Structural analysis of Tb PGFS caused "African trypanosomiasis"

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Parasitic protozoa Trypanosoma causes diseases in both human and domestic animals. In human, the disease is characterized by clinical symptoms such as fever, headache, body pain, sleepiness, and immunosuppression. Bioactive substances produced during the infection cause these symptoms, whose molecular mechanisms remain to be elucidated.

Prostaglandins (PGs) of 2-series are metabolites of arachidonic acid (AA) formed by the oxidative cyclization of the central five carbon atoms within this fatty acid. It is well documented that PGD_2 is one of the endogenous sleep-promoting substances. PGD_2 and PGE_2 play important roles in clinical and pathological manifestations (such as somnolence, immunosupression acute fever, shivering, headache) in mammals. Also, it is often believed that host cells solely produce prostaglandins, which mediate inflammatory responses during infection with parasitic protozoa. Trypanosoma produces PGD_2, PGE_2, and PGF_{2a} ($PGF_{2a} > PGE_2 > PGD_2$), and releases them in the medium and out of parasitized red blood cells.

X-ray data collection for TbPGFS crystals with cofactor NADPH was performed at BL40B2 of the SPring-8 synchrotron radiation source. Although the crystals did not diffract beyond 3.5 Å resolution in house, the resolution was improved up to 2.8 Å resolution by using BL40B2 in the present study. However, the crystals were all decayed after the total of 3 hours of X-ray data collection. We think concentration of D(+)-trehalose is low. This crystal is crystallized by the ammonium sulphate. In this case, we could not use a glycerol well known to cryoprotectant. In our laboratory, these crystals have a good R_{merge} by 15% trehalose. In the point of getting better diffraction data at SPring-8 BL40B2, we want to retry to use the crystal made of the higher concentration ~30% trehalose.

In this time we could determine the space group of the crystals to be $P4_12_12$ by using HKL plot, and the unit cell parameters of $a = b = 114$ Å and $c = 140$ Å. The structure determination in MIR method by using the data collected in house are now in progress. At the same time we would like to collect the high resolution data at SPring-8, so we are now focusing on improving the quality of the crystals under the cryo temperature.

X-ray Fiber Diffraction Study on Natural Rubber

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Rubber from *Hevea brasiliensis* is known as natural rubber (NR). The major component of NR is polyisoprene consisting of the isoprene units C_5H_8 in the *cis*-1,4 configuration. NR exhibits rapid crystallization both by storing the sample at –25 °C (cold crystallization) and by stretching it (strain-induced crystallization). Proteins and fatty acids are major contaminants in NR. Recent study has revealed that the rate of crystallization of NR at –25°C was almost the same before and after deproteinization. However it decreased significantly after the removal of the fatty acids. These findings indicated that fatty acid groups accelerate the rate of cold crystallization of NR. Although several attempts to solve the crystal structure of NR have been done, there was some question on the proposed structural models. For better understanding of the crystallization mechanism of NR, we have been investigating molecular and crystal structure of NR. In the present beam time, we tried to collect high resolution fiber diffraction data of NR.

Four kinds of NR samples, such as non-crosslinked NR, peroxide-crosslinked NR, deproteinized NR (DPNR) and peroxide-crosslinked DPNR, were used in this study. X-ray measurements were performed by using R-AXIS IV^{++} detector with 300 seconds exposure at room temperature, -25°C, -50°C –80°C –120°C and –160°C. We collected about 30 fiber diffraction patterns of NR. One of the fiber diffraction patterns of NR is shown in Fig. 1. All the 38 observed reflections in Fig. 1 could be indexed in terms of an orthorhombic unit cell with dimensions of $a = 12.59(1)$, $b = 9.00(1)$, and c(fiber axis) = 8.24(1) Å. This unit cell is essentially the same as those reported by Bunn and Nyburg. Structure analysis of NR is now under way.

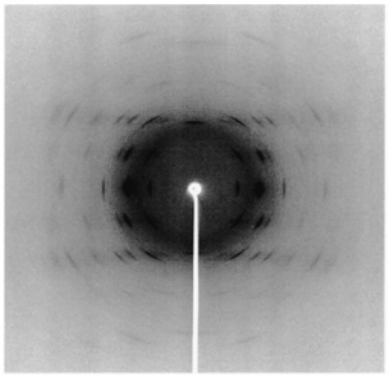

Fig. 1 X-ray fiber diffraction pattern of NR.