MLF Experimental Report	提出日 Date of Report 2011/4/13
課題番号 Project No. 2010B0044 実験課題名 Title of experiment Neutron Crystallographic Study of Protonation states of human hemoglobin 実験責任者名 Name of principal investigator Toshiyuki Chatake 所属 Affiliation Research Reactor Institute, Kyoto University	装置責任者 Name of responsible person Ichiro Tanaka 装置名 Name of Instrument/(BL No.) iBIX/(BL03) 実施日 Date of Experiment 2011/02/07-2011/02/19

試料、実験方法、利用の結果得られた主なデータ、考察、結論等を、記述して下さい。(適宜、図表添付のこと)
Please report your samples, experimental method and results, discussion and conclusions. Please add figures and tables for better explanation.

1. 試料 Name of sample(s) and chemical formula, or compositions including physical form.

(1) Adult human deoxyhemoglobin (DeoxyHb).
A crystal of deoxyhemoglobin (T-state) (Fig.1).*
$P2_12_12_1$, a = 97.1 Å, b = 99.3 Å, c = 65.8 Å
(2) Adult human CO-hemoglobin (COHb).
A crystal of CO-hemoglobin (R-state) (Fig.2_1).*
$P4_12_12$, $a = b$ =54.21 Å, c = 196.38 Å.
*Crystals were grown in deuterated solution.

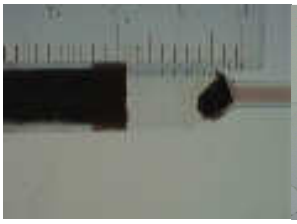

Fig.1 A Crystal of deoxy-hemoglobin (DeoxyHb).

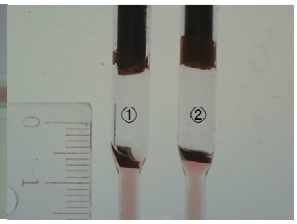

Fig.2 Crystals of carbomonoxy-hemoglobin (COHb).

2. 実験方法及び結果 (実験がうまくいかなかった場合、その理由を記述してください。)
Experimental method and results. If you failed to conduct experiment as planned, please describe reasons.

Experimental and Results: Prior to collection of neutron diffraction data, preliminary neutron experiments were carried out using crystals of DeoxyHb and COHb. A neutron time-of-flight image was taken for 10 h for each crystal (02/07-02/08). Although the crystal of DeoxyHb was very huge; the size of the crystal was more than 30 mm^3, the maximum resolution of the diffraction image was about 3.0~3.5 Å (Fig. 3). Because the size of the crystal was approximately same as the beam size of iBIX (ϕ = 5 mm), the current power of J-PARC (220 kW) is thought to be insufficient for neutron data collection of the present crystal of DeoxyHb. In order to overcome this problem, the improvement of the quality of this kind of crystal and/or higher power of J-PARC would be necessary. In case of the crystal of COHb, a better diffraction image than the crystal of DeoxyHb was obtained. We found diffractionts at 2.4 Å resolution on the diffraction image (Fig. 4). This crystal has a long c-axis (196.38 Å), which exceeds the nominally maximum length of a crystal lattice (135 Å) by ~60 Å, nevertheless the neighboring diffractions of the crystal of COHb seemed to be separated on the display of a graphic a workstation. We concluded that neutron diffraction images of the crystal of COHb could be processed, and we collected the neutron diffraction data set of COHb crystal in the remaining time of this experiment.

2. 実験方法及び結果（つづき） Experimental method and results (continued)

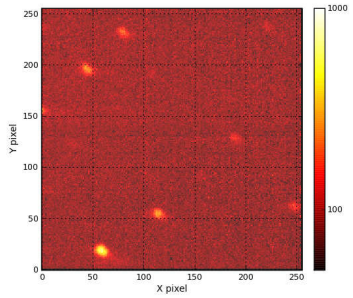

Fig.3 A Diffraction image of the crystal of DeoxyHb.

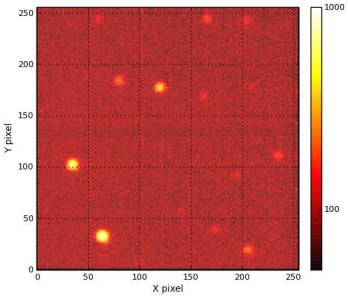

Fig.4 A Diffraction image of the crystal of COHb.

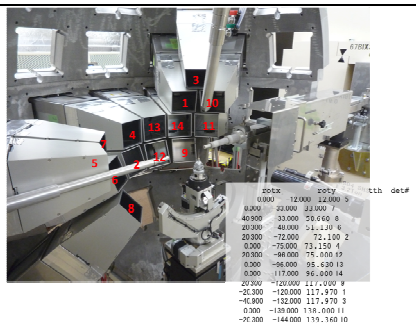

Fig.5 The layout of eighteen detectors in the present data collection.

Neutron data collection was carried out using the crystal of COHb. Fourteen wavelength-shifting fiber detectors were used for the present data collection. The layout of the detectors is shown in Fig. 5. The power of J-PARC was 220 kW during the data collection. The diameter of a collimator was 5 mm. The first data set was collected with the wavelength of 3.46 - 6.16 Å. Eighteen frames were taken with different directions of the crystal. The exposure times were 10.2 h and 9.6 h for 1st - 3rd flames and 4th - 18th flames, respectively. In order to cover the low-resolution area, the second data collection was carried out with the same setting except the exposure time and wavelength. The exposure time of the second data collection and the wavelength were 1.02 h/flame and 5.86 - 8.55 Å, respectively. Assuming that the effective resolution of the present COHb crystal was 2.4 Å, it was estimated that about 95% diffractions were covered in this experiment.

After the data collection of the crystal of COHb, the extent of methoxylation of COHb was measured, because methoxylation of COHb should not occur during diffraction experiment. This measurement was carried out at a chemistry laboratory in MLF building. The capillary was unsealed, and then crystal was solved in deuterated buffer. The solution was diluted and the absorption spectrum of the diluted solution was measured by an absorption spectrometer. After the measurement, NaCN was added in the solution and the absorption spectrum was measured again. There were very small differences between the two spectrums before and after the addition of NaCN, indicating that the methoxylation of COHb was negligibly small. Now, we are processing the neutron diffraction frames to index, integrate and merge intensities.

Conclusion: In the present study, we succeeded in collecting the full data set of neutron diffraction of adult human carbomonoxy-hemoglobin. Hemoglobin has two conformations of the tetrameric structure, composed of two alpha and two beta subunits. They are Relax and Tense states (R-state and T-state). In our previous study, we determined the neutron structure of the T-state adult human deoxyhemoglobin. On the other hand, COHb takes R-state. It is expected that the comparison between protonation behaviors of the two states provides the insight of the mechanism of oxygen-transportation by hemoglobin. In addition, the present study shows the capacity of iBIX, which supports a longer unit cell than the estimate. The nominally maximum length is 135 Å for iBIX, meanwhile the length of the c-axis of the crystal of COHb was 196.4 Å. Neighboring diffractions around 2.4 Å resolution can be separated in this crystal, indicating that iBIX can support a long crystal lattice (~200 Å) if the effective resolution is medium.