Synthesis and characterization of lipophilic catechins.

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Summary

Lipophilic catechins were synthesized by combining lauroyl chloride with (+)-catechin in order to increase the solubility in lipid membranes and explore their effectiveness as an antioxidant *in vivo*. The OH groups of the catechin were randomly acylated and the reaction mixture was separated using HPLC. The structures of three lipophilic catechins (3-acylated, 3', 4'-diacylated and 3,3',4'-triacylated catechins) were determined using ^{1}H NMR. DPPH radical scavenging activities of lipophilic catechins compared to (+)-catechin in ethanol solution were examined using a spectrophotometer and an electron spin resonance (ESR). The 3-acylated catechin was similar to (+)-catechin, but 3', 4'-diacylated catechin and 3,3',4'-triacylated catechin were less effective in scavenging DPPH radicals. Thus, it was suggested that the acylation of the alcoholic OH group at 3-position did not alter the radical scavenging activity of the original catechin, but the acylation of the phenolic OH groups on the B-ring decreased it. 3-Lauroylcatechin synthesized in this study was an effective lipophilic catechin with respect to the DPPH radical scavenging, and was expected to exhibit more diverse pharmacological activities.

Keywords

Tea catechin, Lipophilicity, Chemical structure, Synthesis, Radical scavenging activity

Introduction

Tea catechins show various biological and pharmacological effects. A commonly discussed mechanism of their effects is the radical scavenging activity and it had been considered that catechins could scavenge radicals even in the lipid membranes. However the interaction of catechins and lipid membrane is still obscure to explore the mechanism. It is because catechins are not effectively absorbed from digestive tracts due to their hydrophilic properties, and, therefore, have low solubility in lipid membranes. The galloylated catechins such as ECg and EGCg exhibit a relative higher free radical scavenging capacity than non-galloylated ones, which attributed to the solubility in lipid membrane, suggesting the importance of chemical structure of catechin to exhibit more effective pharmacological activities. It is expected that higher solubility and permeability in lipid membrane are desirable to explore the role of catechins in oxidative reaction *in vivo*. Thus, we synthesized lipophilic catechins by combining lauroyl chloride with (+)-catechin and determined their DPPH radical scavenging activities using a spectrophotometer and an electron spin resonance (ESR). In addition, the relationship between the radical scavenging activity and the chemical structure of catechin was discussed.

Materials and Methods

Synthesis of Lipophilic Catechins

(+)-Catechin (2×10^{-3} mol) (Tokyo kasei, Japan), which was completely dried using a vacuum pump,

was dissolved in dioxan dehydrated with dry molecular sieve and reacted with lauroyl chloride (2 x 10^{-3} mol) in the presence or absence of pyridine (2 x 10^{-3} mol). The reaction mixture was kept for several hours or more under nitrogen gas to advance the reaction.

HPLC Separation of synthesized lipophilic catechins

HPLC (Shimadzu Co. LTD., Japan) equipped with a reversed phase C18 column (20 x 250 mm i.d., 5 μm particle size) (Shiseido Co. LTD., Japan) was used to separate and purify the synthesized lipophilic catechins. The solvent was a mixture of methanol, chloroform and water. Five ml of the samples was applied at a flow rate of 8 ml/min under room temperature.

Measurement of ^{1}H NMR Spectra

To determine the strucrture of synthesized catechins, each sample was dissolved in $CDCl_3$ and the ^{1}H NMR spectra of the solutions were measured using JEOL GX 500 spectrometer.

