Neutron structure factors of in-vivo deuterated amorphous protein C-phycocyanin

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ABSTRACT Neutron powder diffraction measurements of fully deuterated protein C-phycocyanin have been made at three temperatures, 295, 200, and 77 K, using dry and partially hydrated samples. The average coherent structure factors and the corresponding radial distribution functions $d(r)$ are determined. The changes in $d(r)$ functions observed in hydrated samples depend strongly on the level of hydration and most of these changes are due to water-protein interactions. At 0.365 gram D_2O per gram of protein, the water crystallized into hexagonal ice at 200 K and below, but at 0.175 gram D_2O per gram of protein, no crystallization of water was observed. At the higher hydration a peak appears in the radial distribution function which indicates that the average distance of the water molecule in the first hydration shell from the amino acid residues is 3.5 Å.

1. INTRODUCTION

Structural information on the water in protein coming from x-ray and neutron diffraction analysis is of great importance to the understanding of protein hydration. There are excellent reviews on these works (1–9). Several recent surveys center on aspects of protein hydration: the distribution of water around the twenty different amino acid residues (10), the hydration of helices (11), and the helix geometry (12).

Water has been shown to play a crucial role in the stability and in the catalytic function of proteins (13). However, little is known about the nature of the increase in water "structure" due to nonpolar groups. Some molecular dynamics simulation (14–16) attempted to give some interpretation of this "structure." Changed properties of water contained in rather hydrophobic pockets of nanometer size cavities in polymer membranes due to extensive hydrogen bonding has also been observed (17) and x-ray studies of the structure of water in a hydrogel has been reported recently (18). Recently, inelastic neutron scattering was also used to investigate liquid-like motions and the nature of a dynamical transition in myoglobin (19, 20). It was shown that the line shape of the inelastic scattering function approximates the scaling behavior predicted for a simple liquid by mode-coupling theories (21) in the vicinity of the liquid-glass transition identified to be at 200 K.

As an extension of earlier quasi-elastic and inelastic neutron scattering work on protein hydration (22–24) and on the temperature dependence of the structure of bulk water (25) and amorphous ice (26), we have begun to explore the structure factor of a completely deuterated protein hydrated with D_2O. Both the influence of temperature and of the level of hydration on the structure factor of the protein have been studied, particularly, above and below 200 K, the approximate "glass transition temperature."

We report here some results from measurements using high resolution two-axis spectrometer 7C2 at the Orphée Reactor of the Laboratoire Léon Brillouin. We used 0.7 Å incident neutrons which allowed us to investigate the microscopic structure in the range of about 1 to 10 Å.

2. THE STRUCTURE FACTOR OF AMORPHOUS PROTEINS

A protein molecule can be considered as a folded polymer chain consisting of some specific sequence of twenty different amino acid residues. For the protein used in our experiment, all hydrogen atoms have been replaced by deuterium atoms and other constituents of the amino acid residues are carbon, oxygen, nitrogen, and sulphur, which are all coherent neutron scatterers, thus the primary intensity of scattered neutrons comes from the coherent scattering process. The coherent differential scattering cross section of an assembly of N atoms each located at $\mathbf{r}_i$ is given by:

$$\frac{d\sigma_c}{d\Omega} = \left\langle \left| \sum_{i=1}^{N} b_i e^{i\mathbf{Q}\cdot\mathbf{r}_i} \right|^2 \right\rangle \qquad (1)$$

where b_i is the bound coherent scattering length of the i-th nucleus and $\mathbf{Q}$ is the scattering vector with a magnitude $Q = 4\pi \operatorname{Sin}\theta/\lambda$, λ being the wavelength of neutrons and 2θ the scattering angle. The statistical average in Eq. 1 includes average over the orientation and the distribution of conformations of the folded chains at a given temperature and hydration. For a crystalline protein, given the crystallographic structure of the protein, the sum in Eq. 1 can be calculated for a specific conformation existing in the crystal, including the temperature factors of each atom. For an amorphous protein, however, further orientational and conformational averages have to be performed. Computation of the differential

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scattering cross section as given in Eq. 1 for a given crystal structure is tedious but even if we do it, the result cannot be directly compared with the experiment on an amorphous protein.

In order to extract a sensible result from neutron diffraction pattern of deuterated proteins, we may make the following simplifying assumptions: (1) Orientations of amino acid residues are uncorrelated with positions of their center of masses and (2) The form factors are temperature and hydration independent. With these assumptions we can proceed as follows: Let $\mathbf{R}_J$ be the center of mass of the J-th amino acid residue and $\mathbf{X}_{Ji}$ the position of the i-th atom in the J-th residue. Thus

$$\mathbf{r}_{Ji} = \mathbf{R}_J + \mathbf{X}_{Ji} \tag{2}$$

and the sums in Eq. 1 can be factorized as

$$\sum_{J=1}^{N_r} \sum_{i=1}^{n_J} b_{Ji} e^{i\mathbf{Q}\cdot\mathbf{r}_{Ji}} = \sum_{J=1}^{N_r} e^{i\mathbf{Q}\cdot\mathbf{R}_J} F_J(\mathbf{Q}). \tag{3}$$

