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Tryptophan–water interaction in Monellin: Hydration patterns from molecular dynamics simulation

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Abstract

Femtosecond spectroscopy carried out earlier on Monellin and some other systems has given insights into the hydration dynamics of the proteins. In the present work, molecular dynamics simulations have been performed on Monellin to study the hydration dynamics. A method has been described to follow up the molecular events of the protein–water interactions in detail. The time constants of the survival correlation function match well with the reported experimental values. This validates the procedure, adapted here for Monellin, to investigate the hydration dynamics in general.
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1. Introduction

Protein–water interaction is crucial for accomplishing many biological functions of proteins. Probing such interactions has become amenable to many experimental and theoretical computations [1–15]. Earlier, dielectric measurements [13], nuclear overhauser effect (NOE) [14] and nuclear magnetic relaxation dispersion (NMRD) [15] studies, have shown that the relaxation time of water molecules in the close vicinity of a biomolecule ranges from picoseconds to nanoseconds. Recently, natural probe tryptophan, located at the protein surfaces, has been extensively investigated using femtosecond spectroscopy experiments to understand solvation dynamics [16–19]. From such experiments the solvation times have been categorized into bulk-type and protein-layer type, both of which are dynamically involved and reflect the distribution of residence times of water molecules around proteins. Particularly, this bimodality (1.3 and 16 ps) was recovered from femtosecond resolved fluorescence studies on sweet protein Monellin [18], originally isolated from berries of the plant *Dioscoreophyllum cumminsii* [20,21]. From theoretical side, the solvent effect on tryptophan fluorescence wavelength shift in proteins has been rigorously investigated by hybrid quantum mechanical/molecular dynamics (QM/MD) techniques [22]. Bizzaari et al. have explored the protein–solvent interfaces [23] from molecular dynamics (MD) simulations. The anisotropic environment of proteins has been probed explicitly by tracking protein water interactions [24]. In this study we have carried out hydration analysis by MD simulation on Monellin, in order to get the atomic level insights into the hydration dynamics. The results are compared with those obtained from femtosecond resolved fluorescence spectroscopy [18].

The use of MD simulation to re-investigate hydration dynamics probed with excited state of the single tryptophan residue (Trp3) of Monellin relies on the linear response approximation (LRA). The LRA connects the non-equilibrium response function, measured with excited state dynamic stokes shift of Trp3, to equilibrium time correlation function (TCF) (from MD simulation) of the fluctuation $\Delta E(t)-\langle\Delta E\rangle$ through fluctuation dissipation theorem [25–27]. Thus, the trajectories of the water molecules due to spontaneous fluctuations in the environment of Trp3 in the ground and excited states remain similar.

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In a simple theoretical model, based on the assumption of dynamic equilibrium [4], a relationship between the solvation time and the residence time of water molecules around proteins has been discussed [28]. The present MD study focuses on the hydration around Trp3 of Monellin by evaluating TCF and explicitly tracking the interacting water molecules. This procedure can offer insights into the hydration dynamics of any protein with known structure.

2. Methods

2.1. Simulation protocol

The MD simulation was performed for 200 ps on the crystal structure of Monellin (4MON [29]), surrounded by water molecules (TIP3P [30]) using AMBER7 [31] with parm98 parameters [32]. The solvation box was 8 Å from the farthest atom along any axis. Particle Mesh Ewald summation [33] was used for the long-range electrostatics and Van der Waals cut-off was 10 Å. Pressure and temperature relaxation was 0.5 ps^{-1}. In this NTP simulation, the temperature of the system was raised from 0 to 300 K during the first 1 ps and the coordinates were stored after every 0.01 ps.

2.2. Hydration analysis

The residence times were evaluated from the coordinates of 20 000 snapshots obtained from the simulation by the method followed by Sanjeev et al. [24]. All the water molecules were labeled to extract the information on the number of times a particular water molecule interacts with a selected protein atom. The time that a given water molecule is within a predefined distance from a polar atom is considered as the residence time of that water molecule on that polar atom within that distance. The trajectories of water molecules within radii of 4 and 6 Å from the center of the indole ring of Trp3, as suggested by the radial distribution function and the survival time correlation function respectively, were analyzed.

