Crystal structure of the 8 bp-specific restriction enzyme SwaI

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INTRODUCTION

SwaI, a Type IIP restriction enzyme from *Staphylococcus warneri* cleaves the symmetric sequence ATTT|AAAT, producing fragments with blunt ends ('|' = cleavage site). We solved the crystal structure of SwaI alone, of SwaI bound to uncleaved DNA in the presence of Ca^{2+} ions, and of SwaI bound to cleaved DNA in the presence of Mg^{2+} ions. We describe these structures, and compare them to that of PacI, which cleaves the related 8-bp sequence, TTAAT|TAA.

SwaI sequence homologs

SwaI is a homodimer of 2x226-aa. Sequence comparisons using NCBI BLAST produced only four significant hits (E value > 10^{-60}), all to hypothetical proteins from bacteria (Fig. 1)

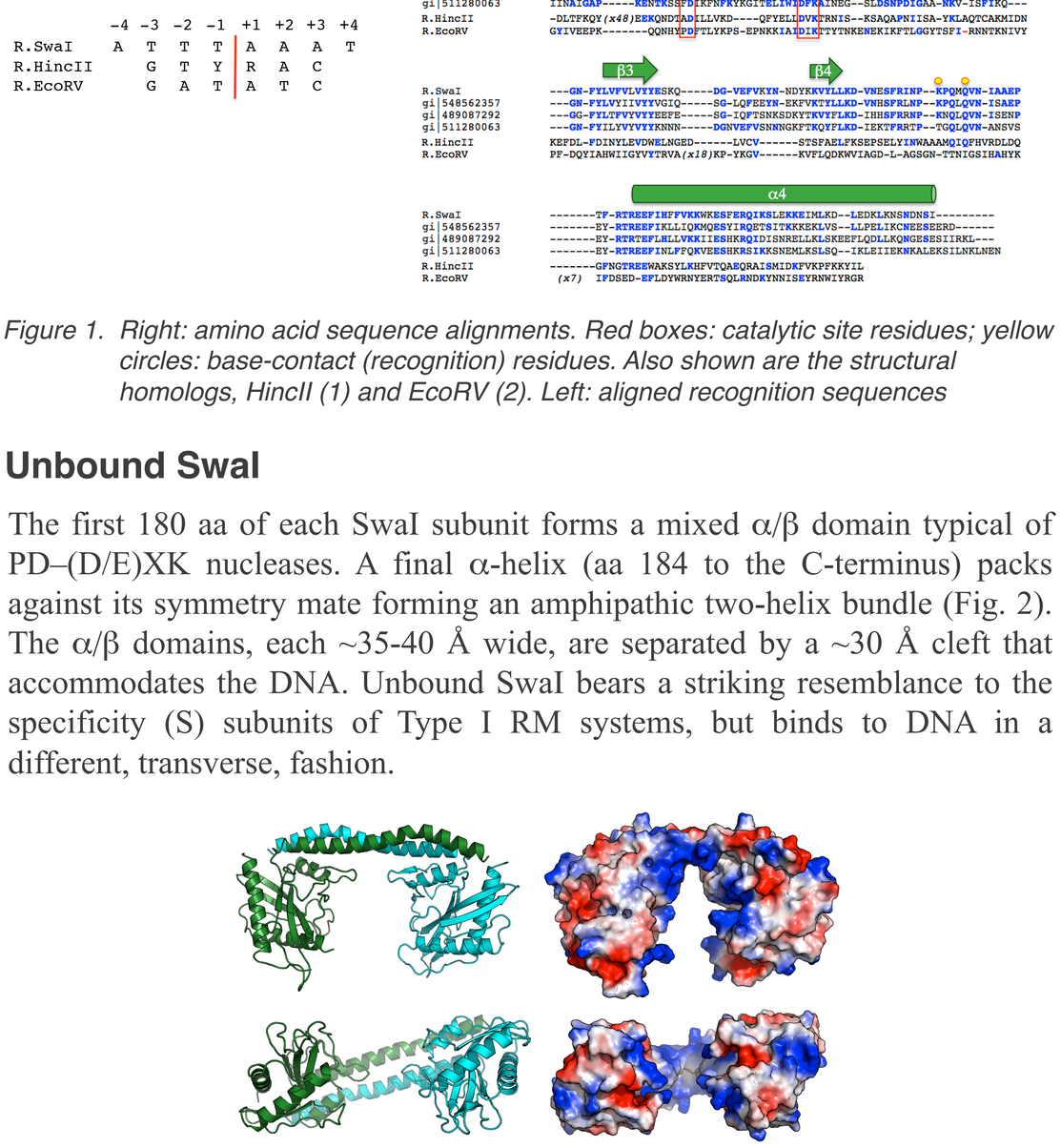


Figure 1. Right: amino acid sequence alignments. Red boxes: catalytic site residues; yellow circles: base-contact (recognition) residues. Also shown are the structural homologs, HincII (1) and EcoRV (2). Left: aligned recognition sequences

Unbound SwaI

The first 180 aa of each SwaI subunit forms a mixed α/β domain typical of PD–(D/E)XK nucleases. A final α-helix (aa 184 to the C-terminus) packs against its symmetry mate forming an amphipathic two-helix bundle (Fig. 2). The α/β domains, each ~35-40 Å wide, are separated by a ~30 Å cleft that accommodates the DNA. Unbound SwaI bears a striking resemblance to the specificity (S) subunits of Type I RM systems, but binds to DNA in a different, transverse, fashion.

