Inhibitory Action of Conjugated C_{18}-Fatty Acids on DNA Polymerases and DNA Topoisomerases

Yoshiyuki Mizushina[a,b,*], Tsuyoshi Tsuzuki[c], Takahiro Eitsuka[c], Teruo Miyazawa[c], Kanako Kobayashi[a], Hiroshi Ikawa[a], Isoko Kuriyama[a], Yuko Yonezawa[a], Masaharu Takemura[d], Hiromi Yoshida[a,b], and Kengo Sakaguchi[e]

[a]Laboratory of Food & Nutritional Sciences, Department of Nutritional Science and [b]High Technology Research Center, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan, [c]Food and Biodynamic Chemistry Laboratory, Graduate School of Life Science and Agriculture, Tohoku University, Sendai 981-8555, Japan, [d]Life Science Research Center, Mie University, Tsu, Mie 514-8507, Japan, and [e]Department of Applied Biological Science, Frontier Research Center for Genomic Drug Discovery, Tokyo University of Science, Noda-shi, Chiba 278-8510, Japan

ABSTRACT: We reported previously that unsaturated linear-chain FA of the *cis*-configuration with a C_{18}-hydrocarbon chain such as linoleic acid (18:2Δ9c,12c) could potently inhibit the activities of mammalian DNA polymerases and DNA topoisomerases, but their saturated forms could not. There are chemically two classes of unsaturated FA, normal and conjugated, but only the conjugated forms show potent antitumor activity. In this report, we study the inhibitory effects of chemically synthesized conjugated C_{18}-FA on mammalian DNA polymerases and DNA topoisomerases as compared with normal unsaturated FA. The conjugated α-eleostearic acid (18:3Δ9*c*,11*t*,13*t*) was the strongest of all the FA tested. For the inhibition, the conjugated form is crucially important. The energy-minimized 3-D structures of the FA were calculated, and both a length of less than 20 Å and a width of 8.13–9.24 Å in the C_{18}–FA structure were found to be important for enzyme inhibition. The 3-D structure of the active site of both DNA polymerases and topoisomerases must have had a pocket to join α-eleostearic acid, and this pocket was 12.03 Å long and 9.24 Å wide.

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We have screened inhibitors of mammalian DNA polymerases (1,2), and found that mammalian DNA polymerase α and β (pol α and β) are inhibited by linear-chain FA with the following characteristics: a hydrocarbon chain containing 18 or more carbons, a free carboxyl end, and the double bonds with the *cis*-configuration (1–3). The mode of binding of linear-chain FA to pol β has been studied extensively using NMR spectroscopy; FA were shown to specifically bind to the proteolytic N-terminal 8-kDa domain fragment of pol β at the DNA-binding groove (4). FA could also strongly inhibit the activities of human DNA topoisomerase I and II (topo I and II), with a degree of inhibition almost the same as that of the polymerases (5). In these studies, we realized the importance of the 3-D structure of FA and the presence of two classes of unsaturated linear FA, normal and conjugated. In this report, we describe the relationship between FA and the mode of inhibition, concentrating on the actions of conjugated FA.

Conjugated FA are positional and geometrical isomers in which conjugated double bonds are present. Among such FA, CLA, a mixture of 9*c*-, 11*t*-, 10*t*-, and 12*c*-octadecadienoic acids (18:2Δ9*c*,11*t* and 18:2Δ10*t*,12*c*, respectively) as major constituents, is known to have various physiological properties (6–10). It has been reported that dietary supplementation of conjugated linoleic acid reduces the incidence of mammary tumors in mice (11). CLA has also been shown to inhibit the proliferation of human breast cancer cells (12), colon cancer cells (13), and lung cancer cells (14) in culture. Additionally, n-3 highly unsaturated FA such as α-linolenic acid have been shown to have anticarcinogenic activity *in vitro* and *in vivo* (15–18). We previously reported the effect of *in vivo* tumor growth suppression by conjugated FA (19,20). Therefore, we prepared conjugated C_{18}-FA converted from linoleic acid (18:2Δ9*c*,12*c*), α-linolenic acid (18:3Δ9*c*,12*c*,15*c*), and stearidonic acid (18:4Δ6*c*,9*c*,12*c*,15*c*). The conjugated FA could also act as inhibitors of DNA polymerases and topoisomerases, and could be even stronger inhibitors than linoleic acid. As expected, some conjugated FA were strong inhibitors. Using computer modeling and 3-D structural analysis, we suggest the 3-D site where the enzyme binds to the conjugated FA. The molecular design method may be useful in creating agents for cancer chemotherapy.

