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Mutation of a Conserved Active Site Residue Converts Tyrosyl-DNA Phosphodiesterase I into a DNA Topoisomerase I-dependent Poison

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Tyrosyl-DNA phosphodiesterase 1 (Tdp1) catalyzes the resolution of 3′ and 5′ phospho-DNA adducts. A defective mutant, associated with the recessive neurodegenerative disease SCAN1, accumulates Tdp1–DNA complexes *in vitro*. To assess the conservation of enzyme architecture, a 2.0 Å crystal structure of yeast Tdp1 was determined that is very similar to human Tdp1. Poorly conserved regions of primary structure are peripheral to an essentially identical catalytic core. Enzyme mechanism was also conserved, because the yeast SCAN1 mutant ($H_{432}R$) enhanced cell sensitivity to the DNA topoisomerase I (Top1) poison camptothecin. A more severe Top1-dependent lethality of Tdp1H_{432}N was drug-independent, coinciding with increased covalent Top1–DNA and Tdp1–DNA complex formation *in vivo*. However, both H_{432} mutants were recessive to wild-type Tdp1. Thus, yeast H_{432} acts in the general acid/base catalytic mechanism of Tdp1 to resolve 3′ phosphotyrosyl and 3′ phosphoamide linkages. However, the distinct pattern of mutant Tdp1 activity evident in yeast cells, suggests a more severe defect in Tdp1H_{432}N-catalyzed resolution of 3′ phospho-adducts.

Keywords: camptothecin; DNA topoisomerase I; enzyme mechanism; Tdp1; X-ray crystallography

Introduction

The enzyme tyrosyl-DNA phosphodiesterase 1 (Tdp1), initially reported to selectively hydrolyze a phosphotyrosyl linkage formed at the 3′ end of DNA,[1] is encoded by the *TDP1* gene and is functionally conserved from yeast to humans.[2,3] In eukaryotes, 3′tyrosyl-DNA adducts arise from the catalytic activity of DNA topoisomerase I (Top1), which unwinds DNA in advance of replication forks and transcription complexes. Top1 transiently cleaves one strand of duplex DNA *via* the nucleophilic attack of the active site Tyr on the DNA phosphodiester backbone to yield a 3′phosphotyrosyl bond.[4–6] The free 5′OH then rotates around the non-scissile strand. The short-lived covalent Top1-DNA intermediate is readily reversed by a second transesterification reaction in which the 5′OH acts as a nucleophile to religate the DNA. Top1 plays a critical role in DNA metabolism, but the covalent Top1-DNA intermediate can be converted into DNA lesions that can trigger cell-cycle arrest and cell death. Camptothecin (CPT) targets Top1 by reversibly stabilizing the enzyme–DNA intermediate, and several CPT analogs are effective

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Abbreviations used: Tdp1, tyrosyl-DNA phosphodiesterase; Top1, topoisomerase I; CPT, camptothecin; 3′PG, 3′phosphoglycolate; SCAN1, spinocerebellar ataxia with axonal neuropathy; MAD, multiple anomalous dispersion; ssDNA, single-strand DNA.

chemotherapeutics.[5,7,8] During S-phase, the collision of advancing replication forks with CPT–Top1–DNA complexes converts the enzyme-linked DNA breaks into potentially lethal DNA damage. Other DNA anomalies, such as oxidative/UV damage, nicks, mismatches or *cis*platin adducts have been shown to stabilize Top1–DNA complexes,[9–11] thereby increasing the likelihood of cytotoxic lesions. The ability of Tdp1 to resolve 3′phosphotyrosyl linkages suggested a role for the enzyme in protecting cells against cytotoxic Top1–DNA lesions.

