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Dynamics of hydration water in protein

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Abstract. — Incoherent quasi-elastic neutron scattering studies of *in vivo* deuterated C-phycocyanin, at different levels of hydration, have been made. We show that the mobility at high temperature, (~ 300 K) of the water molecules near the protein surface can be described by relatively simple models. At full hydration the high temperature data can be interpreted using a model where each water molecule is diffusing in a confined space of 3 Å in radius. At low hydration, and 298 K, the diffusional behaviour is typical of jump diffusion with a residence time 10 times larger than the one in bulk water at the same temperature.

1. Introduction.

Studies of single particle dynamics of hydration water in proteins have been hampered by the fact that about 40 % of the constituent atoms in a typical protein molecule are hydrogen atoms, present in the backbone and in the side chains. The incoherent background is thus too large for an accurate determination of the elastic incoherent structure factor (EISF) of the bound and surface water. One way of circumventing this difficulty is to deuterate the protein molecule. It was shown by Crespi [1] in 1977 that nearly 99 % deuterated phycobiliprotein can be harvested from Synechococcus lividus (blue-green algae) grown in perdeuterated cultures. Furthermore the X-ray crystallographic structure of phycocyanin has been determined to a resolution of 3 Å by Schirmer *et al.* [2], making possible a quantitative study of dynamics of the protein at the molecular level. Some preliminary results and their analysis of a quasi-elastic incoherent neutron scattering experiment of this deuterated C-phycocyanin, at different hydration levels, have been summarized by Middendorf [3]. We have recently studied the low frequency collective modes in dry and D_2O hydrated C-phycocyanin by a coherent inelastic neutron scattering experiment using sufficiently high energy neutrons to be able to see the excitations [4].

Computer simulation is an essential tool for the study of complex statistical mechanical systems where analytical methods have no hope of yielding tractable results. It is also indispensable for interpreting experimental data in such cases. The necessary ingredient for applying this technique is the availability of realistic inter-atomic potential functions for the system. In the last several years fairly reliable atom-atom potential functions between atoms

in the protein, atoms in water (simple point charge model, SPC) and between the two, have been devised [5-10]. Successful simulations of proteins in water have been thus available in the literature [9, 10].

In this paper, we analyse quasi-elastic neutron scattering data obtained with deuterated samples of the protein C-phycocyanin, at different levels of hydration. We show that the mobility at high temperatures (~ 300 K) of the water molecules near the protein surface can be described by relatively simple models.

2. Experiment.

C-phycocyanin may contain up to 0.5 g of H_2O per gram of protein (100 % hydration). We prepared perdeuterated samples of C-phycocyanin $[PC_\alpha - PC_\beta]_6$, $M_w = 244$ KD, at three different levels of hydration : 100 %, 40 % and 20 %. The water content was measured by the increase in weight of the protein sample after exposure to humidity. The samples were wrapped in a thin aluminium foil and placed inside a vacuum tight aluminium circular disc container of 50 mm diameter and 1 mm thickness.

Neutron scattering experiments were performed at the High Flux Reactor of the Institut Laue Langevin at Grenoble. Experiments with 100 % and 40 % hydrated samples were done at the IN6 time of flight spectrometer. The wavelength of the incident neutrons was $\lambda = 5.12$ Å, and the covered Q range extends from 0.254 Å^{-1} to 2.04 Å^{-1}. At small Q, the resolution function has a FWHM = 78.16 μeV and at the largest Q, a FWHM = 114 μeV. The measurements were made for nine temperatures : 333 K, 313 K, 293 K, 273 K, 258 K, 243 K, 223 K, 150 K and 100 K. The IN5 time of flight spectrometer has been used to study the 20 % hydrated sample. ($\lambda = 7.7$ Å, mean resolution FWHM = 31.7 μeV). The measurements were made only at three temperatures : 298 K, 273 K and 258 K.