N_r is the number of amino acid residues and the form factor $F_J(\mathbf{Q})$ is defined as:

$$F_J(\mathbf{Q}) = \sum_{i=1}^{n_J} e^{i\mathbf{Q}\cdot\mathbf{X}_{Ji}} b_{Ji}, \tag{4}$$

where n_J is the number of atoms in the J-th amino acid residue. The differential scattering cross section per protein molecule can then be written as

$$\frac{d\sigma_c}{d\Omega} = \left\langle \left| \sum_{J,J'}^{N_r} F_J(\mathbf{Q}) F_{J'}(\mathbf{Q}) e^{i\mathbf{Q}\cdot\mathbf{R}_{JJ'}} \right| \right\rangle \tag{5}$$

where $\mathbf{R}_{JJ'} = \mathbf{R}_J - \mathbf{R}_{J'}$. To evaluate the averages in Eq. 5, we consider $J = J'$ and $J \neq J'$ separately. For $J = J'$ the average reduces to a simple result:

$$N_r \langle F(\mathbf{Q})^2 \rangle. \tag{6}$$

where

$$\langle F(\mathbf{Q})^2 \rangle = \frac{1}{N_r} \sum_{J=1}^{N_r} \sum_{i,j}^{n_J} b_{Ji} b_{Jj} e^{i\mathbf{Q}\cdot\mathbf{X}_{Jij}} = \bar{n} \langle b^2 \rangle f_1(Q). \tag{7}$$

But for $J \neq J'$, the average can be approximately factorized as:

$$N_r \langle F(\mathbf{Q}) \rangle^2 [S_{rr}(Q) - 1]. \tag{8}$$

where

$$\langle F(\mathbf{Q}) \rangle^2 = \sum_{N_r}^{1} \sum_{J=1}^{N_r} \left(\sum_{i=1}^{n_J} b_{Ji} e^{i\mathbf{Q}\cdot\mathbf{X}_{Ji}} \right)^2 = \bar{n} \langle b^2 \rangle f_2(Q). \tag{9}$$

In general $f_1(Q)$ and $f_2(Q)$ are different functions. $\bar{n}$ is the average number of atoms in an amino acid residue.

In Eqs. 7 and 9 the average is over the orientation of the specific amino acid residue and also over their distribution in the protein. As a result, the vector dependence on Q disappears. The residue–residue structure factor is given by

$$S_{rr}(Q) = \left\langle \frac{1}{N_r} \sum_{J,J'} e^{i\mathbf{Q}\cdot\mathbf{R}_{JJ'}} \right\rangle \tag{10}$$

which is the quantity of considerable interest in our experiment. The coherent scattering cross section can thus be written as

$$\frac{d\sigma_c}{d\Omega} = N_r \{ \langle F(Q)^2 \rangle + \langle F(Q) \rangle^2 [S_{rr}(Q) - 1] \}$$
$$= N \langle b^2 \rangle S(Q) \tag{11}$$

where $N = N_r \bar{n}$ is the total number of atoms in a protein molecule and

$$S(Q) = f_1(Q) + f_2(Q)[S_{rr}(Q) - 1] \tag{12}$$

is the average coherent structure factor of the protein. At very large Q, $S_{rr}(Q)$ tends to 1 and $S(Q)$ is dominated by $f_1(Q)$ which oscillates around an asymptotic value equal to 1; thus $S(\infty) = 1$. At $Q = 0$, $S(Q)$ has the thermodynamic limit

$$S(0) = \rho k_B T \chi_T \tag{13}$$

where ρ is the density, k_B the Boltzmann constant, T the absolute temperature, and χ_T the isothermal compressibility.

It is seen from (12) that the average structure factor $S(Q)$ is a function of $f_1(Q)$, $f_2(Q)$ and $S_{rr}(Q)$. We expect intuitively that $f_1(Q)$ and $f_2(Q)$ are weakly dependent on the temperature and hydration, and they can be calculated precisely when the amino acid sequence of the protein is specified. So, they can be computed approximately from the crystallographic data. If this can be done, then in principle we can extract $S_{rr}(Q)$, the residue–residue correlation function. In this preliminary report, however, we shall look at $S(Q)$ directly.

From the $S(Q)$ structure factor, it is possible to evaluate the real space correlation function $d(r)$ from the transform relation

$$d(r) = 4\pi r \rho [g(r) - 1]$$
$$= \frac{2}{\pi} \int_0^\infty Q[S(Q) - S(\infty)] M(Q) \sin(Qr)\, dQ. \tag{14}$$

The $g(r)$ as well as $d(r)$ contain a weighted sum of all the partial pair correlation functions according to the relative atomic concentrations and the atomic coherent neutron scattering lengths. They include both intermolecular and intramolecular contributions and thus the interpretations are not straightforward. We shall attempt to correlate the peak position in $d(r)$ with the known atomic distances in proteins.

3. SAMPLE PREPARATION

The C-phycocyanin protein is a light-harvesting protein abundant in blue-green algae. Nearly 99% deuterated

samples of this phycobiliprotein were isolated from the cyanobacteria *Synechococcus lividus* grown in perdeuterated cultures (27) (99% pure D_2O) at Argonne National Laboratory, Argonne, IL. This process yielded in vivo deuterated protein that had virtually all its ^1H-C bonds replaced by ^2H-C bonds. 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 dissolving the lyophilized, perdeuterated protein in D_2O and freeze-drying it in a D_2O rich atmosphere before either sealing the samples in the containers or adsorbing the appropriate amount of D_2O.

The amino-acid sequence of C-phycocyanin from several different species of cyanobacteria has been established by Schirmer and his collaborators (28). These authors also used x-ray diffraction patterns of single crystal C-phycocyanin to refine the coordinates of all the non-hydrogen atoms in the protein to a resolution of 2.1 Å (28–30).

At full hydration the protein C-phycocyanin ($[PC_\alpha$-$PC_\beta]_6$, M_w = 244 KD), contains 0.5 gram of D_2O per gram of protein. The water content was measured by the increase in weight of the protein sample after exposing the protein to the vapor of D_2O. We prepared two samples with different degrees of hydration. The first one was 73% hydrated, i.e., it contained 0.365 gram D_2O per gram of protein and the second one was 35% hydrated, i.e., it contained 0.175 gram of D_2O per gram of protein. These samples contain these amount of water in addition to the 4% of water molecules (D_2O), which have to be considered like in many other proteins, as an integral part of the molecule.

