Inhibitory Activities of (−)-Epigallocatechin-3-*O*-gallate against Topoisomerases I and II

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Received March 15, 2001; accepted May 29, 2001

The substitution of gallic acid at the 3 position of (−)-epigallocatechin-3-*O*-gallate (EGCG) increased the inhibition against topoisomerase I from calf thymus gland and topoisomerase II from human placenta, and the substitution of a hydroxyl group at the 3′ position increased the inhibition against the topoisomerase I. These results suggested that the 3 and 3′ positions of the EGCG molecule play important roles in the process of inhibition of topoisomerases I and II. EGCG showed strong inhibition against topoisomerases I from wheat germ, calf thymus gland and Vero cells, and showed weak or no inhibition against topoisomerases I from carcinoma cells such as A549, HeLa and COLO 201 cells. EGCG differentially inhibited the topoisomerases I from different sources.

Key words epigallocatechin-3-*O*-gallate; topoisomerase inhibition; EGCG; catechin

(−)-Epigallocatechin-3-*O*-gallate (EGCG), isolated from tea plant, is well known to inhibit the growth of cancer cells[1] and various enzymes concerning cancer such as topoisomerase II,[2] telomerase,[3] reverse transcriptase,[4] tyrosinase,[5] metal protease,[6] epoxidase,[7] nitric oxide synthase[8] and xanthine oxidase.[9] Topoisomerases I and II are involved in many aspects of DNA metabolism[10] and are the primary cellular target enzymes for a number of clinically important anticancer drugs.[11] Although it has been previously reported that EGCG inhibits topoisomerase II with the formation of a cleavable complex,[2] there has also a report on topoisomerase I inhibition and structure–activity relationships.[12] Here we report on the structure–activity relationship of EGCG against topoisomerases I and II, and the selective inhibitions of EGCG against various topoisomerases I from different sources.

MATERIALS AND METHODS

Catechins, Enzymes, Substrates and Inhibitors EGCG, (−)-epicatechin-3-*O*-gallate, (−)-epigallocatechin and (−)-epicatechin were isolated from commercial oolong tea.[13] Topoisomerase I from calf thymus gland, T4 DNA ligase, *Bam* HI, *Eco* RI, *Hin* dIII and supercoiled pBR322 DNA were purchased from MBI Fermentas. *Alu* I, *Sca* I and *Pst* I were purchased from Gibco BRL. Topoisomerase II (mixture of α and β types) from human placenta and kinetoplast DNA were purchased from Topogen. DNase I from bovine pancreas, DNase II from porcine spleen, RNase A from bovine pancreas, Na^+,K^+-ATPase from porcine cerebral cortex, RNA from yeast extract and doxorubicin hydrochloride were obtained from Sigma. Camptothecin was obtained from Aldrich. For the preparation of topoisomerases I from COLO 201 (human colon carcinoma), HeLa (human cervix carcinoma), A549 (human lung carcinoma) and Vero (African green monkey kidney) cells, cells cultured for 5 d were washed with phosphate buffered saline and harvested by centrifugation. The cells pellets (1×10^6 cells) were resuspended in 200 μl of cold lysis buffer (10 mM Tris–HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride, 5 mM β-mercaptoethanol, 0.5% 3-[(3-cholamidopropyl) dimethyl-ammonio]-1-propanesulfonate and 10% glycerin), and kept on ice for 30 min. The lysate was centrifuged and the supernatant was used as an enzyme solution of the topoisomerase I obtained from each cell. The amount of enzyme added to the enzyme reaction for topoisomerase I was one unit which converted 0.15 μg of supercoiled pBR322 DNA to a relaxed form in 40 min at 37°C.[14] Topostatin was isolated from the culture filtrate of *Thermomonospora alba* strain No. 1520 in our laboratory.[14]

Enzyme Reactions Relaxation activities of topoisomerases I and II were measured by detecting the conversion of supercoiled pBR322 DNA to its relaxed form.[15,16] The decatenation activity of topoisomerase II was measured by detecting the conversion of catenated kinetoplast DNA to minicircle monomers.[15,16] Activities of the restriction enzymes (*Alu* I, *Bam* HI, *Eco* RI, *Hin* dIII, *Pst* I and *Sca* I) and nucleases (DNase I, DNase II and RNase A) were determined by measuring the concentration of undigested supercoiled pBR322 DNA or RNA after the enzyme reactions.[17,18] The assay of T4 DNA ligase was based on the ligation of linearized pBR322 DNA which was cleaved by *Hin* dIII.[17,18] Telomerase activity was measured by a TRAP-*eze*® Telomerase Detection Kit (Intergen Co.). After each enzyme reaction, the incubation mixture was subjected to gel electrophoresis, and DNA or RNA on the gel was measured by a densitometer with a transilluminator (Atto Co., AE-6900M). Na^+,K^+-ATPase activities were determined by measuring the concentration of inorganic phosphate released from ATP according to the malachite green-molybdate colorimetry method.[19] The reaction mixture (200 μl) consisting of 150 mM histidine, 20 mM $MgCl_2$, 100 mM KCl, 650 mM NaCl, 3 mM ATP and 24 μg of Na^+,K^+-ATPase was incubated at 37 °C for 15 min. The reaction was terminated with 800 μl of malachite green reagent (8 mM ammonium molybdate, 0.4 mM polyvinyl alcohol, 0.7 mM malachite green), and then OD at 630 nm of the mixture was measured. The assay conditions for inhibitory activities and electrophoreses have been described in previous papers.[14,17,18,20,21] The inhibitory activity (IC_{50}) was defined as the amount of inhibitor that reduced each enzyme activity by 50%.

