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β-Cyclodextrin/Surface Plasmon Resonance Detection System for Sensing Bitter-Astringent Taste Intensity of Green Tea Catechins

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To develop a methodology for creating a sensor with a receptor for specific taste substances, we focused on constructing a sensing system for the bitter-astringent taste intensity of green tea catechins: (−)-epigallocatechin-3-*O*-gallate (EGCg), (−)-epicatechin-3-*O*-gallate (ECg), (−)-epigallocatechin (EGC), and (−)-epicatechin (EC). ^{1}H NMR titration experiments revealed that β-cyclodextrin was an adequate receptor for sensing the bitter-astringent taste intensity of catechins. A surface plasmon resonance (SPR) system immobilized β-cyclodextrin indicated larger responses for the gallate-type catechins in comparison to the non-gallate-type catechins. These responses corresponded to the tendency of the bitter-astringent taste intensity of the catechins felt by humans. Furthermore, the SPR system detected the larger stability of the complex between the gallate-type catechins and β-cyclodextrin, which was interpreted as the aftertaste produced in humans by the gallate-type catechins. These results demonstrate that the β-cyclodextrin/SPR system can sense the bitter-astringent taste intensity of the green tea catechins similar to human gustation. The methodology presented in this study can be used as a basic strategy for developing taste sensors with specific receptor functions.

KEYWORDS: Cyclodextrin; surface plasmon resonance; bitter-astringent taste; catechins

INTRODUCTION

The taste of food is an important factor in determining its quality. Therefore, the evaluation of food tastes is essential for inspection and control of food quality, grading of food and agricultural products, and analysis of market trends. Although the taste of food is usually evaluated by a sensory test, much effort (e.g., panelist training and use of reference substances) is required to obtain reliable results.

As a solution to this problem, a taste sensor system developed by Toko et al. (*1*, *2*) has revolutionized the taste evaluation of foods (*3*−*9*) and medicines (*10*−*17*). This technology has introduced an objective scale into taste intensity. The taste sensor system detects taste information by sensor electrodes with lipid/polymer membranes prepared for each primary taste. The taste intensities are recorded as membrane potential changes caused by the interaction between the lipid/polymer membranes and the taste substances.

Although actual taste cells also detect tastes through the process of a membrane potential change, the generation pathways of the action potential are diverse. The initiation mechanisms are presumed to be the following (*18*, *19*). In bitterness, sweetness, and umami tastes, taste substances interact with the corresponding seven-transmembrane receptors linked to the G protein, gustducin. In saltiness and sourness, the cations act on membrane ion channels or directly pass through the channels into the taste cell. Therefore, if taste sensors equipped with receptor functions to recognize specific taste substances are developed and used in conjunction with existing lipid/polymer membrane-type taste sensors, more precise taste sensing will be realized.

In the development of such a taste sensor, it is essential to find a receptor molecule that can correlate the chemical structures of taste substances with their taste properties (e.g., primary tastes) and intensities. Furthermore, it is necessary to solve how to detect the interaction between the receptor and the taste substances. However, the detection mechanism does not necessarily have to be electrical as in a taste cell. In the present study, to develop a methodology to solve these problems, we focused on detecting the bitter-astringent taste of green tea catechins. Bitterness and astringency coexist in the taste of the catechins; thus, the taste is often evaluated as a bitter-astringent taste (*20*). In the following sections, the search for an appropriate receptor molecule in the cyclodextrin family, some of which form complexes with catechins (*21*−*23*) and sensing of the taste intensity by a surface plasmon resonance (SPR) system immobilized β-cyclodextrin are discussed.

MATERIALS AND METHODS

Materials and Apparatuses. All chemicals were obtained from commercial suppliers: cyclodextrins (>97%, Wako Pure Chemical Industries Ltd., Osaka, Japan); EGCg **1**, ECg **2**, EGC **3**, and EC **4** (Wako Pure

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Chemical Industries Ltd.); deuterium oxide and tetramethylsilane (Cambridge Isotope Laboratories, Andover, MA); carbon tetrachloride (Wako Pure Chemical Industries Ltd.); sodium dihydrogenphosphate (NaH_2PO_4) and disodium hydrogenphosphate (Na_2HPO_4) (Wako Pure Chemical Industries Ltd.); 6-monodeoxy-6-monoamino-β-cyclodextrin **5** (Sigma-Aldrich, St. Louis, MO); and all reagents except for **5** for SPR experiments (Biacore AB, Uppsala, Sweden). Cyclodextrins were dried at 90 °C for 24 h under reduced pressure before use. Proton nuclear magnetic resonance (NMR) spectra were recorded on a 500 MHz spectrometer. SPR signals were detected by Biacore X100 with sensor chip CM5 (Biacore, research grade).

