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Temperature dependence of the dynamics of superoxide dismutase by quasi-elastic neutron scattering

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Abstract

The atomic dynamics of the enzyme Cu, Zn superoxide dismutase has been investigated by means of quasi-elastic neutron scattering in the temperature range 4–320 K and in an extended momentum range up to 8.5 \AA^{-1} . Below 200 K the integrated elastic scattering intensity can be well described by a quasi-harmonic model, while above this temperature a pronounced decrease of the elastic intensity is observed, together with the onset of a small quasi-elastic component. This behaviour, which is similar to that already observed in other globular proteins, can be attributed to the onset of torsional degrees of freedom which give rise to transitions between slightly different conformational substates of the protein tertiary structure.

1. Introduction

In recent years the complex energy landscape of globular proteins has been studied in detail using various high resolution spectroscopic techniques, as optical [1, 2], Mössbauer [3, 4], and neutron spectroscopies [5–9]. Among these inelastic (INS) and quasielastic (QENS) neutron scattering are of particular relevance since they provide information in space (5–50 Å) and time (10^{-13} – 10^{-7} s) regions which are important for relating functional behaviour of proteins to their microscopic structure and dynamics.

The effectiveness of neutron techniques for biological studies is also due to the fact that the incoherent neutron scattering cross-section of protons 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, neutron scattering provides therefore information on the average dynamics of the polypeptide chains and of the associated water of hydration.

Detailed QENS studies have been performed so far on the whole α -helical protein myoglobin [7, 8]. The analysis of the dependence upon momentum Q and temperature of the ω -integrated intensity under the elastic peak evidenced a drastic change in the atomic dynamics around 180 K. Below this temperature the elastic scattering intensity could be interpreted in the frame of a quasi-harmonic model, while above 180 K it showed a marked decrease accompanied by the onset of a broad quasi-elastic component. This behaviour has been attributed to a “glass-like” dynamic transition occurring when, by increasing temperature, the protein can perform transitions among a manifold of conformational substates of its tertiary structure having slightly different energies. Further data on myoglobin and on other proteins obtained by different techniques

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as Mössbauer [3, 4] and optical [1, 2] spectroscopies agree well with the QENS results and provide further evidence of the existence of such dynamic transitions in globular proteins.

We have recently undertaken a systematic study of the dynamics of an entirely β -sheet protein: Cu, Zn superoxide dismutase (SOD). SOD is well characterized from the crystallographic point of view, and its 3-D structure has been described in detail for different species: ox [10], spinach [11], yeast [12], human [13], and *Xenopus* [14]. Each subunit is formed by eight antiparallel β -stands joined by three external loops. The molecular fold of the enzyme is highly maintained through the phyla, with a substantial conservation of the primary structure. 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 a first QENS experiment performed at the ISIS pulsed neutron source we have used the high resolution backscattering spectrometer IRIS to investigate the temperature dependence of the elastic and quasi-elastic scattering from moderately hydrated SOD [15]. These measurements have shown the existence of a dynamic transition analogous to that observed in myoglobin. The integrated elastic intensity has a marked decrease above ~ 180 K, and the mean square atomic displacements we derived are comparable to those of myoglobin. On the other hand due to the higher structural rigidity of SOD scaffolding the quasielastic contribution is quite smaller: at 300 K and for a 0.25 hydration level (g water/g protein) its intensity is only 10% of the total one. Its width is appreciably Q and temperature independent with an average value of about $50 \mu\text{eV}$.

In this paper we present new results which extend the previous ones towards higher Q -values and therefore enable us to investigate in more detail the anharmonic behaviour of the integrated elastic intensity above 180 K. The results are discussed and compared with those previously obtained for the more flexible myoglobin [7].

