Antioxidant Polymer Particles. Enzymatic Immobilization of Catechin on Polymer Particles

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Catechin-immobilizing polymer particles have been developed by laccase-catalyzed oxidation of catechin in the presence of amine-containing porous polymer particles. The resulting particles showed good scavenging activity toward stable free DPPH radical and ABTS radical cation.

Phenolic compounds including flavonoids and flavanoids are widespread in plants. Many of them are known to possess biological and pharmacological activities such as antioxidative, antibacterial, antiviral, and antimutagenic effects.[1] They are also known to be potent inhibitors for several enzymes.[2]

(+)-Catechin is the simplest compound belonging to the large family of flavanols and often used as a model compound for the biochemical behaviors of the entire class. Catechin showed good radical scavenging activity due to an *o*-dihydroxy B ring structure; the B ring confers higher stability to the radical form and participates in electron delocalization.[3]

Based on unique biological and pharmacological functions of catechins, catechin-containing materials are highly potent for various applications. However, catechins contain many phenolic groups; and hence, the selective modification is generally difficult. Recently, tyrosinase-catalyzed grafting of catechin on chitosan was reported.[4] The resulting catechin-modified chitosan behaved as an associative thickener.

In order to amplify physiological properties of catechins, we have designed polymerized catechins and polymeric catechin-conjugates of various polyamines and synthesized them by utilizing specific enzyme catalysis.[5] We preliminarily reported the great amplification of antioxidant activity and xanthine oxidase inhibition of catechin by the enzymatic polymerization.[6]

Very recently, we have found that laccase catalysis efficiently induced an oxidation of catechin in the presence of poly(allylamine) to produce a polymeric conjugate containing a catechin group. Furthermore, the resulting conjugate showed a good antioxidant property for low-density lipoprotein peroxidation induced by a free radical.[7] In this study, we have expanded this enzymatic conjugation to development of catechin-immobilizing polymer particles exhibiting antioxidant properties.

In this study, laccase derived from *Myceliophthora* was used as a catalyst for the conjugate production. The immobilization was carried out by the laccase-catalyzed oxidation of catechin in the presence of porous acrylic polymer particles having an amino group with average particle diameter of 300 μm (FPHA13L, Mitsubishi Chemical Co., Japan) at room temperature under air.[8] The amount of primary amino group is 0.3 mmol/mL (data from the supplier). During the enzymatic immobilization, the particle color changed from white to orange, which did not disappear after removal of the unreacted monomer and products not immobilized on the particles. Without the enzyme, the appearance change was scarcely observed. Our previous study on the laccase-catalyzed conjugation of catechin on poly(allylamine) showed that a new peak at 430 nm due to the conjugate appeared in the UV–vis spectrum. Therefore, the formation of the colored particles suggests that the immobilization of catechin on the particles took place via the laccase catalysis.[9] Pore volumes of the starting particles and the catechin-immobilizing ones were 1.20 mL/g and 1.09 mL/g, respectively. The decrease of the pore volume strongly supports the formation of the catechin-immobilizing polymer particles.

The method using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging is among the most popular spectrophotometric methods for determining the antioxidant ability of compounds.[10] DPPH, a purple-colored stable free radical, is reduced into the yellow-colored diphenylpicryl hydrazine, as the radical is scavenged by antioxidants through donation of hydrogen. Figure 1 shows effect of the molar feed ratio of catechin for the amino group of the particles.[11] In the range of the low feed ratio, the scavenging activity increased as increasing the feed ratio and above the feed ratio of 0.05, the activity increased slightly. No scavenging activity was found in the particles obtained without laccase. Effect of the particle amount on the scavenging activity was examined by using the particles prepared with the feed ratio = 0.05 (Figure 2).[11] The activity gradually increased as the function of the particle amount. The reuse of the present particles for scavenging of DPPH was examined. Within a range of 4 cycles, the activity scarcely changed (data not shown).