^{1}H NMR Titration Experiments. The ^{1}H NMR chemical shift changes in the protons of the catechins (**1**, **2**, **3**, and **4**) by the addition of the cyclodextrins (α-, β-, and γ-cyclodextrins) were examined according to the following procedure. Stock solutions of the catechins (5.50 mM) and the cyclodextrins (6.11 mM) in deuterium oxide buffered at pD 7.0 with 0.1 M NaH_2PO_4/Na_2HPO_4 were prepared separately (*24*). For each combination between the catechins and the cyclodextrins, 13 NMR tubes were filled separately with different concentrations of the cyclodextrin at 0.000, 0.125, 0.250, 0.500, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, and 5.00 mM against a constant concentration of the catechin (1.00 mM) in a 0.550 mL total solution for each. ^{1}H NMR spectra were obtained for each tube at 300 K. Chemical shifts in ^{1}H NMR spectra are reported on the parts per million scale and are referenced to tetramethylsilane (δ 0.0000 ppm) in carbon tetrachloride as an external standard, which was inserted into an NMR tube (ϕ = 5 mm) with a coaxial cell. The digital resolution of the ^{1}H NMR spectra was 0.31 Hz.

NOESY Experiments. NOESY spectra for the deuterium oxide solution of each catechin (2.0 mM) using pulsed field gradients were acquired at 300 K under the following conditions: relaxation delay, 1.0 s; mixing time, 500 ms; 128 scans; 256 × 256 data points. The data were processed with a sine-bell window function.

Immobilization of 6-Monodeoxy-6-monoamino-β-cyclodextrin 5 onto a Sensor Tip. At 25 °C, solutions were flowed at 10 μL/min into a flow cell attached to a sensor tip according to the following procedure to immobilize 6-monodeoxy-6-monoamino-β-cyclodextrin **5** onto dextran with carboxyl groups on the tip: (1) aqueous solution including 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (0.01 M), sodium chloride (0.15 M), 2,2′,2″,2‴-(ethane-1,2-diyldinitrilo)tetraacetic acid (EDTA) (3.0 mM), and 0.05% v/v Surfactant P20 (solution A) for 260 s; (2) aqueous solution including 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (0.39 M) and *N*-hydroxysuccinimide (NHS) (0.10 M) for 7 min; (3) solution A for 20 s; (4) solution A including **5** (5 mM) for 9 min; (5) solution A for 80 s; (6) 1.0 M aqueous solution of ethanolamine hydrochloride for 7 min; (7) solution A for 60 s.

Detection of Complexation between β-Cyclodextrin and the Catechins by the SPR System. At 25 °C, catechin solutions were flowed at 10 μL/min into a flow cell with the sensor tip (CM5, Biacore) immobilized amino-β-cyclodextrin **5** according to the following procedure: (1) 0.1 M Na_2HPO_4/NaH_2PO_4 buffer solution (pH 6.6) for 60 s; (2) 0.1 M Na_2HPO_4/NaH_2PO_4 (pH 6.6) buffer solution including the catechins for 60 s (the SPR data were recorded at 50 s after the beginning of the catechin solution flow); (3) 0.1 M Na_2HPO_4/NaH_2PO_4 (pH 6.6) buffer solution for 60 s (the SPR data were recorded at 20 s after the beginning of the buffer solution flow); (4) 10 mM glycine−HCl (pH 2) (purchased from Biacore) for 30 s; (5) 0.1 M Na_2HPO_4/NaH_2PO_4 (pH 6.6) buffer solution for 60 s.

