

heteroduplex DNA by branch-migration. HJ-resolving enzymes mediate the resolution of the 4-way junction by introducing symmetrical nicks in opposing strands. Members of this ubiquitous family of structure-specific endonucleases function as dimers and require divalent cations for cleavage. Phage T4 endonuclease VII (EndoVII) is involved in mismatch repair and the resolution of branch points prior to packaging of DNA into the phage head. In contrast to other resolvases, such as Cce1 or RuvC, it exhibits a rather broad substrate specificity ranging from 4-way junctions to single base mismatches. We have solved the crystal structure of an inactive mutant of Endo VII in complex with a synthetic HJ with arms of varying length at 3.2Å resolution. Both the protein and the DNA junction undergo significant conformational changes on binding. The overall geometry of the junction corresponds to the stacked-X type conformation. However, the "stacked" arms form an angle of 136°, the bases at the cross-over point are unstacked, and residues of the central helices of the EndoVII dimer cause a substantial opening of the cross-over region of the junction. Residues at the active site and a bound Mg²⁺ ion are arranged such, that cleavage would occur at the preferred cutting sites, two base-pairs 3' of the cross-over point in the exchanging strands. The geometry of the active site is consistent with the previously proposed catalytic mechanism [1].

[1] Raaijmakers, H., Törö, I., Birkenbihl, R., Kemper, B. & Suck, D. *J.Mol.Biol.*, 2001, 308, 311-323.

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Thioredoxins (Trxs) are protein disulfide reductases found ubiquitously and acting as electron donors for redox enzymes and as regulators of activity of various proteins including transcription factors and photosynthetic enzymes. Despite their biological importance, the mechanism of substrate recognition by Trxs is poorly understood. By mutating Trx and target protein disulfides and activating the remaining target protein cysteine with TNB to form a disulfide, we have been able to stabilize a mimic of the reaction intermediate: a mixed disulfide Trx-substrate protein complex [1]. The substrate protein here being an *in vitro* substrate is also a potential *in vivo* substrate - the barley α -amylase/subtilisin inhibitor (BASI). The crystal structure reveals interactions between a conserved hydrophobic motif in Trx and a sequence of residues from BASI, which suggests that recognition of features around protein disulfides plays an important role for the substrate specificity of Trx. Comparison with glutaredoxin and glutathione transferase structures indicates that these proteins use a mechanism similar to the Trx mechanism to position the substrate in the reactive site. It also reveals a unique structural element in Trx that allows recognition of protein disulfides rather than glutathione. Crystal structures of barley Trx h1 and h2 isozymes show that the isozymes expose different putative protein-protein interaction interfaces and that a functional differentiation among the Trx isoforms could exist in barley.

[1] Maeda K., Häggglund P., Finnie C., Svensson B., Henriksen A. *Structure*, 2006, 14, 1701.