MATERIALS AND METHODS

Materials. The linear-chain FA were named using the nomenclature described by Weete (21). In the following symbols, (A:B_2 ΔC_1*c*,C_2*t*), "A" refers to the number of carbon atoms, "B_2" refers to the number of double bonds, "C_1,C_2" represents the position of each double bond from the carboxyl end of the molecule, and "*c*" and "*t*" refer to the *cis*- and *trans*-configurations of the double bond, respectively. Saturated C_{18}-FA; *n*-octadecanoic acid (stearic acid, 18:0), and the *cis*-configuration of unsaturated C_{18}-FA such as *cis*-9-octadecenoic acid (oleic acid, 18:1Δ9*c*), *cis*-9,12-octadecadienoic acid (linoleic acid, 18:2Δ9*c*,12*c*), *cis*-9,12,15-octadeca-

*To whom correspondence should be addressed at the Department of Nutritional Sciences, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan. E-mail: mizushin@nutr.kobegakuin.ac.jp

Abbreviations: dsDNA, double-stranded DNA; dTTP, [^{3}h]2′-deoxythymidine 5′-triphosphate; NP-40, Nonidet P-40; oligo(dT)$_{12–18}$, oligo(12–18) deoxyribothymidylic acid; pol, DNA-directed DNA polymerase (EC 2.7.7.7); poly(dA), polydeoxyriboadenylic acid; poly(rC), polycytidylic acid; topo, DNA topoisomerase.

trienoic acid (α-linolenic acid, 18:3Δ9*c*,12*c*,15*c*), and *cis*-6,9,12,15-octadecatetraenoic acid (stearidonic acid, 18:4Δ6*c*,9*c*,12*c*,15*c*) were purchased from Nu-Chek-Prep Inc. (Elysian, MN). Nucleotides such as [^{3}H]2′-deoxythymidine 5′-triphosphate (dTTP, 43 Ci/mmol) and chemically synthesized template-primers such as polydeoxyriboadenylic acid [poly(dA)] and oligo(12–18) deoxyribothymidylic acid [oligo(dT)$_{12–18}$] were purchased from Amersham Biosciences (Buckinghamshire, United Kingdom). All other reagents were of analytical grade and were purchased from Wako Ltd. (Osaka, Japan).

Preparation of conjugated C_{18}-FA by alkaline treatment. Conjugated C_{18}-FA were prepared by using alkaline treatment following an AOAC method with slight modifications (22). Potassium hydroxide at a concentration of the 6.6 or 21% (w/w) in ethylene glycol was prepared, and the potassium hydroxide solution was bubbled for 5 min with nitrogen gas. Ten milligrams of linoleic acid, linolenic acid, or stearidonic acid was added to 1 mL of 6.6 or 21% potassium hydroxide solution in a test tube (10 mL volume). The mixture was bubbled with nitrogen gas and then screw-capped and allowed to stand for 5 or 10 min at 180°C. The reaction mixture was cooled and 1 mL of methanol was added. The mixture was acidified to below pH 2 with 2 mL of 6 N HCl. After dilution with 2 mL of distilled water, the conjugated FA was extracted with 5 mL of hexane. The hexane extract was then washed with 3 mL of 30% methanol and with 3 mL of distilled water before being evaporated under a stream of nitrogen gas. Each isomer of converted C_{18}-FA was separated using a Shimadzu HPLC system. The conjugated FA were stored at −20°C after being purged with nitrogen gas. UV/Vis spectrophotometric analysis of the conjugated FA was performed with a Shimadzu UV-2400PC. Spectrophotometric readings confirmed the conjugated FA of diene (at 235 nm), triene (268 nm), and tetraene (315 nm) (22,23).

Enzymes. DNA polymerase α (pol α) was purified from calf thymus by immuno-affinity column chromatography, as described previously (24). Recombinant rat pol β was purified from *Escherichia coli* JMpβ5 as described by Date *et al.* (25). The human pol γ catalytic gene was cloned into pFastBac. Histidine-tagged enzymes were expressed using the BAC-TO-BAC HT Baculovirus Expression System according to the supplier's manual (Life Technologies, Rockville, MD) and purified using ProBoundresin (Invitrogen Japan, Tokyo, Japan) (Mizushina, Y., Yoshida, H., and Sakaguchi, K., unpublished data). Human pol δ and ε were purified from the nuclear fraction of human peripheral blood cancer cells (Molt-4) using the second subunit of pol δ- and ε-conjugated-affinity column chromatography, respectively (26). Human recombinant DNA topoisomerase I (topo I) and DNA topoisomerase IIα (topo II) (2 units/μL each) were purchased from TopoGen, Inc. (Columbus, OH).

