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Cooperative protein structural dynamics of Homodimeric Hemoglobin

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1 Introduction

Proteins serve as molecular machines in performing their biological functions, but the detailed structural transitions 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 transitions between the relaxed (R) and tense (T) forms have been elusive. In this work, we employed picosecond X-ray solution scattering [1] and novel structural analysis [2] to track the details of the structural dynamics of wild-type homodimeric hemoglobin (HbI) from the clam Scapharca inaequivalvis and its F97Y mutant over a wide time range from 100 ps to 56.2 ms.

2 Experiment

Time-resolved X-ray solution scattering data were acquired using the pump−probe method at the NW14A beamline at KEK. Aqueous solution samples of HbI ligated with CO ligands [HbI(CO)2] and its F97Y mutant were prepared. The samples contained in a capillary of 1 mm thickness were excited with 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.

3 Results and Discussion

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 liganded and unliganded 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 the complete photolysis of both subunits does. 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 ability to keep track of the detailed movements of the protein in aqueous solution in real time provides new insights into the protein structural dynamics.

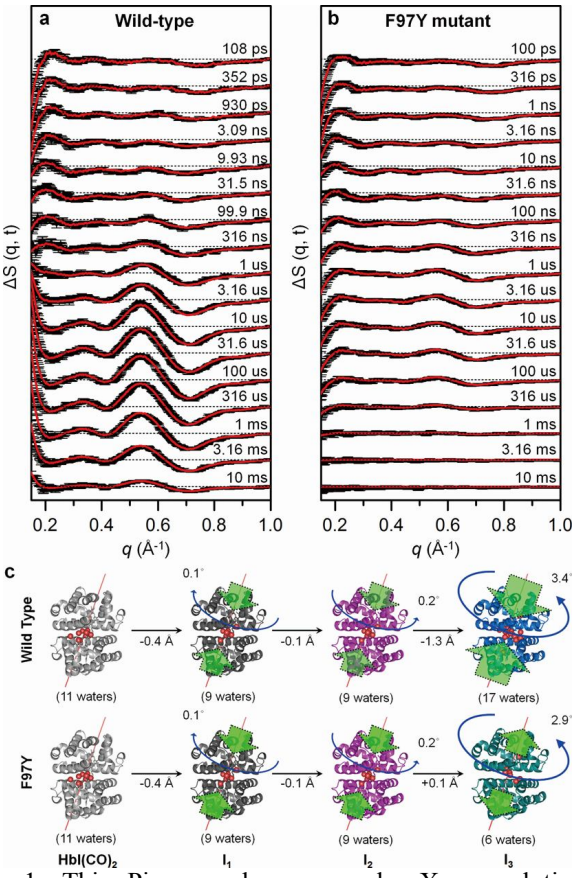

Fig. 1: This Picosecond pump−probe X-ray solution scattering for (a) wildtype $HbI(CO)_2$ and (b) its F97Y mutant. The time delay after photoexcitation is indicated above each curve. Experimental curves (black) are compared with theoretical curves (red) that were generated from data analysis. The extracted structural dynamics are summarized in (c).

References

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