2. Experimental

The protein was partly purchased from Fluka Chemie AG (Buchs, Switzerland) as a lyophilized

powder and furtherly purified by an FPLC (LKB-Pharmacia AB, Uppsala, Sweden) mono Q ion exchange chromatography, and partly purified from bovine erythrocytes [16]. 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. By this treatment, 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 at a 0.2 and 0.27 hydration levels (g D_2O /g protein); the sample was kept in a vacuum-tight holder ($20 \times 20 \times 1$ mm) with thin (0.3 mm) aluminum windows. The QENS experiments were performed at the ISIS pulsed neutron facility (Rutherford-Appleton Laboratory, UK) using IRIS, a high resolution backscattering spectrometer, and MARI, a time-of-flight chopper instrument.

IRIS [17] is an inverted-geometry spectrometer with a 36 m flight path and two crystal analyser arrays in near-backscattering geometry (175°), pyrolytic graphite (PG) and mica. A particular feature of this instrument is to allow the selection of different analyser reflections by simply rephasing the choppers placed in the incident beam. In the present experiment we used the (004) reflection from graphite. The energy resolution was $\Delta E = 50 \mu\text{eV}$ FWHM, and the momentum transfer $\hbar Q = \hbar 4\pi \sin(\theta)/\lambda$ ranged from 0.5 to 3.7 \AA^{-1} . Elastic scans were also performed on MARI, a direct geometry chopper spectrometer on a 100 K CH_4 moderator [18]. They were performed using an incident energy of 50 meV ($\Delta E/E = 1\%$), and covering a momentum range $0.3\text{--}8.5 \text{ \AA}^{-1}$.

Neutron spectra were corrected and normalized according to standard procedures [19] in order to obtain the dynamic structure factor, $S_p(Q, \omega)$, for hydrogens. For the structure factor we have adopted the following empirical expression:

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

Here $A_0(Q)$ is the elastic intensity, $A_i(Q)$ and Γ_i are the amplitudes and widths of the quasi-elastic

components, B is an ω -independent background and $R(\omega)$ is the instrument resolution function which convolutes the data. We assumed that $R(\omega)$ could be well represented by the spectrum of SOD at 4.2 K.

Following the procedure described in more detail in Ref. [15] we have used the Bayesian analysis technique [20] in order to assess the minimum number (N) of quasi-elastic components necessary for a satisfactory fitting of the data, and to determine the optimal estimates for the intensity and line shape parameters. In agreement with our previous findings we obtain 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 quasi-elastic component besides the elastic one. In the present case the accuracy of the parameters of the quasielastic components is lower than that of the previous experiment since its width is comparable with the instrumental resolution. However the values we obtained are in excellent agreement with those of the previous ones.

3. Results and discussion

In Fig. 1 the elastic intensity $I_{el} = S_p(Q, \omega \approx 0)$ measured on IRIS as a function of Q^2 is reported for two temperatures: 180 and 230 K. Similar data obtained in a larger Q -range on MARI at 70 and 300 K are reported in Fig. 2. In both cases the $\ln I_{el}$ versus Q^2 curves can be well fitted by a straight line only at $T \leq 180$ K, while above this temperature the curves show a more marked decrease below $Q^2 \sim 10 \text{ \AA}^{-2}$. In order to analyse quantitatively these deviations from a quasi-harmonic behaviour we have adopted the same simple model proposed by Doster et al. [7] for myoglobin. The anharmonic contribution is attributed to the onset of torsional jumps of protons among distinct sites with slightly different energy, and is modelled assuming for simplicity only two jumping sites separated by a distance d ; this leads to the following expression for the elastic intensity:

$$S_p(Q, 0) = \exp(-Q^2 \langle u_G^2 \rangle) \times \left[1 - 2p_1 p_2 \left(1 - \frac{\sin(Qd)}{Qd} \right) \right] \quad (2)$$

