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Food Components Inhibiting Recombinant Human Histidine Decarboxylase Activity

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Histidine decarboxylase (HDC) catalyzes histamine formation from histidine. Histamine is a bioactive amine acting as a neurotransmitter as well as a chemical mediator. Phenolic food components have been tested for their ability to inhibit recombinant human HDC. Epicatechin gallate (ECG) was found to be a potent inhibitor as it inhibited HDC activity in a competitive manner with $K_i = 10$ μM against L-histidine. Epigallocatechin gallate (EGCG) showed time-dependent inhibition which disappeared under anaerobic conditions. It is probable that time-dependent inhibition could be due to the result of autoxidation of EGCG. The initial burst observed for EGCG suggests that EGCG itself is involved in HDC inhibition as observed for ECG. Our present results have shown that the tested food components can inhibit HDC activity. This inhibition likely affects histamine biosynthesis and possibly leads to controlling the biological action induced by histamine. Therefore, those food components exhibiting HDC inhibitory activity might be potentially useful in controlling histamine-induced biological actions.

KEYWORDS: Histidine decarboxylase; histamine; epicatechin gallate (ECG); epigallocatechin gallate (EGCG) inhibitor

INTRODUCTION

Histidine decarboxylase (HDC) is a specific enzyme (E.C.4.1.1.22) that catalizes the formation of histamine. Histamine plays an important role in various physiological reactions including allergy, gastric acid secretion, capillary dilatation, neurotransmission, and smooth muscle contraction (*1*, *2*). Therefore, any compounds which inhibit HDC activity would be highly beneficial for curing allergy, for example. However, no desirable HDC inhibitors have been reported since the amount of HDC in examined tissues is low and the protein appears to be highly unstable. This lack of availability of HDC has made it difficult to study the interaction of HDC and various compounds.

The expressions of recombinant HDC have been carried out by using *Escherichia coil* (*3*, *4*), yeast (*5*), and cultured cells (*6*−*8*) in recent years. Green tea epigallocatechin gallate (EGCG) was reported as a potent inhibitor for recombinant rat HDC (*9*). EGCG also inhibited recombinant pig-kidney dopa decarboxylase (DDC) (*10*), a pyridoxal 5′-phosphate (PLP) enzyme belonging to the same family as mammalian HDC (*11*). EGCG inactivated DDC activity in both time- and concentration-dependent manners (*10*). The interaction of DDC with EGCG was studied since EGCG is considered as a substrate analogue to DDC. On the other hand, EGCG does not have a chemical structure similar to that of histidine, the substrate of HDC, and the inhibition mechanism of EGCG on HDC is not fully understood.

EGCG has been reported to exhibit various biological activities including antioxidant activity (*12*, *13*), anticancer activity (*12*, *13*), and interaction with biopolymers, resulting in haze and gel formation (*14*, *15*), where high reactivity of hydroxyl groups bonded to the phenyl ring is involved. In recent years, the importance of polyphenols in food components and their involvement in a number of chemical reactions in human body have been reported (*16*). We have explored the role of the polyphenols by examining their specific interaction with HDC. In the present study, we have screened polyphenols in food components that inhibit recombinant human HDC. The phenolic compounds having a chemical structure similar to that of EGCG in general food components are investigated.

MATERIALS AND METHODS

Chemicals. Epicatechin (EC), epicatechin gellate (ECG), epigallocatechin (EGC), and EGCG were purchased from Wako (Osaka, Japan). Caffeic acid was purchased from Cayman (Ann Arbor, MI). Gallic acid was obtained from LKT Laboratories (St. Paul, MN). All other chemicals were purchased from local suppliers at the highest purity available (>95%).

Preparation of Recombinant Human HDC. A recombinant human HDC was obtained from transformed *Saccharomyces cerevisiae* as described previously (*5*). A partially purified HDC was used with a specific activity of ∼0.2 (nmol/min)/mg. To determine the specific

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activity, the protein concentration was estimated by using a Bio-Rad protein assay dye reagent, Bio-Rad Laboratories (Hercules, CA), with bovine serum albumin as the standard. The amount of histamine was estimated by using HPLC analysis as described below.

