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Research Article

Green tea epigallocatechin-3-gallate is an inhibitor of mammalian histidine decarboxylase

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Abstract. (–)-Epigallocatechin-3-gallate, an antiproliferative and antiangiogenic component of green tea, has been reported to inhibit dopa decarboxylase. In this report, we show that this compound also inhibits histidine decarboxylase, the enzymic activity responsible for histamine biosynthesis. This inhibition was proved by a double approach, activity measurements and UV-Vis spectra of enzyme-bound pyridoxal-5′-phosphate. At 0.1 mM (–)-epigallocatechin-3-gallate, histidine decarboxylase activity was inhibited by more than 60% and the typical spectrum of the internal aldimine form shifted to a stable major maximum at 345 nm, suggesting that the compound causes a stable change in the structure of the holoenzyme. Since histamine release is one of the primary events in many inflammatory responses, a new potential application of (–)-epigallocatechin-3-gallate in prevention or treatment of inflammatory processes is suggested by these data.

Key words. Histidine decarboxylase; epigallocatechin-3-gallate; pyridoxal-5′-phosphate; histamine; green tea.

Pyridoxal-5′-phosphate (PLP)-dependent histidine decarboxylase (HDC; EC 4.1.1.22) activities have been detected in Gram-negative bacteria [1] and mammals [2]. The product of HDC catalysis, histamine, is a biogenic amine involved in important physiological and pathological processes: neurotransmission, gastric secretion, anaphylactic reactions, and cell growth [3, 4]. At present, therapies against undesirable effects of histamine are mainly focussed on the blockage of histamine receptors by antagonists. The characterization of the mammalian HDC structure and catalytic mechanism, and their differences with respect to homologous enzymes, could open new perspectives for the development of new and more selective inhibitors acting specifically on the biosynthesis of histamine in different organisms [4]. Since a strong and selective inhibition of HDC is of potential pharmacological interest, it is interesting to test the action of a variety of compounds on this enzyme. This may be followed by measuring their effects on enzyme activity and/or on the spectroscopic properties of the PLP-bound enzyme [5].

(–)-Epigallocatechin-3-gallate (EGCG), a constituent of green tea with strong antioxidant, antiproliferative, and antiangiogenic effects [6], has been shown to inhibit two other PLP-dependent decarboxylases, namely, dopa decarboxylase (DDC; EC 4.1.1.28) and ornithine decarboxylase (ODC; EC 4.1.1.17) [7, 8]. These data prompted us to study the effects of EGCG on both the decarboxylase activity and the spectral properties of mammalian HDC. In this study, we demonstrate that EGCG inhibits both natural and recombinant HDC activities and changes the characteristic spectrum of PLP-bound HDC, suggesting new pharmacological applications for this natural compound.

Materials and methods

An active, recombinant version of rat HDC, previously validated and characterized by us [5, 9], was purified from

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transformed *Escherichia coli* cultures essentially as previously described [5]. In the assays of HDC activity, 6 μM HDC was preincubated for 1 h at 37 °C in the presence of 0.1 mM EGCG or 1 mM α-fluoromethylhistidine, followed by determination of $^{14}CO_2$ release from L-[U-$^{14}CO_2$] according to the procedure described elsewhere [9].

Absorption spectra of 30 μM HDC were obtained with a Shimadzu 1603 spectrophotometer. To avoid interference by free PLP, purified HDC was submitted to exclusion gel chromatography (Sephacryl HiPrep S-200; Amersham-Pharmacia) in 50 mM potassium phosphate buffer (pH 7) immediately before spectroscopic measurements.

As a source of naturally occurring HDC, we used the rat basophilic leukemia cell line RBL-2H3. Cell culture, determination of HDC activity in cell extracts, and study of the expression levels of HDC protein by Western blot were carried out as previously described [10].

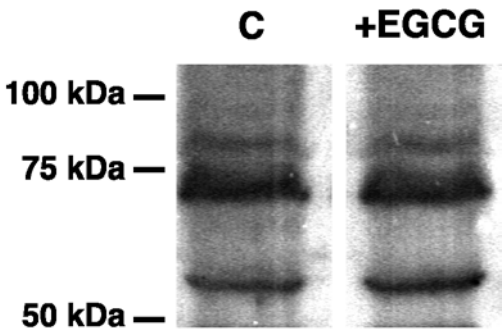


Figure 1. Expression levels of HDC polypeptides in RBL-2H3 cells, both untreated and treated with 0.1 mM EGCG for 90 min, as determined by Western blot. The positions of standard molecular-weight marker proteins are indicated on the left. The pattern of bands in control cells is that previously characterized by us [10].

