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Original Contribution

Structure–activity relationship of the tocopherol-regeneration reaction by catechins☆

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Abstract

The reaction rates (k_r) of 5,7-diisopropyl-tocopheroxyl radical (Toc$^{\bullet}$) with catechins (epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG)) and related compounds (methyl gallate (MG), 4-methylcatechol (MC), and 5-methoxyresorcinol (MR)) have been measured by stopped-flow spectrophotometer. The k_r values increased in the order of MR << MG < EC < MC ~ ECG < EGC < EGCG in ethanol and 2-propanol/H_2O (5/1, v/v) solutions, indicating that the reactivity of the OH groups in catechins increased in the order of resorcinol A-ring << gallate G-ring < catechol B-ring < pyrogallol B-ring. The catechins which have lower oxidation potentials show higher reactivities. The rate constants for catechins in micellar solution showed notable pH dependence with one or two peaks around pH 9–11, because of the dissociation of various phenolic hydroxyl protons in catechins. The structure–activity relationship in the free-radical-scavenging reaction by catechins has been clarified by the detailed analyses of the pH dependence of k_r values. The reaction rates increased remarkably with increasing the anionic character of catechins, that is, the electron-donating capacity of catechins. The mono anion form at catechol B-and resorcinol A-rings and dianion form at pyrogallol B-and gallate G-rings show the highest activity for free-radical-scavenging. It was found that catechins (EC, ECG, EGC, and EGCG) have activity similar to or higher than that of vitamin C in vitamin E regeneration at pH 7–12 in micellar solution.

Keywords: Tea catechins; Epigallocatechin gallate; Vitamin E regeneration; Reaction rate; Antioxidant activity; Structure–activity relationship; Free radicals

Introduction

Catechins, tea flavanols, are well known as representative natural polyphenolic antioxidants [1,2]. Catechins are widely present in fruits and plants in high concentrations and may function as scavengers of active oxygen species in biological systems. Especially, green and black teas contain considerable amounts of catechins (epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG); see Fig. 1) [1–4]. In recent years, the green tea catechins have been recognized to be effective protectants against certain forms of cancer [4–8]. Although the human epidemiology remains inconclusive, catechins display remarkable cancer preventive effects in several animal models. The cancer preventive effects often have been attributed to antioxidant actions. Catechins are found in blood and tissues following oral ingestion [9–11], prevent human plasma oxidation, and act as inhibitors of low-density lipoprotein (LDL) oxidation [12–15]. Green tea polyphenols, including catechins, inhibit oxidant-induced DNA strand breakage in cultured lung cells [16].

Several kinetic studies have been performed for the reaction of flavonoids, including catechins, with active free

Abbreviations: CA, (+)-catechin; EC, (–)-epicatechin; EGC, (–)-epigallocatechin; ECG, (–)-epicatechingallate; EGCG, (–)-epigallocatechin-gallate; MR, 5-methoxyresorcinol; MC, 4-methylcatechol; MG, methyl gallate; LDL, low-density lipoprotein; HPLC, high-performance liquid chromatography; Toc, 5,7-diisopropyltocopheroxyl; ArO, (aroxyl) 2,6-di-*tert*-butyl-4-(4-methoxyphenyl)phenoxyl; $UQ_{10}H_2$, ubiquinol-10; Ru, rutin; Qu, quercetin; LH, unsaturated lipids.

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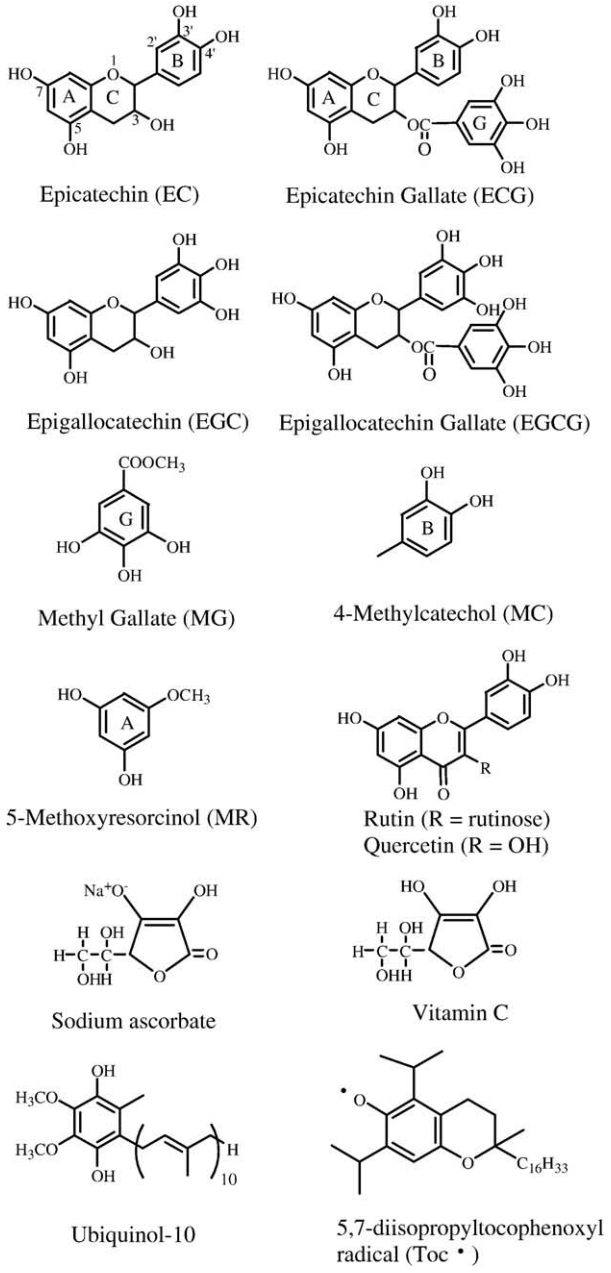

