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Femtosecond Conical Intersection Dynamics of Tryptophan in Proteins and Validation of Slowdown of Hydration Layer Dynamics

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Supporting Information

ABSTRACT: Water motion probed by intrinsic tryptophan shows the significant slowdown around protein surfaces, but it is unknown how the ultrafast internal conversion of two nearly degenerate states of Trp (^1L_a and ^1L_b) affects the initial hydration in proteins. Here, we used a mini-protein with 10 different tryptophan locations one at a time through site-directed mutagenesis and extensively characterized the conversion dynamics of the two states. We observed all the conversion time scales in 40−80 fs by measurement of their anisotropy dynamics. This result is significant and shows no noticeable effect on the initial observed hydration dynamics and unambiguously validates the slowdown of hydration layer dynamics as shown here again in two mutant proteins.

Tryptophan (Trp or W) has been developed as a powerful optical probe to study protein hydration dynamics[1−5] with site-directed mutagenesis.[3−5] The recent series of characterizations of hydration dynamics on various proteins showed the slowdown of the hydrating water motions near protein surfaces.[6−11] The obvious evidence is that, at the blue side of the emission such as at 305 or 310 nm, the femtosecond-resolved fluorescence transients significantly slow down compared with those at the same wavelength in bulk water. It has been suspected that the complexity of excited states (^1L_a and ^1L_b) may smear the initial ultrafast decay dynamics at the blue-side emission in proteins. The ^1L_a (S_2) state in polar environments lies below the ^1L_b (S_1) state due to its larger static dipole moment.[12] Ultrafast internal conversion through conical intersection (CI) was proposed from the higher ^1L_b to lower ^1L_a state[13−15] and observed to occur in ∼40 fs in bulk water.[16,17] The internal conversion from ^1L_a to ^1L_b in gas phase (or in vacuum) has also been observed in 20−100 fs.[18] Typically, when we excite tryptophan in proteins at 290 nm, both states are excited simultaneously.[19] Thus, one critical question is what are the time scales of the CI dynamics of ^1L_b to ^1L_a in proteins and, related to this, how this dynamics affects the initial protein hydration.

To resolve this critical issue, we scanned the GB1 protein (the B1 immunoglobin-binding domain of protein G) by placing Trp at different positions one at a time with site-directed mutagenesis (Figure 1A). GB1 is a small domain protein with 56 amino-acid residues containing only one single tryptophan residue (W43).[20] Nine mutant proteins were made from double mutation by first replacing W43 with F43. Since

