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

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The DNA religation reaction of yeast type II topoisomerase (topo II) was investigated to elucidate its metal-dependent general acid/base catalysis. Quantum mechanical/molecular mechanical calculations were performed for the topo II religation reaction, and the proton transfer pathway was examined. We found a substrate-mediated proton transfer of the topo II religation reaction, which involves the 3′ OH nucleophile, the reactive phosphate, water, Arg781, and Tyr782. Metal A stabilizes the transition states, which is consistent with a two-metal mechanism in topo II. This pathway may be required for the cleavage/religation reaction of topo IA and II and will provide a general explanation for the catalytic mechanism in the topo IA and II.

Keywords: topoisomerase II; QM/MM study; proton transfer; catalytic mechanism; DNA religation

Introduction

DNA topoisomerases are essential enzymes that solve the topological problems of double-stranded DNA during various DNA-related events, such as replication, transcription, and chromosome segregation, by directing the cleavage and religation of DNA strands (Ghilarov & Shkundina, 2012; Schoeffler & Berger, 2008; Vos, Tretter, Schmidt, & Berger, 2011; Wang, 2002). Topoisomerases are the targets of some of the most successful anticancer and antibacterial drugs (Bailly, 2012; Bradbury & Pucci, 2008; Pommier, Leo, Zhang, & Marchand, 2010). Some of these drugs stabilize the topoisomerase–DNA covalent complexes and/or inhibit the religation step, which induces the accumulation of the complexes, toxic intermediates (Pommier et al., 2010). Therefore, considerable attention has been paid to the mechanism underlying the cleavage/religation reaction. There are several DNA topoisomerase families. Type IA and II topoisomerases (topo IA and topo II) possess similar catalytic domains for the cleavage/religation reaction, (Aravind, Leipe, & Koonin, 1998; Berger, Fass, Wang, & Harrison, 1998) and their catalytic mechanisms are expected to be similar (Liu & Wang, 1999). It is believed that this reaction proceeds via a general acid/base catalysis (Supplementary Figure 1) (Deweese & Osheroff, 2010). During the DNA cleavage step, a general base deprotonates the catalytic tyrosine residue to activate the oxygen for nucleophilic attack on the DNA backbone phosphate, and the subsequent substitution of deoxyribose produces its covalent intermediate. A general acid enhances the scission of the P–O3′ bond by protonation of the O3′ atom. The religation reaction is believed to be the reverse of the DNA cleavage reaction (Schmidt, Burgin, Deweese, Osheroff, & Berger, 2010). Despite numerous experimental studies in recent years, it is still not defined for the general acid/base in topo IA and II.

In topo II, possible candidates for the general base involved in DNA cleavage are conserved histidine residues near the catalytic center and the metal-associated water (Deweese & Osheroff, 2009, 2010; Noble & Maxwell, 2002), whereas candidates for the general acid are water and an undefined amino acid in the catalytic site (Deweese & Osheroff, 2009, 2010). In topo IA, a charge relay mechanism was proposed (Perry & Mondragon, 2002; Zhang, Cheng, & Tse-Dinh, 2011). In this mechanism, a conserved glutamic acid in the TOPRIM domain acts as the general acid and base in the cleavage and religation reactions, respectively, and the reactions are promoted by hydrogen bonds between the conserved aspartic acid in the TOPRIM domain and the conserved histidine. However, mutation of the glutamic acid to

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glutamine did not abolish the cleavage/religation activity (Chen & Wang, 1998).

By contrast, a substrate-assisted mechanism has been examined theoretically in DNA polymerase (Wang & Schlick, 2008; Wang, Yu, Hu, Broyde, & Zhang, 2007), which catalyzes a phosphoryl transfer reaction similar to that of religation reaction in topo IA and II. In this mechanism, a proton migrates from the O3′ to a leaving group via the α-phosphate of deoxynucleoside triphosphate. This mechanism can explain why mutational studies failed to identify the general acid and base involved in the cleavage/religation reaction, because external general acids and bases are not required in this mechanism. Thus, in this study, we examined the possible substrate-assisted mechanism for the DNA religation reaction of topo II using quantum mechanics/molecular mechanics (QM/MM) calculations.

Methods
Modelling of the reactant state
The initial coordinates were taken from the crystal structure of yeast topo II–covalent intermediate (PDBID: 3L4K), which is the only one PDB structure containing two divalent ions in its active site (Schmidt et al., 2010). The structure is considered to be the best for the reactant state of the religation reaction, although artificial atoms were introduced into the crystal structure to determine the covalent intermediate state, i.e. Zn2+ and 3′ sulfur atoms. We replaced the Zn2+ and terminal S3′ atoms with Mg2+ and O atoms, respectively.

