AR-NW12A, AR-NE3A/2013G657

# Recognition of phosphorothioate DNA by transcription factor SATB1

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## 1. Introduction

DNAs containing phosphorothioate modifications (psDNAs) are shown to exhibit favorable properties for therapeutic applications [1]. It is known that this modification increases resistance to nucleases and improves cell penetration properties. In addition, it enhances binding affinities with DNA-binding proteins by two to three orders of magnitude. Due to these properties, psDNAs are used as decoy molecules that inhibit particular transcription factors related to diseases. However, there are only a few structural study focused on the specific interaction of psDNA with protein. In this study, we determined the crystal structure of the complex of psDNA and the N-terminal CUT domain (CUTr1) of special AT-rich sequence binding protein 1 (SATB1), and revealed stereospecific interaction of them. For this domain, we have observed an enhanced affinity to psDNA by an ITC experiment.

## 2. Experiment

DNA dodecamers containing racemic mixtures of phosphorothioate linkages (ps) 1 (5'-GCAT$_{ps}$A$_{ps}$T$_{ps}$A$_{ps}$TTAGC-3') and 2 (5'-G$_{ps}$C$_{ps}$TAATATATGC-3') were annealed and mixed with SATB1-CUTr1 domain at a molar ratio 1.5:1.0. Crystals of the complex were produced by sitting-drop vapor diffusion method at 293 K in 50 mM Tris-HCl (pH 8.5), 20% polyethylene glycol MME550, 20% ethylene glycol, and 10 mM $MgCl_2$. Diffraction data up to 1.79 Å and 2.3 Å resolution were obtained under a nitrogen gas stream at 95 K on beam lines AR-NW12A and AR-NE3A, respectively. The diffraction data from the two data sets were scaled and merged, and the structure was determined by a molecular replacement method using the structure of the complex of SATB1-CUTr1 and an unmodified DNA (PDB ID: 2O4A) [2].

## 3. Results and Discussion

The crystal structure of the SATB1-CUTr1/psDNA complex contained two protein molecules binding to the major groove of one double-stranded DNA molecule (Fig. 1). The comparison of this structure with the previous structure with an unmodified DNA showed that overall backbone structures of protein were not affected by the modification in DNA. On the other hand, the ends of the DNA strands were distorted in the modified DNA, probably due to the crystal packing.

The DNAs are mixtures of isomers with regard to the chiral centers at the phosphorothioate linkages, i.e., *S*p and *R*p. Accordingly, distinct electron densities for the isomers were observed at the modified sites, most remarkably at T6 (Fig. 2A). From the density map, occupancies of 0.38 and 0.62 were derived for the *S*p and *R*p isomers, respectively, which shows that the latter is preferable at this site. Noticeably, the SP1 atom of the *R*p isomer formed extensive hydrophobic interactions with protein and DNA atoms, i.e., Cβ of Ser389, Cβ of Gln390, Cβ of Ala391, Cγ of Gln402, and C7 of T6 (Fig. 2B). In the complex with the unmodified DNA, the equivalent site was occupied by a water molecule, which forms hydrogen bonds with the OP2 atom of T6 and the backbone N atom of Ala39 [2]. Thus, it is likely that the oxygen-to-sulfur substitution of T6 nucleotide contributes to the enhanced affinity between protein and DNA, through the favorable hydrophobic interactions.

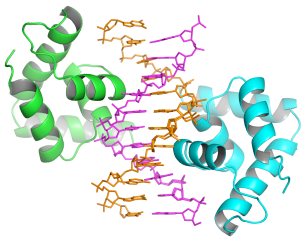

Fig. 1: Overall structure of the SATB1-CUTr1/psDNA complex. DNA strands 1 and 2 are shown in orange and magenta, respectively.

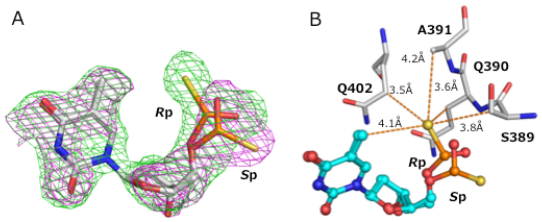


Fig. 2: (A) Omit maps contoured at 2.5 σ of the *R*p and *S*p isomers of phosphorothioate T6 nucleotide, shown in green and magenta, respectively. (B) The hydrophobic interactions between the SP1 atom of the *R*p isomer and the protein and DNA atoms, as indicated by dashed lines.

## References

[1] C. A. Stein, *Chem. Biol.* **3**, 319 (1996).
[2] K. Yamasaki *et al.*, *Nucleic Acids Res.* **35**, 5073 (2007).

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