3. Theoretical models.

INCOHERENT ELASTIC AND QUASI-ELASTIC LINES. — According to a model of Volino and Dianoux [11] the self-dynamic structure factor $S(Q, \omega)$ for a point particle diffusing inside a sphere of radius a is given by

$$S(Q, \omega) = A_0(Qa)\, \delta(\omega) + (1 - A_0(Qa))\, L(\omega) \tag{1}$$

where

$$A_0(Qa) = \left[\frac{3 j_1(Qa)}{Qa} \right]^2 = \left[3 \frac{\sin(Qa) - (Qa)\cos(Qa)}{(Qa)^3} \right]^2 \tag{2}$$

is the EISF expressing the strength of the elastic line arising from confinement of the particle, $j_1(Qa)$ is the first order spherical Bessel function. $L(\omega)$ is a Lorentzian function expressing the typical quasi-elastic line arising from the diffusive motions of the particle. Theoretically $L(\omega)$ is a sum of many Lorentzians with different amplitudes resulting from different eigenfunctions of the diffusion equation. But in practice, due to finite resolution of the instrument, only one average Lorentzian function can be detected. We extend this model to include, p = fraction of immobile protons and deuterons in the protein molecule and q = fraction of strongly bound protons on the surface of the protein. One can then write :

$$S(Q, \omega) = [p + (1-p) A_0(Qa)]\, \delta(\omega) + (1-p)(1 - A_0(Qa))[qL_1(\omega) + (1-q) L_2(\omega)]$$

After resolution broadening by a resolution function $R(\omega)$

$$S_M(Q, \omega) = [p + (1-p) A_0] R(\omega) + (1-p)(1 - A_0)[qL_1(\omega) + (1-q) L_2(\omega)]^* R(\omega). \tag{3}$$

If the strongly bound water molecules have a Lorentzian line $L_1(\omega)$ narrower than the resolution function $R(\omega)$, then to a good approximation we can write :

$$S_M(Q, \omega) = [P + (1-P)A_0]R(\omega) + (1-P)(1-A_0)L_2(\omega)^* R(\omega) \quad (4)$$

where

$$P = p + q(1-p). \quad (5)$$

Fitting to the quasi-elastic was therefore made with a function of the form : $\alpha R(\omega) + \beta L(\omega)^* R(\omega)$ and the three parameters α, β and the half width Γ of the Lorentzian function $L(\omega)$ as a function of Q were extracted. From the parameters α and β, one can then obtain the physical parameter q and EISF $A_0(Qa)$, knowing P.

4. Data analysis and discussion.

The raw data were only corrected for the contribution due to the sample holder. Consequently the signal contains the elastic incoherent scattering contribution due to the bound protons and deuterons in the dry protein besides those from the hydration water.

4.1 100 % AND 40 % HYDRATED C-PHYCOCYANIN. — Figure 1 shows the fitted quasi elastic line using equation (4) for a temperature of $T = 293$ K at scattering angle $\theta = 65.4°$, for the 100 % hydrated sample. From these fits we extracted P and then q which are given in table I. Table I summarizes the characteristics of the 100 % and 40 % hydrated samples, including the fraction q of the immobile protons in the hydration water and the fraction p of the bound protons in the protein, both related to the parameter P (through Eq. (5)). Assuming that full exchange occurs between the labile protons and H_2O vapour, during the procedure of hydration, one can easily calculate the fraction p of bound protons in the protein.

