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X-ray crystallography of enzymes involved in ADP-dependent glycolysis

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Thermococcus litoralis uses a modified Embden-Meyerhof pathway. Its phosphofructokinase (TLPFK) catalyses the phosphorylation of fructose-6-phosphate by ADP, but not by ATP, in the presence of Mg^{2+} to form fructose-1,6-bisphosphte. The gene encoding TLPFK has been cloned and sequenced in *E. coli*. TLPFK had a molecular mass of approximately 125 kDa and was consists of dimer with subunit size of 52 kDa. We obtained crystals of TLPFK diffracting up to ~ 2.5 Å, but usually only up to 3.0 ~ 2.8 Å, on the preliminary data collection at PF-BL6A and BL18B beamlines. In spite of some efforts, we could not obtain crystals of selenomethylated proteins. We prepared derivative crystals, mainly of Pt and Hg atoms, to solve the structure by multiple isomorphous replacement method, as well as some native crystals. All data sets have cell constants $a = b = 84.8 \sim 85.8$ Å, $c = 163.2 \sim 164.4$ Å, space group = $P4_12_12$ or $P4_32_12$. Wavelength, camera length, oscillation width, number of frames, exposure time per frame, resolution limit, and R_{merge} were as follows. K_2PtCl_4: 1.0723/1.7026/1.64928 Å, 180 mm, 1.0 deg, 100 frames, 15 s, 2.47/2.60/2.60 Å, 0.064/0.055/0.059. Native 1: 1.0723 Å, 180 mm, 1.0 deg, 180 frames, 10 s, 2.03 Å, 0.057. $K_2Pt(CN)_4$: 1.0723/1.0726/1.0649 Å, 180 mm, 0.8 deg, 225 frames, 10 s, 2.50 Å, 0.064/0.065/0.066. Native 2: 1.0723 Å, 180 mm, 1.0 deg, 180 frames, 10s, 2.30 Å, 0.051. $HAuCl_4$: 1.0375 Å, 180 mm 1.0 deg, 180 frames, 10 s, 2.60 Å, 0.065. p-Chloromercuribenzoic acid (PCMB): 1.0080 Å, 220 mm, 1.0 deg, 180 frames, 10s, 2.98 Å, 0.058. p-Hydroxymercuriphenylsulfonic acid (PHMBS): 1.0080 Å, 220 mm, 1.0 deg, 180 frames, 10 s, 2.60 Å, 0.061. p-Hydroxymercuribenzoic acid (PHMB): 1.0080 Å, 220 mm, 1.0 deg, 180 frames, 10 s, 2.60 Å, 0.058. Thimerosal: 1.0080 Å, 220 mm, 1.0 deg, 180 frames, 10 s, 3.20 Å, 0.076. Using the program SOLVE, we have obtained electron density with figure of merits of 0.5 ~ 0.7. However, the maps are still ambiguous for smooth chain tracing. Crystallography of TLPFK is still in progress.

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Microfibril angle in woods and its biological significance –how a tree regulates and optimizes its mechanical structure-

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Wood is a fiber composite made of cellulose fibers embedded in a hemicellulose and lignin matrix. These structural materials in the cell walls have many mechanical functions of supporting the tree beside their biological functions. The primary factors are the orientation (microfibril angle: MFA) and lateral dimension of cellulose microfibrils[1], and thus we studied a branch of *Cryptomeria japonica* D. Don by SAXS. The averaged MFAs and lateral diameters were exemplified in Figure 1 together with the corresponding diagrams. The MFA was larger in the lower and basal parts of a branch in general, which was in accord with the formation of compression woods (a response to gravitational force), while the dimension remained constant. The results has to be considered as preliminary, but it seems possible to map out dynamic changes of MFA in a whole tree by modifying sample preparation procedure and a setup for sample translation.

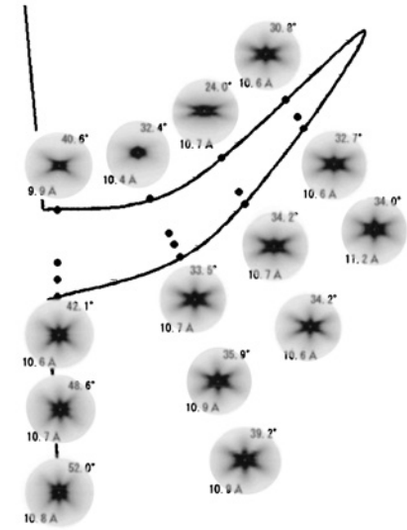

Figure 1. Microfibril angles and radii obtained from various portions of a branch in Sugi (*Cryptomeria japonica*).

[1] for example: M.P. Sarén et al., *J. Struct. Biol.* **136**, 101 (2001), R. Hori et al. *Biomacromolecules*, **3**, 182(2002)