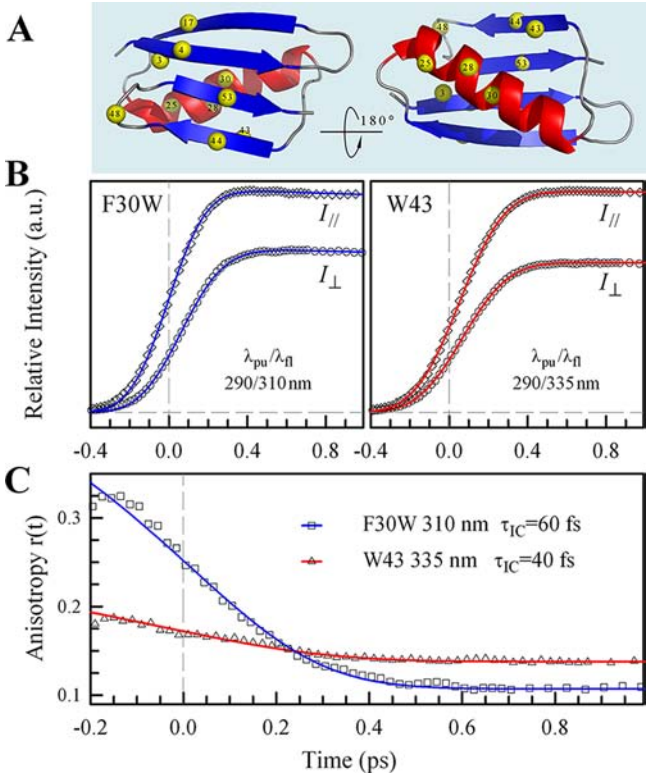

Figure 1. (A) Structure of the native state of GB1 with 9 designed Trp mutants and the wild-type (W43) labeled by yellow balls. (B) Femtosecond-resolved parallel ($I_{//}$) and perpendicular ($I_\perp$) fluorescence transients of mutant F30W gated at 310 nm (left, blue) and wild-type W43 gated at 335 nm (right, red). (C) Corresponding anisotropies of the two mutants in (B). The symbols in (B) and (C) are the experimental data and the solid lines are the best simulations from our model.

the CI dynamics is ultrafast and the absorption of ^1L_b and ^1L_a is overlapped, we examined the fluorescence anisotropy dynamics after initial excitation to understand the CI dynamics and extract their time scales. Because of the nearly perpendicular transition dipoles of the two states,[12a] we should observe the ultrafast change of anisotropy. Upon 290-nm fs-pulse excitation, we actually prepared a coherent superposition of nearly degenerate ^1L_a and ^1L_b states. The evolution of anisotropy

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with time for such coherent states has been well studied by Wynne and Hochstrasser, especially for symmetric molecules.[21] With the ultrafast conversion by CI for a coherent superposition state, the Jonas group has recently carried out a series of theoretical and experimental studies to understand the molecular mechanism.[22] However, since the actual coupling between the dephasing and CI processes for tryptophan is unknown, we proposed two possible models to simulate our experimental results: One is called the sequential model, i.e., the coherent state decays to ^1L_a and ^1L_b states with the dephasing time T_2 and then molecules in ^1L_b are converted into ^1L_a state by CI with the time τ_{IC}. The other model is a parallel one and both the dephasing and CI processes occur at the same time. The detailed kinetics for the two models are given in the Supporting Information. Surprisingly, both models give the similar CI times (τ_{IC}) for each mutant while the sequential model shows the dephasing times (T_2) in 30−70 fs and the parallel model around 300 fs. However, the sequential model gives a better fit (Figure S1). Figure 1B shows the typical fluorescence transients either at 310 or 335 nm with the two different polarization detections (parallel and perpendicular) for the mutant of F30W and wide-type W43. The solid lines are the fitting results using the sequential model (eqs S9 and S10) with the two state dipole ratio (μ_b/μ_a) of 0.455.[23] Figure 1C is the resulting anisotropy dynamics with the solid fitting lines (eq S11). We obtained the internal conversion time (by CI) of 60 fs for F30W and 40 fs for W43 and a similar dephasing time of 50 fs for both proteins. Because of the limited temporal resolution of 360−400 fs determined by the water Raman signal at 320 nm, the initial anisotropy value is not very high (not 0.6−0.7 as expected for a coherent superposition of two nearly degenerate states) and drops to 0.2−0.35. In Figure 1C, the anisotropy promptly decays to a constant value on such ultrafast time window and this value is directly related to $n_a^* f_a / n_b^* f_{ba}$ ($=N_a^0\beta_1$), i.e., proportional to $(2N_a^0\beta_1 - 1)/5(N_a^0\beta_1 + 1)$ (eq S13). n_a^* and n_b^* ($n_a^*/n_b^* = N_a^0$) are the initially excited populations in ^1L_a and ^1L_b states, respectively, which are directly proportional to their extinction coefficients. The constants f_a and f_{ba} ($f_a/f_{ba} = \beta_1$) are relative emission coefficients, at a given wavelength, of the initially excited ^1L_a state and the ^1L_a state that is transferred from the ^1L_b state through CI, respectively. By fitting both the transients and anisotropy dynamics, we obtained the CI dynamics of tryptophan in the 10 proteins and the related initial absorption coefficients of ^1L_a and ^1L_b states at 290 nm. Figure 2A shows the obtained CI time scales of the wild-type and 9 mutants and Figure 2B basically gives the ratio of initial excited ^1L_a and ^1L_b populations (if $f_a = f_{ba}$), i.e., the relative absorption coefficients of the two states at 290 nm. Significantly, all CI dynamics (Figure 2A, inset) occur in 40−80 fs in all the mutant proteins and are independent of the emission maximum, i.e., local environment total polarity. The CI dynamics can vary by a factor of 2 but are all less than 100 fs, within several vibrational periods, similar to the values observed in gas phase[18] and bulk water.[16] The theoretical calculations showed single/double-bond rearrangements and out-of-plane molecular distortions responsible for the CI process,[14] and thus, these structural changes seem not to be affected by the local physical constraints due to the small amplitude motions during CI. The CI dynamics could be mainly affected by the relative energy levels of ^1L_a and ^1L_b states at $t = 0$, which are determined by the local electrostatics of initial configurations upon excitation. Thus, due to no obvious trend of τ_{IC} with