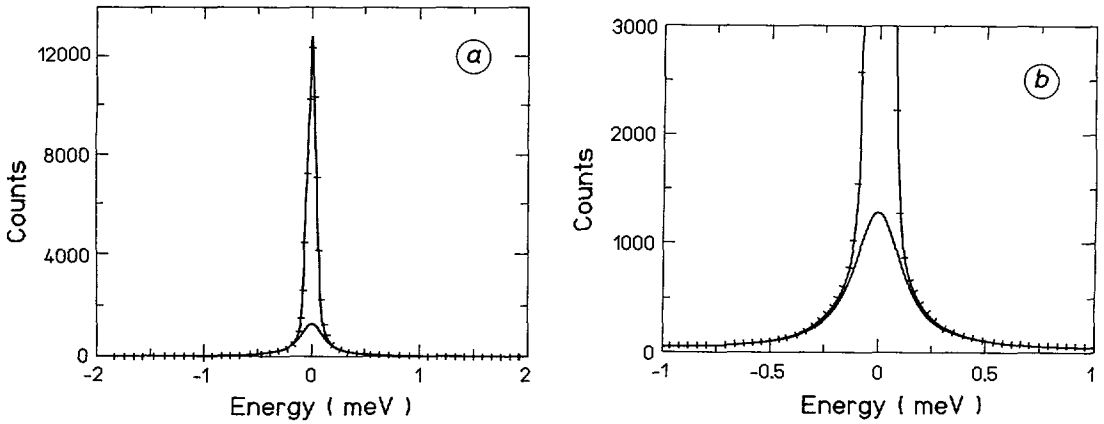

Fig. 1. — A typical quasielastic spectrum for the fully hydrated C-phycocyanin, at $\theta = 65.4°$ and for $T = 293$ K. Symbols (+) are experimental points and the full line is the fit using equation (4).

Figure 2 shows the EISF $A_0(Qa)$ relative to the 100 % hydrated sample for $T = 333$ K. The dots are the experimental data and the solid line is the theoretical prediction of equation (2) assuming $a = 3$ Å. The deviation of experimental EISF from the theory for confinement in a spherical cavity can be traced to the anisotropy of the confining volume. In fact, Dianoux, Pineri and Volino [12] worked out an extension to the theory of reference [11]

Table I.

Hydration level of C-phycocyanin	number of H_2O molecules per protein	number of labile protons	$P(T) = p + q(T)\ (1-p)$	p	$q(T)$
100 % 0.5 g H_2O/g of protein	6 726	3 564	$0.22 + 0.78\ q(T)$	0.22	0.42 $T = 333$ K
40 % 0.2 g H_2O/g of protein	2 711	3 564	$0.42 + 0.58\ q(T)$	0.42	0.83 $T = 333$ K

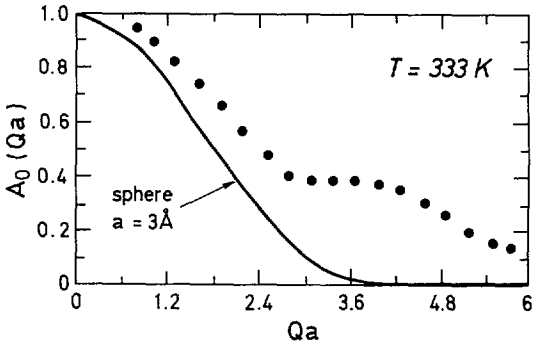

Fig. 2. — The elastic incoherent structure factor $A_0(Qa)$, for $T = 333$ K for the fully hydrated C-phycocyanin. (•) symbols represent experimental points and the full line is the theoretical prediction of equation (2) with $a = 3$ Å.

for a spherical cavity and showed that for a cylindrical cavity of radius R and length L, EISF can indeed decay at much slower rate at large Q, and even show an enhancement, for $L/2\,R$ ratio of 0.5, i.e. in a circular disc case.

For the 100 % hydrated sample, the results show that at high temperature around 58 % of the water molecules are mobile and that their motion can be interpreted within the model previously presented, for molecular diffusion inside a small volume. With decreasing temperature the amount of mobile water molecules decreases and around 230 K all the motions appear frozen (Fig. 3a) within the time scale of the experiment. Figure 3b shows the Lorentzian linewidth, Γ, for the full (100 %) hydrated sample together with the results obtained with a bulk water sample [13]. The linewidth from hydration water shows two distinctly different features from the corresponding bulk water. First, Γ *vs.* Q^2 shows a flat and constant value Γ_0 at small Q. Second, it also shows a flat asymptotic value Γ_∞ at large Q. The first feature can be accounted for from the model of Volino and Dianoux due to the confinements. In fact the model predicts that the plateau value Γ_0 at $Q = 0$ should be 4.33 $[D/a^2]$ and this plateau should persist until $Q_0 = \pi/a$. Estimating those values from figure 2b, we get $\Gamma_0 = 0.0824$ meV $= 1.25 \times 10^{11}$ rad.s^{-1} and $Q_0 = 1.05$ Å^{-1}. These values lead to $a = 3$ Å and $D = 2.6 \times 10^{-5}$ cm^2/s which is close to the diffusion coefficient of bulk water at this temperature, $D_B = 2.1 \times 10^{-5}$ cm^2/s. According to jump diffusion model [14], Γ *vs.* Q^2 curve should approach another plateau value $\Gamma_\infty = 0.16$ meV $= 0.243 \times 10^{12}$ rad.s^{-1} at large Q. The residence time for the jump diffusion is thus calculated to be $\tau =$

