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Structural analysis of catechin derivatives as mammalian DNA polymerase inhibitors ☆

Yoshiyuki Mizushina [a,b,*], Akiko Saito [c], Akira Tanaka [d], Noriyuki Nakajima [e], Isoko Kuriyama [a], Masaharu Takemura [f], Toshifumi Takeuchi [g], Fumio Sugawara [g], Hiromi Yoshida [a,b]

[a] *Laboratory of Food and Nutritional Sciences, Department of Nutritional Science, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan*
[b] *High Technology Research Center, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan*
[c] *Biotechnology Center, Toyama Prefecture, Kosugi, Toyama 939-0398, Japan*
[d] *Department of Bioresources Science, College of Technology, Toyama Prefectural University, Kosugi, Toyama 939-0398, Japan*
[e] *Biotechnology Research Center, Toyama Prefectural University, Kosugi, Toyama 939-0398, Japan*
[f] *Life Science Research Center, Mie University, Kamihama-cho, Tsu, Mie 514-8507, Japan*
[g] *Department of Applied Biological Science, Tokyo University of Science, Noda, Chiba 278-8510, Japan*

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Abstract

The inhibitory activities against DNA polymerases (pols) of catechin derivatives (i.e., flavan-3-ols) such as (+)-catechin, (−)-epicatechin, (−)-gallocatechin, (−)-epigallocatechin, (+)-catechin gallate, (−)-epicatechin gallate, (−)-gallocatechin gallate, and (−)-epigallocatechin gallate (EGCg) were investigated. Among the eight catechins, some catechins inhibited mammalian pols, with EGCg being the strongest inhibitor of pol α and λ with IC_{50} values of 5.1 and 3.8 μM, respectively. EGCg did not influence the activities of plant (cauliflower) pol α and β or prokaryotic pols, and further had no effect on the activities of DNA metabolic enzymes such as calf terminal deoxynucleotidyl transferase, T7 RNA polymerase, and bovine deoxyribonuclease I. EGCg-induced inhibition of pol α and λ was competitive with respect to the DNA template-primer and non-competitive with respect to the dNTP (2′-deoxyribonucleotide 5′-triphosphate) substrate. Tea catechins also suppressed TPA (12-*O*-tetradecanoylphorbol-13-acetate)-induced inflammation, and the tendency of the pol inhibitory activity was the same as that of anti-inflammation. EGCg at 250 μg was the strongest suppressor of inflammation (65.6% inhibition) among the compounds tested. The relationship between the structure of tea catechins and the inhibition of mammalian pols and inflammation was discussed.

Keywords: Tea catechins; (−)-Epigallocatechin gallate; DNA polymerase α; DNA polymerase λ; Enzyme inhibitor; Anti-inflammation

Eukaryotic cells reportedly contain three replicative DNA polymerases (pol α, δ, and ε), a mitochondrial DNA polymerase (pol γ) and at least 13 repair-type DNA polymerases (pol β, δ, ε, ζ, η, θ, κ, λ, μ, σ, ϕ, pol1-like I, and pol1-like II) [1,2]. Selective inhibitors of eukaryotic DNA polymerases (pols), which are reportedly separable into 15 classes, are not only useful as tools and molecular probes to distinguish pols and to clarify their biological and in vivo functions [3], but are also potential anti-cancer agents which could inhibit the

☆ *Abbreviations:* C, (+)-catechin; EC, (−)-epicatechin; GC, (−)-gallocatechin; EGC, (−)-epigallocatechin; Cg, (+)-catechin gallate; ECg, (−)-epicatechin gallate; GCg, (−)-gallocatechin gallate; EGCg, (−)-epigallocatechin gallate; pol, DNA-directed DNA polymerase (EC 2.7.7.7); dTTP, 2′-deoxythymidine 5′-triphosphate; dNTP, 2′-deoxyribonucleotide 5′-triphosphate; HIV, human immunodeficiency virus type-1; dsDNA, double-stranded DNA; EtBr, ethidium bromide, TPA, 12-*O*-tetradecanoylphorbol-13-acetate; DMSO, dimethyl sulfoxide; IE, inhibitory effect.

* Corresponding author. Fax: +81 78 974 5689.
E-mail address: mizushin@nutr.kobegakuin.ac.jp (Y. Mizushina).

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proliferation of cancer cells. We have found many pol inhibitors in dietary compounds such as long chain fatty acids [4–7], conjugated fatty acids [8], steroids [9], some bile acids such as lithocholic acid [10–12], glycolipids [13–17], vitamin A related compounds such as retinal [18], vitamin B6 [19], vitamin D2/D3 [20] and curcumin [21,22]. Most of these inhibitors bound directly to the pol protein, and subsequently inhibited its activities [6,10]. In this report, we found that polyphenols from ethanol extracts of green tea (*Camellia sinensis*) inhibited pols.

