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Dynamic Behavior of Tea Catechins Interacting with Lipid Membranes As Determined by NMR Spectroscopy

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Interaction between tea catechins, such as epicatechin gallate (ECg) and epigallocatechin gallate (EGCg), and isotropic bicelle model lipid membranes was investigated by solution NMR techniques. ^{1}H NMR measurements provided signals from the B-ring and the galloyl moiety in ECg and EGCg that were obviously shifted, and whose proton T_1 relaxation times were shortened upon interaction of the catechins with the bicelles. These results indicate that the B-ring and the galloyl moiety play an important role in this interaction. Nuclear Overhauser effect spectrometry experiments demonstrated that the B-ring and the galloyl moiety are located near the γ-H in the phospholipid trimethylammonium group. On the basis of these findings, we propose that ECg and EGCg interact with the surface of lipid membranes via the choline moiety.

KEYWORDS: Tea catechins; lipid membrane; isotropic bicelle; solution NMR

INTRODUCTION

Tea catechins are plant polyphenols present in the leaves of *Camellia sinensis* and are present in high concentrations in green tea. The major catechins in tea leaves are (−)-epicatechin (EC), (−)-epicatechin 3-gallate (ECg), (−)-epigallocatechin (EGC), (−)-epigallocatechin 3-gallate (EGCg), and (+)-catechin. Their potential physiological activities such as antioxidant activity (*1*), antimutagenicity (*2*), anticarcinogenicity (*3*), antihypercholesterolemia (*4*), antibacterial effects (*5*), inactivation of the influenza virus (*6*), and cutaneous photoprotection from UV radiation (*7*) have received much attention. The various analogues have substantially different activities. In general, ECg and EGCg, which are gallic acid esters of catechins, show higher activities than EC and EGC, lacking the galloyl moiety (**Figure 1**). It has been speculated that the observed different biological activities of catechins towards cells and microorganisms are due to their affinities for lipid membranes. Indeed, published reports indicate that the affinity of catechins possessing the galloyl moiety is higher than those without the galloyl moiety and that *cis*-catechins have higher affinity for lipid bilayers than the corresponding *trans*-catechins (*8*). The amount of each type of tea catechin incorporated into liposomes parallels their partition coefficients in an *n*-octanol/phosphate buffer solution (PBS) system. Thus, it can be inferred that the affinity of tea catechins for lipid bilayers is dependent on their lipophilicity. The lipophilicity of catechins is governed by factors such as the presence of the galloyl moiety, the compound's stereochemical structure, and the number of hydroxyl groups on the B ring (*8*). In addition, the stability of these catechins depends on the number of hydroxyl groups on the B ring: for example, EGC and EGCg, with three hydroxyl groups on the B ring, tend to be oxidized easily compared to EC and ECg, which have two hydroxyl groups on the B ring (*9*). Recently, it was reported that EGCg binds tightly to the 67 kDa laminin receptor located on the surface of membranes (*10*), suggesting that tea catechins bind to specific proteins in the membrane. We propose that catechins interact with the lipid membrane, move freely on the surface of the lipid bilayers, and then bind to receptors and proteins.

Bicelles are frequently used as models of lipid bilayers and are composed of a mixture of long-chain phospholipids (e.g., 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, DMPC) and short-chain phospholipids (e.g., 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine, DHPC) or detergents (**Figure 2**) (*11–13*). The long-chain phospholipids form a planar bilayer and are surrounded at the rims by short-chain phospholipids that shield the long-chain lipid tails from water. The molar ratio of DMPC to DHPC, expressed as *q*, determines the size and shape of bicelles. When *q* is larger than 2.5 at a given total phospholipid concentration, the bicelles can be aligned by a magnetic field and, thus, are often studied by solid-state NMR to reveal the structures of membranes associated with proteins, peptides, and other molecules (*14–16*). Dilute bicellar suspensions have been used to achieve a small degree of orientation of nonmembrane-associated biomolecules for the purpose of obtaining residual dipolar couplings used in the refinement of high-resolution NMR structures (*17–19*). On the other hand, bicelles with a small *q* value (<1), which do not align in magnetic fields, are suitable for high-resolution NMR studies to determine the structures and

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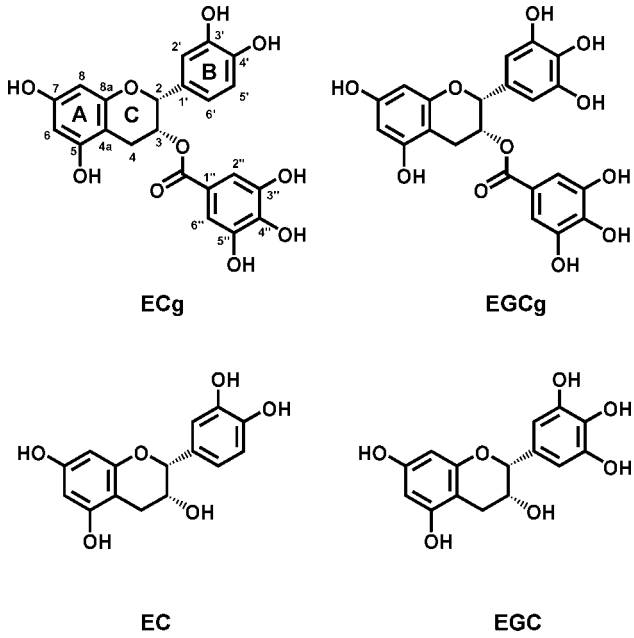

Figure 1. Structures of tea catechins: (−)-epicatechin gallate (ECg), (−)-epigallocatechin gallate (EGCg), (−)-epicatechin (EC), and (−)-epigallocatechin (EGC).

