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Three-Dimensional Structural Model Analysis of the Binding Site of Lithocholic Acid, an Inhibitor of DNA Polymerase β and DNA Topoisomerase II[1]

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The molecular action of lithocholic acid (LCA), a selective inhibitor of mammalian DNA polymerase β (pol β), was investigated. We found that LCA could also strongly inhibit the activity of human DNA topoisomerase II (topo II). No other DNA metabolic enzymes tested were affected by LCA. Therefore, LCA should be classified as an inhibitor of both pol β and topo II. Here, we report the molecular interaction of LCA with pol β and topo II. By three-dimensional structural model analysis and by comparison with the spatial positioning of specific amino acids binding to LCA on pol β (Lys60, Leu77, and Thr79), we obtained supplementary information that allowed us to build a structural model of topo II. Modeling analysis revealed that the LCA-interaction interface in both enzymes has a pocket comprised of three amino acids in common, which binds to the LCA molecule. In topo II, the three amino acid residues were Lys720, Leu760, and Thr791. These results suggested that the LCA binding domains of pol β and topo II are three-dimensionally very similar.

Key words: DNA polymerase β, DNA topoisomerase II, docking simulation, enzyme inhibitor, interaction interface, lithocholic acid.

The purpose of this study was to elucidate the precise molecular interaction of mammalian DNA polymerase β (pol β) and a secondary bile acid, lithocholic acid (LCA), which is known to promote tumorigenesis in rats induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and to be a selective inhibitor of pol β (*1–3*). Since pol β is known to be involved in DNA excision repair (*4*), these observations suggest a possible mechanism for the cancer-promoting activity of LCA, which has long remained obscure (*5*).

In investigating the molecular interaction of LCA and pol β, we found that LCA was also a potent inhibitor of human DNA topoisomerase II (topo II). DNA polymerase catalyzes addition of deoxyribonucleotides to the 3'-hydroxyl terminus of primed double-stranded DNA molecules, and DNA topoisomerase catalyzes the concerted breaking and rejoining of DNA strands and is involved in producing necessary topological and conformational changes in DNA (*6*). Therefore, there are no known functional similarities between these two enzymes, although they are critical to many cellular processes including DNA replication, repair and recombination, and thus may act in harmony with each other.

A flavone compound from *Psoralea corylifolia* was reported to inhibit both pol β and topo II activities (*7*). However, the compound was an intercalating agent, and was thought to bind to the DNA molecule directly, and subsequently to inhibit both activities indirectly. Intercalating agents are generally thought to inhibit these enzyme activities by modifying the three-dimensional structure of the DNA molecule; LCA does not belong to this category.

The crystal structure of pol β was analyzed by Wilson and co-workers (*8–13*), and the NMR structure of the N-terminal 8-kDa domain of pol β has been determined (*14–16*). Fortunately, the precise crystal structure of topo II has also been reported (*17, 18*). Pol β and topo II must have some as yet unknown common structural and/or functional characteristics. To obtain new information about the structure–function relationship between pol β and topo II, we searched for the LCA-binding region of topo II by computer analysis, by comparison of the NMR data of the interaction between the 8-kDa domain of pol β and LCA (*19*). We also simulated the LCA-binding region on topo II by three-dimensional structural model analysis. A similarity in three-dimensional structure was observed between pol β and topo II, although their amino acid sequences are markedly different from each other. This study provided novel informa-

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Abbreviations: LCA, lithocholic acid; pol, DNA polymerase [EC 2.7.7.7], topo II, DNA topoisomerase II.

tion regarding the relationship between the functions and three-dimensional structures of pol β and topo II.

MATERIALS AND METHODS

Materials—Calf thymus DNA was purchased from Sigma (St. Louis, MO, USA). Lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid) (LCA) and all other reagents were of analytical grade and were purchased from Wako (Osaka).

Enzymes—Recombinant rat DNA polymerase β (rat pol β) was purified from *E. coli* JMpβ5 as described by Date *et al.* (*20*). Pol α was purified from the calf thymus by immuno-affinity column chromatography as described previously (*21*). DNA polymerase I (plant α-like polymerase) and II (plant β-like polymerase) from a higher plant, cauliflower inflorescence, were purified according to the methods outlined by Sakaguchi *et al.* (*22*). Human immunodeficiency virus type-1 (HIV-1) reverse transcriptase and the Klenow fragment of DNA polymerase I were purchased from Worthington Biochemical (Freehold, NJ, USA). T4 DNA polymerase, Taq DNA polymerase, T7 RNA polymerase and T4 polynucleotide kinase were purchased from Takara (Kyoto). Calf thymus terminal deoxynucleotidyl transferase and bovine pancreas deoxyribonuclease I were purchased from Stratagene Cloning Systems (La Jolla, CA, USA) Purified human placenta DNA topoisomerase II (topo II) (2 units/μl) was purchased from TopoGen (Columbus, OH, USA).

