5A, NW12A/2005G265

X-ray crystallographic 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 phosphorylated states but also by the presence of other transcriptional regulatory proteins. The molecular mechanism for this regulation is still 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 a native enhancer.

Experiments and discussion

Human Ets1 fragment including the partial exon VII and ets domain was crystallized with space group $P3_1$ (Fig 1). The phosphorylated Ets1 was also crystallized with the same space group. 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).

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.

The Ets1(276-441) fragment including the exon VII inhibited its DNA binding and the phosphorylation of the exon VII by CaMKII enhanced the inhibition. The shorter fragment of Ets1(297-441), which lacks the exon VII in large part, didn't have such an inhibitory effect. Those were confirmed by EMSA.

Fig 1 shows a superimposed view of non-autoinhibitory Ets1(297-441) and autoinhibitory Ets1(276-441). Although the structural analyses were expected to reveal the molecular mechanism, the electron density of the exon VII was not clearly observed.

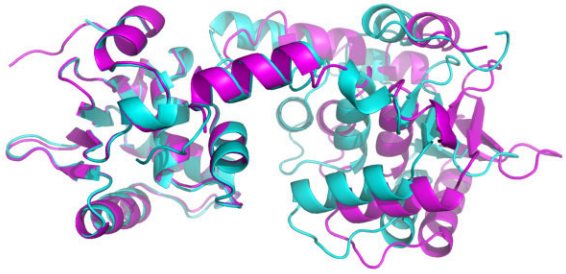
Further studies are required for uncovering how the exon VII and its phosphorylation regulate the Ets1 activity.

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

fig1: Superposition of Ets1(297-441)(***cyan***) and Ets1(276-441)(***magenta***) structures.



References

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