Low-frequency collective modes in dry and hydrated proteins

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ABSTRACT We have observed Brillouin-like low frequency collective modes in the scattering of 1 Å neutrons from a fully *in vivo* deuterated protein. These modes are tentatively interpreted as due to short-lived coherent excitations propagating with velocities between 2,000 and 4,000 m/s in elements of the secondary structure and patches of closely associated water.

1. INTRODUCTION

The molecular dynamics of biopolymer-water interactions can be studied in different ways. One approach examines the changes induced by hydrating the "dry" macromolecule in steps i.e., by adding more and more water until the fully hydrated state is reached. Here water may be thought of as a "perturbant," although it must be remembered that the initial, nominally dry state always requires a certain minimum number of water molecules. Experimentally this is the domain of sorption studies (Rupley, et al., 1983; Finney et al., 1982) and also much of the simulation work performed to date has necessarily been concerned with low hydration levels. Another possibility is to start from the relatively well-known properties of the bulk liquid, i.e., pure water or a weak electrolyte (buffer), and to investigate the perturbations resulting from adding a biomolecular component (Neilson and Enderby, 1986).

Substantial progress has been made in recent years with numerical simulations of biopolymer-water interactions, and in the most advanced cases (such as small proteins surrounded by several 100 waters) the trajectories $r_i(t)$ of essentially all atoms have been calculated for times up to a few 100 ps (McCammon and Harvey, 1987). The predictive power of such simulations is enormous, but the number of assumptions and parameters entering into them makes it imperative to pursue experimental work capable of yielding genuine spatiotemporal data. For proteins, simulation results have been compared principally with mean-square displacements derived from crystallography; comparison with dynamic structure factors measured by neutron scattering has been attempted so far only for a single protein (Cusack, 1986). Here cold neutrons were used for the measurement of the dynamic structure factor. Owing to the low speed of the incident neutrons (i.e., lower than speed of sound in the medium under study) used, the measurement was incapable of detecting the collective modes having propagating speed >774 m/s. However this experiment is capable of measuring the density of states through the process of incoherent scattering over the energy range up to 200 cm^{-1}.

As an extension of earlier neutron scattering work on protein hydration (Middendorf et al., 1984; Middendorf, 1984) and on collective excitations in pure water (Teixeira et al., 1985), we have begun to study Brillouin-like low frequency bands in the scattering of thermal neutrons from a covalently deuterated protein hydrated with D_2O. We report here some results from an experiment using three-axis spectrometer IN8 at the Institut Laue-Langevin. We used 1 Å incident neutrons which have a velocity of 4,000 m/s, and were thus able to detect the collective modes having speed of propagation in the vicinity of 2,000 to 4,000 m/s.

2. SAMPLE PROPERTIES AND EXPERIMENTAL DETAILS

Our initial objective was to see whether short-wavelength collective excitations of the kind already studied for pure D_2O could also be observed in a protein-water system. In proteins every second atom, on average, is a proton; it is desirable therefore to work with a perdeuterated sample so that the incoherent scattering is minimised. It is important further to choose a crystallographically characterised protein. Both requirements are well satisfied in the case of *C*-phycocyanin, a light-harvesting protein abundant in blue-green algae.

Biosynthetically deuterated samples of this phycobiliprotein were isolated from *Synechococcus lividus* grown in perdeuterated cultures at Argonne National Laboratory, Argonne, IL, (Crespi, 1977). The crystallographic structure of phycocyanin has been determined to a resolution of 5 Å by Fisher et al. (1980) and more recently to 3 Å by Schirmer et al. (1985). *C*-phycocyanin has been shown to exist in various aggregation states, the basic building block being a heterodimer of molecular weight 29,000-30,000 and a volume of ~38,000 $Å^3$. The $(\alpha\beta)_3$ molecule is shaped like an oblate ellipsoid (diameter 110 Å, thickness 40 Å) with a central solvent channel of 10 Å radius and radial clefts. There

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are three prolate ellipsoidal regions arranged symmetrically in 120° sectors around this channel, each corresponding to a $\alpha \beta$-unit.