Enzyme Assays—The activity of DNA polymerases was measured by the methods described previously (*23, 24*). Relaxation activity of topo II was determined by detecting the conversion of supercoiled plasmid DNA to its relaxed form (*25, 26*). The activities of T7 RNA polymerase, T4 polynucleotide kinase and bovine deoxyribonuclease I were measured in each of the standard assays according to Nakayama and Saneyoshi (*27*), Soltis and Uhlenbeck (*28*), and Lu and Sakaguchi (*29*), respectively.

Thermal Transition of DNA—Thermal transition profiles of double-stranded to single-stranded DNA with or without each of the LCA were determined with a spectrophotometer (U3210, Hitachi, Tokyo) equipped with a thermoelectric cell holder. Calf thymus DNA (6 μg/ml) was dissolved in 0 1 M sodium phosphate buffer (pH 7.0) containing 1% dimethylsulfoxide (DMSO) The solution temperature was equilibrated at 78°C for 10 min, and then increased by 1°C at 2-min intervals for each measurement point. Any change in the absorbance of LCA itself at each temperature point was automatically subtracted from that of DNA plus LCA in the spectrophotometer.

Search for the Lithocholic Acid Interaction Interface on DNA Topoisomerase II—To identify the LCA binding site of topo II, LCA-binding amino acid residues of the N-terminal 8-kDa domain of pol β were identified by ^{1}H-^{15}N HMQC NMR mapping results for the pol β 8-kDa domain with or without LCA (*19*). The major shifted amino acids were Lys60, Leu77, and Thr79. These amino acids were traced on the three-dimensional structure of topo II by computer analysis. The computer analysis employed two methods: the evolutionary trace method (*30, 31*) and the geometrical method by template-based analysis (*32–34*). Both methods were performed with Insight II/Binding Site Analysis (Molecular Simulations, San Diego, CA, USA, 1999).

Lithocholic Acid Docking Modeling—The molecular docking of LCA and the 8-kDa domain of pol β or topo II was done using the Affinity program within the Insight II modeling software (Molecular Simulations Inc., San Diego, CA, USA, 1999). The calculations used a CVFF force-field in the Discovery program and a Monte Carlo strategy in the Affinity programs (*35*).

RESULTS AND DISCUSSION

Effects of Lithocholic Acid on Various DNA Metabolic Enzymes—Lithocholic acid (LCA) contains a terpenoid ring carboxyl group in the molecule and is ionized at neutral pH as shown in Fig. 1. The length of the LCA molecule is 11.68 Å (Fig. 1B). As reported by Ogawa *et al.* (*3*), LCA inhibited eukaryotic DNA polymerases, and among the DNA polymerases α, β, γ, δ, and ε, the β type was most sensitive to the inhibition. Interestingly, LCA could also potently inhibit human DNA topoisomerase II (topo II) activity at the same concentration (Table I). Fifty percent inhibition of the activities of DNA polymerase β (pol β) and topo II by LCA was observed at doses of 11 and 15 μM, respectively Calf pol α was also moderately inhibited by LCA, and the IC_{50} value was 52 μM. On the other hand, the activities of mammalian pol γ, δ, and ε, higher plant (cauliflower) DNA polymerase I (α-like polymerase) and II (β-like polymerase), prokaryotic DNA polymerases such as the Klenow fragment of *E. coli* DNA polymerase I, T4 DNA polymerase, and Taq DNA polymerase, human immunodeficiency virus type-1 (HIV-1) reverse transcriptase, calf thymus terminal deoxynucleotidyl transferase, DNA metabolic enzymes such as T7 RNA polymerase, T4 polynucleotide kinase, and bovine deoxyribonuclease I were not inhibited by LCA. LCA significantly inhibited the activities of pol β and topo II. LCA should therefore be referred to as a pol β–specific and/or topo II–specific inhibitor.

Influence of Lithocholic Acid on the Hyperchromicity of Double-Stranded DNA—Sun *et al.* (*7*) reported that a high concentration (nearly 400 μM) of daidzein, a natural flavone from *Psoralea corylifolia*, daidzein, inhibited not only the activity of SV-40 DNA polymerase but also that of topo

TABLE I **IC_{50} values of lithocholic acid on the activities of various DNA polymerases and other DNA metabolic enzymes.**

Enzyme	IC_{50} values of LCA (μM)
Calf DNA polymerase α	52
Rat DNA polymerase β	11
*Bovine DNA polymerase γ	>100
*Rat DNA polymerase δ	>100
*Rat DNA polymerase ε	>100
Plant DNA polymerase I (α like)	>100
Plant DNA polymerase II (β like)	>100
E. coli DNA polymerase I (Klenow fragment)	>100
T4 DNA polymerase	>100
Taq DNA polymerase	>100
HIV-1 Reverse transcriptase	>100
Calf Terminal deoxynucleotidyl transferase	>100
T7 RNA polymerase	>100
T4 Polynucleotide kinase	>100
Bovine Deoxyribonuclease I	>100
Human DNA topoisomerase II	15

LCA was incubated with each enzyme (0.05 unit) The enzymatic activity was measured as described previously (*19, 23, 24*). Enzyme activity in the absence of the compounds was taken as 100 %
*From Ogawa *et al.* (Ref. *3*)

II, although the effects were weak. The flavone first intercalates into the DNA molecule as a template-primer, and subsequently inhibits both activities indirectly through induction of a change in the conformation of the DNA.