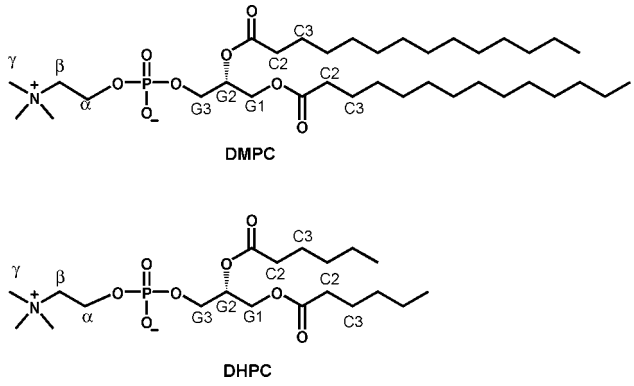

Figure 2. Structures of phospholipids: DMPC (long chains) and DHPC (short chains).

dynamics of substances which interact and form complexes with membranes (*20*, *21*).

Spectroscopic studies indicate that catechins interact with lipid membranes. Solid-state ^{31}P and ^{2}H NMR experiments with DMPC liposomes showed not only that EGCg interacts with lipid bilayers and affects the head-group motions of the phospholipids but also that EGCg has at least one conformation in the lipid bilayer that exhibits rotational motion (*14*). Furthermore, fluorescence quenching experiments with 2-(9-anthroyloxy)stearic acid (2-AS) and 12-(9-anthroyloxy)stearic acid (12-AS) in the liposomes showed that the catechins are located on the surface of the lipid bilayers because the fluorescence emission of 2-AS was quenched whereas that of 12-AS was not (*8*). This conclusion is also consistent with spin probe experiments showing that (+)-catechins interact with lipid membranes and affect their structure (*22*). The conformations of catechins complexed with caffeine (*23*), cyclodextrin (*24*), and proline-rich proteins (*25*) were determined by NMR methods such as nuclear Overhauser effect spectrometry (NOESY). However, detailed structural information regarding the configurations of catechins in lipid membranes has not been available. Through the use of solution NMR techniques and isotropic bicelles ($q = 0.5$), the present study provides molecular-level insights into how catechins interact with lipid bilayers.

MATERIALS AND METHODS

Materials. (−)-EC, (−)-EGC, (−)-ECg, and (−)-EGCg were kindly provided by Mitsui Norin Co. Ltd. (Shizuoka, Japan). The phospholipids, DMPC and DHPC, were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL), and used without further purification. Tris(hydroxymethyl-d_3)amino-d_2-methane (Tris-d_{11}) was obtained from Sigma-Aldrich, Inc. (St. Louis, MO). Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Deuterium oxide (D_2O), tetramethylsilane, and all other chemicals were obtained from Kanto Chemicals Co. (Tokyo, Japan).

Tea Catechin Solutions for ^{1}H NMR Measurements. Aqueous solutions of four tea catechins (EC, EGC, ECg, and EGCg) were prepared in D_2O (1.5 mM). Since trace amounts of insoluble components were present in the ECg sample, this sample was centrifuged and the supernatant was used for experiments. ECg solutions in D_2O (1.5 mM) were prepared at pH 7.0; three additional solutions at pH 2.4, 8.0, and 10.0 were also prepared by adding DCl or NaOD. pH values were determined with a glass electrode calibrated with standard aqueous buffer solutions; conversion of these pH values into the corresponding pD values was not considered in this study (*26*).

Isotropic Bicelle Preparation in the Presence of a Tea Catechin. DMPC dissolved in chloroform and tea catechins (ECg, EGCg, EC, or EGC) dissolved in methanol were combined in a round-bottomed flask at a molar ratio of tea catechins to DMPC of 0.48:1. ECg and EGCg were also prepared at a molar ratio of 0.24:1. DHPC dissolved in chloroform was then added to the flask (DMPC/DHPC = 1:2 [mol/mol], $q = 0.5$). The bicelles and the tea catechin in chloroform/methanol were stirred thoroughly with a vortex mixer; the solvent was evaporated using a rotary evaporator; then, the sample was placed on a vacuum pump for several hours in order to remove all residual solvent. Afterwards, D_2O was added to the flask to provide a final lipid concentration of 8% (w/v); then, the aqueous lipid suspension was vortexed. After stabilizing the sample for 1 h without agitation, ^{1}H NMR spectra of each suspension were acquired. ECg in the presence of the bicelle suspension was prepared also in a D_2O solution (pH 5.8) and in Tris-DCl buffers (20 mM Tris-d_{11}, pH 7.0) containing 50, 100, or 150 mM NaCl.

