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Interaction of tea catechins with phospholipids - Roles in their tastes and biological activities

Interaction of Tea Catechins with Phospholipids - Roles in Their Tastes and Biological Activities

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ABSTRACT

Green tea is well known for its various physiological effects, but there are few studies relating the tastes of tea catechins to their biological activities. We have focused on the interaction of tea catechins with phospholipids to clarify the relationship. The galloyl-type catechins, epicatechin gallate (ECg) and epigallocatechin gallate (EGCg) usually show beneficial properties including chemopreventive, antimutagenic and antioxidant actions more potently than the nongalloyl-type catechins, epicatechin (EC) and epigallocatechin (EGC). The difference in their chemical structures is correlated with their affinity for phospholipid membranes and incorporated amounts into liposomes. This indicates that the interaction of the galloyl-type catechins with phospholipids are always stronger than the nongalloyl-type catechins. Molecular-level insights into how tea catechins interact with lipid membranes were acquired using NMR spectroscopy. Based on solution and solid-state NMR studies with isotropic bicelles and liposomes as models of phospholipid membranes, it is proposed that ECg and EGCg interact with the surface of lipid membranes via the choline moiety of the phospholipids. Since the galloyl-type catechins have been reported to be more bitter and astringent than the nongalloyl-type catechins, both of their tastes and biological activities could be ascribed to the interaction.

Key words: catechins, NMR, phospholipids, taste, interaction

INTRODUCTION

Tea is the second highest drink consumed in the world, next to water. The research on tea and health has been conducted worldwide, particularly in Asia. Green tea is well known for its various physiological effects, and much research has focused on tea catechins, but there are few studies relating the mechanism of the biological activities of the catechins to their tastes. Among tea catechins (Figure 1), the galloyl-type catechins, epicatechin gallate (ECg) and (-)-epigallocatechin gallate (EGCg) has been reported to have beneficial properties including chemopreventive, anticarcinogenic and antioxidant actions. We have proposed that the potency of the biological activities of the galloyl-type catechins should be ascribed to their affinities for phospholipids. The galloyl-type catechins have been reported to be more bitter and astringent than nongalloyl-type catechins, such as epicatechin (EC) and epigallocatechin (EGC)[(1)]. Tastes of food components whose sensation are received through taste buds on the upper surface of the human tongue is categorized into five basic ones: sweet, bitterness, sour, salty, and umami. On the other hand, the

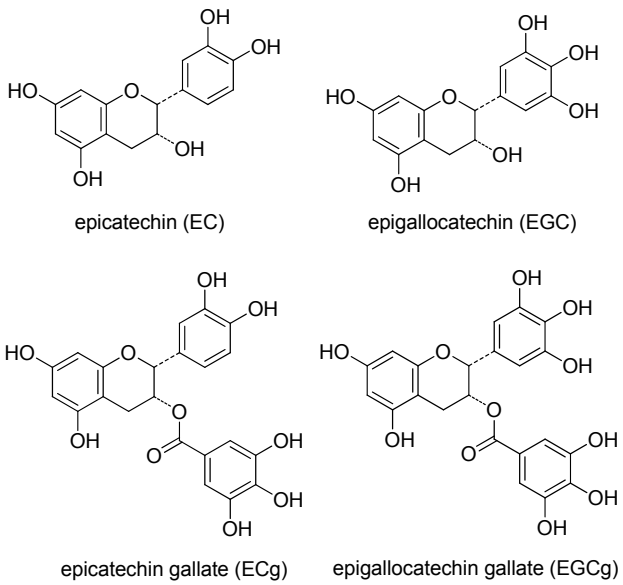

Figure 1. Structures of tea catechins.

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taste buds are not involved in the sensation of astringency[2]. Recently it is reported that bitterness of the galloyl-type catechins are sensed through a certain taste receptor hTAS2R39[3], while mechanisms of astringency of tea catechins have not fully elucidated.

In this paper, studies on the interaction of tea catechins with phospholipids and proteins are reviewed and the relation between the interaction and their astringency is discussed

INTERACTION BETWEEN TEA CATECHINS AND PHOSPHOLIPIDS

The interaction of tea catechins with biological membranes is supposed to be necessary to exert various activities such as antioxidant activities, antibacterial activities and taste. We have clarified that the galloyl-type catechins have high affinity for model biological membranes based on the amount of catechin incorporated into liposomes[4,5], the binding constants obtained by quartz-crystal microbalance (QCM)[6], and partition coefficients for phospholipids using HPLC with an immobilized artificial membrane column[7]. These studies always show the following order of affinity: ECg > EGCg > EC > EGC. This order was closely correlated with the order of their partition coefficients *(log P)* measured by the PBS/n-octanol system[4]. This means that the affinity for phospholipids, phospholipophilicity, of catechins has been linked to their hydrophobicity. If a catechin molecule has high affinity for the bilayers, the chance that the molecule interacts with membrane-associated proteins or incorporates into cells is increased, resulting in exertion of their biological activities.

