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Pigments in Green Tea Leaves (*Camellia sinensis*) Suppress Transformation of the Aryl Hydrocarbon Receptor Induced by Dioxin

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Environmental contaminants such as dioxins enter the body mainly through diet and cause various toxicities through transformation of the aryl hydrocarbon receptor (AhR). We previously reported that certain natural flavonoids at the dietary level suppress the AhR transformation induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In this study, we identified lutein and chlorophyll a and b from green tea leaves as the novel antagonists for AhR. These active compounds suppressed AhR transformation dose-dependently with the 50% inhibitory concentration (IC_{50}) values against 0.1 nM TCDD-induced AhR transformation at 3.2, 5.0, and 5.9 μM, respectively. (−)-Epigallocatechin gallate, which is the most abundant flavonoid in green tea leaves, also showed stronger suppressive effects than did other major tea components, with the IC_{50} value of 1.7 μM. Thus, these pigments of green tea leaves have the potential to protect from dioxin toxicity through the suppression of AhR transformation.

KEYWORDS: Green tea; catechin; lutein; aryl hydrocarbon receptor; TCDD; dioxins

INTRODUCTION

Dioxins, the environmental contaminants, cause serious health concerns because of their potent toxicities, lipophilicity, and resistance to degradation (*1*, *2*). Dioxins express toxicities, such as body weight loss, cancer promotion, immunosuppression, and birth defects, through binding to the cytosolic aryl hydrocarbon receptor (AhR) and subsequent transformation of the receptor (*3*−*5*). The transformed AhR forms a heterodimer with another basic helix-loop-helix protein called AhR nuclear translocator, and travels into the nucleus (*6*−*8*). This heterodimer eventually interacts with a specific DNA sequence, dioxin responsive element (DRE), and works as a transcriptional factor (*6*, *9*) to express a battery of genes including drug-metabolizing enzymes such as cytochrome P450 1A subfamily (CYP1A), quinone oxidoreductase, and glutathione *S*-transferase (*10*, *11*). Accordingly, AhR transformation is recognized as the initial step of the events involved in dioxin toxicities, and the suppression of the transformation is expected to protect us from the toxicities.

Since AhR is one of the orphan receptors, numerous studies have been carried out to search for the agonists and antagonists of AhR, besides dioxins and polycyclic aromatic hydrocarbons. As agonists of AhR, tryptophan and its metabolites (*12*), beta-apo-8′-carotenal, canthaxanthin, and astaxanthin (*13*) are reported to induce the AhR transformation and its downstream events, CYP1A1 and CYP1A2 expression. Indigo and indirubin are also possible agonists of AhR, and they interact with human AhR in vitro (*14*). As antagonists of AhR, 3′,4′-Dimethoxyflavone (*15*) and LY294002 [2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one] (*16*) have been reported to suppress expression of *CYP1A1* mRNA and its enzyme activity through the AhR-dependent pathway. Natural flavonoids also suppress the transformation of AhR (*17*) and CYP1A1 activity (*18*, *19*). In addition, resveratrol (*20*) and curcumin (*21*) are reported to act as antagonists of AhR. Thus, it is important to search for novel AhR ligands. From the viewpoint of the prevention of toxicities by dioxins, it is critical to search for natural compounds possessing the antagonistic effects toward AhR. Because dioxins unexpectedly enter the body mainly through diet (*22*, *23*), antagonists of AhR should be components of food to prevent or reduce the toxicities.

Previously, we have reported that flavones and flavonols have an ability to suppress AhR transformation at the dietary level, while catechins show a moderate effect. The IC_{50} value of galangin, apigenin, kaempferol, and quercetin against 1 nM TCDD-induced AhR is less than 3 μM, while that of (−)-epigallocatechin gallate is 10 times larger (*17*). Another report showed that green tea extract inhibited *CYP1A1* gene expression

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(*24*), but the green tea extract used was an artificial mixture enriched with catechins (more than 81%) and different from commercially available ones, which contain only 15−25% catechins as dry weight matter (*25*). A recent report also showed that antagonistic compounds isolated from green tea extract were catechins (*26*), although the isolation method was focused on flavonoids. Thus, there is a possibility that plants including green tea leaves contain novel ligand(s) of AhR. In this investigation, we have isolated and identified antagonists of AhR from green tea leaves and determined their activities in vitro.