4. EXPERIMENTAL PROCEDURE AND DATA TREATMENT

The experiments were performed at the Orphée reactor, at the laboratoire Léon Brillouin, Saclay, France, on the 7C2 spectrometer which is equipped with a BF_3 position-sensitive detector with 640 cells. The angular step between two adjacent cells is equal to 0.2° which leads to a maximum diffraction angle 2θ of 128°. We selected an incident wavelength of λ = 0.703 Å by means of a Cu 111 monochromator that allowed us to cover a range of magnitude of scattering wavevectors ($Q = 4\pi \sin\theta/\lambda$) extending from 0.7 to 16 $Å^{-1}$. The protein samples were held in containers of thin-walled vanadium (0.1 mm thick) with an internal diameter equal to 6 mm and placed inside a cryostat equipped with a vanadium tail. The experiments were done at several temperatures between 295 and 77 K. For the "dry" C-phycocyanin sample and the two hydrated C-phycocyanin (35% and 73% hydration) samples, the following sequence of measurements: "sample+container" at each required temperature, "empty container," standard "vanadium bar," "empty cryostat," and "cadmium rod" having the same

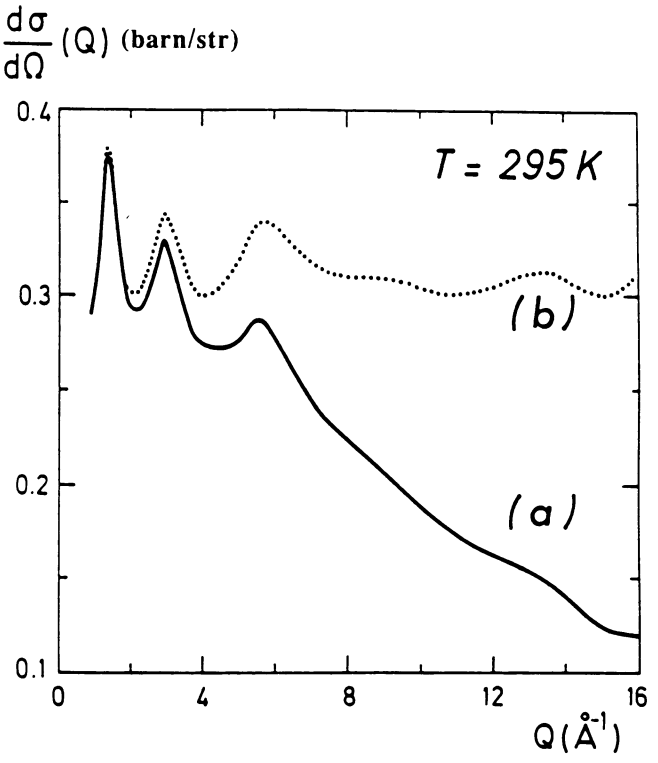

FIGURE 1 (*a*) Differential scattering cross-section of a dry deuterated protein C-phycocyanin at 295 K. (*b*) Differential scattering cross-section of a dry deuterated protein C-phycocyanin corrected for inelasticity effects.

dimensions as the sample, were made. The experimental spectra were corrected for container scattering and sample attenuation and the absolute differential scattering cross sections for the samples were evaluated at each temperature. Fig. 1 *a* shows the differential scattering cross section $d\sigma^{abs}/d\Omega$ of a dry protein sample at 295 K. The curve is characterized by a steady fall-off in the overall intensity as the momentum transfer Q is increased. This effect is commonly observed for low mass atomic materials containing particularly H or D atoms.

In order to correct for the inelasticity effect we used an empirical method based on the Placzek formalism and consisting of the polynomial expansion

$$\frac{d\sigma(Q)}{d\Omega} = \frac{d\sigma^{abs}(Q)}{d\Omega}(1 + AQ^2 + BQ^4 + --). \quad (15)$$

The coefficients A and B were determined in such a way that the oscillations in the resulting $d(r)$ function are minimal at small r. This empirical method has been successfully applied to molecular systems (31, 32) and more recently to liquid water (33). Fig. 1 *b* shows the differential scattering cross section corrected for the inelasticity effect for the dry protein sample at 295 K. The values of the structure factor may be obtained in the two limits $Q \to 0$ and $Q \to \infty$. Indeed $S(0) = \rho k_B \chi_T$ is evaluated from the isothermal compressibility χ_T and the density ρ; at large values of Q, one has $S(\infty) = 1$. Compressibili-

ties of globular proteins are lower than those of liquids and solid polymers but larger than those of metals and covalent solids (34). χ_T is less than 5×10^{-6} Atm^{-1} for pressures up to 400 Atm and PH 9.1 for the ribonuclease protein in the native state (35). Using the above estimate of χ_T (we do not know the temperature dependence) the calculated values of $S(0)$ varies from .05 to .01 in the temperature range 295 to 77 K. This value of $S(0)$ is much lower than that observed for bulk water.

After inelastic corrections, the structure factor is:

$$S(Q) = \left(\frac{d\sigma}{d\Omega}(Q) - C\right) \Big/ \left(\frac{d\sigma}{d\Omega}(\infty) - C\right)$$

where C is the Q-independent contribution of multiple and incoherent scattering. The real space correlation function $d(r)$ may then be obtained by Fourier transformation of $S(Q)$. Moreover, in order to avoid spurious oscillations in $d(r)$ due to limited range of data for $S(Q)$ (up to 16 $Å^{-1}$) we have multiplied $S(Q)$ by a modification function: $M(Q) = (\text{Sin}\ (\pi Q/Q_{max}/(\pi\ Q/Q_{max}))^2$ putting $Q_{max} = 12$ $Å^{-1}$ (See Eq. 14).