The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical cation assay is often also used for evaluation

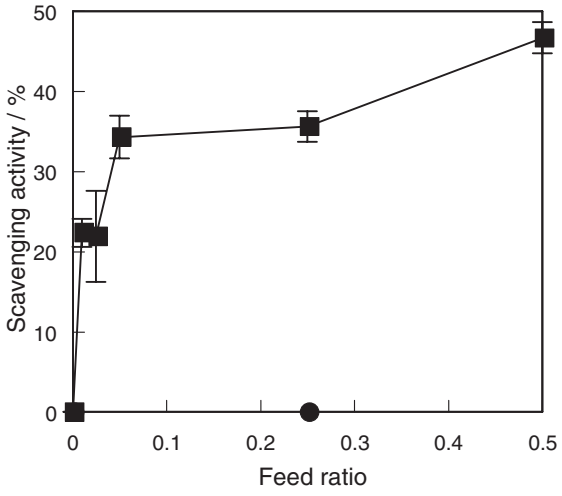

Figure 1. DPPH scavenging activity of polymer particles (10 mg) prepared (■) by laccase and (●) without laccase.

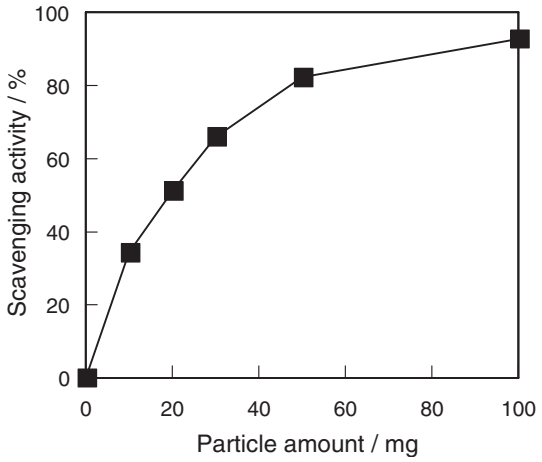

Figure 2. Effects of particle amount on DPPH scavenging activity by using particles prepared with the feed ratio of 0.05.

of antioxidant activity.[12] The present particles showed good scavenging activity toward ABTS radical cation (Figure 3); under the measured conditions, more than 90% radical cation was scavenged.[13] Above the feed ratio of catechin larger than 0.05, the activity was almost constant. Similar behaviors were observed in the scavenging of DPPH by the present particles (Figure 1).

In conclusion, the immobilization of catechin on polymer particles was successfully achieved by using laccase catalysis. The resulting particles efficiently scavenged DPPH radical and ABTS radical cation. Furthermore, the DPPH scavenging activity hardly changed within 4 cycles of the reuse. Since the present particles can be easily applied for packed column systems to remove radicals and reactive oxygen species, they possess large potential applications as radical scavenger in various fields such as food and biomedical industries. Further studies on applications of the flavonoid-immobilizing particles are under way in our laboratory.

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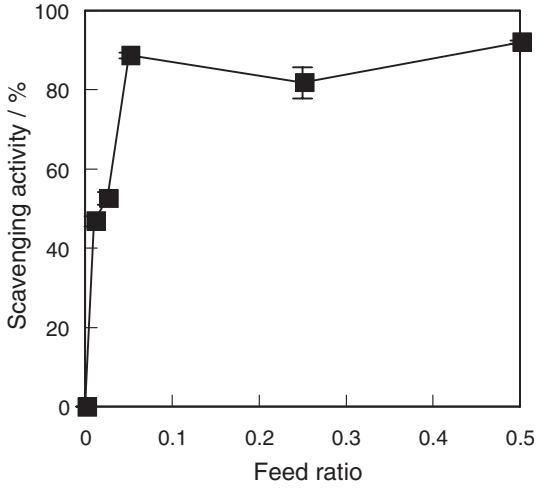

Figure 3. ABTS radical cation scavenging activity of polymer particles (10 mg).

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8 A typical procedure of the immobilization is as follows. Catechin (1.45–145 mg) in 2 mL of methanol was added to 15 mL of acetate buffer (pH 5) including 2 g of polymer particles. The reaction was started by the addition of laccase solution (10 units). The mixture was kept under gentle stirring at room temperature for 24 h under air. The particles were separated by filtration and washed successively with methanol, 0.1 N NaOH solution, and water.

9 The immobilized amount of catechin was too small to detect by elemental analysis.

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11 A particle sample was mixed with 100 μM of DPPH in ethanol solution (3.5 mL). After gentle stirring for 10 min, absorbance at 517 nm was recorded using a UV–vis spectrophotometer. All analyses were run in triplicate and the results were averaged.

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13 A particle sample (10 mg) was mixed with 0.5 mM ABTS cation radical in 5 mM phosphate buffered saline (3 mL). After gentle stirring for 10 min, absorbance at 734 nm was recorded using a UV–vis spectrophotometer. All analyses were run in triplicate and the results were averaged.

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