

P022 Three metal ions participate in the reaction catalysed by T5FEN

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Flap endonucleases (FENs) are a family of divalent metal ion dependent nucleases, which are present in all kingdoms of life. They catalyse the hydrolysis of phosphodiester bonds, an essential activity in DNA replication and repair, and accelerate the reaction by a factor of 10^{17} . The mechanism of phosphodiester bond hydrolysis is currently highly debated, with a two-metal-ion mechanism the most commonly proposed. However, substrate free structures of FENs reveal two active site metal ions separated typically by $>4\text{\AA}$ which is incompatible with the mechanism.

To elucidate the roles of metal ions within the FEN reaction a detailed evaluation of the magnesium ion response of T5FEN was performed. The investigation revealed that overall the wild-type T5FEN catalysed reaction requires at least three magnesium ions, implying that an additional ion is bound in the presence of substrate. Mutation of carboxylates that ligand metal site two altered the magnesium ion requirement for substrate association but not catalysis. Analyses of calcium inhibition reactions were consistent with a requirement for two viable cofactors (Mg^{2+} or Mn^{2+}). This makes it plausible that FEN derives its major rate acceleration from a two-metal-ion-mechanism but that its overall metallobiochemistry is more complex and requires the action of three metal ions.