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The galloyl moiety of green tea catechins is the critical structural feature to inhibit fatty-acid synthase

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Abstract

It has been reported that inhibition of fatty-acid synthase (FAS) is selectively cytotoxic to human cancer cells. Considerable interest has developed in identifying novel inhibitors of this enzyme complex. Our previous work showed that green tea (−)-epigallocatechin gallate can inhibit FAS *in vitro*. To elucidate the structure–activity relationship of the inhibitory effects of tea polyphenols, we investigated the inhibition kinetics of the major catechins and analogues. Ungallated catechins from green tea do not show obvious inhibition compared with gallated catechins. Another gallated catechin, (−)-epicatechin gallate, was also found as a potent inhibitor of FAS and its inhibition characteristics are similar to (−)-epigallocatechin gallate. Furthermore, the analogues of galloyl moiety without the catechin skeleton such as propyl gallate also showed obvious slow-binding inhibition, whereas the green tea ungallated catechin not. Atomic orbital energy analyses suggest that the positive charge is more distinctly distributed on the carbon atom of ester bond of galloyl moiety of gallate catechins, and that gallated forms are more susceptible for a nucleophilic attack than other catechins. Here we identify the galloyl moiety of green tea catechins as critical in the inactivation of the ketoacyl reductase activity of FAS for the first time.

Keywords: Galloyl moiety; Steady-state kinetics; Inhibition; Fatty-acid synthase; Gallated catechins

1. Introduction

Animal fatty-acid synthase (FAS; E.C.3.2.1.85) comprises two identical, 260–270 kDa subunits juxtaposed head to tail, each containing an acyl carrier protein and seven enzymatic active sites, including acetyl transacylase, malonyl transacylase, β-ketoacyl synthase, β-ketoacyl reductase, β-hydroxyacyl dehydratase, enoyl reductase, and thioesterase. This multifunctional enzyme complex is encoded by a single gene. Animal FAS complexes are commonly referred to as type I and discrete monofunctional FAS enzymes found in prokaryotes and plant are referred to as type II. Animal FAS catalyzes the *de novo* synthesis of fatty-acids from acetyl-CoA and malonyl-CoA in the presence of the reducing substrate NADPH through the reaction which elongates the acetyl group by C_2 units derived from malonyl-CoA in a stepwise and sequential manner [1,2]. The overall reaction catalyzed by the animal FAS can be summarized by the following equation:

$$\text{Acetyl-CoA} + 7\text{Malonyl-CoA} + 14\text{NADPH} + 14\text{H}^+ \rightarrow \text{Palmitic acid} + 7\text{CO}_2 + 8\text{CoA} + 14\text{NADP}^+ + 6\text{H}_2\text{O}$$

The amino acid sequence of the human FAS has 79 and 63% identity with those of the rat and chicken enzymes, respectively, and most partial specific activities and enzymatic characteristics of human FAS are similar to chicken FAS. Most information about FAS is derived from non-human animal studies [3]. Traditionally fowl FAS is a good model for the kinetic study of animal FAS since fowl FAS mainly exists in liver so that it is relatively easy to obtain the enough quantity to meet the great need of enzyme.

It has been discovered that FAS is selectively highly expressed in certain human cancers, including carcinoma of the breast, prostate, colon, ovary, and endometrium compared to normal human tissues, and therefore is a putative tumor marker, which led to the consideration of

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Abbreviations: EGCG, (−)-epigallocatechin gallate; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin gallate; EC, (−)-epicatechin; C, (+)-catechin; FAS, fatty-acid synthase.

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FAS as a target for anticancer therapy [4–7]. In addition, inhibitors of FAS such as cerulenin and C75 were reported to be selectively cytotoxic to many human cancer cells [8]. Increased malonyl-CoA, resulting from inhibition of FAS, is a potential mediator of this cytotoxicity [9]. The inhibitors of FAS have such significant biological functions, but they are scarce and novel. Therefore, it is significant to identify the new inhibitors and investigate their inhibition mechanism *in vitro* and *in vivo*.

Epidemiological studies suggest that the consumption of green tea may help prevent cancers in humans [10]. The precise mechanisms are not known although there are several hypotheses proposed. The main components of green tea are catechins including (−)-epigallocatechin gallate (EGCG; 9–13%), (−)-epicatechin gallate (ECG; 3–6%), (−)-epigallocatechin (EGC; 3–6%), (−)-epicatechin (EC; 1–3%), (+)-catechin (C; less than 1%) and a small quantity of other flavanol derivatives [11]. Gallated catechins, especially EGCG, are known to inhibit growth of cancer cells and induce apoptosis of various types of tumor cells [12–16]. The mechanisms through which drinking green tea can prevent cancer growth are most likely complex, and may be determined by many biological factors. Our previous work showed that green tea EGCG is a potent inhibitor of FAS. Inhibition is characterized by a rapid, reversible phase followed by a slower, irreversible phase. The inhibitory effects of EGCG on FAS are highly related to β-ketoacyl reductase [17]. In the present work, we show that green tea ECG is also a potent inhibitor and its inhibition characteristics are similar as EGCG. The green tea ungallated catechins do not markedly inhibit FAS compared to the gallated catechins. The catechins and analogues without the galloyl moiety do not show obvious slow-binding inhibition, whereas the analogue with galloyl moiety such as propyl gallate does. We identify for the first time that the galloyl moiety of green tea catechins is the critical structural feature for inactivation of FAS. This discovery of the important structural feature will provide some valuable information for the design of new inhibitors of FAS.

