Structural studies on peroxisomal β-oxidation multifunctional enzyme type 1 (MFE1)

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Rat peroxisomal multifunctional enzyme type 1 (perMFE-1) is a monomeric protein that catalyses the second and the third reaction in the β-oxidation of fatty acyl-CoA molecules in separate active sites of the enzyme. perMFE-1 possesses enzyme activities for the hydratase-1/isomerases (H1/I) and (3*S*)-hydroxyacyl-CoA dehydrogenases (HAD) reactions. Five domains have been recognised in perMFE-1. The H/I reaction is located in the N-terminal part of the polypeptide (domain A) and the HAD activity (domains C and D) is between the B and E domains, which have a structural role and participate in intracellular targeting of the protein [1].

Crystallographic studies have been initiated to resolve the structure of the perMFE-1. The results obtained by crystallographic methods are used together with kinetic data on the wild type and variant enzymes to further elucidate the mechanisms of catalysis as well as substrate and cofactor trafficking from one active site to the other.

The HAD domain together with the E domain has been crystallised [2]. The crystallisation conditions consist of 0.2M sodium citrate, pH 5.6, 20mM ammonium acetate and 27% poly ethylene glycol 4000. Crystals reached the size of 0.35 x 0.2 x 0.2 mm in 30 days. Data has been collected from unliganded crystals to 2.45Å resolution using a rotating anode X-ray source. The unliganded crystal has primitive tetragonal space group with unit cell parameters $a=b=125.9$, $c=60.2$Å. In order to obtain an initial electron density map, the unliganded crystals were soaked with concentrated (1M) KBr solution and then back-soaked in cryo protection solution consisting of well solution in which the water was replaced with 10% glycerol. Fluorescence scan revealed clear peak indicative of bound Br ions and data sets were collected at peak, inflection point and high energy remote wavelengths at the BW7A beam line at DESY, Hamburg, respectively. The unit cell dimensions were $a=b=178.79$ and $c=60.47$Å suggesting a different tetragonal space group with two monomers in the asymmetric unit. Further analysis for finding the Br binding sites is ongoing.

References

[1] T.R. Kiema, J.P. Taskinen, P.L. Pirila, K.T. Koivuranta, R.K. Wierenga and J.K. Hiltunen (2002) Organization of the multifunctional enzyme type 1: interaction between N- and C-terminal domains is required for the hydratase-1/isomerase activity. *Biochem. J.* **367**:433-41.

[2] J.P. Taskinen, T.R. Kiema, K.T. Koivuranta, R.K. Wierenga, and J.K.Hiltunen (2002) *Acta Crystallogr. D*, **58**:690-693.