Lyophilised, fully D_2O-exchanged samples of perdeuterated C-phycocyanin (dPC) were hydrated for 6 d over a saturated D_2O-salt giving 91% relative humidity; then pure D_2O was added to adjust the water content to 0.50 g/g, following the procedure described in Yang and Rupley (1979), to prepare concentrated lysozyme samples. The nominally dry sample was estimated to contain 4% D_2O in addition to those crystallographically located molecules that are in integral part of the structure. The sample container was analogous to the one used for D_2O (Teixeira et al., 1985) but thinner (3 mm) in order to avoid the effects due to multiple scattering.

The three-axis spectrometer IN8 was equipped with a special He-filled chamber to improve its low-angle performance, as in similar work on D_2O. For neutrons with incident wavelength 1 Å, this allowed us to scan the $\omega = -3$ to $+3$ THz energy transfer region ($-100 < \hbar\omega < 100$ cm^{-1}) with a resolution of 0.67 THz (20 cm^{-1}) down to $Q_{min} \simeq 0.35$ $Å^{-1}$. For both the dry and hydrated sample, constant-Q scans were performed at $Q = 0.3, 0.4, 0.5$, and 0.6 $Å^{-1}$. The spectra recorded showed weak side bands roughly symmetrical with respect to the central elastic line (see Fig. 1). We performed empty container runs at each Q value. They represent very small contributions as compared with the protein scattering. We subtracted these runs, taking into account the measured transmissions. The energy shifts relative to $\hbar\omega = 0$ tended to increase with increasing Q and hydration. They are plotted in Fig. 2 *a*) together with the dispersion curve determined for pure D_2O. The linewidth Γ are plotted vs. Q^2 in Fig. 2 *b*. Although the counting statistics of individual bands was quite poor owing to small samples and limited instrument time, the data set as a whole shows that the shifts observed for the D_2O-hydrated sample (50% g/g) are consistently larger than those for the "dry" phycocyanin sample ($\simeq$5% D_2O) by factors between 1.5 and 2.

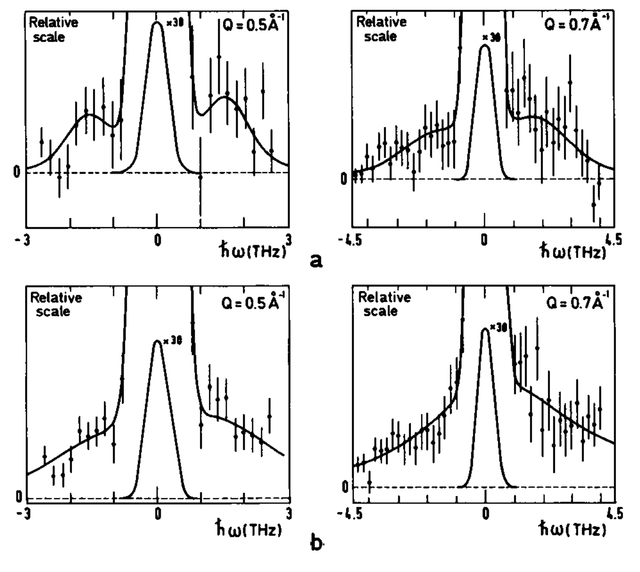

FIGURE 1 Scattered intensity I vs. $\hbar\omega$ from constant-Q scans at $Q = 0.5$ $Å^{-1}$ and $Q = 0.7$ $Å^{-1}$ for *a* dry phycocyanin, (*b*) D_2O-hydrated phycocyanin. Full curves represent least-square fits according to a damped harmonic-oscillator model (compare reference Teixeira et al., 1985 for details). The typical χ^2 values were ~1.3

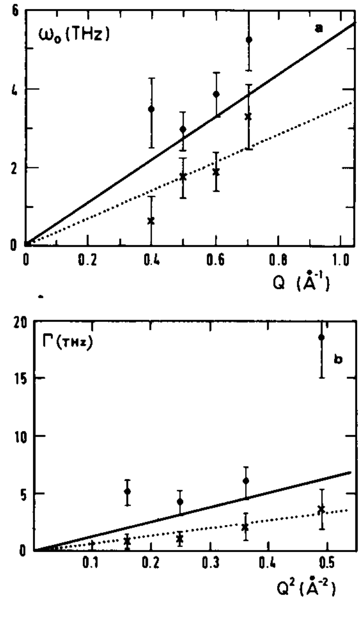

FIGURE 2 (*a*) Representation of the dispersion curve. (*b*) Linewidth, Γ, plotted against Q^2. (*Full line*) pure D_2O (Teixeira et al., 1985). (*Crosses*) dry C-phycocyanin. The dotted line (*a*) gives a velocity equal to 2,260 $m.s.^{-1}$. (*Full dots*) D_2O-hydrated c-phycocyanin.

