

Time-resolved Resonance Raman Observation of Quaternary Structural Changes of Dimeric Hemoglobin

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Homodimeric hemoglobin from *Scapharca inaequalvis* (HbI) shows cooperative oxygen binding. Although the structure of the HbI subunit is similar to that of human adult hemoglobin (HbA), the contact sites in the oligomer are completely different between the two proteins. It is interesting to compare allosteric dynamics of HbI with those of HbA. Moreover, comparison of the dynamics between wild type (WT) and mutants of the 97th phenylalanine (F97) provides insights into the allostery of HbI since F97 was suggested to play a key role in oxygen affinity and cooperativity (Figure 1). Here, we employed the time-resolved resonance Raman spectroscopy to explore the structural changes after the photodissociation of the carbon monoxide (CO)-bound HbI in nanosecond to microsecond range. The structural dynamics of two HbI mutants, F97Y and F97L, were also studied and compared with those of WT HbI.

Figure 2 shows temporal changes of the iron-histidine stretching [$\nu(\text{Fe-His})$] frequencies of WT, F97L, and F97Y. The $\nu(\text{Fe-His})$ frequency showed a 3-cm⁻¹ downshift with a single time constant of 1.2 μs . This is in contrast to the $\nu(\text{Fe-His})$ frequency change observed for HbA, which showed a triphasic downshift of 8 cm⁻¹.¹ In contrast to WT, the $\nu(\text{Fe-His})$ frequency changes of F97L and F97Y were small (~ 1 cm⁻¹). Because both F97L and F97Y mutants exhibit a weaker cooperativity than WT, it is highly likely that the $\nu(\text{Fe-His})$ frequency change of WT is mainly due to the quaternary changes of HbI. The crystallographic data indicated that the orientation of the F97 side chain is different between CO-bound and unbound states. The $\nu(\text{Fe-His})$ frequency change is associated with the reorientation because the histidine bound to the heme iron is located one-turn next to F97 in the same helix.

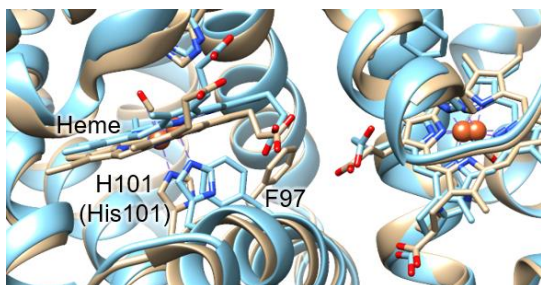


Figure 1. Comparison of crystal structures of CO-bound (light brown) and unbound (blue) HbI.

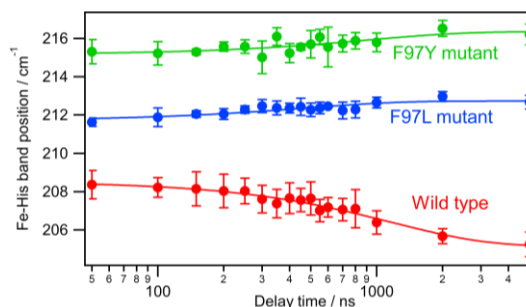


Figure 2. Temporal changes of the iron-histidine stretching [$\nu(\text{Fe-His})$] frequencies of WT, F97L, and F97Y.

1) S. Chang, M. Mizuno, H. Ishikawa, Y. Mizutani, *Phys. Chem. Chem. Phys.*, **2018**, 20, 3363.