5. RESULTS AND DISCUSSION

Fig. 2 gives $S(Q)$ plots for the dry C-phycocyanin protein samples, at different temperatures from 295 down to 77 K. The structure factor of the dry protein, at 295 K, (Fig. 2 *a*) shows characteristics of amorphous systems (36), with three main peaks located respectively at 1.34, 2.96, and 5.65 $Å^{-1}$. When the temperature is decreased to 200 and 77 K (c.f. Fig. 2 *b* and *c*), significant changes in $S(Q)$ occur only at the level of the first peak, whose height decreases but by a lesser amount below 200 K. Beyond 4 $Å^{-1}$ there is hardly any temperature dependence of $S(Q)$, since in this region, the intramolecular structure factor $f_1(Q)$ of the residues dominates $S(Q)$ and within statistical accuracy the three curves can be superimposed (see Fig. 2 *d*).

For a 35% hydrated C-phycocyanin it appears that there is a splitting of the first peak. When going down in temperature from 295 to 77 K, no change occurs at the level of the second maximum located at 1.56 $Å^{-1}$, while the height of the first maximum located at 1.37 $Å^{-1}$ decreases (see Fig. 3 *b*). There is no significant shift in the positions of the other peaks located at 2.91 and 5.79 $Å^{-1}$, and as for the dry sample, the high Q-range of $S(Q)$ is dominated by the intramolecular contribution (see Fig. 3 *c*). This behavior, when crossing the glass transition temperature, has already been observed in the molecular glass former orthoterphenyl (37).

When increasing the level of hydration of the C-phycocyanin protein, the structure factor $S(Q)$ of the 73% hydrated sample exhibits a different behavior at 295 K (see Fig. 4 *a*). The first peak is now located at 1.96 $Å^{-1}$ which means that the contribution of the hydration water becomes dominant, the first diffraction peak of bulk water being located at about 1.95 $Å^{-1}$ at 295 K

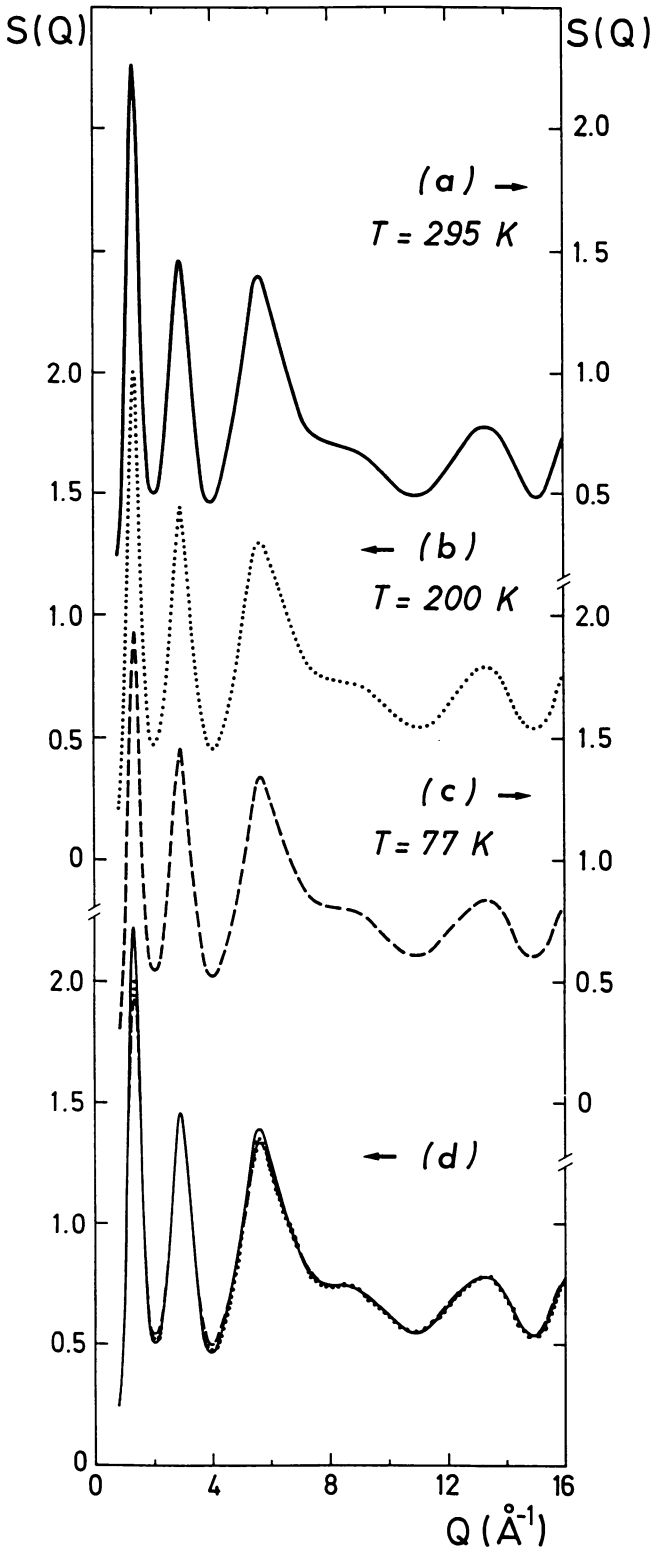

FIGURE 2 $S(Q)$ plots for a dry deuterated protein C-phycocyanin at different temperatures (*a*) 295 K, (*b*) 200 K, (*c*) 77 K. (*d*) is the superposition of plots (*a*), (*b*), and (*c*).

(25). At 200 K the nucleation of water gives rise to a hexagonal ice, characterized by the presence of a triplet (Fig. 4 *b*).

