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Structural analysis of bacterial transporter protein

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Introduction

The MexAB-OprM efflux pump of *Pseudomonas aeruginosa* exports xenobiotics including antibiotics out of cells contributing to multiantibiotic of this hospital pathogen. The pump assembly consists of the proton conducting transporter MexB[1], the membrane fusion protein MexA[2], and the outer membrane protein OprM[3].

An aim of this study is to obtain atomic level three-dimensional structure of these medically important and scientifically interesting transporter proteins and contribute for better understanding of multi-drug resistance.

Experiments and Results

We collected the MexA data of Lutetium derivative crystals using an ADSC Q210 detector and synchrotron radiation with 1 Å wavelength and 350mm distance at 100 Kelvin.

The data were processed using HKL2000 program package. MexA was belonging to monoclinic space group $P2_1$ with unit cell parameters of a = 130.0Å, b = 180.4Å, c = 214.2Å β = 107.0. Single isomorphous replacement with the anomalous scattering (SIRAS) method using the $Lu(O_2C_2H_5)_2$ as heavy atom was applied computing the site of it by SHELEX and SHARP. Initial phase was calculated by this derivative crystal and the native one which had been previously collected the data in BL6A. Then the data of another native refined up to final resolution 2.40Å using the improvement software, CNS and refmac5. Crystallographic data shows Table 1.

Table 1	Lutetium data
Resolution (Å)	20.0-3.4 (3.52-3.40) [b]
R_{merge} [a]	0.032 (0.072)
Observed reflections	463,515 (40807)
Independent reflections	244,854 (23653)
No. of rejected reflections	3,609
Completeness	98.9 (95.5)
Multiplicity	1.9
$I/\sigma(I)$	25.7(9.9)

[a] $R_{merge} = \Sigma\Sigma j|<I(h)>-I(h)j|/\Sigma\Sigma j<I(h)>$, where $<I(h)>$ is the mean intensity of symmetry-equivalent reflections. Friedel pairs were merged as individual data.

[b] Values within parenthesis are for the highest resolution shell.

The crystal structure of MexA appeared as a spiral assembly of the 13 protomer by contiguous joining of the hexamer and heptamer forming a rod at the middle and a funnel-top structure at both ends[4](Fig.1).

We had 240 frame data of another component at AR-NW12. Wavelength, crystal to camera distance, temperature was the same as Lutetium derivative. The data were processed using HKL2000 program package. MexB was belonging to hexagonal space group P63 with unit cell parameters of a = b = 115.2Å, c = 231.7Å. The crystals diffracted beyond 3.38 Å. Detailed analysis of crystals are now in progress.

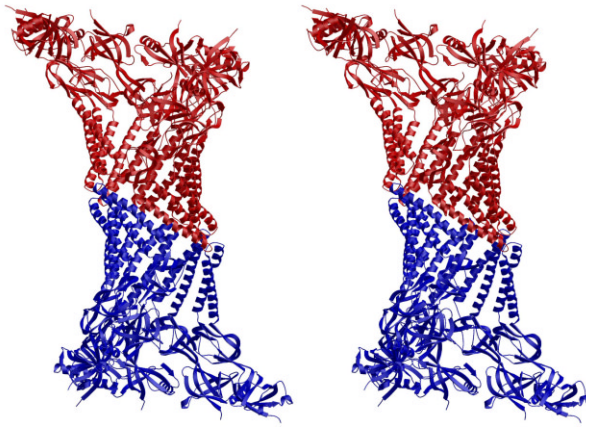

Fig.1 Stereoview of MexA 13mer

References

[1] L. Guan et al., J. Biol. Chem. Vol.274, No.15, pp. 10517 -10522(1999)
[2] H. Yoneyama et al., J. Biol. Chem. Vol.275, No.7, pp. 4624-4638(2000)
[3] A. Nakajima et al., J. Biol. Chem. Vol.275, No.39, pp. 30064-30068 (2000)
[4] H. Akama et al., J. Biol. Chem. in press(2004)

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