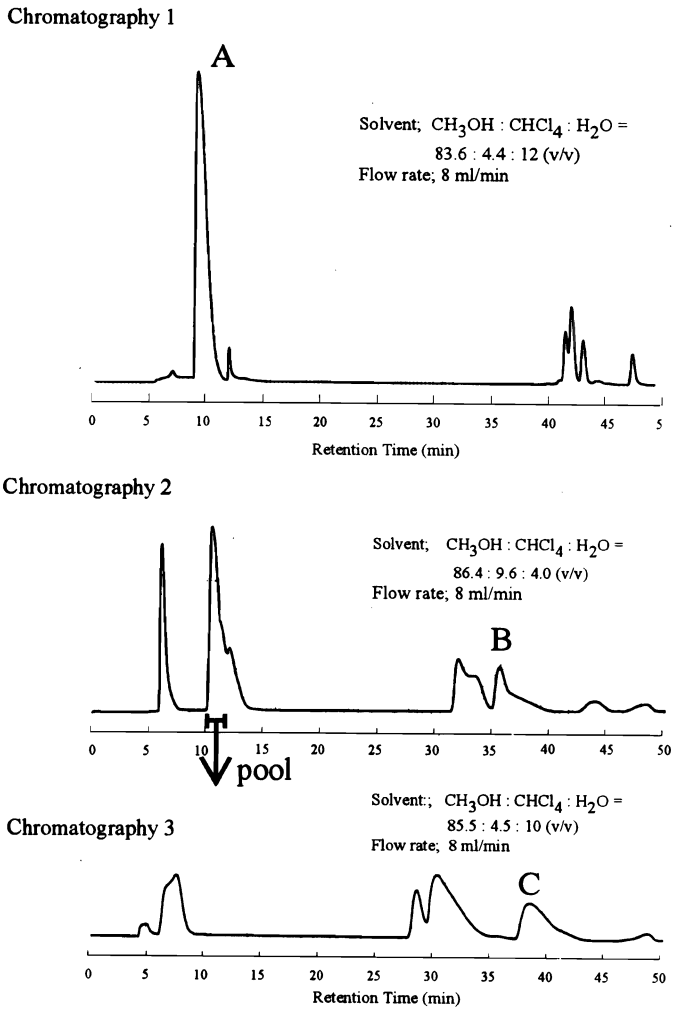

Fig. 1. HPLC chromatograms of the reaction mixture.

Measurement of scavenging activity on DPPH radical

Ethanol solutions of (+)-catechin or acylated catechins (2 x10^{-5} M) were mixed with DPPH (1 x 10^{-4} M) dissolved in ethanol, and the change of the absorbance at a specific wavelength (519 nm) was followed with time using Hitachi F3000 spectrophotometer. The scavenging capacity of catechins on DPPH radical was calculated by the following equation:

Scavenged % = $[(A_{control} - A_{sample})/A_{control}] \times 100$,

where, $A_{control}$ = absorbance of DPPH, A_{sample} = absorbance of DPPH mixed with a catechin.

In addition, the change of the ESR signal intensity of the mixed solutions, where 2 x10^{-5} M catechin was applied, measured at room temperature using a JEOL FR30 spectrometer, operating at 9.5 GHz with 100 kHz field modulation. The modulation width was 0.25 mT and the output power was 4 mW.

Results and Discussion

An importance of chemical structure of catechin on the radical scavenging activity has been reported [1]. We tried to synthesize lipophilic catechins from the viewpoint that they have higher solubility in lipid