2. Experimental procedures

2.1. Materials

The preparation, storage and use of FAS from chicken liver were performed as described previously [18]. The final purified enzyme was homogeneous on polyacrylamide gel electrophoresis (PAGE) in the presence and absence of SDS. Acetyl-CoA, malonyl-CoA, NADPH and all catechins (from green tea) were purchased from Sigma. C75 was provided by Procter & Gamble Co. *p*-Cresol, 4-methyl catechol, and gallic acid were purchased from Aldrich. All other reagents were local products of analytical grade.

2.2. Assay of fatty-acid synthase

The enzyme and substrates concentrations were determined by absorption measurements with the following extinction coefficients: chicken liver FAS, $4.83 \times 10^5\ M^{-1}\ cm^{-1}$ at 279 nm; acetyl-CoA, $1.54 \times 10^4\ M^{-1}\ cm^{-1}$ at 259 nm, pH 7.0; malonyl-CoA, $1.46 \times 10^4\ M^{-1}\ cm^{-1}$ at 260 nm, pH 6.0; NADPH, $6.02 \times 10^3\ M^{-1}\ cm^{-1}$ at 340 nm and $1.59 \times 10^4\ M^{-1}\ cm^{-1}$ at 259 nm, pH 9.0 [19]. FAS activity of the overall reaction, β-ketoacyl reduction and enoyl reduction was determined with a Hitachi UV557 Double wavelength/Double beam spectrophotometer at 37° by following the decrease of NADPH at 340 nm. The overall reaction mixture contained potassium phosphate buffer, 100 mM, pH 7.0; EDTA, 1 mM; acetyl-CoA, 3 μM; malonyl-CoA, 10 μM; NADPH, 35 μM and chicken liver FAS 10 μg in a total volume of 2.0 mL. The ketoacyl reduction reaction mixture contained ethyl acetoacetate, 40 mM; NADPH, 35 μM; 1 mM EDTA and the enzyme 10 μg in 100 mM phosphate buffer, pH 7.0. The enoyl reduction reaction mixture contained ethyl crotonate, 40 mM; NADPH, 35 μM; 1 mM EDTA and 70 μg FAS in 10 mM phosphate buffer, pH 6.3 [20].

2.3. Measurement of inhibition of fatty-acid synthase

Inhibition of FAS in the inhibitor solution with different concentrations was measured and repeated three times. IC_{50} values were obtained from the dose–response curves of inhibitors. Time course of inactivation was determined by taking aliquots to measure the residual activity at the indicated time intervals after the enzyme solution was mixed with the inhibitor. The time-dependent inhibition is often an irreversible process to form the covalent bond between the inhibitor and enzyme known as slow-binding inactivation. The vehicles without inhibitors were used as control in these experiments. FAS activity in control does not change in 4 hr during these experiments. The apparent first-order rate constant, k_{obs} can be obtained from the semilogarithmic plot of inactivation time course.

Centrichromatography to estimate the reversibility of the inhibition was carried out as follows: The enzyme solutions were mixed with the inhibitors and aliquots were taken to Sephadex G25 column to centrifugation to exclude the small molecules at the indicated time intervals, and then the activities of eluted fractions were measured. Centrifuged fractions of the same enzyme solution without inhibitors were used as control.

2.4. Chemical calculation

All calculations were performed with the GAUSSIAN98 software package version A.9 (Gaussian, Inc.). The initial geometries were constructed with help of MOLDEN. The chemicals were completely optimized by Density functional theory B3LYP with 3–21g(d) basis set without any

restriction. Atomic orbital energy analysis is performed by the applications of linear combination of atomic orbital (LCAO). The lower value of gap energy between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) indicates the higher susceptibility toward nucleophilic attack. Mulliken population analysis shows the charge distribution of atoms [21].

3. Results

3.1. Comparison of inhibitory effects of tea polyphenols and C75 on FAS

Major tea polyphenols of green tea can be divided into two groups, ungallated and gallated catechins. Ungallated catechins, such as EGC, EC, and C, do not have a galloyl moiety, whereas gallated catechins, ECG and EGCG are gallated. Catechins have an A ring that has a characteristic hydroxylation at the 5 and 7 positions and a B ring that is usually 3′4′ or 3′4′5′ hydroxylated (Fig. 1).