RESULTS AND DISCUSSION

Binding Study on the Complexation between Cyclodextrins and Catechins. The major catechins in green tea are EGCg **1**, ECg **2**, EGC **3**, and EC **4**, which are classified as gallate-type catechins (**1** and **2**) or non-gallate-type catechins (**3** and **4**) by the existence of a galloyl group on the oxygen atom at the C3 position (**Figure 1**). Humans feel the taste of the gallate-type catechins to be more bitter-astringent than that of the non-gallate-type catechins (*20*). Therefore, the receptor molecule for sensing the bitter-astringent taste intensity of the green tea catechins must have a higher binding ability for the gallate-type catechins than for the non-gallate-type catechins. β-Cyclodextrin is known to be a good receptor for some

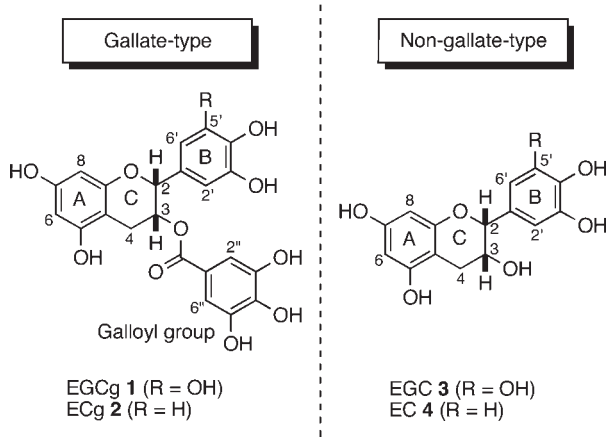

Figure 1. Four major green tea catechins.

catechins (*21*). However, the inclusion phenomena of the cyclodextrins for the green tea catechins have not been studied systematically and comprehensively.

To investigate which cyclodextrins were the most feasible for sensing the bitter-astringent taste intensity of the green tea catechins, the binding abilities of α-, β-, and γ-cyclodextrins for the four catechins (**1**−**4**) were examined by ^{1}H NMR titration experiments in deuterium oxide solution (pD 7.0) at 300 K. The concentration of the cyclodextrins was increased from 0 to 5.00 mM against a constant concentration of the catechins (1.00 mM). **Figures 2** and **3** show the chemical shift changes ($\Delta\delta^{cat}_{obs}$) in each proton of the catechins against the total concentration of β- and γ-cyclodextrins, respectively. $\Delta\delta^{cat}_{obs}$ is the difference between the observed ^{1}H NMR chemical shift of the catechin with the cyclodextrins and that without the cyclodextrins. The protons on the A-ring (C6−H and C8−H) were not plotted in the figures because they were substituted by the deuterium from deuterium oxide. The plots for C2−H in **Figure 2a**,**c** are not shown because the NMR signals for these protons overlapped the residual proton of the solvent signal. The proton signals of the gallate-type catechins broadened with the addition of β-cyclodextrin. Broadened signals were prominently observed for C2−H, C3−H, C4−Hβ, C2′−H, and C6′−H of EGCg and for C2−H, C3−H, C4−Hβ, C2′−H, C5′−H, and C6′−H of ECg. Here, the proton on C4, which gave a cross peak to C2−H in the NOESY spectrum, was assigned as C4−Hβ. The broadening would be due to the motion of the gallate-type catechin molecules becoming restricted in β-cyclodextrin, as Ishizu et al. described in their paper on the complexation of (+)-catechin and β-cyclodextrin (*25*). The broadening of the ^{1}H NMR signals did not occur for the combination of the non-gallate-type catechins and β-cyclodextrin or for any of the combinations of the catechins and γ-cyclodextrin.

All of the ^{1}H NMR signals of the gallate-type catechins shifted downfield with increasing concentration of β-cyclodextrin except for C2−H and C3−H. Interestingly, there was little change in the chemical shift of C4−Hα (**Figure 2a**,**b**). In the protons of EGC (**Figure 2c**), the C3−H and C4−Hs shifted upfield and downfield, respectively, and C2′−H and C6′−H shifted very little. In the protons of EC (**Figure 2d**), all proton signals but C4−Hβ and C5′−H shifted upfield. However, the downfield shift of C5′−H was small. In the case of γ-cyclodextrin (**Figure 3**), although the protons of the catechin tended to shift upfield, C5′−H of ECg shifted downfield and C2′−H, C6′−H, C2″−H, and C6″−H of EGCg, C4−Hβ, C2′−H, and C6′−H of ECg, C3−H and C4−Hβ of EGC, and C4β−H and C5′−H of EC did not shift much. In the case of α-cyclodextrin, chemical shift changes were not observed for any protons of the catechins. From this result, it appeared that α-cyclodextrin does not include the catechins.