The topo II model was immersed in a TIP3P water box. A 500 ps molecular dynamics (MD) simulation was performed with T = 300 K and P = 1 bar to construct the hydrogen bond network in the active center with (i) positional restraints for all the heavy atoms except for water molecules and (ii) a distance restraint between the O2P oxygen of the reactive phosphate and the O3′ H atom. All MD simulations were performed using GROMACS 4.5.4 (Hess, Kutzner, van der Spoel, & Lindahl, 2008) with the ff99SBildn force field (Lindorff-Larsen et al., 2010).

QM/MM calculation
A water droplet model was used for the QM/MM calculations. The droplet model contained approximately 50,000 solvent water molecules, 15 Na+ ions, 16 Mg2+ ions, and approximately 25,000 atoms of the DNA and topo II. During the QM/MM calculations, important atoms in the active site were included in the QM region, whereas the remaining atoms were handled by MM. The QM region included the DNA backbone, two Mg ions, eight active site water molecules, and the side chains of Glu449, Asp526, Asp528, Arg781, and Tyr782 (a total of 134 QM atoms, see Figure 1(a)). The QM/MM calculations were carried out using the NWChem program package (Kendall et al., 2000). The QM level of theory employed was hybrid DFT (B3LYP) with the 6–31+G* and 4–31G basis sets for Mg ions and all other atoms, respectively, whereas the MM level was handled by the ff99 force field (Wang, Cieplak, & Kollman, 2000). We have used hydrogen cap atoms in the QM/MM boundary region. More details of computational methods and set-ups are provided in the Supporting Information.

Results
Modelling of the reactant state
The hydrophilicity of the active site of the cleavage/religation reaction should allow binding of several water molecules in the active site. Unfortunately, they were not resolved in the crystal structure because of the limited
resolution (2.98 Å). Furthermore, the terminal 3’ oxygen of the substrate DNA was substituted by sulfur to trap the covalent intermediate (Schmidt et al., 2010). In order to hydrate the active site and generate an appropriate hydrogen bond between the terminal 3’ oxygen and the O2P of the reactive phosphate, we performed a sufficiently long MD simulation of 500 ps with restraints, as described in the Methods section. Then, a QM/MM geometry optimization was performed without any restraints. The QM/MM optimized structure showed that one and four water molecules were coordinated to metals A and B, forming 5- and 6-coordinated structures (see Figure 1(b). Stereoview figures are provided in the Supporting Information). Interestingly, the distance between the terminal O3’ and the O2P of the reactive phosphate was reduced from 3.85 to 2.78 Å, and the distance between Mg A (2+) and O3’ was increased from 2.05 to 2.40 Å. These distance changes are attributed to the reconstruction of the active site after the replacement of artificial atoms.

**QM/MM simulation for the DNA religation reaction**

Starting from the QM/MM optimized structure for the reactant, possible reaction pathways were searched using the QM/MM geometry optimization with harmonic potential restraints. The most favorable pathway had two characteristic activation barriers in the first and last reaction steps. The overall energy profile is shown in Figure 2.

We found three intermediate states (I1–I3) and four transition states (T1–T4) between the reactant (R) and product (P) states. Schematic illustrations and the threedimensional structures of these key states are shown in Figure 3(a) and Supplementary Figure 2. The first step (R→I1) was initiated by the nucleophilic attack of the terminal O3’ on the reactive phosphate, and a proton transfer occurs from the terminal 3’ OH to the O2P oxygen of the reactive phosphate. The calculated activation energy of ΔE° = 18.9 kcal/mol is the highest energy barrier in the overall reaction. The calculated activation energy is comparable to the experimental value of ΔG° = 20 kcal/mol, which is estimated by the experimental reaction rate constants (Mueller-Planitz & Herschlag, 2008).

Figure 3(b–c) show the three-dimensional structures of the high energy transition states, TS1 and TS3. In TS1, the proton transfer was already completed and the hydrogen bond distance between O3’ and the H of O2P was 1.50 Å. Another characteristic feature of this step was a significant decrease in the Mg A–O3’ distance from 2.40 to 2.06 Å, which was associated with a stabilization of the free O3’ via its direct coordination to Mg A. TS1 was the highest energy state; hence, Mg A promoted the overall religation reaction and the nucleophilic attack of the terminal O3’ against the reactive phosphate. This was consistent with the experimental observations that metal ions were required in the religation reaction of topo II and topo IA (Goto, Laips, & Wang, 1984; Tse-Dinh, 1986).