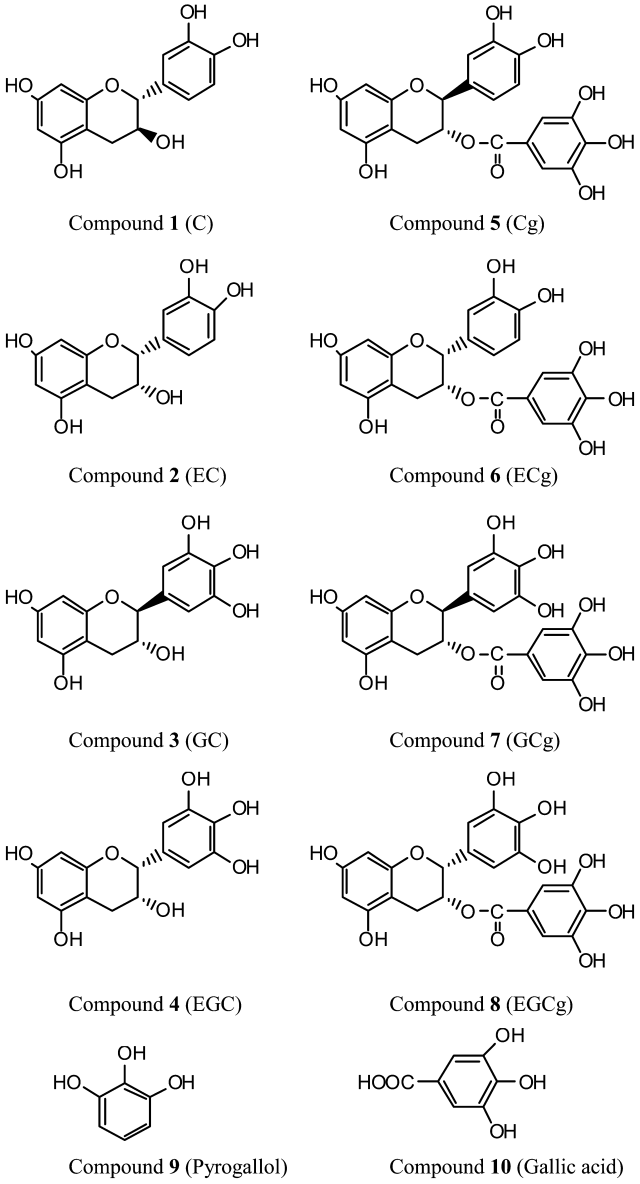

Fig. 1. Structures of catechin derivatives. Compound **1**, (+)-catechin (C); compound **2**, (−)-epicatechin (EC); compound **3**, (−)-gallocatechin (GC); compound **4**, (−)-epigallocatechin (EGC); compound **5**, (+)-catechin gallate (Cg); compound **6**, (−)-epicatechin gallate (ECg); compound **7**, (−)-gallocatechin gallate (GCg); compound **8**, (−)-epigallocatechin gallate (EGCg); compound **9**, pyrogallol; and compound **10**, gallic acid.

Green tea obtained from the leaves of the plant *Camellia sinensis* is one of the most popular beverages in the world. The major polyphenolic compounds in green tea are catechin derivatives (i.e., flavan-3-ols), including (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECg), and (−)-epigallocatechin gallate (EGCg) (Fig. 1). Their composition varies depending on the season of harvest and manufacturing process. Green tea leaves usually contain about 1% EC, 2–3% EGC, 1–2% ECg, and 5–8% EGCg [23]. The biological effects of tea and tea polyphenols including anti-oxidative activity toward low-density lipoproteins [24], anti-carcinogenicity [25], and anti-bacterial actions [26–28] have been examined extensively both in vitro and in vivo. Catechin derivatives have also been receiving attention for their protective effects against cardiovascular disease and cancer [29–33].

The inhibitory activities of commercially available catechin derivatives against pols were investigated that were caused on account of cancer cell proliferation and suppression of TPA (2-*O*-tetradecanoylphorbol-13-acetate)-induced inflammation. We discuss the structure-function relationships, the inhibitory effects on pol and the anti-inflammatory activity of catechin derivatives. We also consider the possible mechanisms of action of the compounds based on the distribution of electrostatic potential on the molecular surface.

Materials and methods

Materials. Eight tea catechin derivatives (i.e., flavan-3-ols), (+)-catechin (C, compound **1**), (−)-epicatechin (EC, compound **2**), (−)-gallocatechin (GC, compound **3**), (−)-epigallocatechin (EGC, compound **4**), (+)-catechin gallate (Cg, compound **5**), (−)-epicatechin gallate (ECg, compound **6**), (−)-gallocatechin gallate (GCg, compound **7**) and (−)-epigallocatechin gallate (EGCg, compound **8**), and these part compounds such as pyrogallol (compound **9**) and gallic acid (compound **10**), were purchased from Sigma (St. Louis, MO, USA) (Fig. 1). The compounds, purified using HPLC system, were of analytical grade. Nucleotides such as [^{3}H]dTTP (43 Ci/mmol) and chemically synthesized template-primers, poly(dA), poly(rA), and oligo(dT)$_{12-18}$, were purchased from Amersham Biosciences (Buckinghamshire, UK). All other reagents were of analytical grade and purchased from Nacalai Tesque (Kyoto, Japan).