Whether LCA inhibited the activities through intercalation should be examined To determine whether LCA binds to DNA, the melting temperature (T_m) of double-stranded DNA (dsDNA) in the presence of 15 μM LCA was measured using a spectrophotometer equipped with a thermoelectric cell holder (Fig. 2). As described in "MATERIALS AND METHODS," calf thymus dsDNA was dissolved at 6 μg/ml in 0.1 M sodium phosphate buffer (pH 7.0) containing 1% DMSO. At this concentration of LCA, the thermal transition of Tm was not observed, whereas 15 μM ethidium bromide (EtBr), a typical intercalating agent, caused the thermal transition (Fig. 2). These compounds at 100 μM also induced no thermal transition of Tm (data not shown). Thus, LCA did not bind to the dsDNA, suggesting that it must inhibit the enzyme activities by a direct interaction.

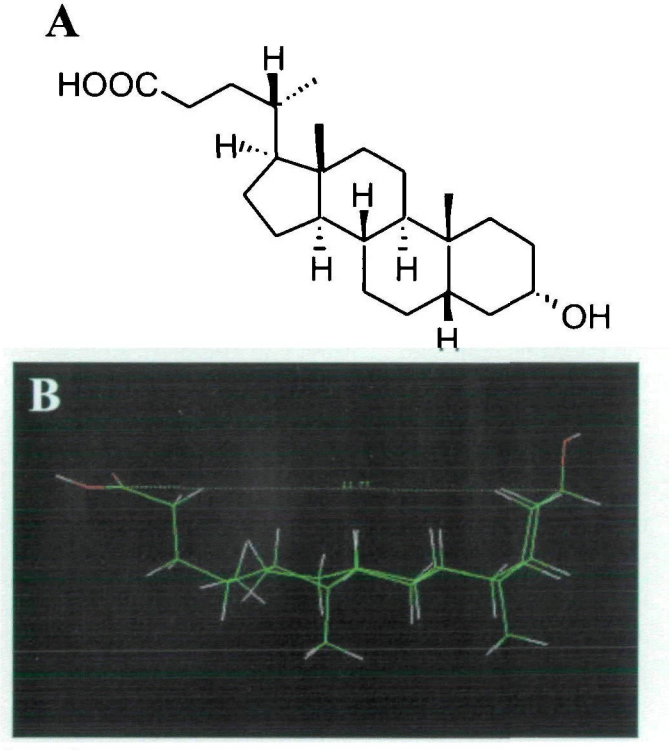

Fig 1 **Structure of lithocholic acid (ionized at neutral pH).** (A) Chemical structure of LCA. (B) The three-dimensional structure of LCA

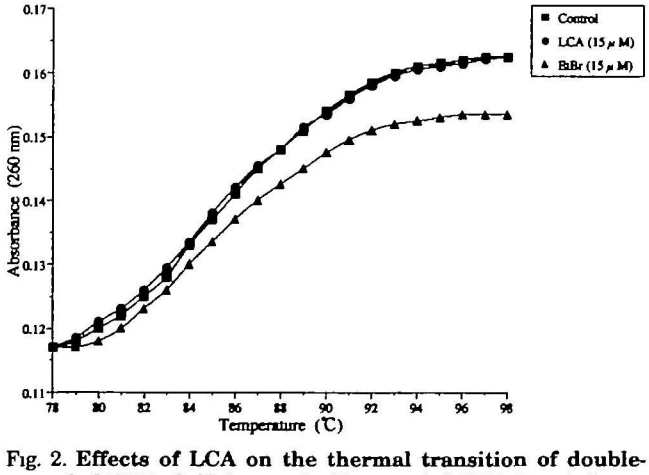

Fig. 2. **Effects of LCA on the thermal transition of double-stranded DNA.** Calf thymus double-stranded DNA (6 μg/ml) was incubated alone (control, ■) or with 15 μM lithocholic acid (LCA, ●) or 15 μM ethidium bromide (EtBr, ▲) in 0 1 M Na-phosphate buffer (pH 7.0)

Mapping of Lithocholic Acid Interaction Interface of the N-Terminal 8-kDa Domain of DNA Polymerase β—Pol β used in this study has been extensively studied, and its amino acid sequence and secondary and tertiary structures have been reported (*8–13, 36*). The enzyme can be divided into two domain fragments by controlled proteolysis: an 8-kDa N-terminal fragment and a 31-kDa C-terminal frag-

