Measurement of Coherent Debye-Waller Factor in In Vivo Deuterated C-Phycocyanin by Inelastic Neutron Scattering

M.-C. -Bellissent-Funel,* A. Filabozzi,# and S. H. Chen§

*Laboratoire Léon Brillouin (CEA-CNRS), CE-Saclay, 91191 Gif-sur-Yvette Cedex, France; #Dipartimento di Fisica, Università di Roma "Tor Vergata" and Istituto Nazionale Fisica della Materia, 00133 Roma, Italy; and §Department of Nuclear Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 USA

ABSTRACT Quasielastic neutron scattering measurements of dry and 35% D_2O hydrated amorphous protein powder of C-phycocyanin were made as a function of temperature ranging from 313K down to 100K. The protein is grown from blue-green algae cultured in D_2O and is deuterated up to 99%. The scattering is thus dominated by coherent scattering. Within the best energy resolution of the time-of-flight instrument, which is 28 μeV FWHM, the scattering appears entirely elastic. For this reason we are able to extract a coherent Debye-Waller factor by making an independent measurement of the static structure factor. We observe a considerable difference in the q dependence of the Debye-Waller factor between the dry and hydrated proteins; furthermore, there is an interesting temperature dependence of the Debye-Waller factor that is quite different from that predicted for dense hard-sphere liquids.

INTRODUCTION

The dynamics of globular proteins has attracted much attention in the last decade (Brooks et al., 1988; McCammon and Harvey, 1987; Smith, 1991). Two popular methods of studying dynamics of proteins include computer molecular dynamics (CMD) and incoherent neutron scattering. In the former category a large system including solvent molecules is necessary for the simulation to be realistic. In particular, valuable insights into slow dynamics and large-amplitude anharmonic motions have been learned from a careful CMD study of Steinbach and Brooks (1993), by varying the amount of hydration of the protein myoglobin. The essential finding of these authors is that the slow dynamics and anharmonicity of the protein molecule, which are essential for it to function as an enzyme, require a certain amount of hydration (defined as full hydration). However, above the full hydration, the presence of additional solvent molecules does not really influence the slow dynamics. On the experimental side, the incoherent quasielastic and inelastic neutron scattering have been used effectively to identify the so-called glass transition in myoglobin and lysozyme as a function of temperature (Doster et al., 1989, 1990; Cusack and Doster, 1990). It was found that in both cases the anharmonicity of proteins markedly increases above 220K, as reflected in the temperature dependence of the mean square fluctuations averaged over all protein atoms, deduced from the incoherent Debye-Waller factor. The low-frequency vibrational density of states of hydrated myoglobin extracted from an incoherent inelastic neutron scattering measurement shows substantial deviation from the standard Debye form of harmonic solids above the glass transition (Cusack and Doster, 1990). This also points to the fact that the oscillations of atoms in a functioning protein show a marked deviation from that of a harmonic solid.

In contrast to the above-mentioned experiments, the coherent scattering from proteins, which directly probes the density fluctuations, has rarely been made in the past, mainly because of the lack of availability of a perdeuterated protein molecule. In this regard we were fortunate to obtain several grams of 99% deuterated C-phycocyanin sample from Dr. H. Crespi of the Argonne National Laboratory (Crespi, 1977).

With this sample we were able to perform a series of incoherent quasielastic neutron scattering studies of dynamics of H_2O on the surface of the protein (Bellissent-Funel et al., 1992a, b; Chen and Bellissent-Funel, 1994) as well as inside pores of a porous silica glass of Vycor (Bellissent-Funel et al., 1995). In these studies a well-defined elastic component in the scattering is observed that has a q dependence given by an incoherent Debye-Waller factor. It is interesting to note that this incoherent Debye-Waller factor can be interpreted as implying a confinement of water molecules in the radius of a few angstroms. This could be a result of a cage effect occurring in densely hydrogen bonded patches of water on the surface (Chen et al., 1996). Furthermore, the dynamics of water as reflected in the quasielastic component of the scattering is considerably slowed down as compared to that of bulk water.

We also performed both an inelastic neutron scattering experiment (Bellissent-Funel et al., 1989) and an elastic diffraction experiment (Bellissent-Funel et al., 1993). The inelastic neutron scattering experiment identifies short-wavelength collective modes in the hydrated protein that propagate at a much higher sound speed than ordinary low-frequency sound. In the elastic diffraction experiment, a dramatic change in the radial distribution function of surface water is observed in hydrated samples, depending

Received for publication 10 April 1996 and in final form 15 January 1997.

Address reprint requests to Dr. M. C. Bellissent-Funel, Lab Leon-Brillouin (CEA-CNRS), CE Saclay, 91191 Gif-sur-Yvette, France. Tel.: 33-1-6908-6066; Fax: 33-1-6933-1487; E-mail: mcbel@bali.saclay.cea.tr.

0006-3495/97/04/1792/08 $2.00

mostly on the level of hydration rather than on temperature, due to water-protein interactions.

In this paper, we want to compare the coherent Debye-Waller factor of a dry protein with that of a protein covered with a near-monolayer of water molecules. It has been demonstrated from a molecular dynamics simulation (Steinbach and Brooks, 1993) and shown experimentally (Rupley and Careri, 1991) that one monolayer of water molecules is sufficient to allow a protein to function. The additional water molecules above a monolayer do not significantly alter the dynamics of the protein. Thus the study of a hydrated powder of protein allows us to investigate the dynamics of the protein itself without complications contributed by bulk water. In the future, the same experiments could be repeated in solution.

In this experiment we report a quasielastic coherent neutron scattering study of the perdeuterated C-phycocyanin protein. In combination with the previous static structure factor measurement, we are able to extract the coherent Debye-Waller factor from the measured elastic portion of the dynamic structure factor. To our knowledge this is the first time that a coherent Debye-Waller factor of a dry and hydrated protein has been measured in an experiment. The coherent Debye-Waller factor gives the fraction of the elastically scattered neutrons as a function of q (Bragg wavenumber of the scattering). Because the peaks in the static structure factor more or less reflect the prominent local structure of the solid, the fraction of elastic scattered neutrons is expected to increase at such q values (pseudo-Bragg diffraction). Hence the coherent Debye-Waller factor is predicted to have peaks commensurate with that of the static structure factor for a dense hard-sphere liquid (Bengtzelius et al., 1984). We shall see in the following that our finding is at variance with this prediction.