Isotropic bicelles and liposomes were used as models of phospholipid membranes for solution and solid-state NMR spectroscopy, respectively. In our solution NMR study using bicelles, we revealed that catechins interact with the surface of phospholipid membranes, and the B ring and galloyl moiety of ECg and EGCg are closely located near the trimethylammonium group (γ position) of phospholipids, as determined by NOESY experiments[8]. Furthermore, direct evidence of the molecular interaction between catechins and phospholipid bilayers using liposomes has been obtained by solid-state ^{2}H NMR analyses[9,10]. Based on these studies it is proposed that ECg and EGCg interact with the surface of lipid membranes via the choline moiety of phosphatidyl choline.

Recently, we synthesized [^{13}C]-ECg, in which the carbonyl carbon of the galloyl moiety was labeled by ^{13}C isotope, and analyzed it by solid-state NMR spectroscopy. In the solid-state ^{13}C NMR analysis, the accurate intermolecular-interatomic distance between the labeled carbonyl carbon of [^{13}C]-ECg and the phosphorus of the phospholipid was determined to be 5.3 ± 0.1 Å by ^{13}C–^{31}P rotational echo double resonance (REDOR) measurements (Figure 2)[11]. This result confirms that ECg molecules are located not in the hydrophobic core of the layer but on the surface of the phospholipid layer, because the galloyl moiety is very close to the γ position in the choline moiety and to the phosphate group. These results also indicate that the galloyl moiety contributes to increasing the hydrophobicity of catechin molecules, and consequently to high affinity of the galloyl-catechins for phospholipid membranes, as well as to stabilization of catechin molecules in the phospholipid membranes.

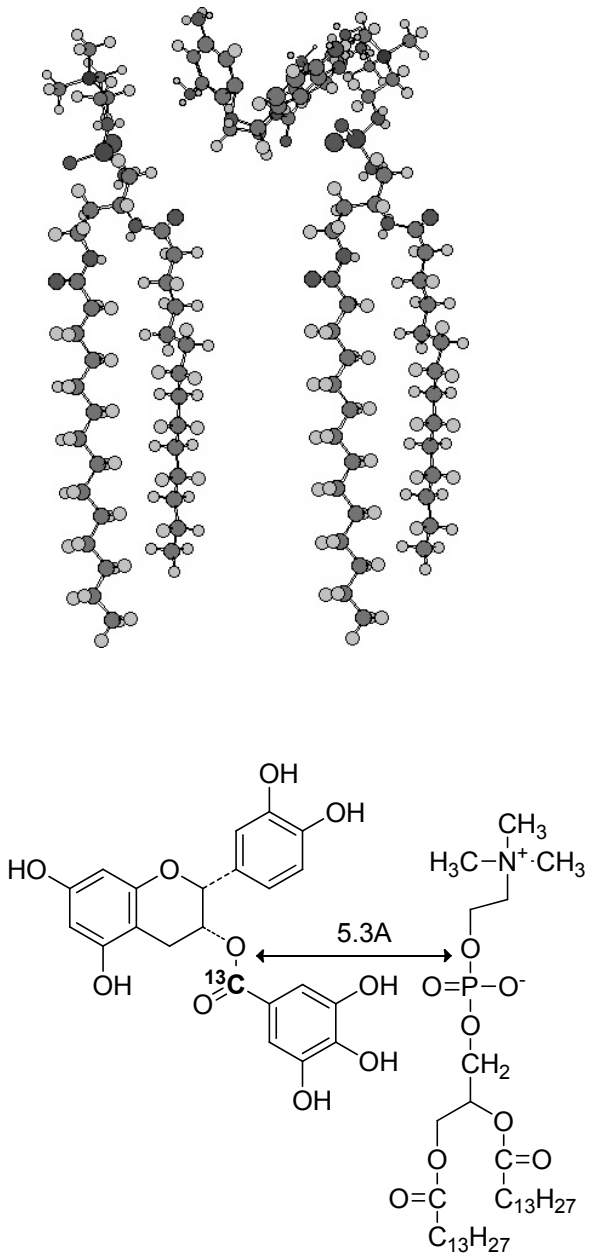

Figure 2. Interaction of ECg with phospholipids dimyristoyl phosphatidyl choline (DMPC). The upper figure shows a ball-and-stick model, and the lower figure shows the distance between the labeled carbonyl carbon of ECg and the phosphorus of the phospholipid. Data taken from Uekusa *et al.*[11]