HDC Inhibition Assay. For HDC inhibition assay, the food compounds were dissolved in EtOH or H_2O at a concentration of 0.2−200 mM. The final assay mixture was prepared as reported by Ohmori et al. (*17*). Each mixture contained 0.2 mM dithiothreitol, 1% (v/v) polyethylene glycol 300, 0.01 mM PLP, 3.2 mM L-histidine, a testing food compound, and enzyme in 100 mM potassium phosphate buffer (pH 6.8). The final volume of the assay was 800 μL, including 40 μL of the solution of the food compound to be tested. Each assay mixture was incubated at 37 °C for 1 h, and the reaction was terminated by adding 40 μL of 60% perchloric acid.

HDC Activity Determination. Histamine produced during the reaction was measured on an HPLC system (model LC-6A, Shimazu (Kyoto, Japan)) equipped with postcolumn derivatization with *o*-phthalaldehyde (OPA) (*18*). A histamine Pak (Toso (Tokyo, Japan)) column was used to separate histamine from an abundant amount of histidine by applying 150 mM sodium dihydrogen phosphate in 10% methanol (pH 5.5) at a flow rate of 0.4 mL/min. The OPA solution was prepared as follows: OPA was dissolved in ethanol to 5%, which was then added to the solution containing 200 mM boric acid, 0.2% mercaptoethanol, and 0.1% Brij, to a final concentration of 0.08%. The pH of the solution was adjusted to 10.4 with 8 N KOH. The OPA solution was delivered to mix with an eluent from the column, and then peaks for the OPA derivatives were monitored by a fluorescence detector (121-fluorometer, GILSON (Middleton, WI)) with the excitation filter at 355 nm and emission filter at 440 nm. The flow rate of the OPA solution was 0.5 mL/min. HPLC analysis was performed at room temperature (25 ± 2 °C). A recorded peak arising from histamine appeared ∼30 min after the injection. Calibrations were performed by injections of histamine solution at known concentrations. Quantification of histamine was made on the basis of the peak area. The HDC activity of each sample was calculated by the following equation: HDC activity (%) = [(histamine peak area of sample) − (histamine peak area of blank)]/[(histamine peak area of control) − (histamine peak area of blank)] × 100. Duplicate samples were analyzed in each experiment.

Kinetic Experiments of HDC Inactivation by EGCG and ECG. For determining K_i, the concentration of L-histidine was set to a range from 0.05 to 1.6 mM. For a time-dependent test, HDC was preincubated with various concentrations of EGCG or ECG for a set time period at 37 °C. Incubation was initiated by the addition of L-histidine, and the mixture was reacted for 1 h at 37 °C. An anaerobic experiment was carried out in capped vials by bubbling nitrogen gas into the sample briefly through a thin needle and then sealing the vials.

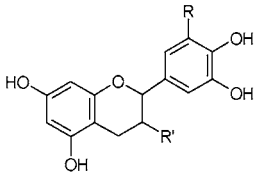
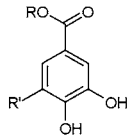
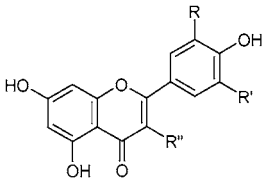
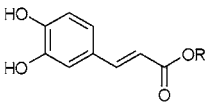
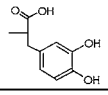
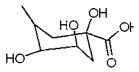
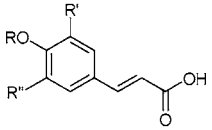
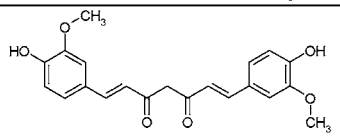
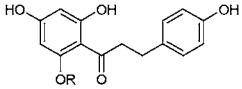
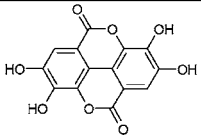
Data Analysis. For data analysis, an enzyme kinetics module in Sigmaplot version 9.01 (Systat Software Inc. (Richmond, CA)) was used.

