Cooperative Protein Structural Dynamics of Homodimeric Hemoglobin

Proteins serve as molecular machines in performing their functions, but the detailed structural propagations are difficult to observe in their native aqueous environments in real time. For example, despite extensive studies, the solution-phase structures of the intermediates along the allosteric pathways for the propagations between the relaxed (R) and tense (T) forms have remained elusive. In this work, we employed picosecond X-ray solution scattering [1] and novel structural analysis [2] to track the detailed structural dynamics of wild-type homodimeric hemoglobin (HbI) from the clam *Scapharca inaequivalvis* and its F97Y mutant over a wide time range.

The allosteric structural transition of hemoglobin induced by ligand binding is an important process that is directly related to the biological function and reactivity of the protein. Because of the heteromeric nature of human tetrameric hemoglobin (HbA), the structural transition between allosteric sites involving cooperative ligand binding and subsequent tertiary and quaternary structural changes is complex. As a result, it has been difficult to characterize the structure and kinetics of singly, doubly, or multiply liganded species that are transiently formed along the allosteric pathways. In this regard, HbI has a simpler homodimeric structure and thus is a convenient model system for studying allosteric structural changes. However, even for this simpler system, the allosteric process involving cooperative ligand binding and subsequent tertiary and quaternary structural change is complex, and its detailed structural dynamics have yet to be understood completely.

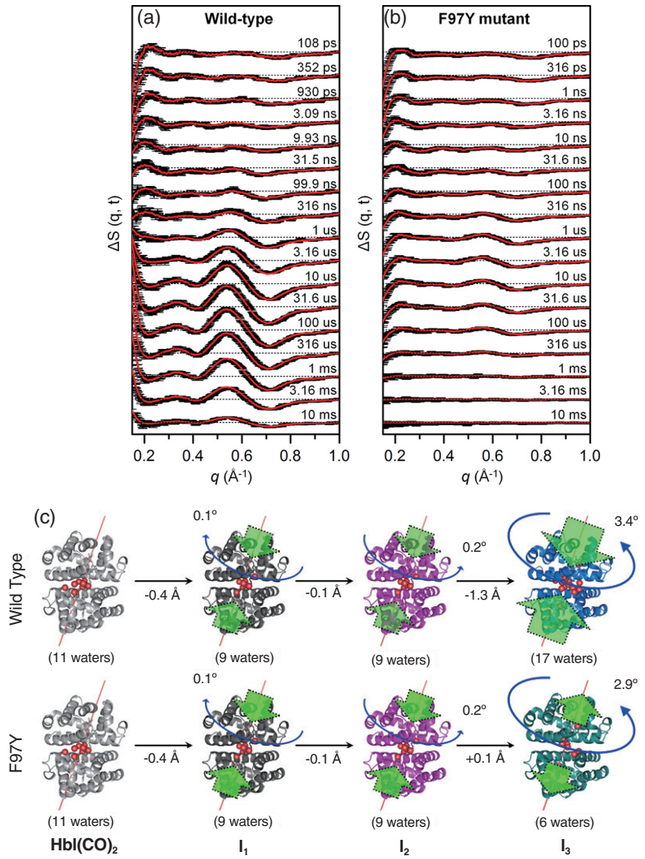

Figure 1: The picosecond pump-probe X-ray solution scattering data for (a) wild-type $HbI(CO)_2$ and (b) its F97Y mutant are shown. The time delay after photoexcitation is indicated above each curve. Experimental curves (black) are compared with theoretical curves (red) that were generated from data analysis. The extracted structural dynamics are summarized in (c). The green and blue arrows in (c) are used to indicate the relative magnitudes and directions of the changes in the heme-heme distance and subunit rotation angle relative to $HbI(CO)_2$.

In this work, to investigate directly the structural dynamics of HbI in the solution phase, we applied pump-probe X-ray solution scattering (which is globally sensitive to secondary, tertiary, and quaternary structural changes of proteins in solution) to visualize the detailed allosteric structural transition of HbI in solution in real time. By applying a novel structural analysis using Monte Carlo simulations to the measured X-ray solution scattering data, we describe in detail the structural dynamics involved in the allosteric transitions of wild-type HbI and its F97Y mutant.

Time-resolved X-ray solution scattering data were acquired using the pump-probe method at the NW14A beamline at PF-AR. Aqueous solution samples of HbI ligated with CO ligands [$HbI(CO)_2$] and its F97Y mutant were prepared using a previously established protocol [3]. The samples contained in a capillary of 1 mm thickness were excited with ~35 ps laser pulses at 532 nm. Time-resolved scattering curves were collected at 40–70 pump-probe time delays between the laser pump pulse and the X-ray probe pulse in the range from 100 ps to 56.2 ms as well as at a reference time delay of -5 μs. To attain a signal-to-noise ratio good enough for data analysis, about 20 images were acquired and averaged at each time delay. The measured time delays were spread evenly on a logarithmic time scale. Taking the difference between the scattering curve measured at each time delay point and the reference scattering curve measured at -5 μs yielded the difference scattering curve $\Delta S(q, t)$ as shown in Fig. 1(a) and 1(b).

From kinetic analysis, we identified three structurally distinct intermediates and their kinetic pathways common for both the wild type and the mutant. The data revealed that the singly ligated and unligated forms of each intermediate share the same structure, providing direct evidence that the ligand photolysis of only a single subunit induces the same structural change as that induced by the complete photolysis of both subunits. In addition, by applying novel structural analysis to the scattering data, we elucidated the detailed structural changes in the protein, including changes in the heme-heme distance, the quaternary rotation angle of subunits, and interfacial water gain/loss. The structural transitions from $HbI(CO)_2$ to I_2 via I_1 are identical for the wild type and the mutant. In contrast, for the transition from I_2 to I_3, interfacial water molecules enter in the wild type and exit in the mutant, and the extent of the structural change is smaller for the mutant. Especially, the heme-heme distance of I_3 (F97Y) is not reduced relative to that of I_2, whereas I_3 (wild type) exhibits a smaller heme-heme distance than that of I_2. A summary of the extracted structural dynamics is provided in Fig. 1(c).

The ability to keep track of the detailed movements of the protein in aqueous solution in real time will provide new insights into the structural dynamics of proteins.

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