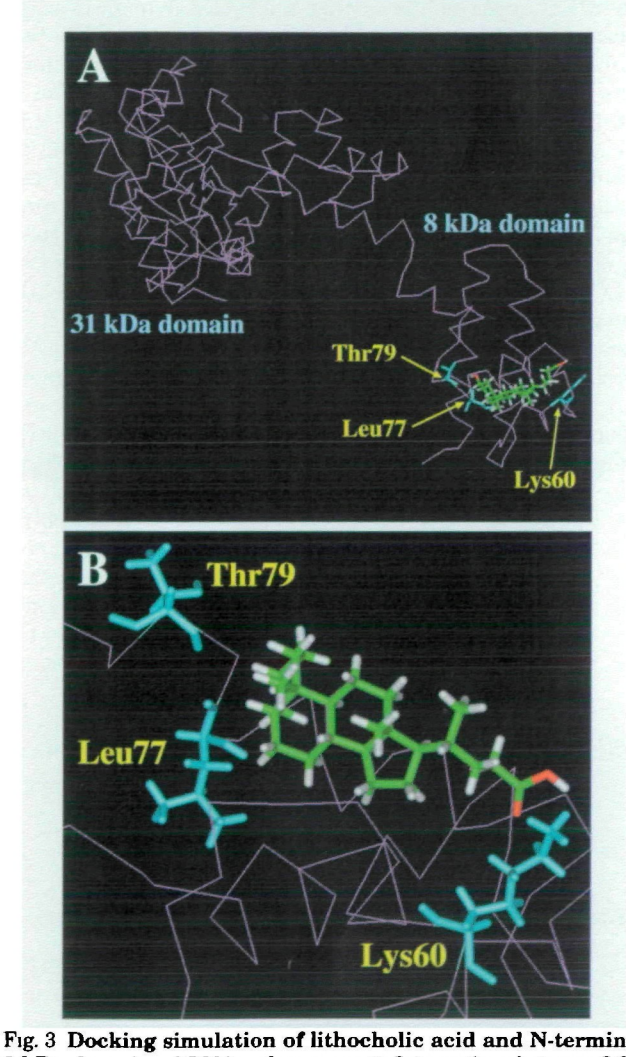

Fig. 3 **Docking simulation of lithocholic acid and N-terminal 8 kDa domain of DNA polymerase β.** Interactions between LCA and 8-kDa domain of pol β (A and B). The amino acid residues Lys60, Leu77, and Thr79, which were significantly shifted as the cross-peaks from ^1H-^{15}N HMQC NMR experiments, are depicted in blue (*19*) The Cα-backbone of the X-ray crystal structure of pol β is shown in pink. The carbons, oxygens and hydrogens of the structure of LCA are indicated in green, red and white, respectively. The Protein Data Bank code of pol β is 1BNO This figure was displayed using Insight II (Molecular Simulations).

ment (*37, 38*). The 31-kDa domain is the catalytic part involved in DNA polymerization, and the 8-kDa domain is the template DNA-binding domain. We prepared the whole enzyme of pol β with a molecular weight (M.W.) of 39 kDa, and the two domain fragments of 8 and 31 kDa. Both fragments were obtained by controlled proteolysis, and purified by FPLC Superose 12 chromatography to near homogeneity (see Fig. 4 in Ref. *24*).

LCA directly inhibited binding of the 8-kDa domain of pol β to the DNA template, and indirectly influenced DNA polymerization on the 31-kDa catalytic domain as determined by analyzing the products of poly(dA)-oligo$(dT)_{16}$ used as the template-primer (*19*). To obtain new information about the structure-function relationship between pol β and topo II, the remainder of this report is devoted to a search for the LCA-binding region of 8 kDa domain of pol β and topo II using computer three-dimensional structural model analysis.

NMR analysis indicated that the 8-kDa domain of pol β (residues 1–87) was formed by four α-helices, packed as two antiparallel pairs (Fig 3A) (*14, 15*). The pairs of α-helices cross one another at 50° giving them a V-like shape. The 8-kDa domain contains a "helix–hairpin–helix" motif (*14, 15*). The complex is in fast exchange on the time-scale of NMR, permitting us to follow the chemical shift changes of the backbone NH and ^{15}N signals of the 8-kDa domain on complex formation by recording a series of ^{1}H–^{15}N HMQC spectra of uniformly ^{15}N-labeled 8-kDa domain in the presence of LCA. Of the 79 amides in residues 5–86 of the 8-kDa domain, 75 were assigned in the LCA complex (*15*). These chemical shift changes could be explained in terms of LCA contact. Surface residues displaying chemical shift changes were predominantly, although not entirely, clustered on one side of the domain. The LCA-binding interface of the 8-kDa domain was a single region consisting mostly of Lys60 in helix-3, Leu77 in helix-4 and Thr79 in the 79-87 unstructured linker segment (Fig 3, A and B) (*19*). In the docking simulation, the binding energy between NH_3^+ in Lys60 and COO^- in LCA was –58.009 kcal/mol through a salt bridge, and the binding force consisted of coulomb (–61.489 kcal/mol) and van der Waals (+3.480 kcal/mol) forces (Table II). The binding energy between LCA and the backbone of Leu77 (H---CO) and between LCA and the backbone of Thr79 (O---NH) was –5.582 kcal/mol. The distances between LCA and Lys60, Leu77, and Thr79 were 1.57 Å, 1.57 Å, and 2.05 Å, respectively (Fig. 3B) We expected that residues between the Lys60 site and the Leu77/Thr79 site would be important for LCA binding

Prasad *et al.* reported that template DNA [*i.e.*, p$(dT)_8$]-binding activity was impaired in site-directed mutants of Phe25, Lys35, Lys60, or Lys68 (*16*). The helix-3–hairpin–helix-4 motif and residues in an adjacent Ω-type loop connecting helix-1 and helix-2 form the ssDNA interaction surface (*16*). Since LCA bound to the ssDNA-binding region of the 8-kDa domain and competed for binding with template DNA (*19*). The only amino acid residue shifted by both LCA binding and ssDNA binding was Lys60 in helix-3 Leu77 and Thr79 are different from the other DNA-binding sites (*i.e.*, Phe25, Lys35, and Lys68). LCA probably competes with template DNA at residue Lys60 and binds to the site, and this subsequently inhibits the ssDNA-binding activity on the 8-kDa domain.

