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Structural basis of the oxygen dissociation mechanism of the giant hemoglobin in the presence of the allosteric effectors

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1 Introduction

Allosteric oxygen-binding property of hemoglobin (Hb) is well studied for the past a half century. The molecular mechanism of the cooperative binding of oxygen molecules to each subunit has been understood based on the crystal structures of the fully-oxygenated (oxy) and deoxygenated (deoxy) forms of mammalian Hbs [1]. However, it remains to be clarified structures of oxy-deoxy intermediate forms without any artificial modification of the Hb molecule.

We have reported that an extracellular giant Hb form marine tube worm, *Lamellibrachia satsuma* is able to shift from the oxy to deoxy form in keeping the crystalline state [2]. However, Hb of *L. satsuma* show little susceptibility for allosteric effectors such as calcium or magnesium ions. To elucidate the effects for cooperative oxygen-binding mechanism for the giant Hbs, we have performed the crystal structure analyses of the oxy-deoxy intermediate forms of the giant Hb from another marine worm, *Oligobrachia mashikoi* (*Oligobrachia* Hb), which show significant enhancement under existence of calcium ion.

2 Experiment

Oligobrachia Hb was prepared and crystallized as oxy form as described [3]. The oxy-deoxy intermediate crystals were obtained from the oxy crystals through the soaking method. The crystals were transferred to the cryoprotectant solution containing PEG 400 in a step-wise manner, increasing to a final concentration of 20% (v/v). In the last step, the crystals were soaked in the cryoprotectant solution containing 50-100 mM sodium hydrosulfite. The soaking time for the final solution was varied from 10 to 120 sec., and then immediately flash-frozen in a nitrogen gas stream at 95 K. Oxygen saturation fraction was measured by absorption spectra of each crystal.

X-ray diffraction experiments were performed at beamelines 1A and 17A at PF, KEK. The data were processed and scaled using XDS [4] and were truncated using the CCP4 program suite [5]. The structure were solved by the molecular replacement method using MOLREP [6]. Several cycles of manual model rebuilding and refinement were performed by using the program COOT [7] and REFMAC5 [8], respectively.

3 Results and Discussion

When soaking the oxy-crystals in the final solution, it was observed that the color of the crystals gradually changed from bright red to deep purple under microscope observation. However, due to variations in crystal volume, the correlation between the soaking time and the oxygen saturation fraction will be weak. Therefore, the oxygen saturation fraction of individual crystals was identified by microspectroscopy. Partial oxygen saturation fractions were also confirmed by the obtained electron density for the bound oxygen at the heme pocket, because the electron densities are clearly weaken.

Comparisons of the oxy-structure with the intermediate structure revealed that local structural changes correlate with the large quaternary changes. For example, the side chain conformation of Arg residue near the heme (Figure 1) is clearly associated with the quaternary structural changes. The electron densities for the calcium ions are very weak or disappeared for the oxy-deoxy intermediate which have deoxygenated quaternary structure. These results confirm our previous estimation [9] that the calcium ions bound to the interface between subunits play a key role for the allosteric transition of the structure of the giant Hbs.

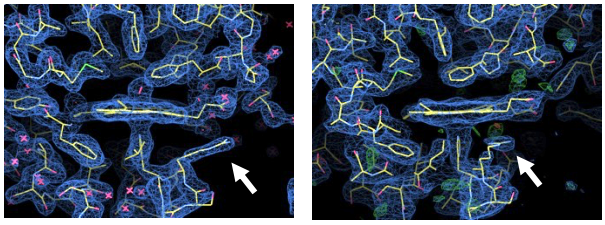

Fig. 1: Comparison between the fully-oxygenated (left) and 63% oxygenated structure (right). The quaternary structures are that of oxy, and deoxy forms, respectively.

References

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