Enzymes. DNA polymerase α (pol α) was purified from calf thymus by immuno-affinity column chromatography as described previously [34]. Recombinant rat pol β was purified from *Escherichia coli* JMpβ5 as described by Date et al. [35]. Human pol γ catalytic gene was cloned into pFastBac. Histidine-tagged enzyme was expressed using the BAC-TO-BAC HT Baculovirus Expression System according to the directions provided (Life Technologies, MD) and purified using ProBoundresin (Invitrogen Japan, Tokyo, Japan) (Mizushina et al., in preparation). Human pol δ and ε were purified by the nuclear fractionation of human peripheral blood cancer cells (Molt-4) using the second subunit of pol δ- and ε-conjugated affinity column chromatography, respectively [36]. Recombinant human pol η and ι tagged with His6 at their C-terminal were expressed in SF9 insect cells using the baculovirus expression system and were purified as reported [37,38]. A truncated form of pol κ (i.e., hDINB1DC) with a 6× His-tag attached at the

C-terminal was overproduced using the BAC-to-BAC Baculovirus Expression System kit (Gibco-BRL) and purified as reported [39]. Recombinant His-pol λ was overexpressed and purified according to a method outlined previously [40]. Pol I (α-like) and II (β-like) from a higher plant, cauliflower inflorescence, were purified according to the methods outlined by Sakaguchi et al. [41]. HIV reverse transcriptase (recombinant) and the Klenow fragment of pol I from *E. coli* were purchased from Worthington Biochemical (Freehold, NJ, USA). T4 pol, Taq pol, and T7 RNA polymerase were obtained from Takara (Kyoto, Japan). Calf thymus terminal deoxynucleotidyl transferase and bovine pancreas deoxyribonuclease I were purchased from Stratagene Cloning Systems (La Jolla, CA, USA).

DNA polymerase assays. Activities of pols were measured using the methods described previously [4,5]. For pols, poly(dA)/oligo$(dT)_{12–18}$ and dTTP were used as the DNA template-primer and dNTP substrate, respectively. For HIV reverse transcriptase, poly(rA)/oligo$(dT)_{12–18}$ and dTTP were used as the template-primer and nucleotide substrate, respectively. For terminal deoxynucleotidyl transcriptase, oligo$(dT)_{12–18}$ (3′-OH) and dTTP were used as the template-primer and nucleotide substrate, respectively.

Catechin derivatives 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 amount, 0.05 U) in 50 mM Tris–HCl (pH 7.5) containing 1 mM dithiothreitol, 50% glycerol, and 0.1 mM EDTA, and kept 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 pol which was incubated at 74 °C for 60 min. The activity without the inhibitor was taken as 100%, and the level of activity at each concentration of inhibitor was determined relative to this value. One unit of pol activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol of deoxyribonucleotide triphosphates (i.e., dTTP) into synthetic template-primers (i.e., poly(dA)/oligo$(dT)_{12–18}$, A/T = 2/1) in 60 min at 37 °C under the normal reaction conditions for each enzyme [4,5].

Other enzyme assays. Activities of T7 RNA polymerase and bovine deoxyribonuclease I were measured using standard assays as described by Nakayama et al. [42] and Lu and Sakaguchi [43], respectively.

DNA intercalating measurement. The intercalation profiles of dsDNA with or without EGCg were determined with fluorescence emission spectra using RF-1500 (Shimadzu, Kyoto). Calf thymus dsDNA (2 μg/ml) was dissolved in 0.1 M sodium phosphate buffer (pH 7.0) containing 0.5 μM EtBr and 1% DMSO at 25 °C. The emission spectra were measured upon excitation of 520 nm. Any change in the absorbance of the compound itself at each wavelength point (540–630 nm) was automatically subtracted from that of DNA plus the compound in the fluorescence meter.

Anti-inflammatory assay. The mouse inflammatory test was performed according to Gschwendt's method [44]. This experiment complied with the regulations concerning animal experimentation and the care of experimental animals of Kobe-Gakuin University. Briefly, a solution of the test compound in acetone (250 μg/20 μl) was applied to the inner part of the ear. Thirty minutes after the test compound was applied, a solution of TPA (0.5 μg/20 μl of acetone) was applied to the same part of the ear. To the other ear of the same mouse, methanol and a TPA solution were applied as a control. After 7 h, a disk (6 mm in diameter) was obtained from the ear and weighed. The IE is presented as the ratio of the increase in weight of the ear disks: IE: {[(TPA only)-(tested compound plus TPA)]/[(TPA only) − (vehicle)] × 100}.