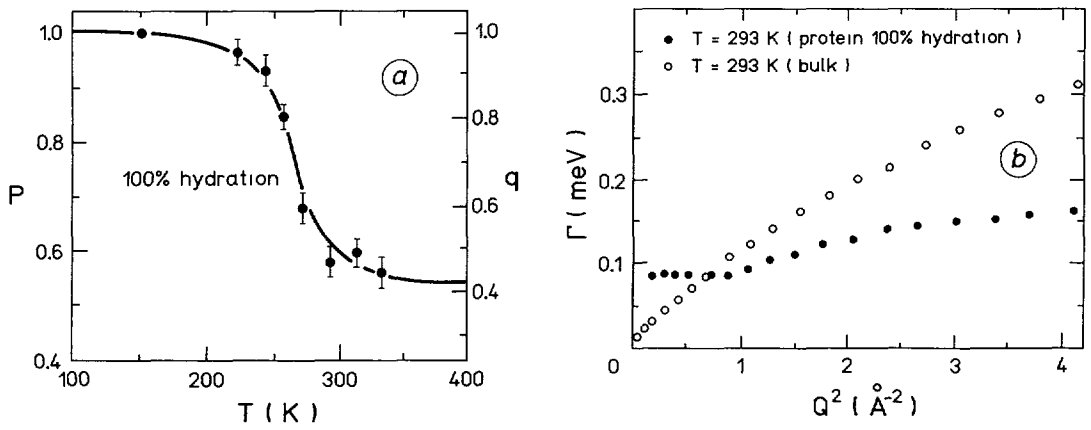

Fig. 3. — a) The variation of the measured P parameter and of the fraction of « immobile » water q *versus* T for the fully hydrated C-phycocyanin. b) HWHM of the Lorentzian quasielastic line Γ *versus* Q^2, for $T = 293$ K for the fully hydrated C-phycocyanin and for the bulk water.

$1/\Gamma_\infty = 4.1$ ps. The interpretation of the curves 3a and 3b suggests that several water molecules (around 42 % at $T = 333$ K) are bound to the protein. They are either immobile or diffusing at rates not accessible to the instrumental resolution. Experimentally they appear like an extra contribution to the elastic peak due to the protons of the protein.

In the simplified picture described above, at 333 K, only 58 % of the water molecules have a significant mobility. We attempted to confirm this observation from the analysis of the quasielastic neutron scattering data of the 40 % hydrated sample.

Assuming that the first layer of water forms on the surface of the protein in the same way as in the fully hydrated sample, one can expect a completely elastic scattering, under the same experimental conditions. This is actually the case : the scattered intensity follows the elastic structure factor of the protein and no diffusion broadening of quasi elastic peak is observed within the resolution function of the IN6 instrument (see part 2). Thus the parameter P that measures the amount of « immobile » water is close to 1, at all temperatures.

4.2 20 % HYDRATED C-PHYCOCYANIN. — The 20 % hydrated sample corresponds to a situation where almost certainly all the water molecules are bound to the substrate. In order to study the water dynamics in this sample we used a spectrometer with higher resolution (see part 2). Table II gives the characteristics q and p of the 20 % hydrated sample, which have been previously defined. In figure 4a, the number q of strongly bound protons is plotted *versus* temperature. It is worth noting that the curves in figures 3a and 4a are not directly comparable as they are obtained under different experimental conditions and the definition of the parameter q contains the instrumental resolution. Although we have a limited amount of data, it is clear that at intermediate temperatures (around 273 K), q is increased substantially in the less hydrated sample, suggesting again that the mobility of water molecules depends strongly of their vicinity to the protein substrate. In the case of this sample, the EISF was done with a higher resolution instrument IN5 so $A_0(Qa)$ is extracted more accurately. The wing of $A_0(Qa)$ resembles the functional form of $\left[\frac{3j_1(Qa)}{Qa}\right]^2$ and assuming $a = 3$ Å.