NMR Experiments. ^{1}H NMR spectra were recorded on a JEOL JNM-α400 NMR spectrometer operating at a resonance frequency of 399.7 MHz with 12 Hz spinning at 310 K. Typically, an excitation pulse length of 6.5 μs and a pulse delay time of 2.0 s were used. Differential homonuclear Hartmann–Hahn (HOHAHA) selective excitation spectra were acquired using the MLEV17 sequence with a spin locking power of 12.5 kHz preceded by a 2.5 ms trim pulse; mixing times were 100 and 120 ms. Correlation spectroscopy (COSY) spectra were acquired with typical acquisition parameters: 90° pulses of 11.8 μs, a pulse delay time of 2.0 s, 32 scans, and 256 and 512 data points were acquired in the f_1 and f_2 axes, respectively. The sine-bell function was adopted as a window function. Chemical shift values of the samples were referenced with respect to DSS at 0 ppm, used as an internal standard. Alice2 (JEOL) and Ramo 1D (*27*) were used as the analytical software.

Proton T_1 Relaxation Times of Free Tea Catechins in the Presence or Absence of Bicelles. ECg and EGCg (1.5 mM) in D_2O in the presence or absence of bicelles were used for T_1 measurements without removing dissolved O_2. T_1 values were determined by the inversion–recovery method (the 180°–τ–90° pulse sequence technique) at 310 K. Typical acquisition parameters used were as follows: 90° pulse length of 11.85 μs, 64 scans, and a pulse delay time of 40 s ($>5T_1$). The pulse delay time was determined by estimating the T_1 value by

$$T_1 = t_{null}/\ln 2 \quad (1)$$

where t_{null} is the time producing no signal intensity by the inversion–recovery method. The τ value representing a pulse interval between

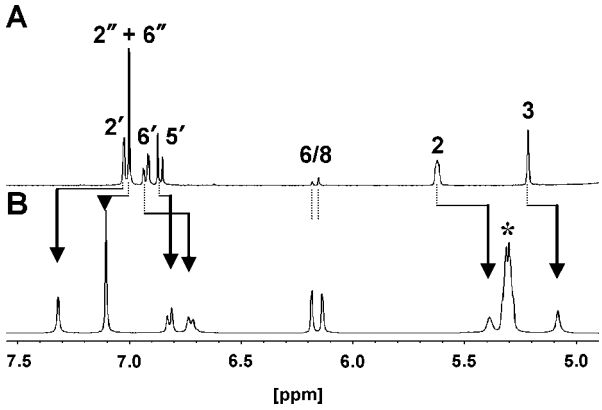

Figure 3. Lowfield region of ^{1}H NMR spectra of ECg in the absence (**A**) and the presence (**B**) of bicelles in D_2O at 310 K. Some signals of ECg were shifted by the interaction with the bicelles, shown by arrows. The asterisk (∗) mark is the signal from bicelles (G2).

the 180° and 90° pulse was varied from 50 ms to 40 s by 16 points in total. Signal intensities were plotted and fitted by eq 2 using KaleidaGraph (Synergy Software) to calculate T_1 values by least-squares regression

$$M_z(t) = M_0[1 - 2\exp(-\tau/T_1)] \quad (2)$$

where $M_z(t)$ is the z component of the magnetization vector at time (t) and M_0 is the thermal equilibrium of the z component of the magnetization vector.

NOESY Experiments on Bicelle/Tea Catechin Samples. NOESY spectra were acquired at 310 K. Typical acquisition parameters used were as follows: 90° pulse length of 12.1 μs; pulse delay time of 3.0 s; mixing times of 50, 200, and 400 ms; 64 scans; and 256 and 512 data points acquired in the f_1 and f_2 axes, respectively. For the ECg solution without bicelles, NOESY spectra were measured with mixing times of 200, 600, and 1000 ms and a pulse delay time of 6.0 s. The sine-bell function was adopted as a window function.

RESULTS

^{1}H NMR Measurements of Tea Catechins in the Absence and Presence of Isotropic Bicelles. ^{1}H NMR chemical shifts of the tea catechins ECg, EGCg, EC, and EGC in the presence of bicelles were obtained in D_2O at 310 K. Signal assignments were made on the basis of the results of 1D-HOHAHA and COSY spectral measurements and were consistent with previously reported results (*28*). No proton signals from hydroxyl groups were observed due to H–D exchange with D_2O.