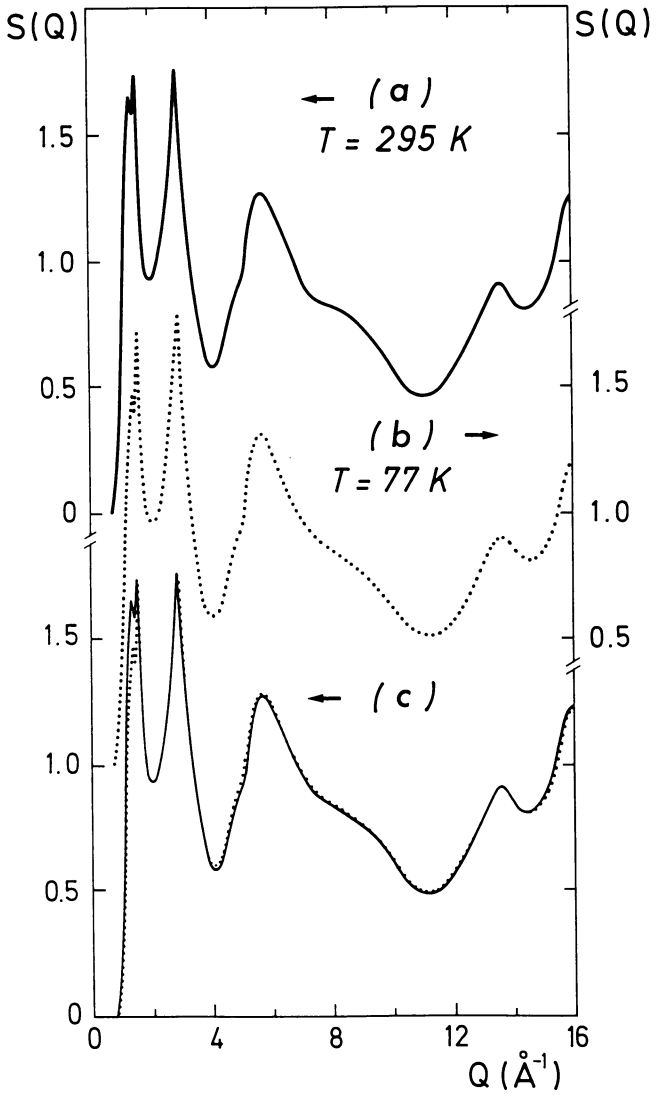

FIGURE 3 $S(Q)$ plots for a 35% hydrated protein C-phycocyanin (a) at 295 K, and (b) at 77 K. (c) is the superposition of plots (a) and (b).

The d(r) function includes both intra-residue and inter-residue correlations. The extraction of the inter-residue correlations, which is ultimately more interesting, needs some models for the amino acid residue form factors f_1 and f_2. In this paper, we present only the total d(r) functions and their qualitative interpretations. Fig. 5 gives an example of the d(r) functions for the 73% hydrated C-phycocyanin sample in comparison with that of dry C-phycocyanin, at T = 295 K.

In Fig. 5 the solid line gives the d(r) function for dry protein at T = 295 K. The peak at 1.26 Å gives some indication of the most probable distance and arises from the weighted sum of all atomic distances in protein corresponding to distances such as 1.24 Å of the carboxyl bond, 1.43 Å of the carbon–oxygen single bond, 1.54 Å of the carbon–carbon single bond, 1.47 Å of the carbon–nitrogen single bond, 1.27 Å of the carbon–nitrogen double bond, 0.994 Å of the nitrogen–hydrogen single bond, and 1.26 Å of the delocalized carbon–oxygen double bond. The peak at 2.5 Å corresponds roughly to the peptide bond distances (38). The molecular conformation of C-phycocyanin is characterized by the predominance of α helices (29); this is confirmed by the peaks observed at 4.9 and 5.83 Å. An increase in elastic intensity between 3.7 and 5.4 Å in the myoglobin has been attributed to the presence of α-helices in this protein (39). For example, in an idealized α-polypeptide helix, 3.6 residues/turn × 1.5 Å/residues (rise per turn) = 5.4 Å/turn (40) gives a characteristic length "the pitch of the helix" associated with the secondary structure of protein. The helices in the protein are often distorted from these idealized arrangements. For example, the terminal residues are distorted—forming hydrogen bonds or sometimes a more open structure of a π helix. In our case, we can at best say that the peaks at 4.9 and 5.83 Å correspond to characteristic pitch lengths of helices.

In Fig. 5, the d(r) function for a 73% hydrated protein at 295 K is compared with that of the dry protein at the

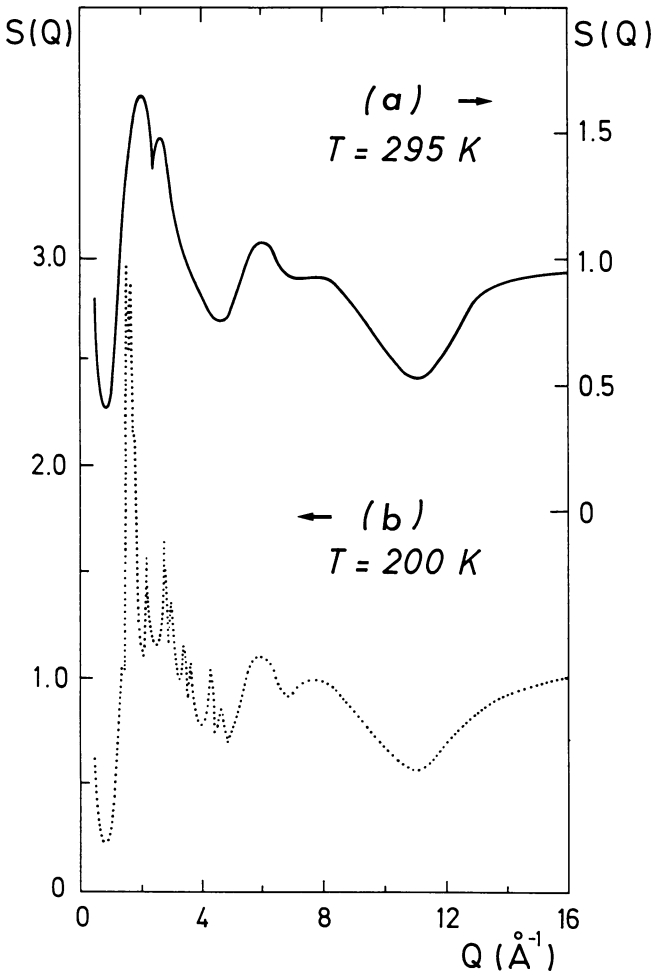

FIGURE 4 (a) $S(Q)$ of a 73% hydrated protein C-phycocyanin at 295 K. (b) $S(Q)$ of a 73% hydrated protein C-phycocyanin at 200 K. The nucleation of water as hexagonal ice is evident from the Bragg peaks seen here.

