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Direct observation of protein residue solvation dynamics

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Abstract

Dynamics of solvation of chromophores attached to a protein by amino acid residues in comparison to that by water molecules has remained a long-standing problem in the field of protein solvation dynamics. An attempt to unravel the existing controversy has been made by studying the solvation dynamics of dansyl labeled proteolytic enzyme α-chymotrypsin in both native and denatured state. In the native state the dansyl probe at the surface of the protein interacts largely with the hydration water while in the denatured state the solvation relaxation of the probe in the randomly oriented polypeptide chain is mainly governed by the polar amino acid residues of the protein. A significant structural perturbation of the protein upon denaturation due to which the probe finds itself in a non-polar environment of the peptide residues is also evident from steady-state fluorescence, circular dichroism (CD) and dynamic light scattering (DLS) experiments. High-resolution streak camera has been employed in order to study dynamic fluorescence stokes shift of the dansyl probe due to hydration water and protein residues. The time scale of solvation by polar peptide residues is found to be an order of magnitude slower than that by bulk type water molecules. In order to show the effect of environmental restriction on the solvation dynamics, the protein in both native and denatured states have been encapsulated inside reverse micelles of varying degree of hydration (w_0). Simple theoretical models have been proposed in order to qualitatively understand the experimental findings. This study might invoke further research in the field of protein solvation.

Keywords: Protein solvation; Native/denatured states; Picosecond dynamics; Streak camera; Dynamic light scattering

1. Introduction

Water plays an important role in biomolecular recognition at specific sites of proteins and nucleic acids [1]. Especially, the specific and nonspecific dynamical interactions of a biological macromolecules with the water molecules (hydration water) in their close vicinity are essential for restoring biomolecular structure and functionality within a narrow range of temperature, pH and ionic strength [1,2]. In the recent past a significant efforts has been made in order to measure and understand the biomolecular surface hydration [3–7]. One of the early indications about the time scale of the dynamics of hydration came from dielectric measurements on protein solution [7]. The measurements show four distinct relaxation time constants (8.3, 40 ps, 10 ns and 80 ns) of myoglobin solution in contrast to that of bulk water (8.2 ps) at 298 K. However, the lack of spatial resolution in the dielectric relaxation studies has brought complications in the interpretation of the results. The exploration of the hydration dynamics using nuclear magnetic resonance (NMR) technique belongs to two classes of experiments [3], those involve the nuclear overhauser effect (NOE) and the nuclear magnetic relaxation dispersion (NMRD). The ability to study the dynamics of hydration at a particular site of a biomolecule is a major strength of the NOE method. Nevertheless, the intrinsic limitation of time resolution of the NOE has made dynamics accessible up to sub-nanosecond scale, reporting 500–300 ps [5,6]. On the other hand, from the frequency dependence of a typical NMRD experiment it has been possible to report the time scale in the range of 10–50 ps [3,4]. In contrast to the NOE techniques the lack of spatial resolution in the NMRD experiments has been considered as [4] one of the major limitations.

Spatial and temporal limitations of the earlier experiments impose constraints on the exploration of complete picture of biomolecular hydration dynamics, and simultane-

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ously stimulated molecular dynamics (MD) simulation [8] and femtosecond-resolved fluorescence studies [2,9,10] to enter into the field. In an MD study on the hydration dynamics of a protein plastocyanin, it was observed that the rotational relaxation of the water molecules on average significantly slows down in the close proximity of the protein surface [8]. Femtosecond-resolved fluorescence studies on an extrinsic dye probe in a protein pocket explored the solvation dynamics of polar amino acid residues and/or rigid water molecules on slower time scales [9,10]. However, from these studies the time scales of surface waters of a biomolecule was not evident. Local structural perturbation of protein molecules due to docking of the extrinsic dye is also another issue for the discussion. In this context the tryptophan residues at the surface of protein molecules were found to be attractive probes for the surface hydration dynamics. The study on single tryptophan containing proteins has ruled out the complications due to spatial heterogeneity in the data analysis. In a hybrid quantum mechanical–classical MD method [11] the physical origin of fluorescence signal of tryptophan residues in various proteins has been clearly described. The potential danger of using arbitrary excitation wavelength of tryptophan residues in order to extract environmental dynamics has also been vividly discussed in a recent work [12].