Figure 3 shows ^{1}H NMR spectra for ECg in the absence (**A**) and the presence (**B**) of bicelles in D_2O at 310 K. Interaction with the bicelles resulted in a considerable shift of the ECg B-ring (2′-, 5′-, and 6′-H) and the galloyl moiety (2″- and 6″-H) signals. The C-ring (2- and 3-H) protons also showed substantial upfield shifts. In contrast, the chemical shifts of the A ring (6-/8-H) changed 0.01–0.03 ppm, and although their intensities diminished in aqueous solutions, the intensities did not diminish in the presence of bicelles. 4α- and 4β-H signals were not observed because they overlapped large signals from the lipid alkyl chains of the bicelles. In general, the line widths of all the signals broadened in the presence of the bicelles. ^{1}H NMR spectra of the other tea catechins (EC, EGC, and EGCg) with bicelles were measured in a similar manner. The results for EGCg were similar to those of ECg; that is, signals from the B and C rings and the galloyl moiety shifted over 0.05 ppm, whereas signals from the A ring shifted 0.03 ppm (**Figure 4A**). When EC interacted with the bicelles, chemical shift changes were observed for the B-ring protons and 2-H on the C ring,

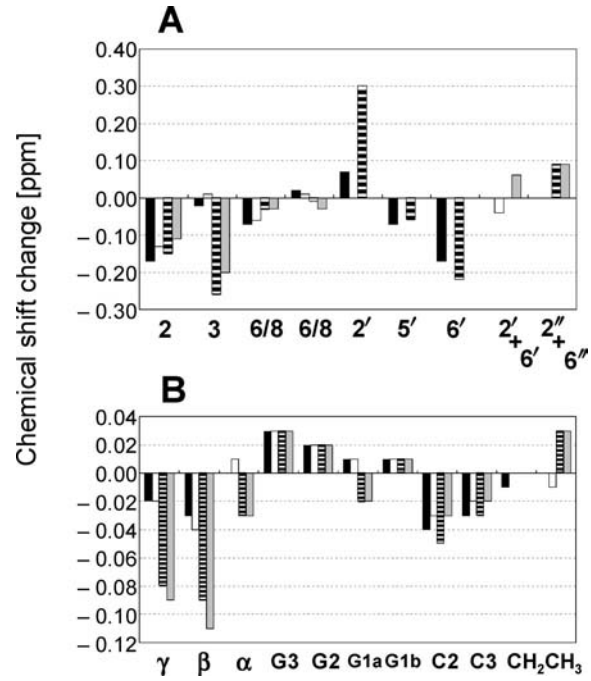

Figure 4. Comparision of chemical shift change values: EC (solid), EGC (open), ECg (hatched), and EGCg (gray). The changes of catechin signals in the presence of bicelles are compared to the free state (**A**). 4α- and 4β-H signals were not observed in the presence of bicelles because they were overlapped with the large signals of lipid alkyl chains. The changes of the bicelles' (DMPC and DHPC) signals in the presence of catechins are compared to those in the absence of catechins (**B**).

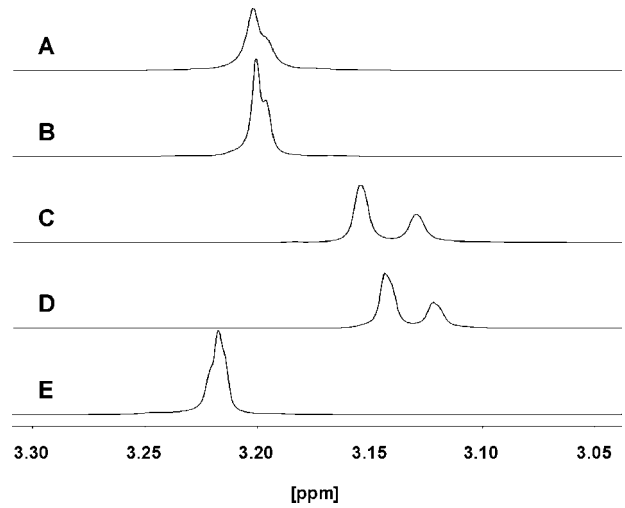

Figure 5. Comparison of ^{1}H NMR spectra magnifying the γ-H signals of the phospholipids in D_2O at 310 K: bicelles with EC (**A**), EGC (**B**), ECg (**C**), EGCg (**D**), and free bicelles in the absence of the tea catehins (**E**).

although the 2′-H shift was much smaller than in ECg. Only 0.01–0.06 ppm shifts for EGC in the presence of bicelles were observed, except for 2-H of the C ring (0.13 ppm).

The changes of chemical shifts of phospholipids' protons were observed when the bicelles (DMPC and DHPC) interacted with catechins. Following interaction with ECg or EGCg, the β- and γ-H signals showed significant changes; chemical shifts of the G1-G3-, C2- and C3-H signals also changed (**Figure 4B**), and alkyl chain protons of the phospholipids exhibited small changes in chemical shift. Interaction with EC and EGC resulted in smaller chemical shift changes of the β- and γ-H signals compared to that observed with ECg and EGCg. Following the