Computational analysis. A compound model was constructed and simple-minimized. Compound models were simulated with force field parameters based on the consistent valence force field (CVFF). 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, San Diego, CA, USA). Electrostatic potentials on the surface of compounds were analyzed with WebLab ViewerLite (version 3.2, Accelrys, San Diego, CA, USA) software.

Results and discussion

Effects of catechin derivatives on the activities of mammalian DNA polymerase α and β

We tested the effects of tea catechin derivatives (compounds **1**–**10**, Fig. 1), purchased commercially, on calf pol α and rat pol β. The relative activity of each pol at a set concentration (100 μM) of the compounds is shown in Fig. 2. Compounds **1** (C) and **2** (EC) did not influence pol α and β activities. Compounds **9** (pyrogallol) and **10** (gallic acid), which are the structural parts of flavan-3-ol, also did not inhibit pol activities. Compounds **3** to **8** inhibited both pols, with the effect on pol α being stronger than that on pol β. Those compounds which at 100 μM inhibited by less than 50% the relative activity of pol α were GC, EGC, Cg, ECg, GCg, and EGCg. Compound **8** (EGCg) had the strongest inhibitory effect on pol α of all the catechin derivatives tested. Therefore, we concentrated on the properties of EGCg in the latter part of this study.

Effects of EGCg on the activities of DNA polymerases and other DNA metabolic enzymes

As shown in Fig. 3, 10 μM of EGCg inhibited the activities of all the mammalian pols tested except pol β. Notably, calf pol α and human pol λ were strongly inhibited with the relative activities of 23.5% and 14.5%, respectively. EGCg also inhibited human immunodeficiency virus type-1 (HIV) reverse transcriptase, but the effect was weaker than those for pol α and λ. On the other hand, the activities of higher plant (cauliflower) pol I (α-like) and II (β-like), prokaryotic pols such as the Klenow fragment of *E. coli* pol I, T4 pol, and Taq DNA pol, and DNA metabolic enzymes such as calf thymus terminal deoxynucleotidyl transferase, T7 RNA polymerase, and bovine deoxyribonuclease I were not influenced by EGCg. The compound should therefore be classified as an inhibitor of mammalian pols and HIV reverse transcriptase.

Effects of EGCg on the activities of mammalian DNA polymerases

Fig. 4 shows the dose–response curves for the inhibition by EGCg of mammalian pol α and λ . The compound was effective against both calf pol α and human pol λ activities, and the effect was dose-dependent, with 50% inhibition at doses of 5.1 and 3.8 μM, respectively. The inhibitory effect was strongest against pol λ among the mammalian DNA polymerases tested (Table 1 and

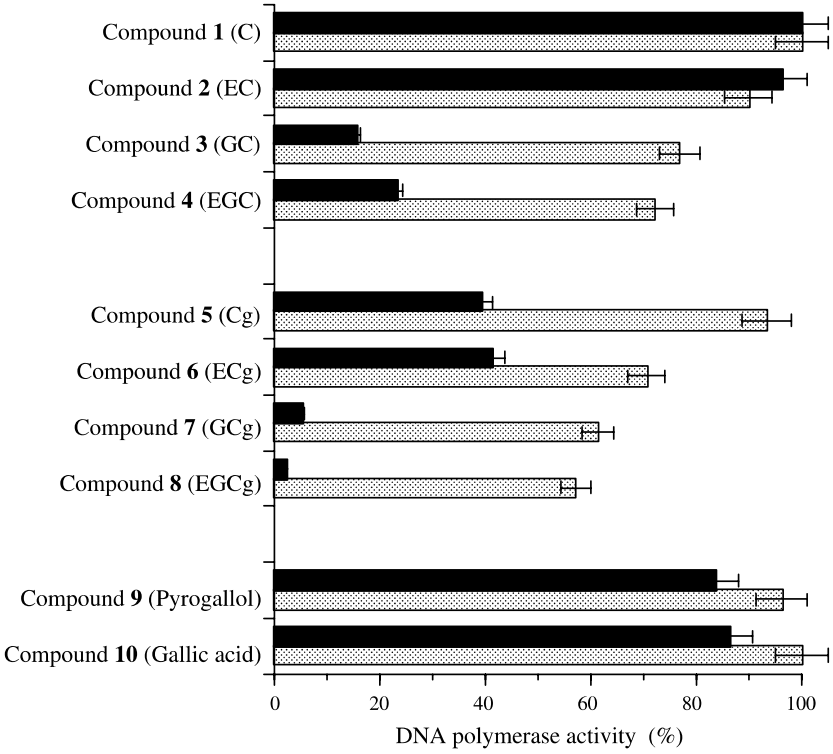

Fig. 2. Effects of catechin derivatives on the activities of mammalian DNA polymerase α and β compounds **1** to **10** (100 μM each) were incubated with calf pol α (black bar, 0.05 U) and rat pol β (gray dotted bar, 0.05 U). Percent relative activity is shown. The enzymatic activity was measured as described in the text. Pol activity in the absence of the compound was taken as 100%.

