail by crystallographic analysis of five different species, namely ox (Tainer et al., 1982), spinach (Kitagawa et al., 1991), yeast (Dijnovic et al., 1992), human (Parge et al., 1992) and *Xenopus* (Dijnovic et al., 1993). The central structural core of the SOD subunit is a flattened Greek-key β-barrel motif consisting of eight antiparallel β-strands joined by three external loops. The molecular fold of the enzyme is highly maintained throughout the phyla, with substantial conservation of the primary structure. The metal cluster forming the enzyme-active site is constituted by a copper and a zinc atom coupled together by a bridging imidazolate side chain. A recent optical absorption study of the copper ion native Cu,Zn SOD, in the temperature range 10–300 K, has indicated the existence of local microheterogeneity (i.e., several different conformations) and the occurrence of a structural rearrangement at the level of the metal site at ~180 K (Cupane et al., 1994).

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To obtain information on the the overall dynamics of this protein, incoherent QENS measurements on moderately hydrated SOD powders have been undertaken in a wide temperature range. In this paper the results obtained on SOD are discussed and compared with those previously obtained for the more flexible myoglobin (Doster et al., 1989).

MATERIALS AND METHODS

The protein was partly purchased from Fluka Chemie AG (Buchs, Switzerland) as a lyophilized powder and further purified by an FPLC (LKB-Pharmacia AB, Uppsala, Sweden) mono Q ion exchange chromatography, and partly purified from bovine erythrocytes (McCord and Fridovich, 1969). The quality of the sample was controlled before and after neutron experiment by measuring the enzyme activity and the electron paramagnetic resonance and optical spectra of the samples. All the parameters were not modified by the treatment. After purification the protein was exhaustively dialyzed against water to remove any salt and was then fully D_2O exchanged, the final pH being 6.0. In this way, owing to the large n-p incoherent cross section (79.7 barn), the scattering contribution from the protons in the polypeptide chains was enhanced with respect to that from water molecules. The measurements were performed on samples at two different hydration levels: 0.25 and 0.41 (g D_2O/g protein) corresponding to a concentration of 0.13 and 0.08 M, respectively. The samples were kept in a vacuum-tight holder (20 × 20 × 1 mm) with thin (0.3 mm) aluminum windows. The explored temperature range was 4–320 K.

Quasielastic neutron scattering

The backscattering spectrometer IRIS at the ISIS spallation neutron source (Rutherford Appleton Laboratory, U. K.) was used to measure sets of quasielastic spectra from SOD. IRIS is an inverted-geometry spectrometer with a 36 m flight path and two crystal analyzer arrays in near-backscattering geometry (175°): pyrolytic graphite (PG) and mica. A detailed description of the instrument can be found in Carlile and Adams (1992). The analyzers produce a set of 102 spectra (2000 points each) with energy resolution $\Delta E = 11$ μeV (mica006) and 15 μeV (PG002) full width at half maximal (FWHM), respectively. The momentum transfer $Q = 4\pi\sin(\theta)/\lambda$ ranges from 0.3 to 1.85 Å^{-1}, and its resolution ΔQ varies from 0.02 Å^{-1} (lowest Q) to 0.04 Å^{-1} (high Q). The ratio of energy window to resolution width is ~100 for both analyzer arrays.

Data analysis

Time-of-flight raw neutron spectra were corrected and normalized according to standard procedures (Windsor, 1981) to obtain the dynamic structure factor for protons $S_p(Q,\omega)$. Fig. 1 shows a set of $S_p(Q,\omega)$ curves measured at 290 K; the 51 spectra recorded have been binned into 24 groups to improve the counting statistics. They show a dominating elastic peak on top of a smaller quasielastic component, and have been therefore analyzed in terms of an elastic component and a sum of N quasielastic contributions with Lorentzian lineshape according to the following expression:

$$S_p(Q,\omega) = \left[A_0(Q)\delta(\omega) + \sum_{i=1}^{N} A_i(Q) \frac{\Gamma_i}{\pi(\omega^2 + \Gamma_i^2)} \right] \otimes R(\omega) + B \quad (1)$$

where $A_0(Q)$ is the elastic intensity, $A_i(Q)$ and Γ_i are the amplitudes and widths of the quasielastic components, B is an ω-independent background, and $R(\omega)$ is the instrument resolution function that convolutes the data. We assumed that $R(\omega)$ could be well represented by the spectrum of SOD at 4.2 K.