RESULTS

The chemical structures of phenolic compounds tested in the present study for screening are summarized and listed in **Table 1**. The inhibitory effect (<80% HDC activity) was detected for ECG, EGCG, myricetin, EC, EGC, and rosmarinic acid at 1 mM (**Figure 1**). Gallic acid, methyl gallate, phlorizin, caffeic acid, and chlorogenic acid inhibited HDC at 10 mM, while the others did not inhibit HDC even at higher concentrations (>10 mM). ECG and EGCG inhibited HDC activity most effectively among the examined compounds (**Figures 1** and **2**). Since ECG and EGCG dissolved in H_2O inhibited HDC activity more effectively than those dissolved in EtOH, the experiments were performed with the aqueous solutions of ECG and EGCG.

The kinetic analysis was carried out for ECG and EGCG. **Figure 3** shows double reciprocal plots for the inhibition reaction of ECG and EGCG by altering the inhibitor concentration and substrate concentration, which indicates that ECG and EGCG compete with L-histidine with an inhibition constants $K_i = 10 \pm 4$ and 38 ± 8 μM, respectively. **Figure 4a** shows a time-dependent test for EGCG at various concentrations. With

Table 1. Compounds Used in the Present Study

Compound	structure	
Epicatechin gallate (ECG)		R = H R' = gallate
Epigallocatechin gallate (EGCG)		R = OH R' = gallate
Epicatechin (EC)		R = H R' = OH
Epigallocatechin (EGC)		R = OH R' = OH
Gallic acid		R = H, R' = OH
Methyl gallate		R = CH_3 R' = OH
Protocatecluic acid		R = H, R' = H
Myricetin		R = R' = R" = OH
Quercetin		R = R" = OH R' = H
Kaempherol		R = R' = H R" = OH
Luteorin		R = R" = H R' = OH
Apigenin		R = R' = R" = H
Rutin		R = H, R' = OH R" = α-L-Rha (1→6)-β-D-Glc
Caffeic acid		R = H
Rosmarinic acid		R = 
Chlorogenic acid		R = 
Ferulic acid		R = CH_3 R' = H, R" = OH
p-coumaric acid		R = R' = R" = H
Sinapic acid		R = H, R' = R" = -O-CH_3
Curcumin		
Phlorizin		R = 1-β-D-Glc
Ellagic acid		

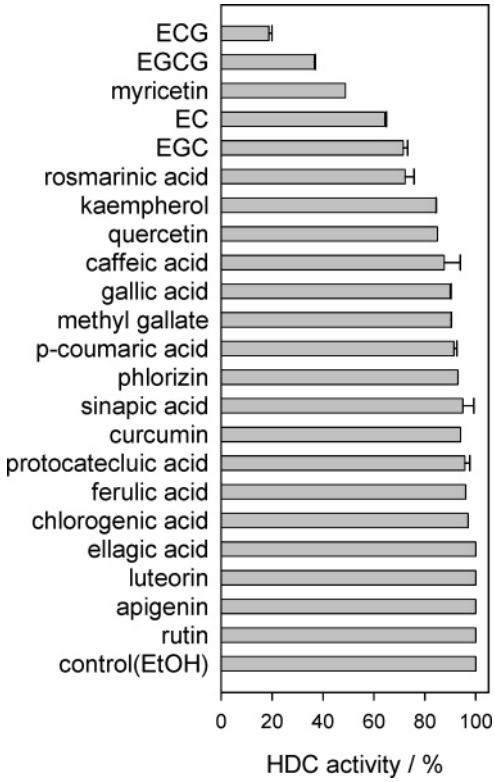


Figure 1. HDC activity in the presence of various food compounds. The assay mixture contained 0.2 mM dithiothreitol (DTT), 1% (v/v) polyethylene glycol 300 (PEG), 0.01 mM PLP, 3.2 mM L-histidine, each food compound, which was dissolved in EtOH, and the enzyme in 100 mM potassium phosphate buffer (pH 6.8). The final volume was 800 μL including 40 μL of the solution of the food compound at a final concentration of 1 mM. For the control, 40 μL of EtOH was contained in the final volume of the assay mixture. The mixture was reacted at 37 °C for 1 h.