Fig. 4). Pol α is known to be a replicative polymerase and pol λ is a repair-related and/or recombination polymerase [1]. The observations indicate that EGCg is a selective inhibitor of pol α and λ, although these pols differ in amino acid sequence and three-dimensional structure.

Mode of inhibition of DNA polymerase α and λ by EGCg

Next, to elucidate the mechanism of inhibition, the extent of the inhibition as a function of the DNA template-primer or dNTP (2′-deoxyribonucleotide 5′-triphosphate) substrate concentration was studied. Table 1 shows the results of the kinetic analyses of EGCg. Poly(dA)/oligo(dT)$_{12-18}$ and dTTP were used as the DNA template-primer and dNTP substrate, respectively. Double reciprocal plots of the results show that the inhibition by EGCg of pol α activity was competitive with respect to the DNA template-primer and non-competitive with respect to the dNTP substrate. In the case of the DNA template-primer, the apparent maximum velocity (V_{max}) was unchanged at 55.6 μM, whereas 126%, 171%, and 264% increases in Michaelis constant (K_m) were observed in the presence of 1, 2, and 3 μM EGCg, respectively. The K_m for dTTP was 1.65 μM, and the V_{max} for the substrate decreased from 29.2 to 20.4 pmol/h in the presence of 3 μM EGCg. The inhibition constant (K_i) value, obtained from Dixon plots, was found to be 2.28 μM and 5.60 μM for the DNA template-primer and the dNTP substrate, respectively. Similarly, the inhibition of pol λ by EGCg was competitive with respect to the DNA template-primer, since there was no change in the apparent V_{max} (66.5 pmol/h), while the K_m increased from 2.50 to 8.33 μM for the DNA template-primer, in the presence of zero to 3 μM EGCg. In contrast, the apparent K_m for dTNP substrate was unchanged at 1.18 μM, whereas a 2.9-fold decrease in the V_{max} was observed in the presence of 3 μM EGCg. The inhibition was therefore non-competitive with respect to dTTP. The K_i value was found to be 1.41 and 2.55 μM for the DNA template-primer and dNTP substrate, respectively. The affinity of EGCg might be higher at the DNA template-primer binding site than at the nucleotide substrate binding site of pol α and λ. When activated DNA was used as the template-primer instead of synthesized DNA (i.e., poly(dA)/oligo(dT)$_{12-18}$), the mode of inhibition by each compound was unchanged (data not shown). EGCg may directly interact with or bind to the DNA template-primer binding sites on pol α and λ, thereby decreasing its affinity for the dNTP substrate. Similar results were observed with other tea catechin derivatives, and the inhibition of pol α and λ was competitive with respect to the DNA template-primer and non-competitive with respect to the dTNP substrate (data not shown). These results suggested that the

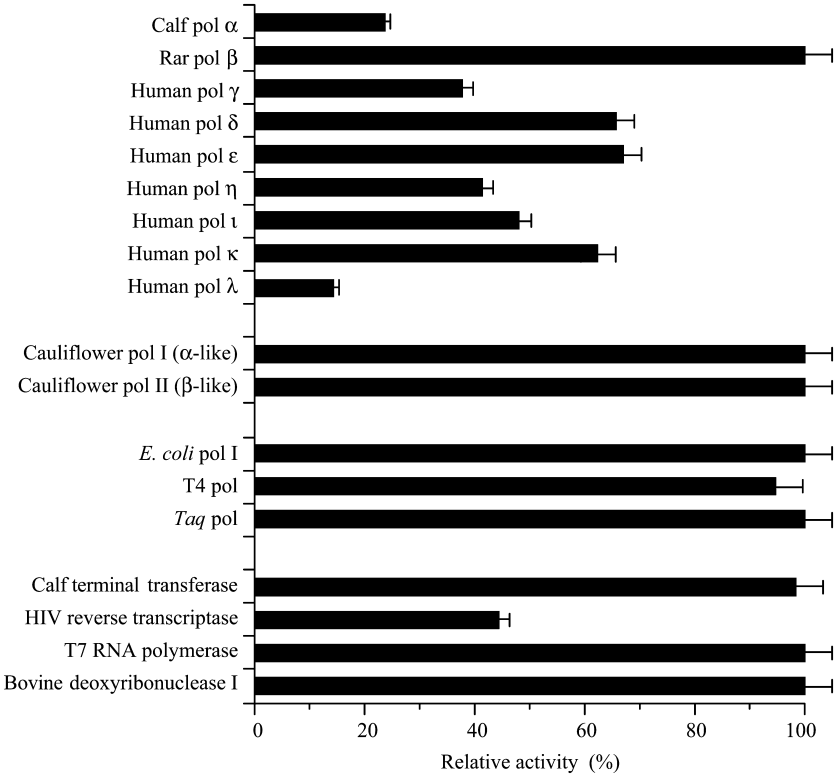

Fig. 3. Effects of EGCg on the activities of various DNA polymerases and other DNA metabolic enzymes. EGCg (10 μM) was incubated with each enzyme. Percent relative activity is shown. The enzymatic activity was measured as described in the text. Enzymatic activity in the absence of the compound was taken as 100%.

