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# Crystal structure analysis of cooperative oxygen-binding of the giant hemoglobins

Nobutaka Numoto*
Medical Research Institute, Tokyo Medical and Dental University (TMDU),
Bunkyo-ku, Tokyo 113-8510, Japan

## 1 Introduction

Hemoglobin (Hb) is well known protein because of its allosteric oxygen-binding regulation. The mechanism has been interpreted as the structural changes based on the crystal structures of the oxygenated (oxy) and deoxygenated (deoxy) forms [1]. However, it remains to be determined the structure of intermediate forms 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 both oxy and deoxy forms [2]. We found that deoxy crystals of V2Hb could be obtained from the oxy crystals in keeping the crystalline structure by a soaking method. These findings encourage us to initiate structural studies for the intermediate forms between the oxy and deoxy states of V2Hb, which should provide a more accurate understanding of the allosteric mechanism of Hbs.

## 2 Experiment

V2Hb was prepared and crystallized as oxy form as described [2]. The crystals of the oxy-deoxy intermediate states 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 mM sodium hydrosulfite. The soaking time for the final solution was varied from 3 to 300 sec., and then immediately flash-frozen in a nitrogen gas stream at 95 K.

X-ray diffraction experiments were performed at beamelines 5A and NW12A at PF and PF-AR, KEK. The data were processed and scaled using the HKL2000 package [3] and were truncated using the CCP4 program suite [4]. The structure were solved by the molecular replacement method using MOLREP [5]. Several cycles of manual model rebuilding and refinement were performed by using the program COOT [6] and REFMAC5, [7] respectively.

## 3 Results and Discussion

The obtained structures reveal that in the case of the soaking time was longer than 10 sec., the electron densities of the oxygen molecules at some heme pockets (oxygen binding sites) were very week or disappeared. These facts suggest that we obtained an ‘intermediate’ structures between oxy and deoxy states of V2Hb. The ternary structural changes at the Val E11, which is observed in the previous reports of oxy and deoxy structures, are observed in our ‘intermediate’ structures along with the disappearance of the oxygen molecule. In contrast, little quaternary rearrangements were observed (Figure 1) before a complete dissociation of all the oxygen molecules from all the subunits.

It was difficult to estimate the accurate oxy/deoxy ratio of the Hb from the electron densities for the oxygen binding sites, but the oxy/deoxy ratio can be determined more accurately by the absorption spectrum. A combination of the X-ray crystallographic and spectroscopic method is now in contemplation.

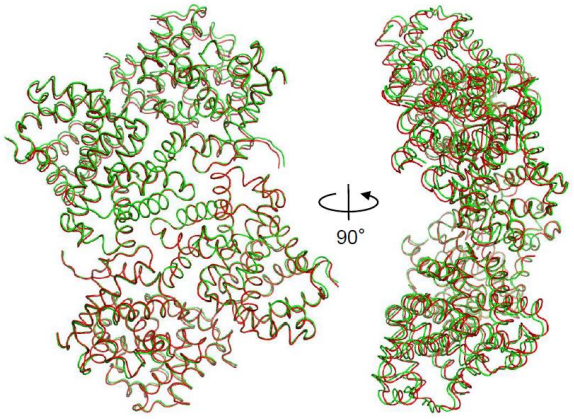


Fig. 1: Quaternary changes between the oxy (red) and the intermediate (green) form of V2Hb. The structures are represented for the content of the asymmetric unit (i.e., one third of the whole molecule).

## References

[1] M. F. Perutz, Nature **228**, 726 (1970).
[2] N. Numoto *et al*., *Acta Cryst.* **D70**, 1823 (2014).
[3] Z. Otwinowski and W. Minor, *Methods Enzymol.* **276**, 307 (1997).
[4] M. D. Winn *et al., Acta Cryst.* **D67**, 235 (2011).
[5] A. Vagin and A. Teplyakov, *Acta Cryst.* **D66**, 22 (2010).
[6] P. Emsley *et al., Acta Cryst.* **D66**, 486 (2010).
[7] G. N. Murshudov *et al., Acta Cryst.* **D67**, 355 (2011).

* numoto.str@mri.tmd.ac.jp