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Internal motions in proteins: A combined neutron scattering and molecular modelling approach

M-C BELLISSENT-FUNEL
Laboratoire Léon Brillouin (CEA-CNRS), CEA-Saclay, 91191 Gif-sur-Yvette Cedex, France
E-mail: mcbel@llb.saclay.cea.fr

Abstract. It is well-known that water plays a major role in the stability and catalytic function of proteins. Both the effect of hydration water on the dynamics of proteins and that of proteins on the dynamics of water have been studied using inelastic neutron scattering. Inelastic neutron scattering is the most direct probe of diffusive protein dynamics on the picosecond–nanosecond time-scale. We present here results relative to a photosynthetic globular protein, the C-phycocyanin, that can be obtained in protonated and deuterated forms. Diffusive motions have been studied using the protonated C-phycocyanin, protein. Molecular dynamics simulation and analytical theory have been combined to analyse the data and get a detailed description of diffusive motions for protein. The simulation-derived dynamic structure factors are in good agreement with experiment. The dynamical parameters are shown to present a smooth variation with distance from the core of the protein. The collective dynamics has been investigated using the fully deuterated C-phycocyanin protein. Both the experimental and calculated spectra exhibit a dynamic relaxation with a characteristic time of about 10 ps.

Keywords. Proteins; hydration; molecular dynamics; neutron scattering; numerical simulations.

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1. Introduction

A wide variety of internal motions exist in biological macromolecules [1]. At physiological temperatures, motions are partly vibrational, partly diffusive [2]. Diffusive motions are of particular interest, in the context of dynamical or 'glass transition' determined between 180 K and 220 K for many soluble proteins [3].

Figure 1 gives an example of the dynamical transition occurring at a temperature close to 220 K for the C-phycocyanin protein. Below 220 K there are only vibrations, above 220 K the diffusive motions are activated [4].

The description of internal diffusion in proteins is complicated by a variety of existing motions. These involve groups of atoms undergoing a plethora of continuous or jump-like diffusion. Neutron spectroscopy permits the investigation of motions in the time range from 10^{-14} to 10^{-9} s (time-of-flight, backscattering, spin echo techniques). Because of the large incoherent cross-section of hydrogen nuclei (about 40 times larger than the cross-section of other elements) and the fact that hydrogen

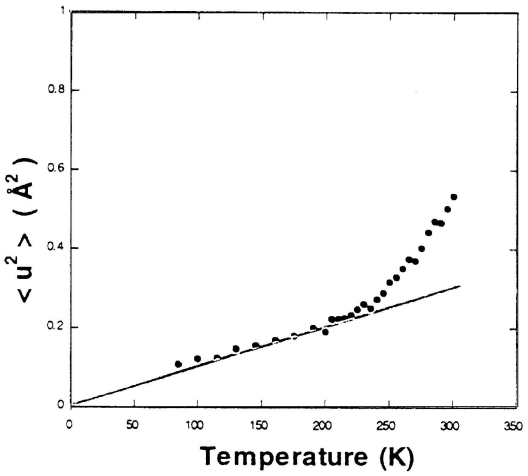

Figure 1. Evolution of the mean square displacements $\langle u^2 \rangle$ as a function of the temperature for a hydrated C-phycocyanin protein ($h = 0.5$ g D_2O/g protein) [4].

atoms are distributed 'quasi-homogeneously' in the biological macromolecule, this technique is a powerful tool for the study of all internal motions. Our strategy is to combine the neutron results with that of computer molecular dynamics (CMD) simulations. This strategy offers a unique opportunity to validate the potential of MD simulations and get a detailed knowledge of protein dynamics. Dynamics of water that plays an essential role in biology becomes also accessible on a detailed way. CMD simulations are analysed with theoretical laws developed for polymeric systems, from which geometry of motions and distribution of relaxation times of various parts of the protein are obtained. Mode coupling theory (MCT) that is well appropriate to describe supercooled liquids allowed us to provide with a model for confined or interfacial water [5–7].

Our purpose was to study diffusive motions in protein, above the dynamical transition temperature, in order to access relaxation times and geometry of motions. The adopted strategy has been to combine quasi-elastic neutron scattering and molecular dynamics simulation. Both techniques cover similar time range (few picoseconds to nanoseconds) and space range (few Å).