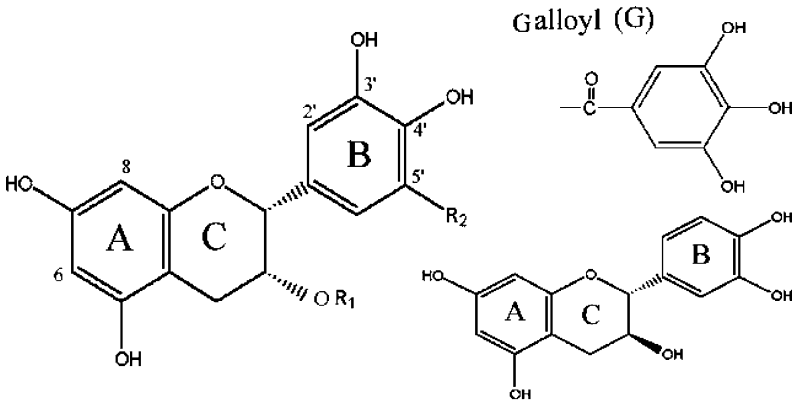
The inhibitory effects on FAS of these compounds were measured and compared with that of C75, a novel inhibitor synthesized by Kuhajda *et al.* of Johns Hopkins University [22] at the same concentration in the reaction system (Fig. 2). The FAS activity did not change in control sample. After the preincubation with FAS for 3 hr, gallic acid, C, EC and EGC did not show noticeable inhibition of FAS (>90% residual activity of FAS), whereas C75, EGCG and ECG exhibited marked inhibition of FAS. The residual activity of FAS inactivated by EGCG or ECG was approximately 21%, and the residual activity of FAS inactivated by C75 was 27%.

3.2. Inhibition kinetics of ECG

ECG exhibited similar FAS inhibition characteristics to EGCG. ECG lacks a 5′ hydroxyl group on the B ring of catechin skeleton compared to EGCG (Fig. 1). Therefore, this hydroxyl group is not important for the inhibition of FAS. 42 ± 2 μM ECG inhibited 50% of the overall reaction activity of FAS and 68 ± 2 μM ECG inhibited 50% of the ketoacyl reduction reaction activity of FAS. Inhibition of ketoacyl reduction was considerable, but inhibition of enoyl reduction was much less than inhibition of the overall reaction and ketoacyl reduction. Therefore, it is plausible that the inhibition is highly related to β-ketoacyl reductase.

The inactivation time course of ECG is also similar to EGCG [17]. The inactivation rate of ketoacyl reduction was comparable to that of the overall reaction but the inactivation rate of the enoyl reduction was much less under the same conditions. The apparent rate constants of inactivation of FAS by ECG in the presence of one of the three substrates are measured to test whether the time-dependent inhibition is related to the substrate binding sites. NADPH slowed the apparent inactivation rate of the overall reaction about 3.25-fold, while malonyl-CoA and acetyl-CoA had no obvious effect on the inactivation rate. Only β-ketoacyl reductase and enoyl reductase have NADPH binding sites among the seven enzymatic active sites on FAS [1]. Since ECG had obvious inhibitory effects not on enoyl reduction but ketoacyl reduction, ECG may interact on the NADPH binding sites of β-ketoacyl reductase.

We tested the dependence of the inactivation rate on ECG concentration. The plot of k_{obs}, the apparent first-order rate constant vs. [ECG], shown in Fig. 3, is hyperbolic, indicating that the inhibition of ECG also follows the



	R1	R2
(-)-Epicatechin (EC)	H	H
(-)-Epigallocatechin (EGC)	H	OH
(-)-Epicatechin gallate (ECG)	G	H
(-)-Epigallocatechin gallate (EGCG)	G	OH

Fig. 1. Structures of five major tea polyphenols.

