Structural Impact of the Leukemia Drug 1-β-D-Arabinofuranosylcytosine (Ara-C) on the Covalent Human Topoisomerase I-DNA Complex

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Human topoisomerase I solves the DNA topological problems that arise from a wide variety of nuclear processes including replication, transcription, and recombination (1, 2). The enzyme nicks one strand of duplex DNA using a transesterification reaction that produces a transient 3’-phosphotyrosine linkage and guides the relaxation of either positive or negative superhelical tension by a proposed “controlled rotation” mechanism (3). The enzyme then catalyzes a second transesterification, stabilizing the covalent protein-DNA complex and trapping the enzyme on DNA (7, 8). In this way, CPT converts topoisomerase I into a cellular poison. Human topoisomerase I is also affected by several forms of DNA damage, including abasic lesions, wobble base pairs, and base pair mismatches (9–13). Such lesions can impact each stage of topoisomerase I’s catalytic cycle, including DNA binding, single-strand DNA cleavage, and religation.

1-β-D-Arabinofuranosylcytosine (Ara-C)1 is a potent antineoplastic drug used in the treatment of acute leukemia. Previous biochemical studies indicated the incorporation of Ara-C into DNA reduced the catalytic activity of human topoisomerase I by decreasing the rate of single DNA strand religation by the enzyme by 2–3-fold. We present the 3.1 Å crystal structure of human topoisomerase I in covalent complex with an oligonucleotide containing Ara-C at the +1 position of the non-scissile DNA strand. The structure reveals that a hydrogen bond formed between the 2’-hydroxyl of Ara-C and the O4’ of the adjacent −1 base 5’ to the damage site stabilizes a C3’-endo pucker in the Ara-C arabinose ring. The structural distortions at the site of damage are translated across the DNA double helix to the active site of human topoisomerase I. The free sulfhydryl at the 5’-end of the nicked DNA strand in this trapped covalent complex is shifted out of alignment with the 3’-phosphotyrosine linkage at the catalytic tyrosine 723 residue, producing a geometry not optimal for religation. The subtle structural changes caused by the presence of Ara-C in the DNA duplex may contribute to the cytotoxicity of this leukemia drug by prolonging the lifetime of the covalent human topoisomerase I-DNA complex.

Human topoisomerase I plays a vital role in maintaining DNA stability and is known to travel with active replication and transcription complexes in human cells (4, 5). Human topoisomerase I is the sole target of the camptothecins (CPT), a potent class of antinecancer drugs used to treat late-term solid malignancies (3, 4, 6). Camptothecin effectively targets the religation phase of topoisomerase I catalysis by stabilizing the covalent protein-DNA complex and trapping the enzyme on DNA (7, 8). In this way, CPT converts topoisomerase I into a cellular poison. Human topoisomerase I is also affected by several forms of DNA damage, including abasic lesions, wobble base pairs, and base pair mismatches (9–13). Such lesions can impact each stage of topoisomerase I’s catalytic cycle, including DNA binding, single-strand DNA cleavage, and religation.

1-β-D-Arabinofuranosylcytosine (Ara-C)1 is a nucleoside analogue used in the treatment of acute leukemia (14, 15). Ara-C and the standard cytosine DNA base differ by the presence of a 2’-hydroxyl on the arabinose ring of the drug (Fig. 1). Ara-C is thought to inhibit DNA polymerases central to replication and repair processes, and thus to slow the growth of malignant cells (16, 17). The detailed impact of Ara-C on human cells, however, is poorly understood. Incorporation of Ara-C into DNA causes localized alterations in the DNA duplex, including changes in sugar pucker, base stacking, and backbone torsion angles (17, 18). Biochemical studies using human topoisomerase I have revealed that Ara-C incorporation at the +1 position of the intact (non-scissile) strand adjacent to the site of single-strand DNA cleavage induces a 4–6-fold increase in covalent topoisomerase I-DNA complexes caused by a 2–3-fold decrease in the rate of religation by the enzyme (12). Because the stabilization of covalent topoisomerase I-DNA complexes converts the enzyme into a cellular poison, the cytotoxicity of Ara-C may be enhanced by this ability to impact the action of topoisomerase I.