A series of publications [13–16] followed by a review [2] explored dynamical time scales of surface hydration of various kinds of proteins including a sweet protein Monellin [14] by using tryptophan as fluorescent probe at the surfaces of the protein molecules. The constructed hydration correlation function from dynamical fluorescence stokes shift (FSS) of the tryptophan (Trp3) at the surface of Monellin showed two distinct time constants of 1.3 and 16 ps. In a simple theoretical model the observed hydration times were correlated with residence times of the water molecules at the surface (hydration layer) of the protein [17]. A more rigorous dynamical exchange model considering the diffusion of bulk water to the hydration layer is advanced in the literature [18]. The observed decay in the hydration correlation function ($C(t)$) was concluded to be *mainly* due to water molecules for the following experimental findings: (i) X-ray structure of native [19] and solution structure of single chain Monellin (SCM) [20] show significant surface exposure of the Trp3. From the X-ray structural analysis of (4MON) it is also evident that total number of residues within a radius of 4–6 Å from the Trp3 is 4 (Gly1, Lys44, Met42, Gln10) out of which the number of polar/charged residues is only 2 (Lys44 and Gln10). The NMR structure (1MNL) of the SCM shows existence of two residues (Glu2 and Gly1) within a radius of 4–6 Å from the Trp3 out of which only one residue (Glu2) is polar. In a recent MD simulation study from this group identified 58 unique water molecules interacting directly with Trp3 appeared within 4 Å from the center of the Trp3 indole ring during a simulation period of 140 ps. Thus the solvation relaxation of the Trp3 has an overwhelming influence from the surrounding water molecules compared to that from 2 to 3 polar amino acid side chains of Monellin. (ii) The solvation relaxation of a fluorescent probe inside a protein cavity [9,10], where polar residues of the protein molecule are expected to contribute mainly in the relaxation process shows much longer time constants. (iii) A typical relaxation time of a residue in the protein Monellin is evidenced from solvation of the probe Trp3 in the denatured Monellin [14] and rotational relaxation of the Trp3 in the native protein. The solvation dynamics of the Trp3 in the denatured Monellin shows a longer component of 56 ps in a 100 ps experimental window and assigned to be due to relaxation of random coiled structure of the protein around the tryptophan moiety. The study [14] does not rule out the possibility of inclusion of much longer solvation time constants beyond the experimental time window. The rotational relaxation time of the probe as evidenced from temporal fluorescence anisotropy measurement shows decay with a time constant of 32 ps (37%) and remains constant (63%) thereafter up to 300 ps. In the case of significant contribution of polar side chain motions in the relaxation process, slower time scales are expected in the decay of hydration correlation function.

However, in a recent MD study on the protein Monellin the role of water molecules in the slower (16 ps) hydration relaxation process has been argued. The MD study [21] made a point that the 16 ps component recovered from the femtosecond-resolved FSS experiment [14] was too slow to be from water molecules alone as NMRD [22] and other MD simulation [23,24] studies of aqueous proteins reveal up to seven times slower water dynamics at the surfaces of the proteins. Note that 16 ps component is 12 times slower than that of bulk water and reveals the dynamics of water molecules at a particular site (Trp3) of the protein Monellin. On the other hand the NMRD and MD studies reveal overall picture of water dynamics at the protein surfaces. In this context it should be mentioned that our MD simulation study [25] explored the equilibrium dynamics of water molecules in the close vicinity of the Trp3 of Monellin. The study [25] recovered a time constant of 14.3 ps in the dynamics of water molecules within a radius of 6 Å from the Trp3, which is in close agreement with the femtosecond-resolved FSS study [14]. In another recent work [26] the observed 16 ps component is assigned to be due to *highly quenched conformer*, as the component shows positive (decay) amplitude even at longer wavelengths. The argument is further supported by an experimental observation of similar decay in the fluorescence of tryptophan in a small 22-mer peptide, where the origin of the decay is proposed to be due to a rotamer of the tryptophan [27]. However, single tryptophan residue at the interface of the protein phospholipase A_2 (PLA2) shows [16]: (a) longer rise component (∼10 ps) in the fluorescence decays and hydration relaxation in the similar time scale (∼14 ps); (b) persistence of the temporal fluorescence anisotropy up to 300 ps experimental time window. The observations clearly rule out the involvement of either quenched state and/or rotamer in the observed hydration dynamics at the protein surfaces *in general*. Note that the lack of rise component in the detected fluorescence is not essential to rule out relaxation, as significant overlap of early and later time emission spectra may surpass that signature.

In the context of the above discussion it is extremely important to observe time scales of solvation of a probe by protein residues in contrast to those by mainly water molecules. Here, we have explored the time scales of solvation of a probe by polar protein residues. The fluorescent probe dansyl is covalently attached to a polypeptide chain of a protein α-chymotrypsin and