Proposal Number [C04B12B2-1007N] BL12B2

Structure-based drug design

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Our research is to solve structures of PPAR, a key molecular target of type II diabetes, in complex with novel agonists discovered and developed in our division and consequently these structures could provide insight on how to design compounds with more potency. Protein PPAR were co-crystallized with compound SP990 and compound SP999. These crystals diffract to 2.6Å and 2.6Å in Spring8 station SP12B2. The data collection parameters for these datasets are summarized in table 1. All datasets have been processed by HKL2000 in station SP12B2. The data process results are shown in table2.

In addition to PPAR project, anti-SARS drug discovery project is also our main project. Various active compounds have been co-crystallized with SARS protease and the data of complex crystals have been collected in Spring8. All results are shown in table1 and table2.

Table 1. The summary of data collection parameters

Sample	Distance (mm)	Wavelength (Å)	Oscillation range(°)	Beam position (x,y)	Total frames
PPAR with SP990	222.98	1.0	1.0	(94.388,93.798)	151
PPAR with SP999	220.0	1.0	1.0	(94.388,93.798)	172
SARS protease with CSV940	130.0	1.0	1.0	(94.388,93.798)	145
SARS protease with CSV951	110.0	1.0	1.0	(94.388,93.798)	126

Table 2 . The summary of data process results

Data collection statistics	PPAR with SP990	PPAR with SP999	SARS protease with CSV940	SARS protease with CSV951
Space group	P21	P21	C2	P21
(a, b, c)	(58.26,88.81,56.42)	(56.57,89.10,58.99)	(107.78, 82.91,53.63)	(52.35, 96.27, 67.77)
(α,β, γ)	(90.00,89.98,90.00)	(90.00,90.54,90.00)	(90, 104.926, 90)	(90, 103.021, 90)
Resolution range(Å)	30.0-2.53	30.0-2.8	30.0-1.85	30.0-1.8
Completeness(%)	99.1	98.6	98.6	99.6
R_{merge} (%)	5.1	7.2	4.7	6.6
I/σ(I)	24.0	14.9	23.904	15.84

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Structural Studies of GSP Synthetase by X-ray Crystallography

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Glutathionylspermidine (Gsp) is a metabolite converted from glutathione by an ATP-cleaving Gsp synthetase activity common in *Escherichia coli* and protozoal parasites of *Trypanosoma* family. In trypanosomatids, a subsequent glutathionylation at N^8 of Gsp forms N^1, ^{8}-bis (glutathionyl)-spermidine, trypanothione, is believed to be the major surveillance thiol involved in the oxidant defense mechanisms. These parasites are known to cause significant human disease affecting millions of people in South America (Chagas' disease) and Africa (African sleeping sickness). Thus the Gsp synthetase is an attractive target for drug design. With its physiological role remains unclear, the 70 kDa *E. coli* Gsp synthetase is a bifunctional protein which catalyzes both amide bond formation and breakdown between spermidine and glutathione. *E. coli*, however, utilizes a GSH-based system for oxidant defense, the Gsp synthetase/ amidase represents an intriguing juxtaposition of two important classes of molecule.

In the current project, we would like to prepare the recombinant GSPS in bacteria expression system to a large-scale. These GSPS will be crystallized and subjected to X-ray diffraction analysis in order to obtain their crystal structures by the MAD (multiplewavelrngth-anomalous diffraction) method. In addition, we attempt to explain the catalysis mechanism of GSPS, based on the solved GSPS structure.

We already got the crystals of GSPS and its Se-Met derivative. In the present experiments, we successfully obtained the fluorescence spectra of the crystals of Se-substituted GSPS (Figure 1), which are useful for us to select the precise wavelengths for data collections. We have collected the enough data with high resolution in order to solve the GSPS structure by the MAD or SAD method. We are very grateful to NSRRC for supporting enough x-ray beam time. We also thank Dr. Yu-San Huang and Mr. Chien-Chang Tseng for assistance of data collections.

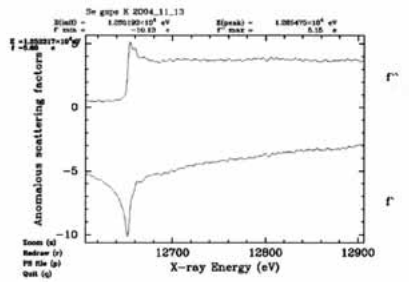

Figure 1