On the pol β protein, one molecule of LCA competes with one molecule of the template-primer DNA and subsequently interferes with binding of the template-primer to the 8-kDa domain, and LCA binding indirectly inhibits catalytic activity on the 31-kDa domain (*19*). The action of LCA was similar to the effect of the long-chain fatty acids reported previously (*23, 24, 39, 40*), although LCA and the fatty acids are structurally unrelated. Both the hydrophilic end (carboxyl group) and hydrophobic sites (methyl chain) in the fatty acids played crucial roles in the inhibition (*23, 24, 40*). The carboxyl group at carbon position 24 in LCA is thought to be important for the inhibition of pol β, because the inhibition disappeased when the carboxyl group was converted to a carboxyl ester (*19*) The carboxyl group, *i.e.*, hydrophilic site, of LCA must be required for inhibition.

Identification of Lithocholic Acid-Binding Residues in DNA Topoisomerase II—As described above, LCA inhibited the activity of topo II without binding to the DNA substrate (Table I and Fig. 2). These results strongly suggested that LCA binds directly to topo II. We searched for the LCA-binding site of topo II by referring to the spatial positions of LCA-binding amino acids on pol β by computer analysis.

TABLE II **The binding energy of lithocholic acid and DNA polymerase β or DNA topoisomerase II.**

Enzyme	LCA-interacting amino acid	Force	Energy (kcal/mol)
8-kDa domain of DNA polymerase β	Lys60	Coulomb	–61 489
		van der Waals	+3 480
		Total	–58 009
	Leu77 & Thr79	Coulomb	–4 481
		van der Waals	–1 101
		Total	–5 582
	All	Coulomb	–72.974
		van der Waals	–10.253
		Total	–83.227
DNA topoisomerase II	Lys720	Coulomb	–58.261
		van der Waals	+1 485
		Total	–56.776
	Leu760 & Thr791	Coulomb	–3.301
		van der Waals	–0.262
		Total	–3.563
	All	Coulomb	–80.269
		van der Waals	–19.354
		Total	–99.623

Fortunately, both pol β and topo II have been structurally characterized by single crystal X-ray diffraction analysis, although the topo II analyzed was from budding yeast (*17, 18*). The crystal structure of a large fragment of yeast topo II was studied in detail by Berger *et al* (*17*) Since this is the only three-dimensional structure of topo II available, and since the amino acid sequences of topo II, especially in their DNA-binding domains, are highly conserved in eukaryotes, we used this structure for our analysis. The DNA binding domains show 50.1% homology and 80 4% similarity between *S. cerevisiae* (residues 500–990) and human (residues 512–999) [accession numbers M13814 and NP-001058]. The topo II molecule was shown to be a heart-shaped dimeric protein with a large central hole with overall dimensions of 120 × 85 × 55 Å. The polypeptide chain folds into two subfragments designated as A′ and B′: A′ contains residues 682–1178, while B′ contains residues 420–633 The active sites of the dimer associate covalently with DNA-break-points. The amino acid sequence in the DNA-binding site is completely different from that of the 8-kDa domain of pol β, lacking even a box of similar amino acid sequence.

The geometrical method for template-based finding of topo II was highly constrained by distance constraints between each of the three Cα-atoms of LCA-binding amino acids of pol β (*i.e.*, Lys60, Leu77, and Thr79) (Fig. 3). There were three geometrically selected groups of three amino acids in topo II. Group I consisted of Lys789, Thr744, and Leu748; group II was Lys720, Thr791, and Leu760, and group III was Lys700, Thr785, and Leu761. The amino acid residues of each group were searched for by the evolutionary trace method adjusted to maximize either specificity or sensitivity, and thereby the focus of the trace was shifted from residues that are functionally essential to the Lys720 and Leu760 residues of group II, which regulate specific functional features in the PIR database (Fig. 4). Thr791 in group II and all residues of groups I and III were not conserved in the data base. The evolutionary trace method is a systematic, transparent and novel predictive technique that identifies active sites and functional interfaces in proteins with known structures (*30, 31*). This method identifies conserved and specific residues in different members of the same family, and therefore can be used to find the relationships between function and sequence of different members. In Fig. 4, the asterisks (*) and colons (:) show the conserved and the similar amino acids, respectively, and indicate the amino acids in the catalytic site and the functional interface on topo II. The amino acid residues from 710 to 812 in topo II were well conserved (Fig. 4).