In order to limit the contribution of bulk water, powders of protein hydrated at a level $h = 0.5$ g water/g protein have been used. According to the work of Rupley and Careri [8] for instance, the enzymatic function of lysozyme is activated for a minimum level $h = 0.25$ g water/g protein. It is known that the full water coverage of the protein is obtained for $h = 0.3$ g water/g protein.

2. C-phycocyanin protein

The photosynthetic C-phycocyanin (CPC) protein is available in the protonated (h-CPC) and deuterated (d-CPC) forms. The d-CPC form has been harvested, by

Crespi [9], from *Synechococcus lividus* (blue-green algae) grown in perdeuterated culture, providing a 99% deuterated protein. The structure of C-phycocyanin (h-CPC) is now known with a 1.66 Å resolution [10], allowing dynamical studies and simulation, at a molecular level, of the surface water as well as of the protein itself.

3. Neutron scattering experiments

To maximise the contribution from the protein motions under controlled conditions, the incoherent neutron scattering experiments have been performed using fully deuterated solvent and hydrogen–deuterium exchanged proteins. This deuteration procedure serves to ensure that the solvent is fully deuterated (and not partially hydrogenated by exchange of hydrogen from the protein) and that no change in the deuteration of the protein or solvent occurs during the experiment. The consequent partial deuteration of the photosynthetic protein is limited to the exchangeable hydrogens, which means that all the hydrogens left are essentially in the protein. Under these conditions the scattering is dominated by the protein hydrogens. The powder protein sample was hydrated to $h = 0.52$ g H_2O/g protein, containing about 170 mg of protein [11]. Experiments were performed on the Mibemol time-of-flight spectrometer at the Orphée Reactor of LLB, Saclay. The incident wavelength was 6 Å, the experimental resolution 96 μeV (full-width half-maximum) and the q-range was 0.46 Å^{-1} to 1.95 Å^{-1}. The time of data acquisition was about 12 h. The sample cell was a flat aluminium of 1.5 mm optical thickness [4,11].

In order to study the collective dynamics, neutron spin echo measurements have been performed with the deuterated C-phycocyanin protein hydrated with D_2O. The spectrum deduced from molecular dynamics simulation shows a reasonable agreement with the experimental one, showing that the main contribution comes from backbone and side-chain motions [12]. Both the experimental and calculated spectra exhibit a dynamic relaxation with a characteristic time of about 10 ps.

In the following we report only the results of incoherent quasi-elastic neutron scattering experiments and the comparison with CMD.

4. Molecular dynamics simulations

The molecular dynamics simulation was performed on a simple model consisting of a single hydrated C-phycocyanin $\alpha\beta$ monomer together with its three chromophores and water molecules. For the hydration water the TIP3P model was used. The program used was CHARMM with force field and version 22 [13]. The simulation was performed in the microcanonical ensemble. The non-bonded cut-off distance was 14 Å. Smoothing was performed with the switch method from 9 to 13 Å. Bonds containing hydrogens were constrained with SHAKE, allowing a 2 fs time-step.

To hydrate the protein, a box of water with the standard liquid water density and 90×60×45 Å dimensions was equilibrated with CHARMM. The water molecules with oxygen atoms within 2.6 Å or further than 4.7 Å from any protein heavy atom were deleted. Five non-structural water molecules, found in interior pockets but not crystallographically, were also eliminated. Using this procedure about one and

a half water layers remain, corresponding to a hydration of 0.6 g/g. The final model contained 1100 water molecules forming a system of 8417 atoms.

The system was subjected to 5000 steps of adopted-basis Newton–Raphson minimisation, then heated in 5 K increments for 10 ps, then equilibrated for 100 ps. The production dynamics was for 1 ns [4,11]. The RMS deviation of the backbone atoms from the X-ray crystal structure was 1.4 Å at the end of the simulation indicating that the protein has preserved its three-dimensional structure.

5. Results and discussion

Powders at different hydration levels, as well as solutions, have been investigated by incoherent quasi-elastic neutron scattering. In particular, the quasi-elastic component of the spectrum reveals dynamical aspects related to diffusive motions that might be functionally important by participating to the general flexibility of the protein. From these components one has inferred that diffusive motions of protein protons occur within a confined volume and that about 25% of the protons in the protein are involved in short-time (10 ps time range) diffusive motions. These protons belong to the surface residues of protein. For a hydrated C-phycocyanin protein ($h = 0.52$ g/g), the volume of diffusion is close to that of a sphere of radius 2 Å [4,11]. The same findings are obtained for hydrated powders of parvalbumin [14]. At higher resolution (100 ps time range), backbone motions are observed [15].