MATERIALS AND METHODS

Materials. Dried green tea leaves (*Camellia sinensis*) were manufactured in the Shizuoka Prefecture in Japan. TCDD was purchased from AccuStandard (New Haven, CT). As the authentic compounds, catechins ((+)-catechin, (−)-epicatechin, (−)-gallocatechin, (−)-epigallocatechin, (−)-catechin gallate, (−)-epicatechin gallate, (−)-gallocatechin gallate, and (−)-epigallocatechin gallate) were purchased from Kurita Kogyo (Tokyo, Japan). Luteolin, kaempferol, kaempferol-3-glucoside, β-cryptoxanthin, and zeaxanthin were obtained from Extrasynthèse (Genay, France), and quercetin was purchased from Wako Pure Chemical (Tokyo, Japan). Lutein, chlorophyll a, chlorophyll b, lycopene, and astaxanthin were from Sigma (St. Louis, MO), and β-carotene was purchased form Nacalai Tesque (Kyoto, Japan). For electrophoretic mobility shift assay, oligonucleotide probe for dioxin responsive element (*27*) (DRE; 5′-GAT CCG GAG TTG CGT GAG AAG AGC CA-3′ (coding) and 5′-GAT CTG GCT CTT CTC ACG CAA CAC CG-3′ (noncoding)) was synthesized. All other reagents used were of the highest grade available from a commercial source.

Extraction and Fractionation of Green Tea Leaves. To identify the green tea components having suppressive effects on AhR transformation, dried green tea leaves (500 g) were extracted three times with 1 L of 75% ethanol for 24 h, and the extract was evaporated and used as the ethanol extract. The ethanol extract was suspended in 1 L of distilled water and partitioned stepwise with 2 L of *n*-hexane, chloroform, ethyl acetate, and *n*-butanol, three times each. Each fraction obtained was quantitatively recovered, evaporated, and assayed for antagonistic effects on AhR transformation. The *n*-hexane-partitioned fraction was further fractionated by column chromatography and high-performance liquid chromatography (HPLC) in the following way: An aliquot of 200 μL of the *n*-hexane-partitioned fraction (100 mg/mL methanol solution) was applied to a 300- × 20-mm i. d. MCI gel column in methanol as the immobile phase. The column was eluted with 500 mL of methanol and then with 200 mL of acetone. The elute with methanol was fractionated into four subfractions (referred to as the methanol fraction-1−4) by measuring the absorbance at both 210 and 350 nm, while the elute with acetone was collected in a lump fraction. This column chromatography was repeated 5 times under the same conditions, and each subfraction was collected and combined. Each subfraction was dried in vacuo, dissolved in methanol at a concentration of 100 mg/mL, and subjected to the preparative HPLC equipped with a photodiode array detector (Waters 2695 and 2996 PDA system). The HPLC conditions were as follows: Column, 250- × 20-mm i. d., 5-μm Js80H ODS column (YMC Co., Ltd., Kyoto, Japan) maintained at 40 °C; elution was performed with 100% methanol at a flow rate of 8 mL/min; injection volume of each fraction was 200 μL; and the detection was UV at 210 nm. The HPLC was carried out repeatedly under the same conditions, and each peak was collected and combined.

Instrumental Analysis. The active compounds isolated from green tea leaves were purified by rechromatography with HPLC using a Waters 600E Multisolvent Delivery System and a 486UV/Vis detector (Nihon Waters K. K., Japan), and mass spectra (MS) were obtained using a JEOL JMS-SX 102 mass spectrometer (JEOL Ltd., Japan). ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-A 400 at 400.00 and 100.4 MHz, respectively (JEOL Ltd.), and chemical shifts were given in δ (ppm) with tetramethylsilane used as an internal standard.

Determination of Polyphenols Content in Green Tea Extract. To determine the contents of polyphenols, chlorophylls, and lutein in green tea extract, a hot-water extract was separately prepared in addition to the ethanol extract. These extracts were dissolved in methanol at 1 mg/mL, and aliquots of 10 μL were injected into the HPLC. Analytical conditions were as follows: for detection of polyphenols, a 250- × 4.6-mm i. d. Wako pack C18HG column maintained at 40 °C; mobile phase, 22% methanol solution in 0.1% phosphate buffer; flow rate, 1 mL/min; and wavelength at 230 and 280 nm; and for chlorophylls and lutein, a 250- × 4.6-mm i. d. Js80H column (YMC Co., Ltd.) maintained at 30 °C or room temperature; mobile phase, 100% methanol; flow rate, 1 mL/min; and monitoring with a Waters 486 UV/Vis at 210 nm.

Preparation of the Cytosol Fraction from Rats. Animal treatments in the present study conformed to *The Guidelines for the Care and Use of Experimental Animals, in Rokkodai Campus, Kobe University*. Livers from male Sprague−Dawley rats (6 weeks old, 140−170 g, obtained from Japan SLC, Shizuoka, Japan) were subjected to preparation of the cytosol fraction as described previously (*17*). After measuring the protein contents (*28*), the cytosolic fraction was used for estimation of the antagonistic effects of green tea extract or its components on AhR transformation.

