X-ray Crystallography of RdxA, a nitroreductase from *Helicobacter pylori*

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Classic nitroreductases, first identified from enterobacterium *Enterobacter cloacae*, have the ability to reduce aromatic nitrocompounds to their nitroso equivalents which are labile and readily degraded. Nitroreductases follow ping pong bi bi reaction mechanism in which electrons from NAD(P)H, the first susbstrate, are transferred to the nitrocompound via co-factor flavin mononucleotide. Nitroreductases can be classified on the basis of the affinity to the first substrate, NAD(P)H specific nitroreductases and NADPH specific ones. In *E. coli*, NADPH specific nitroreductase is dominant over NADPH specific one.

RdxA is a NADPH specific nitroreductase from human pathogen *H. pylori* which causes gastric ulcer and even stomach cancer. *H. pylori* can be eradicated with antibiotics including a nitrocompound metronidazole but strains resistant to metronidazole have been widespread. A recent study showed that the null mutation in RdxA is responsible for the resistance against metronidazole and the molecular basis of the suseptibility is of great interest. To understand the substrate specificity and reaction mechanism of RdxA, we purified and crystallized recombinant RdxA.

The crystals of RdxA was soaked with crystallization buffer containing 30% of glyrcerol and flash-cooled by gas nitrogen prior to the data collection. The crystals diffracted upto 2.4Å of resolution. Diffraction data were processed by Mosflm and scaled using Scala. The overall R_{merge} was 8.5% for the 2.4Å dataset. By using the atomic coordinates of the nitroreductase from *E. cloacae*, we could locate the homodimeric RdxA in the asymmetric unit with *R*-factor 48.6%. We performed maximum likelihood refinement implemented in Crystallography and NMR System to get improved electron density maps. Now we are under construction of the atomic model of RdxA.

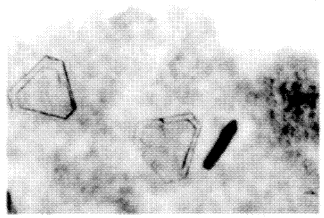

Fig 1. The crystals of RdxA

Structural analysis of the calcium binding protein MRP14 by X-ray crystallography

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The MRP14 is a protein that is specifically expressed in human myeloid origin cell, and the expression is specified in the stages of acute or chronic inflammatory states such as rheumatoid arthritis or sarcodosis. This protein has two Ca^{2+}-binding motifs per monomer and belongs to the S100 family of proteins, the largest subfamily of EF-hand proteins. S100 proteins take part in various cellular events such as cell-cycle regulation or Ca^{2+} transport. In order to understand such biological functions of a S100 family from the structural view point, we determined the crystal structure of MRP14 protein.

The recombinant MRP14 (114 AA / monomer) was prepared using *E.coli* overexpression system. The purified MRP14 was crystallized using the hanging drop vapor diffusion method at 20°C. Crystals with dimensions of 0.05 x 0.4 x 1.0 mm^3 have been obtained in a few days. Se-Met substituted crystal for the MAD phasing was crystallized under same conditions.

X-ray diffraction measurements were carried out under cryogenic condition (100K) using flash-cooling technique on the BL41XU station of SPring-8. The Se-Met crystal belongs to space group *P* 21 with cell dimensions of a = 57.62 Å, b = 178.59 Å, c = 61.24 Å and β = 113.45°. The asymmetric unit contains 4 dimers each of which has 10 Se-Met residues. MAD data with three wavelengths were collected up to 2.3 Å resolution using MAR-CCD detector. MAD data were processed using the program MOSFLM for integration and the program SCALA (CCP4) for scaling. A summary of the data collection is given in TABLE. Eight of 40 Se sites were found by the SHELX97 program. Initial phases for the 8 sites were calculated and 20 additional sites were located by difference-Fourier methods using SHARP program. After phase improvement by NCS-averaging using the program SOLOMON, structure was built into the electron density map. Twenty nine residues of C-terminus were not built because of poor electron density map at this region that is on surface of the molecule. The structure refinements are now in progress.

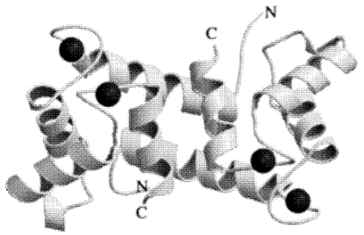

FIGURE : Crystal structure of MRP14 (~ res. 85) with bound Ca^{2+}.

	Peak	Edge	Remote
Wavelength (Å)	0.97916	0.97939	0.89999
Resolution (Å)	40-2.3 (2.42-2.30)	40-2.3 (2.42-2.30)	40-2.3 (2.42-2.30)
No. of reflections	261277 (20381)	239355 (20212)	284544 (40391)
Unique reflections	49818 (7043)	49806 (7035)	50075 (7288)
Completeness	99.4 (99.4)	99.4 (99.4)	99.9 (99.9)
Redundancy	5.2 (2.9)	5.2 (2.9)	5.7 (5.5)
I/σ	11.1 (2.7)	11.3 (2.9)	10.5 (2.4)
Rmeas (%)	5.8 (32.0)	5.6 (30.6)	6.2 (36.9)
Ranom (%)	6.3 (14.8)	6.9 (14.5)	- (-)

TABLE : Summary of data processing.