DNA polymerase assays. Activities of DNA polymerases were measured by methods described previously (2,3). For DNA polymerases, poly(dA)/oligo(dT)$_{12–18}$ and [^{3}H]-2′-deoxythymidine 5′-triphosphate were used as the template-primer DNA and nucleotide substrate, respectively. Prepared conjugated C_{18}-FA were dissolved in DMSO at various concentrations and sonicated for 30 s. Aliquots of 4 μL of sonicated samples were mixed with 16 μL of each enzyme (final, 0.05 units) in 50 mM Tris-HCl (pH 7.5) containing 1 mM DTT, 50% glycerol, and 0.1 mM EDTA, and held at 0°C for 10 min. These inhibitor-enzyme mixtures (8 μL) were added to 16 μL of each of the enzyme standard reaction mixtures, and incubation was carried out at 37°C for 60 min, except for Taq DNA polymerase, which was incubated at 74°C for 60 min. The activity without the inhibitor was considered 100%, and the remaining activities at each concentration of inhibitor were determined relative to this value. One unit of DNA polymerase activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol of dTTP into synthetic template-primers [i.e., poly(dA)/oligo(dT)$_{12–18}$, A/T = 2:1] in 60 min at 37°C under normal reaction conditions for each enzyme (2,3).

DNA topoisomerase (topo) assays. Relaxation activities of DNA topoisomerases were determined by detecting the conversion of supercoiled plasmid DNA to its relaxed form. The topo II reaction was performed in 20-μL reaction mixtures containing 50 mM Tris-HCl buffer (pH 8.0), 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM DTT, pBR322 plasmid DNA (200 ng), 2 μL of inhibitor solution (10% DMSO), and one unit of topo II. The reaction mixtures were incubated at 37°C for 30 min and terminated by adding 2 μL of loading buffer consisting of 5% sarkosyl, 0.0025% bromophenol blue, and 25% glycerol. The mixtures were subjected to 1% agarose gel electrophoresis in Tris-acetate-EDTA running buffer. The agarose gels were stained with ethidium bromide, and DNA was visualized on a UV transilluminator. Zero-D scan (version 1.0; M&S Instruments Trading Inc., Tokyo, Japan) was used for densitometric quantitation of the plasmid DNA products. Relaxation activity of topo I was analyzed in the same manner as described above, except that the reaction mixtures contained 10 mM Tris-HCl (pH 7.9), pUC19 plasmid DNA (200 ng), 1 mM EDTA, 150 mM NaCl, 0.1% BSA, 0.1 mM spermidine, 5% glycerol, and one unit of topo I. One unit was defined as the amount of enzyme capable of relaxing 0.25 μg of DNA in 15 min at 37°C.

Computational analysis of C_{18}-FA. A compound model was constructed and simple-minimized. Compound models were simulated with force field parameters based on the Consistent Valence Force Field. Group-based cutoffs, 0.95 nm for van der Waals and 0.95 nm for Coulomb interactions, were introduced. The temperature was set at 298 K. Calculations based on simulation images were carried out using INSIGHT II (Accelrys Inc., San Diego, CA).

RESULTS AND DISCUSSION

Conjugated C_{18}-FA-mediated inhibition of mammalian DNA polymerases and human topo. First of all, we tested whether several classes of linear-chain conjugated C_{18}-FA have the ability to inhibit calf DNA pol α, rat pol β, and human DNA topo I and II. Twenty micromoles of saturated FA with an 18-hydrocarbon chain

(i.e., stearic acid, 18:0) inhibited pol α activity (Fig. 1A), with an IC_{50} value of 15.0 μM, but had no inhibitory effect on pol β, topo I, or topo II (Figs. 1B–D). On the other hand, monounsaturated linear-chain C_{18}-FA of the *cis*-configuration (i.e., oleic acid, 18:1Δ9*c*) significantly inhibited the activities of all enzymes tested. Only the conjugated FA inhibited the activities of pol β, topo I, and topo II, which could be molecular targets for anticancer chemotherapy. We therefore systematically tested the inhibitory effect of the three purified $C_{18:2}$-conjugated FA (18:2Δ9*c*,11*c*, 18:2Δ9*t*,11*t*, and 18:2Δ10*t*,12*c*) converted from linoleic acid (18:2Δ9*c*,12*c*), the six purified $C_{18:3}$-conjugated FA [jacaric acid (18:3Δ8*c*,10*t*,12*c*), calendic acid (18:3Δ8*t*,10*t*,12*c*), punicic acid (18:3Δ9*c*,11*t*,13*c*), α-eleostearic acid (18:3Δ9*c*,11t,13t), β-eleostearic acid (18:3Δ9*t*,11*t*,13*t*)] converted from linolenic acid (18:3Δ9*c*,12*c*,15*c*), and a purified $C_{18:4}$-conjugated FA (18:4Δ9*c*,11*t*,13*t*,15*c*) converted from stearidonic acid (18:4Δ6*c*,9*c*,12*c*,15*c*), toward the DNA polymerases and topoisomerases.