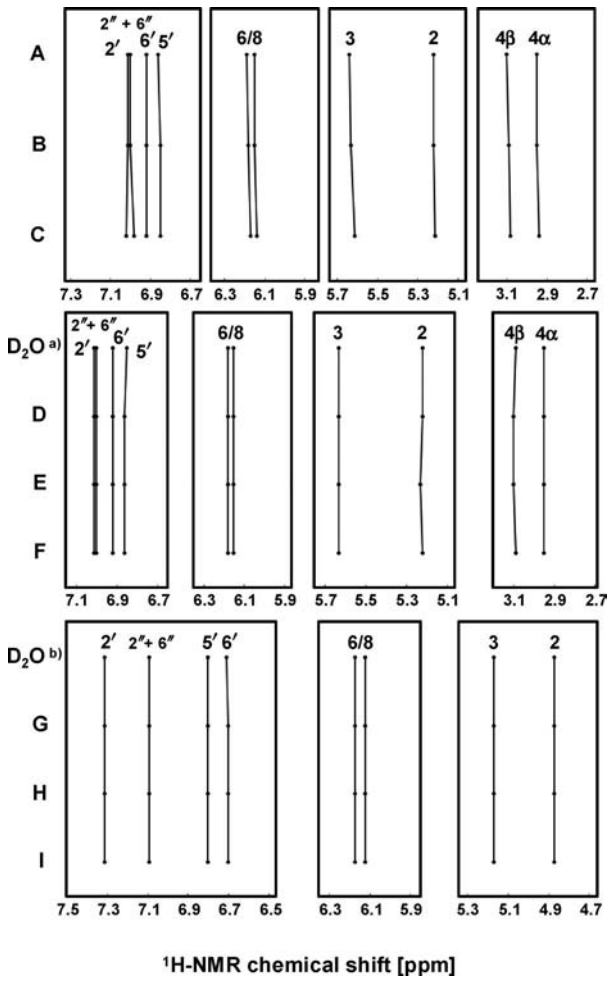

Figure 6. Chemical shifts of the ECg signals under various pH's and salt concentrations. ECg (free) dissolved in D_2O at pH 2.4 (**A**), 7.0 (**B**), and 8.0 (**C**) and in Tris-DCl (20 mM Tris-d_{11}, pH 7.0) buffer containing NaCl at 50 mM (**D**), 100 mM (**E**), and 150 mM (**F**). ECg with bicelles dissolved in Tris-DCl buffer containing NaCl at 50 mM (**G**), 100 mM (**H**), and 150 mM (**I**). Superscripts a and b refer to pH 7.0 and pH 5.8, respectively.

addition of ECg or EGCg, the γ-H signals (from the trimethylammonium group) of the phospholipids split into a doublet (**Figure 5C**,**D**). The ratio of the integratated value of the split signals was approximately 2:1, while the signal in the absence of tea catechins was a broad singlet (**Figure 5E**). In the case of EC and EGC, small changes in the chemical shifts were observed, but there was no obvious signal splitting (**Figure 5A**,**B**).

Figure 6 shows the effects of pH and ionic strength on ECg signal chemical shifts in the presence and absence of bicelles. ECg solutions in D_2O at pH 5.8 and 7.0 were prepared in the presence or absence of bicelles. This pH variation (**Figure 6A**–**C**) caused all ECg signals to shift slightly in the same direction (<0.03 ppm). In contrast, at pH 10.0, the color of the sample solution gradually turned red during the NMR experiment, and no signals were observed (data not shown). The observed color change could be due to the degradation or polymerization of ECg at basic pH (*29*). NaCl concentrations of 50, 100, or 150 mM in a Tris-DCl (20 mM Tris-d_{11}, pH 7.0) buffer had little effect on ECg chemical shift values in the absence (**Figure 6D**–**F**) and presence (**Figure 6G**–**I**) of bicelles over a period of 5 h. These results indicate that the chemical shift changes of tea catechins in the presence of bicelles, shown in **Figures 3** and **4**, cannot be ascribed to changes in pH or ionic strength in the local environment of the catechins interacting with the bicelles.

Table 1. T_1 Relaxation Times (Seconds) of the Protons in ECg and EGCg Solutions in the Presence and Absence of Bicelles Determined by the Inversion Recovery Method[a]

	ECg		EGCg	
	free	ECg + bicelles	free	EGCg + bicelles
2	0.78 ± 0.16	0.83 ± 0.06	0.89 ± 0.04	0.80 ± 0.04
3	0.70 ± 0.11	0.77 ± 0.08	0.74 ± 0.01	0.75 ± 0.02
4α	0.32 ± 0.02	overlapped[b]	0.36 ± 0.04	overlapped[b]
4β	0.23 ± 0.03	overlapped[b]	0.27 ± 0.00	overlapped[b]
6/8	3.37 ± 1.36[c]	0.99 ± 0.06[c]	2.11 ± 1.18[c]	0.82 ± 0.03[c]
	3.21 ± 1.23[c]	1.09 ± 0.25[c]	1.70 ± 0.47[c]	0.84 ± 0.07[c]
2′	1.83 ± 0.67	1.01 ± 0.11	2.03 ± 0.17	0.84 ± 0.04
5′	1.57 ± 0.46	0.88 ± 0.05		
6′	1.12 ± 0.18	0.91 ± 0.10	2.03 ± 0.17	0.84 ± 0.04
2″, 6″	3.38 ± 0.88	1.10 ± 0.15	5.31 ± 0.92	0.87 ± 0.06

[a] Values are the average with standard deviations of three experimental data. [b] Signals were not assigned because of the large signal of lipid alkyl chains. [c] 6- and 8-H signals were not identified.