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RESULTS AND DISCUSSION

The effects of some catechins on topoisomerase I from calf thymus gland and topoisomerase II from human placenta were examined and summarized in Table 1. EGCG showed strong inhibition against topoisomerases I and II. (−)-Epicatechin-3-*O*-gallate substituted a hydrogen at the 3′ position of EGCG, and strongly inhibited topoisomerase II, but showed weak inhibition against topoisomerase I. (−)-Epigallocatechin substituted a hydroxyl group at the 3 position of EGCG, and showed weak inhibition against topoisomerases I and II. (−)-Epicatechin substituted a hydrogen at the 3′ position of (−)-epigallocatechin, showed weaker inhibition against topoisomerase II than that of (−)-epigallocatechin, and did not inhibit topoisomerase I, even at 1000 μM. These results showed that the substitution of gallic acid at the 3 position of EGCG increased the inhibition against topoisomerases I and II, and the substitution of a hydroxyl group at the 3′ position increased the inhibition against topoisomerase I. It was suggested that the inhibitory potency against topoisomerases I and II depended on the hydroxyl group at the 3′ position and the gallic acid at the 3 position of the catechin molecule.

Table 1. Inhibitory Activities and Structures of Catechins

Compound	Position		Inhibition (IC_{50}, mM)[a] against	
	3′	3	Topoisomerase I	Topoisomerase II
(−)-Epigallocatechin-3-*O*-gallate	OH	*O*-Gallate	5	3
(−)-Epicatechin-3-*O*-gallate	H	*O*-Gallate	136	2
(−)-Epigallocatechin	OH	OH	475	111
(−)-Epicatechin	H	OH	>1000	345
Gallic acid	—	—	>1000	196

a) Values represent the means obtained from three independent experiments.

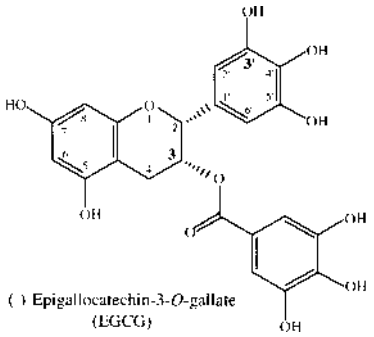

Table 2. Inhibitory Spectra of Topoisomerase Inhibitors

Enzyme	Inhibition (IC_{50}, mM)[a]			
	EGCG[b]	Topostatin	Camptothecin	Doxorubicin
Topoisomerase I from wheat germ[c]	1	1	3	>100
Topoisomerase I from Vero cell[c]	4	17	27	>100
Topoisomerase I from calf thymus gland[c]	5	17	17	>100
Topoisomerase I from A549 cell[c]	13	55	4	>100
Topoisomerase I from HeLa cell[c]	52	29	9	>100
Topoisomerase I from COLO 201 cell[c]	>100	50	17	>100
Topoisomerase II from human placenta[c]	1	4	>100	1
Topoisomerase II from human placenta[d]	2	4	>100	1
*Alu*I from *Arthrobacter luteus*	97	>100	>100	24
*Bam*HI from *Bacillus amyloliquefaciens*	59	>100	>100	>100
*Eco*RI from *Escherichia coli*	65	>100	>100	>100
*Hin*dIII from *Haemophilus influenzae*	0.1	17	>100	96
*Pst*I from *Providencia stuartii*	50	19	>100	>100
*Sca*I from *Streptomyces caespitosus*	>100	7	>100	25
RNase A from bovine pancreas	>100	>100	>100	>100
DNase I from bovine pancreas	>100	>100	>100	>100
DNase II from porcine spleen	>100	>100	>100	>100
T4 ligase from *Escherichia coli*	>100	>100	>100	73
Telomerase from COLO 201 cell	0.3	>100	>100	
Na^+,K^+-ATPase from porcine cerebral cortex	3	7	>100	>100

a) Values represent the means obtained from three independent experiments. *b*) (−)-Epigallocatechin-3-*O*-gallate. *c*) Relaxation activity. *d*) Decatenation activity.

The inhibitory effects of EGCG on various topoisomerases and DNA-related enzymes were examined and are summarized in Table 2. For comparison, topostatin as an inhibitor of topoisomerases I and II,[14,20,21] camptothecin as an inhibitor of topoisomerase I[22] and doxorubicin as an inhibitor of topoisomerase II[23] were also examined. In respect to topoisomerase I inhibition, EGCG strongly inhibited topoisomerases I from wheat germ, calf thymus gland and Vero cells, and showed weak or no inhibition against the enzymes from carcinoma cells such as A549, HeLa and COLO 201 cells. It is interesting that EGCG can recognize differences in topoisomerases I from different sources. EGCG may be a useful tool for the analysis of topoisomerase isozymes. Flavone compounds such as genistein and quercetin are known as topoisomerase II inhibitors which inhibit the ATPase activity of the enzyme.[24] Similarly, EGCG may impair the ATPase reaction of topoisomerase II because Na^+,K^+-ATPase was inhibited at a low concentration (3 μM). Among the DNA-related enzymes tested, *Hin* dIII and telomerase were fairly affected at the concentration of 0.1 and 0.3 μM, respectively, whereas other enzymes such as RNase, DNase, DNA ligase and some restriction endonucleases were not inhibited, even at a high concentration of EGCG. The inhibitory mechanism of EGCG may differ from that of other topoisomerase inhibitors because EGCG showed a different inhibitory spectrum.

It would be interesting to establish the structure–activity relationship of EGCG since the catechin is an inhibitor having multi-potentiality for enzyme inhibition. Our data suggests that the 3 and 3′ positions of the EGCG molecule play important roles in the process of topoisomerases I and II inhibition, and that EGCG can differentially inhibit topoisomerases I from different sources.

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