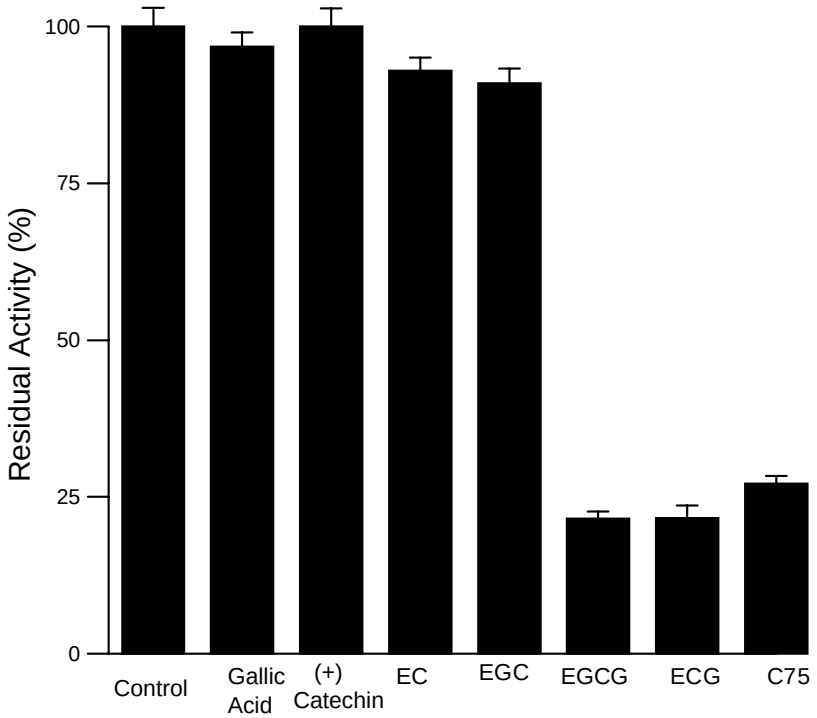


Fig. 2. Comparison of the inhibitory effects of tea polyphenols and C75 on FAS. The residual activities of FAS were measured after the enzyme was incubated with tea polyphenols and C75 for 3 hr. Data are expressed as the mean ± SEM of three independent measurements. The concentration of each reagent in preincubation system was 0.5 mM. The concentration of FAS was 1.95 μM.

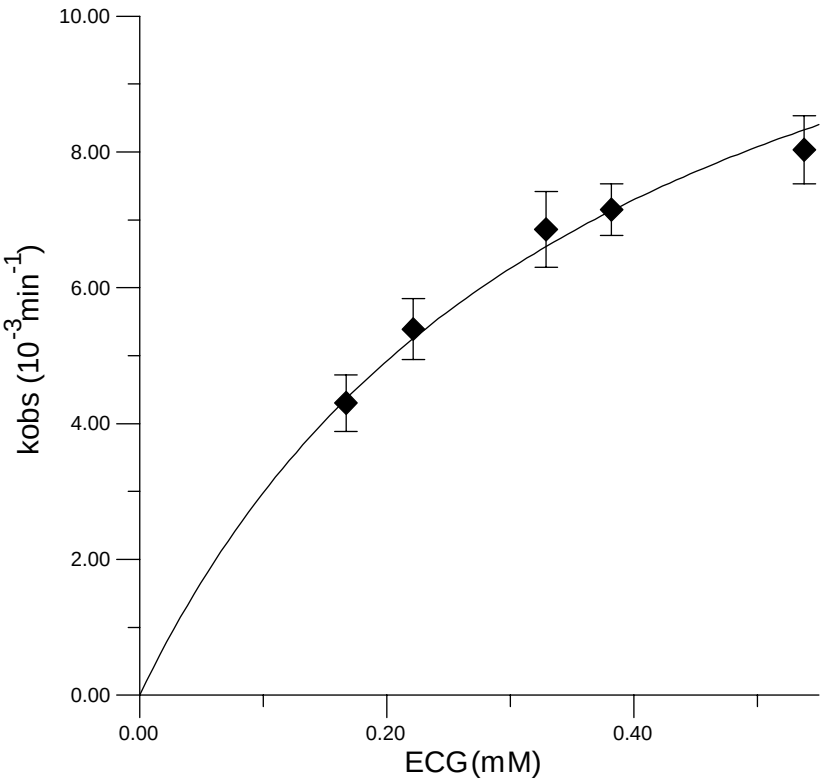


Fig. 3. The dependence of the apparent first-order rate constant (k_{obs}) on ECG concentration. The concentration of FAS in the inactivation system was 1.4 μM. k_{obs}, defined as the apparent first-order rate constant, is expressed as the mean ± SEM of three independent measurements. The activity of FAS preincubated with vehicles as control did not change during the experiment.

Table 1
Residual activity of inactivated FAS by ECG at different periods after centrichromatography

Time of inhibition by ECG	Residual activity of FAS (%)	
	Control group[a]	Test group[b]
0 s	100	100
1 min	69	99
1.5 hr	7.6	8.1

[a] At the indicated time the residual activity of inactivated FAS without centrichromatography.
[b] At the indicated time the inactivated FAS was taken to centrichromatography and the elution after centrifuge was assayed.

saturation kinetic with the two-step inhibition mechanism, a rapid equilibrium step to form a reversible enzyme-inhibitor complex (E···I) followed by a slower, irreversible first-order inactivation step [17]. K_s, the dissociation constant of E···I reversible complex at the first step of 0.373 mM and k, the first-order irreversible inactivation rate constant at the second step of 0.0141 min^{-1} were calculated from the data in Fig. 3 by the data analysis method shown in our previous work [17].