DYNAMICS OF TEA CATECHINS IN THE PHOSPHOLIPID BILAYERS

After the contact of tea catechins with the surface of phospholipids, they supposed to move in or on the phospholipids bilayers. So far, dynamics of catechins and phospholipids during their interaction have not been fully elucidated. We measured diffusion coefficients of the catechins in aqueous solutions in the presence or absence of bicelles. Solutions of the catechins (EC, EGC, ECg, and EGCg) in D_2O (1.5 mM) and isotropic bicelles (dimyristoyl phosphatidylcholine (DMPC) : dihexanoyl phosphatidyl choline (DHPC) = 1 : 2 [mol/mol]) interacting with catechins (DMPC : catechin = 1 : 0.48, final lipid concentration of 8% w/v) were prepared. Diffusion coefficients were measured by diffusion-ordered spectroscopy (DOSY) to evaluate their translation diffusion coefficients (D) (Table 1). In the presence of the bicelles, the catechins exhibited smaller D values comparable with those of the bicelles. In particular significant reductions in D values were observed in the case of the galloyl-type catechins. These results are consistent with the previously reported affinities of the catechins for phospholipid liposomes, indicating that the galloyl-type catechins tightly bind to the bicelles. ^{31}P analysis indicated that ECg changes the gel-to-liquid-crystalline phase transition temperature of DMPC bilayers as well as the dynamics and mobility of the phospholipids[11]. It has been already reported that astringent substances such as catechins changed the membrane potentials remarkably[12]. These results suggest that the presence of the galloyl-type catechins change dynamics of the lipid bilayers, which might cause astringency.

Table 1. The diffusion coefficients (D) [10^{-10} m^2/s] of the catechins in the absence and presence of bicelles and the phospholipid (γ protons) embedded therein.

	D (catechin)		D (phospholipid)
	control	in the presence of bicelles	CH_3
EC	6.8 ± 0.2	4.2 ± 0.2	4.0 ± 0.2
EGC	6.9 ± 0.2	4.5 ± 0.5	3.9 ± 0.4
ECg	6.5 ± 0.9	1.6 ± 0.6	1.5 ± 0.6
EGCg	7.2 ± 0.6	1.8 ± 0.6	1.7 ± 0.6

BINDING AFFINITY OF TEA CATECHINS FOR PROTEINS

It has been demonstrated and reviewed[2] that polyphenols bind various proteins including salivary histidine-rich proteins, casein, gelatin, bovine serum albumin, mucins and that these proteins are involved in the sensation of astringency. Comparing tea catechins by HPLC analysis with the human serum albumin (HSA) column, we showed that the galloyl-type catechins have higher binding affinities for HSA (K_{HSA}) than the nongalloyl-type catechins[13]. We also showed that association constants (K_a) of the galloyl-type catechins toward HSA immobilized on QCM were greater than those of the nongalloyl-type catechins[14] (Table 2). These results suggest that the galloyl-type catechins nonspecifically bind to various proteins.

Table 2. K_{HSA} of the catechins by HPLC with HSA column and the association constants (*Ka*) of the catechins toward HSA immobilized on QCM. Data taken from Ishii *et al.*[12] and Minoda *et al.*[13]

	K_{HSA}	$Ka[M^{-1}]$
EC	0.6	2.1×10^3
EGC	0.8	2.8×10^3
ECg	8.6	1.4×10^5
EGCg	10.9	2.5×10^5

On the other hand, it is well known that binding of EGCG to the 67-kDa laminin receptor with a nanomolar *K (d)* value[15] and bitterness of the galloyl-type catechins are sensed through a certain taste receptor[3]. These are the examples of specific interaction of catechins to certain proteins. Further studies are necessary to clarify the role of nonspecific binding to the proteins to exert astringency of the galloyl-type catechins.

CONCLUSIONS

Affinity of the galloyl-type catechins (ECg and EGCg) for phospholipid bilayers are higher than those of the nongalloyl-type catechins (EC and EGC). The key interaction involved as a driving force for the insertion of the catechins to the phospholipid bilayers is hydrophobic interaction. We propose that when these catechins exert their biological activities, they interact with the phospholipid membranes, moving randomly on their surface and consequently bind to membrane-associated proteins, such as receptors, or pass through the membrane into the cell via channels or passive transport.

The galloyl-type catechins are more astringent than the nongalloyl-type catechins, suggesting that the interaction of the galloyl-type catechins with phospholipid bilayers could be involved to cause their astringency by changing dynamics of the lipid bilayers.

Affinity of the galloyl-type catechins for various proteins are supposedly higher than those of the nongalloyl-type catechins. Nonspecific binding of the galloyl-type catechins could be also involved to cause their astringency.

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