Results and discussion

Very few effective inhibitors of HDC activity have been described so far and only the irreversible inhibitor α-fluoromethylhistidine has proved to inhibit HDC completely [4, 11]. Worth noting is that EGCG has been shown to inhibit other PLP-dependent decarboxylases, namely, ODC and DDC [7, 8]. Table 1 shows that EGCG produces a remarkable inhibition of purified recombinant rat HDC activity. Furthermore, rat HDC activity naturally occurring in an extract of RBL-2H3 cells was also inhibited by EGCG to a similar degree. In fact, these inhibition values are very similar to that previously shown for EGCG on DDC, where 1 h of incubation in the presence of 0.122 mM EGCG produced 75% inhibition of DDC activity [7]. On the other hand, EGCG can also inhibit HDC activity in vivo. Pretreatment of rat RBL-2H3 cells with 0.1 mM EGCG for 90 min yielded cell extracts with an HDC activity 21 ± 7% ($p < 0.01$) lower than that of control, untreated cells. Our results indicate that the first effect of EGCG on HDC is direct inhibition of the enzymatic activity, since the expression level of HDC protein remained unchanged (fig. 1). This is remarkable, since early effects are especially important during anti-inflammatory treatments.

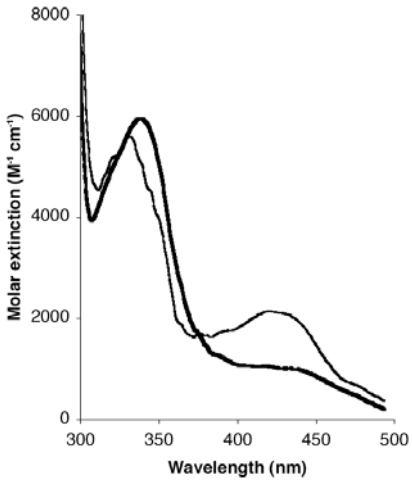
Table 1. Inhibition of recombinant and natural rat HDC by ECGC and the irreversible inhibitor α-fluoromethylhistidine.

Treatment	Recombinant HDC activity inhibition (%)	Natural HDC activity inhibition (%)
0.1 mM EGCG	67 ± 13*	57 ± 9*
1 mM α-fluoromethylhistidine	98 ± 3*	99 ± 1*

Activity assays were carried out as described in Materials and methods. Data are given as percentages of inhibition of the activity of control, untreated samples and are means ± SD of five independent determinations (in the case of α-fluoromethylhistidine effects on recombinant HDC) or two to three independent experiments with duplicate samples (the remaining treatments). * Significantly different to control values ($p < 0.01$), according to Student's t test.

As a catechol-type polyphenol, EGCG has been considered as a 'substrate analog' for the reaction catalyzed by DDC. However, EGCG shows no relevant structural similarity to either histidine or ornithine, substrates of the reactions catalyzed by HDC and ODC, respectively. In addition, DDC and HDC are not homologous to ODC [4, 12]. As far as we know, there is no report indicating that mammalian HDC (or mammalian ODC) could accept a catechol moiety into its catalytic site; on the contrary, mammalian HDC seems to have a catalytic site more specific than that of DDC [5 and references therein]. Furthermore, there is a previously demonstration that the active site of HDC does not decarboxylate the DDC substrate, DOPA [13]. Thus, it would be unlikely that EGCG reach the enzyme-bound cofactor. Nevertheless, the major point in common among the three mentioned amino acid decarboxylases is the role of PLP as an essential cofactor for their catalysis. If a compound induces changes in either the conformation or the configuration of the cofactor within the catalytic site, this can be followed by changes in the typical spectra of PLP-bound enzymes [5, 14].