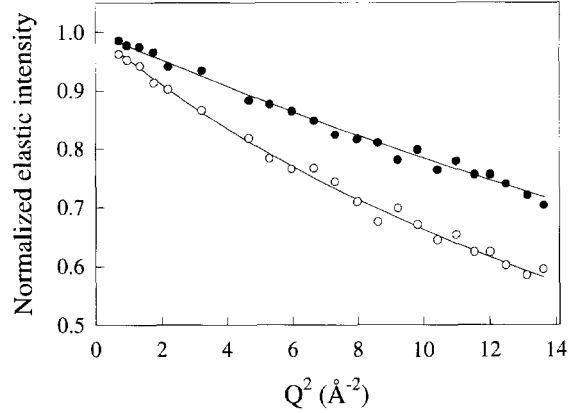


Fig. 1. Normalized elastic intensity $\ln I_{el}$ versus Q^2 for SOD at 0.2 hydration and for two temperatures: 180 K (full circles), and 230 K (open circles). The data have been obtained with IRIS using the 004 reflection from the pyrolytic graphite analysers. The fit of the data to Eq. (2) is also shown as a continuous line.

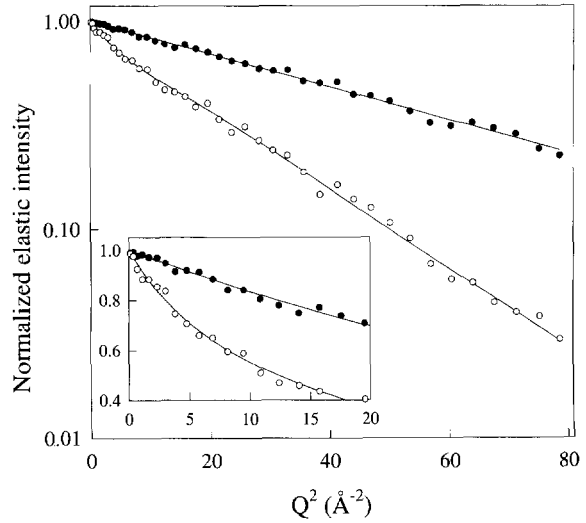


Fig. 2. Normalized elastic intensity $\ln I_{el}$ versus Q^2 for SOD at 0.2 hydration and for two temperatures: 70 K (full circles), and 300 K (open circles). In the inset the region below 20 \AA^{-2} is expanded to evidence the anharmonic behaviour of I_{el} . The data have been obtained with the chopper MARI using an incident energy of 50 meV. The fit of the data to Eq. (2) is also shown as a continuous line are also indicated.

where $\langle u_G^2 \rangle$ is due to the sum of all the quasi-harmonic (Gaussian) contributions to the total atomic displacement, p_1 and p_2 denote the occupation probabilities for the two sites of different

Table 1

Total mean square atomic displacement $\langle u_p^2 \rangle$, Gaussian contribution $\langle u_G^2 \rangle$ to the mean square atomic displacement, product of the probabilities p_1 and p_2 and jump distance d derived from the fit of the integrated elastic intensities to Eq. (2)

Instrument	Hydration (g D ₂ O/ g protein)	T (K)	$\langle u_p^2 \rangle$ (Å ⁻²)	$\langle u_G^2 \rangle$ (Å ⁻²)	$p_1 \cdot p_2$	d (Å)
MARI	0.27	70	0.013	0.013		
MARI	0.27	295	0.079	0.043	0.06	1.3
IRIS	0.20	180	0.022	0.022		
IRIS	0.20	230	0.046	0.036	0.02	1.3

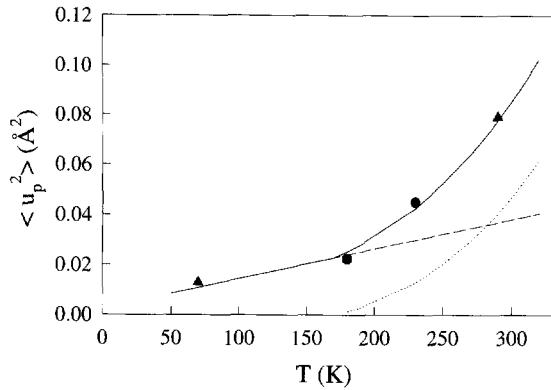


Fig. 3. Temperature dependence of the mean square proton displacement obtained from the analysis of the integrated elastic scattering intensities assuming the two state model described in the text. IRIS data (●), MARI data (▲). The solid line shows $\langle u_p^2 \rangle$ the parameters reported in Table 1. The dashed line is the quasi-harmonic contribution $\langle u_G^2 \rangle$ extrapolated from low temperature. The dotted line shows the contribution due to the conformational dynamics $p_1 p_2 d^2/3$.

energy. The second factor in Eq. (2) contributes significantly to the Q -dependence of the elastic intensity only for $Qd < 1$.