In pol β and topo II, the three amino acid residues that were placed at the same three-dimensional position were Lys60, Leu77, and Thr79 in pol β, and Lys720, Leu760, and Thr791 in topo II. The geometrical method indicated that the distances between the Cα-atoms of the amino acids in pol β were almost the same as those in topo II (Fig 5, A and B). The longest distance between Lys60 and Thr79 of pol β was calculated as 19.67 Å, almost the same as that between Lys720 and Thr791 of topo II (18 89 Å) The difference in the distances between the two enzymes was within 0 93 Å. The length of the LCA molecule was 11.68 Å (Fig. 1B). Therefore, LCA must enter the pocket formed by these three amino acids in both pol β (Fig. 5C) and topo II (Fig. 5D)

Docking Modeling of the Lithocholic Acid Binding to DNA Polymerase β or DNA Topoisomerase II—As described above, the mixture of both geometrical and evolutionary

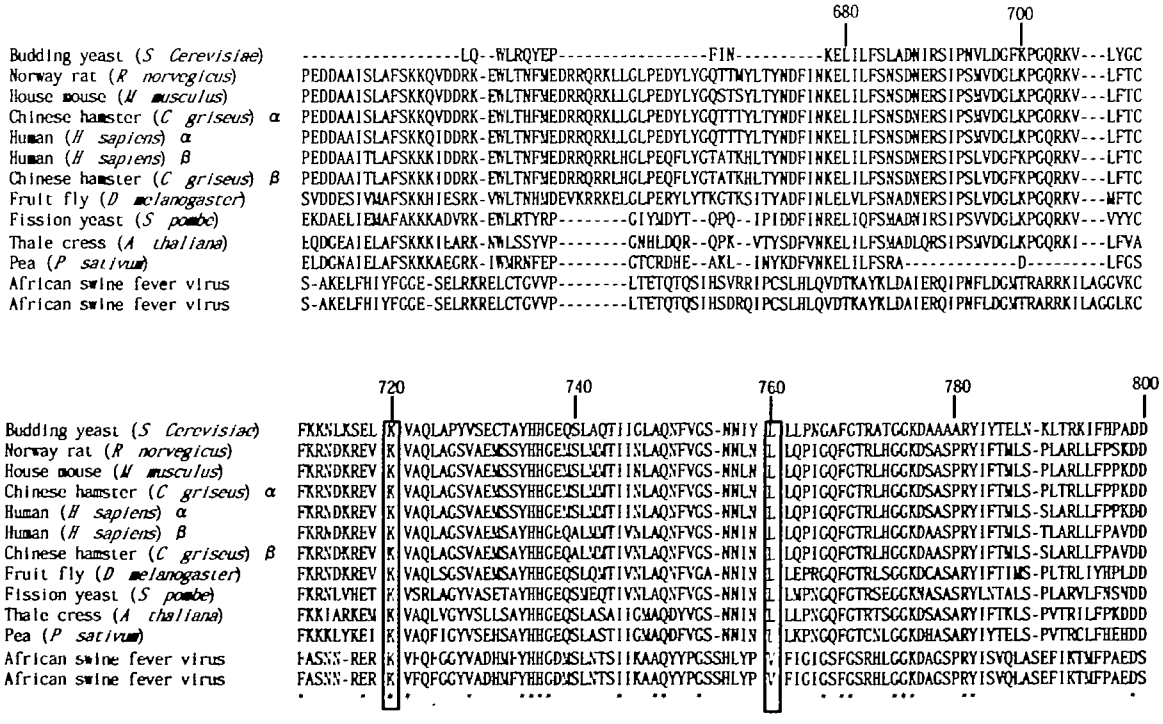

Fig. 4 **Evolutionary tracing of LCA-binding amino acids on eukaryotic type II DNA topoisomerase (amino acid residues 666 to 800).** Asterisks (*) and colons (:) show conserved and similar amino acids, respectively The evolutionary trace method was used to determine the amino acids in the catalytic site and the functional interface on topo II (*30, 31*)