Estimation of the Antagonistic Effects of Green Tea Components on AhR Transformation. To estimate the antagonistic effects of the green tea components on AhR transformation, the cytosolic fraction (4.0 mg protein/mL) in HEDG buffer (25 mM HEPES, 1.5 mM ethylenediaminetetraacetic acid (EDTA), 1.0 mM dithiothreitol, 10% glycerol, pH 7.4) was incubated with various concentrations of green tea extract or its components dissolved in DMSO at 20 °C for 10 min and then with 0.1 or 1 nM TCDD or with DMSO (10 μL/mL) alone as a vehicle control for a further 2 h. The resultant mixture was subjected to an electrophoretic mobility shift assay in the manner described in the next section.

Determination of Transformed AhR by Electrophoretic Mobility Shift Assay. Transformed AhR was determined by electrophoretic mobility shift assay using a DRE oligonucleotide probe corresponding to the 26-bp AhR binding site as described previously (*17*). The prepared double-strand oligonucleotide was 5′-end-labeled with T4 polynucleotide kinase (Takara Biochemicals, Otsu, Japan) and [γ-^{32}P]-ATP (Amersham Pharmacia Biotech, Buckinghamshire, England). Free nucleotides were removed from the labeled DRE probe on a Sephadex G-25 spin column (Roche Diagnostics, Co., Indianapolis, IN). The reaction mixture for the binding consisted of 10 μg of protein from the cytosolic fraction, 250 ng of poly[dI-dC] (Roche Diagnostics, Co.) in 12 μL of HEDG buffer containing 150 mM KCl, and was incubated for 15 min at room temperature. ^{32}P-Labeled DRE probe (25 kcpm, 10 fmol) was added, and the mixture was incubated for a further 15 min at room temperature. The entire volume of the mixture was loaded onto a 4% nonstacking native polyacrylamide gel containing 0.25 × TBE buffer (25 mM Tris, 22.5 mM borate, 0.25 mM EDTA) and was electrophoresed in the same buffer at 60 V for 30 min before loading and for 90 min after loading. After electrophoresis, the gels were dried and exposed to X-ray films. Density of AhR/DRE complex was determined by using the Digital Imaging System Is-9000 (Alpha Innotech, San Leandro, CA).

RESULTS

Evaluation of the Suppressive Effects of Green Tea Extract on AhR Transformation. To confirm whether crude green tea extract can suppress AhR transformation, green tea leaves were extracted with 75% ethanol to obtain almost all of the low-molecular weight components. As shown in **Figure 1A**, the ethanol extract suppressed AhR transformation induced by 1 nM TCDD in a dose-dependent manner, while the ethanol extract itself did not induce the transformation (**Figure 1A**, Lane 8). This indicates that green tea extract possesses suppressive effects toward TCDD-induced AhR transformation and that total antagonistic activities in the extract overcome total agonistic ones. To determine the 50% inhibitory concentration (IC_{50}) value, the density of each specific band was analyzed, and a log of the concentration of the ethanol extract against the ratio of transformed AhR was plotted (**Figure 1B**). The IC_{50} value

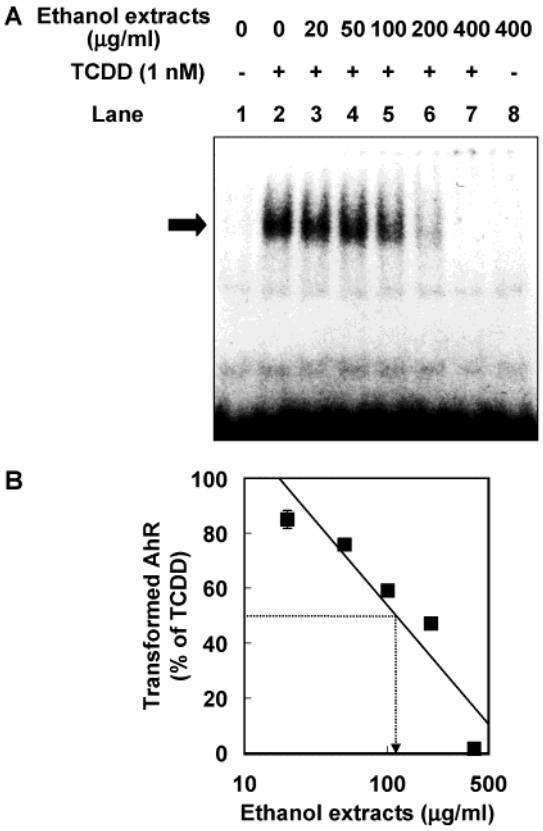

Figure 1. Suppressive effects of the ethanol extract from green tea leaves on AhR transformation. (**A**) Representative EMSA result, the arrow indicates AhR/DRE complex. (**B**) Quantified density of AhR/DRE complex. Data are represented as the mean ± SE from the independent triplicate experiments. The IC_{50} value of the ethanol extract against 1 nM TCDD was determined by plotting a log of the concentration of the ethanol extract against the ratio of transformed AhR.

of green tea extract against the 1 nM TCDD-induced AhR transformation was 110 μg/mL.

