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Crystal structure analysis of a tandem array of two engrailed homeodomains

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1 Introduction

DNA-binding proteins with small molecular sizes are expected to utilize for molecular tools that include genome editing. The engrailed homeodomain (EHD) has a potential to become a molecular module, because it consists of about 60 amino acid residues and recognizes six base pairs. However, the 6bp length is not enough for targeting a particular site in a large genome sequence. Therefore, an array of two EHDs (EHD_2) connected by glycine linker was constructed to recognize a longer sequence [1]. Although wild-type EHD_2 unexpectedly binds to one EHD target sequence (6bp), the mutant $(EHD[R53A])_2$ can recognize two EHD target sequences (12bp). To investigate the molecular basis in the DNA recognition, we performed X-ray crystallographic analysis of $(EHD[R53A])_2$-DNA complex [2].

2 Experiment

The 12mer DNA with two EHD target sequences $(TAATCC)_2$ was used for preparing crystals of the $(EHD[R53A])_2$-DNA complex. Crystals of the $(EHD[R53A])_2$-DNA complex were obtained using the crystallization solution containing PEG4000 as a precipitant. X-ray diffraction experiment was performed at the BL-17A beamline and two diffraction data sets were collected with a single crystal at wavelengths of 0.98 Å and 1.90 Å (Table 1). The data set (λ = 0.98 Å) was used for structure determination by the molecular replacement method with the monomeric EHD structure (PDB; 2HDD) as a search model [3]. The other (λ = 1.90 Å) was used for building the model of the 12mer DNA based on the anomalous scattering from phosphate atoms in the backbone.

3 Results and Discussion

The final model consists of two EHD[R53A]s and one DNA duplex (Fig. 1). The order of two EHD[R53A]s could not be determined because the glycine linker of $(EHD[R53A])_2$ was not observed in the electron density map. The entire DNA molecule was modeled with a double conformation guided by the anomalous difference map. Approximately 5% decrease was achieved in both R_{work} and R_{free} factors when the DNA model was changed from a single conformation to the double conformation. The final R_{work} and R_{free} factors were converged to 18.8% and 23.5%, respectively.

One of the $(EHD[R53A])_2$ binds to the target region of DNA, but the other is not bound to the target. The target binding domain conserved the base-specific interactions with the target sequence as observed in the wild-type EHD [3]. The analysis of symmetry-related molecule in the crystal suggests that the crystal packing inhibited the binding to the target sequence for the non-target binding domain. On the other hand, the mutation and DNA-binding studies showed that both domains bind to the target sequence. Therefore, we consider that in solution both domains of the $(EHD[R53A])_2$ bind to the target sequence in a similar manner as observed in the target binding domain.

Table 1: Diffraction data statistics

Wavelength (Å)	0.98	1.90
Space group	$P2_1$	$P2_1$
a, *b*, *c* (Å)	39.8, 61.4, 40.0	39.8, 61.5, 40.0
α, β, γ (°)	90, 106.5, 90	90, 106.5, 90
Resolution (Å)	50-1.6	50-2.6
(highest shell)	(1.63-1.60)	(2.72-2.60)
Completeness (%)	99.9 (100.0)	99.0 (92.3)
Multiplicity	3.4 (3.5)	19.8 (17.5)
$\langle I/\sigma(I)\rangle$	15.7 (2.2)	94.9 (27.8)
R_{meas} (%)	3.4 (33.8)	7.7 (15.1)
$CC_{1/2}$	0.999 (0.921)	1.000 (0.997)

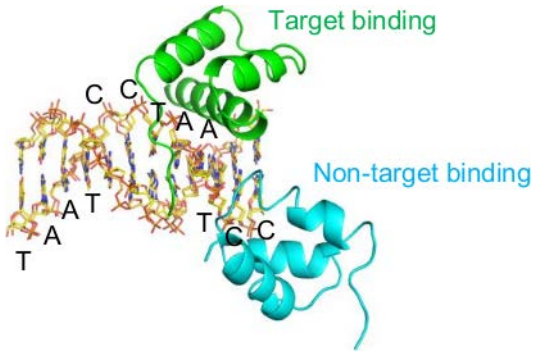

Fig. 1: Structure of the $(EHD[R53A])_2$-DNA complex

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References

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