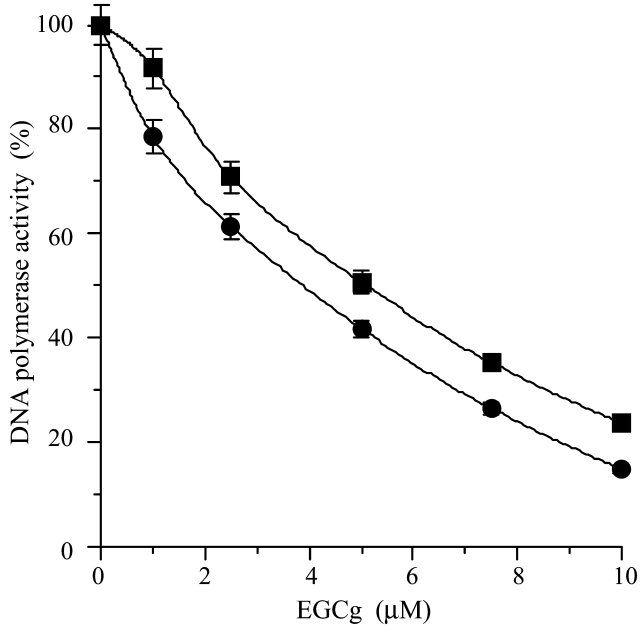

Fig. 4. Dose–response curves of the inhibition of mammalian DNA polymerase α and λ by EGCg. Calf pol α (square, 0.05 U) and human pol λ (circle, 0.05 U) were pre-incubated with the indicated concentrations (0–10 μM) of EGCg, and then enzymatic activities were assayed as described in the text. Pol activity in the absence of the compound was taken as 100%.

tea catechin binding sites on pol α and λ are structurally similar, and that this compound-binding region on pols is more influential than these competitive sites with regard to enzymatic inhibition.

Influence of EGCg on the binding of double-stranded DNA

To determine whether tea polyphenols (flavan-3-ol derivatives) such as EGCg bind to DNA, fluorescence emission spectra of EtBr (ethidium bromide) were measured in the presence of dsDNA (double-stranded DNA) and the compound (Fig. 5). As described under Materials and methods, calf thymus dsDNA at 2 μg/ml was dissolved in 0.1 M sodium phosphate buffer (pH 7.0) containing 1% dimethyl sulfoxide (DMSO). When EtBr was intercalated with dsDNA, the fluorescence of the EtBr–dsDNA complex increased, and the maximum emission wavelength was 590 nm. At a high concentration (i.e., 100 μM) of EGCg, no decrease in the fluorescence of EtBr was observed. Thus, EGCg did not bind and intercalate with the dsDNA, suggesting that it must inhibit the enzymes by interacting with them directly. The same results were obtained with other tea catechin derivatives (data not shown).

Table 1
Kinetic analysis of the inhibition by EGCg of the activities of DNA polymerase α and λ, as a function of DNA template-primer dose and nucleotide substrate concentration

Enzyme (0.05 U)	Substrate	Compound concentration (μM)	K_m (μM)	V_{max} (pmol/h)	K_i (μM)	Inhibitory mode
Pol α	DNA template-primer[a]	0	13.0 (± 1.0)	55.6 (± 3.0)	2.85 (± 0.40)	Competitive
		2	16.4 (± 1.2)			
		4	22.2 (± 1.5)			
		6	34.3 (± 1.4)			
	dNTP substrate[b]	0	1.65 (± 0.31)	29.2 (± 1.4)	4.95 (± 0.72)	Non-competitive
		2		25.6 (± 1.4)		
		4		22.7 (± 1.3)		
		6		20.4 (± 0.9)		
	DNA template-primer[a]	0	2.50 (± 0.20)	66.5 (± 2.8)	1.41 (± 0.20)	Competitive
		1	3.23 (± 0.22)			
		2	4.55 (± 0.40)			
		3	8.33 (± 0.48)			
Pol λ	dNTP substrate[b]	0	1.18 (± 0.10)	52.6 (± 2.5)	2.55 (± 0.33)	Non-competitive
		1		32.3 (± 1.7)		
		2		23.3 (± 1.2)		
		3		18.2 (± 1.2)		

[a] Poly(dA)/oligo(dT)$_{12-18}$ (=2/1).
[b] dTTP.