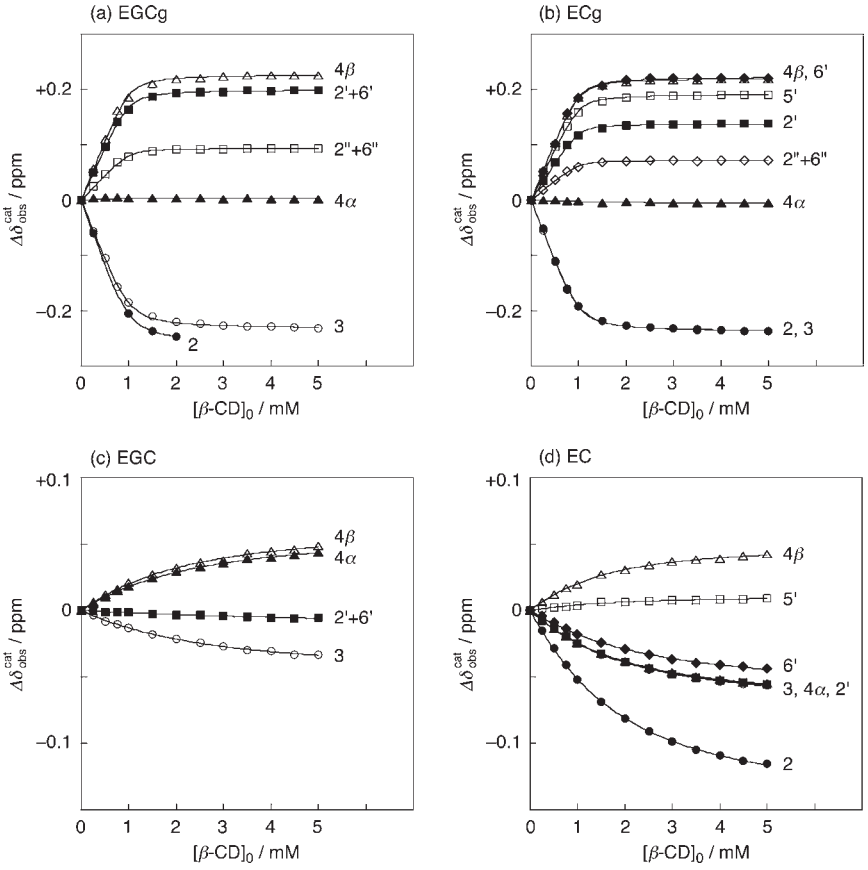

Figure 2. ^{1}H NMR titration curves between β-cyclodextrin and the catechins in D_2O at 300 K. $\Delta\delta_{obs}^{cat}$ is the difference between the observed ^{1}H NMR chemical shift of the catechins with and without β-cyclodextrin. $[\beta\text{-CD}]_0$ is the total concentration of β-cyclodextrin. The numbers in the graphs identify the protons of the catechins according to their attached carbon number.

To determine the binding constants of each catechin for β- and γ-cyclodextrins, the fitting curves for the ^{1}H NMR titration plots in **Figures 2** and **3** were calculated by nonlinear least-squares regression procedures according to eq 1 using KaleidaGraph (Synergy Software) (eq 1 is the standard binding isotherm for the formation of a 1:1 complex) (*26*). In eq 1, $[R]_0$ and $[C]_0$ are the total concentrations of the cyclodextrins and the catechins, respectively, K_b is a binding constant, and $\Delta\delta_{11}^{cat}$ is the difference between the ^{1}H NMR chemical shift of the catechins forming the 1:1 complex with the receptors and the free catechins.