Fig. 1. Molecular structures of EC, ECG, EGC, EGCG, MG, MC, MR, rutin, sodium ascorbate (Na^+AsH^-), vitamin C, ubiquinol-10, and 5,7-diisopropyltocopheroxyl ($Toc^\bullet$).

radicals, such as $N_3^\bullet$, $HO^\bullet$, $O_2^{-\bullet}$, *t*-$BuO^\bullet$, and $LOO^\bullet$, by using pulse radiolysis techniques [17–19] and HPLC and oxygen consumption methods [20–22]. Owing to the various dissociable phenolic hydroxyl groups in catechins, it is expected that the reaction rates between catechins and active free radical show notable pH dependence [23,24]. Therefore, it is necessary to measure the free-radical-scavenging rates of catechins at various pH in aqueous solutions and in organic solvents, in order to clarify the mechanism of antioxidant action of catechins in biological systems.

Synergistic effect of vitamin E and C has been studied extensively [25,26]. The mixtures of vitamin E and these catechins may function synergistically as antioxidants in various tissues, as well as those of vitamins E and C. In fact, suppression of the α-tocopherol consumption by flavonoids (morin, fisetin, and quercetin) was previously reported in the case of oxidative modification of human LDL treated with macrophage or metal ion [27,28]. Further, the flavonoids in wine [29] and in liposome [20] were reported to act synergistically with tocopherol to inhibit lipid peroxidation. Consequently, the measurements of the regeneration rate of tocopheroxyl radical with catechins are important.

In a previous work, preliminary kinetic study was performed for the reaction of CA (catechin) and EC with 5,7-diisopropyltocopheroxyl ($Toc^\bullet$) radicals (see Fig. 1) in homogeneous and micellar solutions (reaction (1)) [30].

$$Toc^\bullet + \text{catechin} \xrightarrow{k_r} TocH + \text{catechin}^\bullet \quad (1)$$

The second-order rate constants (k_r) of CA and EC obtained in micellar solutions showed notable pH dependence with a maximum around pH 9.5. CA is a tetrabasic acid and can exist in five different molecular forms, depending on the pH value [23,24]. By comparing the k_r values with the mole fraction (f) of each molecular form of CA, the reaction rate k_{r1} for the undissociated form (CAH_2), k_{r2} for the monoanion (CAH^-), and k_{r3} for the dianion (CA^{2-}) of B-ring of CA were determined. It has been found that CA and EC have activity similar to that of vitamin C in vitamin E regeneration. Similar measurements were performed for the reaction of flavone and its derivatives (chrysin, flavonol, apigenin, rutin, and quercetin) [24].

In the present work, we have measured the rate constants (k_r) for the reaction of catechins (EC, ECG, EGC, and EGCG) and related compounds (methyl gallate (MG), 4-methylcatechol (MC), and 5-methoxyresorcinol (MR)) (see Fig. 1) with $Toc^\bullet$ radicals in ethanol, 2-propanol/water (5/1, v/v), and aqueous Triton X-100 micellar solution (5.0 wt %) (pH 4–12) (reaction (1)). MG, MC, and MR are considered to be a model of gallate G-ring, catechol B-ring, and resorcinol A-ring in catechins, respectively. The k_r values obtained in micellar solution showed notable pH dependence.

Materials and methods

EC, EGC, ECG, and EGCG were obtained from Funakoshi (Japan). MC was obtained from Wako Chemicals (Japan). MG (Tokyo Kasei Organic Chemicals, Japan) and MR (Aldrich) are commercially available. The $Toc^\bullet$ radical is fairly stable and was prepared by PbO_2 oxidation of the corresponding 5,7-diisopropyltocol in ethanol or in 2-propanol/water (5/1, v/v) solutions under a nitrogen atmosphere [31]. In the case of the reaction in micellar solution, $Toc^\bullet$ radical was prepared by the reaction between 2,6-di-*tert*-butyl-4-(4-methoxyphenyl)phenoxyl radical ($ArO^\bullet$)