The ethanol extract was then stepwisely partitioned with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol, and the suppressive effect of the obtained fractions on AhR transformation was examined (**Figure 2**). The *n*-hexane and the ethyl acetate fractions showed strong effects with IC_{50} values of 41 and 42 μg/mL, respectively, while the other fractions showed weak effects with the IC_{50} values of $>$ 100 μg/mL. Hence, we searched for the antagonistic compounds in the *n*-hexane and the ethyl acetate fractions.

Isolation of Antagonists of AhR From *n*-Hexane Fraction. The *n*-hexane fraction is rich in various pigments such as chlorophylls, pheophytins, carotenoids, and so on. It has not yet been investigated whether these pigments in tea leaves can suppress AhR transformation. Consequently, the *n*-hexane fraction was subjected to further fractionation into subfractions using column chromatography (**Figure 2**). Among the subfractions, an acetone subfraction showed the most suppressive effects on AhR transformation, and the methanol fraction-1 also showed moderate effects (**Figure 3A**). Each subfraction was subjected to preparative HPLC and separated into 4 parts from the methanol fraction-1, 13 from the methanol fraction-2, 10 from the methanol fraction-3, 8 from the methanol fraction-4, and 4 from the acetone fraction (**Figure 2**). Each obtained part was dried in vacuo, dissolved in DMSO containing 20% acetone, and examined for the suppressive effect on AhR transformation. As a result, the HPLC-fractions 1-2, 2-4, 3-3, 4-1, a-2, a-3, and a-4 showed strong effects (**Figure 3**, parts **B**−**F**). These HPLC fractions were purified by rechromatography with HPLC and subjected to identification by instrumental analyses.

Identification of the Antagonists of AhR in Green Tea Extract. A major compound in the HPLC fractions 3-3 and 4-1 was identified as lutein by ^{1}H-NMR, ^{13}C-NMR, and FAB-MS (*m*-nitrobenzyl alcohol matrix): $m/z = 568[M]^+$, and obtained NMR data was consistent with literature ones (*29*−*31*). The compounds in the HPLC fractions a-2 and a-4 were identified as chlorophyll b and chlorophyll a from the molecular ion peak in their FAB-MS, respectively (*32*, *33*). Also, their R_f values on thin-layer chromatography were the same as those of authentic samples. Regarding the HPLC fractions 1-2 and a-3, they mainly contained caffeine and pheophytins, respectively (data not shown), but the authentic compounds did not show any effects. Probably, minor compounds in these fractions showed the strong suppressive effects on AhR transformation. In the case of the HPLC fraction 2-4, the active compound(s) could not be identified because this fraction contained at least several compounds.

Antagonistic Effects of Lutein and Chlorophyll a and b on AhR Transformation. Because we identified lutein and chlorophyll a and b in green tea leaves, their antagonistic effects on AhR transformation were further investigated using the corresponding authentic compounds. Lutein showed suppressive effects on AhR transformation in a dose-dependent manner against 1 and 0.1 nM TCDD (**Figure 4**, parts **A** and **B**, respectively). Chlorophyll a and b also suppressed the transformation dose-dependently (**Figure 4**, parts **C** and **D**, respectively). The IC_{50} values of lutein and chlorophyll a and b against 0.1 nM TCDD-induced AhR transformation were 3.2, 5.0, and 5.9 μM, respectively. Moreover, these compounds did not show the agonistic effects toward AhR (data not shown).

Among these compounds, lutein is a novel antagonist for AhR. Thus, we examine the effects of other commercially available carotenoids on AhR transformation. As shown in **Figure 5**, β-cryptoxanthin and zeaxanthin also suppressed AhR transformation to about 50% at 50 μM. At the same concentration, lutein completely suppressed AhR transformation (**Figure 4B** and **Figure 5**). These results indicate that lutein has a unique property for AhR in carotenoids.

Contents of Polyphenols in Green Tea Extract and Their Antagonistic Effects on AhR Transformation. The ethyl acetate-partitioned fraction from green tea extract suppressed the TCDD-induced AhR transformation, as did the *n*-hexane-partitioned fraction. Green tea extract contains various flavonoids, mainly catechins, and their aglycones are expected to be distributed in the ethyl acetate-partitioned fraction because of their chemical properties (*17*). When we determined the contents of catechins and caffeine in each fraction from green tea extract, catechins were mainly contained in the ethyl acetate fraction, and caffeine was in the chloroform fraction (**Table 1**). In the current study, green tea leaves were extracted with 75% ethanol and further fractionated to determine their effects on AhR transformation. However, green tea is generally drunk as a hot-water extract, although powdered green tea is also drunk as a suspension (e.g., in a tea ceremony or in commercial beverages). To compare the contents of compounds between the ethanol extract and the hot-water extract, each of them was subjected to an HPLC analysis. The ethanol extract contained all compounds that were contained in the hot-water extract (**Table 2**). For example, the content of (−)-epigallocatechin gallate in the ethanol extract was 4 times as much as that in the