As shown in Figure 1, at 20 μM, conjugated C_{18}-FA of the *cis*-configuration were more potent inhibitors than unsaturated C_{18}-FA of the *cis*-configuration. Notably, the inhibitory effect of 18:2Δ10*t*,12*c* was stronger than that of linoleic acid, and similarly, the $C_{18:4}$-conjugated FA was a stronger inhibitor than the $C_{18:4}$ FA. Among $C_{18:3}$-FA, α-eleostearic acid was the strongest inhibitor of the DNA polymerases and topoisomerases. On the other hand, no *trans*-conjugated FA

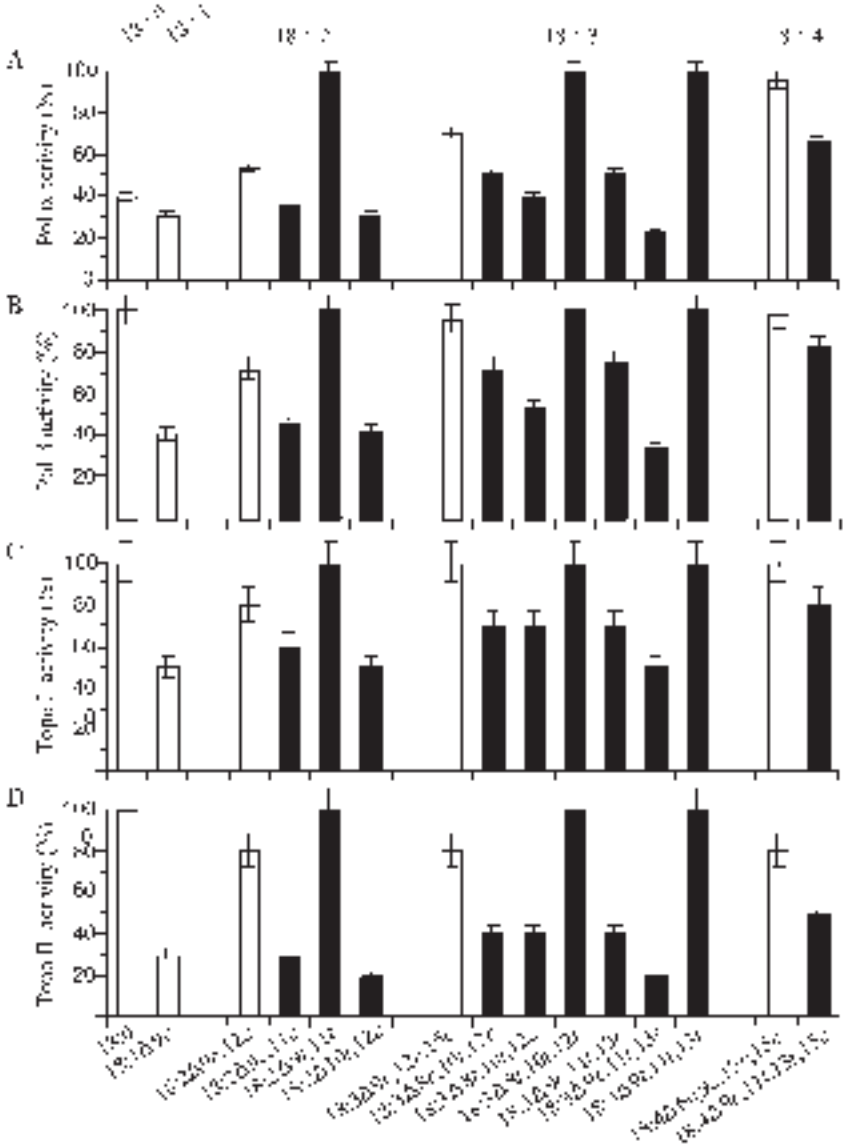

FIG. 1. Inhibitory effect of conjugated linear-chain C_{18}-FA (20 μM each) on DNA polymerases and DNA topoisomerases. (A) calf pol (DNA-directed DNA polymerase) α; (B) rat pol β; (C) human topo (DNA topoisomerase) I; (D) human topo II. The white bars are normal FA and the black bars are conjugated FA. Enzyme activity in the absence of FA was taken as 100%. Data are shown as means ± SEM of three independent experiments.

completely inhibited the activities of DNA polymerases and topoisomerases. These results suggest that conjugated unsaturating carbohydrate bonds of the *cis*-configuration in these FA play a critical role in the pol α, pol β, topo I, and topo II inhibitions, but the number of conjugated unsaturating carbohydrate bonds seems to have no relation to the inhibitory effect.