To test this two-step inhibition mechanism, the reversibility of inhibition on FAS by ECG was measured. After centrichromatography, the activity of FAS recovered from 69 to 99% when inhibition occurred after 1 min, while the activity could not recover when inactivation occurred after 1.5 hr (Table 1). These results indicate that the initial inhibition is reversible, and that the subsequent inactivation is irreversible. This observation is consistent with characteristics of the above two-step inhibition.

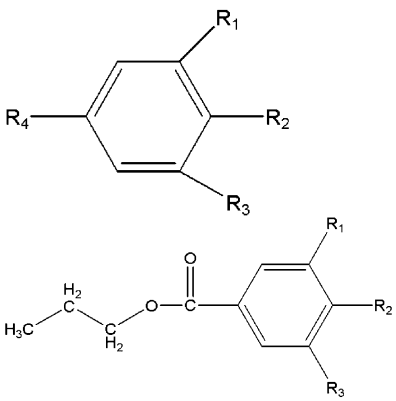
3.3. Inhibition kinetics of analogues and ungallated catechins

The gallated forms of green tea catechins have obvious inhibitory effects on FAS, whereas ungallated not. It is plausible that galloyl moiety of green tea catechins may be the important structural feature. To elucidate this hypothesis, we studied the steady-state kinetics of analogues with and without galloyl moiety. The IC_{50} values of some catechins and analogues are summarized in Table 2. IC_{50} values of ungallated catechins are much higher than gallated catechins. C showed more obvious inhibitory effects than EC. Because C and EC are optical isomers, their optical configuration differences probably result in the differences of inhibition abilities. The analogues that have phenolic hydroxyl groups exhibited weak inhibition of the overall reaction and ketoacyl reduction of FAS, whereas those without phenolic hydroxyl groups such as propyl benzoate and propyl acetate not. Therefore, it is conceivable that the phenolic hydroxyl groups of B ring and gallate group are involved in the inhibition. The IC_{50} values of the analogues of the B ring without the galloyl moiety are approximately 175- to 600-, 5- to 17- and 2- to 7-fold of that of ECG, C, and EC, respectively. It is plausible that the A and C rings of catechins may increase the inhibition to some extent since the ungallated catechins have more obvious inhibitory effects on FAS than the analogues of the B ring without the galloyl moiety.

The analogues that contain both the ester bond and the phenolic hydroxyl group such as propyl gallate and propyl *p*-hydroxylbenzoate exhibited the stronger inhibition than the B ring analogues and ungallated catechins. It is

Table 2
The IC_{50} values of some catechins and the analogues of the B ring and the gallate group

The reagents	IC_{50} (mM)		Structures			
	Overall reaction	Ketoacetyl reduction				
Gallated catechin						
EGCG[a]	0.052 ± 0.002	0.10 ± 0.003			Fig. 1	
ECG	0.042 ± 0.002	0.068 ± 0.002				
Ungallated catechins						
(+)-Catechin	1.6 ± 0.4	7.4 ± 0.2			Fig. 1	
(−)-Epicatechin	3.8 ± 0.04	9.38 ± 0.2			Fig. 1	
The analogues of the B ring			R1	R2	R3	R4
Phenol	26 ± 5	38 ± 3	H	OH	H	H
Catechol	7.4 ± 0.9	21 ± 2	OH	OH	H	H
p-Cresol	16 ± 2	21 ± 4	H	OH	H	CH_3
p-Hydroxylbenzoic acid	22 ± 2	53 ± 8	H	OH	H	COOH
3,4-Dihydroxylbenzoic acid	9.0 ± 1	30 ± 8	OH	OH	H	COOH
Gallic acid	21 ± 2	26 ± 1	OH	OH	OH	COOH
The analogues of the gallate group			R1	R2	R3	
Propyl gallate	0.5 ± 0.08	1.4 ± 0.9	OH	OH	OH	
Propyl *p*-hydroxylbenzoate	1.1 ± 0.2	2.6 ± 0.4	H	OH	H	
Propyl benzoate	>30	30	H	H	H	
Propyl acetate	>30	>30	$CH_3CH_2CH_2OCOCH_3$			



[a] These data were from our previous work [17].

plausible that the ester bond strengthens the inhibitory effect. Therefore, the IC_{50} values of gallated catechins are about two orders of magnitude lower than the catechins without the galloyl moiety.

In a concentration range of 5–20 mM, the time-dependent inhibition was not observed among all the analogues without galloyl moiety. It is plausible that these analogues only reversibly associate with FAS, and cannot form covalent bonds with FAS. EC and C also did not exhibit time-dependent inhibition at about two orders of magnitude higher concentrations than gallated catechins. Because EC and C lack the gallate ester group compared to ECG and EGCG (Fig. 1), it is likely that the time-dependent inhibition may be highly related to the galloyl moiety. We investigated the time-dependent inhibition kinetics of the gallate analogues including propyl gallate, propyl *p*-hydroxybenzoate, propyl benzoate and propyl acetate. Interestingly, in a concentration range of 10–30 mM propyl *p*-hydroxybenzoate, propyl benzoate and propyl acetate did not exhibit obvious time-dependent inhibition, whereas only propyl gallate did at a concentration of about 2–3 mM even though it has not the catechin skeleton (Fig. 4). Inactivation rate of FAS by 5 mM propyl gallate is similar to about 0.5 mM gallated catechins, whereas 5 mM ungallated catechins did not show the obvious slow-binding inactivation to FAS.