THEORY

Because both dry and hydrated C-phycocyanin proteins are amorphous solids, we can write down the dynamic structure factor more or less in parallel with the well-known theory applicable to coherent scattering from a crystalline solid (Lovesey, 1987). We therefore write

$$S(q, \omega) = f(q)S(q)\delta(\omega) + (1 - f(q))S(q)L(\omega), \quad (1)$$

where the first term represents the elastic contribution and the second term all of the inelastic contributions, including the one-phonon and multi-phonon terms. This dynamic structure factor satisfies the sum rule

$$\int_{-\infty}^{+\infty} S(q, \omega)\mathrm{d}\omega = S(q), \quad (2)$$

where $S(q)$ is the static structure factor, if the frequency integral of $L(\omega)$ is normalized to unity; $f(q)$ in this equation can be interpreted as the coherent Debye-Waller factor.

SAMPLE PREPARATION

The C-phycocyanin protein

The protein of interest is C-phycocyanin, which contains light absorption centers central to the type II photosynthesis performed by cyanobacteria *Synechococcus lividus* (blue-green algae). As part of their effort to understand the photosynthetic process, Zuber and his collaborators have established the amino acid sequence of C-phycocyanin from several different species of cyanobacteria (Schirmer et al., 1985). Based on this work, Schirmer and collaborators used their measured X-ray diffraction patterns of molecular crystals formed from C-phycocyanin to refine the coordinates of all of the nonhydrogen atoms in the protein to a resolution of 2.1 Å (Schirmer et al., 1987); the best resolution of 1.66 Å has been recently obtained by Duerring et al. (1991). The crystallized C-phycocyanin unit cell ($P6_3$, $a = b = 154.6$ Å, $c = 40.5$ Å) contains a protein hexamer. C-phycocyanin can exist in various aggregation states, the basic building block being an ($\alpha\beta$) heterodimer of molecular weight 29,000–30,000. It has been shown that the secondary structure of this protein is 62% α-helical. The $(\alpha\beta)_6$ molecule is shaped like an oblate ellipsoid (diameter 110 Å, thickness 40 Å) with a central solvent channel and radial clefts.

Sample preparation

The C-phycocyanin is abundant in blue-green algae. Nearly 99% deuterated samples of this phycobiliprotein were isolated from cyanobacteria, which were grown in perdeuterated cultures (Crespi, 1977) (99% pure D_2O) by Dr. Crespi at Argonne National Laboratory. This process yielded deuterated protein that had virtually all of its ^{1}H-C bonds replaced by ^{2}H-C bonds. The protein has been lyophilized following the protocol described in detail by Debreczeny (1994). Deuterium in the weaker H-N and H-O bonds will tend to exchange with atmospheric hydrogen after extraction; however, we minimized the amount of hydrogen in these bonds by dissolution of the lyophilized protein in D_2O and freeze-drying it in a D_2O-rich atmosphere before either sealing the sample in the container (dry protein) or adsorbing the appropriate amount of D_2O to reach an hydration of 35%, corresponding to 0.175 g D_2O/g protein. The hydrated sample contains these amounts of water in addition to the 4% water molecules (D_2O), which have to be considered, like in many other proteins, as an integral part of the molecule. Under these conditions, the protein is covered by almost a monolayer of water molecules.

The more easily obtained powder form extracted directly from the cyanobacteria does not contain repeated unit cells, but rather is made of individual hexamers with random orientations and a uniform density of 0.3 g/ml. Such a sample is similar to an amorphous solid; neutron diffraction data (Bellissent-Funel et al., 1993) of the dry deuterated protein showed several broad diffraction peaks characteristic of noncrystalline solids (Fig. 1 *a*); the comparison with

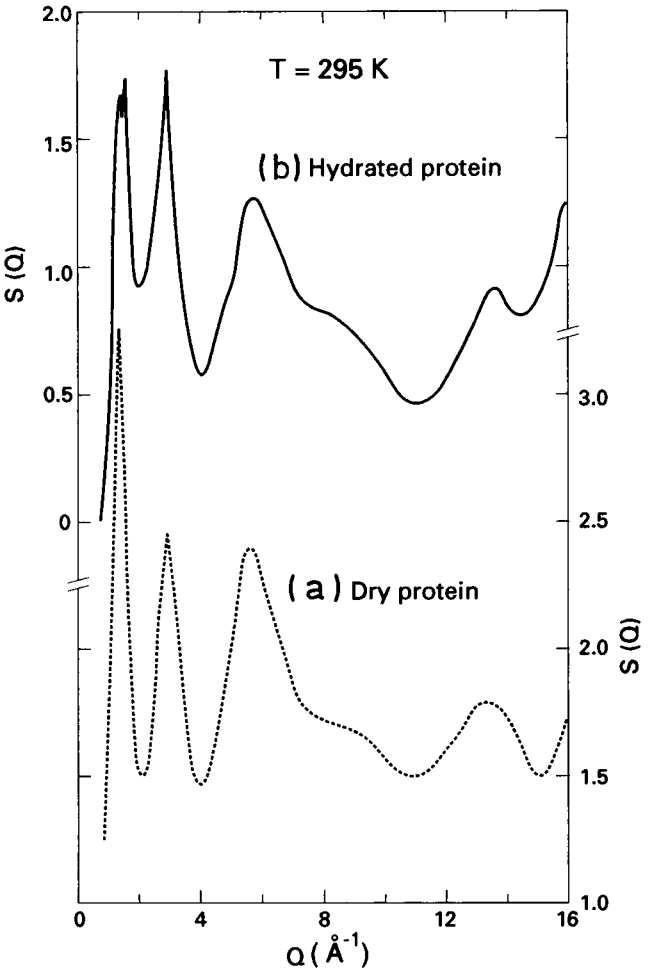

FIGURE 1 Neutron structure factors $S(q)$ at T = 295K. (*a*) Dry protein; (*b*) hydrated protein.

