 MLF Experimental Report	提出日 Date of Report 24 October, 2014
課題番号 Project No. 2012B0106 実験課題名 Title of experiment Neutron diffraction analysis of trigonal ferro cytochrome c of horse heart 実験責任者名 Name of principal investigator Yoko Sugawara 所属 Affiliation Kitasato University	装置責任者 Name of responsible person Kusaka, Katsuhiro 装置名 Name of Instrument/(BL No.) IBARAKI biological crystal diffractometer/(BL-03) 実施日 Date of Experiment 27 February – 16 March, 2013

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.
<p>Oxidized form of cytochrome c from horse heart (104 amino acid residues with a heme)</p>

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.
<p>Cytochrome c, a class of heme-containing proteins, mediates the transfer of electrons in the redox systems in mitochondria. It was proposed that hydration water molecules would participate in the inter-molecular electron transfer process. In order to make the hydration and hydrogen bonding schemes around the heme moiety clear, we are carrying out X-ray and neutron crystallographic analysis of oxidized form of cytochrome c from horse heart.</p> <p>We had prepared crystals of trigonal (space group $P3_121$, $a = 80.8$, $c = 90.6$ Å) and tetragonal (space group $P4_3$, $a = 58.4$, $c = 42.1$ Å) cytochrome c with approximate volumes of 1 mm^3 using ammonium sulfate-sodium nitrate and ammonium sulfate-sodium chloride as precipitants, respectively. The test neutron data collection revealed that the tetragonal crystal was found to be better in crystallinity (Project Number: 2012A0097). Therefore full neutron intensity data collection using a tetragonal crystal with approximate volume of 2.4 mm^3 ($2.0 \times 1.2 \times 1.0 \text{ mm}$) was carried out.</p>

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

The single crystal sealed in a quartz capillary tube was irradiated by the pulsed white neutron, and Bragg reflections were collected with TOF technique at room temperature using 30 detectors. The accelerator power was 300 kW. Wavelength of the incident neutron was 3.0 – 6.0 Å for the 1st frame and 5.5 – 8.0 Å for the 2nd frame. Twenty data sets were collected for each frames, and the exposure time of 1st frame was 18 hours per one crystal orientation and that for 2nd frame was 1.8 hours. Total beam time was 16 days.

Data reduction was carried out using STARGazer. Intensities of 8647 symmetrically independent reflections up to 2.0 Å resolution were collected. Structure refinement was carried out using Phenix starting from the X-ray structure of tetragonal cytochrome c (PDB-ID: 1HRC), and R and R_{free} values at the present stage are 0.23, 0.24, respectively. The nuclear density distribution was calculated by maximum entropy method (MEM) using 2765 reflections ($|F_o| > 10\sigma(F_o)$) up to 2.0 Å resolution. The MEM map around the heme moiety is shown in Fig.1. Several hydrogen atoms are recognized in the nuclear density map as negative densities.

The quality of the data was insufficient to discuss the precise hydration scheme around the heme moiety. We are continuing trials to improve quality and size of crystals. In 2015, the accelerator power will increase to 600 kW and we are going to carry out remeasurements.

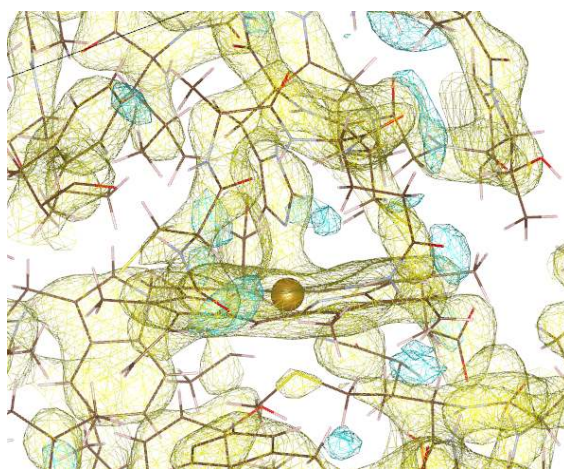


Fig. 1 The nuclear density distribution around the heme moiety calculated by maximum entropy method (MEM). (Yellow cage, positive region ($0.08 \text{ cm}^{-14}/\text{\AA}^3$); light blue cage, negative region ($-0.04 \text{ cm}^{-14}/\text{\AA}^3$.)