The simulation-derived scattering function $S(q, \omega)$ is in good agreement with experiment (figure 2). In both cases the quasi-elastic intensity, which appears as a broadening of the elastic peak, increases with q.

Intermediate scattering functions $I_{\mathrm{inc}}(q, t)$ have been estimated for both backbone and side-chain residues (figure 3). They have been interpreted with an analytical model derived by extending a simple description of confined diffusion. The determination that the side-chain motion in globular proteins contains a strong liquid-like non-vibrational confined diffusion component at physiological temperatures is consistent with the use of this model for proteins [14,16,17].

The intermediate scattering function $I_{\mathrm{inc}}(q, t)$ has been fitted with the commonly used form [18]:

$$I_{\mathrm{inc}}(q, t) = (1 - A(q)) \cdot \phi(q, t) + A(q). \tag{1}$$

In eq. (1), $A(q)$ and $(1-A(q))\cdot\phi(q, t)$ are the elastic and quasi-elastic components of $I_{\mathrm{inc}}(q, t)$, respectively. $A(q)$ models the elastic incoherent structure factor (EISF). $A(q)$ is determined by the geometry of the space accessed by the atoms of the system [18].

The time dependence of the quasi-elastic component is given by $\phi(q, t)$ (eq. (2)). It was found to be impossible to fit $I_{\mathrm{inc}}(q, t)$ with a single exponential time dependence. Therefore, $\phi(q, t)$ is modelled here with a Kolhrausch–William–Watts stretched exponential:

$$\phi(q, t) = \exp\{-[t/\tau(q)]^{\beta(q)}\}, \tag{2}$$

where τ is the relaxation time and β the stretched exponent giving the spread of the distribution of relaxation times.

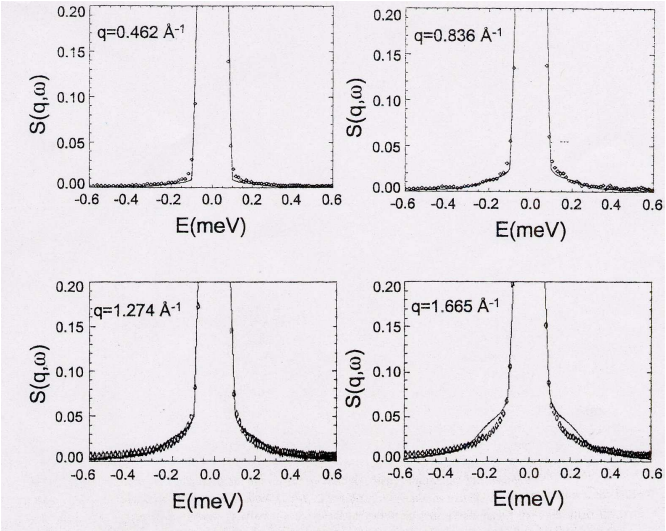

Figure 2. Experimental and simulation-derived $S(q, \omega)$ for C-phycocyanin hydrated with D_2O [4,11].

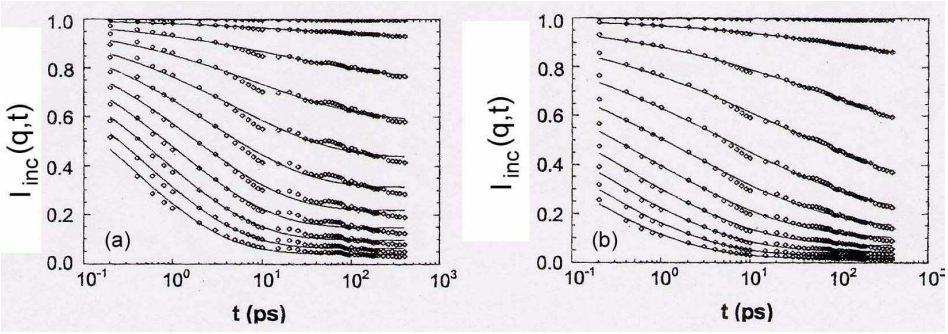

Figure 3. Simulation-derived intermediate scattering function together with the fit using a stretched exponential function (see eq. (4) from ref. [7]). (**a**) Backbone atoms. (**b**) Side-chain atoms [4,11].