Proton T_1 Relaxation Times of Tea Catechins in the Presence and Absence of Bicelles. Table 1 summarizes the proton T_1 relaxation times, obtained by the inversion–recovery method, of ECg and EGCg solutions in the presence and absence of bicelles. In the absence of bicelles, the T_1 values differed considerably in the order: T_1 [galloyl moiety] > T_1 [B ring] > T_1 [A ring] > T_1 [C ring]. T_1 values of the EGCg protons were similar to those of ECg except that the galloyl moiety protons exhibited longer T_1 values. Due to possible tautomerism (*24*, *30*), T_1 values for 6-/8-H bound to the A ring were not obtained accurately. Interaction of ECg and EGCg with the bicelles shortened the T_1 relaxation times of the galloyl moiety and B-ring protons. The 6-/8-H signals of ECg/EGCg were observed when the compounds interacted with the bicelles, and their T_1 values were similar to those of other ECg/EGCg protons.

NOESY Experiments. NOESY experiments were used to investigate the conformations of tea catechins (ECg, EGCg, EC, and EGC) and to confirm their interactions with phospholipids. NOESY experiments are suitable for estimating both intramolecular and intermolecular proton distances up to 5 Å (*31*). NOESY spectra were first measured with various mixing times (50, 200, and 400 ms, data not shown). By increasing the mixing time, many long-range NOEs were observed. We found that a mixing time of 200 ms was optimal for observing the cross peaks in the bicelle/catechin samples. **Figure 7** shows the NOESY spectra of ECg and EGCg interacting with bicelles in D_2O at 310 K. In the ECg/bicelle sample (**Figure 7A**), intermolecular cross peaks were observed between the γ-H of the phospholipids and 2″- and 6″-H on the galloyl moiety of ECg (medium), 2′-H on the B ring (weak), and 6-/8-H (weak). Several ECg intramolecular cross peaks were observed between 2′-H and 2-H (weak), 2′-H and 3-H (weak), 2′-H and 2″- and 6″-H (strong), 6′-H and 2-H (weak), and 6′-H and 3-H (weak). EGCg–bicelle interactions (**Figure 7B**) produced inter- and intramolecular cross peaks similar to those observed with ECg. On the other hand, obvious correlations between the EC/EGC signals and the bicelles were not observed in the NOESY spectra, although intramolecular cross peaks were observed.

DISCUSSION

The results obtained from our ^{1}H NMR measurements indicate that ECg and EGCg interact with isotropic bicelles,

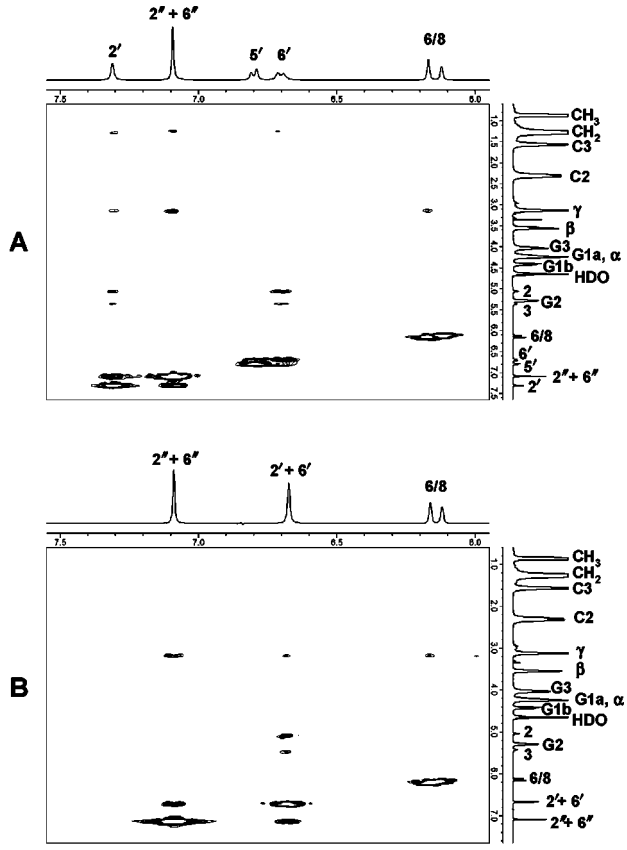

Figure 7. NOESY spectra of ECg (**A**) and EGCg (**B**) that have interacted with bicelles, with a 200 ms mixing time in D_2O at 310 K.