Figure 2 shows the dose–response curves for each of the normal or conjugated unsaturated linear-chain C_{18}-FA against calf pol α and rat pol β. The inhibition by these C_{18}-FA was dose dependent. Among $C_{18:2}$- and $C_{18:3}$-FA, conjugated FA were stronger pol α and β inhibitors than nonconjugated FA. The inhibitory effect of α-eleostearic acid was the strongest, with 50% inhibition for pol α and β observed at doses of 10.7 and 13.4 μM, respectively. Table 1 shows IC_{50} values for mammalian pol α to ε and human topos I and II. The inhibitory effect of five mammalian DNA polymerases by normal and conjugated C_{18}-FA was the same as that of human DNA topoisomerases, suggesting that pol α to ε and topo I and II have the same inhibitory mechanism.

Double reciprocal plots of the results indicated that the inhibition of both calf pol α and rat pol β by α-eleostearic acid was through competition with the DNA template primer [i.e., poly(dA)/oligo$(dT)_{12–18}$] (data not shown), suggesting that α-eleostearic acid might compete with the template primer to bind to the catalytic site of the DNA polymerases.

To determine the effects of a nonionic detergent on the binding of α-eleostearic acid to the DNA polymerases and topoisomerases, a neutral detergent, Nonidet P-40 (NP-40), was added to the reaction mixture at a concentration of 0.1%. In the absence of α-eleostearic acid, the enzyme activity was taken as 100%. As shown in Table 2, the inhibitory effect on pol α, pol β, topo I, and topo II by α-eleostearic acid at 25 μM was largely reversed by the addition of NP-40 to the reaction mixture, suggesting that the binding to the enzyme by α-eleostearic acid is hydrophobic. To determine whether the effects of the compound were due to nonspecific adhesion to the enzymes or to selective binding at specific sites, we also tested whether an excess amount of a substrate analogue, polycytidylic acid [poly(rC)] (100 μg/mL), or a protein, BSA (100 μg/mL), could prevent the inhibitory effects of α-eleostearic acid (Table 2). Poly(rC) and BSA showed little or no influence on the effects of α-eleostearic acid, suggesting that the binding to the DNA polymerases and topoisomerases occurs selectively.

3-D modeling of conjugated C_{18}-FA. The conjugated C_{18}-FA, α-eleostearic acid, was the strongest inhibitor of DNA polymerases and topoisomerases and was considered to bind directly to the template-primer binding site in the DNA polymerases. Therefore, the inhibition of DNA topoisomerase by α-eleostearic acid occurs in a similar manner between chemical structures of the enzyme protein and the FA, as previously described for the DNA polymerases and normal FA (2,3). Based on the previous computer modeling data, we carried out a 3-D structural analysis of the linear-chain conjugated C_{18}-FA, and speculated on the model of the DNA polymerases and topoisomerases with the FA. The energy-minimized 3-D C_{18}-FA obtained by computer modeling are shown in Figure 3. Oleic acid and α-eleostearic acid formed a V-shaped curve, with molecular widths of 8.50 and 9.24 Å, respectively (Table 3). The hydrocarbon chain in the saturated and *trans*-unsaturated FA molecule was linear because of the zero or low width, and did not inhibit the enzyme activities