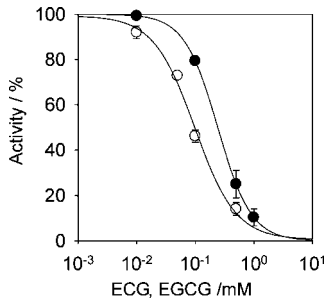


Figure 2. Concentration dependence of ECG (○) and EGCG (●) on the HDC activity. The assay mixture containing 0.2 mM DTT, 1% (v/v) PEG, 0.01 mM PLP, 3.2 mM L-histidine, various concentrations of ECG or EGCG, and the enzyme in 100 mM potassium phosphate buffer (pH 6.8) was incubated at 37 °C for 1 h. The final volume was 800 μL including 40 μL of the aqueous solution of ECG or EGCG. For the control, 40 μL of H_2O was contained in the final volume of the assay mixture.

increasing preincubation time, HDC activity decreased in a pseudo-first-order kinetic manner, as observed for the inhibition of DDC (*10*). Since polyphenols are highly susceptible to oxidation and are known antioxidants, time-dependent inactivation might be induced by autoxidation of EGCG. To determine whether autoxidation of EGCG affects inhibition, HDC activity was compared under aerobic and anaerobic conditions in the presence and absence of EGCG. As shown in **Figure 4**, EGCG inhibited HDC activity under anaerobic conditions but not in a time-dependent manner. Interestingly, ECG did not show a time-dependent inhibition under both aerobic and anaerobic conditions (**Figure 5**). Inhibition profiles were almost the same under aerobic and anaerobic conditions (**Figure 5**).

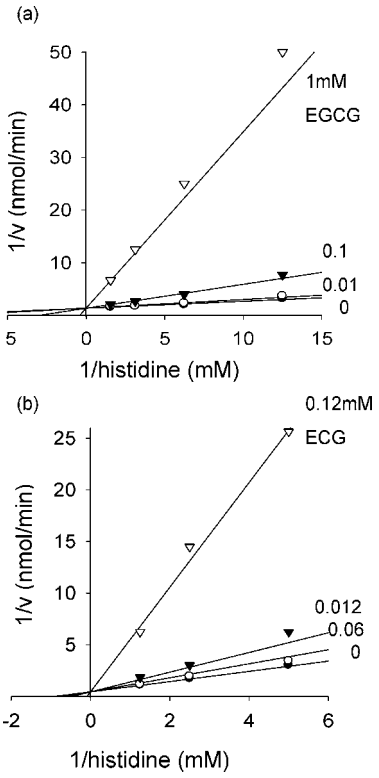


Figure 3. Double reciprocal plots of activity versus L-histidine in the presence of various concentration of (**a**) EGCG and (**b**) ECG. The assay mixture containing 0.2 mM DTT, 1% (v/v) PEG, 0.01 mM PLP, 0.05–1.6 mM L-histidine, various concentrations of ECG or EGCG, and the enzyme in 100 mM potassium phosphate buffer (pH 6.8) was incubated at 37 °C for 1 h.

DISCUSSION

Among 22 food compounds examined in the present study, ECG and EGCG were the most effective inhibitors for HDC. Both ECG and EGCG inhibited competitively against L-histidine. The inhibition of EGCG was time-dependent, suggesting that the inactivation is irreversible, while the inhibition of ECG was not time-dependent under our experimental conditions. The time-dependent inhibition of EGCG disappeared under anaerobic conditions, indicating that oxygen molecules may participate in the time-dependent inactivation.

