MLF Experimental Report	提出日 Date of Report 2015/2/22
課題番号 Project No. 2014B0250 実験課題名 Title of experiment Hydration effect on electron transfer process in cytochrome *c* and DNA probed by muon labelling method 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 Yasuhiro Miyake 装置名 Name of Instrument/（BL No.） Muon D1 実施日 Date of Experiment 2014/12/20 9:00 – 2014/12/22 7:00

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.

a) Cytochrome c (104 amino acid residues with a heme from horse heart): wet and dry

b) Lysozyme (129 amino acid residues form hen egg white): dry

c) Cytochrome c reductase (a protein complex with Fe- hemes and a FeS cluster from horse heart): dry

d) Catalase (527 amino acid residues with a heme from bovine liver): dry

Water (H_2O) contents of the samples were adjusted to approximately 5 % (a dry sample) and 20% (a wet sample).

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.

In order to investigate the electron transfer process of redox system of proteins, we measured the μSR time spectra of the following four samples.

1. Dried sample of cytochrome *c* (water content approximately 5 %)
2. Wetted sample of cytochrome *c* (water content approximately 20 %)
3. Dried sample of lysozyme (water content approximately 5 %)
4. Dried sample of cytochrome c reductase (water content approximately 5 %)
5. Dried sample of catalase (water content approximately 5 %)

Cytochrome c and cytochrome c reductase are the members of the respiratory chain in mitochondria, and lysozyme is selected as a reference protein which does not participate in electron transfer system. Catalase is an enzyme responsible for the degradation of hydrogen peroxide, and. contains a Fe-heme group in its active center. Catalase is not the member of electron transfer system but its Fe(III) in a heme is able to be reduced to Fe(II).

In order to obtain information just after a pulse radiation, we used the single pulse by removing the second pulse arriving at 600 ns after the first pulse.

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

We had carried out measurements of temperature dependence of the dry cytochrome c and wet and dry lysozyme c on May, 2014 (2014A0225). However, conditioning of the new detector system had not been completed and the measured data were distorted in some extent. We re-measured the dry and the wet samples of cytochrome c and the dry sample of lysozyme. The distortion due to counting loss was partly improved, but it is still difficult to determine reliable Γ values. Therefore the μSR data were compared qualitatively (Fig. 1 and 2). The μSR spectra of cytochrome c, cytochrome c reductase and catalase resemble each other. The relaxation of cytochrome c seems to be a little larger than that of catalase. We are going to analyze the difference among these samples based on the precise data in order to confirm that the μSR properties of cytochrome c reflects the electron transfer processes correlated with the redox reaction of Fe-heme. At the same time, the first principle calculations are in progress in order to know the stopping site of muon, charge states and possible dynamic information about moving electron in the proteins.

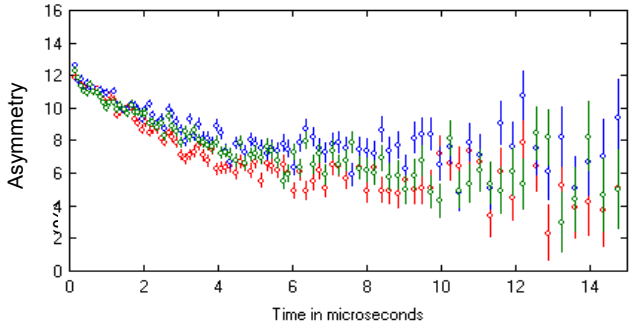

Fig.1 Zero field data of cytochrome c (red), cytochrome c reductase (green) and catalase (blue) in dry forms at 100K.

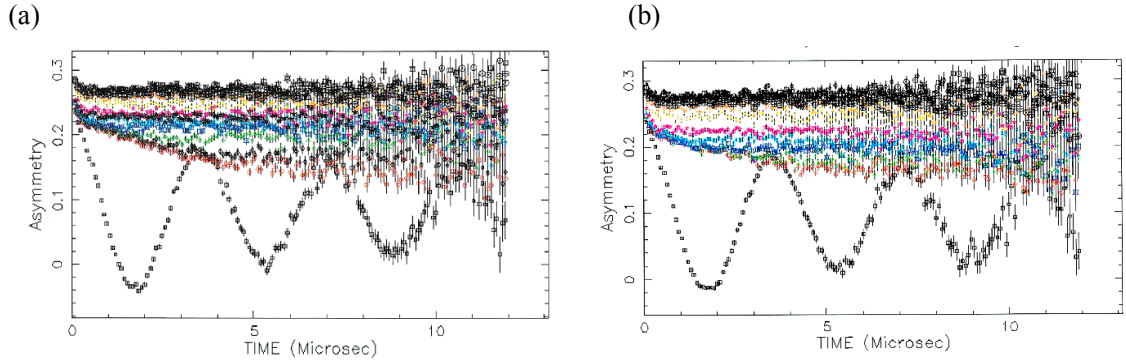

Fig.2 Magnetic field dependence (LF 0-3000G with TF 20G) of cytochrome c (a) and catalase (b) in dry forms at 100K.