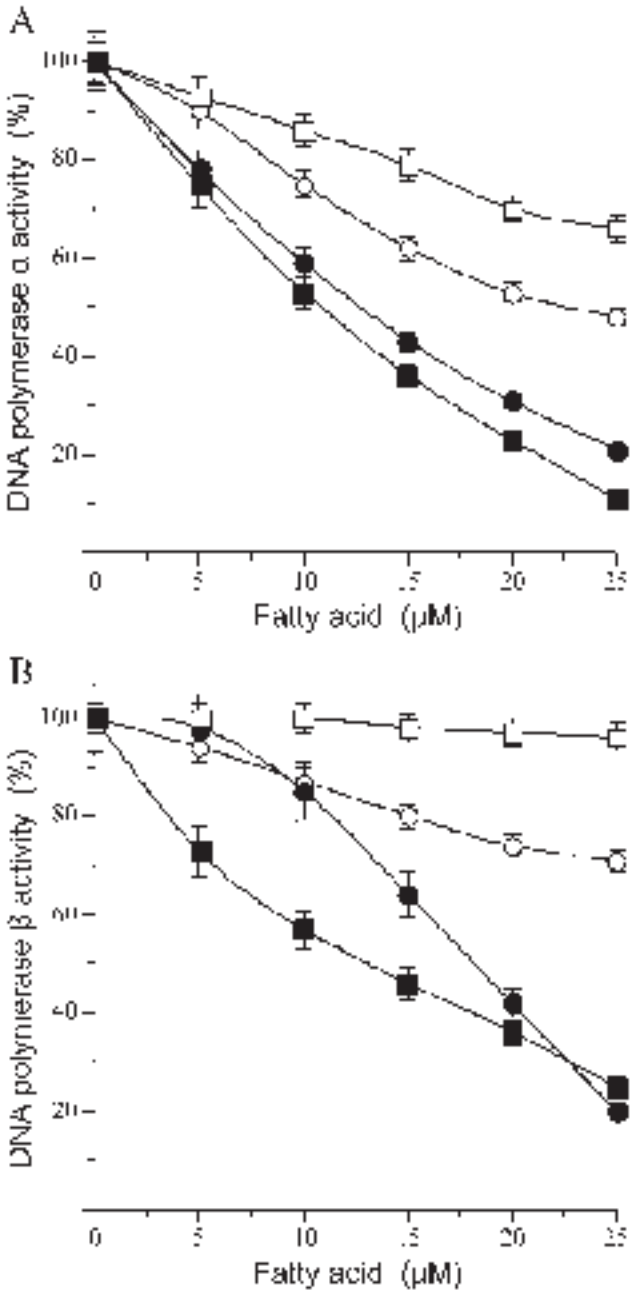

FIG. 2. Dose–response curves of conjugated linear-chain C_{18}-FA for DNA polymerases. (A) Calf pol α; (B) rat pol β. $C_{18:2}$-FA are linoleic acid (18:2Δ9*c*,12*c*, open circle) and 18:2Δ10*t*,12*c* (closed circle). $C_{18:3}$-FA are linolenic acid (18:3Δ9*c*,12*c*,15*c*, open square) and α-eleostearic acid (18:2Δ9*c*,11*t*,13*t*, closed square). The DNA polymerase activity in the absence of FA was taken as 100%. Data are shown as means ± SEM of three independent experiments. For abbreviations see Figure 1.

TABLE 1
IC_{50} Values (μM) of Enzymatic Inhibition Against Mammalian DNA Polymerases and Human DNA Topoisomerases

	Mammalian DNA polymerases				
FA	Pol α	Pol β	Pol γ	Pol Δ	Pol ε
18:0	15.0 ± 0.6	>100	52.5 ± 1.8	50.2 ± 2.2	62.8 ± 2.2
18:1Δ9*c*	11.7 ± 0.5	33.5 ± 1.7	10.4 ± 0.4	33.8 ± 1.8	36.3 ± 1.4
18:2Δ9*c*,12*c*[a]	22.5 ± 0.7	56.0 ± 2.7	22.2 ± 0.9	33.8 ± 1.5	39.7 ± 1.6
18:2Δ10*t*,12*c*	12.8 ± 0.5	18.0 ± 1.2	10.6 ± 0.4	22.0 ± 1.3	29.3 ± 1.0
18:3Δ9*c*,12*c*,15*c*	40.4 ± 1.2	>100	31.7 ± 1.4	68.3 ± 2.8	66.7 ± 2.0
18:3Δ9*c*,11*t*,13*t*[a]	10.7 ± 0.4	13.4 ± 0.8	10.6 ± 0.3	14.0 ± 0.8	22.0 ± 0.7
18:4Δ6*c*,9*c*,12*c*,15*c*	>100	>100	>100	>100	>100
18:4Δ9*c*,11*t*,13*t*,15*c*[a]	46.5 ± 1.9	83.1 ± 3.0	>100	71.0 ± 3.3	>100