2.3. Survival time correlation function

The dynamical behavior of interfacial water can be described by evaluating the residence time of the water molecules in the first, or successive, hydration shells of protein atoms exposed to the solvent. This is achieved by defining a 'survival time correlation function', $C_R(t)$, as

$$C_R(t) = \frac{\langle N(\tau)N(t+\tau)\rangle - \langle N\rangle^2}{\langle N^2\rangle - \langle N\rangle^2} \quad (1)$$

where R is the distance of water oxygen atoms from a selected protein atom, $N(\tau)$ is the number of water molecules coming within the radius R at a given snapshot τ (which varies from zero to simulation length) and $\langle N\rangle$ is the average number of water molecules within the radius R for all the snapshots. $C_R(t)$ measures the probability that a water molecule remains in a given radius at a time t, without being exchanged with the bulk water. The relaxation trend of $C_R(t)$ provides information about the local dynamics of the water molecules in the hydration shells. A fit of the survival time correlation function is obtained by using a double exponential,

$$C_R(t) = A_1 e^{-(t/\tau_s)} + A_2 e^{-(t/\tau_l)} + y_0 \quad (2)$$

where τ_s and τ_l are the short and the long time decay constants, respectively. The persistent component (y_0) in the time window is defined as offset in Table 1. The decays correspond to the dynamics of the exchange of the solvent molecules, that stay in the hydration shell, with the bulk water.

Table 1
Short and long time constants extracted by a best fit according to Eq. (2), of the survival time correlation function of water hydration shell at various radial distances from Trp3

Radial distance from Trp3 (Å)	A_1 (%)	τ_s (ps)	A_2 (%)	τ_l (ps)	Offset (%)
4	27	0.23	69	5.2	4
5	9	0.14	77	9.0	14
6	9	1.3	63	14.3	28
10	3	4.5	30	36.2	67

3. Results and discussion

Fig. 1 shows the protein in the box of water molecules (1a) and the Trp3 residue surrounded by the water molecules with short residence times (<30 ps; 1b) and by the water molecules with long residence times (>30 ps; 1c). This figure depicts that the number of water molecules around Trp3 with longer residence times, which contribute to the slower hydration relaxation process [16,28], is small. The radial distribution function (RDF) of the water molecules around the protein Monellin (Fig. 2) has been calculated for three different cases: (1) all residues (all atoms), (2) the Trp3 residue (all atoms) and (3) NE1 atom of Trp3 indole ring of Monellin. In the first two cases, the RDFs reveal two peaks. The first peak (1.8–1.9 Å) is due to hydrogen bonds between the oxygen atoms of water molecules and hydrogen atoms of Monellin. The second peak (2.8–3.5 Å) is due to the interaction of water molecules and the polar atoms of Monellin. The third case, with only one large peak, is mainly due to the favorable interaction of water molecules with the single nitrogen atom of the Trp3 indole ring. This RDF pattern is in agreement with earlier studies [10] and hence 4 Å cutoff was used as the first hydration layer.

In Fig. 3, $C_R(t)$ (Eq. (1)) is plotted as a function of time for four water shells (radius (R) ranging from 4 to 10 Å from the center of the Trp3 indole ring). All the curves show the same trend for the survival time correlation function which consists of a fast initial decay, on the time scale of <5 ps, followed by a slower decay. A fitting procedure