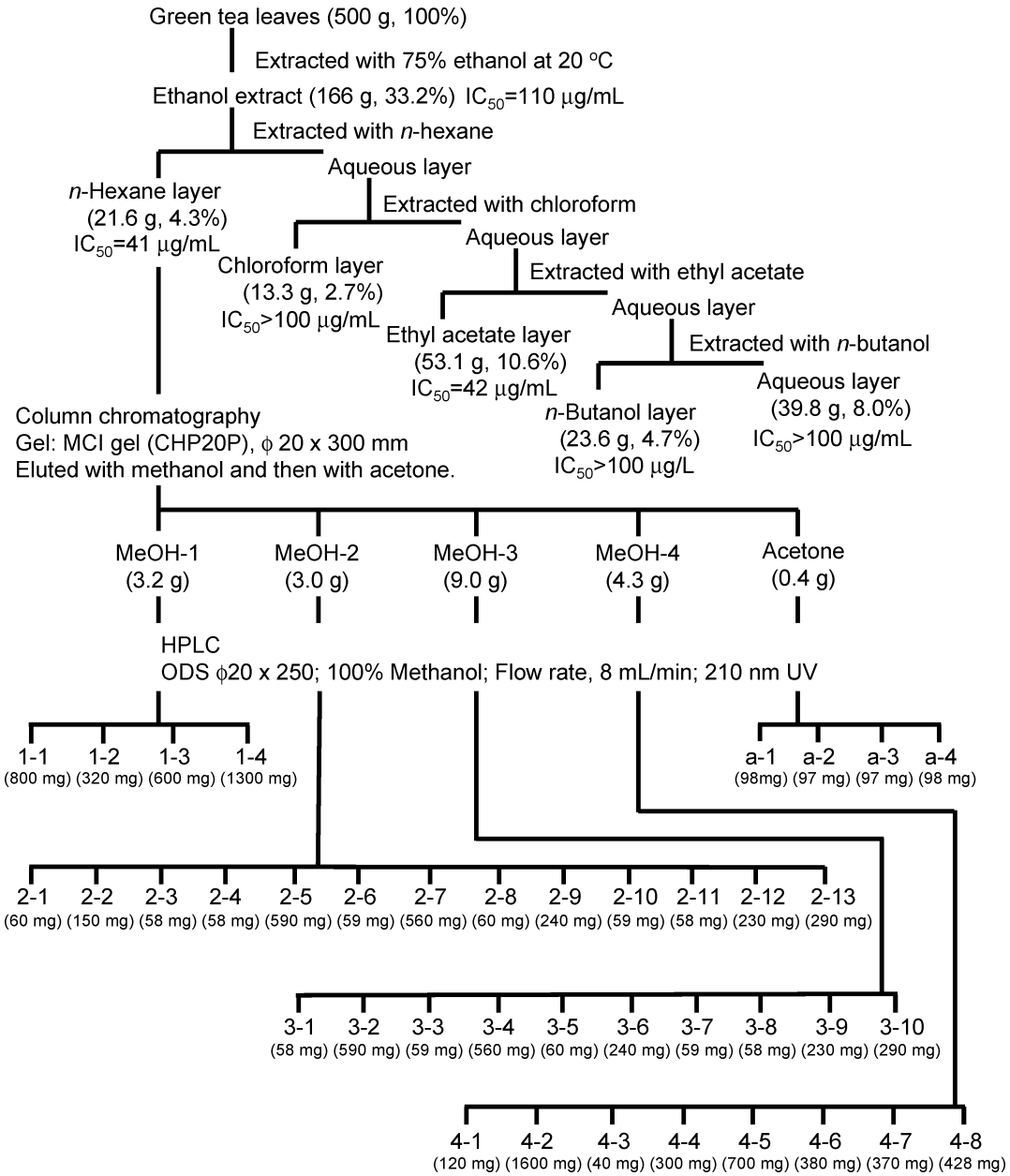

Figure 2. Extraction and fractionation procedures of green tea leaves. Weight and percent in parentheses are the yield of each fraction. The IC_{50} values were determined against 1 nM TCDD-induced AhR transformation.

hot-water extract. (−)-Epicatechin gallate, kaempferol, chlorophyll a, and lutein were detected in the ethanol extract but not in the hot-water extract. These results indicate that catechins, the major polyphenols in green tea leaves, are contained abundantly in the ethanol extract and distributed in the ethyl-acetate-partitioned fraction.