	Human topisomerases	
FA	Topo I	Topo II
18:0	>100	>100
18:1Δ9*c*	22.5 ± 2.0	7.5 ± 0.5
18:2Δ9*c*,12*c*	35.0 ± 3.0	45.0 ± 3.5
18:2Δ10*t*,12*c*[a]	20.0 ± 2.0	5.0 ± 0.5
18:3Δ9*c*,12*c*,15*c*	>100	50.0 ± 4.0
18:3Δ9*c*,11*t*,13*t*[a]	20.0 ± 2.0	5.0 ± 0.5
18:4Δ6*c*,9*c*,12*c*,15*c*	>100	60.0 ± 0.4
18:4Δ9*c*,11*t*,13*t*,15*c*[a]	37.5 ± 3.5	40.0 ± 2.5

[a]Conjugated FA. Data are shown as means ± SEM of three independent experiments. Pol, DNA-directed DNA polymerase; topo, DNA topoisomerase.

(Table 3, Fig. 1). Because the hydrocarbon chains in their FA fold up at the position of the double bonds in the *cis*-configuration, di- and more unsaturated FA of the *cis*-configuration lost molecular length and did not form a V-shaped curve as seen in α-eleostearic acid. Therefore, both the molecular width and length of the FA appeared to be important for enzyme inhibition. The molecular lengths and widths of normal and conjugated linear-chain C_{18}-FA are compared in Table 3. A molecular length of more than 12 Å and molecular width of 8.5 to 9.5 Å influenced the activities of DNA polymerases and topoisomerases. The conjugated position of double bonds in the FA must affect these enzymes directly.

Several anticancer agents in clinical use have been shown to be potent inhibitors of DNA topoisomerases. For example, adriamycin (Doxorubicin), amsacrine (m-AMSA), and ellipticine have shown significant activity as inhibitors of topo II. The plant alkaloid camptothecin and its synthetic derivatives such as CPT-11 are extensively studied topo I inhibitors. All of these agents inhibit the rejoining reaction of topoisomerases by stabilizing a covalent topoisomerase–DNA complex termed the "cleavable complex." To determine whether conjugated FA bind to DNA, the melting temperature (T_m) of double-stranded DNA (dsDNA) in the presence of more than 100 μM of α-eleostearic acid was measured. The thermal transition of T_m was not observed, whereas 15 μM of ethidium bromide, a typical intercalating agent, caused the thermal transition (data not shown). Thus, none of the conjugated FA bound to the dsDNA, suggesting that it must inhibit the enzyme activities by interacting with the enzymes directly.

TABLE 2
Effects of Polyoxyriboadenylic Acid [poly(rC)], Bovine Serum Albumin (BSA), or Nonidet P-40 (NP-40) on the Inhibition of DNA Polymerase and Topoisomerase Activities by α-Eleostearic Acid (18:2Δ9*c*,11*t*,13*t*)[a]

	Relative activity (%)			
Compounds added to the reaction mixture	Pol α	Pol β	Topo I	Topo II
Without the compounds				
None (control)	100 ± 1.0	100 ± 2.0	100 ± 5.0	100 ± 5.0
+100 μg/mL poly(rC)	100 ± 0.5	100 ± 1.0	100 ± 2.5	100 ± 2.5
+100 μg/mL BSA	100 ± 2.0	100 ± 2.5	100 ± 5.0	100 ± 5.0
+0.1% NP-40	100 ± 2.5	100 ± 3.0	100 ± 5.0	100 ± 5.0
With 25 μM α-eleostearic acid				
25 μM α-eleostearic acid	11.0 ± 0.4	12.3 ± 0.5	50.0 ± 3.0	10.0 ± 1.0
25 μM α-eleostearic acid + 100 μg/mL poly(rC)	9.8 ± 0.3	12.5 ± 0.5	50.0 ± 2.5	10.0 ± 1.0
25 μM α-eleostearic acid + 100 μg/mL BSA	10.5 ± 0.8	13.3 ± 0.7	50.0 ± 4.0	10.0 ± 1.5
25 μM α-eleostearic acid + 0.1% NP-40	95.6 ± 3.5	98.0 ± 4.2	100 ± 6.5	100 ± 5.5

[a]100 μM poly(rC) and 100 μg/mL BSA or 0.1% NP-40 were added to the reaction mixture. In the absence of α-eleostearic acid, DNA polymerase activity was taken to be 100%. Data are shown as means ± SEM of three independent experiments. For abbreviations see Table 1.