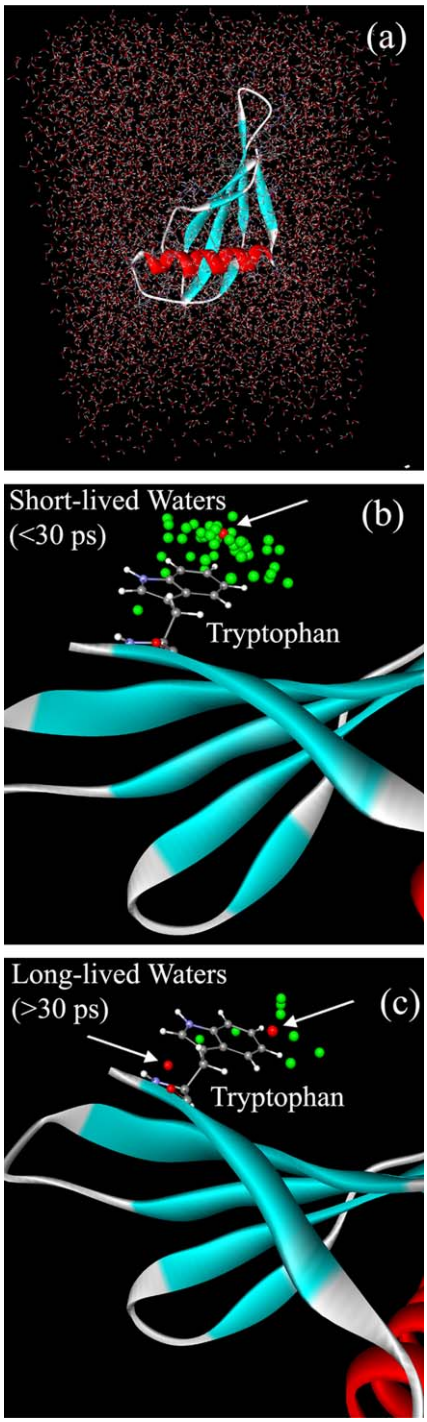

Fig. 1. (a) Monellin in the solvation box. The initial coordinates have been obtained by X-ray-structure (Protein Data Bank entry 4MON). Monellin is centered in a box of 4495 water molecules. Single tryptophan (Trp3) of Monellin (b) with short-lived and (c) long-lived water molecules. The temporal trajectories of water molecules indicated by arrows are discussed in the text.

for the data shown in Fig. 3 (solid lines), by Eq. (2) describes the biexponential trend of $C_R(t)$ for all the four water shells. The fitting parameters (Table 1) show that the values of τ_s, describing the short-time decay of $C_R(t)$, are of the order of 1 ps or less for all the water shells (except for 10 Å) considered. Values of τ_l, describing the

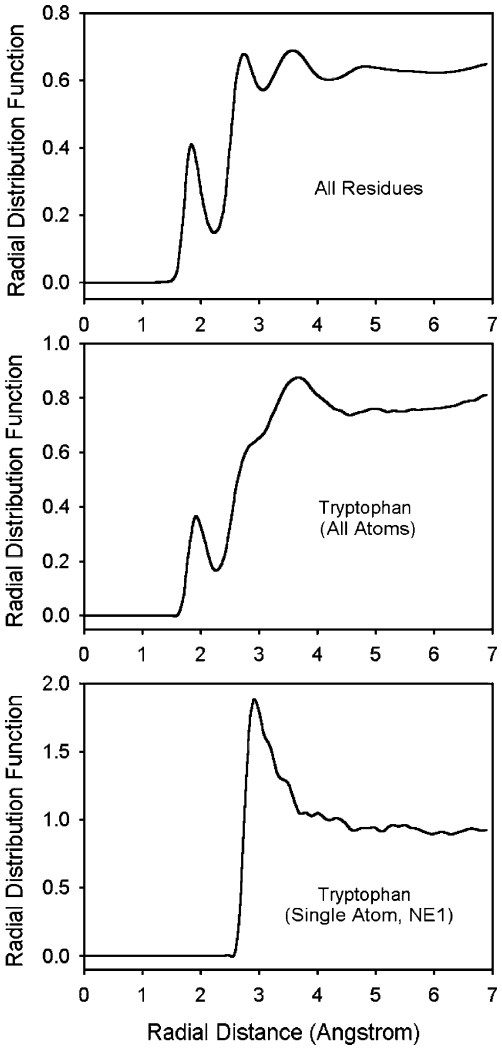

Fig. 2. Radial distribution of water molecules around Monellin as a function of the distance between water oxygen atoms and the protein atoms, including hydrogen.

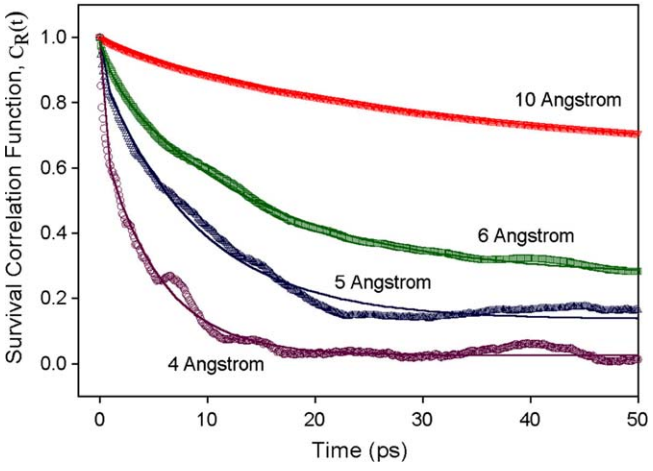

Fig. 3. Survival time correlation function, $C_R(t)$, of water molecules in various hydration shells with different radii from the single tryptophan (Trp3) of Monellin.