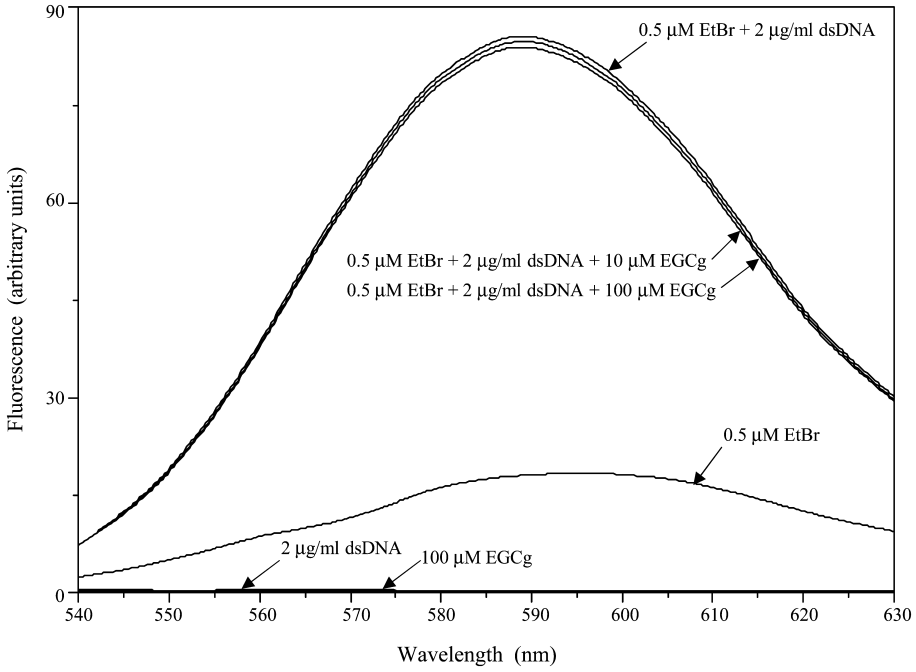

Fig. 5. Fluorescence emission spectra of EtBr–dsDNA complex in the presence of EGCg. 0, 10 or 100 μM EGCg was incubated with 2 μg/ml of calf thymus dsDNA and 0.5 μM EtBr in 0.1 M Na-phosphate buffer (pH 7.0). The excitation wavelength was 520 nm.

Anti-inflammatory effects of catechin derivatives

We reported previously a relation between mammalian pol inhibitors and anti-inflammatory activity [21,22]. Using the mouse inflammatory test, we examined the anti-inflammatory properties of the tea catechin derivatives. An application of TPA (0.5 μg) to the ear induced edema, the weight of an ear disk 7 h after the application having increased 241%. As shown in Table 2, EGCg (250 μg) achieved the strongest reduction in TPA-induced inflammation among the compounds tested, and the inhibitory effect (IE) was 65.6%. IEs of GC, EGC, Cg, ECg, GCg, and EGCg were more than 25%, but other compounds had little or no effect on the inflammation.

Next, the suppression of inflammation by tea catechin derivatives was compared with the inhibitory

Table 2
Anti-inflammatory activity of catechin derivatives toward TPA-induced edema on the mouse ear

Compound	Inhibitory effect (%) (±SE)
1 (C)	11.1 (± 4.9)*
2 (EC)	13.3 (± 4.3)*
3 (GC)	30.8 (± 2.8)*
4 (EGC)	32.5 (± 2.6)*
5 (Cg)	27.0 (± 2.5)*
6 (ECg)	25.2 (± 2.8)*
7 (GCg)	58.0 (± 2.9)*
8 (EGCg)	65.6 (± 4.2)*
9 (pyrogallol)	9.3 (± 3.8)*
10 (gallic acid)	8.2 (± 3.2)*

SE is shown in parentheses. The compound (250 μg) was applied to one of the ears and, after 30 min, TPA (0.5 μg) was applied to both ears. Edema was evaluated after 7 h. The inhibitory effect is expressed as the percentage of edema. Five mice were used for each experiment.
* Significantly different, $P < 0.05$ with Student's t test.

effects on mammalian pols. The inhibition of pol α by these compounds had a high correlation with the anti-inflammatory activity (Tables 1 and 2). TPA is a compound that promotes tumorigenesis [45] and is generally used as an artificial inducer of inflammation [46,47]. TPA-induced inflammation can be distinguished from acute inflammation and is accompanied by fibroblastic proliferation and granulation. These results suggested that the molecular basis of the so-called promotion of oncogenesis involves a biochemical process which requires pols. Therefore, EGCg could have anti-inflammatory activity based on its inhibition of mammalian pols.