and that this interaction affects the chemical shifts of these catechins (**Figure 4**). In particular, the chemical shifts of 2-H, 3-H, the B ring protons, and the protons of the galloyl moiety changed considerably. These changes in chemical shift are independent of pH and ionic strength (**Figure 6**). The present results also show that bicelle protons experience altered chemical shifts upon interaction of the bicelle with ECg and EGCg (**Figure 4**). The addition of ECg or EGCg changes the chemical shifts of the bicelle γ- and β-H and splits the γ-H signal into the two signals (**Figure 5**). Furthermore, the T_1 values of the B-ring protons and the galloyl protons are substantially shortened in the presence of bicelles (**Table 1**). These results indicate that the galloyl moiety of ECg/EGCg, located close to the B ring, is also located near the γ-H of the phospholipids. Intermolecular NOEs observed between γ-H of the phospholipids and 2″-and 6″-H on the galloyl moiety of ECg/EGCg confirmed the interaction of the galloyl moiety with the trimethylammonium group (**Figure 7**). As a result, the chemical shift changes of 2′-, 5′-, and 6′-H and 2″- and 6″-H of ECg would be caused by the interaction with the trimethylammonium group of lipids. Also, the changes in chemical shift might be brought about conformational changes in ECg and EGCg since strong correlations between 2′-H (2′- and 6′-H) and 2″- and 6″-H, in addition to some weak intermolecular correlations, were observed, although using the ECg solution in the absence of bicelles resulted in significant intramolecular cross peaks not being obtained for those protons due to close chemical shifts (data not shown). Judging from the proton chemical shifts (see the Supporting Information), it would be expected that the conformation of ECg interacting with bicelles in water would be different from its conformation in a methanol-d_4 solution (data not shown). Because of possible rotational motion of the EGCg B ring, its 2′- and 6′-H did not provide distinct signals, but they did provide strong correlations with 2″- and 6″-H. Although we assume that the B ring and the galloyl ring of ECg also could be rotating or flipping about the C–C bond axis, ECg in the presence of bicelles resulted in the NOE between 2″- and 6″-H being observed with 2′-H, but not between 5′- and 6′-H on the B ring with 2′-H. This means that the lifetime of 2′-H located within 5 Å of 2″- and 6″-H of the galloyl moiety might be longer than that of 5′- and 6′-H, and it suggests that the B ring and the galloyl moiety are closely located in the presence of bicelles. The chemical shifts of 2- and 3-H of ECg/EGCg also changed substantially, plausibly due to not only the interaction with the bicelles but also the possible structural changes in the B ring and the galloyl moiety resulting from their interaction. Although changes in the chemical shifts of signals from EC and EGC were also observed, clear intermolecular NOEs were not seen in this experimental condition, suggesting that, although EC and EGC might interact with the bicelles, their lifetimes are much shorter than those of ECg and EGCg. A model of plausible interaction of ECg with lipid bilayers is drawn in **Figure 8**. From the above-mentioned observation, ECg and EGCg interact with the surface of lipid membranes via the choline moiety. The galloyl rings would be closely located to the trimethylammonium group of the phospholipids, and the B ring situated nearby the galloyl group and the A ring could be also within 5 Å from it (**Figure 8**). A previous study with liposome systems showed that catechins possessing the galloyl moiety have a higher affinity for lipid bilayers than those lacking this moiety (*32*). **Figure 4B** clearly shows that the magnitude of the β- and γ-H chemical shift changes is related to the amount of catechin incorporated into the liposome.

Another possible cause of the change in chemical shift could be a ring current effect, which would determine whether the shift is upfield or downfield. Protons on the catechins, except for the four C-ring protons, are shifted downfield, as are those on the aromatic rings. Interaction between ECg and lipid molecules caused the 2′-H of the B ring to be located closer to the galloyl moiety, as demonstrated by NOESY experiments. The ^{1}H NMR spectrum of ECg in the presence of bicelles (**Figure 3**) shows 2′-H and 2″- and 6″-H signals shifted downfield compared with chemical shifts observed in the absence of bicelles. It is presumed that 2′-H is deshielded by being located close to the plane of the ring of the galloyl moiety. On the other hand, 5′- and 6′-H are shifted upfield, suggesting that the protons are shielded by being located above the plane of the ring of galloyl moiety. On the basis of these results, we propose that the plane of the B ring may be located perpendicular to the galloyl moiety and that 2′-H is near the galloyl moiety. Although we assume that the B ring and the galloyl moiety are rotating, the lifetime at the above-mentioned conformation might be longer than the others. Furthermore, the lipid α-, β-, and γ-H were shifted upfield (**Figure 4B**), suggesting that the plane of the galloyl moiety of ECg/EGCg may be inserted parallel to the membrane normal, resulting in the γ-H of the phospholipids being shielded by the ring current.

The addition of ECg and EGCg resulted in the γ-H signals of the bicelles being split into a doublet whose integrated values were approximately 2:1. The molar ratio of DMPC and DHPC used in this study was 2:1, so it can be assumed that the two γ-H signals split by the interaction arose from DMPC and DHPC, respectively. Since the DHPC signal was also shifted upfield, the catechins may be transiently located on the edge of the bicelles.

