Supplemental Information

Mechanism and Specificity of DNA Strand Exchange Catalyzed by Vaccinia DNA Topoisomerase Type I

Mary R. Stahley and James T. Stivers

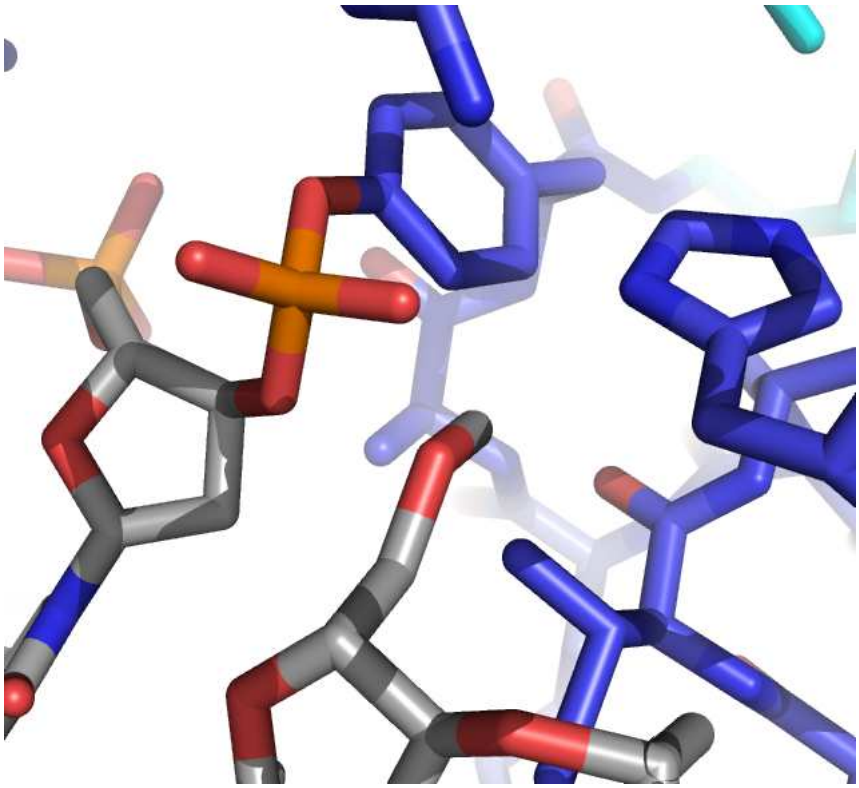


Fig. S1. Model of 5′-OMe substitution at the 5′ position of the -1 nucleotide of the cleaved DNA strand. The 5'-OMe modified downstream strand was constructed from structural coordinates of variola topoisomerase in covalent complex with vanadate, upstream DNA, and downstream DNA (PDB ID 3IGC). The modified downstream strand is displayed in the context of the variola topoisomerase structure in covalent complex with upstream DNA (PDB ID 2H7F).

Single Stranded Binding. vTopo was tested for its ability to bind single strands in the absence of an overhang duplex under the same conditions described for duplex DNA and covalent complex strand binding measurements. The increase in anisotropy was monitored upon vTopo addition to a 5'-FAM labeled 12mer strand (Fig. S1A). A similar experiment was performed to measure $32/32^{FAM}$ duplex DNA binding to the Y274F mutant Topo that cannot form a covalent adduct.

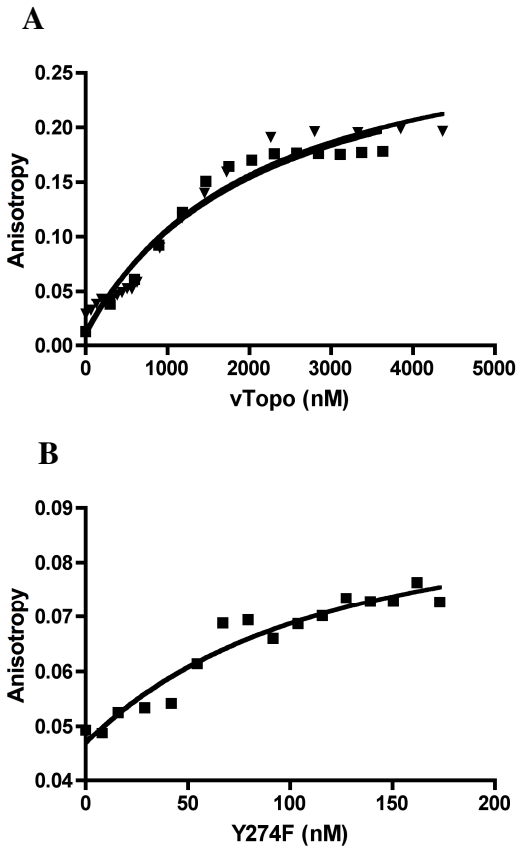
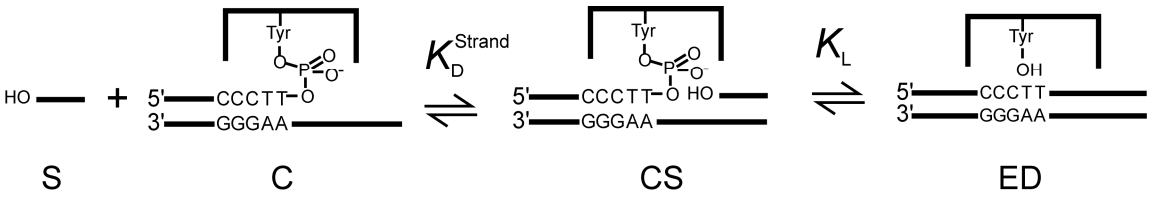