Previously, we have reported that flavonoids, including catechins, suppress the AhR transformation induced by 1 nM TCDD (*17*). In the present study, we examined the suppressive effects of catechins and other polyphenols contained in green tea leaves on the AhR transformation induced by 0.1 nM TCDD, a lower concentration close to the physiological conditions. As shown in **Table 2**, (−)-catechin gallate and (−)-epigallocatechin gallate showed the strong effects, with the IC_{50} values of 0.5 and 1.7 μM, respectively, while others showed moderate or weak effects. (−)-Epigallocatechin gallate at this concentration can exist in the body (*34*), whereas the amount of (−)-catechin gallate is small in green tea extract (**Tables 1** and **2**). Other flavonoids, including luteolin, quercetin, and kaempferol, also strongly suppressed the transformation, with IC_{50} values of 0.52, 0.84, and 0.63 μM, respectively. The other compounds, kaempferol-3-glucoside, caffeine, gallic acid, *n*-butylgallate, theanine, and theobromine, did not affect AhR transformation. These results suggest that catechins, especially (−)-epigallocatechin gallate, are major effective compounds in the ethyl acetate-partitioned fraction.

DISCUSSION

Dioxins, the environmental contaminants, express toxicities through the transformation of AhR (*3*−*5*) and enter the body mainly through diet (*22*, *23*). It is important to search for natural antagonists of AhR in food. Previous reports showed that green tea extract and its major compounds, catechins, suppressed AhR transformation and its downstream event, *CYP1A* gene expression (*24*, *26*). In this study, we have confirmed that green tea extract suppresses the transformation of AhR in a dose-

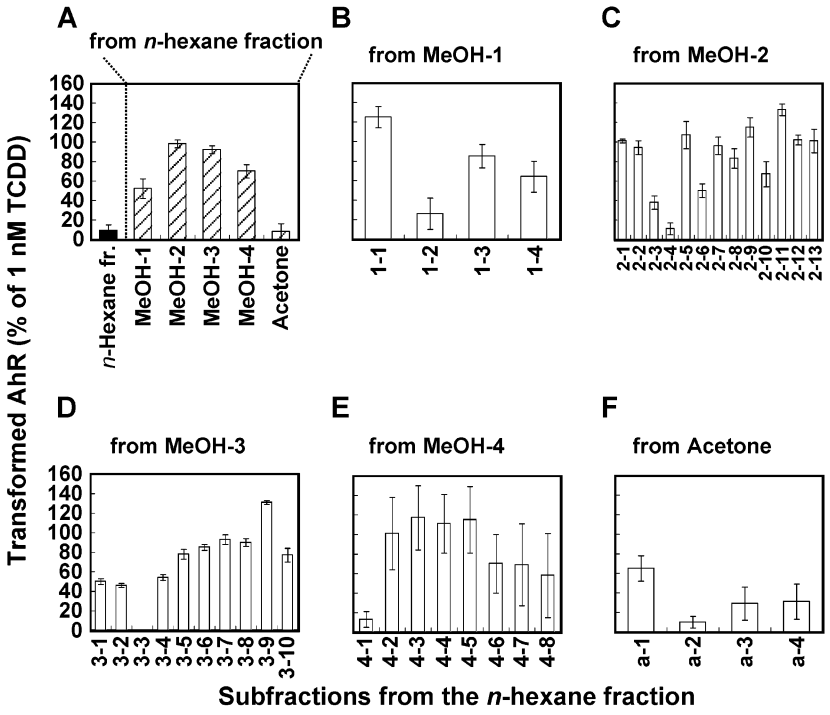

Figure 3. Suppressive effects of the subfractions from the *n*-hexane fraction on the AhR transformation. Suppressive effects of subfractions at 10 μg/mL (**A**) or 5 μg/mL (**B**–**F**) were determined against 1 nM TCDD-induced AhR transformation. Data are shown as the percent of transformed AhR (% of TCDD) and represented as the mean ± SE from the independent triplicate experiments.