The first intermediate state (I1) of the religation reaction was stabilized by another hydrogen bond between the O5’ of the (−1) phosphate and the H atom of the reactive phosphate that migrated from O3’. The relative QM energy of I1 was calculated as E° = 7.7 kcal/mol (Figure 2, Supplementary Figure 2).

In the second step, the H atom of the reactive phosphate moved from the O5’ of the (−1) phosphate to the O atom of water 1. Water 1 was stabilized close to the reactive phosphate via hydrogen bonds with the (−1) phosphate and Arg781 (Supplementary Figure 2). This step had a low activation barrier of 3.6 kcal/mol, and the resulting intermediate I2 was slightly better stabilized than I1 (E° = 5.5 kcal/mol).

The third step was a proton transfer from the O2P of reactive phosphate to the water 1, and this reaction also had a small activation barrier. In I2, the distance between the O2P of reactive phosphate and the oxygen of water 1 was 2.46 Å (Supplementary Figure 2). In this case, the close contact between the O atoms facilitated a low activation barrier proton transfer, known as a low-barrier hydrogen bond (LBHB). A remarkable structural feature of I2 was that the water 1 was sandwiched by two phosphate groups, reactive phosphate and (−1) phosphate. The stable hydrogen bonds around water 1 supported the LBHB interaction. In I3, a hydronium ion of water 1 is formed, which was stabilized by the tetra- and pentacoordinated phosphates with formal charges of −1 and −2, respectively. The highly charged pentacoordinated phosphate was stabilized by three cation groups, the hydronium ion, Mg A on the opposite side of the hydronium ion, and Arg781. Arg781 closed to the proton-donor O2P of the reactive phosphate where R₃H = 2.45 Å to R₉H = 2.11 Å (Supplementary Figure 2).

The H atom of Arg781 was involved in this salt bridge and it transferred to Tyr782 during the next step. The last step (I3–TS4–P) involved a concerted double proton transfer and the dissociation of Tyr782. The
double proton transfer occurred between: (1) the hydronium ion and Arg781 and (2) Arg781 and Tyr782. The calculated energy barrier of this step was 8.5 kcal/mol, which was the second highest step in the overall DNA religation reaction. The evolution of this step was monitored based on the interatomic distances and the relative QM energy along the reaction path (Figure 4). It was shown that the hydrogen bonds required for the double proton transfer were formed in TS4. In TS4, one N atom of Arg781 side chain was changed its hybridization from a planar (sp²) form to a tetrahedral (sp³) form, as shown in Figure 3(c). Furthermore, the salt bridge was weakened between the hydronium ion and the O2P of the highly charged pentacovalent phosphate, i.e. from $R_{H-O} = 1.31 \text{ Å}$ ($R_{O-O} = 2.39 \text{ Å}$) and $\theta_{O-H-O} = 162$ to $R_{H-O} = 1.71 \text{ Å}$ ($R_{O-O} = 2.52 \text{ Å}$) and $\theta_{O-H-O} = 135$ for I3 and TS4 (Supplementary Figure 2 and Figure 3(c)), respectively. Therefore, the energy barrier of TS4 was
attributed to two factors: the formation of tetrahedral NH₃ in Arg781 and the weakening of the hydronium ion–pentacovalent phosphate salt bridge. These structural changes led to the formation of hydrogen bonds that allowed the concerted double proton transfer. After the formation of these hydrogen bonds in TS4, the two proton transfer reactions and the dissociation of Tyr782 occurred in a completely concerted manner (Figure 4).

The optimal reaction pathway described above used an indirect proton transfer path via water 1 and Arg781 from the pentacovalent phosphate to Tyr782. The energy barrier of the direct proton transfer path was calculated as 23.16 kcal/mol, which was too high; hence, this direct pathway could not be the route of the proton migration.

**Discussion**

A two-metal mechanism has been used in polymerases and phosphodiesterases (Yang, Lee, & Nowotny, 2006). In this mechanism, metal A assists the deprotonation of the nucleophile and metal B enhances the protonation of the leaving group, and both ions contribute to stabilize the excess charge of the pentacovalent transition state. Several experimental studies of topo II have suggested the active role of two metal ions during the cleavage/religation reaction (Deweese, Guengerich, Burgin, & Osheroff, 2009; Noble & Maxwell, 2002; West et al., 2000).

Schmidt et al. proposed a unified two-metal mechanism for the cleavage/religation reaction of topo IA and topo II (Schmidt et al., 2010) based on the crystal structure of a covalent intermediate of topo II, where the metal B was located far from the reactive phosphate. In the unified catalytic mechanism, the two metal ions were expected to have a key role in the general acid/base catalysis where one metal ion, Mg A, stabilizes a pentacovalent transition state directly and promotes the leaving and deprotonation of the 3′ hydroxyl during the cleavage and religation reactions, respectively, whereas another metal in the active site, Mg B (2+), anchors the substrate DNA.

