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Names and affiliations of applicants (* indicates experimentalists):

Shin-ichi Adachi*, Sam-Yong Park*, Tetsutaro Iizuka
RIKEN/SPring-8, Japan
Hideki Morimoto*, Khoon Tee Chong
Osaka University, Japan
Keith Moffat*, Vukica Srajer*, Tsu-yi Teng*, Zhong Ren*, Wilfried Schildkamp", Claude Pradervand*
The University of Chicago, USA
Michael Wulff*, Dominique Bourgeois*, Thomas Ursby*
ESRF, France

Report:

After very successful ns time resolved studies of carbonmonoxy myoglobin (MbCO) photolysis in April 95 and May 96 (Srajer et al., 1996), we started another ns time resolved study of carbonmonoxy hemoglobin (HbCO). We had the first beamtime in March 97 for this project, and we are very happy to be able to collect some results of photolysis of CO in Mg-Fe hybrid HbCO single crystal in ns time scale.

The analysis of the data collected in March 97 is almost finished and major achievements so far are as follows.

1. Photolyzed CO molecule in the heme pocket of Mg-Fe hybrid Hb was clearly observed at time delay of -2 ns between laser and X-ray pulse.

We collected datasets of ground state (not photolyzed, HbCO state) and excited state (photolyzed Hb* state) of Mg-Fe hybrid HbCO. In this hybrid HbCO crystal, two iron atoms in alfa subunits are replaced by magnesium, and CO molecules are bound to the iron atoms in beta subunits. This crystal is known to stay in its T-state (Park et al, 1996), thus with this crystal, we can observe ligand binding process in T-state Hb. We calculated difference Fourier map between ground and excited states by using the dataset

at the time delay of ~2ns after laser, and we observed negative electron density just above the heme iron which caused by photodissociation of CO molecule. In addition, we could observe positive electron density near His E7 in the distal heme pocket, which is possible canditate of photodissociated CO molecule. It is interesting to note that the position of posible photodissociated CO molecule in the heme pocket is significantly different from that observed in MbCO crystal, and we are examining the position in more detail.

2. The time course of ligand rebinding process was measured in nano- to submicrosecond time scale.

We collected datasets at the time delay of -2, 10, 50, and 500 ns after laser. We could observe decrease of the electron density which is observed above the heme iron in the time range of 10 - 50 ns, which could be caused by the rebinding of CO molecule to the heme iron. We expected much slower rate of ligand rebinding in T-state Hb, and we need to check the reproductivity of the result. In addition, the signal-to-noise ratio of the difference Fourier maps at 10, 50, and 500 ns were not so good that we couldn't clarify the structural changes in detail. We are now planning to optimize or increase the degree of photolysis to improve the data quality, to maximize the difference signal and to extract more accurate structure factor amplitudes.

Park, S.-Y., Nakagawa, A., and Morimoto, H. (1996) J. Mol. Biol. 255, 726-734.
Srajer, et. al. (1996) Science 274, 1726-1729.