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X-ray crystallographic analysis of Runx1-CBFβ-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.

To study how the enhancer complex is assembled, we focused on the T cell-enriched protein complex formed on TCRα enhancer that includes multiple transcription factors such as Runx1, Ets1, CBFβ, CREB, etc.

Those transcription factors bind to the TCRα enhancer in a highly synergistic manner. Detail of such molecular process is unknown but is thought to be quite complicated. For example, Ets1 activity is negatively regulated by the regulatory region (the exon VII) just beside the DNA binding domain (so called the ETS domain) and its phosphorylations. The DNA binding of Runx1 (so called the Runt domain) counteracts this intramolecular inhibition of Ets1 in a stereospecific manner.

To study how the transcription factor assembly is formed, we have been trying to perform crystallographic analyses for the Runx1-CBFβ-Ets1-DNA complex, which is thought to be the most important part of the complex formed on the TCRα enhancer. We have attempted to crystallize several kinds of complexes including various protein fragments.

Experiments and discussion

The several kinds of Runx1-CBFβ-Ets1-DNA complexes including different fragments of the components were crystallized all with a space group of $P2_12_12_1$ (fig 1).

Diffraction images were collected with synchrotron radiation at BL-17A in Photon Factory and processed using HKL2000. The diffraction images were quite anisotropic so that further optimization would be required for structural determination at a higher resolution. The data processing statistics are described bellow (table 1).

The phase was successfully calculated by the molecular replacement method using the crystal structure of the Runx1-CBFβ-DNA complex that we had previously reported, as a search model.

We have been also performing functional analyses such as EMSA (Electrophoresis Mobility Shift Assay), SPR (Surface Plasmon Resonance) and transactivation experiments, and we have found out that the synergistic effects would depend on what fragments of Ets1 or Runx1 were used. The functional aspects of this complex might be explained by the 3D structures of the complexes including different protein fragments.

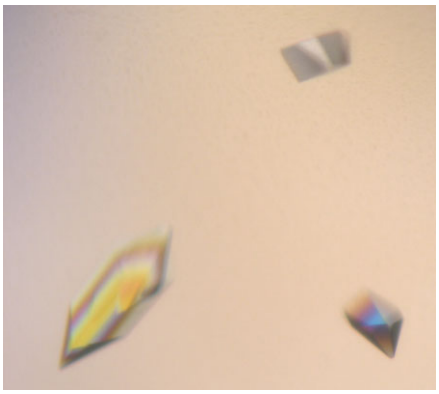

Fig.1: Crystals of Runx1-CBFβ-Ets1-DNA complex.

Table1: Diffraction data statistics of Runx1-CBFβ-Ets1-DNA complex.

Beam-line	BL-17A
Wavelength	1.0
Resolution (Å)	50-3.4
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	
a, b, c	78.7, 99.0, 194.2
No. of reflections	
Observed	147604
Unique	21075
Completeness (%)	98.0 (86.1)[#]
$I/\sigma(I)$	27.6 (13.6)[#]
R_{merge} (%)	7.5 (10.1)[#]

[#] Numbers in parentheses refer to data for high resolution outer shell 3.52-3.40 Å

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