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X-ray analysis of human Ets1

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Introduction

Ets1 is a transcriptional regulatory protein that plays important roles in differentiation of haematopoietic cells and is also involved in cancer development, especially tumor angiogenesis and metastasis. Ets1 is comprised of 441 amino acids including highly conserved ets domain which is responsible for DNA binding, and the flanking regulatory region, called exon VII, which controls DNA binding activity. Ets1 activity is regulated not only by the regulatory region (the exon VII) and its phosphorylation states but also by the presence of other transcriptional regulatory proteins. The molecular mechanism for this regulation remains unknown. Our aim is to elucidate the mechanism from the crystallographic approaches. We are engaged in crystallographic analyses for Ets1, phosphorylated Ets1 and Ets-containing higher ordered protein-DNA complex, an enhanceosome built on native enhancers.

Experiments and discussion

Human Ets1 fragment including the exon VII and ets domain has been crystallized with space group $P3_1$ (Fig 1). Diffraction images were collected with synchrotron radiation at BL5 and NW12 in Photon Factory and processed using HKL2000. The qualities of diffraction images were dramatically improved by post-crystallization treatments [1]. The data processing statistics are described bellow (table 1).

Fig 1. Crystals of the Ets1 fragment

The phase was calculated by the molecular replacement method using the crystal structure of the ets domain that we had already obtained as a search model.

This Ets1 fragment exhibited self-inhibited activity in electrophoretic mobility shift assay (EMSA) using some target DNA sequences. Because the shorter fragment of Ets1 without the exon VII didn't have such an inhibitory effect in EMSA, the exon VII might regulate the DNA binding activity of Ets1. Although the structure model is under refinement, this structure might explain the DNA binding regulation of the exon VII.

Table 1: Diffraction data statistics of Ets-1 (276-441)

Beam-line	NW12A
Wavelength	1.000
Resolution (Å)	50-2.6
Space group	$P3_1$
Unit-cell parameters (Å)	
a, b, c	57.5 57.5 106.9
No. of reflections	
Observed	64008
Unique	23473
Completeness (%)	99.2 (96.0)[#]
$I/\sigma(I)$	30.0(2.2)[#]
R_{merge} (%)	5.9 (32.5)[#]

[#] Numbers in parentheses refer to data for high resolution outer shell 2.69-2.60 Å

References

[1] Begaña Heras and Jennifer L. Martin, Acta Crystallogaphica Section D Biological Crystallography, D61, 1173-1180 (2005).

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