same temperature. The d(r) function shows changes in structure due to the presence of water of hydration. In particular, the peak at 1.26 Å seen in dry protein is shifted to a lower value of 1.05 Å. This peak at 1.05 Å arises now not only from the weighted sum of all atomic pair distances in protein as described above, but takes into account the intramolecular distances of hydration water. At high level of hydration it appears that this distance (1.05 Å) is close to the distance of 1 Å characteristic of the O–D intramolecular distance of bulk water (25). The peak of the polypeptide bond at 2.5 Å is always present and a definite peak appears at 3.5 Å, which is due to hydration of protein and which can be considered as the manifestation of the interactions between the water molecules and the amino acid residues. As it appears in Fig. 6, the d(r) functions exhibit some changes in the intermolecular part ($r > 3$ Å) when lowering the temperature down to 77 K. In particular, we observe an out-of-phase behavior of the large r oscillations of the d(r) function when compared to that at 295 K. Such a behavior of d(r) is similar to that of supercooled water, which tends to the structure of amorphous ice at low temperature (26).

In case of 35% hydration (see Fig. 7) the perturbation to the structure of the protein due to water of hydration is not detectable. It has been remarked (20) that a small amount of water suppresses rotational and translational motion of the whole molecule, emphasizing internal fluctuations (i.e., density fluctuations). This latter mechanism ensures that the water does not significantly affect the static structure of the protein. Similar behavior is observed by us in the case of low hydration (35%) of the protein and is further confirmed by the absence of the peak at 3.5 Å, which appears in the case of 73% hydrated protein as seen previously.

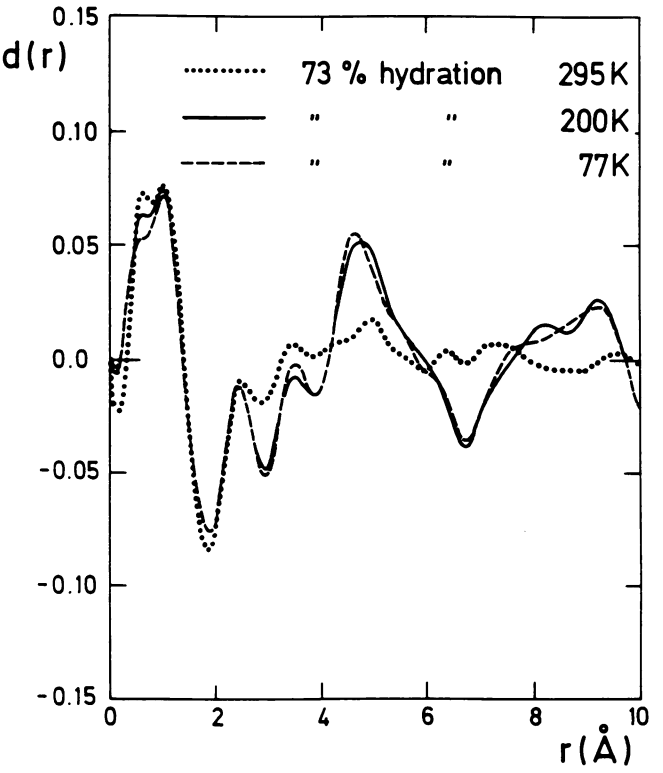

FIGURE 6 d(r) function for a 73% hydrated protein C-phycocyanin at 295, 200, and 77 K.

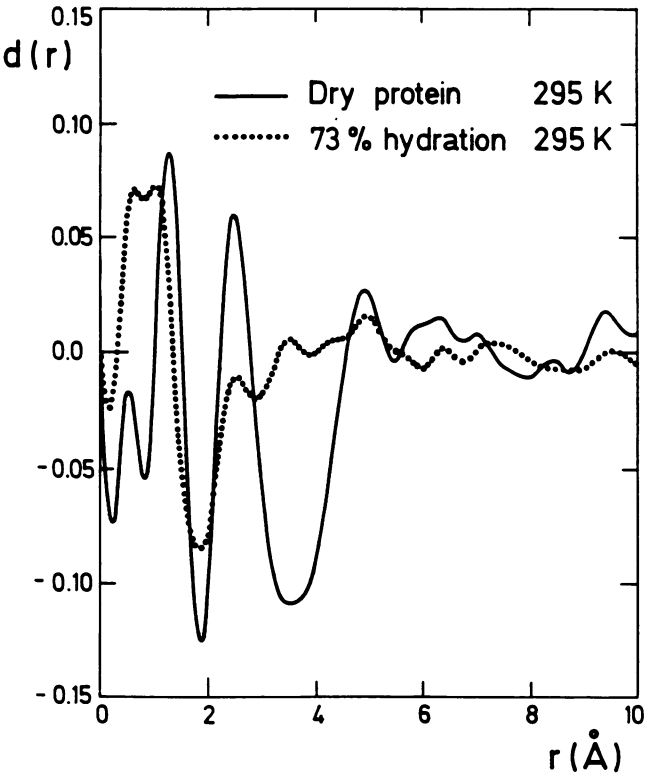

FIGURE 5 d(r) function for a dry deuterated protein C-phycocyanin and for a 73% hydrated protein C-phycocyanin at 295 K.

