Structural Investigations of Tyrosyl-DNA Phosphodiesterase (Tdp1)

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INTRODUCTION

Tyrosyl-DNA phosphodiesterase (Tdp1) is a DNA repair enzyme that catalyzes the hydrolysis of a phosphodiester bond between a tyrosine residue and a DNA 3' phosphate. The only known example of such a linkage in eukaryotic cells occurs normally as a transient link between a type IB topoisomerase and DNA. Thus human Tdp1 is thought to be responsible for repairing lesions that occur when topoisomerase I becomes stalled on the DNA in the cell. The structure of hTdp1 is a monomer composed of two similar domains that are related by a pseudo-twofold axis of symmetry (Figure 1) [1]. Each domain contributes conserved histidine and lysine residues to form a single active site. The tertiary structure and active site composition of Tdp1 confirm the earlier conclusion that that the protein is a member of the phospholipase D (PLD) superfamily [2]. Tdp1 appears to catalyze phosphodiester bond cleavage by a mechanism similar to that of other PLD superfamily members, where a conserved histidine residue acts as a nucleophile in the first step of the enzymatic reaction. Tdp1 has an asymmetrical, elongated substrate-binding cleft that appears well suited to binding a protein moiety on one side of the cleft and a single strand of DNA on the other.

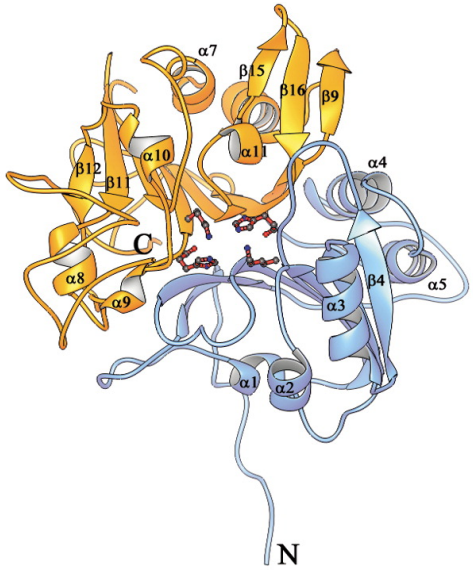

Figure 1. Ribbon Representations of the Structure of Human Tdp1, colored to show the two-domain structure of the protein. The N-terminal domain of Tdp1 (residues 149–350) is colored blue, and the C-terminal domain (residues 351–608) is colored yellow. The view is parallel to the pseudo-2-fold axis of symmetry between the domains. The active site residues His263, Lys265, His493, and Lys495 are depicted as red ball and stick structures.

INSIGHTS FROM VANADATE TRANSITION STATE MIMICS

We have reported the three-dimensional structures of human Tdp1 bound to the phosphate transition state analogs vanadate and tungstate [3]. Each structure showed the inhibitor covalently bound to His263, confirming that this residue is the active nucleophile in the first step of the catalytic reaction. Vanadate in the Tdp1-vanadate structure adopted trigonal bipyramidal geometry that mimics the transition state for hydrolysis of a phosphodiester bond, while Tdp1-tungstate displayed unusual octahedral coordination. In both cases, glycerol from the cryoprotectant solution became liganded to the vanadate or tungstate inhibitor molecules in a bidentate 1,2 diol fashion (Figure 2A). Attempts to co-crystallize Tdp1 with vanadate and single-stranded oligonucleotides yielded similar structures of vanadate-glycerol complexes when glycerol was used as a cryoprotectant, but no DNA could be seen in the electron density maps. Most likely, the formation of complexes of Tdp1, vanadate and substrates was hampered by interference from glycerol. Since the hydroxyl groups of glycerol might have been competing with the 3'OH of the DNA oligonucleotides as ligands for vanadate, subsequent co-crystallization experiments employed a cryoprotectant lacking free hydroxyl groups, PEG 250 dimethyl ether.

