Metal ions bound at the active site of the junction-resolving enzyme T7 endonuclease I

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Homologous genetic recombination is important in the repair of double-strand breaks in DNA, in the rescue of stalled replication forks, and in the creation of genetic diversity. The central intermediate in this process is the four-way (Holliday) DNA junction. This must be ultimately resolved by nucleases that are selective for the structure of the junction.

Bacteriophage T7 DNA undergoes genetic recombination during infection. The phage-encoded junction-resolving enzyme is endonuclease I. Mutants in the gene encoding this enzyme are deficient in recombination and accumulate branched DNA intermediates.

We have recently presented the crystal structure of a defective mutant of T7 endonuclease I (E65K). The putative active site contains three acidic side chains (Glu 20, Asp 55 and Glu 65) and a lysine (Lys 67). These correspond closely to the active site residues of a number of type II restriction enzymes particularly *Bgl*I. The crystal structure of *Bgl*I was solved as a complex with a DNA oligonucleotide, and two calcium ions were observed bound in the active site. We have therefore proposed that two metal ions are bound in the active site of T7 endonuclease I, and that both participate in the cleavage of the phosphodiester backbone.

In this study we have sought direct evidence for the binding of metal ions to the active site of wild-type T7 endonuclease I. None was observed in our earlier crystal structure of the enzyme, but this may be a result of the E65K mutation that inverted the electrostatic charge of one of the active site residues. We have now solved the crystal structure of the wild-type protein, with and without bound manganese ions.

The crystal structure of the wild-type protein in the absence of bound manganese is very similar to that of the E65K mutant. However, electron density maps clearly showed that lysine 65 had been substituted by glutamic acid. Diffusion of manganese ions into the crystals of the wild-type protein revealed two peaks of electron density per active site, defining two metal ion binding sites. These peaks were situated in similar positions to the calcium ions found in the *Bgl*I structure (Figure1). The manganese ion located near Ca1 is fully occupied and is co-ordinated by three protein ligands, whilst that located near Ca2 appears partially occupied, and is co-ordinated by only one protein ligand.

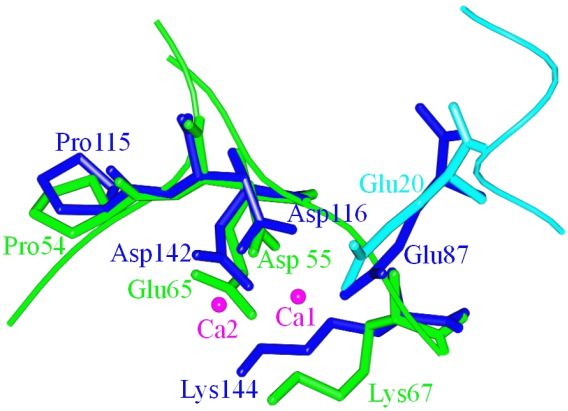

Figure 1
Superimposition of T7 endonuclease I wild-type and the restriction enzyme *Bgl*I in the vicinity of the active site. Amino acids from endonuclease I are illustrated in green and light blue whilst those from *Bgl*I are shown in dark blue. The calcium ions in the *Bgl*I structure are depicted in pink.

In the light of these results we can refine our view of the probable mechanism of endonuclease I. At this time, we have insufficient evidence to propose a complete mechanism, but we can identify some probable

components. It is highly likely that the hydrolytic water molecule is co-ordinated by the metal ion bound in site 1, which would need to be located correctly to attack in-line. A number of potential functions could be ascribed to the metal ion bound to site 2. This could carry out electrophilic catalysis, possibly in conjunction with Lys 67, helping to stabilise the negatively charged transition state. It could polarize the P-O bond, and a co-ordinated water molecule could act as a general acid to protonate the leaving group.

Ultimately the structure of an endonuclease I - DNA complex will probably be required before we can conclusively identify the full mechanism by which endonuclease I hydrolyses specific phosphodiester bonds within the four-way DNA junction, but the major functionalities are probably now identified.

References

Hadden, J.M., Convery, M.A., Déclais, A-C., Lilley, D.M.J. & Phillips, S.E.V. (2001). Crystal structure of bacteriophage T7 endonuclease I: A Holliday junction resolving enzyme. *Nature Structural Biology*, **8**, 62-67.

Déclais A.C, Hadden J.M, Phillips S.E.V & Lilley D.M. (2001). The active site of the junction-resolving enzyme T7 endonuclease I. *J. Mol. Biol*., **307**, 1145-58.

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