ECG can be a potent inhibitor for HDC as the K_i value of 10 μM suggests. Typically, PLP-dependent enzymes form an internal aldimine between the aldehyde group of PLP and the active site Lys residue via a Schiff base (*19*, *20*). During catalysis, an internal aldimine is converted into an external aldimine, which is a complex of substrate and PLP. Our present results indicate that ECG preferably binds to the substrate binding site where ECG competes with L-histidine. Since ECG has a strong affinity for HDC (K_i = 10 μM) as compared to L-histidine (K_m = ~300 μM (*4*, *17*)), the presence of ECG may prevent the formation of the external aldimine from proceeding in the catalysis. It is probable that HDC has a relatively large substrate binding pocket which can accommodate a large compound such as ECG. As judged by the chemical structure of ECG, it does not have any functional amino group for the formation of the Schiff base with PLP. Therefore, it is hard to assume that ECG binds to PLP. Whether or not a direct interaction between ECG and PLP occurs during the inhibition

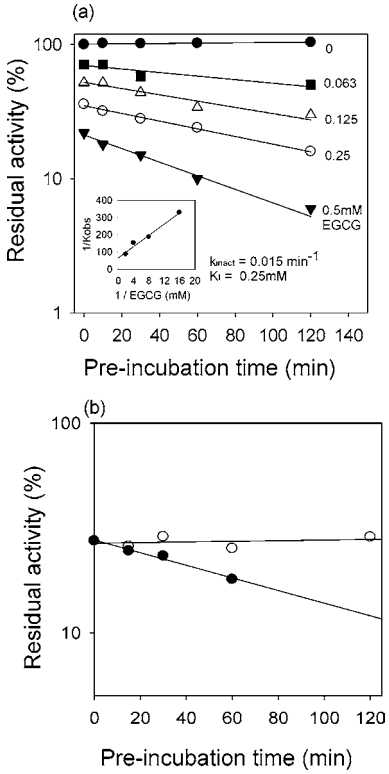


Figure 4. (**a**) Time-dependent inhibition of HDC by EGCG. Inset: double reciprocal plot of the apparent rate of inactivation versus EGCG. (**b**) HDC activity in the presence of 0.5 mM EGCG under aerobic (●) and anaerobic (○) conditions. The mixture containing 0.2 mM DTT, 1% (v/v) PEG, 0.01 mM PLP, various concentrations of EGCG, and the enzyme in 100 mM potassium phosphate buffer (pH 6.8) was preincubated at 37 °C. At time intervals, 40 μL of L-histidine was added to 760 μL of the mixture to 3.2 mM concentration, and then the mixture was incubated at 37 °C for 1 h.

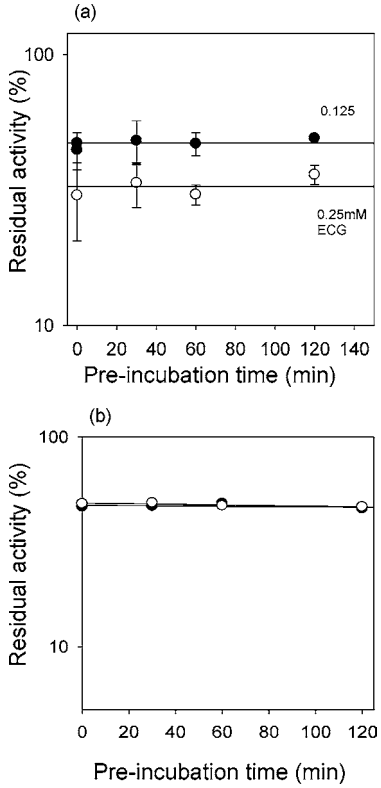


Figure 5. (**a**) Non-time-dependent inhibition of HDC by ECG. (**b**) HDC activity in the presence of 0.1 mM ECG under aerobic (●) and anaerobic (○) conditions. The mixture containing 0.2 mM DTT, 1% (v/v) PEG, 0.01 mM PLP, various concentrations of ECG, and the enzyme in 100 mM potassium phosphate buffer (pH 6.8) was preincubated at 37 °C. At time intervals, 40 μL of L-histidine was added to 760 μL of the mixture to 3.2 mM, and then the mixture was incubated at 37 °C for 1 h.

is still in question. It may be possible to consider binding of a hydroxyl group of ECG to the aldehyde group of PLP although no such hypothesis and reports have been found in the literature. To clarify and to understand the inhibition mechanism of ECG, a crystallographic study is necessary.