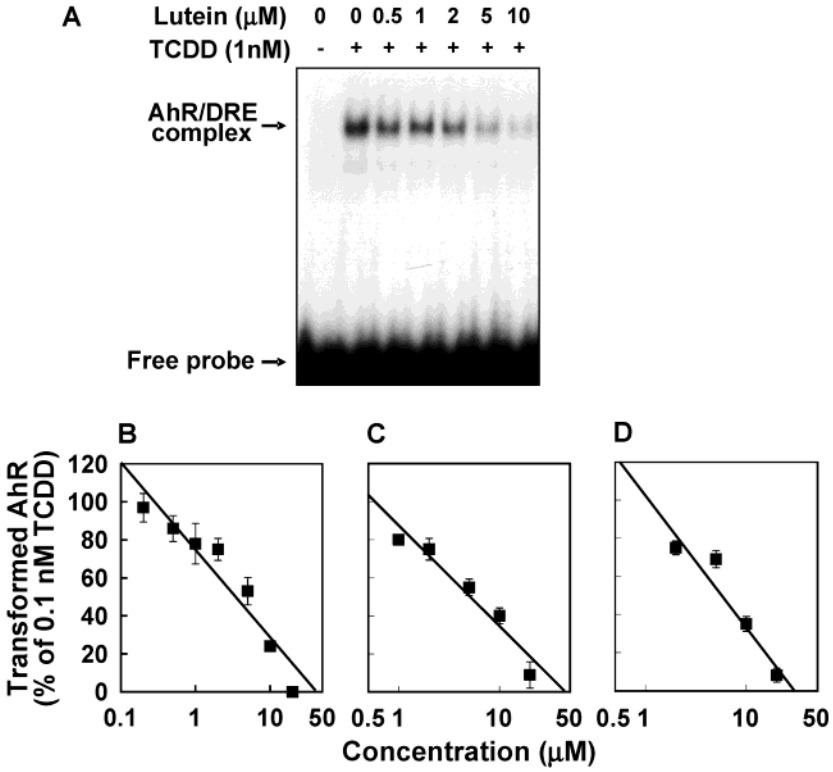

Figure 4. Dose-dependent suppressive effects of lutein or chlorophylls at the indicated concentrations on AhR transformation. The representative EMSA result of lutein against 1 nM TCDD is shown in panel **A**, and the arrow indicates AhR/DRE complex. The quantified density of the AhR/DRE complex of each compound against 0.1 nM TCDD is shown in panels **B**–**D**. Data are represented as the mean ± SE from the independent triplicate experiments. The IC_{50} values of lutein and chlorophyll a and b against 0.1 nM TCDD were determined by plotting a log of the concentration of the compounds against the ratio of transformed AhR.

dependent manner, and found that lutein and chlorophyll a and b are the novel antagonists of AhR from the *n*-hexane-partitioned fraction of green tea extract. These pigments, in addition to catechins, especially (−)-epigallocatechin gallate, contribute to the suppressive effects of green tea extract on AhR transformation.

Lutein is a common carotenoid in the plant kingdom and has various biological functions such as antioxidant activity, anti-

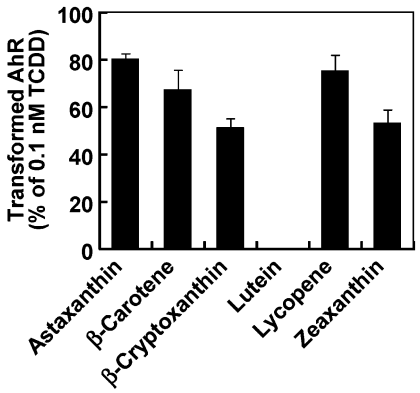

Figure 5. Suppressive effects of carotenoids on the AhR transformation. Suppressive effects of carotenoids at 50 μM were determined against 0.1 nM TCDD-induced AhR transformation. Data are shown as the percent of transformed AhR (% of TCDD) and represented as the mean ± SE from the independent triplicate experiments.

Table 1. Contents of Catechins in the Organic Solvent-Partitioned Fraction from the Ethanol Extract of Green Tea Leaves[a]

	fraction				
compounds	*n*-hexane	chloroform	ethyl acetate	*n*-butanol	aq
(+)-catechin	nd[b]	nd	trace	trace	nd
(–)-epicatechin	nd	trace	9.0	nd	nd
(–)-gallocatechin	nd	trace	1.4	trace	nd
(–)-epigallocatechin	trace	trace	23.2	10.5	nd
(–)-epicatechin gallate	nd	trace	10.3	nd	nd
(–)-epigallocatechin gallate	trace	1.1	58.7	3.2	nd
caffeine	trace	77.9	trace	nd	nd

[a] Contents of various catechins and caffeine were determined by HPLC analysis and represented as w/w percent in each organic solvent-partitioned fraction. [b] nd, not detected.

carcinogenic activity, and protection against the development of age-related macular degeneration (*35*−*37*). Several carotenoids, excluding lutein, have been reported to be ligands of AhR. Gradelet et al. (*13*) reported that β-apo-8′-carotenal, canthaxanthin, and astaxanthin induce CYP1A1 and 1A2 in the rat. Some synthetic retinoids have also been reported to induce CYP1A1 via AhR transformation (*38*). In the present study, we have isolated and identified lutein as a novel antagonist with the IC_{50} value against 0.1 nM TCDD of 3.2 μM (**Figure 4** and **Table 2**). Although the mechanism of the suppression has not yet been elucidated, the suppressive effect is similar to that of catechins having gallate moieties (**Table 2**). Other xanthophylls such as β-cryptoxanthin and zeaxanthin also showed a moderate suppressive effect, whereas β-carotene, lycopene, and astaxanthin showed a weaker effect. These results suggest that the polyene structure is not so important, but the hydroxyl groups in 6C rings are needed to exhibit the effects.