Human topoisomerase I is 765 amino acids (91 kDa) and is composed of four domains: N-terminal (residues 1–200), core (201–635), linker (636–712), and C-terminal domain (713–765). Several crystal structures of human topoisomerase I DNA complexes have been determined (3, 8, 13, 19–21). Core subdomains I and II form the “CAP” of human topoisomerase I that contacts one side of the DNA, while core subdomain III, the “CAT,” and the C-terminal domain contact the opposite side of the DNA. The CAP and CAT regions of the enzyme together wrap completely around the DNA duplex and position the

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† The atomic coordinates and structure factors (code 1NH3) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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§ The abbreviations used are: Ara-C, β-D-arabinofuranosylcytosine; r.m.s.d., root mean square deviation; PDB, protein data bank.

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active site residues within hydrogen bonding distance of the scissile DNA phosphate group. Four of five active site residues, Arg-488, Lys-532, Arg-590, and His-632, are located in core subdomain III, while the catalytic Tyr-723 resides in the C-terminal domain (3, 19, 20).

Structures of covalent human topoisomerase I-DNA complexes containing an intact 3′-phosphotyrosine linkage have also been reported (8, 19). In these trapped covalent protein-DNA complexes, the scissile phosphate contained a bridging phosphorothiolate linkage, which, upon cleavage by topoisomerase I, generates a free 5′-sulfhydril unable to participate in strand religation (8, 19, 22). The use of 5′-bridging phosphorothiolate linkages to trap covalent complexes has been successfully employed to examine several enzymes that form transient 3′-phosphotyrosine linkages, including eukaryotic type IB topoisomerases, viral topoisomerases, and bacterial and phage tyrosine recombinases and integrases (8, 19, 22–28). Detailed biochemical studies have shown that the presence of a 5′-bridging phosphorothiolate linkage has a marginal effect on the rate of cleavage by such enzymes (down to 2-fold), but lowers the rate of religation by at least 10,000-fold (24). In addition, x-ray crystallographic studies have revealed that when the active form of human topoisomerase I (with the Tyr-723 residue intact) is used for crystallization, a bridging phosphorothiolate linkage is required to obtain crystals (8, 19).

We determined the 3.1 Å crystal structure of a human topoisomerase I in covalent complex with a 22-base pair oligonucleotide containing Ara-C at the +1 position of the non-scissile DNA strand to elucidate the structural impact of Ara-C on this enzyme. This is only the third structure of a covalent topoisomerase I-DNA complex reported to date (8, 19). We find that Ara-C introduces numerous subtle structural changes, including changes in sugar pucker and base position, that contribute to a new positioning of the free 5′-sulfhydril away from the 3′-phosphotyrosine linkage. Thus, the single-strand religation reaction catalyzed by the enzyme is decreased, producing a longer lived covalent protein-DNA complex.

**MATERIALS AND METHODS**

**Protein Purification and DNA Oligonucleotides**—The core domain of human topoisomerase I (58 kDa, residues 175–659 including a nuclear localization signal) was expressed using baculovirus in Spodoptera frugiperda (Sf9) insect cells as described (29). The 6.3-kDa C-terminal domain of human topoisomerase I (residues 713–765) was expressed as 

![1-β-D-arabinofuranosyleytosine](image1.png) 

**Ara-C**

![5′-AAAAGACTT^GGAAAAATTTTT-3′](image2.png) 

**scissile**

3′-TTTTTCTGAA CCTTTTTAAAAA-5′ **intact**

where ^ corresponds to the site of cleavage and the bold, underlined C indicates the position of the Ara-C base. The G base in italics contained a bridging phosphorothiolate linkage, which upon cleavage by human topoisomerase I generates a free 5′-sulfhydril rather than a hydroxyl (22–28). This method of trapping human topoisomerase I in covalent complexes with duplex DNA has been used previously in structural studies of this enzyme (8, 19), as well as in several detailed mechanistic and biological studies of topoisomerases and related tyrosine recombinases (22–28). Note that the Ara-C nucleotide is in the intact DNA strand, and the G nucleotide containing the 5′-bridging phosphorothiolate linkage is in the scissile DNA strand.