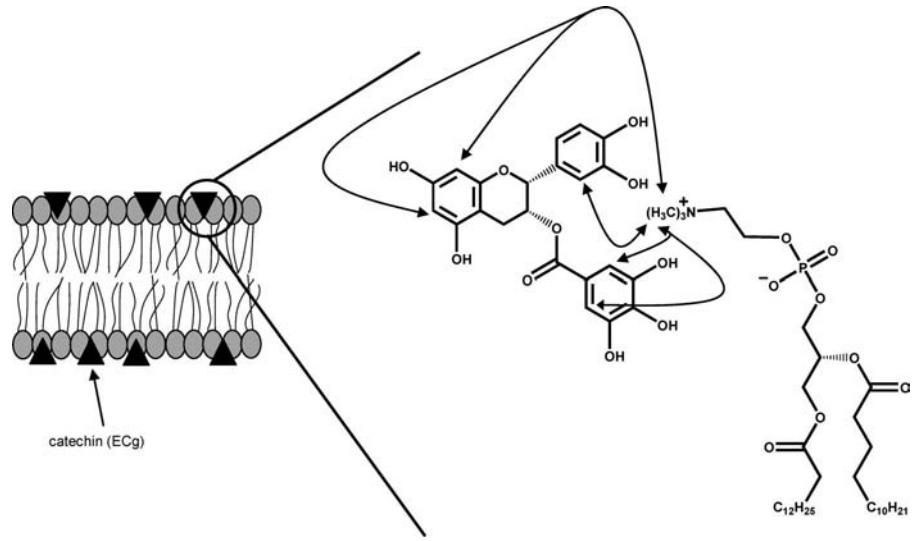

Figure 8. Schematic representation of the possible interaction between ECg and bicelles. ECg is shown with a solid triangle. The intermolecular NOEs observed in NOESY experiments with a 200 ms mixing time are shown by arrows. ECg interacts with the surface of lipid membranes via the choline moiety.

Flavonoids such as flavone, chrysin, luteolin, myricetin, and luteolin-7-glucoside show a broad distribution in POPC model membranes with an increasing number of hydroxyl groups (*33*). Although catechins are also clarified as flavonoids, they are unique polyphenols which dissolve in water in tea extracts. Our results indicate that catechins interact with the lipid headgroups of bicelles, supporting previous fluorescence experiments using 2-AS and 12-AS (*8*), and ^{31}P solid-state NMR studies (*14*).

The signals of the A-ring protons (6-/8-H) shifted 0.01–0.03 ppm, although their intensity clearly increased upon interaction with the bicelles. In the absence of bicelles, the signal intensities of the two A-ring protons of catechins in D_2O gradually became smaller with time, possibly arising from deuteriation due to tautomerism (*24*, *30*). However, when catechins interacted with bicelles in D_2O, the intensity of 6-/8-H was unchanged from the original integral values. These results, including the observation of the cross peaks between 6-/8-H and γ-H of bicelles, suggest that the A ring is located in the hydrophilic region of the lipid bilayer, where the contact with the aqueous solvent is relatively limited compared with ECg solution, without bicelles. Similar results were observed in an acidic solution (pH 2.4), where the intensity of the A-ring proton in ECg in the absence of bicelles decreased rapidly with time, although the same signals in the presence of bicelles retained their original intensities.

Small molecules such as catechins are believed to tumble rapidly in solution. However, specific ECg moieties showed distinct T_1 relaxation times in D_2O. In particular, the T_1 relaxation times of the galloyl moiety were longer than those of other protons (**Table 1**), suggesting that the galloyl moiety could exhibit motion relative to the ring axis in addition to molecular tumbling. Interaction of ECg and EGCg with bicelles resulted in a broadening of all signals from the catechins, and fine structures such as coupling were not visible. This line broadening could be attributed to restricted molecular motion of tea catechins in the bicelles.

It is important to understand the conformational preferences of catechins and their intra- and intermolecular interactions in lipid membrane. The results of this study represent a step towards elucidating in detail the relationship between the chemical structure and the biological activity of catechins and suggest requirements for the active form. A further work is ongoing to examine the structure of ECg/EGCg under the interaction of lipid bilayers. The techniques used in the present study with isotropic bicelles will be useful for characterizing the interactions between lipid membranes and other plant polyphenols, such as quercetin and daidzein.

ABBREVIATIONS USED

ECg, epicatechin gallate; EGCg, epigallocatechin gallate; EC, epicatechin; EGC, epigallocatechin; NOE, nuclear Overhauser effect; 2-AS, 2-(9-anthroyloxy)stearic acid; 12-AS, 12-(9-anthroyloxy)stearic acid; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser effect spectroscopy; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DHPC, 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PBS, phosphate buffered saline.

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Supporting Information Available: Proton chemical shift data, δ [ppm] (*J*, Hz), for tea catechins (ECg, EGCg, EC and EGC) in the presence and absence of bicelles (Table S1), and for bicelles in the presence and absence of the catechins (Table S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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