long-time decay of $C_R(t)$, are very different depending on the water shell. The time constants $\tau_s = 1.3$ ps and $\tau_l = 14.3$ ps for $R = 6$Å are found to be in good agreement with those determined by Peon et al. [18] ($\tau_s = 1.3$ ps and $\tau_l = 16$ ps) by femtosecond spectroscopy on Monellin, using Trp3 as fluorophore. As evidenced from the offset (y_0), residence time of water molecules in the largest shell, instead, appear to be high. For example, 67% offset value in the decay of $C_R(t)$ of a hydration shell with 10 Å radius indicates that a significant number of total water molecules (67%) at time $t = 0$ are not exchanging with water molecules outside the shell in the time window up to 50 ps. These results also reveal the fact that the larger the shell, the slower the exchange with bulk water.

For the largest water shell ($R = 10$ Å), involving a large number of water molecules moving far from the protein surface, the long-time relaxation is almost linear in the semilog plot (data not shown). The longer values of τ_l and the larger offset in the decay of $C_R(t)$ of the larger hydration shells indicate that the water molecules keep exchanging with themselves within the water shell (here 10 Å) without being exchanged with the water molecules outside the shell [23]. As the water shells progressively come nearer the protein surface, a single exponential decay is no longer followed. Instead, a bimodal solvation of Monellin is observed from our analysis. Thus, the relaxation in the hydration shell near the Trp3 residue occurs through two different types of solvent dynamics. It should be mentioned that in a previous work, the decay of $C_R(t)$ is described in a stretched exponential function [23]. However, the deviation from the exponential approximation in the decays of $C_R(t)$ was found to be negligibly small for the shells with larger radii. In the present work the dynamics of the survival correlation function is well described by biexponential function, even for the hydration shell of 4 Å radius.

Water residence times provide useful insights into the structural and dynamical behavior of interfacial water molecules in the hydration shell of protein atoms exposed to solvent. An explicit analysis of protein–water interaction in terms of the residence times can give insights into the solvation dynamics [16]. Here, we have followed the trajectories of water molecules which come in contact with Trp3 during the simulation. Dynamical events of these water molecules at the atomic level were captured by analyzing the residence times as outlined in Section 2. 58 unique water molecules interacting directly with Trp3 appeared within 4 Å from the center of the Trp3 indole ring (first hydration layer) during the simulation and the trajectories of all these water molecules were analyzed over the entire simulation length for two different hydration shells (4 and 6 Å). Fig. 4 shows the trajectories of three water molecules having different characteristics. If a shell of radius 10 Å is considered around Trp3, it is seen that water number 2330 visits this shell several times within 140 ps of simulation length, whereas water number 2712 stays in the shell for a long time and water number 2781 never goes out of the shell in the same time length. This observation can be directly related to the residence times of the water molecules: in a 10 Å shell, water number 2330 has a short residence time, whereas water number 2712 and water number 2781 have long residence times. As the time constants τ_s and τ_l for 6 Å shell were in agreement with the experimental time constants obtained from femtosecond spectroscopy [18], a radius of 6 Å from Trp3 was chosen to get the residence times of all the 58 water molecules.

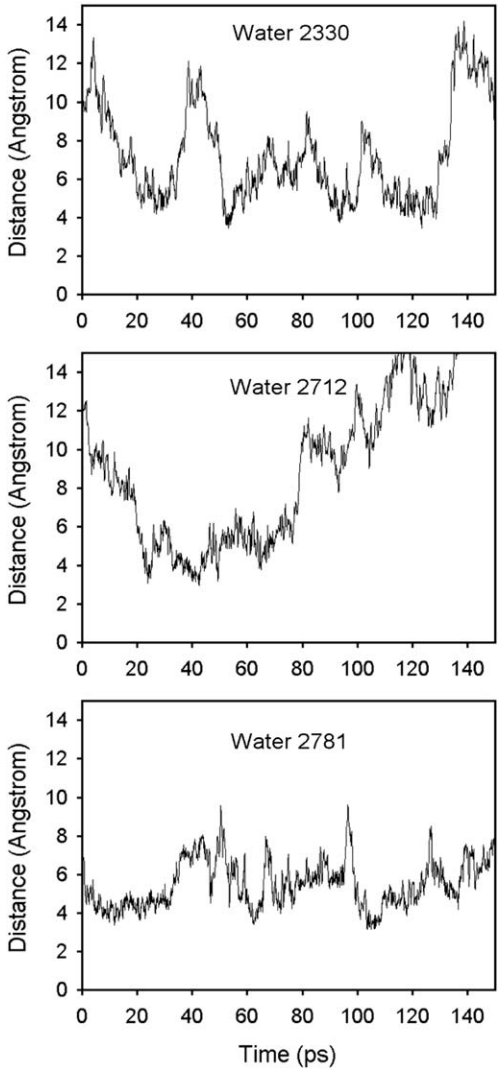

Fig. 4. The temporal trajectories of water molecules in the vicinity of the single tryptophan (Trp3) of Monellin.