To assess the minimum number (N) of quasielastic components necessary for a satisfactory fitting of the data, and to determine the optimal estimates for the intensity and lineshape parameters, we made use of a Bayesian analysis technique (Sivia and Carlile, 1992). This method has already been successfully applied to the analysis of QENS data, and a detailed description of the algorithm can be found in Sivia et al. (1992). It allows one to calculate a posterior conditional probability ($P(N|\{data\})$) for a number N of quasielastic components in Eq. 1. The dependence of this probability on N is shown in Fig. 2 for the SOD sample at 0.25 hydration and at different temperatures. It can be seen that below 200 K the elastic line alone ($N = 0$) is sufficient for a satisfactory fitting, while above this temperature the data support a model with one quasielastic component besides the elastic one. On the basis of the above analysis, models with more than one quasielastic component can be ruled out at all the measured temperatures because they do not improve significantly the probability P. In the following we will analyze and discuss the temperature and hydration behavior of the physical parameters of the model.

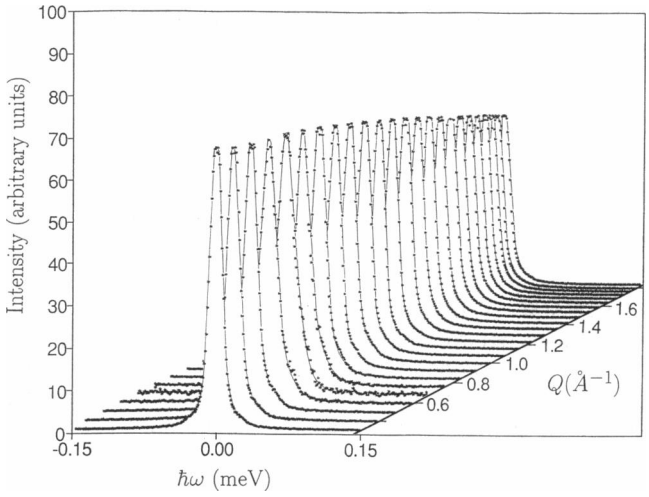

FIGURE 1 Set of quasielastic spectra for an IRIS run for SOD at 0.25 hydration and at 290 K (Q range 0.3–1.85 Å^{-1}). Only the ω interval from −150 to +150 μeV is shown for better clarity.

RESULTS AND DISCUSSION

In Fig. 3 the elastic intensity $S_p(Q,\omega \approx 0)$ as a function of Q^2 is reported for four selected temperatures. In the Q range

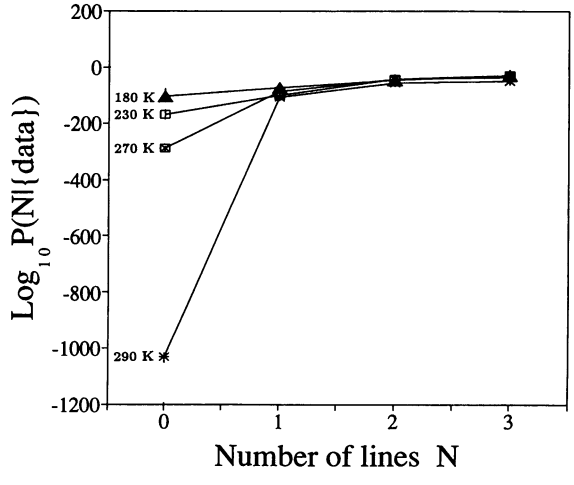

FIGURE 2 The logarithm (to base 10) of the posterior conditional probability $P(N|data)$ versus the number N of quasielastic components. The values refer to spectra for SOD at 0.25 hydration and at different temperatures: (△) 180 K, (□) 230 K, (⊠) 270 K, and (*) 290 K.

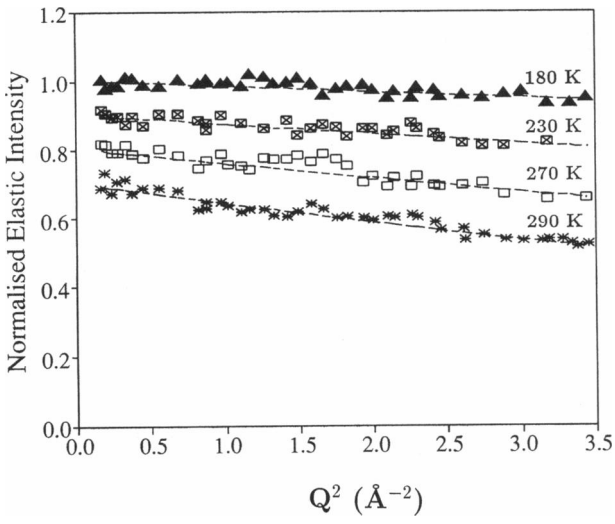

FIGURE 3 Normalized elastic intensity versus Q^2 for SOD at 0.25 hydration and for different temperatures: (△) 180 K, (⊠) 230 K, (□) 270 K, and (*) 290 K. The linear fits are also indicated. The curves are shifted with respect to each other for better visibility.