**Crystallization and Data Collection**—Crystals of reconstituted human topoisomerase I in covalent complex with the 22-bp DNA duplex containing an Ara-C site of damage at the intact +1 position were grown by sitting drop vapor diffusion and cryoprotected as described (19). Crystals belong to space group P3\(_2\). Data were collected at Brookhaven National Laboratory, beamline X12B, using x-ray radiation of 1.1 Å wavelength, and a MAR CCD detector. Data were processed, scaled, and reduced using HKL2000 (30).

**Structure Determination and Refinement**—The structure was determined by molecular replacement with AMoRe (31) using the crystal structure of the human topoisomerase I reconstituted covalent complex as a search model (PDB code 1A31; Ref. 19). 10% of the diffraction data were set aside for the R\(_{free}\) statistic prior to any structural refinement. Partial hemihedral twinning was detected, with a twinning fraction of 8.8% and a twinning operation of (h, −h, −k, −l). The data were detwinned readily using the method of Yeates (32) as implemented in CNS. The model was refined by rigid body, positional, thermal displacement parameter, and simulated annealing methods in CNS using torsion angle dynamics and the maximum likelihood function target. Model rebuilding and manual adjustments into the electron density maps were performed using the program O (33). Final refinement included an overall B-factor, solvent correction, and 33 well-ordered water molecules. The final structure yielded no Ramachandran outliers as calculated by PROCHECK (34). Molecular graphics figures were created with Molscript, Raster3D, DINO, and Podray (35–37). Coordinates have been deposited with the RCSB and assigned code 1NH3.

**RESULTS**

**Overall Structure**—The crystal structure of reconstituted human topoisomerase I in covalent complex with a 22-base pair oligonucleotide containing Ara-C at the +1 position of the non-scissile strand was determined to 3.1 Å resolution and refined to crystallographic R and R\(_{free}\) values of 0.24 and 0.31, respectively (Table I). Human topoisomerase I-DNA complex crystals are notoriously difficult to handle (3, 13, 19–21). Indeed, in this case crystal fragility allowed the collection of
Human Topoisomerase I-Ara-C Complex

Crystalllographic analysis of human topoisomerase I-Ara-C complex

| TABLE I  |
|-----------------|-----------------|
| Resolution (Å) (highest shell) | 100 – 3.1 (3.2–3.1) |
| Space group | P3_2 |
| Unit cell dimensions | a=b=73.0, c=186.3, α=β=90°, γ=120° |
| No. of total reflections | 169,504 |
| No. of unique reflections | 13,878 |
| Mean redundancy | 12.2 |
| R_i<ref> || (highest shell) | 15.5 (41.5) |
| Complement (%) (highest shell) | 69.3 (54.7) |
| Mean I/σ (highest shell) | 5.6 (1.4) |
| \( R_{\text{crys}} \) (%) | 30.9 |
| \( R_{\text{free}} \) (%) | 24.3 |
| Twin operator | h, -h-k, -l |
| Twin fraction | 0.088 |
| r.m.s.d. | |
| Bond length (Å) | 0.0118 |
| Bond angles (°) | 1.55 |
| Dihedral angles (°) | 23.1 |
| Improper angles (°) | 1.59 |
| Number of protein atoms | 3825 |
| Mean B value, protein atoms (Å²) | 61.2 |
| Number of DNA atoms | 877 |
| Mean B value, DNA atoms (Å²) | 52.4 |
| Number of solvent atoms | 33 |
| Mean B value, solvent atoms (Å²) | 44.2 |

\( R_{\text{crys}} = \sum |I-\langle I\rangle|/\sum I \), where I is the observed intensity and \( \langle I\rangle \) is the average intensity of multiple symmetry-related observations of that reflection.