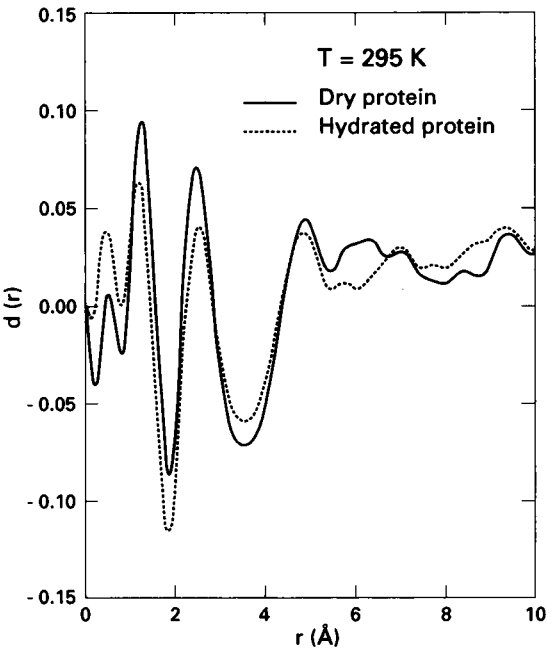

FIGURE 2 Pair correlation functions $d(r)$ at T = 295K. for dry protein (*full line*) and for hydrated protein (*dotted line*).

the hydrated sample is given in Fig. 1 *b*, where the effect of hydration on the neutron structure factor consists mainly in some splitting of the first diffraction peak. Fig. 2 compares the pair correlation functions relative to dry and hydrated protein at 295K. In the case of 35%, the water-protein interactions are not easily detectable, as it appears for a more hydrated protein sample. In the later case the water-protein interactions are manifested by the presence of a peak at 3.5 Å (Bellissent-Funel et al., 1993).

EXPERIMENTAL PROCEDURE AND DATA TREATMENT

Two experiments have been performed, using high-resolution time-of-flight spectrometers, one on the dry sample at ILL using IN6, the other on the 35% hydrated sample at Laboratoire Léon Brillouin using MIBEMOL. In the first case an incident neutron beam of λ = 5.12 Å has been used. With this wavelength a resolution (full-width at half-maximum) ranging from 78 μeV at low q values to 114 μeV at high q values and a wave vector range from 0.4 to 2.0 Å^{-1} have been obtained. In the second case, the same measurements were made with both high and low resolution: one with incident neutrons of λ = 6 Å (FWHM = 96 μeV), with wave vectors ranging from 0.32 to 1.93 Å^{-1}, and the other with neutrons of λ = 9 Å (FWHM = 28 μeV), with wave vectors ranging from 0.21 to 1.28 Å^{-1}.

An aluminum can shaped like a rectangular plate has been used as a sample holder. The can has window thickness of 0.5 mm and a surface area of 30 × 30 mm^2. The sample thickness is 2.5 mm, and it is oriented at 45° with respect to the incident beam.

Measurements have been performed at temperatures of T = 100, 243, 273, 313K for the dry sample and at temperatures of T = 180, 200, 220, 295K for the hydrated sample. The empty cell and the vanadium standard have also been measured. The experimental spectra were corrected for container scattering and sample attenuation, and from that the dynamic structure factor $S(q, \omega)$ has been deduced. We show in Figs. 3 and 4 the $S(q, \omega)$ for the dry and 35% hydrated proteins, respectively.

We analyzed the data by assuming that the dynamic structure factor is composed of an elastic component and a quasielastic one with a Lorentzian lineshape. This function is then convolved with the resolution function $R(\omega)$:

$$S(q, \omega) = [A(q) \cdot \delta(\omega) + A_1(q) \cdot L(\omega, \Gamma)] \otimes R(\omega). \quad (3)$$

The amplitudes for the first and second terms are $A(q)$ and $A_1(q)$, respectively. $L(\omega, \Gamma)$ is a Lorentzian of width Γ, and $R(\omega)$ is the instrument resolution as measured by the

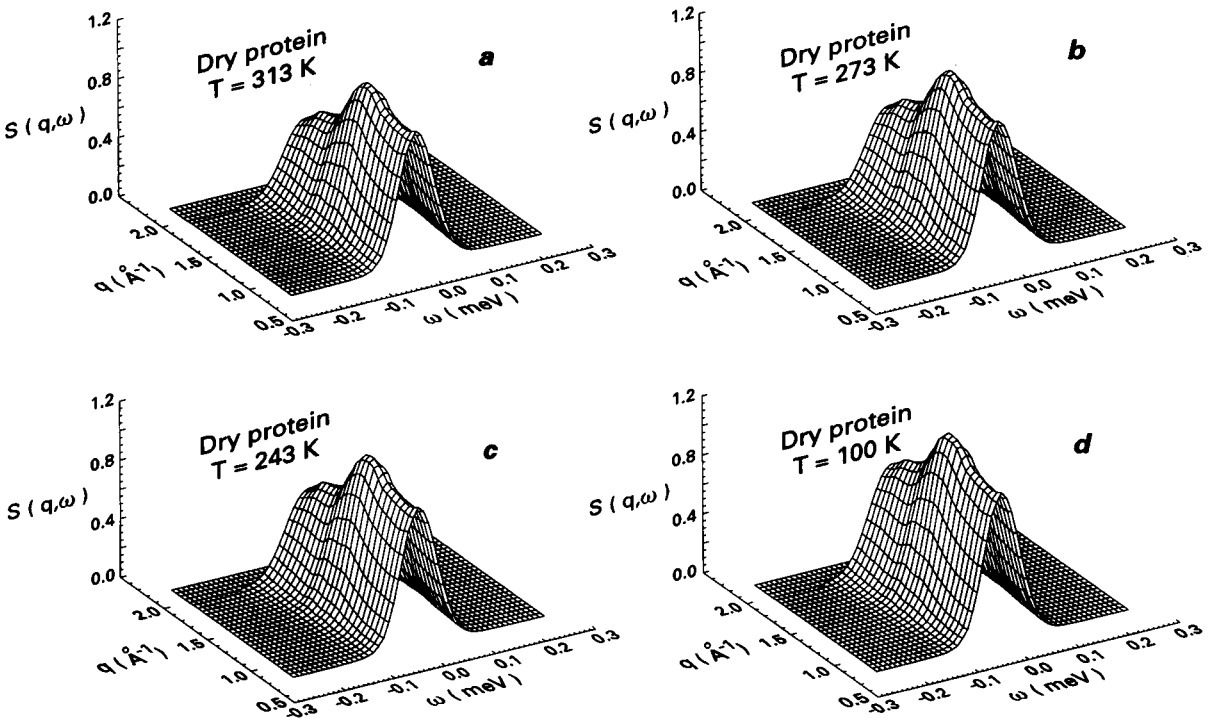

FIGURE 3 $S(q, \omega)$ surfaces of dry protein at different temperatures. (*a*) Dry protein at $T = 313$K; (*b*) dry protein at $T = 273$K; (*c*) dry protein at $T = 243$K; (*d*) dry protein at $T = 100$K. Note that the ω-broadening of all $S(q, \omega)$ functions is resolution limited. The data were taken at a neutron wavelength of $\lambda = 5.12$ Å; the energy resolution function has a FWHM = 98 μeV. The q dependence of the $S(q, \omega = 0)$ is essentially independent of temperature in dry protein.