Figure 4b shows the Lorentzian linewidth Γ (HWHM) plotted *versus* the square of the momentum transfer, Q^2. The behaviour of the 20 % hydrated sample is typical of jump diffusion even at distances relatively large (of the order of 2.3 Å). The initial slope gives a

Table II.

Hydration level of C-phycocyanin	number of H_2O molecules per protein	number of labile protons	$P(T) = p + q(T)\ (1-p)$	p	$q(T)$
20 % 0.1 g H_2O/g of protein	1 335	3 564	$0.59 + 0.41\ q(T)$	0.59	0.51 $T = 298$ K

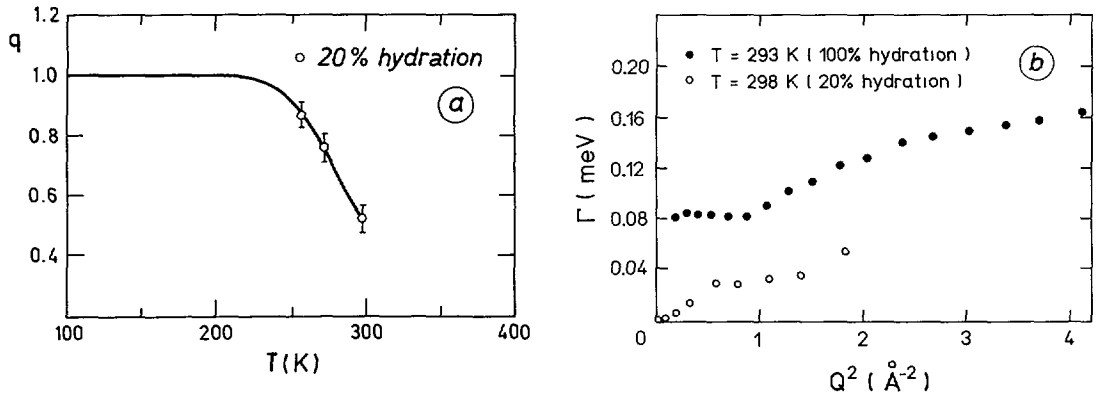

Fig. 4. — a) The variation of the fraction of « immobile » water q *versus* T, for the 20 % hydrated C-phycocyanin. b) HWHM of the Lorentzian quasielastic line Γ *versus* Q^2, for two levels of hydration of the protein. (Notice that the two curves are obtained with different resolutions.)

diffusion coefficient equal to 0.64×10^{-5} cm^2 s^{-1}, at 298 K, 4 times lower than in bulk water. Also, the residence time equal to 14 ps is about 10 times larger than the one of bulk water at the same temperature comparing with data obtained with supercooled water [13].

5. Conclusion.

We conclude that the mobility of water bound to the protein C-phycocyanin can be described with relatively simple models at high temperature (~ 300 K) and shows the following main features :

a) The external layers have faster dynamics than the internal more bounded layers. The water molecules in these external layers move by jumps between adjacent sites, separated by distance of the order of 3 Å. The residence time is about 4 times longer than in bulk water, but because of the larger jump length, the diffusion is comparable to bulk water although limited to short distances. This bulk water represents 58 % of the total amount of water in a full hydrated sample.

b) Water molecules in the internal layers show a jump diffusion with a diffusion coefficient 4 times smaller and a residence time 10 times longer than in bulk water. These movements could correspond to 20 % of the water in a full hydrated sample. It appears consequently that the separation of bound water in two classes is rather artificial, the mobility increasing from complete immobility to almost bulk water mobility (at least over short distances) in a continuous although rapid way, i.e. within very few layers. Then the mobility can be strongly dependent on the regions of the protein where the water molecule is attached.

c) In all cases, below 230 K, all the diffusion movements appear frozen. This temperature is close to the minimum temperature of supercooling samples, i.e. to homogeneous nucleation of ice embryos.

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