\( R_{\text{free}} = \sum |F_{\text{cal}}-F_{\text{obs}}|/\sum F_{\text{cal}} \) for 10% of the data not used at any stage of structural refinement.

\( R_{\text{free}} = \sum |F_{\text{cal}}-F_{\text{obs}}|/\sum F_{\text{cal}} \), where \( F_{\text{cal}} \) and \( F_{\text{obs}} \) are the observed and calculated structure factors.

The overall structure of this complex (the Ara-C structure) is similar to the previously reported reconstituted human topoisomerase I-DNA complex with a non-damaged DNA duplex (PDB code 1A31; Ref. 19; Fig. 2). The quality of the electron density is good given the limited resolution and completeness of the diffraction data (Fig. 3). The CAP region of the enzyme’s core domain (residues 203–432) sits above the DNA and projects two α-helices out along the DNA helical axis, while the CAT (residues 433–635, 719–765) sits below the DNA and assembles the active site around the scissile DNA phosphate. Together, the CAP and CAT wrap completely around the DNA duplex. The C-terminal domain is packed between the CAT and the DNA and contains the fifth active site residue, the nucleophilic Tyr-723. The linker domain (residues 636–712) has been observed in previous crystal structures to extend from the CAT and C-terminal domains in the direction of the downstream region of the DNA (3, 8, 20). This domain is not present in the reconstituted protein construct used for these studies; the reconstituted complex is composed of the human topoisomerase I core domain (residues 175–659) and C-terminal domain (713–765). The root mean square deviation (r.m.s.d.) between the Ara-C structure and 1A31 is 0.89 Å over all protein atoms (Table II). Shifts in position of up to 2.6 Å between individual protein residues (e.g., Ala-326 in the CAP region) were observed when the Ara-C and 1A31 structures are compared. Such shifts are within the range observed when the protein regions of other human topoisomerase I DNA complexes are compared (13, 20, 21).

The DNA duplex in the Ara-C structure is similar in overall structure to that observed in 1A31 (Fig. 2). In addition, like all previously described topoisomerase I-DNA complexes, the DNA molecules pack head-to-tail in a pseudocontinuous helix. The r.m.s.d. over all nucleic acid atoms between the two structures is 1.2 Å (Table II). The ends of the DNA duplex shift in position by up to 5.4 Å (Fig. 2); similar shifts in position of the ends of the DNA duplexes have been observed in previous human topoisomerase I structures (13, 20, 21).

Impact of Ara-C on DNA Base Pairing—An Ara-C:G base pair at the +1 position of the non-scissile strand. The CAP region is composed of core subdomains I (red) and II (green), and the CAT region is composed of core subdomain III (blue) and the C-terminal domain (cyan). The –10 and +12 positions in the DNA duplex (yellow) are labeled and the Ara-C-G base pair at the +1 position of the Ara-C complex DNA oligonucleotide is indicated in magenta. Superimposed in gray is the structure of human topoisomerase I in covalent complex with a non-damaged DNA oligonucleotide reported previously (19). Shifts in position in the bases at the ends of the DNA duplex are indicated.

**Fig. 2.** Crystal structure of human topoisomerase I in covalent complex with 22 base pair duplex DNA containing Ara-C at the +1 position of the non-scissile strand. The CAP region is composed of core subdomains I (red) and II (green), and the CAT region is composed of core subdomain III (blue) and the C-terminal domain (cyan). The –10 and +12 positions in the DNA duplex (yellow) are labeled and the Ara-C-G base pair at the +1 position of the Ara-C complex DNA oligonucleotide is indicated in magenta. Superimposed in gray is the structure of human topoisomerase I in covalent complex with a non-damaged DNA oligonucleotide reported previously (19). Shifts in position in the bases at the ends of the DNA duplex are indicated.
The r.m.s.d. over all 12 equivalent

