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X-ray crystallographic analysis of Runx1-CBFbeta-Ets1-DNA complex assembled on the enhancer of T cell receptor alpha chain gene

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Introduction

Transcription is initially regulated through assembly of protein complex formed on an enhancer, called an enhanceosome.

To reveal how an enhanceosome formation is regulated, we studied the T cell-specific enhanceosome formed on the *TCRα* enhancer that contains multiple transcription factors such as Runx1, Ets1, CBFβ, CREB, etc. These transcription factors synergistically bind to the *TCRα* enhancer. Ets1 activity is negatively regulated by the regulatory region (the exon VII) flanking its DNA binding domain (so called the ETS domain) and phosphorylations of the exon VII suppress more strongly DNA binding activity of Ets1. Runx1, which binds to *TCRα* enhancer with Ets1 side by side, counteracts the self-inhibition of DNA binding activity of Ets1, leading to the cooperative DNA binding. To elucidate the mechanism, we performed crystallographic analyses for the Ets1-Runx1-CBFβ-DNA complex, the most important part of the enhanceosome formed on the *TCRα* enhancer. We have solved the crystal structures of the complexes containing various lengths of Ets1 and Runx1 fragments.

Experiments and discussion

The Ets1-Runx1-CBFβ-DNA complexes comprised of different lengths of fragments were crystallized all with a space group of $P2_12_12_1$.

Diffraction images were collected with synchrotron radiation at NW12A or BL-17A in Photon Factory and processed using HKL2000. We successfully obtained higher resolution data than previous ones by improving quality of the crystals.

The phase was determined by the molecular replacement program Phaser using the crystal structure of the Runx1-CBFβ-DNA complex. Refinement of the model was carried out using CNS.

Unexpectedly, no direct interactions between Ets1 and Runx1 is observed, although functional analyses such as EMSA (Electrophoresis Mobility Shift Assay), SPR (Surface Plasmon Resonance) and transactivation experiments showed the cooperative DNA binding and transactivation activities between Ets1 and Runx1. We hypothesized that mechanism of the cooperation between Ets1 and Runx1 would involve the DNA in the complex. We have devoted ourselves to the functional analyses of this complex using site-directed mutagenesis based on the crystal structure. To elucidate the insight of the mechanism, some of those mutant complexes have been crystallized along with the same procedures as the wild-type complex. We show here the diffraction and refinement statistics of Ets1-Runx1(K167A)-CBFβ-DNA complex (table 1).

Table1: Diffraction and refinement statistics of Ets1-Runx1(K167A)-CBFβ-DNA complex.

Beam-line	NW12A
Wavelength	1.0000
Resolution (Å)	50.0-2.90 (3.00-2.90)
Space group	$P2_12_12_1$
Unit-cell (Å)	
a, b, c	78.5, 100.6, 194.4
Completeness (%)	97.8 (82.2)
$I/\sigma(I)$	32.1 (2.7)
R_{merge} (%)	0.059 (0.369)
Refinement Statistics	
Resolution range (Å)	42.36-2.90 (3.08-2.90)
No.reflections	34,189
R_{work}/R_{free}	0.219 (0.371) / 0.264 (0.395)
No. atoms	
Protein	5,790
DNA	1,218
Water	12
Rms Deviations	
Bond length (Å)	0.010
Bond angles (°)	1.3

Values in parentheses are for the outermost resolution shell.

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