vanadium standard. In fact, no quasielastic contribution has been found in both the dry and hydrated proteins, and in the low- and high-resolution cases. Thus we were able to calculate directly the $A(q)$ amplitude for both samples. Because the dynamic structure factor was not measured on an absolute scale, only the relative magnitude of $A(q)$ is obtained.

RESULTS AND DISCUSSION

In this section we describe the elastic component of the dynamic structure factor of the protein sample, both dry and hydrated, as a function of temperature. The dynamic structure factor $S(q, \omega)$ is proportional to the differential scattering cross section, which is written as

$$\frac{d^2\sigma^s}{d\Omega\, d\omega} = \frac{\sigma^v}{4\pi} \frac{\rho_v}{\rho_s} \frac{I^s}{I^v},$$

where σ^v is the total scattering cross section of vanadium, ρ_s and ρ_v are the respective densities of the sample and of the vanadium, and I^s and I^v are the intensities, respectively, scattered by the sample and the vanadium. The scattering cross section we measured has a normalization factor that is independent of q and temperature, but difficult to determine in practice. However, we are only interested in the q and temperature dependence of coherent Debye-Waller factors. So a reasonable way to present the data without being affected by this unknown constant factor is to plot the coherent Debye-Waller factor on a logarithmic scale.

In Fig. 3 the $S(q, \omega)$ surfaces of the dry protein at different temperatures ($T = 313$, 273, 243, and 100K) are shown in a 3-D plot as a function of momentum transfer q and energy transfer ω. The shape of all $S(q, \omega)$ functions is essentially the same at the four temperatures. As far as the ω broadening is concerned, we note that it is resolution limited. In fact, the data were taken with incident neutrons of wavelength $\lambda = 5.12$ Å, and the energy resolution function had a FWHM = 98 μeV. Furthermore, the q dependence of the $S(q, \omega = 0)$ is weakly dependent on temperature for the dry protein.

The $S(q, \omega)$ surfaces of hydrated protein at four different temperatures ($T = 295$, 220, 200, and 180K) are reported in Fig. 4. The overall shape of the $S(q, \omega)$ functions appears to