not available, we compared the DNA duplex from our Ara-C

resonance spectroscopy (NMR) and one crystal structure (17,

one solution structure by nuclear magnetic

have been reported, one solution structure by nuclear magnetic

Determined by NMR (Fig. 5). The r.m.s.d. over all 12 equivalent

base pairs between these two duplexes is 2.3 Å. The DNA bases

superimpose well, while the backbone is more deviant between

the two structures. The ribose at the −2 scissile position in the

Ara-C complex shifts in position by 0.7 Å from the equivalent

ribose in the NMR structure; this base is at the terminus of the

NMR duplex, however. The ribose ring of the +1 scissile strand

in the Ara-C structure shifts by 2.6 Å relative to the equivalent

ribose in the NMR structure, a change likely caused by the

break in the DNA backbone at this position. The arabinose

sugar of the Ara-C base shifts in position by 0.9 Å. While both

rings exhibit a C3′-endo pucker, it is more extreme in the

topoisomerase I complex. Intrastrand hydrogen bonds between

the Ara-C 2′-OH and the ribose O4′ of the adjacent DNA base

are also formed in both structures. Thus, it appears that the

nick in the DNA backbone of the Ara-C topoisomerase I covalent

dNA complex stabilizes the C3′-endo pucker in the Ara-C

arabinose sugar ring.

Active Site—The active site in the Ara-C structure is similar

overall to those observed in human topoisomerase I-DNA crystal

structures elucidated previously (3, 13, 19–21). Arg-488,

Lys-532, Arg-590, and Tyr-723 share an overall r.m.s.d. of 0.6 Å

with the equivalent residues in 1A31 (Fig. 6). His-632 is not

ordered in this structure, similar to the structure of a reconstituted

covalent human topoisomerase I complex reported previously (19).
The free 5′-sulphydryl group exhibits the largest structural change at the active site, shifting in position by 2.6 Å relative to the 1A31 structure. Thus, in the Ara-C complex, the 5′-sulphydryl is 3.7 Å from the 3′-phosphotyrosine linkage, while in the 1A31 structure, the equivalent group is only 2.5 Å from the 3′-phosphotyrosine linkage. Indeed, a rotation of the 5′-sulphydryl by 120 degrees in 1A31 places it in an ideal position to perform an in-line attack on the covalent phosphotyrosine bond. In the Ara-C complex, a similar alignment cannot be formed without more significant structural changes. This observation helps to explain the decreased rate of religation by human topoisomerase I using DNA substrates containing an Ara-C lesion at the intact +1 position (12).

The shift in the position of the free 5′-end of the nicked DNA strand in the Ara-C complex appears to be caused by the subtle alteration in the +1 DNA base mediated by the arabinose Ara-C sugar. The new position of the free 5′-sulphydryl in the Ara-C complex brings it within 3.5 Å of the side chain of Asn-722, the amino acid immediately N-terminal in sequence to the catalytic Tyr-723. Asn-722 shifts in position by 0.5 Å relative to the 1A31 structure; indeed, in the 1A31 complex, Asn-722 is 4.5 Å from the free 5′-sulphydryl (19). The proximity

Table II

| Residues or number of atoms | r.m.s.d. |
|-----------------------------|---------|
| All protein atoms           | 215–765 | 0.89 |
| All Cα atoms                | 215–765 | 0.91 |
| CAP protein atoms           | 215–434 | 0.86 |
| CAT protein atoms           | 455–628 | 0.81 |
| C-term protein atoms        | 720–765 | 0.82 |
| All DNA atoms               | 877     | 1.21 |
| P positions                 | 44      | 1.29 |

Two structures of DNA duplexes containing an Ara-C lesion have been reported, one solution structure by nuclear magnetic resonance spectroscopy (NMR) and one crystal structure (17, 18). While the atomic coordinates of the crystal structure are not available, we compared the DNA duplex from our Ara-C complex to the 12-base pair DNA duplex containing Ara-C determined by NMR (Fig. 5). The r.m.s.d. over all 12 equivalent