$$\Delta\delta_{obs}^{cat} = \Delta\delta_{11}^{cat}[1 + K_b[C]_0 + K_b[R]_0 - \{(1+K_b[C]_0+K_b[R]_0)^2 - 4K_b^2[C]_0[R]_0\}^{1/2}]/2K_b[R]_0 \quad (1)$$

The binding constants were given by averaging the values calculated for each proton of the catechins. However, the values for the above protons, which had exceedingly small chemical shift changes, were removed from the calculations, because the binding constants calculated from the titration plots of those protons included large errors. As shown in **Table 1**, the binding constants of β-cyclodextrin for the gallate-type catechins were extremely large (295×10^2 M^{-1} for EGCg and 322×10^2 M^{-1} for ECg) and were much greater than those for the non-gallate-type catechins (580 M^{-1} for EGC and 813 M^{-1} for EC). Although a tendency in the affinity of γ-cyclodextrin for the catechins was similar to that of β-cyclodextrin, the binding constants between γ-cyclodextrin and the catechins were considerably smaller than those between β-cyclodextrin and the catechins. On the basis of these results, β-cyclodextrin was selected as the receptor molecule for sensing the bitter-astringent taste intensity of green tea catechins.

Detection of the Bitter-Astingent Taste Intensity of the Green Tea Catechins by Means of the β-Cyclodextrin/SPR System. To detect the complexation between β-cyclodextrin and the catechins, an SPR method was used. Although the SPR system has been used as a biosensor detector for various chemical and biological species, its application to taste sensing is novel (*27*). The immobilization of β-cyclodextrin onto a sensor tip in a flow cell of an SPR system was achieved by the coupling of 6-monodeoxy-6-monoamino-β-cyclodextrin **5** to the methylcarboxyl groups of dextran immobilized on the sensor tip (*28*). The carboxyl groups were activated with EDC and NHS in the coupling reaction. The unreacted carboxyl groups were capped with ethanolamine.

The relationship between the concentration of each catechin and the SPR response is shown in **Figure 4**, where the values indicated on the *y*-axis define 0.1° of the angle shift in the SPR signal as 1000 RU. The SPR responses for the gallate-type catechins (**1** and **2**) were larger than those for the non-gallate-type catechins (**3** and **4**). The values were 323 RU for EGCg, 326 RU for ECg, 90 RU for EGC, and 90 RU for EC at 5.0 mM catechin solutions. The SPR responses reflected the binding abilities of β-cyclodextrin for the catechins. In addition, **Figure 4** shows the β-cyclodextrin/SPR system has thresholds of < 0.1 and 1 mM for the gallate-type and non-gallate-type catechins, respectively. Humans have thresholds of 0.44, 0.41, 1.2, and 1.6 mM for EGCg, ECg, EGC, and EC, respectively (*20*, *29*). Therefore, these results reveal that the sensitivity of the β-cyclodextrin/SPR system for the bitter-astringent taste of the green tea catechins is equal to or higher than that of human gustation.

In addition, the taste of the gallate-type catechins is characterized by a bitter-astringent aftertaste (*30*). The β-cyclodextrin/SPR system also was able to sense this aftertaste. After flowing

