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Studies of Tyrosyl-DNA Phosphodiesterase: Vanadate as a Tool for Exploring Mechanism, Macromolecular Assemblies and Inhibitor Design. D.R. Davies, H.I. Interthal, J.J. Champoux, W.G.J. Hol, Dept. of Biochemistry, Univ. of Washington, Seattle, WA 98195 USA.

Tyrosyl-DNA phosphodiesterase (Tdp1) catalyzes the hydrolysis of a phosphodiester bond between a tyrosine residue and a DNA 3' phosphate and functions as a DNA repair enzyme that cleaves stalled topoisomerase I-DNA complexes. We have crystallized a quaternary complex containing Tdp1, vanadate, a DNA oligonucleotide, and a tyrosine-containing peptide that mimics the transition state for hydrolysis of the Tdp1 substrate. This structure provides insights into the nature of the macromolecular assembly formed during DNA repair between Tdp1, DNA, and a fragment of topoisomerase I. The ability of vanadate to accept a variety of different ligands has been further exploited to produce several different quaternary complexes with a variety of oligonucleotides, and peptides or a tyrosine analog, in efforts to explore the binding properties of the Tdp1 DNA and peptide binding clefts. The versatility of this system suggests that the formation of quaternary complexes around vanadate could be adapted to become a useful method for structure-based inhibitor design, and has the potential to be generally applicable to other enzymes that perform chemistry on phosphate esters.

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