Despite the complexity of the substrate of this phosphodiesterase, vanadate succeeded in linking human Tdp1, a tyrosine-containing peptide and a single-stranded DNA oligonucleotide into a quaternary complex that mimics the transition state for the first step of the catalytic reaction [4]. In a single glace, this structure revealed the substrate binding mode and the mechanism of the first catalytic step for the enzyme (Figure 2B). Single-stranded DNA is bound in the narrow, positively charged groove of the substrate-binding cleft, although uncharged polar side chains are largely responsible for specific interactions with the DNA. The peptide moiety is bound in the larger, more open half of the substrate-binding cleft, but is held in place with very few specific hydrogen bonds. The trigonal bipyramidal geometry of the vanadate moiety is consistent with the transition state of an S_N2 nucleophilic attack on phosphate, where the tyrosine-containing peptide is the leaving group.

These structures illustrate the versatility and utility of vanadate as a tool for the exploration of mechanism, substrate binding, and inhibition of phsophoryl-transfer enzymes. In all cases, vanadate complexes self-assembled during co-crystallization of Tdp1 with vanadate and its ligands. To our knowledge, the quaternary structure consisting of Tdp1, vanadate, ssDNA, and the topoisomerase I-derived peptide is the largest and most complex structure of its kind solved to date.

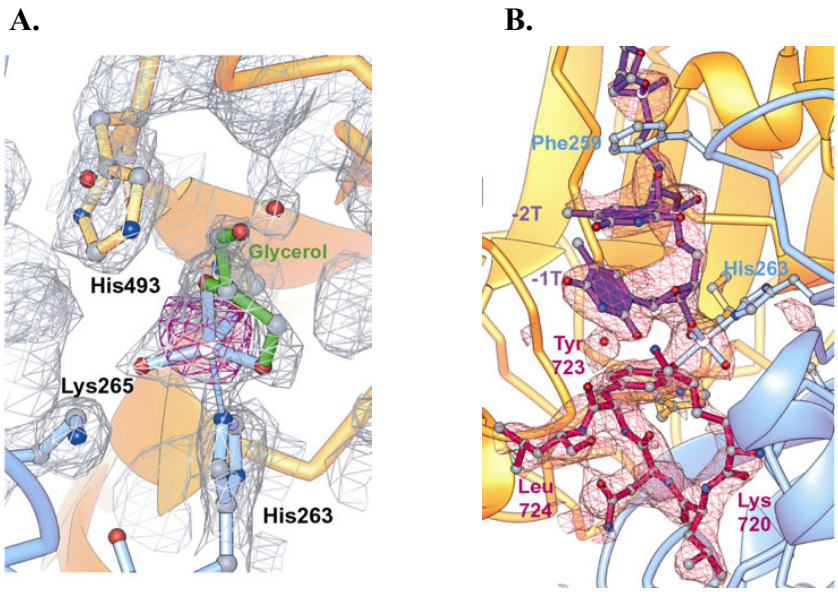

Figure 2. Two structures that illustrate the versatility of vanadate for assembling transition state mimics for Tdp1. (A) Electron density associated with the vanadate-glycerol complex in the active site of human Tdp1. The Tdp1-vanadate structure is colored as in Fig. 1. The vanadium atom is colored white and bonds for the glycerol moiety are depicted in green. Gray contours represent the 1.5 sigma level for the final $2F_o$-F_c electron density map. Purple contours represent the 2.0 sigma level of the anomalous difference map, showing experimental confirmation of the presence of vanadium in the complex. (B) Difference electron density for the Tdp1-vanadate-peptide-DNA complex. His263, Lys265, His493, Lys495 and Phe259 are displayed as ball-and-stick structures. The substrate analog is also displayed in ball-and-stick, with the bonds of the peptide moiety in magenta, the vanadate in blue, and the DNA in purple. The topoisomerase I-derived peptide follows the numbering system of the full-length topoisomerase I enzyme. Contours represent the 2.5-sigma level in the initial F_o-F_c electron density map calculated for the molecular replacement solution with apo-Tdp1 used as the search model.

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