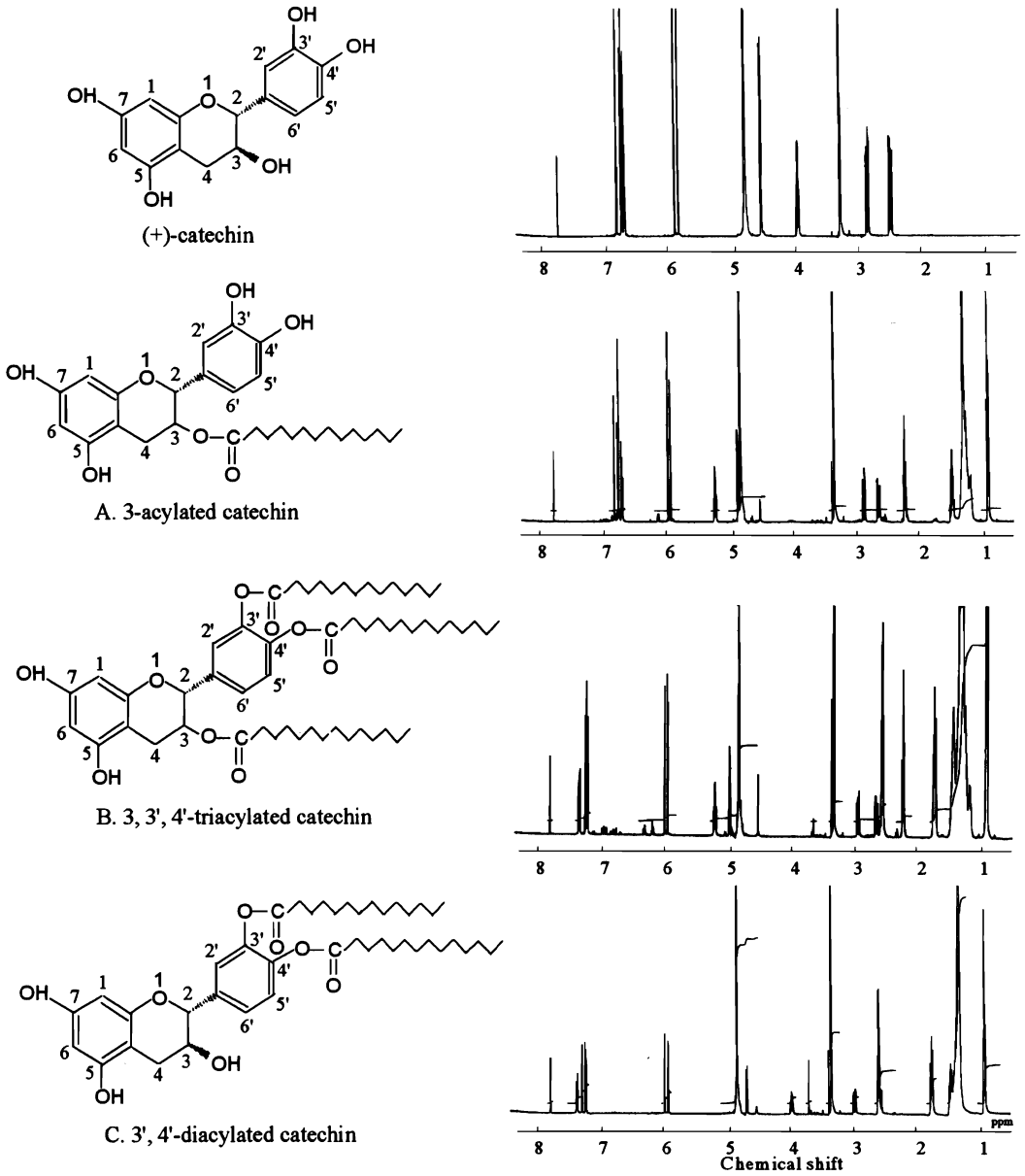

Fig. 2. ^{1}H NMR spectra of (+)-catechin and lipophilic catechins.

membranes, which leads to exhibit stronger radical scavenging activity resulting in more effective pharmacological activities. To synthesize lipophilic catechins, the OH groups of the catechin were randomly acylated by combining lauroyl chloride with (+)-catechin, and the reaction mixture was separated and purified using HPLC. The chromatograms were shown in Figure 1. The structures of three lipophilic catechin (A.3-acylated catechin, B.3,3',4'-triacylated catechin and C.3',4'-diacylated catechin) were successfully determined using ^{1}H NMR. They were shown in Fig. 2 with their NMR spectra. Integration curves in the alkyl region indicated that A, B and C possess one, three and two alkyl chains, respectively. The change of the chemical shifts of the protons in A, B and C rings, caused by the acylation suggested that the assignment of the acylated positions was undoubted. Figure 3 shows the change of the absorbance of the DPPH solution after mixing with each catechin. The quantity of DPPH radical scavenging increased with time in all the samples and the order was 3,3',4'-triacylated catechin≦3',4'-