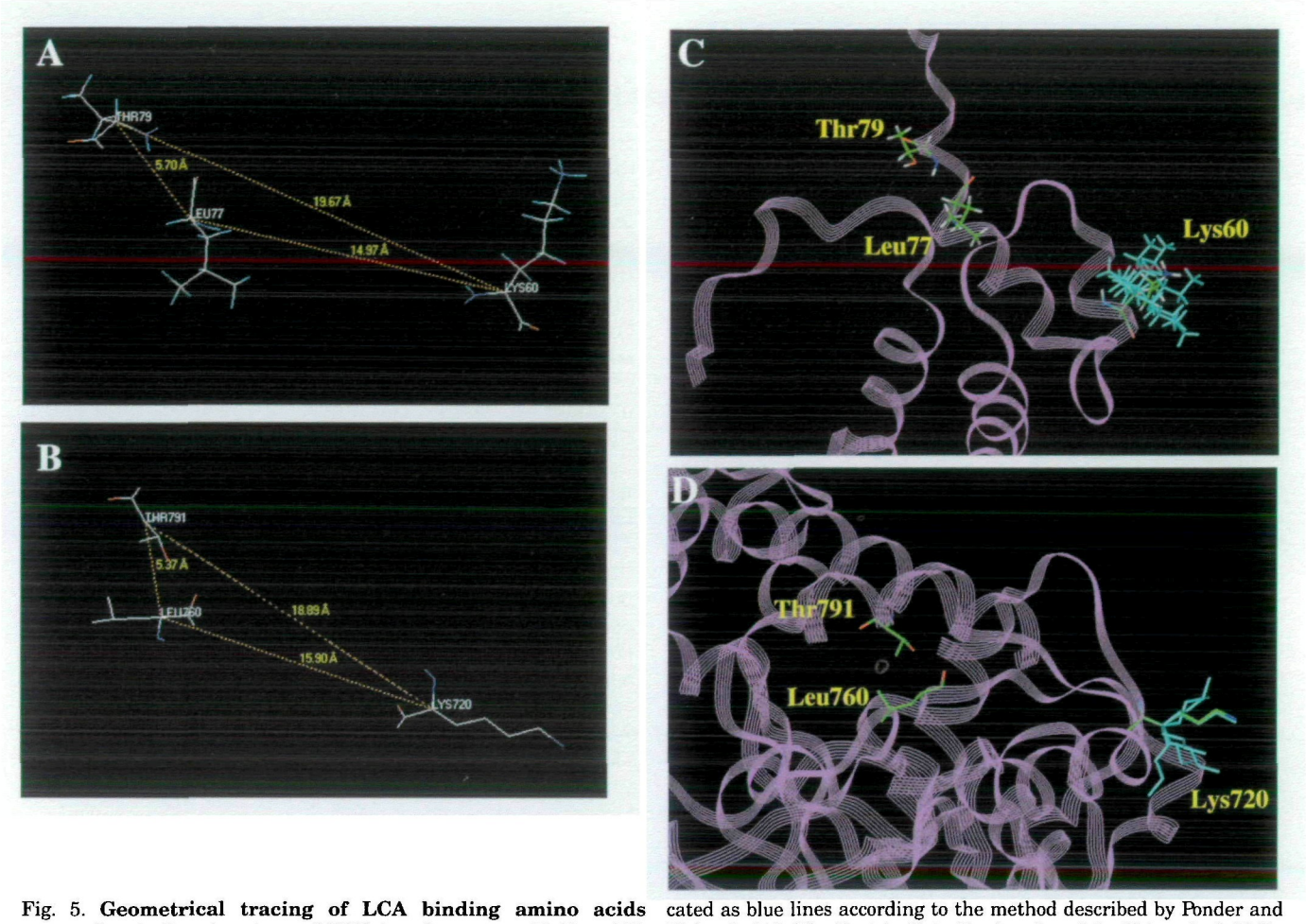

Fig. 5. **Geometrical tracing of LCA binding amino acids mapped onto the structure of DNA topoisomerase II.** (A and B) The distances between Cα-carbons of LCA-binding amino acids of pol β (A) and topo II (B) are shown. (C and D) LCA-binding amino acids mapped onto the ribbon structure of pol β (C) and topo II (D) are shown. The flexibility of Lys60 in pol β and Lys720 in topo II is indicated as blue lines according to the method described by Ponder and Richards (*41*). The Protein Data Bank codes of the N-terminal 8 kDa domain of pol β and topo II are 1BNO and 1BJT, respectively. This figure was displayed using Insight II (Molecular Simulations, San Diego, CA, 1999).