EGCG was reported to be less stable than ECG: a gradual decrease of EGCG concentration was observed in solutions of decaffeinated green tea extracts during the experimental time (*21*). Mochizuki et al. reported that EGCG was oxidized more rapidly than ECG in weak alkaline conditions, where autoxidation of EGCG occurred in the air-saturated 0.1 M Tris buffer at pH 9.0 (*22*). Epidermal growth factor receptor was inactivated by EGCG, and autoxidation of EGCG might be the cause of the inactivation (*23*). In that paper, they hypothesized that superoxide, •EGCG, and other radicals generated from EGCG autoxidation could attack and inactivate the receptors. The same interpretation might be applied to the inactivation of EGCG on HDC, although the details still need to be studied.

Autoxidation of EGCG has been reported under various in vitro conditions, in which dimers and oligomers have been identified as autoxidation products of EGCG (*24*, *25*). It should be noted, however, that these oxidized products have not yet been detected in vivo. EGCG itself is found in plasma and tissue of human, rat, and mouse (*26*−*29*). If oxidation of EGCG is prevented, for example, by the action of superoxide dismutase, an antioxidative enzyme, any in vitro events induced by the autoxidation of EGCG might not be visible in vivo. When EGCG is studied as a potential inhibitor for the targeted enzyme, it should be examined whether the observed inhibition is due to autoxidation of EGCG. Other polyphenol compounds that exhibit a biological activity similar to that of and resemble the structure of EGCG might behave like EGCG, and their biological activities could be the results of autoxidation.

Besides autoxidation, the chemical structure of EGCG might be an important factor for HDC inhibition since inhibition was observed even under anaerobic conditions (**Figure 4b**). The initial burst as observed for the time-dependent experiments carried out under aerobic conditions (**Figure 4a**) also suggests the involvement of EGCG in the HDC inactivation. The inhibition profile of EGCG for the recombinant pig-kidney DDC was similar to that for HDC; however, the initial burst was not observed (*10*). This suggests that inactivation of DDC by EGCG is mainly due to the autoxidation of EGCG and is somewhat different from that of HDC. In the case of HDC, the structural elements common between EGCG and ECG might be involved in the early inactivation stage of HDC. The inhibitory rates of EGCG and ECG are higher than those of EGC and EC, suggesting that the galloyl ester group is involved in HDC inhibition. Since the inhibition of gallic acid was much weaker than that of EGCG and ECG, an association of gallic acid to the 3′-position at the flavanol might be important in inhibiting HDC activity.

Finally we note the beneficial property of the food components in inhibiting HDC activity. In addition to ECG and EGCG, nine examined phenolic food components significantly inhibited HDC activity in the present study; they are myricetin, epicatechin, epigallocatechin, rosmarinic acid, gallic acid, methyl gallate, phlorizin, caffeic acid, and chlorogenic acid. Since histamine plays an important role in various biological actions,

inhibition of HDC activity likely affects histamine biosynthesis and possibly leads to control of the biological action induced by histamine. α-(Fluoromethyl)histidine, a suicide inhibitor of HDC (*30*), is reported to influence the biological actions controlled by histamine (*31*−*34*). Thus, any food components exhibiting HDC inhibitory activity might be potentially useful in controlling the histamine-induced biological actions. To achieve the use of phenolic compounds as beneficial food ingredients, the following still need to be considered: The effective concentration of phenolic compounds in the present study is not extremely low. In addition, it is uncertain whether or not such compounds are absorbed in the same form (*35*) and how compounds are transported into the cells.

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