6. CONCLUSION

The results reported here may be regarded as the first determination of the structure factor of an amorphous protein, the effects of hydration and temperature on the total structure function d(r) being studied explicitly.

The observed major changes in d(r) of hydrated protein when compared to the dry protein come mainly from hydration rather than temperature. At the lower hydration, low temperatures do not affect significantly the overall structure of the protein and the bound water molecules, and we have not observed crystallization of water in the case of a 35% hydrated sample. At the higher hydration, from the analysis of the d(r) functions, it appears that the contribution of hydration water becomes more important, and the effects of temperature manifest themselves by the formation of hexagonal ice in 73% hydrated sample at low temperature. New experiments on the protein samples as a function of closely spaced temperatures around 200 K, and of lower and higher

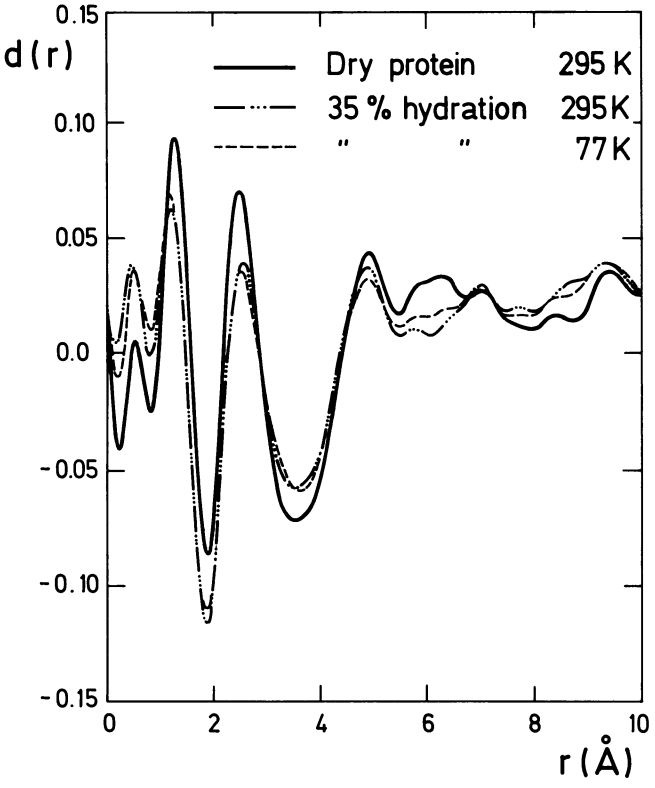

FIGURE 7 Comparison of d(r) functions for a dry and a 35% hydrated protein at the room temperature and at 77 K.

levels of hydration, are planned in order to investigate in more detail the hydrophobic hydration.

The most dramatic effect of hydration water on the protein surface is the appearance of the peak at 3.5 Å in the d(r) function, which is absent in dry and 35% hydrated samples. This effect is clearly evident in the computer simulations (15, 16) which show an increase in clustering of water molecules close to the protein surface within distances of 3–4.25 Å (mainly away from the nonpolar atoms in the protein) and some evidence is given by recent experiments on crystals of carbonmonoxymyoglobin (41). These water molecules do not interact strongly with the protein (as do those in contact with the polar groups) but their diffusive motion is restricted nevertheless due to their contact with the nonpolar groups, which does not allow possibly the same number of hydrogen-bonding neighbors as in bulk water. Work is in progress for attempting to extract $S_{\pi}(Q)$ as outlined in section 2 using f_1 and f_2 calculated from the known atomic coordinates in the crystalline form.

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REFERENCES

1. Baker, E. N., and R. E. Hubbard. 1984. Hydrogen bonding in globular proteins. *Prog. Biophys. Mol. Biol.* 44:97–179.
2. Edsall, J. T., and H. A. Mac Kenzie. 1983. Water and proteins. II. The location and dynamics of water in protein systems and its relation to the stability and properties, *Adv. Biophys.* 16:53–183.
3. Finney, J. L. 1979. The organisation and function of water in protein crystals. *In* Water: A Comprehensive Treatise. Vol. 6. F. Franks, editor. Plenum Press, New York. 47–122, 411–436.
4. Kossiakof, A. A. 1985. The application of neutron crystallography to the study of dynamic and hydration properties of proteins. *Annu. Rev. Biochem.* 54:1195–1227.
5. Saenger, W. 1987. Structure and dynamics of water surrounding biomolecules. *Annu. Rev. Biophys. Biophys. Chem.* 16:93–114.
6. Savage, H. 1986. Water structure in crystalline solids: Ices to proteins. *Water Sci. Rev.* 2:67–148.
7. Savage, H., and A. Wlodawer. 1986. Determination of water structure around biomolecules using X-ray and neutron diffraction methods. *Methods Enzymol.* 127:162–183.
8. Schoenborn, B. P., Editor. 1984. Neutrons in Biology. Plenum Press, New York. 472 pp.
9. Westhof, E., and D. L. Beveridge. 1990. Hydration of nuclei acids. *In* Water Science Reviews. Vol. 5. F. Franks, editor. Cambridge University Press, Cambridge, UK. 24–136.
10. Thanki, N., J. M. Thornton, and J. M. Goodfellow. 1988. Distributions of water around amino acid residues in proteins. *J. Mol. Biol.* 202:637–657.
11. Karle, I. L., and P. Balaram. 1989. Prediction of peptide and protein structures. *UCLA Symp. Mol. Cell. Biol.* (*New Series*). 86:71–85.
12. Barlow, D. J., and J. M. Thornton. 1988. Helix geometry in proteins. *J. Mol. Biol.* 201:601–619.
13. Rupley, J. A., and G. Careri. 1991. Protein hydration and function. *Adv. Protein Chem.* 41:37–172.
14. Geiger, A., A. Rahman, and F. H. Stillinger. 1979. Molecular dynamics study of the hydration of Lennard-Jones solutes. *J. Chem. Phys.* 70:263–276.
15. Rossky, P. J., and M. Karplus. 1979. Solvation. A molecular dynamics study of dipeptide in water. *J. Am. Chem. Soc.* 101:1913–1936.
16. Levitt, M., and R. Sharon. 1988. Accurate Stimulation of Protein Dynamics in Solution. *Proc. Natl. Acad. Sci. USA (Biophys.).* 85:7557–7561.
17. Wiggins, P. M. 1988. Water structure in polymer membranes. *Prog. Polym. Sci.* 13:1–35.
18. Bosio, L., G. P. Johari, M. Oumezzine, and J. Teixeira. 1992. X-ray and neutron scattering studies of the structure of water in a hydrogel. *Chem. Phys. Lett.* 188:113–118.
19. Doster, W., S. Cusack, and W. Petry. 1989. Dynamical transition of myoglobin revealed by inelastic neutron scattering. *Nature (Lond.).* 337:754–756.
20. Doster, W., S. Cusack, and W. Petry. 1990. Dynamic instability of liquidlike motions in a globular protein observed by inelastic neutron scattering. *Phys. Rev. Lett.* 65:1080–1083.
21. Gotze, W. 1989. Aspects of Structural Glass Transitions. *In* Liquid, Freezing and Glass Transition. Part 1. J. P. Hansen, D. Levesque, and J. Zinn-Justin, editors. North-Holland Science Publishers, Amsterdam. 287–503.
22. Bellissent-Funel, M.-C., J. Teixeira, S. H. Chen, B. Dorner, H. D. Middendorf, and H. L. Crespi. 1989. Low-frequency collective modes in dry and hydrated proteins. *Biophys. J.* 56:713–716.
23. Bellissent-Funel, M.-C., J. Teixeira, K. F. Bradley, S. H. Chen, and