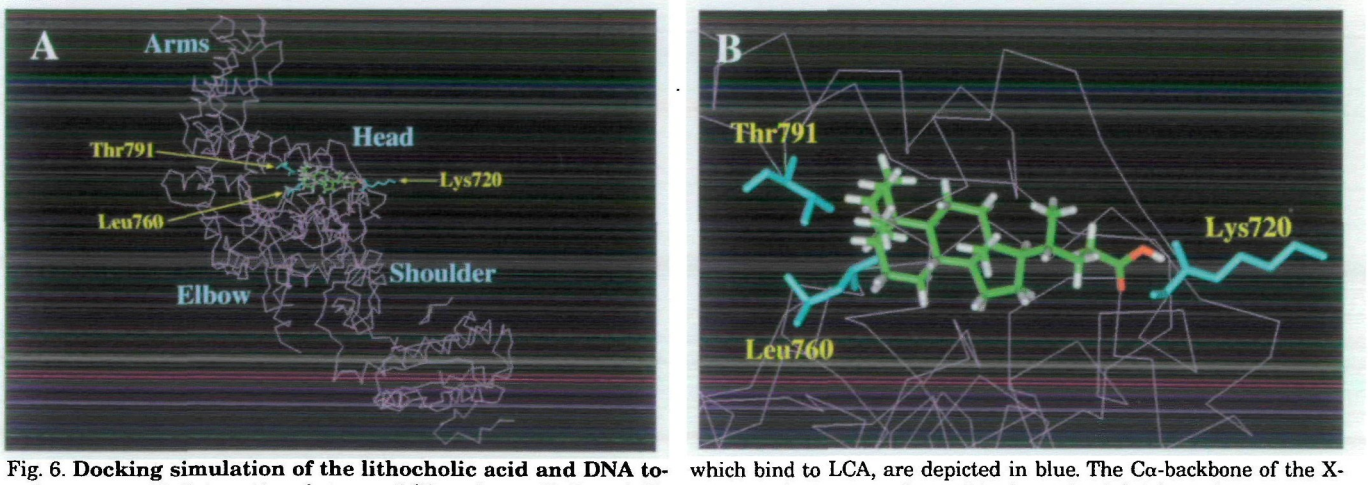

Fig. 6. **Docking simulation of the lithocholic acid and DNA topoisomerase II.** Interactions between LCA and topo II (A and B). Evolutionary tracing and the geometrical method were carried out with Insight II/Binding Site Analysis (Molecular Simulations, San Diego, CA, 1999), and amino acid residues Lys720, Leu760, and Thr791, which bind to LCA, are depicted in blue. The Cα-backbone of the X-ray crystal structure of topo II is shown in pink. The carbons, oxygens and hydrogens of the structure of LCA are indicated green, red and white, respectively. The Protein Data Bank code of topo II is 1BJT. This figure was displayed using Insight II (Molecular Simulations).

tracing is a new method for uncovering functionally important residues in proteins. Modeling of the LCA interaction interface on topo II obtained by computer analysis was compared with pol β modeling using the results of NMR analysis of the 8-kDa domain of pol β with LCA. In the ^{1}H–^{15}N HMQC NMR experiments, LCA on the 8-kDa domain was bridged with Lys60, Leu77, and Thr79, and intercalated smoothly into the pocket between helix-3 and helix-4 in the 62–68 turn (Fig. 3 and Fig. 5C).

The results of computer simulation of the binding mode between pol β and LCA are shown in Fig. 3, and those between topo II and LCA are shown in Fig. 6. In this docking simulation, LCA on topo II was bridged with Lys720, Leu760, and Thr791, and intercalated smoothly into the pocket (Figs. 5D and 6) The distance between LCA and the surface residues of topo II was within 2.11 Å. The binding force between LCA and topo II consisted of the salt bridge between NH_3^+ of Lys720 and COO^- of LCA and the hydrogen bond between the hydroxyl group of LCA and the backbone functional group of Leu760 or Thr791. The binding energies of Lys720-LCA and Leu760-LCA-Thr791 were –56.776 and –3.563 kcal/mol, respectively (Table II). The LCA-Lys720 binding was much tighter than that of LCA-Leu760 or LCA-Thr791. The total binding energy of LCA and pol β (–83.227 kcal/mol) was almost the same as that of LCA and topo II (–99.623 kcal/mol).

Lys60 in the 8-kDa domain of pol β binds to both LCA and DNA (*16, 19*). The "head" region in topo II is considered to be the DNA-binding site (*17, 18*), and this region has the three LCA-binding amino acid residues (Fig. 6A). Therefore, one (probably Lys720) or more of these amino acids may bind to the DNA. Figure 5 shows the flexibility of the residues of Lys60 in pol β (or Lys720 in topo II), calculated by the method of Ponder and Richards (*41*). This flexibility was employed in the docking simulations above.

To obtain further information on the binding of LCA and topo II, mutant proteins in which each of the three relevant amino acid residues in topo II (*i.e.*, Lys720, Leu760, and Thr791) is replaced should be used in the future studies.

The present results reveal a three-dimensional structural relationship between pol β and topo II in their DNA-binding sites. As described in the Introduction, LCA, one of the major secondary bile acids, promotes carcinogenesis in rat colon epithelial cells induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, which methylates DNA (*1, 2*). Epidemiological studies have shown that dietary factors are important in the etiology of colon cancer. High intake of fat, which elevates secretion of fecal bile acids, is correlated with a high incidence of colon cancer (*42, 43*). In fact, such dietary habits in Western populations cause a higher incidence of colon cancer and higher levels of fecal bile acids than in other populations (*44*). The molecular mechanism responsible for the cancer-promoting activity of this bile acid remains obscure. Our studies may provide an insight into the mechanism of this effect, because LCA selectively inhibited the activities of pol β and topo II, both of which are critical for DNA replication, repair and recombination, and subsequently pol β and topo II emerged as important cellular targets for LCA-induced oncogenesis. LCA causes cancer as a topo II inhibitor, while many other inhibitors of topo II are anticancer drugs, such as etoposide, teniposide, doxorubicin, daunorubicin, idarubicin, and mitoxanthrone. Reportedly, DNA topoisomerase inhibitors are often carcinogenic (*45–47*). They cause the secondary carcinomas, which develop several years after their use in treatment of leukemia/lymphoma of children (*45–47*). Since pol β is known to participate in the short-patch base excision repair pathway (*48–50*), the LCA-induced oncogenesis may be related to the inhibition of the repair system. There is a conflict of interest here. A more detailed explanation of how the two enzymes might act in treating LCA-induced oncogenesis will enhance the significance of their study.

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