Our results show that Mg A promotes both deprotonation of the nucleophile and protonation of the leaving group, i.e. Mg A stabilized the deprotonated O3′ in TS1 with the close coordination value of R_{MgA-O3′}=2.06 Å and the highly charged pentacoordinated phosphate in TS4. The coordination geometries of Mg A in each transition states are consistent with two-metal mechanism proposed by Schmidt et al.

It is notable that phosphoryl transfer reactions have been classified into associative and dissociative mechanisms (Lassila, Zalatan, & Herschlag, 2011) depending on the dissociation time of the leaving group. As shown in Figure 3, the religation reaction of topo II is a typical case of the associative mechanism.

The arginine residue involved in substrate-mediated proton transfer is strictly conserved between topo IA and topo II (Narula et al., 2011; Okada et al., 2000), although the role of the arginine residue is not well understood. In yeast topo II, the substitution of Arg781 with alanine reduced the catalytic activity greatly (Liu & Wang, 1998), whereas an experimental study of the human enzyme showed that a lysine substitution rescued the catalytic activity (Okada et al., 2000). Topo IA behaved similarly in the corresponding arginine (Arg321) mutant (Chen & Wang, 1998; Narula et al., 2011). Furthermore, a mutation of arginine to a bulky hydrophobic residue resulted in a complete loss of religation activity of topo IA and an accumulation of covalent intermediates (Narula et al., 2011). Based on our results, these experiments can be explained as follow. The participation of surrounding water molecules and the lysine side chain mediate the proton transfer pathway in the less active alanine and the active lysine mutants, respectively, whereas the bulky hydrophobic side chain completely disrupts the pathway. Thus, the religation reaction of topo IA and topo II can be explained by the substrate-mediated proton relay (SMPR) mechanism elucidated in the present study.

Interestingly, the DNA cleavage reaction remains active in topo IA, although its religation activity is abolished completely by the bulky hydrophobic substitutions (Narula et al., 2011). This implicates that another proton transfer pathway such as the charge relay mechanism is present in topo IA.

The catalytic activity of topo IA has a bell-shaped pH-dependency but this trend is not observed in a His365 mutant (Perry & Mondragon, 2002). This indicates that His365 participates in general acid-base catalysis, although His365 does not make direct contact with the reactive phosphate in the crystal structure of topo IA. These results implicate the charge relay mechanism (Perry & Mondragon, 2002).

In the charge relay mechanism, O3′ is protonated and deprotonated during the cleavage and religation by the glutamic acid in the TOPRIM domain, whereas the pKa
of the glutamic acid is controlled by a hydrogen bond network formed by the active site of aspartic acid and histidine (Perry & Mondragon, 2002). However, the charge relay mechanism is inconsistent with the fact that the mutation of Glu to Gln still maintains the DNA relaxation activity in topo IA (Chen & Wang, 1998; Perry & Mondragon, 2002).

Based on these experimental results and our computational results, the charge relay mechanism and the SMPR mechanism alone cannot explain the DNA cleavage reaction of topo IA fully. In conclusion, we suggest that both proton pathways must coexist in the topo IA DNA cleavage reaction.

No clinically used drugs are targeted at topo IA, but it was recently identified as a target for antibiotics that are expected to lead to the accumulation of toxic covalent intermediates (Tse-Dinh, 2009). Thus, the disruption of the proton transfer pathway in the religation reaction of topo IA may be an attractive strategy for the rational design of novel antibiotics.

Conclusion

We found a SMPR mechanism of the religation reaction in topo II, and the SMPR mechanism can be applied to the cleavage and religation reaction in both topo IA and topo II. In the SMPR mechanism, Mg A promotes both deprotonation of the nucleophile and protonation of the leaving group, i.e. Mg A stabilizes the transient negative charges during the nucleophilic attack and promotes the dissociation of the leaving group. The proton relay pathway in the SMPR mechanism is formed by the terminal O3′, reactive phosphate, water, Arg781, and Tyr782. This pathway is consistent with a series of Arg781 mutations in topo IA and topo II.

For the cleavage reaction of topo IA, the experimental results and our calculations suggested the coexistence of the charge relay mechanism and the SMPR mechanism. These mechanisms imply their irreversibility, which might be related to their highly sophisticated biological functions. We also expect that this irreversible proton transfer pathway will provide new insights for drug design.

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Supplementary material

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