explored in the present experiment the behavior is linear both at low and high temperatures, but above ~200 K a marked decrease of the intensity is observed, indicating the activation of further degrees of freedom. A similar behavior has already been revealed in previous Mössbauer and QENS experiments on various globular proteins (Doster et al., 1990; Parak et al., 1981), and has been interpreted in terms of a "glassy" model of protein conformational substates (Frauenfelder et al., 1979). This model predicts quasiharmonic behavior in the low temperature regime leading to an elastic intensity describable in terms of a Debye-Waller factor, i.e., $S_p(Q,\omega \approx 0) = \exp(-Q^2 \langle u_p^2 \rangle)$, where $\langle u_p^2 \rangle$ is a mean square displacement for protons. The glass model predicts also deviations from the Gaussian behavior for both the Q- and temperature-dependence of the elastic intensity when temperature is raised above that corresponding to the onset of transitions between conformational substates of the polypeptidic chains ("glass-like" transition). A simple model able to account for the observed anharmonicity can be formulated in terms of torsional jumps of protons among distinct sites with slightly different energy (Doster et al., 1989). Assuming for simplicity only two jumping sites separated by a distance d, it is possible to write the elastic intensity as:

$$S_p(Q,0) = \exp(-Q^2 \langle u_G^2 \rangle) \left[1 - 2p_1p_2\left(1 - \frac{\sin(Qd)}{Qd}\right)\right] \quad (2)$$

where $\langle u_G^2 \rangle$ is due to the sum of all the Gaussian contributions to the total atomic displacement, and p_1 and p_2 denote the occupation probabilities for the two sites of different energy. The second factor in Eq. 2 contributes significantly to the Q-dependence of the elastic intensity only for $Qd < 1$. The data reported in Fig. 3 do not show any appreciable deviation from a linear behavior, and thus we may conclude that for SOD $d < 2\pi/Q_{max}$, i.e., $d < 3.4$ Å; it is worth noting that in the case of myoglobin the jumping distance d was found to be 1.5 Å (Doster et al., 1989).

Making use of Eq. 2, and ignoring the term in square brackets we have derived values for the total mean square proton displacement $\langle u_p^2 \rangle$. Its temperature dependence, reported in Fig. 4 for the two selected hydration levels, clearly indicates that the total mean square displacement strongly depends on the hydration level as already shown by Mössbauer experiments on myoglobin (Goldanskii and Krupyanskii, 1989). The dependence of $\langle u_p^2 \rangle$ for myoglobin at 0.33 hydration, measured in a QENS experiment with energy resolution similar to the present one (Doster et al., 1989), is also reported for comparison. They indicate that the not-exchangeable protons (i.e., the CH_3 and CH_2 groups) have comparable mean square displacement in both SOD and myoglobin independent of the different secondary structure of the two proteins.

The good quality and high statistics of IRIS data allowed us to perform an accurate lineshape analysis of the quasielastic component. This component is absent below 200 K; it then rises rapidly as shown in Fig. 5, reaching ~25% of the total intensity at 320 K. On the other hand its linewidth is remarkably both T- and Q-independent with average values 48 ± 5 μeV (FWHM) for both the 0.25 and the 0.41 hydration levels. An increase in the quasielastic component on increasing temperature was also observed in the QENS experiments on myoglobin (Doster et al., 1989); however in that case the linewidth of the quasielastic component (~1–2 meV) was much higher than in the case presented here. In the case of SOD the measured linewidth corresponds to correlation times of about 10 ps which are much longer than those of myoglobin (correlations times ≈ 0.3–0.5 ps) indicating an overall higher rigidity of the SOD scaffolding.

From the analysis of the intensity of the elastic and quasielastic components we have also derived the elastic in-

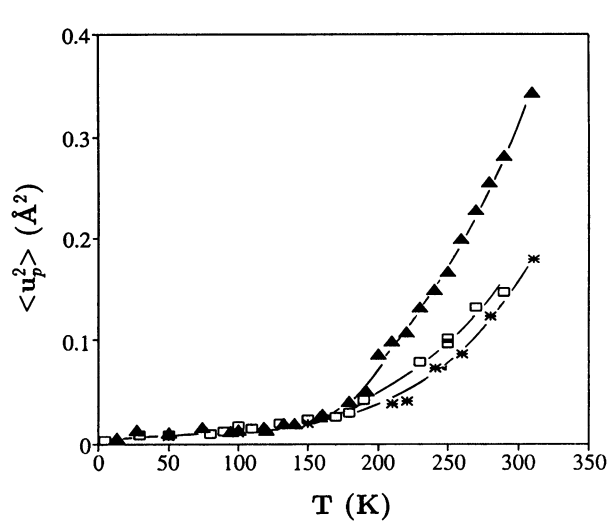

FIGURE 4 Temperature dependence of the total mean square proton displacement $\langle u_p^2 \rangle$. (△) SOD at 0.41 hydration; (□) SOD at 0.25 hydration; (*) myoglobin at 0.33 hydration (from Doster et al., 1989).