The q dependence of $1/\tau(q)$ is shown in figure 4a for the ideal case of free unconfined diffusion and diffusion in a sphere [19]. Comparison of figure 4b with the results of simulation in figure 4a confirms that the hydrogen atoms in the protein undergo confined diffusion. The simulation-derived $\tau(q)$ profile is consistent with a description of diffusion in spheres with a distribution of radii. $A(q)$ can be fitted with a model incorporating a distribution of sphere radii rather than one single radius. The distributions are different for backbone and side chains. For backbone, one gets a single population centred on 1.0 Å while for side chains one gets two populations respectively at 1.0 Å and 1.7 Å.

From a partition of the protein into concentric shells, we have determined the dependence of the three parameters $r_{\rm av}$ (mean sphere size of diffusion), τ, β, on the

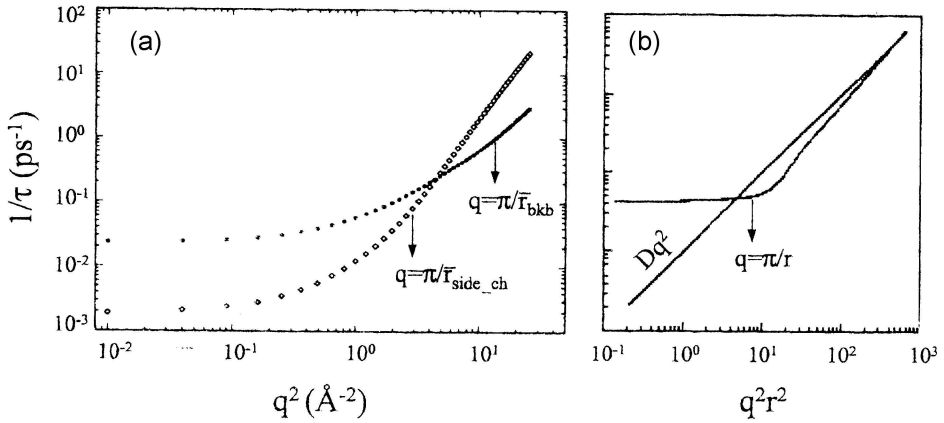

Figure 4. $1/\tau$ vs. q^2 [4,11]. (**a**) Backbone (filled circles) and side chains (open diamonds) of the protein. (**b**) Ideal cases: free-diffusion (Dq^2 law) and diffusion in a sphere [19].

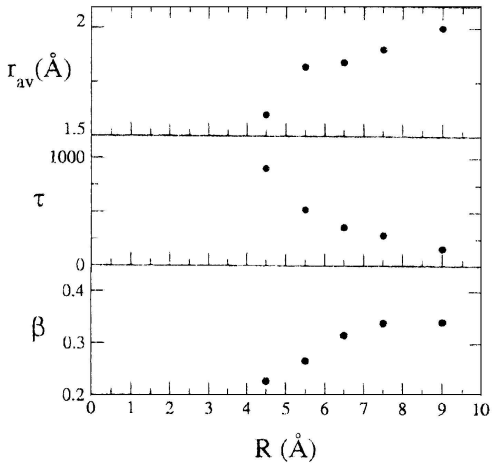

Figure 5. Smooth variation of dynamical parameters, $r_{\rm av}$ (mean sphere size of diffusion), τ, β, with distance, R, from the centre-of-mass of the protein [4,11].

distance from the protein core as presented in figure 5. The parameters have shown to present a smooth variation with distance from the core of the protein. This is reflected in a progressive increase of the mean sphere size of diffusion and in the narrowing and shift to shorter times of the relaxation times distribution. The 40% decrease of the stretch factor β as one goes deeper into the protein is consistent with spreading of relaxation times over larger and larger intervals. This is accompanied by a gradual five-fold increase of τ, from 160 to 800 ps.

6. Conclusion

Some physical picture of the dynamics of a globular protein arises from the analysis combining incoherent quasi-elastic neutron scattering experiments and CMD. First, specific differences are seen between the backbone and side-chain dynamics. The average sphere radius for the backbone atoms is significantly smaller than for the side chains. The time dependence for the backbone is less non-exponential, indicating a narrower spread of relaxation times.

As we have shown previously, the model of dynamics emerging from the present data can readily be represented by a shell model describing the gradual change in dynamical parameters. This smooth, 'depth-dependent' model of dynamics may have important consequences for protein function. It may allow local reorganisation of the structure for efficient ligand binding without affecting the internal stability.

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