The recombinant rat HDC spectrum has been previously characterized by us [5]. The spectrum presents two maxima, corresponding to the enolimine major form (335 nm) and the ketoenamine (425 nm) tautomers of the PLP-HDC 'internal' aldimine. Figure 2A shows that, in fact, the addition of 0.1 mM EGCG alters the absorbance spectrum of HDC. Two major changes are produced. First, the maximum at 425 nm corresponding to the ketoenamine tautomer of the PLP-HDC 'internal' aldimine disappears. On the other hand, there is an upshift of the maximum at 335 nm (corresponding to the enolimine tautomer of the



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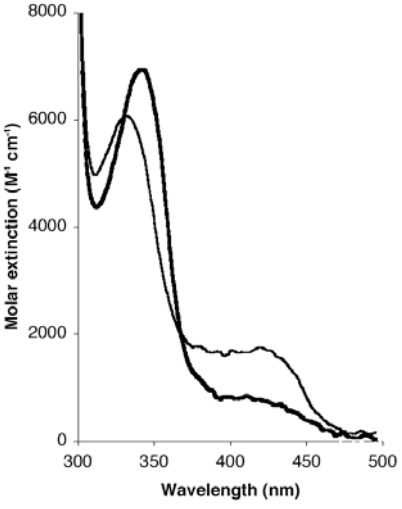

B

Figure 2. Absorption spectra of purified recombinant rat HDC in the absence (narrow lines) or presence (thick lines) of EGCG (*A*) or α-fluoromethylhistidine (*B*).

PLP-HDC aldimine) up to 345 nm. The spectrum differs significantly from that obtained in the presence of histidine methyl ester, a reversible inhibitor of HDC that forms an external aldimine PLP-substrate analog adduct within the active site, as previously shown [5]. The changes in the spectrum of HDC induced by EGCG (shown in fig. 1A) are very similar to the previously described effects of EGCG on the spectrum of DDC, where a maximum at 420 nm decreased and a maximum at 335 shifted up to 342 nm in the presence of 0.1 mM EGCG [7]. Catechol-type polyphenols have been shown to inhibit many enzymes, mainly oxidoreductases. In these cases, the inhibition has been proposed to be caused by scavenging reactive oxygen species required for enzyme reaction [6, 15–17]. For two enzyme activities inhibited by EGCG, molecular modeling has served to predict the binding manner of the inhibitor to the enzyme [16, 18]. To date, there is no further available information on the mechanism of inhibition exerted by these compounds. Worth taking into account is that the spectral changes induced by EGCG on HDC mimic those produced by the suicide, irreversible inhibitor α-fluoromethylhistidine (fig. 1B). The only maximum in the HDC spectrum in the presence of EGCG is similar to that previously obtained by Bhattacharjee and Snell [19] with α-fluoromethylhistidine-treated Gram-negative HDC. According to these authors, this peak is mainly due to an adduct PLP-inhibitor covalently bound to the enzyme. Other authors working with α-fluoromethylhistidine-treated mammalian HDC suggest that the inhibitor can produce different adducts, some of them covalently linked to the enzyme [20]. Therefore, the spectral data obtained with the purified enzyme could suggest some kind of covalent modification of the holoenzyme by EGCG, supporting the previous suggestion for a covalent modification of DDC by EGCG [7].

Current knowledge about biogenic amines has risen from different biomedical areas. These natural amino acid-derived polycations have evolved to play distinct physiological roles. However, from a biochemical point of view, nature uses some similar patterns to manage the intracellular concentrations of these compounds. Humans also want to control amine levels, since these compounds are related to very important pathologies. Therefore, useful strategies for controlling the metabolism or biological effects of any of these amines could also be beneficial for intervention in the biochemistry of the others. The results shown here for HDC demonstrate, for the first time, that this enzyme is inhibited by submillimolar concentrations of EGCG. This observation opens new questions about the exact nature of the interaction between EGCG and both HDC and DDC, leading (directly or indirectly) to very stable and similar PLP conformational/configurational changes. Furthermore, it generates new potential applications for this natural compound. For example, given the present data, evaluation of EGCG in prevention of inflammation or in anti-inflammatory treatments could be worthwhile. In fact, histamine is associated with multiple inflammatory responses and the development of new anti-inflammatory compounds is currently an important growing topic of research [21]. Other authors, working with in vivo models, have described that EGCG also inhibits the most important pro-inflammatory signal transduction pathway [22]. These results together with the direct and short-term inhibition of histamine synthesis presented in this paper point to EGCG as a promising compound for anti-inflammatory therapy.

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