ence of the Ara-C base causes subtle changes in the DNA duplex that are localized largely at the +1 base pair (Fig. 4A).
The Ara-C and G bases shift by 1.4 and 0.75 Å, respectively, from the positions of the A and T bases in the 1A31 structure. The arabinose ring of the Ara-C nucleotide and the ribose ring of the G nucleotide shift in position by up to 3.7 and 1.7 Å, respectively, relative to the positions of the ribose rings in 1A31. The DNA bases surrounding the +1 base pair remain relatively fixed in position, however, particularly in the scissile strand and the upstream region of the intact strand (Fig. 4A). The 2′-hydroxyl of the Ara-C arabinose sugar ring extends in the opposite direction from the equivalent group in a standard ribose sugar (Fig. 1). The Ara-C arabinose ring exhibits a C3′-endo pucker, as predicted based on thermodynamic/energetic considerations (Ref. 39; Fig. 4A). The deoxyribose ring of the +1 adenine base in the intact strand of 1A31, in contrast, exhibits the C2′-endo pucker typical of B-form DNA bases. The C3′-endo pucker in the Ara-C arabinose ring is stabilized by a 2.5 Å intrastrand hydrogen bond between the Ara-C 2′-hydroxyl and the O4′ of the −1 adenine (Fig. 4B). Thus, the Ara-C base exhibits a distinct sugar pucker that alters the +1 base pair of the DNA duplex in the Ara-C structure. This change impacts the position of the 5′-end of the cleaved DNA strand (see below), which helps to explain the decrease in the rate of religation by human topoisomerase I observed with this form of DNA lesion (12).

Two structures of DNA duplexes containing an Ara-C lesion have been reported, one solution structure by nuclear magnetic resonance spectroscopy (NMR) and one crystal structure (17, 18). While the atomic coordinates of the crystal structure are not available, we compared the DNA duplex from our Ara-C complex to the 12-base pair DNA duplex containing Ara-C determined by NMR (Fig. 5). The r.m.s.d. over all 12 equivalent

base pairs between these two duplexes is 2.3 Å. The DNA bases superimpose well, while the backbone is more deviant between the two structures. The ribose at the −2 scissile position in the Ara-C complex shifts in position by 0.7 Å from the equivalent ribose in the NMR structure; this base is at the terminus of the NMR duplex, however. The ribose ring of the +1 scissile strand in the Ara-C structure shifts by 2.6 Å relative to the equivalent ribose in the NMR structure, a change likely caused by the break in the DNA backbone at this position. The arabinose sugar of the Ara-C base shifts in position by 0.9 Å. While both rings exhibit a C3′-endo pucker, it is more extreme in the topoisomerase I complex. Intrastrand hydrogen bonds between the Ara-C 2′-OH and the ribose O4′ of the adjacent DNA base are also formed in both structures. Thus, it appears that the nick in the DNA backbone of the Ara-C topoisomerase I covalent DNA complex stabilizes the C3′-endo pucker in the Ara-C arabinose sugar ring.
of the Asn-722 side chain to the free 5'-end of the nicked DNA strand in the Ara-C complex indicates that a hydrogen bond is possible between these two groups. Asn-722 is conserved in all known sequences of eukaryotic topoisomerases I and has been implicated in the catalytic action of the enzyme. For example, mutations of this residue in both human and *Saccharomyces cerevisiae* topoisomerase I impact the enzyme’s sensitivity to CPT and phases of its catalytic cycle (40).

**DISCUSSION**

The leukemia drug Ara-C contains a arabinose sugar ring rather than the ribose standard to DNA and RNA bases. As such, its 2'-hydroxyl group is oriented in a manner distinct from the equivalent RNA cytosine base (Fig. 1). Ara-C is thought to elicit its antineoplastic effects by acting as a competitive inhibitor of DNA polymerases α and β (12, 17, 41).

Even at low concentrations, however, the drug becomes incorporated into DNA and disrupts DNA metabolism (12, 17, 41). Pourquier et al. (12) have shown that the presence of an Ara-C base at the +1 position of the intact strand (opposite the site of single-strand cleavage) slows the rate of DNA strand religation by human topoisomerase I 2–3-fold (12). The extended lifetime of the covalent topoisomerase I-DNA complex may contribute to antineoplastic effects of Ara-C by enhancing chromosomal instability. Indeed, human leukemia cells that lack detectable levels of topoisomerase I are resistant to the effects of Ara-C (12).