The potential of Tdp1 to reduce the efficacy of CPT-based drugs generated considerable clinical interest, but subsequent studies have failed to establish Tdp1 as a significant determinant of cell sensitivity to CPT. In the absence of other genetic alterations, deletion of *TDP1* (*tdp1*Δ) has little effect on yeast cell sensitivity to Top1 poisons, and Tdp1 protein levels do not predict tumor response to CPT analogs.[12–17] Nevertheless, it has been demonstrated that *tdp1*Δ cells are hypersensitive to elevated levels of covalent Top1–DNA complexes induced by a self-poisoning Top1T_{722}A mutant, and to CPT when the cells express high levels of wild-type Top1.[13,18] In addition, over-expression of Tdp1 in mammalian cell lines confers some resistance to CPT.[19,20] The former study demonstrated a low level of Tdp1-dependent resistance to the DNA topoisomerase II (Top2) targeted drug etoposide, and this is consistent with our recent report that Tdp1 catalyzes the removal of a Top2 peptide covalently attached to DNA by a 5′phosphotyrosyl linkage.[21] Thus, Tdp1 may participate in the repair of Top1 and Top2-induced DNA damage. Additional studies have expanded the spectrum of potential Tdp1 substrates to include a 3′phosphohistidyl bond, such as that induced by mutant Tdp1 covalently linked to DNA, as well as 3′phosphoglycolates (3′PGs), which can be induced by oxidative damage, ionizing radiation or radiomimetic drugs such as bleomycin.[22–24] Although contradictory reports exist about the relative efficiency of yeast Tdp1-catalyzed resolution of 3′ phosphotyrosyl *versus* 3′PG termini,[14,22] these results nevertheless suggest a more general role for Tdp1 in resolving a variety of 3′ and 5′ phospho-DNA adducts.

Based on the presence of two conserved HKD motifs (H*x*K$(x)_4$D), it was suggested that Tdp1 is a member of the phospholipase D superfamily,[2] and this was confirmed by the crystal structure of the human enzyme (hTdp1).[25] Subsequent structural analyses of two complexes helped to clarify the substrate specificity and catalytic mechanism of hTdp1.[26,27] The adduct is released from DNA by a ping-pong reaction involving two sequential nucleophilic attacks by the paired His residues within the two HKD motifs (His263 and His493 in hTdp1).[2,26,28] This catalytic mechanism proved significant when it was shown that a homozygous mutation of His493 to Arg in hTdp1 (h*tdp1*$H_{493}R$) is the molecular basis of the neurodegenerative disease spinocerebellar ataxia with axonal neuropathy (SCAN1).[29] Subsequent studies designed to understand the implications for DNA repair in long-lived neuronal cells that exhibit essentially no replication, revealed that human Tdp1 is a component of a large complex that mediates single-strand DNA (ssDNA) break repair.[30–32] SCAN1 cell lines accumulate more CPT or H_2O_2-induced DNA damage compared to wild-type Tdp1 or heterozygous cell lines.[31]

Despite extensive studies of hTdp1, little is known of the role that Tdp1 may play in maintaining genome stability and in affecting DNA repair. We therefore chose to exploit the facile genetics of the yeast *Saccharomyces cerevisiae* to address outstanding questions of enzyme mechanism and function *in vivo*.[7] To provide a basis for these studies and to enable detailed comparisons to hTdp1, we determined the crystal structure of yeast Tdp1 (yTdp1) to 2.0 Å resolution. Although hTdp1 and yTdp1 have little sequence similarity outside the active site, the two structures are generally very similar. We also report that expression of the yeast SCAN1 mutant (y*tdp1*$H_{432}R$) enhanced cell sensitivity to the Top1 poison CPT. In addition, the consequences of mutating His432 to Asn in yTdp1H_{432}N are even more severe, but only in the context of elevated Top1 levels, consistent with the increased formation of covalent Top1–DNA and Tdp1–DNA complexes *in vivo* that induce potentially cytotoxic lesions. In contrast, neither of the His432 mutants enhanced the cytotoxicity of bleomycin. These *in vivo* findings suggest a restricted role for His432 in the general acid/base catalytic mechanism of Tdp1 in the resolution of 3′ phosphotyrosyl adducts, but not 3′ phosphoglycolates induced by bleomycin.

Results

Domain analysis, crystallization and structure analysis

During crystallization trials of full-length yTdp1, protein degradation was observed from the N terminus, but limited trypsin digestion revealed that the N-terminal 67 residues could be removed to generate a stable molecule. This truncated protein (Tdp1-Δ1) was crystallized and the structure solved to 2.8 Å resolution using selenomethionine-based multiple anomalous dispersion (MAD) methods. The structure revealed that 11 and five additional residues could be removed from the N terminus and the C terminus, respectively, and Tdp1-Δ2 yielded superior crystals that generated the final 2.0 Å structure. Data collection, phasing and refinement statistics are presented in Table 1. There are four Tdp1 molecules in the P1 asymmetric unit and they are identical apart from loop regions. All four molecules contain five peripheral regions that are not visible in the electron density map and are presumably disordered (Figure 1(a) and (b)).