Figure 2. SwaI without DNA. Cartoon representation (left) and surface representation (right. Red: electronegative, blue: electropositive)

Bound SwaI

SwaI undergoes a significant conformational change upon binding to the recognition sequence, largely through bending of the C-terminal helices. The gap between the paired α/β domains closes as the protein wraps around the DNA and contacts the phosphodiester backbone, and the bases in both DNA grooves (Fig. 3a). This is accompanied by a sharp bend at the center of the DNA, narrowing the major groove across the central four base pairs and widening the minor groove (Fig. 3b)

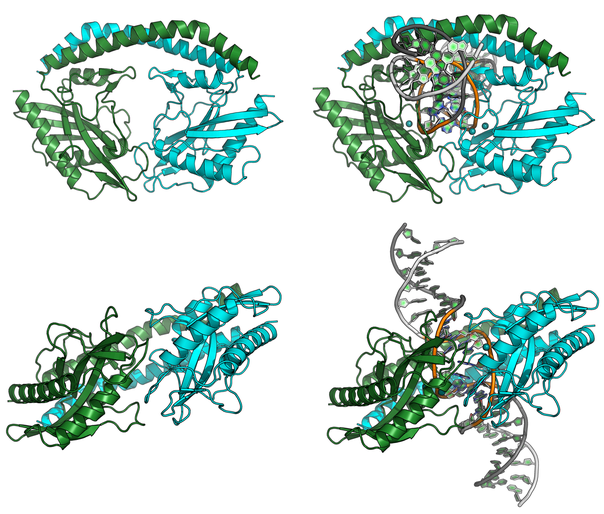
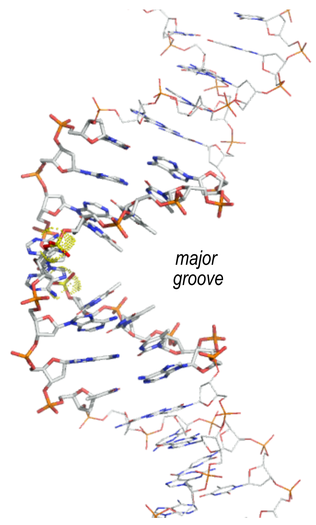


Figure 3a. SwaI bound to specific DNA. In the left panels, the DNA is hidden for clarity



3b. SwaI-bound DNA ('side' view; protein hidden for clarity)

SwaI catalytic site

The catalytic site of SwaI belongs to the PD-(D/E)XK superfamily (3), and comprises W75-D76…D93-F94-K95 (red boxes, Fig. 1). Two metal ions are present and several water molecules, one of which is positioned and oriented to act as the nucleophile in conjunction with the general base, K95 (Fig. 4). Mutants D76A, D93A and K95A were constructed and found to be catalytically inactive, supporting the functional roles of these aa.

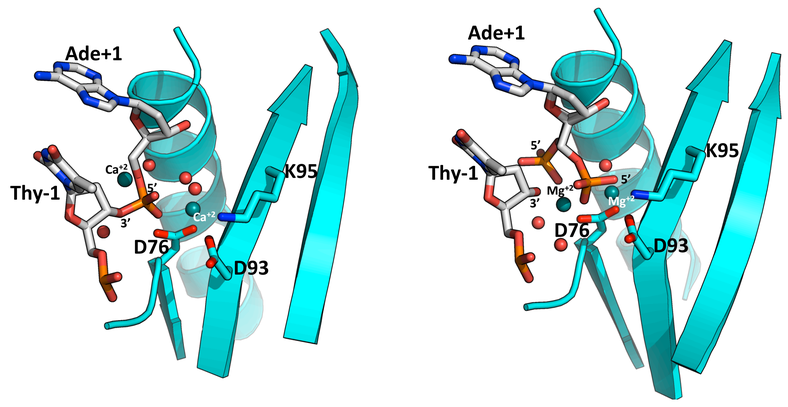


Figure 4. SwaI catalytic site. Left: uncleaved DNA in the presence of Ca^{2+} ions. Right: cleaved DNA in the presence of Mg^{2+} ions

DNA Recognition

Sequence recognition by SwaI is 'economical' in the extreme. N105 and Q170 (major groove) with K72 (minor groove) contact the outer base pairs, 4 and 3. D107 and K166 (major groove) with R35 (minor groove) contact the inner base pairs, 2 and 1 (Fig. 5, and yellow circles, Fig. 1).