3. DISCUSSION

A detailed quantitative interpretation of the data presented here is hardly possible because of the poor statistics and the narrow Q-range, although this instrument is the state of the art of the present generation of the triple-axis spectrometer. Two semi-quantitative facts are nevertheless worth noting and discussing. The first is that in both the dry and the "wet" samples we observe evidence of some dispersion. This shows that the modes we observed are collective modes. In the absence of more precise information, we have provisionally fitted a linear dispersion relation to the dry protein data and find that the acoustic propagation velocity determined in this way is 2,200 m/s (± $\simeq$ 10%). This value is definitely lower than the propagation speed of the short wavelength excitations established (much more reliably) as 3,300 m/s in previous work on pure D_2O (Teixeira et al., 1985). On the other hand the collective modes observed in the hydrated protein have the propagation speed closer to that of D_2O.

To appreciate the value of such data, it is important to be aware of the striking disparity between the volume of theoretical work on phonons in biomolecular systems and the paucity of experiments that have actually yielded

Q-ω-dependent information. For proteins, in particular, much simulation work and several analytical studies have been devoted to quantifying phonons and phonon-like nonlinear excitations (Davydov, 1974; Scott, 1983; Chou, 1985; Lawrence, 1987). However, acoustic phonons showing clear dispersion have been observed only in fibrous proteins by optical methods probing long-wavelength modes ($Q < 0.001$ Å^{-1}). For collagen, the dependence on hydration has been examined in some detail (Harley et al., 1977). It was found that the water of hydration "softens up" the long-wavelength excitations probed, in the sense that their velocity decreases from just under 4,000 m/s to ~2,500 m/s. The hydration-dependent properties of sound waves in DNA fibers have likewise been investigated in considerable detail, and a neutron study mapping out part of the dispersion surface has been published recently (Grimm et al., 1987).

At the simplest level, the results reported here may be regarded as the first direct measurement of the short wavelength collective modes in a globular protein over distances corresponding to the linear dimension of elements of the secondary structure, i.e., over 10–20 Å. This interpretation does not seem unreasonable for the dry sample where the polypeptide structure must be responsible for the effects seen. We know from the work of Schirmer et al. (1985) that ≃62% of this protein consists of α-helical polypeptide segments, and the crystal structure further suggests a relatively regular network of hydrogen bonds along the channel and in the clefts mentioned above. Our momentum transfer window, although narrow, matches the spatial extent of these characteristic features of the 3D structure.

The result for the wet sample, on the other hand, is not easy to understand, mainly because intuitively and on the basis of other work one would expect a general softening of the vibrational spectrum of a protein-water system with increasing hydration. It must be remembered however that short-wavelength collective modes with ps lifetimes are not probed by the techniques used hitherto, and that—as demonstrated by the results on pure D_2O—intuition may not be too helpful in this space-time domain. Essentially all the water we observe must be closely associated with the protein, i.e., belongs to the primary and secondary hydration shells. This water is known to be "structured" to some extent by the protein surface, and less mobile than bulk water. A possible qualitative interpretation is that the short-lived "patches" of contiguously hydrogen-bonded water molecules in the water of hydration surrounding a protein are more solid-like than those in bulk water, and therefore capable of sustaining somewhat "stiffer" collective modes.

These preliminary results demonstrate that the coherent neutron scattering data from experiments performed at a Q-range corresponding to molecular distances with thermal neutrons can be potentially useful in studying the collective modes in dry proteins and the coupling between liquid and biopolymer modes. We hope to be able to substantiate the observations reported here by future measurements using larger samples and examining intermediate hydration levels.

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