X-ray crystallographic studies on hematopoietic and lipocalin type PGDS

Masashi Miyano 3522†*, Hideo Ago 3579†, Daisuke Irikura 3586 #, Eiji Inagaki 3580†, Noriyuki Habuka 3581†, Elena Pinzar 3587 #, Yoshihide Kanaoka 3585#, Yoshihiro Urade 3584 #, and Osamu Hayaishi#

†Central Pharmaceutical Research Institute, Japan Tobacco Inc., Osaka 569-1125.
#Department of Molecular Behavioral Biology, Osaka Bioscience Institute, Osaka 565-0874.

Prostaglandin D synthase (PGDS) is the key enzymes for production of the D and J series of prostanoids. There are two distinct types of PGDS in mammals. One from the immune system is hematopoietic PGDS which requires GSH as a co-factor, and the other from the brain is GSH-independent lipocalin type. cDNAs of both types of enzyme were cloned from various origins and expressed in *E. coli* (1, 2, unpublished data). To elucidate the structure-basis of the mechanism of the highly specific isomerization catalysis from unstable PGH_2 to PGD_2 and to design the highly specific inhibitor, we obtained several crystals of both types of PGDS. Rat hematopoietic PGDS was solved the 3-D structure at 2.3Å as a σ-class glutathion S-transferase (2).

In the way of structural studies on PGDS, we tried to collect hematopoietic PGDS from other origin and a lipocalin PGDS at 100 K using SPring-8 BL41XU due to their large cell unit and small size of crystal.

A) Human hematopoietic PGDS is ca. 80 % homologous to that of rat enzyme. We collected the diffraction images using 40 x 80mm IP and an *off-line* reader equipped at BL41XU at λ=0.7Å, since the diffraction data of the enzyme had been collected only up to 3.5 Å using *in-house* system. The images were processed using Denzo. The crystal parameters are as follows; space group $P4_32_12$, $a=b=47.9$Å, $c=352.7$Å. The merged data was available up to 2.5Å, while the crystal was smaller than that of used for *in-house* data collection. The cell unit was significantly changed by more than 1Å due to for the data collection using cryo-protectant at 100 K. The structure was solved by means of molecular replacement using the structure of rat enzyme as a search model by AMoRE of CCP4 suite. There is one dimer in an asymmetric unit. The refinement and manual model-rebuilding are under way.

B) Sevral lipocalin-type crystals were snapped at 100 K. However, the mosaicity of all tried crystals was too high to collect diffraction data. The ice ring was observed in images. The conditions of the crystallization and the cryo-experiment are needed to further improve data collection.

REFERENCES

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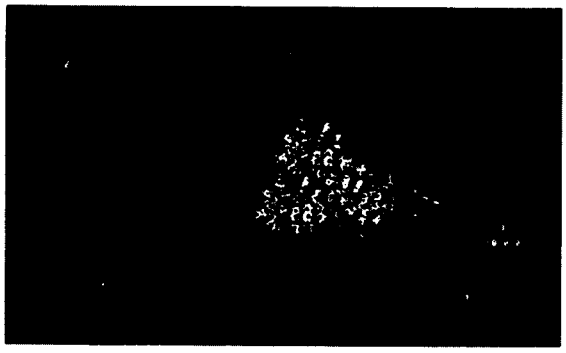

Crystal packing of human hematopoietic PGDS