Supporting Information

Substrate mediated proton relay mechanism for the religation reaction in topoisomerase II

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Supporting Tables and Figures

Supporting Figure 1. Schematic illustrations of the general acid/base catalysis in topo II. HA and B represent a general acid and base respectively.

Supporting Figure 2. The reactant (R), intermediate (I1-I3), and product (P) states are shown with important hydrogen bonds. Schematic illustrations are shown in Figure 3 (a).

Supporting Figure 3. Stereoviews of the QM atoms in the R state (a), TS1 (b) and TS4 (c). Non-stereoviews of these states are shown in Fig 1, Fig3 (b) and (c), respectively.

Supporting table 1. Searched reaction coordinate for each state.

R to I1	Two-dimensional potential search was performed using (I) the distance between H3'(-1) and O3'(-1) and (II) the distance between P(+1) and O3'(-1)
I1 to I2	The distance between H3'(-1) and N(Arg781)
I2 to I3	The distance between H3'(-1) and O(W1)
I3 to P	The distance between H3'(-1) and N(Arg781)

Supporting table 2. Changes of the important distances.

	R	I1	I2	I3	P	TS1	TS4
MGA-O3'(-1)	2.40 Å	2.09 Å	2.11 Å	2.10 Å	2.24 Å	2.06 Å	2.09 Å
MGA-O1P(+1)	1.91 Å	1.97 Å	1.97 Å	1.96 Å	1.95 Å	2.03 Å	1.96 Å
O3'(-1)-P(+1)	2.91 Å	2.06 Å	1.97 Å	2.01 Å	1.81 Å	2.60 Å	2.03 Å
O(Tyr782)-P(+1)	1.66 Å	1.74 Å	1.80 Å	1.82 Å	2.32 Å	1.66 Å	1.83 Å
O2P(+1)-H3'(-1)	1.89 Å	0.99 Å	1.05 Å	1.31 Å	2.55 Å	1.09 Å	1.71 Å
O3'(-1)-H3'(-1)	0.99 Å	2.49 Å	3.41 Å	3.72 Å	5.01 Å	1.50 Å	4.09 Å
O5'(-1)-H3'(-1)	3.07 Å	1.97 Å	3.16 Å	3.32 Å	5.00 Å	2.81 Å	3.79 Å
N(Arg781)-H3'(-1)	4.70 Å	3.81 Å	2.79 Å	2.77 Å	1.08 Å	4.15 Å	2.04 Å
H3'(-1)-O(W1)	4.57 Å	2.99 Å	1.45 Å	1.11 Å	1.58 Å	3.82 Å	1.01 Å
H(Arg781)-O2P(+1)	2.75 Å	2.58 Å	2.45 Å	2.11 Å	2.72 Å	2.75 Å	1.97 Å
N(Arg781)-H(Arg718)	1.04 Å	1.03 Å	1.01 Å	1.01 Å	1.62 Å	1.04 Å	1.01 Å
H(Arg781)-O(Tyr782)	3.42 Å	2.95 Å	2.80 Å	2.53 Å	1.01 Å	3.09 Å	2.29 Å
O3'(-1)- O2P(+1)	2.78 Å	2.67 Å	2.69 Å	2.81 Å	2.74 Å	2.46 Å	2.83 Å
O2P(+1)-O(W1)	3.25 Å	2.81 Å	2.46 Å	2.39 Å	2.70 Å	3.03 Å	2.52 Å

Computational details

Molecular dynamics simulation. To construct the hydrogen bond network in the active site, a 500 ps molecular dynamics simulation was performed. In this simulation, in order to avoid the disruption of the hydrogen bonding network observed in the crystal structure, we applied positional restraints for all the heavy atoms except for water molecules. All MD simulations were performed with $T=300\text{K}$, $P=1\text{bar}$, time step = 2fs. The long range electrostatic interactions are treated by the particle-mesh Ewald (PME) method with a 10 Å non-bonded cutoff. RESP charges for the tyrosine residue that is covalently linked to DNA are calculated using Antechamber module of AMBER and Gaussian09 at the B3LYP/6-31g level. Missing residues in the initial structure were not modeled, and simply capped by ACE and NME group.

Reaction path way searching. Starting from the QM/MM optimized structure of the reactant, possible reaction pathways were searched under the harmonic potential restraints. The putative transition states are found at the highest energy point along with the reaction coordinate. The reaction coordinate for each step is summarized in table S1.