Three-dimensional structures of catechin derivatives

To obtain information about the molecular basis of the difference in inhibition between EGCg and its nine

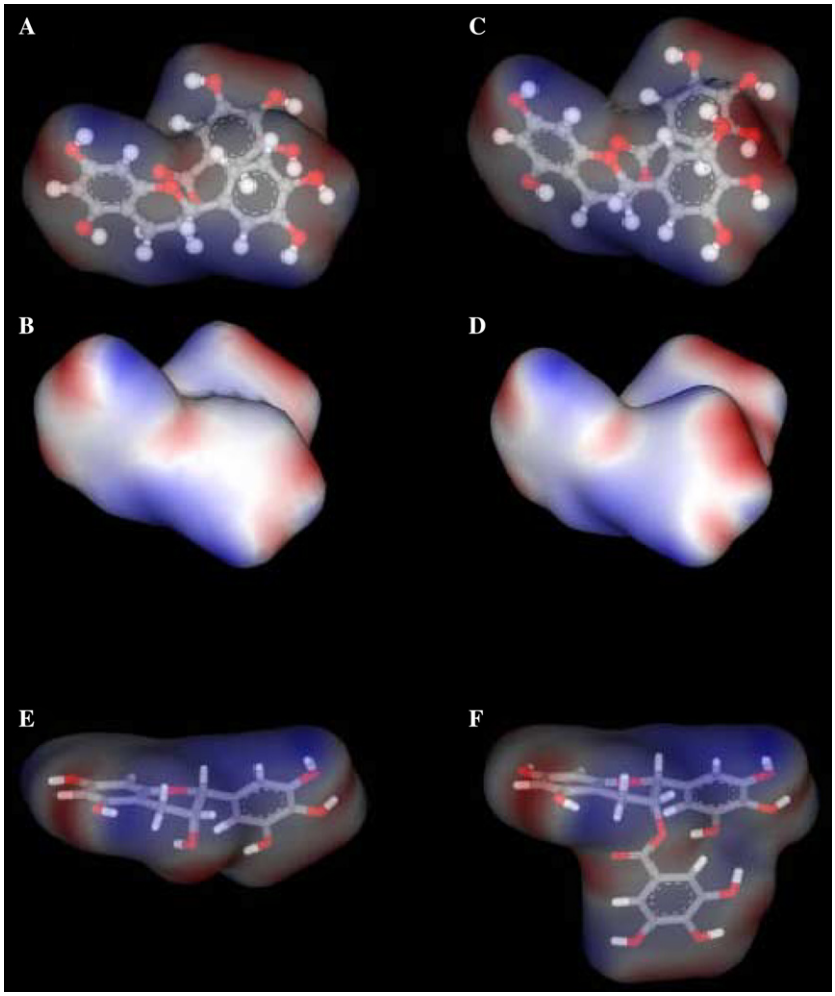

Fig. 6. Computer graphics of catechin derivatives. (A,B) Compound **6** (ECg); (C,D, and F) compound **8** (EGCg); (E) compound **4** (EGC). Ball-and-stick models (A,C) and stick models (E,F) of compounds were built using the graphics program INSIGHT II (Accelrys, San Diego, CA, USA). Carbons, oxygens, and hydrogens are indicated in gray, red, and white, respectively. (B,D) Electrostatic potentials over molecular surfaces were analyzed using WebLab ViewerLite software (version 3.2, Accelrys, San Diego, CA, USA). Blue areas are positively charged, red areas are negatively charged, and white areas are neutral (see Materials and methods).

derivatives, computational analyses were performed using molecular simulation and surface analysis software (Fig. 6). The three-dimensional molecular structures of ECg, EGC, and EGCg are shown in Figs. 6A–F, respectively.

The inhibitory effect of EGCg on pol α was more than 10-fold stronger than that of ECg (Fig. 2), and the anti-inflammatory effect of EGCg was 2.6-fold that of ECg (Table 2). The only structural difference between ECg and EGCg is the hydroxyl group at the 5′-position on the B-ring (Figs. 1 and 6A and C). The electrostatic potential at each point on a constant electronic density surface (approximating the van der Waals surface for each arrangement) was represented graphically in red corresponding to the regions where the electrostatic potential was most negative and blue corresponding to the most positive regions. As shown in Fig. 6D, EGCg supported the enhancement of negative electrostatic potential on the O atom in the hydroxyl group on the B-ring. These results suggested that the electrostatic charge of this hydroxyl group is important for the inhibition of these activities.

Next, EGC and EGCg were compared. The inhibitory effect on pol α and anti-inflammatory effect of EGCg were more than 6- and 2-fold stronger than that of EGC, respectively (Fig. 2 and Table 2), and the major structural difference between EGC and EGCg is the galloyl group at the 3-position on the C-ring (Figs. 1 and 6A and C). Figs. 6E and F indicated that the galloyl group in EGCg supported the enhancement of both negative and positive electrostatic potentials on the O atom in the hydroxyl group and H atom, respectively, and might enhance the inhibitory effects.

Because the biological effect of EGCg was as strong as that of GCg (Fig. 2 and Table 2), the epimer at 2-position was suggested not to have influenced the activity.

The following features of the molecular structure of tea polyphenols based on flavan-3-ol might be important for the inhibition of both mammalian pols and inflammation; (1) the hydroxyl group at the 5′-position on the B-ring and (2) the galloyl group at the 3-position on the C-ring. Tea catechin derivatives, especially EGCg, could, therefore, be considered possible candidates for anti-cancer agents.

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