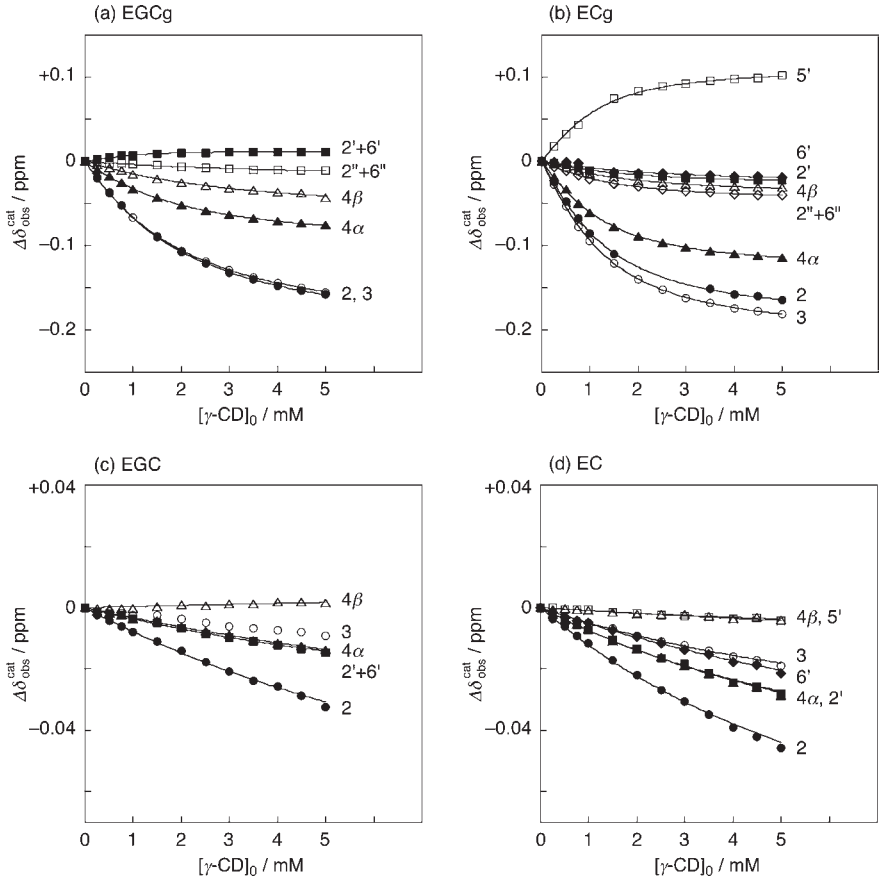

Figure 3. ^{1}H NMR titration curves between γ-cyclodextrin and the catechins in D_2O at 300 K. $\Delta\delta^{cat}_{obs}$ is the difference between the observed ^{1}H NMR chemical shift of the catechins with and without γ-cyclodextrin. $[\gamma\text{-CD}]_0$ is the total concentration of γ-cyclodextrin. The numbers in the graphs identify the protons of the catechins according to their attached carbon number.

Table 1. Binding Constants of the Complexes between Cyclodextrins and Catechins at 300 K in D_2O

	β-cyclodextrin/$\times 10^2$ M^{-1}				γ-cyclodextrin/$\times 10^2$ M^{-1}			
catechin proton	EGCg **1**	ECg **2**	EGC **3**	EC **4**	EGCg **1**	ECg **2**	EGC **3**	EC **4**
C2−H	—[a]	225 ± 23	—[a]	7.88 ± 0.17	6.71 ± 0.14	13.8 ± 0.52	0.88 ± 0.16	1.35 ± 0.19
C3−H	198 ± 21	212 ± 14	5.01 ± 0.41	7.58 ± 0.28	7.01 ± 0.15	14.5 ± 0.30	—[b]	1.23 ± 0.20
C4−Hα	—[b]	—[b]	5.95 ± 0.26	7.74 ± 0.17	6.94 ± 0.16	15.7 ± 0.38	0.63 ± 0.15	1.16 ± 0.15
C4−Hβ	289 ± 52	321 ± 25	6.43 ± 0.45	11.3 ± 0.61	3.72 ± 0.44	—[b]	—[b]	—[b]
C2′(6′)−H	310 ± 51		—[b]		—[b]		0.98 ± 0.17	
C2′−H		430 ± 96		7.85 ± 0.24		—[b]		1.24 ± 0.18
C5′−H		347 ± 67		8.95 ± 1.56		18.6 ± 1.16		—[b]
C6′−H		302 ± 43		5.65 ± 0.42		—[b]		0.83 ± 0.16
C2″(6″)−H	382 ± 86	416 ± 90			—[b]	12.7 ± 0.52		
mean	295 ± 52	322 ± 51	5.80 ± 0.37	8.13 ± 0.49	6.10 ± 0.22	15.0 ± 0.58	0.83 ± 0.16	1.16 ± 0.18

[a] These binding constants could not be determined because the ^{1}H NMR signals were hidden under the residual proton signal of D_2O. [b] These binding constants could not be determined because the chemical shift changes were exceedingly small.

the catechin solution into the flow cell of the SPR system, the running solution (0.1 M Na_2HPO_4/NaH_2PO_4 buffer solution) was flowed into the flow cell at 10 μL/min for 60 s. **Figure 5** shows the SPR responses at 20 s after flowing the running solution against the concentration of the catechins. An SPR response was observed for the gallate-type catechins at >0.05 mM. However, such a response was not detected for the non-gallate-type catechins. This tendency is similar to a human sense for the aftertaste of the catechins. Therefore, it is possible to interpret these SPR responses as the aftertaste of the gallate-type catechins. It is presumed that these phenomena result from the difficulty of the dissociation of the gallate-type catechins from β-cyclodextrin.