Fig. 5a shows the distribution of the residence times within 6 Å of all the 58 water molecules. As can be seen from this figure, a few water molecules show long residence times (>30 ps). These water molecules are more tightly bound to the Trp3 residue. Water molecules with short residence times (<30 ps) continuously exchange with the bulk water around the Trp3 residue. In order to look at the dynamics of such water molecules we have followed their interaction with Trp3 over the entire simulation length (Fig. 5b). The trajectories of these water molecules within 4 Å from Trp3 are also shown in this plot. On an average,

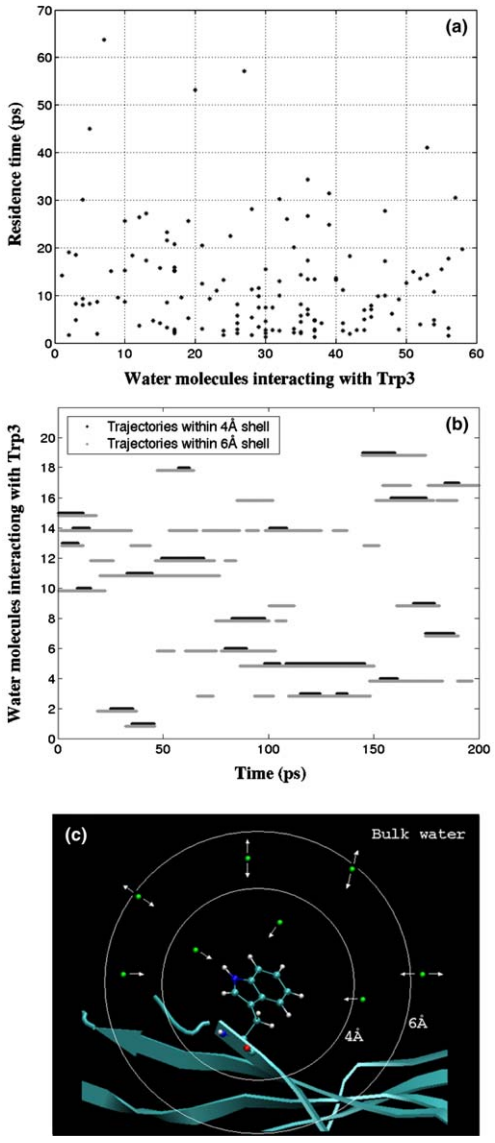

Fig. 5. (a) Distribution of residence time of water molecules within a shell of 6 Å from the single tryptophan (Trp3) of Monellin. (b) Trajectories of some of these water molecules with residence time >5 ps black and gray lines represent the trajectories within a shell of 4 and 6 Å, respectively. (c) Schematic representation derived from (b); water molecules interacting with Trp3 with a significant residence time are represented by single arrow; water molecules exchanging with the bulk water and also visiting the 4 Å shell are represented by double arrow.

at a given time, there are about 2 and 4–5 water molecules with residence times >5 ps within 4 and 6 Å, respectively. These dynamical events of solvation have been schematically represented in Fig. 5c. From these detailed analysis we are able to show the connection between the solvation time and the dynamics of water molecules with significant residence times which exchange within the shells of 4, 6 Å and the bulk water.

4. Conclusions

The present study on the dynamical behavior of the aqueous solvent around Monellin followed by molecular dynamics simulation reveals relaxation properties of hydration water molecules. Two types of solvation trajectories have been observed in the protein, the bulk type and the protein layer type, which is consistent with the bimodality as observed from the femtosecond spectroscopic experiments. The timescales of the solvation reported in our study reveal the dynamical picture of the hydration of the protein. These timescales evaluated around the fluorophore Trp3 residue in the protein correlate well with the experimentally determined values. Further, the molecular events contributing to the solvation dynamics have been interpreted in terms of the distribution of residence times and the exchange of interacting solvent molecules within and outside the hydration shells. Thus, the method proposed in this letter can be effectively used in characterizing the hydration patterns and dynamics of other proteins.

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