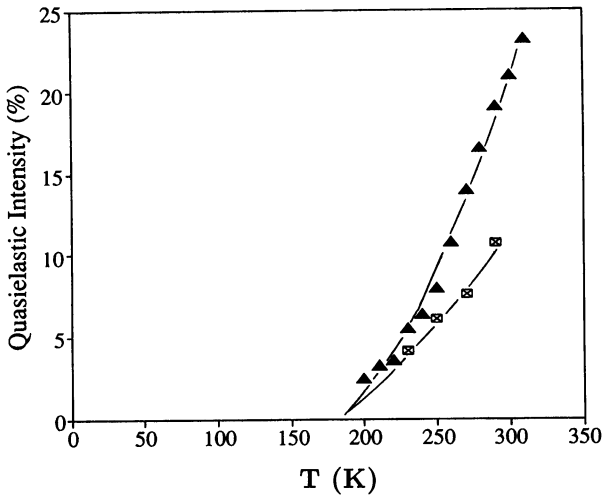

FIGURE 5 Temperature dependence of the intensity of the quasielastic component (in % of the total intensity): (△) SOD at 0.41 hydration; (⊠) SOD at 0.25 hydration.

coherent structure factor (EISF) from the ratio

$$\frac{I^{el}(Q)}{I^{el}(Q) + I^{qel}(Q)} \quad (3)$$

of the integrated intensities corresponding to the elastic and quasielastic parts of the spectra, respectively (Bée, 1988). The dependence of the EISF on Q^2 is shown in Fig. 6 for some selected temperatures. From these curves, assuming that EISF $\propto \exp(-Q^2 \langle R_p^2 \rangle)$, we have derived the average radius $((\langle R_p^2 \rangle)^{1/2})$ of the restricting volume for proton motion; it increases from 0.11 Å at 230 K, to 0.15 Å at 270 K, and reaches 0.18 Å at 290 K. It can therefore be concluded that as temperature is increased the dynamic characteristics of the motions giving rise to the quasielastic component do not change appreciably; on the other hand the amplitude of these motions

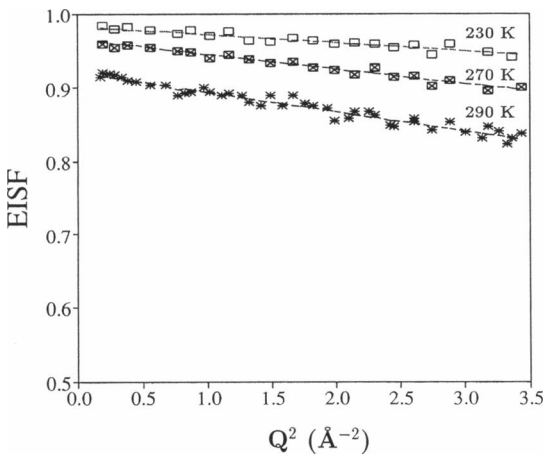

FIGURE 6 Elastic incoherent structure factor (EISF) versus Q^2 for a SOD sample at 0.25 hydration, and at different temperatures: (□) 230 K, (⊠) 270 K, and (*) 290 K.

increases significantly, and this is clearly reflected in the increase of the quasielastic intensity and in the behavior of the EISF, which indicates that in going from 230 to 290 K the volume available for proton motion is almost doubled.

CONCLUSIONS

A first result that clearly emerges from this study is the presence, also in SOD, of a glass-like dynamic transition that appears therefore to be a common feature of globular proteins irrespective of their structural differences. On the other hand the high structural rigidity of SOD scaffolding leads to a smaller quasielastic contribution as compared with a more flexible protein such as myoglobin.

These results indicate also the need to extend the QENS measurements to a wider Q-range to better quantify the anharmonic contributions to the mean square atomic displacements. A further dynamic behavior we intend to investigate is that relative to the low frequency interdomain motions that are expected to contribute to the low energy region ($\omega < 10$ meV) of the inelastic spectrum.

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