Fig. S2. (A) Binding of wild-type vTopo to a 5'-FAM labeled 12 mer single stranded DNA (100 nM). A $K_D = 2.5 \pm 0.5$ μM was obtained by fitting to a quadratic equation of the form in eq 1. Data from two replicate experiments are shown. (B) Binding of Y274F vTopo to a $32/32^{FAM}$ duplex DNA (25 nM). A $K_D = 100 \pm 50$ nM was obtained by fitting to a quadratic equation of the form in eq 1.

Determination of the Ligation Equilibrium Constant ($K_L = k_r/k_{cl}$). Upon addition of a 5'-OH downstream strand (S) to purified covalent complex (CS), strand binding (K_D) and ligation (K_L) equilibria are established (Scheme S1):



Scheme S1. Strand binding and ligation equilibria

For tight binding strands, very little dissociated covalent complex (C + S) will be present under the conditions used (10 μM strand). However, with the weakly associated -3TC and -6GA mismatched strands, overhang covalent complexes (C), and dissociated strands (S), are also present at equilibrium in these cases. To determine the ligation equilibrium, K_L = [ED]/[CS], the concentration of the non-covalent vTopo complex (ED) and the strand-bound covalent complex (CS) need to be determined. In this equilibrium, CS is the sum of the concentrations of all annealed, unligated forms of the covalent complex. Since covalent complex with the dissociated strand (C) and the strand-bound covalent complex (CS) comigrate on a SDS-PAGE gel, the relative amounts of C and CS in this single band was determined using the measured dissociation constant for the strand ($K_{D,}^{strand}$, see derivation below).

In the derivation below the known quantities are: (1) total amount of DNA (DNA_T), (2) the total amount of strand (S_T), (3) the dissociation constant of the OMe version of the strand (K_D^{strand}), (4) the observed ligation rate (k_{lig}^{obs}), and (5) the observed ligation endpoint. The above quantities can be used, as shown in eqs S1-S16 below, to determine the ligation rate, (k_{lig}), the cleavage rate (k_{cl}) and the ligation equilibrium (K_L).

$$[C]+[CS]+[ED]=[DNA_T] \tag{S1}$$

$$[ED]=(Endpo\,\mathrm{int})\times[DNA_T] \tag{S2}$$

$$K_D^{St}=\frac{[S][C]}{[CS]} \tag{S3}$$

$$[S]=[S_T]-[CS] \tag{S4}$$

$$[C]=[DNA_T]-[CS]-[ED] \tag{S5}$$

$$K_D^{St}=\frac{([S_T]-[CS])([DNA_T]-[CS]-[ED])}{[CS]} \tag{S6}$$

$$K_D^{St}=\frac{[DNA_T][S_T]-[CS][S_T]-[ED][S_T]-[DNA_T][CS]+[CS]^2+[ED][CS]}{[CS]} \tag{S7}$$

$$0=-[CS]K_D^{St}-[CS][S_T]-[DNA_T][CS]+[ED][CS]+[DNA_T][S_T]-[ED][S_T]+[CS]^2 \tag{S8}$$

$$0 = [CS]^2 + ([ED] - K_D^{St} - [S_T] - [DNA_T])[CS] + ([DNA_T] - [ED])[S_T] \quad \text{(S9)}$$

$$[CS] = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \quad \text{(S10)}$$

where $a = 1$ (S11)

and $b = [ED] - K_D^{St} - [S_T] - [DNA_T]$ (S12)

and $c = ([DNA_T] - [ED])[S_T]$ (S13)

$$K_L = \frac{[ED]}{[CS]} = \frac{k_{lig}}{k_{cl}} \quad \text{(S14)}$$

$$k_{lig,obs} = k_{cl} + k_{lig} \quad \text{(S15)}$$

$$k_{lig} = \frac{k_{lig,obs}}{\frac{1}{K_L} + 1} \quad \text{(S16)}$$