



	Experiment title: Nanosecond time-resolved crystallography of hemoglobin by using ESRF single bunch mode	Experiment number: LS-731
Beamline: ID09	Date of experiment: From: 18-Sep-97 to: 20-Sep-97	Date of report: 23-Feb-99
Shifts: 6	Local contact(s): Dominique Bourgeois	<i>Received at ESRF:</i> 02 MARS 1999

Names and affiliations of applicants (* indicates experimentalists):

Shin-ichi Adachi*, Sam-Yong Park*, Tetsutaro Iizuka

RIKEN Harima Institute, Japan

Hideki Morimoto, Khoon Tee Chong

Osaka University, Japan

Keith Moffat, Vukica Šrajer, Tsu-yi Teng, Zhong Ren, Wilfried Schildkamp, Claude Pradervand

The University of Chicago, USA

Michael Wulff, Dominique Bourgeois, Thomas Ursby

ESRF, France

Report:

Based on our success of nanosecond time-resolved study of carbonmonoxy myoglobin (MbCO) (Šrajer et al., 1996) and carbonmonoxy magnesium-iron hybrid hemoglobin (Mg-Fe hybrid HbCO) (LS-596 March 97) photolysis, our next target is the photolysis study of carbonmonoxy hemoglobin A (HbACO) crystal in R-state. This is definitely more challenging task because 1. geminate rebinding rate of the R-state HbA is expected to be much faster than that of T-state Hb, and 2. this experiment also aims to monitor quaternary structural change of HbA caused by the R-T transition in the crystal. We had a beamtime in September 97 for this project, and tried to observe photolysis and geminate rebinding process of CO molecule in the HbA crystal.

Unfortunately, photolysis was not successful this time; we could not observe any photolyzed species in the heme pocket of HbA. The possible reasons for the unsuccessful result are as follows.

1. Misalignment of the laser
2. crystals too large to excite
3. rebinding rate might be too fast to monitor in nanosecond range

Thus, we need to optimize the experimental conditions next time. We will align the laser beam more precisely, and we will prepare HbACO crystals whose size is more suitable for

efficient photolysis. Furthermore, a femtosecond pulse laser was installed at ID09 after our previous experiment and this laser would be useful to monitor geminate reaction in HbACO whose reaction rate is expected to be faster than the cases of MbCO or Mg-Fe hybrid HbCO.

In addition, we are planning to improved the data quality, to maximize the difference signal and to extract more accurate structure factor amplitude of our previous Mg-Fe hybrid HbCO results.

Šrajer, V., Teng, T. Y., Ursby, T., Pradervand, C., Ren, Z., Adachi, S., Schildkamp, W., Bourgeois, D., Wulff, M., and Moffat, K. (1996) *Science* 274, 1726-1729.