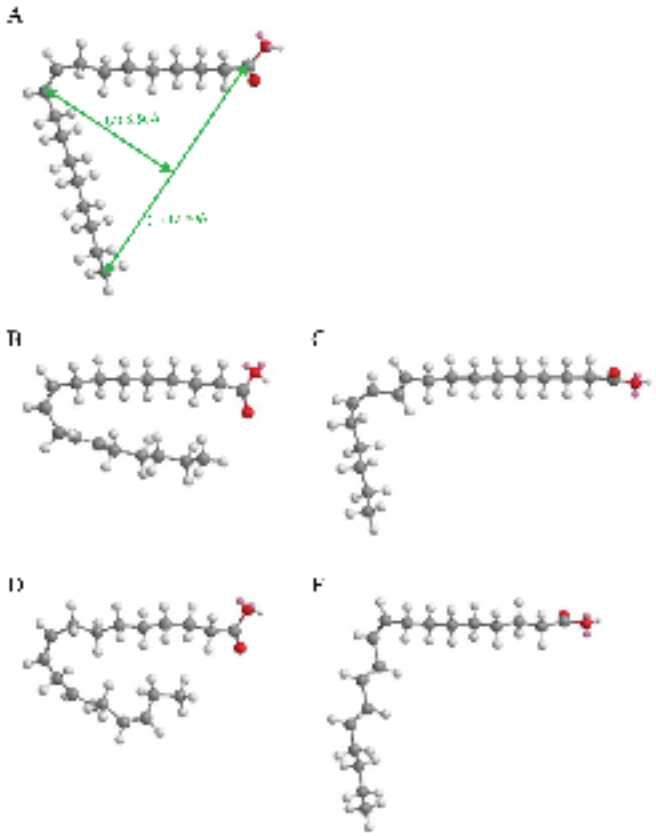

FIG. 3. Computer graphics of linear-chain C_{18}-FA. (A) Oleic acid (18:1Δ9c); (B) linoleic acid (18:2Δ9*c*,12*c*); (C) 18:2Δ10*t*,12*c*; (D) linolenic acid (18:3Δ9*c*,12*c*,15*c*); (E) α-eleostearic acid (18:2Δ9*c*,11*t*,13*t*). The length (a) and width (b) of the FA molecular structure are shown. The carbons, oxygens, and hydrogens of the compounds are indicated in gray, red, and white, respectively. These figures were prepared using Insight II (Accelrys, San Diego, CA). For abbreviations see Figure 1.

Suzuki *et al.* (27) reported that *cis*-unsaturated C_{18}-FA were to be of the noncleavable complex type of topo II inhibitors. The conjugated C_{18}-FA may be the same mode of topo II inhibition as the *cis*-unsaturated FA.

Inhibitors of DNA polymerases could also be anticancer agents, as reported previously (28,29), and polymerases and topoisomerases have recently emerged as important cellular targets for chemical intervention in the development of anticancer agents. The chemical frames of FA containing conjugated double bonds could, moreover, be used for screening new anticancer chemotherapy agents. They can be pursued by three-dimentionally using data on the structural heterogeneity of the FA binding pockets of the target enzymes, since the conjugated FA can be chemically synthesized in great variety. Therefore, the computer-simulated drug design of compounds, especially of inhibitors of the polymerases and topoisomerases, could be of great interest and may in theory be a promising approach to developing new agents for anticancer chemotherapy.

TABLE 3
Molecular Length and Width of the FA Calculated by 3-D Computer Simulation

FA	Length (Å)	Width (Å)
18:0 (stearic acid)	21.40	0
18:1Δ9*c* (oleic acid)	12.80	8.50
18:2Δ9*c*,12*c* (linoleic acid)	4.45	10.58
18:2Δ9*c*,11*c*[a]	4.68	10.28
18:2Δ9*t*,11*t*[a]	20.29	4.75
18:2Δ10*t*,12*c*[a]	14.64	8.68
18:3Δ9*c*,12*c*,15*c* (α-linolenic acid)	5.54	10.30
18:3Δ8*c*,10*t*,12*c*[a]	6.16	11.00
18:3Δ8*t*,10*t*,12*c*[a]	12.66	8.13
18:3Δ8*t*,10*t*,12*t*[a]	20.61	5.34
18:3Δ9*c*,11*t*,13*c*[a]	5.79	11.24
18:3Δ9*c*,11*t*,13*t*[a]	12.03	9.24
18:3Δ9*t*,11*t*,13*t*[a]	20.54	5.12
18:4Δ6*c*,9*c*,12*c*,15*c* (stearidonic acid)	Uncalculated	
18:4Δ9*c*,11*t*,13*t*,15*c*[a]	14.49	8.66

[a]Conjugated FA. These data were obtained using INSIGHT III (Accelrys, San Diego, CA). A restrained molecular dynamics method was used to calculate the energetically minimum structures of compounds.

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