In summary, to develop a taste sensor with a receptor function, we searched for adequate receptors for sensing the bitter-astringent taste of the green tea catechins by ^{1}H NMR titration and examined the detection of the taste intensity by the SPR system immobilized the receptor. Consequently, it was determined that the β-cyclodextrin/SPR system was able to detect the intensity of the bitter-astringent taste of the catchins just as humans feel the taste. Although the SPR method has been used for substrate-specific detection of pathogens, toxins, antibodies, allergens, proteins, and other biological species (*27*), the present study revealed that SPR is also applicable to a taste sensor system, which has some global selectivity targeting

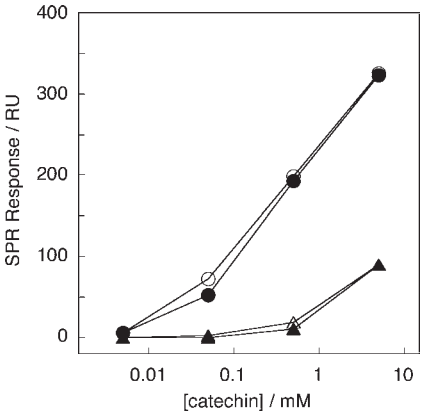

Figure 4. Relationship between the SPR response and the concentration of the catechins under flowing catechin solutions: ●, EGCg; ○, ECg; ▲, EGC; △, EC. The SPR data were recorded 50 s after the beginning of the catechin solution flow. [Catechin] is the concentration of the catechin solution.

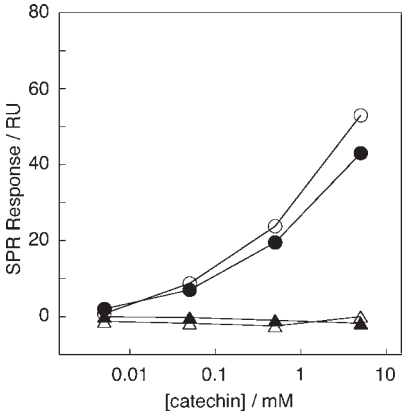

Figure 5. Relationship between the SPR response and the concentration of the catechins under flowing 0.1 M Na_2HPO_4/NaH_2PO_4 buffer solution following the catechin solutions: ●, EGCg; ○, ECg; ▲, EGC; △, EC. The SPR data were recorded 20 s after the beginning of the buffer solution flow. [Catechin] is the concentration of the catechin solution.

more than one taste substance even if it is a system with a receptor function (*1*).

Furthermore, incorporating molecular design into the synthesis of a new receptor will make sensing other taste substances possible. The evaluation of the affinity between a receptor and a taste substance before immobilization of the receptor into an SPR system will make the development of sensors more efficient, because the synthesis of the receptor molecule with the linker moiety for the immobilization takes more effort than making the receptor without the linker. For this purpose, instead of the NMR titration method, an absorbance titration (*26*) or a microcalorimetric method (*31*) must also be used. Finally, to obtain taste information, the receptor with a linker moiety such as an amino group has only to be synthesized and immobilized in an SPR system. The methodology presented in this study can be used as a basic strategy in developing taste sensors with a receptor function.

ABBREVIATIONS USED

SPR, surface plasmon resonance; NMR, nuclear magnetic resonance; EGCg, (−)-epigallocatechin-3-*O*-gallate; ECg, (−)-epicatechin-3-*O*-gallate; EGC, (−)-epigallocatechin; EC, (−)-epicatechin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, 2,2′,2″,2‴-(ethane-1,2-diyldinitrilo)tetraacetic acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; NHS, *N*-hydroxysuccinimide.

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