Time courses of the overall reaction, ketoacyl reduction and enoyl reduction of FAS in the presence of propyl gallate are shown in Fig. 4. The inactivation rate of overall reaction was almost the same as that of ketoacyl reduction, and propyl gallate had no effect on enoyl reduction. The k_{obs} values, the apparent first-order rate inactivation constants of 0.0090 and 0.0088 min^{-1} for the overall reaction and ketoacyl reduction respectively were obtained from Fig. 4. The similarity of the inactivation rates indicates the slow-binding inactivation of the overall reaction is mainly due to that of ketoacyl reduction. The apparent rate constants of inactivation of FAS by propyl gallate in the presence of one of the three substrates were also measured to test whether the time-dependent inhibition is related to the substrate binding sites. NADPH preincubation with FAS slowed the inactivation rate by propyl gallate 3.8-fold for the overall reaction and 4.4-fold for ketoacyl reduction, but acetyl-CoA and malonyl-CoA had no effect. Therefore, the inactivation by propyl gallate is related not to acetyl- and malonyl-CoA binding sites of FAS but to NADPH binding sites. Similar to gallated catechins propyl gallate had no effects on enoyl reduction activity of FAS. So the binding sites of β-ketoacyl reductase are highly related to the inactivation of FAS by propyl gallate. All these results accordingly indicate that the galloyl moiety probably specifically acts on NADPH binding sites of β-ketoacyl reductase resulting in loss of the overall reaction activity.

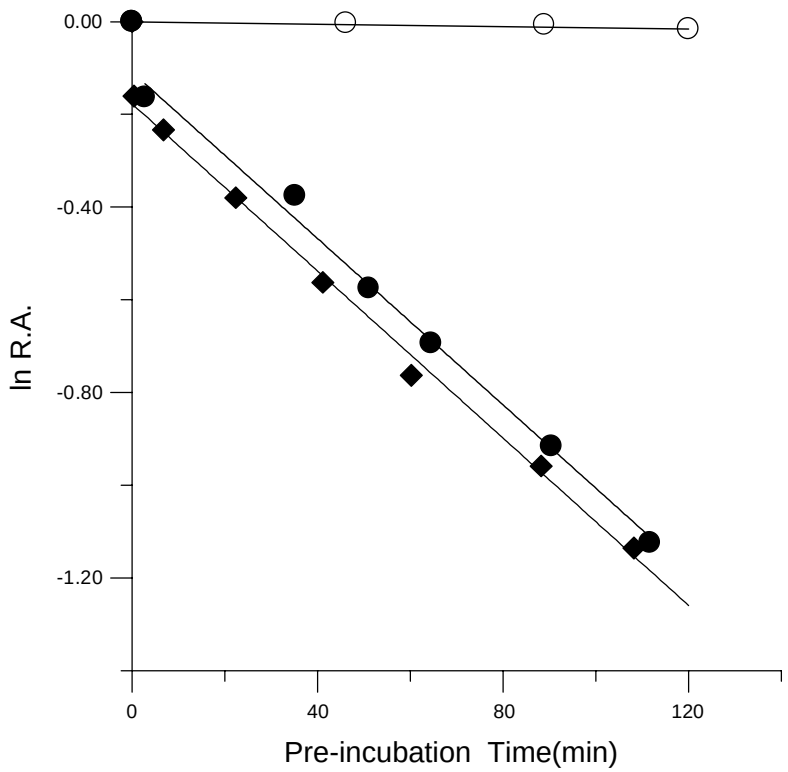


Fig. 4. First-order plot of the inactivation of FAS by propyl gallate. The aliquots of the propyl gallate inactivation system were taken to assay residual activity of the overall reaction (■), β-ketoacyl reduction (●) and enoyl reduction (○) at the indicated time intervals. The linear slope of the plot can be obtained as the apparent first-order rate constant. Conditions of these experiments were the same as those described in Fig. 3 legend. The concentration of propyl gallate was 5 mM. R.A. is the abbreviation of residual activity, which can be calculated as $A_t - A_\infty / A_0 - A_\infty$.

