Poster Presentation

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Partial oxygen-dissociation of crystalline giant hemoglobin

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Allosteric oxygen-binding of hemoglobin (Hb) has been widely discussed based on the quaternary structural changes elucidated by the crystal structures of the oxygenated and deoxygenated states. However, it remains to be determined the structure of intermediate states between the oxy and deoxy forms without any artificial modification of the Hb molecule. A tubeworm, Lamellibrachia satsuma has extracellular giant hemoglobins with a molecular mass of about 400 and 3,600 kDa. Recently, we have determined the crystal structure of the 400 kDa Hb (V2Hb) in the oxy state, and then we successfully obtained the deoxygenated crystals of V2Hb from oxy crystals by the soaking methods [1]. These findings encourage us to initiate structural studies for the intermediate states between the oxy and deoxy forms of V2Hb, which should provide a more accurate understanding of the allosteric mechanism of Hbs. The deoxy crystals of V2Hb were obtained from oxy crystals through the soaking in a solution containing 50 mM sodium hydrosulfite, and incubated for a few minutes. We tested various soaking times from 3 s to 180 s and then immediately flash-frozen under a nitrogen gas stream. The obtained structures reveal that in the case of the soaking time was longer than 10 s, the electron densities of the oxygen molecules at some heme pockets (oxygen binding sites) were very week or disappeared. These 'intermediate' structures show almost the same quaternary structure as that of the oxy structure. This fact suggests that quaternary rearrangement of V2Hb might arise just before a complete dissociation of all the oxygen molecules from all the subunits.

[1] *N. Numoto, T. Nakagawa, R. Ohara, et al., Acta Cryst. D, 2014, in press.*

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