MLF Experimental Report	提出日 Date of Report 2015/03/08
課題番号 Project No. 2014B0228 実験課題名 Title of experiment Visualization of the electron transfer associated with biochemical reaction process in trypsin-BPTI complex 実験責任者名 Name of principal investigator Tamiko Kiyotani 所属 Affiliation Showa Pharmaceutical University	装置責任者 Name of responsible person Yasuhiro Miyake 装置名 Name of Instrument/（BL No.） D1 実施日 Date of Experiment 2015/12/19-20

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) Chymotrypsin – LBPI* complex, Protein made from C, N, O, S, H (Molecular weight: 25,000 Da-9,000 Da)
2) Trypsin – LBTI* complex, Protein made from C, N, O, S, H (Molecular weight: 23,800 Da-9,000 Da)

* LBTI: Lima Bean Trypsin Inhibitor

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.

A first muon spin rotation and relaxation (μSR) experiments on enzyme, serine protease, Chymotrypsin-LBTI and Trypsin-LBTI complexes, have been carried out.

The samples were prepared as follows. Chymotrypsin and LBTI were dissolved in deionized water for the complex, and then the dissolution product was lyophilized during 12 – 24 hours. The lyophilized powder sample was kept in a container with saturated ammonium sulfate solution of 60 - 80%rh, until the percentage of the moisture content in the samples was about 20% by weight. Trypsin-LBTI complex was also prepared by the same procedure.

The two samples were packed in sample cells and μSR measurement has been carried by using the standard cryostat with exchange gas atmosphere. The data collection was carried out under the temperature range of 215-300 K and the magnetic field of the zero magnetic field (ZF), transverse magnetic field (TF), and longitudinal magnetic field (LF) between 10-3500 G. The μSR measurement of the pure silver was carried out for data correction of the instrument artifact under the same condition of the protein sample measurement.

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

The data analysis of the μSR measurement is currently in progress. Some preliminary results would be reported here. WiMDA, which is a standard analysis program of μSR measurement, was used for the data processing and analysis. We have tried to analyze the data using Risch-Kehr (R-K) theory, but an abnormal decrease of Asymmetry data derived from some artifacts of D1 instrument was found to appear between 0 and 4 microseconds, and the data analyses by using WiMDA were not available for this reason. We have tried to correct the Asymmetry results of Chymotrypsin-LBTI complex by using the Asymmetry results of Ag. We might have succeeded in obtaining the right Asymmetry results of samples. The corrected Asymmetry was shown in Fig.1(a) with the uncorrected one in Fig.1(b). The preliminary results of the Asymmetry of Chymotrypsin-LBTI complex are as follows: The corrected data were analyzed by using R-K function, and we found the fitting with R-K function has been succeeded by using two separate time regions, that are, from zero to about 2 μsec and about 2 to 10 μsec. The both results are shown in Fig.2(a) and (b), respectively. In two time regions, the different relaxation constants, Γ, were obtained. The former value is more than 10 times larger than the latter. The fitting results are shown in Fig.2. The magnetic field dependence of the Γ was shown in Fig.3.

The reason why the R-K fitting has been succeeded by using two different relaxation constant might come from the internal magnetic field generated by the proton and electron transfer derived from the enzymatic reaction. This must be confirmed by using Chymotrypsin-MIP complex, where the enzymatic function is completely inhibited.

We have succeeded in correcting abnormal Asymmetry derived by the instrument artifact, but strictly speaking it must be checked by using the artifact free instrument, such as RIKEN-RAL spectrometer whether the correction is complete or not.

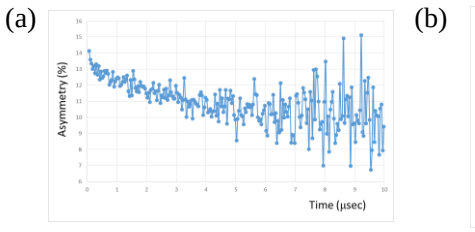

Fig.1 (a) Corrected and (b) Uncorrected μSR spectra of Chymotrypsin-LBTI at 0 G at room temperature.

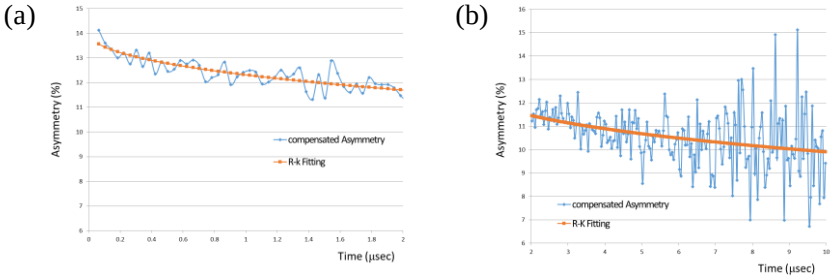

Fig.2 The fitting by R-K function (a) from zero to about 2 μsec and (b) from about 2 to 10 μsec.

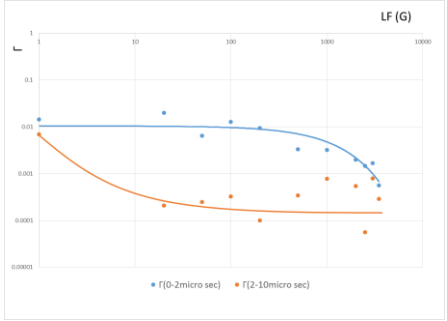

Fig.3. The magnetic field dependence of the Γ.