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Crystallographic analysis of Methyl-CpG-binding protein (MECP2)-DNA complex

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Introduction

DNA methylation is one of the most common epigenetic mechanisms for regulating gene expression. DNA methylation occurs at 5' cytosine of CpG dinucleotides, most of which are clustered in the CpG islands. Methylation of CpG islands leads to transcriptional silencing of the gene located downstream of methyl CpG.

The mechanism for the gene silencing involves proteins with high affinity for methylated CpGs, such as the methyl-CpG-binding protein MECP2. MECP2 contains methylated CpG binding domain (MBD) and a transcriptional repression domain, which interacts with various co-repressor complexes.

Mutation in MECP2 causes Rett syndrome in most cases, which is a progressive neurodevelopmental disorder that occurs almost exclusively in females, indicating that MECP2 has a crucial role in neurodevelopment. Understanding of the regulation of gene expression by MECP2 is beginning to be gained at molecular level. Our aim is to elucidate phenotype and genotype relationship by crystallographic analysis of MECP2 and its DNA complex.

Experiments and discussion

The purified MECP2 MBD was mixed with equal-molar DNA including methylated CpG and transcription factor C/EBP binding sequence, followed by adding the caat enhancer binding protein (C/EBP). The C/EBP, which is a bzip type transcriptional regulatory protein, was utilized for effective crystal packing [1]. For the Multi-wavelength Anomalous Diffraction (MAD) method, a protein-DNA complex including Br-labeled DNA was prepared. The resultant complex has been crystallized. Diffraction images were collected with synchrotron radiation at the beam lines BL5 and NW12 in Photon Factory and processed using HKL2000. The crystal belongs to the space group $C222_1$ and diffracted at 2.4 Å resolution (table 1). The phase of the MAD data was calculated with SOLVE/RESOLVE.

Although electron density of C/EBP and DNA was observed, that of MECP2 was almost absent. This might be due to disorder of the MECP2 molecule in the crystal. We are optimizing the condition for crystallization including the designs of MECP2 fragment and DNA.

Table 1: Data-collection statistics of MeCP2 MBD-DNA-C/EBP complex

Beam-line	NW12A		
Wavelength	0.91930 (peak)	0.91960 (edge)	0.90430 (remote)
Resolution (Å)	50.0-2.4	50.0-2.4	50.0-2.4
Space group	$C222_1$	$C222_1$	$C222_1$
Unit-cell parameters (Å)			
a	113.9	113.9	113.9
b	167.2	167.2	167.2
c	75.3	75.3	75.3
No. of reflections			
Observed	202756	202407	202064
Unique	53770	53604	28385
Completeness (%)	96.8 (82.4)[#]	97.9 (88.9)[#]	98.1 (90.2)[#]
$I/\sigma(I)$	24.8 (2.1)[#]	27.4 (2.7)[#]	26.2 (2.4)[#]
R_{merge} (%)	7.1	6.4	6.6

[#] Numbers in parentheses refer to data for high resolution outer shell 2.48-2.40 Å

References

[1] Tahir H. Tahirov et al., Cell. 104, 755-767 (2001).

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