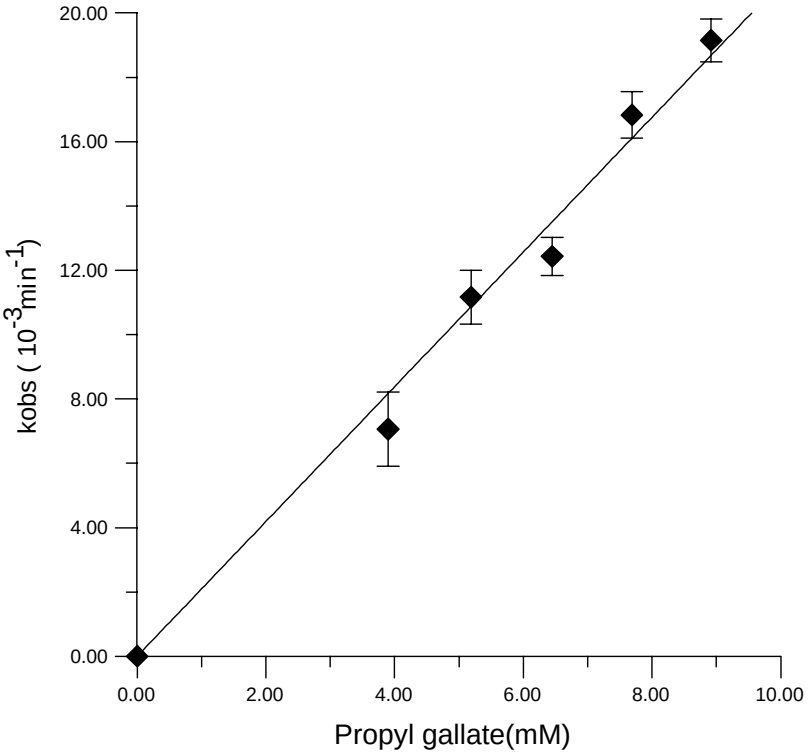


Fig. 5. The dependence of k_{obs} on propyl gallate concentration. Conditions of these experiments were the same as those described in Fig. 3 legend except that the concentration of each reagent was varied as indicated.

We tested the dependence of the inactivation rate on propyl gallate concentration. A plot of k_{obs} vs. [propyl gallate] is a straight line with a linear coefficient of 0.998 (Fig. 5). This indicates that the inactivation of FAS by propyl gallate is a second-order reaction, a simple bimolecular reaction between enzyme and inhibitor shown as below.

$$E + I \xrightarrow{k} EI$$

The reaction rate is fitted to the equation: $v = k[I][E]$. When the concentration of inhibitor is much more than that of enzyme as is likely in this case, $k_{obs} = k[I]$, in which [I] is the concentration of inhibitors, the second-order rate constant k of 2.1×10^{-3} min^{-1} mM^{-1} of the inactivation of FAS by propyl gallate was calculated from the data in Fig. 5.

3.4. Chemical calculation of tea polyphenols and the gallate analogues

The optical configuration of (+)-catechin is different from other major tea polyphenols (Fig. 1). We analyzed the tea polyphenols with similar optical configurations including EC, EGC, ECG, and EGCG. The tea gallated catechins such as EGCG and ECG have lower gap energies between the HOMO and the LUMO than those without galloyl moiety (Table 3). The carbon atoms of the galloyl moiety of gallated catechins have a Mulliken positive charge 2- to 3-folds that of carbon atoms at other sites. The results suggest that carbon atoms of the galloyl moiety of ECG and EGCG have higher susceptibility toward a nucleophilic attack than those of other catechins. It is possible that EGCG and ECG can act on electronegative site of FAS more easily than other tea polyphenols. The slow-binding

Table 3
The calculation results of catechins and analogues of the gallate group

Chemicals	HOMO (eV)	LUMO (eV)	H–L gap (eV)	Mulliken charge on carbon atoms
The catechins				
EC	−0.200	0.0129	0.213	0.230–0.300
EGC	−0.209	0.00214	0.211	0.230–0.300
ECG	−0.207	−0.0486	0.158	0.708
EGCG	−0.205	−0.0354	0.170	0.679
The gallate analogues				
Propyl gallate	−0.212	−0.0325	0.180	0.705
Propyl *p*-hydroxylbenzoate	−0.230	−0.0319	0.198	0.699

inhibitor propyl gallate and its analogue propyl *p*-hydroxybenzoate which does not exhibit the time-dependent inhibition were also analyzed. Interestingly the Mulliken charge values of carbon atoms of the ester bond of these two chemicals are similar, whereas propyl gallate has a lower gap energy than propyl *p*-hydroxybenzoate. The hydroxyl groups at the position 3 or 5 of the gallate group may increase the susceptibility toward a nucleophilic attack resulting in the lower gap energy. The results might suggest that the relative lower gap energy might indicate higher probabilities for slow-binding inactivation among these structural analogues.

4. Discussion

Since the crystal structure of animal FAS has not yet been determined due to the size of this enzyme complex, it is difficult to directly study inhibition mechanisms and design specific inhibitors. Our work provides some valuable information about the inhibition mechanism of FAS by catechins through the steady-state kinetics and chemical calculation, although we have not been able to elucidate the exact binding mode.