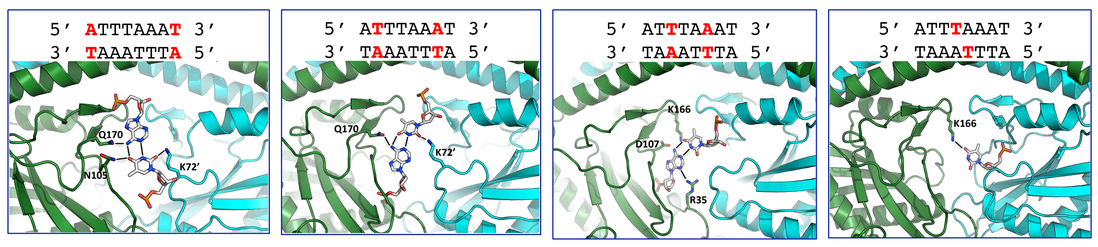


Figure 5. Amino acid contacts at (left to right) base pairs 4, 3, 2, and 1

A dramatic disruption of the central base pairs accompanies DNA binding. A:T bp +1 and -1 un-pair, and the adenines switch positions in the helix (Fig 6). Instead of forming H-bonds with amino acids, the two adenines sandwich between the side chains of R35a and b, and participate in cation-p interactions

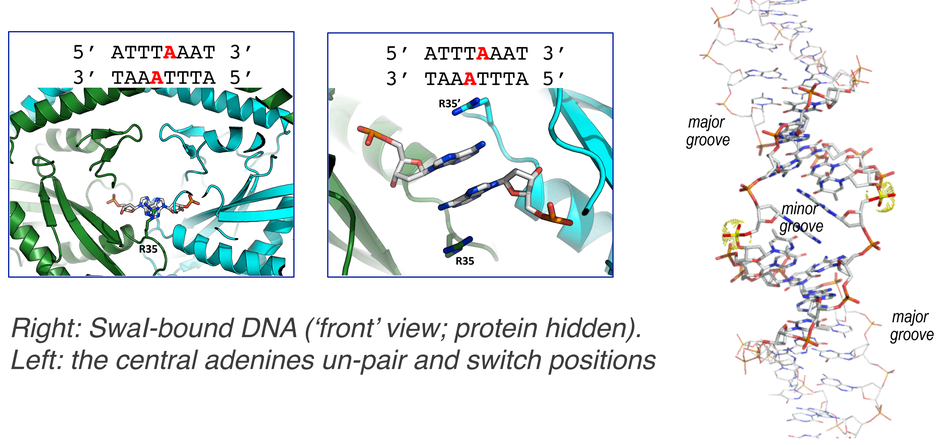


Figure 6. Right: SwaI-bound DNA ('front' view; protein hidden). Left: the central adenines un-pair and switch positions

Comparison with PacI

SwaI is horseshoe-shaped and encircles the DNA (Figs. 2 and 3). PacI (2x142 aa) is smaller and elongated (4), and track along the major groove (Fig. 7). Both enzymes distort their target sequences upon binding, but in different ways

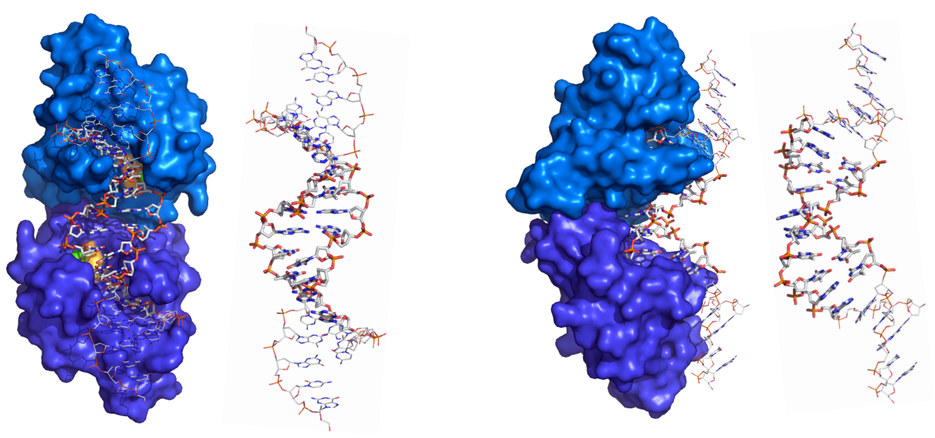


Figure 7. PacI (TTAAT/TAA) co-crystal structure. Left panels: 'front' view of complex, and of the bound DNA with protein hidden. Right panels: 'side' view of the same (4)

REFERENCES

1. Etzkorn, C. and Horton, N.C. (2004). *J. Mol. Biol.*, **343**, 833-849.
2. Winkler, F.K., Banner, D.W., Oefner, C., Tsernoglou, D., Brown, R.S., Heathman, S.P., Bryan, R.K., Martin, P.D., Petratos, K. and Wilson, K.S. (1993. *EMBO J.*, **12**, 1781-17945.
3. Kosinski, J., Feder, M. and Bujnicki, J.M. (2005). *BMC Bioinformatics*, **6**, 172.
4. Shen, B.W., Heiter, D.F., Chan, S.H., Wang, H., Xu, S.Y., Morgan, R.D., Wilson, G.G. and Stoddard, B.L. (2010). *Structure*, **18**, 734-743.