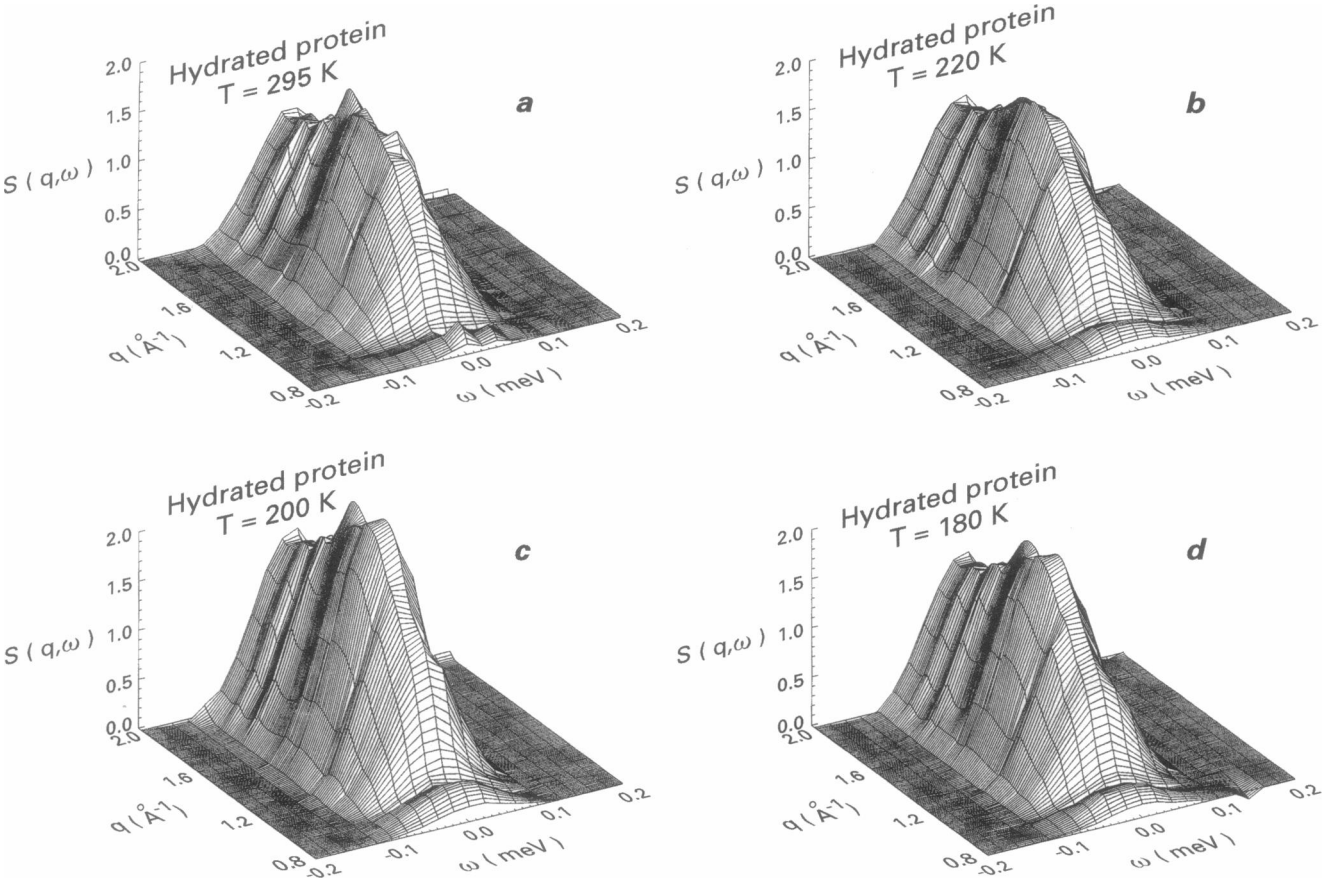

FIGURE 4 $S(q, \omega)$ surfaces of hydrated protein at different temperatures. (*a*) Hydrated protein at $T = 295$K; (*b*) hydrated protein at $T = 220$K; (*c*) hydrated protein at $T = 200$K; (*d*) hydrated protein at $T = 180$K. Note that the ω broadening of the $S(q, \omega)$ functions is resolution limited. The data were taken at a neutron wavelength of $\lambda = 6$ Å; the energy resolution function has a FWHM = 96 μeV. Another set of data taken at $\lambda = 9$ Å with a resolution of FWHM = 28 μeV shows the same energy-limited peaks. The q dependence of the $S(q, \omega = 0)$ has a substantial temperature dependence in hydrated protein.

be more "structured" in comparison with that of the dry protein. As in the case of the dry protein, the ω broadening of all $S(q, \omega)$ functions is resolution limited. The data were taken with incident neutrons of wavelength $\lambda = 6$ Å, and the energy resolution function had a FWHM = 96 μeV. To investigate this aspect, another set of data was taken at $\lambda = 9$ Å, with a resolution of FWHM = 28 μeV, but it shows the same energy-limited peaks. As far as the q dependence of the $S(q, \omega = 0)$ is concerned, it shows a substantial temperature dependence in the case of hydrated protein. One may note the appearance of a broad peak around $q = 0.9$ Å^{-1}, as the temperature is decreased, and the change in intensity of the sharp peak around $q = 1.6$ Å^{-1}.

In Fig. 5 we make a selected presentation of $S(q, \omega)$ of dry protein at $T = 313$K (Fig. 5 *a*) and of hydrated protein at $T = 295$K (Fig. 5 *b*). The dynamic structure factors are shown at fixed q values as a function of ω. The q values range from 0.85 to 1.70 Å^{-1}, with an interval $\Delta q = 0.05$ Å^{-1}. The ω range is -0.25 to 0.25 meV in the dry protein case and -0.2 to 0.2 meV in the hydrated protein case. The neutron structure factors $S(q)$ at each q value as measured in a separate diffraction experiment (Bellissent-Funel et al., 1993) are shown as crosses for comparison. Because the ω widths of all of the peaks are identical, the height of the peak is strictly proportional to the area. Therefore, the height should be roughly proportional to $S(q)$. It can be observed from Fig. 5, *a* and *b*, that this is qualitatively true for the hydrated protein, but there are noticeable differences in the whole q range for the dry protein. This is the basis for our extraction of a q-dependent Debye-Waller factor from the data (see Figs. 6 and 7).

From the comparison of Eqs. 1 and 3, one can obtain the Debye-Waller factor $f(q)$ by dividing the elastic amplitude $A(q)$, given by the fit of the measured $S(q, \omega)$, with the measured $S(q)$ at the corresponding temperatures. In Figs. 6 and 7 the elastic amplitude $A(q)$ for the dry and hydrated proteins, respectively, is shown at two temperatures (highest and lowest one in each case). The static structure factor $S(q)$ measured in a different experiment at the corresponding temperature is also reported, together with the obtained Debye-Waller factor $f(q)$. The temperature correspondence chosen is shown in Table 1. To make this choice it has been taken into account that the dynamics of a protein can generally be considered as "frozen" under 220K (Cusack and

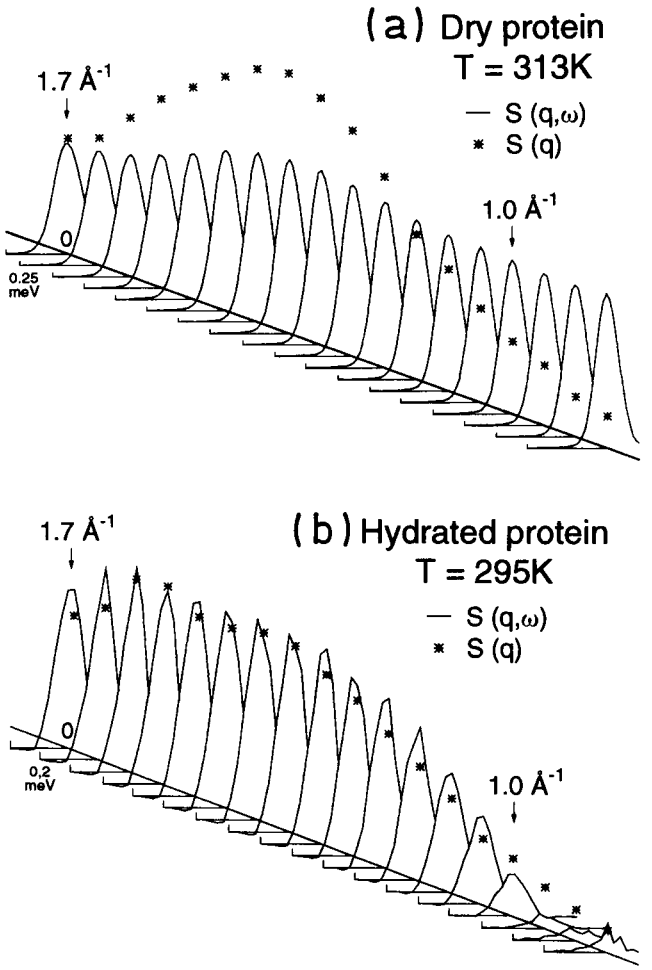

FIGURE 5 Selected presentation of $S(q, \omega)$ versus ω at fixed q values of dry protein at $T = 313$K and of hydrated protein at $T = 295$K. The q values are in the range 0.85–1.70 Å^{-1} with an interval $\Delta q = 0.05$ Å^{-1}. The $S(q)$ at $T = 295$K at each q value, measured in a separate experiment, is shown by asterisks for comparison. (*a*) The dry protein case. The ω range shown is from -0.25 to 0.25 meV. (*b*) The hydrated protein case. The ω-range shown is from -0.2 to 0.2 meV.