diacylated catechin < 3-acylated catechin < (+)-catechin. The capacity of 3-acylated catechin was similar to that of (+)-catechin, but 3', 4'-diacylated and 3,3',4'-triacylated catechin showed very weak activities. In addition, effect of lipophlic catechin on ESR signal intensity of DPPH radicals was examined when DPPH was mixed with (+)-catechin or lipophilic catechin (Fig. 4). These results were in agreement with those in Fig. 3, showing that the number and the position of the acylation strongly affected the radical scavenging capacity. The acylation of alcoholic OH group at 3-position does not affect the capacity because it scarcely has the original activity. However, blocking of phenolic OH groups on the B-ring seriously the capacity. This is consistent with the fact that the superior capacity of catechin to scavenge free radicals can be attributed to the multiple numbers of hydroxyl groups, and in particular the contribution of the ortho-di-hydroxyl groups positioned on the B-ring of the catechin [1,2].

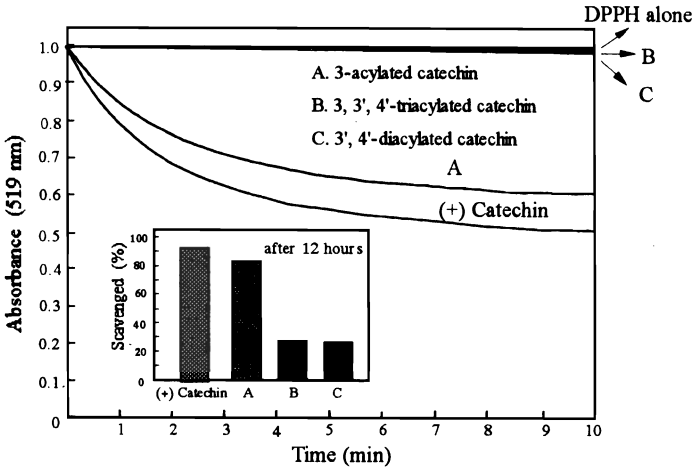

Fig. 3. DPPH radical scavenging activity of lipophilic catechins.

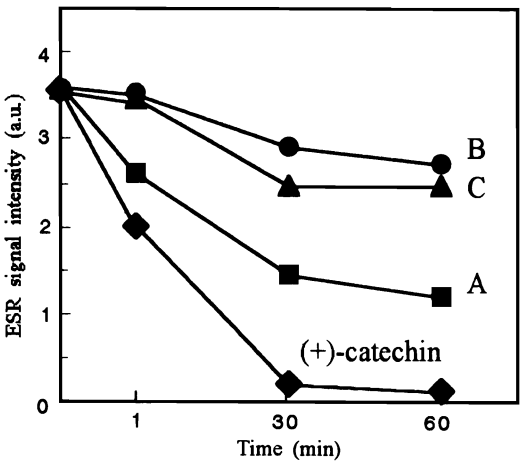

Fig. 4. Effect of lipophilic catechins on ESR signal intensity of DPPH radical.

The effective antioxidant properties *in vivo* are determined not only by the radical scavenging activity of the molecule itself, but also by the interaction with cell membranes as stated above. (+)-Catechin and 3-lauroyl catechin had almost same radical scavenging activity in a uniform solution, but they have different affinity to lipid membrane. In conclusion, it is expectable that 3-lauroylcatechin may exhibit strong antioxidative activity to lipid peroxidation and is usable as a new lipophilic antioxidant. Our next objective is to determine the protective effect of lipophilic catechins against oxidative damage, *e.g.*, lipid peroxidation, irradiation and cancer, to evaluate pharmacological activities.

References

1) Qiong Guo, et al., Biochimica et Biophysica Acta, 1427 (1999) 13-23.

2) Chun Hu and David D. Kitts, Molecular and Cellular Biochemistry, 218 (2001) 147-155.