In this study, chlorophylls, as well as lutein, were identified as the novel antagonists of AhR. Chlorophylls as well as carotenoids, widely distributed in the plant kingdom, are not considered to be incorporated per se into our body, although uptake of chlorophyll derivatives by human intestinal cells has been demonstrated (*39*). Chlorophylls are reported to inhibit absorption and accelerate excretion of dioxins in rat (*40*) and in human intestinal Caco-2 cells (*41*). Chlorophyllin, a copper/sodium salt of chlorophyll, forms a molecular complex with heterocyclic amines, which have been reported to interact with AhR (*42*, *43*). Moreover, bilirubin induced CYP1A1 in hepatocytes via AhR-dependent action (*44*, *45*). These results suggest that the compounds having a porphyrin ring might have the potency to interact with TCDD and/or AhR and suppress the transformation.

Table 2. Contents and IC_{50} Values against 0.1 nM TCDD-Induced AhR Transformation of Selected Compounds in the Ethanol and the Hot-Water Extracts from Green Tea Leaves[a]

compounds	ethanol extract (%)	hot-water extract (%)	IC_{50} value (μM)
(+)-catechin	1.3	1.1	69
(–)-epicatechin	4.6	1.6	93
(–)-gallocatechin	1.8	1.2	34
(–)-epigallocatechin	13.6	3.5	24
(–)-catechin gallate	nd[b]	nd	0.5
(–)-epicatechin gallate	5.0	nd	20
(–)-gallocatechin gallate	nd	nd	8.3
(–)-epigallocatechin gallate	21.6	5.1	1.7
luteolin	0.33	0.25	0.52
quercetin	nd	nd	0.84
kaempferol	0.06	nd	0.63
kaempferol-3-glucoside	N. D.[c]	N. D.	N. E.[d]
chlorophyll a	0.023	nd	5.0
chlorophyll b	nd	nd	5.9
lutein	0.015	nd	3.2
caffeine	9.5	9.4	N. E.
gallic acid	N. D.	N. D.	N. E.
n-butylgallate	N. D.	N. D.	110
theanine	N. D.	N. D.	N. E.
theobromine	N. D.	N. D.	N. E.

[a] Compounds in the ethanol and the hot-water extract were detected by HPLC analysis. [b] nd, under the detection limit. [c] N. D., not determined. [d] N. E., no effect is up to 100 μM.

Catechins, flavonols, and flavones are natural antagonists of AhR, as we have previously reported (*17*), and these pigments were contained mainly in the ethyl acetate fraction (**Table 1**). The results in the present study demonstrated that the IC_{50} value of (−)-epigallocatechin gallate against the 0.1 nM TCDD-induced AhR transformation was 1.7 μM (**Table 2**). (−)-Epigallocatechin gallate at this concentration can exist in the body, since Nakagawa et al. (*34*) reported that the plasma concentration of (−)-epigallocatechin gallate rose to 4.4 μM after an intake of 525 mg. Thus, (−)-epigallocatechin gallate would suppress the AhR transformation at the physiological level. Previous reports have also demonstrated that not only green tea extract but also catechins suppress AhR transformation and *CYP1A1* gene expression (*17*, *24*, *26*, *46*), but their suppressive effects were weaker than those in this study. This difference is due to TCDD concentration because higher concentrations were used. Our previous report showed that the lower concentrations of these compounds were needed to suppress the lower concentration of TCDD (*17*). The level of TCDD in the environment is much lower than those in the experiments, including the current study. Therefore, flavonoids, including catechins, are attributive compounds for the prevention of dioxin toxicity.

Among the antagonistic pigments, (−)-catechin gallate, luteolin, quercetin, and kaempferol showed potentially strong effects from the IC_{50} values against 0.1 nM TCDD, though these strong antagonistic compounds were scarcely present in green tea leaves (**Table 2**). Similarly, lutein is contained in the ethanol extract from green tea leaves but not detected in the hot-water extract. These compounds are abundantly present not only in green tea leaves but also in common plants. After intake of vegetables and fruits, plasma levels of flavonols and lutein were around 0−0.14 μM and 0.2−0.5 μM, respectively (*47*−*49*).

Therefore, intake of vegetables and fruits leads to an increase in the physiological or intestinal levels of these antagonistic pigments and contributes to protecting us from dioxin toxicity.

Our findings are based on only in vitro assays using rat liver cytosol, and the actual effects on dioxin toxicity need future animal studies. Maliakal et al. (*50*, *51*) demonstrated that tea consumption modulates both phase I and phase II enzyme activities in rats, suggesting that tea components have the potency to interact with AhR in vivo. Because the transformation of AhR is the initial step of dioxin toxicity, intake of green tea can possibly protect against dioxin toxicity.

ABBREVIATIONS USED

AhR, aryl hydrocarbon receptor; DRE, dioxin responsive element; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; EMSA, electrophoretic mobility shift assay; DMSO, dimethyl sulfoxide

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