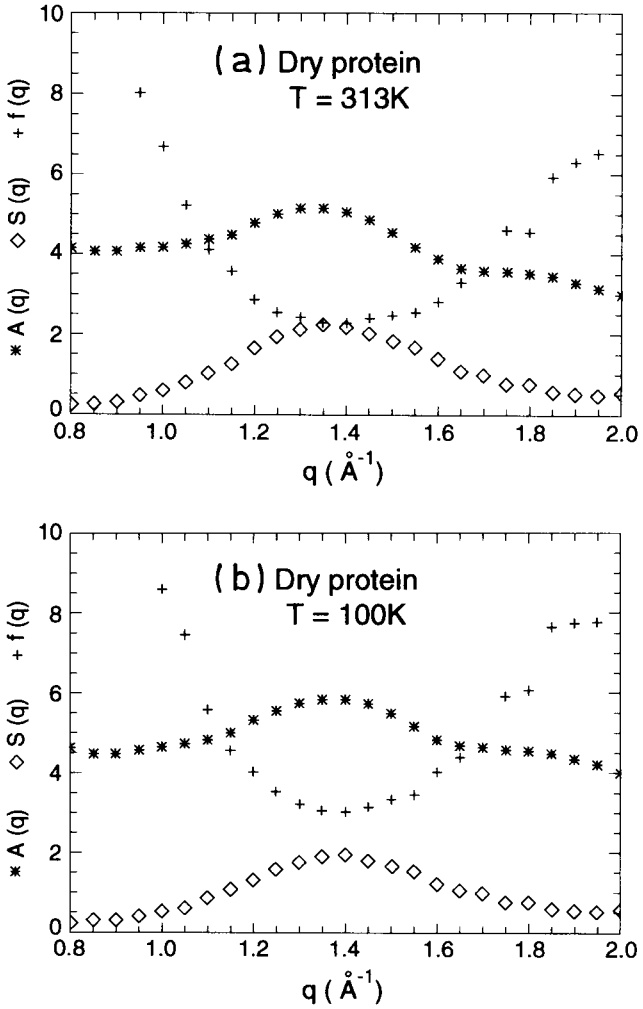

FIGURE 6 The elastic amplitude $A(q)$ for the dry protein is analyzed by dividing it with the measured $S(q)$ at the corresponding temperatures (see Table 1) to obtain the Debye-Waller factor $f(q)$. (*a*) $A(q)$ at $T = 313$K is shown by asterisks; $S(q)$ at $T = 295$K ($S(q)$ in dry protein is nearly temperature-independent) is shown by diamonds; and $f(q)$ is shown by crosses. (*b*) $A(q)$ at $T = 100$K is shown by asterisks; $S(q)$ at $T = 77$K is shown by diamonds; and $f(q)$ is shown by crosses.

Doster, 1990). On the other hand, in the case of the dry protein the structure factor $S(q)$ is nearly temperature independent. As we notice from Fig. 6, *a* and *b*, in the case of the dry protein, $A(q)$ and $S(q)$ exhibit the same behavior as function of q, so that the $f(q)$ resulting from their ratio is structureless at both temperatures. From Fig. 7, *a* and *b*, we can see that in the case of the hydrated protein, $A(q)$ and $S(q)$ display different features for q less than 1 Å^{-1}. This leads to $f(q)$ functions that exhibit broad peaks located at $q = 1.1$ Å^{-1} for $T = 180$K.

In Fig. 8 *a* we present a summary of $f(q)$ values obtained for the dry protein at all temperatures; the same summary for the hydrated protein is shown in Fig. 8 *b*. One may notice that the $f(q)$ values in both cases are temperature dependent. For the hydrated protein there is some apparent inversion in the temperature dependence of $f(q)$ between 200K and 180K. Whereas the $f(q)$ curves for dry protein are devoid of structure, the $f(q)$ curves for hydrated protein display two peaks, located at 0.85 Å^{-1} and 1.1 Å^{-1}. The amplitude of the first one may be amplified because the $A(q)$ values at 220, 200, and 180K have been divided with the only existing data at 77K. Beyond $q = 1.1$ Å^{-1}, the amplitudes of the peaks are more reliable because $S(q)$ values in this q range are smooth and weakly temperature dependent.

CONCLUSION

In this paper we analyze the coherent quasielastic neutron scattering data from a perdeuterated protein C-phycocyanin with or without hydration by D_2O. Within the range of energy resolution used, the scattering appears completely elastic. There is no detectable quasielastic component. Using a simple model for the dynamic structure factor given in

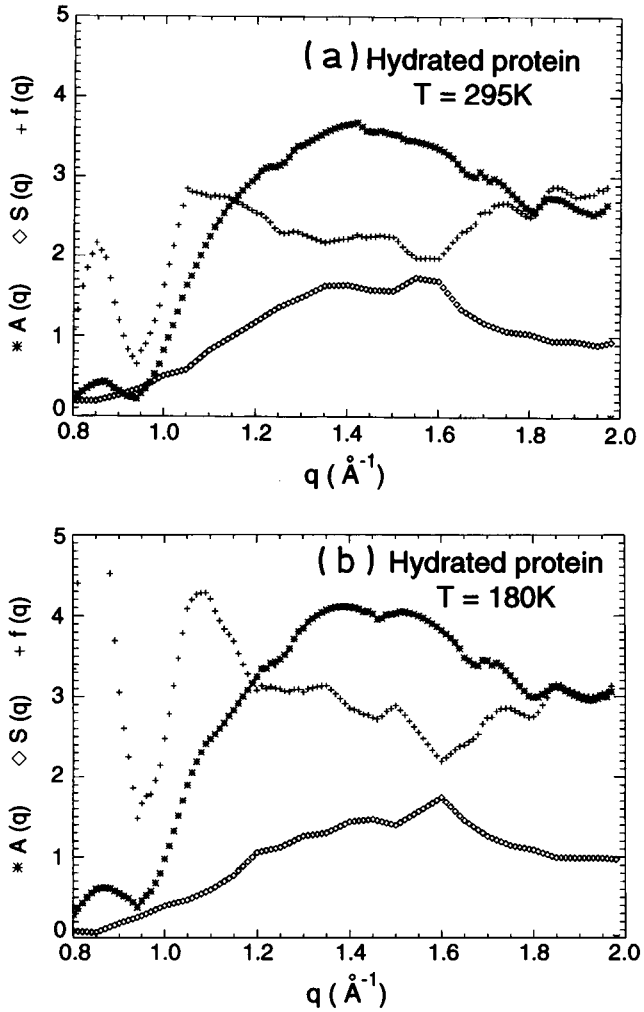

FIGURE 7 The elastic amplitude $A(q)$ for the hydrated protein is analyzed by dividing it with the measured $S(q)$ at the corresponding temperatures (see Table 1) to obtain the Debye-Waller factor $f(q)$. (*a*) $A(q)$ at T = 295K is shown by asterisks; $S(q)$ at T = 295K is shown by diamonds; and $f(q)$ is shown by crosses. (*b*) $A(q)$ at T = 180K is shown by asterisks; $S(q)$ at T = 77K is shown by diamonds; and $f(q)$ is shown by crosses.