Least-square fits of Eq. (2) to the data shown in Figs. 1 and 2 provide $p_1 p_2$, $\langle u_G^2 \rangle$, and d . The values obtained are reported in Table 1. From these values one can estimate overall mean square displacements $\langle u_p^2 \rangle$, which represent the initial slopes of the curves in Figs. 1 and 2. From Eq. (2) one obtains

$$\langle u_p^2 \rangle = \langle u_G^2 \rangle + \frac{p_1 p_2 d^2}{3}. \quad (3)$$

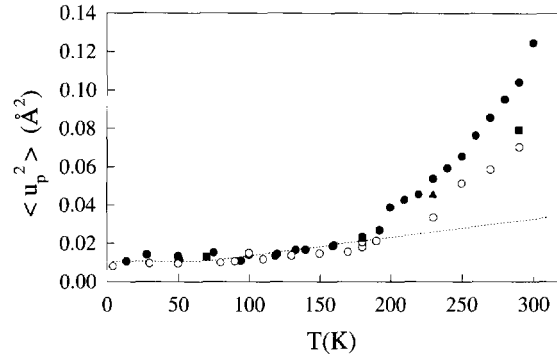


Fig. 4. Temperature dependence of the total mean square proton displacement $\langle u_p^2 \rangle$ from different QENS experiments on SOD. Present paper: IRIS, PG004 analyser reflection and 0.2 protein hydration (▲), MARI, $E_0 = 50$ meV and 0.27 protein hydration (■). From Ref. [15]: IRIS, PG002 analyser reflections, 0.25 (○) and 0.41 (●) hydration respectively. The quasi-harmonic behaviour which is almost the same for all the datasets is indicated by a dotted line.

The temperature dependence of $\langle u_p^2 \rangle$, $\langle u_G^2 \rangle$, and $p_1 p_2 d^2/3$ is reported in Fig. 3, together with the measured $\langle u_p^2 \rangle$ from both IRIS and MARI experiments. All these parameters are weakly dependent upon hydration in the range 0.2–0.27 so that a unique curve from Eq. (3) can fit both datasets.

In Fig. 4 the present $\langle u_p^2 \rangle$ data are plotted together with those previously obtained by us on the same protein, at 0.25 and 0.41 hydration levels [15]. This comparison indicates that the Gaussian contribution to the mean square displacement $\langle u_p^2 \rangle$ is almost hydration independent so that a common curve can reasonably fit all the data below ~ 200 K. On the other hand the contribution which originates from the second term in Eq. (3) changes appreciably with hydration. The difference in Fig. 4 between the data at 0.2 and 0.41 hydration respectively can be accounted for by an increase in the probability term $p_1 p_2$ of about 50%.

The present data confirm therefore the results obtained previously with a higher energy resolution, but in a more limited Q -range. SOD shows a ‘glass-like’ dynamic transition similar to that already observed in other globular proteins. This appears therefore to be a common feature of globular proteins irrespective of their structural differences. The parameters obtained by fitting the

Q -dependence of the integrated elastic intensity with a simple anharmonic model are not very different from those obtained for myoglobin. This may indicate that the mean square atomic displacement is mostly sensitive to the 'local' features of the hydrogen motions, and therefore is only slightly affected by the overall high structural rigidity which is a characteristic of SOD scaffolding. This on the other hand is reflected in the characteristics of the quasi-elastic contribution which shows a quite small intensity and a small broadening as well up to relatively high hydration levels [15].

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