H. L. Crespi. 1992. Single-particle dynamics of hydration water in protein. *Physica B* 180&181:740–744.

24. Bellissent-Funel, M.-C., J. Teixeira, K. F. Bradley, and S. H. Chen. 1992. Dynamics of hydration water in protein. *J. Physique I (France)*. 2:995–1001.
25. Bellissent-Funel, M.-C. 1991. Recent structural studies of liquid D_2O by neutron diffraction. *In* Hydrogen-Bonded Liquids. J. C. Dore and J. Teixeira, editors, Kluwer Academic Publishers, Dordrecht, The Netherlands. 1–3, 117–128.
26. Bellissent-Funel, M.-C., J. Teixeira, and L. Bosio. 1987. Structure of High-Density Amorphous Water. II. Neutron Scattering Study. *J. Chem. Phys.* 87:2231–2235.
27. Crespi, H. L. 1977. Biosynthesis with deuterated microorganisms. *In* Stable Isotopes in the Life Sciences. IAEA, Vienna, 111–121.
28. 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.
29. Schirmer, T., W. Bode, R. Huber, W. Sidler, and H. Zuber. 1985. X-ray crystallographic structure of light-harvesting biliprotein C-phycocyanin from the thermophilic cyanobacterium *Mastigocladus laminosus* and its resemblance to globin structures. *J. Mol. Biol.* 184:257–277.
30. Duerring, M., R. Huber, and W. Bode. 1988. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 236:167.
31. Deraman, M., J. C. Dore, J. G. Powles, J. H. Holloway, and P. Chieux, 1985. Structural studies of liquid hydrogen fluoride by neutron diffraction. I. Liquid DF at 293 K. *Mol. Phys.* 55:1351–1367.
32. Damay, P., F. Leclercq, and P. Chieux. 1990. Geometry of ND_3 group in a metallic $Ca(ND_3)_6$ compound and in solid and liquid deuteroammonia as measured by neutron scattering. *Phys. Rev. B.* 41:9676–9682.
33. Bellissent-Funel, M.-C., L. Bosio, and J. Teixeira. 1991. The inelasticity correction for liquid water in neutron scattering. *J. Phys. Condens. Matter.* 3:4065–4074.
34. Gavish, B., E. Gratton, and C. J. Hardy. 1983. Adiabatic compressibility of globular proteins. *Proc. Natl. Acad. Sci. USA.* 80:750–754.
35. Brandts, J. F., R. J. Oliveira, and C. Westort. 1970. Thermodynamics of protein denaturation. Effect of pressure on the denaturation of ribonuclease A. *Biochemistry.* 9:1038–1047.
36. Moss, S. C., and D. L. Price. 1985. Random packing of structural units and the first sharp diffraction peaks in glasses. *In* Physics of Disordered Systems. D. Adler, H. Fritzsche, and S. R. Ovshinsky, editors. Plenum Press, New York. 77–95.
37. Bartsch, E., H. Bertagnolli, P. Chieux, A. David, and H. Sillescu. 1993. Temperature dependence of the static structure factor of ortho-terphenyl in the supercooled liquid regime close to the glass transition. *Chem. Phys.* 169:373–378.
38. Blundell, T. L., and L. N. Johnson. 1976. The principles of protein structure. *In* Protein Crystallography. Vol. Molecular Biology; An International Series B. Horecker, N. O. Kaplan, J. Marmur and H. A. Scheraga, editors. Academic Press, New York. Ch 2. pp. 18–58.
39. Martel, P., and H. Lin. 1989. Large-scale oscillatory motions in myoglobin. *J. Biol. Phys.* 17:137–144.
40. Mathews, C. K., and K. E. van Holde. 1990. The three-dimensional structure of proteins. *In* Biochemistry. The Benjamin/Cummings Publishing Co., Reading, MA. Ch. 6, 171–212.
41. Cheng, X., and B. P. Schoenborn. 1990. Hydration in protein crystals. A neutron diffraction analysis of carbonmonoxymyoglobin. *Acta Cryst. B.* 46:195–208.