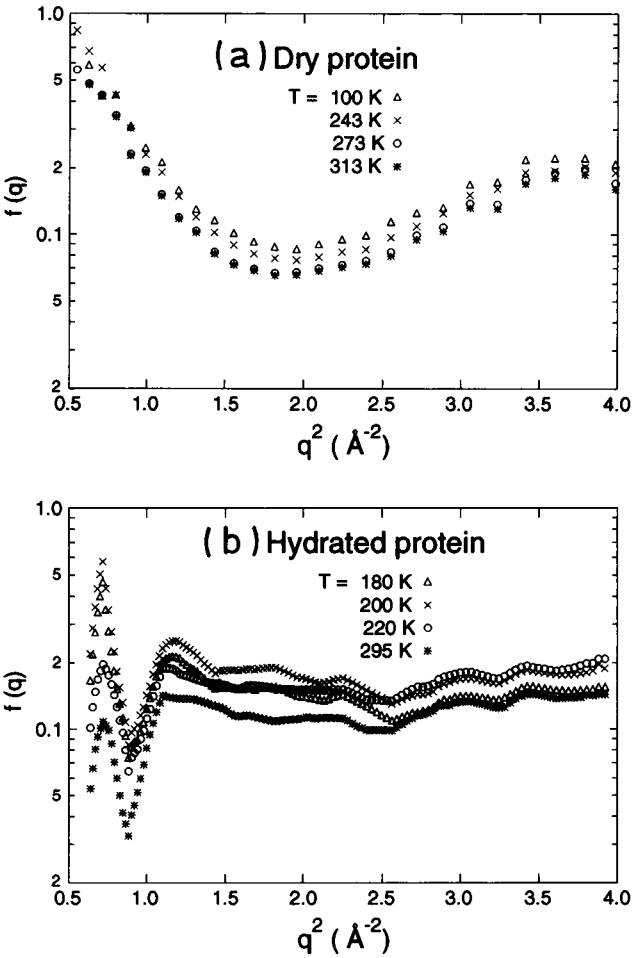

FIGURE 8 Summary of $f(q)$ values obtained for all temperatures, for both dry and hydrated proteins (see Table 1 for the corresponding temperatures of $S(q)$). (*a*) $f(q)$ obtained for the dry protein at T = 100K (*triangles*), T = 243K (*crosses*), T = 273K (*open circles*), T = 313K (*asterisks*). Notice that $f(q)$ values in the case of dry protein are weakly temperature dependent and devoid of structure. (*b*) $f(q)$ obtained for the hydrated protein at T = 180K (*triangles*), T = 200K (*crosses*), T = 220K (*open circles*), T = 295K (*asterisks*). Notice that $f(q)$ values in the case of hydrated protein are noticeably temperature dependent, with a possible inversion in the temperature dependence between 200 and 180K.

Eq. 1, we are able to extract the coherent Debye-Waller factors for both the dry and hydrated proteins. Whereas the Debye-Waller factors for the dry protein show a monotonic decay at low q, the Debye-Waller factor for the hydrated protein show several peaks, incommensurate with the peaks of the static structure factors, with a noticeable temperature dependence above and below 220K, which is more marked than that for dry protein. It is clearly different from those of a hard sphere system (Bengtzelius et al., 1984), where the oscillations in the coherent Debye-Waller factor follow that of the static structure factor $S(q)$, showing peaks at the same q values. This result is not surprising, considering the difference in the structure factor between the two in terms of the respective locations of the peak positions. Finally, following the paper by Petry et al. (1991) about the dynamics of the orthoterphenyl molecular glass, we have plotted in Fig. 9, *a* and *b*, the variation of the coherent Debye-Waller factor as a function of temperature for dry and hydrated

TABLE 1 Temperatures of the $S(q, \omega)$ for the dry and hydrated protein presented in this paper and corresponding temperatures of the static structure factor $S(q)$, measured in a different diffraction experiment, used to obtain $f(q)$

	$S(q, \omega)$	$S(q)$
T_{dry}	100	77
	243	77
	273	295
	313	295
$T_{hydrated}$	180	77
	200	77
	220	77
	295	295

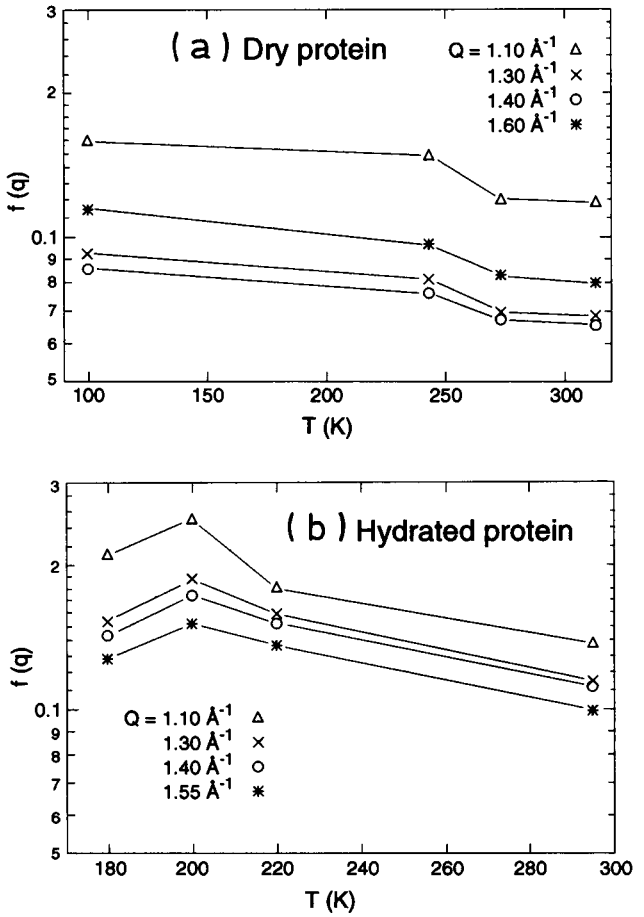

FIGURE 9 Variation of the coherent Debye-Waller factor $f(q)$ as a function of temperature, at several values of q close to the main peak of the structure factor. (*a*) For dry protein; (*b*) for hydrated protein.