In the present work, we demonstrate that the galloyl moiety of green tea catechins is critical for inhibition of FAS by the following evidences. Firstly, the catechins without galloyl moiety did not show obvious slow-binding inactivation compared to gallated catechins. In addition, the reversible inhibitory effects of ungallated catechins on FAS activity were much poorer than gallated catechins. Secondly, ECG, another gallated catechin, exhibited the similar inhibition characteristics to EGCG. Thirdly, the analogues without galloyl moiety did not show the slow-binding inactivation but the reversible weak inhibition just like the ungallated catechins. Lastly and most importantly, the analogue with galloyl moiety showed obvious slow-binding inactivation although it has not the catechin skeleton. It is plausible that catechin skeleton only strengthens the inhibitor effects and lowers the effective concentration.

We also found phenolic hydroxyl groups have some influences on the FAS activity. Since the analogues without galloyl moiety did not show the time-dependent inhibition often considered as irreversible slow-binding inactivation, the phenolic groups on the B ring and galloyl moiety of catechins may be involved in the reversible inhibition. The galloyl moiety of green tea gallated catechins, probably the carbon atom of the ester bond, is involved in the irreversible slow-binding inactivation that is primarily associated with β-ketoacyl reductase. Atomic orbital energy analyses show that the lower gap energies of the gallated catechins which indicate a higher susceptibility toward a nucleophilic attack are consistent with the slow-binding inactivation abilities of green tea catechins. It is possible that the irreversible chemical modification of FAS resulting in a total loss of its activity is a covalent reaction between the electronegative groups of amino acid residues such as the guanidinium group, amino group and hydroxyl group of β-ketoacyl reductase and the carbon atom of the ester bond of gallated catechins. Based on the two-step inhibition behaviour of gallated catechins and the above analysis, we assume that the mechanism of inhibition of FAS by gallated catechins is as follows: (1) inhibitors rapidly associate reversibly with the NADPH binding site or adjacent area of β-ketoacyl reductase of FAS for reversible inhibition. (2) Subsequently, the ester bond of the gallate group of inhibitors covalently reacts with some essential group of β-ketoacyl reductase of FAS resulting in the irreversible loss of enzyme total activity.

The specific antitumor activity of the inhibitors of FAS has led to their consideration as the potential lead compounds for the anticancer drug development [22,23]. However, inhibitors of FAS are scarce. It is a new approach to find this novel inhibitor from the natural resources. Our work showed that the gallated catechins (EGCG and ECG) exhibit potent inhibition of FAS similar to C75 (Fig. 2). It is reported that the IC_{50} values of cerulenin is 53.7 μM, which is a little higher than ECG [24]. Cerulenin was known to mimic the condensation transition state to inhibit β-ketoacyl ACP synthase [25]. C75 was synthesized following the inhibition mechanism of cerulenin [22]. It is plausible that C75 acts specifically on β-ketoacyl synthase just as does cerulenin. The green tea gallated catechins are totally different inhibitors from C75 and cerulenin with regard to the molecular structure and inhibition mechanism. With the characteristics of saturation inhibition kinetics, gallated catechins only require lower concentrations than C75 to inhibit FAS completely.

For many years, it has been shown through epidemiological studies that green tea is a cancer-preventative agent [26]. A growing body of evidence *in vitro* and *in vivo* indicates that components of green tea offer protection against cancer [10]. The cancer-preventative effect of tea polyphenols is probably complex and is affected by many biological factors. Our work provides a new possible explanation for the anticancer effects of green tea. The green tea gallated catechins may inhibit FAS of the tumor tissues resulting in the inhibition of the growth of the cancer cells just as C75 does. Recently, it has been reported that FAS has a role in the control of feeding behavior, and that the treatment of mice with FAS inhibitor C75 can result in the inhibition of feeding and dramatic weight loss. The accumulation of the substrate malonyl-CoA through the inhibition of FAS appears to inhibit the expression of neuropeptide Y which promotes ingestion [27,28]. There are reports that treatment of EGCG or consumption of tea beverage can reduce body weight [29,30]. Although it was supposed that this effect is related to the thermogenesis of caffeine and catechins, gallated catechins may also act as inhibitor of FAS through the same pathway of C75. It needs more investigation to

elucidate the possible link between anticancer and fat-reducing effects of green tea and inhibition of FAS by green tea gallated catechins.

Given the potential of green tea catechins to inhibit FAS, these compounds may have utility in clinical therapy as they are relatively abundant and non-toxic based upon the long term and wide spread use of tea as a beverage, and are bioavailable in humans [31]. Our work has described the critical structural characteristics of gallated catechins and the mechanisms by which they inhibit FAS, thereby providing guidance for the design of potent new inhibitors of FAS.

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