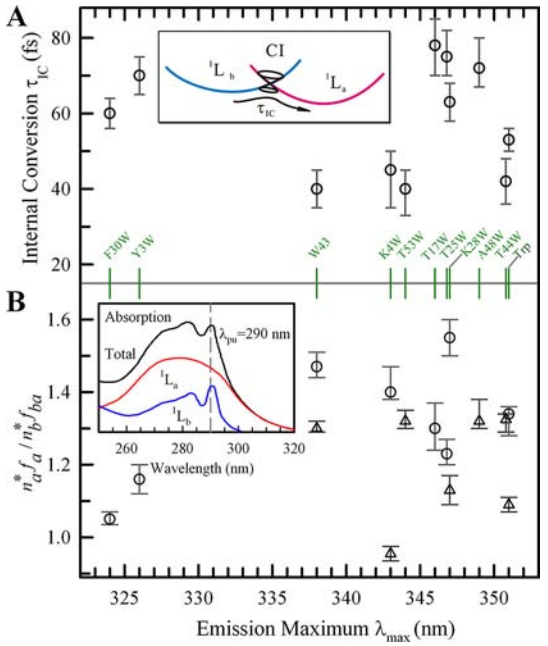

Figure 2. (A) Derived internal conversion time scales (τ_{IC}) of all the 9 mutants and the wild-type of GB1 as well as Trp in bulk water with respect to the emission maxima. The inset shows a sketch of conical intersection (CI) between ^1L_a and ^1L_b states of Trp. (B) Distributions of the fitting parameter $n_a^* f_a / n_b^* f_{ba}$ for 10 GB1 proteins and free Trp in water. Circles and triangles represent the results from 310 and 335-nm measurements, respectively. The inset shows the total absorption spectrum of Trp with deconvolution of relative contributions of ^1L_a and ^1L_b states from ref 19. All mutants are shown in the middle with green ticks corresponding to the data points.

emission maxima (Figure 2A), the initial energy-level ordering of ^1L_a states determined by the ground-state equilibrium configurations in the 10 proteins at $t = 0$ can be different from the final energy-level ordering after environment relaxation (solvation) which determines the emission maxima of the proteins, as shown in the inset of Figure 3A, reflecting the different stabilizations of the excited state by the equilibrated ground-state ($t = 0$) and excited-state ($t = \infty$) configurations. The dephasing times (T_2) of all 10 proteins are also similar in 30−70 fs.

Figure 2B shows all initial ratios of the excited ^1L_a and ^1L_b populations larger than 1.0, close to the reported value of 1.2 in ref 19 at 290-nm excitation (Figure 2B, inset), and the clear difference of the anisotropy plateaus at 310 and 335 nm. For each protein, the plateau constant at 310 nm is always larger than that at 335 nm, indicating that the ratio of f_a/f_{ba} at the shorter wavelength is always larger than that at the longer wavelength. Note that f_a/f_{ba} indicates the difference of emission coefficients of the initial excited ^1L_a state and the transferred ^1L_a state at the same emission wavelength (310 or 335 nm), reflecting that the emission at the same wavelength could be from the different vibronic ^1L_a states and that the transferred ^1L_a is not at the same energy level of the initial excited ^1L_a state, consistent with the CI mechanism.[13−15]