protein at several values of q close to the peak of the structure factor. Although the number of investigated temperatures is small, the coherent Debye-Waller factor exhibits a sudden drop at the kinetic glass transition temperature, as a glass former molecular liquid. It has been shown that there is a glass transition temperature occurring at 200 and 220K.

M-CB-F and SHC thank NATO for financial support that made this research collaboration possible. The research of SHC is supported by a grant from the Materials Science Division of the U.S. Department of Energy. The research of AF is supported by a grant from the Laboratoíre Léon Brillouin and EEC. Finally, we are grateful to Dr. Henry Crespi for providing us deuterated protein.

REFERENCES

Bellissent-Funel, M. C., S. H. Chen, and J. M. Zanotti. 1995. Single-particle dynamics of water molecules in confined space. *Phys. Rev. E.* 51:4558–4568.

Bellissent-Funel, M. C., J. Lal, K. F. Bradley, and S. H. Chen. 1993. Neutron structure factors of in-vivo deuterated amorphous protein C-phycocyanin. *Biophys. J.* 64:1542–1549.

Bellissent-Funel, M. C., J. Teixeira, K. F. Bradley, and S. H. Chen. 1992a. Dynamics of hydration water in protein. *J. Phys. I. France.* 2:995–1001.

Bellissent-Funel, M. C., J. Teixeira, K. F. Bradley, S. H. Chen, and H. L. Crespi. 1992b. Single-particle dynamics of hydration water in protein. *Physica B.* 180, 181:740–744.

Bellissent-Funel, M. C., J. Teixeira, S. H. Chen, B. Dorner, H. D. Middendorf, and H. D. Crespi. 1989. Low-frequency collective modes in dry and hydrated proteins. *Biophys. J.* 56:713–716.

Bengtzelius, U., W. Gotze, and A. Sjolander. 1984. Dynamics of supercooled liquids and the glass transition. *J. Phys. C.* 17:5915–5934.

Brooks, C. L., III, M. Karplus, and M. Pettitt. 1988. Proteins. A theoretical perspective of structure, dynamics and thermodynamics. *Adv. Chem. Phys.* 71. John Wiley & Sons, New York.

Chen, S. H., and M. C. Bellissent-Funel. 1994. Hydrogen Bond Networks. NATO Advanced Study Institute, Series C/ Mathematical and Physical Science, Vol. 435. M. C. Bellissent-Funel and J. C. Dore, editors. Kluwer Academic, Dordrecht, The Netherlands. 307–336.

Chen, S. H., P. Gallo, and M. C. Bellissent-Funel. 1996. Slow dynamics of water in supercooled states and near hydrophilic surfaces. *In* Non-Equilibrium Phenomena in Supercooled Fluids, Glasses and Amorphous Materials. World Scientific Publishers, London.

Crespi, H. L. 1977. Biosynthesis with deuterated microorganisms. *In* Stable Isotopes in the Life Sciences. IAEA, Vienna. 111.

Cusack, S., and W. Doster. 1990. Temperature dependence of the low frequency dynamics of myoglobin. *Biophys. J.* 58:243–251.

Debreczeny, M. P. 1994. Comparison of the rate constants for energy transfer in the light harvesting protein, C-phycocyanin, calculated from Forster's theory and experimentally measured by time-resolved fluorescence spectroscopy. Ph.D. thesis. University of California, Berkeley.

Doster, W., S. Cusack, and W. Petry. 1989. Dynamical transition of myoglobin revealed by inelastic neutron scattering. *Nature.* 337: 754–756.

Doster, W., S. Cusack, and W. Petry. 1990. Dynamical instability of liquidlike motions in a globular protein observed by inelastic neutron scattering. *Phys. Rev. Lett.* 65:1080–1083.

Duerring, M., G. B. Schmidt, and R. Huber. 1991. Isolation, crystallization, crystal structure analysis and refinement of constitutive C-phycocyanin from the chromatically adapting cyanobacterium *Fremyella diplosiphon* at 1.66 Å resolution. *J. Mol. Biol.* 217:577–592.

Lovesey, S. W. 1987. Theory of Neutron Scattering from Condensed Matter, 3rd Ed. Clarendon Press, Oxford.

McCammon, J. A., and S. C. Harvey. 1987. *Dynamics of Proteins and Nucleic Acids.* Cambridge University Press, Cambridge.

Petry, W., E. Bartsch, F. Fujara, M. Kiebel, H. Sillescu, and B. Farago. 1991. Dynamic anomaly in the glass transition region of orthoterphenyl. A neutron scattering study. *Z. Phys. B. Condensed Matter.* 83:175–184.

Rupley, J. A., and G. Careri. 1991. Protein hydration and function. *Adv. Prot. Chem.* 41:37–172.

Schirmer, T., W. Bode, and R. Huber. 1987. Refined three-dimensional structures of two cyanobacterial C-phycocyanins at 2.1 and 2.5 Å resolution. *J. Mol. Biol.* 196:677–695.

Schirmer, T., W. Bode, R. Huber, W. Sidler, and H. Zuber. 1985. X-ray crystallographic structure of the light-harvesting biliprotein C-phycocyanin from the thermophilic cyanobacterium *Mastigocladus laminosus* and its resemblance to globin structures. *J. Mol. Biol.* 184: 257–277.

Smith, J. C. 1991. Protein dynamics: comparison of simulations with inelastic neutron scattering experiments. *Q. Rev. Biophys.* 24:227–291.

Steinbach, P. J., and B. R. Brooks. 1993. Protein hydration elucidated by molecular dynamics simulation. *Proc. Natl. Acad. Sci. USA.* 90: 9135–9139.