We determined the 3.1 Å resolution crystal structure of human topoisomerase I in covalent complex with a 22-base pair DNA duplex containing Ara-C at the +1 position of the intact strand (Fig. 2). The structure reveals that the Ara-C non-standard 2'-hydroxyl introduces numerous subtle structural changes, particularly the +1 base pair (Fig. 4A). The 2'-hydroxyl of Ara-C forms a hydrogen bond with the O4' of the −1 sugar, which stabilizes the C3'-endo pucker exhibited by the arabinose ring of Ara-C (Fig. 4B). These structural changes cause the +1 base pair of the duplex to shift in position relative to the previous structure and is now positioned within 3.5 Å of the side chain of Asn-722.
to the equivalent base pair in a covalent topoisomerase I DNA complex without a site of damage reported previously (1A31; Ref. 19). This, in turn, appears to cause the free 5′-sulphydryl (which replaces the 5′-hydroxyl in this trapped covalent complex; 8, 19, 23–28) in the nicked DNA strand to shift away from the covalent phosphotyrosine linkage and form a hydrogen bond with the side chain of Asn-722, an interaction not observed in previous topoisomerase I covalent complexes (Figs. 4A and 6). Taken together, these results indicate that the subtle change of the duplex opposite the single-strand DNA base appears to stabilize this interaction, allowing us to visualize involving non-damaged DNA. The change caused by the Ara-C-hydroxyl into place for the religation phase of catalysis-as making the 5′-hydroxyl of the nicked strand. Indeed, after relaxation slows, Asn-722 may have ample opportunity to hydrogen bond with the free 5′-hydroxyl in this trapped covalent complex without a site of damage reported previously (1A31; Ref. 19). This, in turn, appears to cause the free 5′-sulphydryl/H11032/Ref. 19). This, in turn, appears to cause the free 5′-end of the nicked strand away from the covalent 3′-phosphotyrosine linkage. These results likely explain the impact on topoisomerase I activity reported by Pourquier et al. (12).

This Ara-C structure provides additional insight into the catalytic mechanism of human topoisomerase I. As the active site residues are brought into place upon DNA binding, Asn-722 does not appear to contact the DNA, as observed in several non-covalent topoisomerase I DNA complexes (3, 13, 20, 21). However, as the downstream region of DNA undergoes relaxation by the proposed controlled rotation mechanism, Asn-722 may have ample opportunity to hydrogen bond with the free 5′-hydroxyl of the nicked strand. Indeed, after relaxation slows, Asn-722 may play a crucial role via hydrogen bonding in guiding the 5′-hydroxyl into place for the religation phase of catalysis. This interaction is likely to be transitory in reactions involving non-damaged DNA. The change caused by the Ara-C base appears to stabilize this interaction, allowing us to visualize it in the structure presented here.

The importance of Asn-722 in human topoisomerase I and the equivalent Asn-726 in S. cerevisiae topoisomerase I in the catalytic cycle and camptothecin sensitivity of the enzyme have been established by several careful biochemical studies. For example, mutation of Asn-722 to histidine in human topoisomerase I increases the rate of DNA cleavage, while mutation to aspartic acid decreases the DNA binding affinity of the enzyme (38). An N722S mutation in human topoisomerase I, in contrast, does not impact the catalytic activity of the enzyme but does reduce its sensitivity to camptothecin (40). We provide structural evidence in this and previous work that sites of DNA damage impact the ability of Asn-722 to align the active site of human topoisomerase I both before and after single-strand DNA cleavage by the enzyme (13). This residue may play a similar role with other DNA lesions that impact human topoisomerase I, including etheno adenine adducts, wobble base pairs, and uracil mismatches. In summary, we show that relatively subtle modifications caused by the presence of a single 2′-hydroxyl group on the opposite side of the substrate DNA duplex can alter the structure of the human topoisomerase I active site and impact the catalytic action of the enzyme.

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