

P016 FEN endonuclease and exonuclease activities are rate limited by different steps

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Flap endonucleases (FENs) are essential for genomic stability as they catalyse the exonucleolytic and structure-specific endonucleolytic hydrolysis of nucleic acids at key steps of metabolism. The FEN enzyme family is highly conserved, and FEN gene mutations result in cancer development.

T5FEN reactions were monitored using fluorescently labelled substrates, followed by separation of products by denaturing HPLC and product quantification. Leaving group pK_a had little effect on single-turnover (k_{ST}) or overall (k_{cat}/K_M) reaction rate. The effects of changing solvent viscosity on k_{cat}/K_M were quantified by addition of sucrose. Exonuclease activity at pH 9.3 was diffusion controlled, implying that steps following FEN-substrate association are faster than the dissociation rate. At pH 6.5 the exonucleolytic reaction was partially diffusion controlled, suggesting FEN-substrate dissociation occurred at similar rate as forward steps. In contrast, the endonuclease k_{cat}/K_M was independent of solvent viscosity, likely due to the additional step accommodating the 5'-single stranded flap being slower than diffusional encounter. These data help explain a frequent FEN mutational phenotype, such as those associated with human cancer, where altering the DNA binding site produces much greater effects on exonucleolytic rather than endonucleolytic activity.