Knowing the CI dynamics of tryptophan in the proteins, we simulated two solvation dynamics, ultrafast and fast, to mimic the fluorescence transients at 305 nm with two different solvation time scales in Figure 3A and to examine how the CI dynamics affects the solvation dynamics. One assumes the solvation dynamics is 120 fs (70%) and 3 ps (20%) and the

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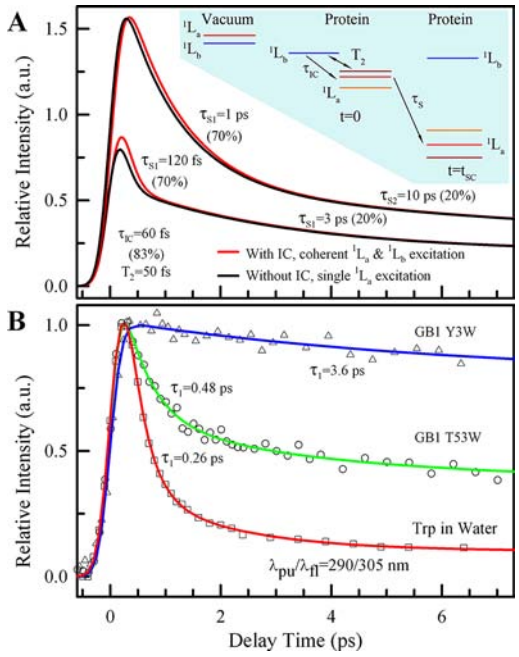

Figure 3. (A) Comparison of simulated transients with and without IC in short time range. With IC, the dephasing dynamics is also included. The solvation parameters used in the simulation are shown beside the transients. The inset shows the schematic, relative energy levels of ^1L_a and ^1L_b states in proteins before ($t = 0$) and after ($t = t_{sc}$) solvation, as well as in vacuum. At $t = 0$, three dynamics, dephasing (T_2), CI (τ_{IC}) and solvation (τ_S) start to occur. (B) Normalized femtosecond-resolved fluorescence transients of two GB1 mutants Y3W and T53W as well as Trp in bulk water gated at the blue-side emission (305 nm), showing drastically different initial decay behaviors and reflecting the slowdown of hydration layer dynamics.

other one is 1 ps (70%) and 10 ps (20%). Both simulated transients have the same lifetime components, 500 ps (5%) and 3 ns (5%). Clearly, with the CI and dephasing dynamics, the overall solvation dynamics with the ultrafast solvation component (120 fs) shows a minor change with a slightly increase in amplitude. For the fast solvation (1 ps), the simulations show nearly the similar transients with and without the CI dynamics. Thus, the CI dynamics will not smear the ultrafast solvation behavior and could apparently "enhance" such ultrafast relaxation process at least in amplitude. Hence, in studies of any protein hydration/solvation probed by Trp, if we observe the slow fluorescence decay transients at 305 nm, the observed slow dynamics should truly reflect the slowdown of hydrating water motions around the protein. In Figure 3B, we show the fluorescence transients at 305 nm for two mutant proteins of GB1 (Y3W and T53W) in comparison with the transient in bulk water at the same experimental conditions. For T53W, the fluorescence emission maximum is at 344 nm. The probe is exposed to hydration water at the protein surface and can detect several layers of hydration water.[2−4] For Y3W, the emission peak is at 325.8 nm. The probe is nearly buried in the protein and can only measure inner water layer(s) at the water−protein interface.[2−4] Clearly, the initial fluorescence decay dynamics at 305 nm slows down to 0.48 and 3.6 ps, and thus, at the protein surface, the protein hydration dynamics, compared with the free-water dynamics, unambiguously slows down and is not affected by the CI dynamics. Thus, the extensive characterization of the CI dynamics of Trp (^1L_a and ^1L_b states) in the proteins here validates the slowdown of hydration layer dynamics[24] and reflects the nature of water−protein interfacial interactions confined around nanoscale protein surfaces.

■ ASSOCIATED CONTENT

Supporting Information

Detailed descriptions of the two models used in data analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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