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Binding effect of polychlorinated compounds and environmental carcinogens on rice bran fiber

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Abstract

To accelerate the fecal excretion of polycyclic biphenyl (PCB), polychlorinated dibenzofurans (PCDFs), polychlorinated-*p*-dioxines (PCDDs) and various mutagens and carcinogens, their binding effect on rice bran fiber (RBF) was investigated for nine heterocyclic amines, six nitroarenes, 4-nitroquinoline-*N*-oxide, benzo[*a*]pyrene, furylfuramide, two kinds of flavonoid compounds and formaldehyde and ascorbic acid. PCBs, PCDFs and PCDDs suspended in nonane were incubated with RBF (10 mg/ml) at 37°C and after centrifugation, unbound chemicals in the supernatant were analyzed by high-performance liquid chromatography (HPLC) and gas chromatography (GC). The binding effects on RBF were enhanced more than other dietary fibers (DFs), which were tested including corn, wheat bran, spinach, Hijiki (a kind of seaweed), sweet potatoes and burdock fibers. It was found that the binding effects were related to lignin contents. Binding of 3-amino-1(or 1,4)-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-p-1 and Trp-p-2), food-derived carcinogens and 1-nitropyrene (1-NP), suspended in methanol, to RBF occurred within 10 min of incubation at 37°C at pH 5–7, and decreased below pH 4; binding of food-derived carcinogens was pH dependent. The binding effects to RBF and pulp lignin were obtained at ratio of over 90%, while corn fiber and cellulose were at ratios of 4–30%. Polycyclic aromatic compounds were related to the number of rings, showing high binding effects to chemical structures with triple rings. Binding of 1-NP and PCB to RBF was not influenced in any aerobic and anaerobic bacterial cultures. It was also found that RBF was capable of binding even conjugates containing mutagens such as glucuronides and sulfates, as well as metabolites in urine. It was suggested, therefore, that mutagens and carcinogens were available for the fecal excretion of residual chemicals and their metabolites, and also for the fecal excretion of PCBs, PCDFs and related compound residues in patients of Yusho disease, who suffered food poisoning due to rice oil contaminated with PCB in Japan.

Keywords: Dietary fiber; Mutagens; Food-derived carcinogens; Dioxins; Lignin

1. Introduction

Dietary fiber (DF) is principally composed of plant cell walls containing various carbohydrates. Recently, DF has been shown to play an important role in gastrointestinal fermentation, and desmutagens or antimutagens [1] have a variety of functions as chemical inactivators, enzymatic inducers, scavengers or antioxidants. In an earlier study, DF was focused on as an inhibitor of colorectal [2–4] and breast cancer [5], binding of steroid hormones [6], bile acid [7,8], iron [9] and pesticides [10], as well as a chemopreventive of adduct formation by heterocyclic amines [11,12].

Dietary fiber consists of hemicellulose, cellulose, pectin, lignin and soluble DF. Soluble DFs, usually polysaccharides, were suggested to enhance the incidence of colorectal cancer [13]. In Japan, increasing numbers of colon and prostate cancers may be due to diet changes, that is, a more American diet high in saturated fats, white flour, refined sugar, starches and red meat chemical additives. Synthetically, manufactured hybrid foods are also a major source of human toxicity [14].

Various organic and inorganic chemicals are possibly bound to DF in the body and quickly excreted in stool. In 1986, Yusho disease occurred in Japan. It was basically caused by polychlorinated biphenyl (PCB) contamination in rice oil. Since the occurrence, it was reported in many studies that in addition to PCB, polychlorinated dibenzofurans

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(PCDFs) contaminated the adipose tissue of patients at a level 100 times that of normal subjects [15]. At present, many researchers agree that the main therapy for Yusho disease is to quickly excrete PCB and PCDF residue in blood and adipose tissue [16]. Fecal excretion of PCBs and PCDFs was achieved with squalane, 2,6,10,15,19,23-hexamethyl tetracosane by oral administration in rats and dogs [17] and by combination treatment with cholestyramine and rice bran fiber (RBF) in rats [18]. After in vitro RBF experimental treatment, we treated patients with Yusho disease in Taiwan [15].

It was found that RBF showed a characteristic binding to chemical mutagens and carcinogens. The present study aimed to reveal the binding effect (a) of various mutagens and carcinogens, including polychlorinated compounds, (b) for fecal excretion and (c) to evaluate the possible application of RBF.

2. Materials and methods

2.1. Chemicals

Environmental mutagens and carcinogens, 1-nitropyrene (1-NP), 1,3-, 1,6- and 1,8-dinitropyrenes, 3-nitrofluoranthene, and 3,7- and 3,9-dinitrofluoranthenes, were synthesized in our laboratory as reported previously [19]. Food-derived mutagens and carcinogens, 3-amino-1,4-methyl-5*H*-pyrido[4,3-*b*]indole (Tri-p-2), 2-amino-6-methyl-6-methyldipyrido[1,2-α:3′,2′-*d*]imidazole (Glu-P-1) and 2-aminodipyrido[1,2-α:3′ ,2′ -*d*]imidazole (Glu-p-2), 2-amino-3-methylimidazo[4,5-*f*]quinoxaline (IQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-9*H*-pyrido[2,3-*b*]-indole (AαC), were kindly donated by Dr. Wakabayashi, from the National Cancer Center Institute, Tokyo, Japan. Other chemicals used in this study, benzene, naphthalene, benzo[*a*]pyrne, coumarin, perylene, chrysene, anthracene, phenanthrene, pyrene, fluoranthene, quercetin, rhamnetin, formaldehyde, ascorbic acid, furylfuramide (AF2) and charcoal-activated powder were purchased from Wako Industries. These chemicals were suspended in methanol. In addition, PCB KC500 and 600 were purchased from Wako Industries, and 3,4,3′, 4′-tetrachlorobiphenyl (TCB), 2,4,5,2′,4′,5′-, 2,3,4,5,3′,4′-hexaCB, 2,3,4,5,2′,4′,5′-, 2,3,4,5,6,2′ ,5′ -heptaCB, 2,3,7,8-, 1,3,6,8-tetrachlorodibenzofuran (TCDF), 2,3,4,7,8-, 1,2,4,6,7-pentaCDF, 1,2,3,4- and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin were kindly obtained from Prof. Masuda, Dai-Ichi Pharmaceutical College, Fukuoka, Japan, and were suspended in concentrations from 0.1 to 0.5 ppm of nonane (Wako Industries).

2.2. Purification of RBF

Purification of RBF was performed by a modified method of Prosky et al. [20] and fractionated to neutral (NDF) and acid detergent (ADF) fibers based on the method of Van Soest et al. [21–23]. Crude rice bran (1.5 kg) was passed through a 24-mesh stainless screen to obtain RBF. The sieved powders were completely washed with water to remove the aliphatic portion using a washing machine (Matsushita Denki Sangyo). The materials were dehydrated, suspended in 10-fold water of RBF and boiled for 1 h. In addition, the materials were washed with water 10 times. The RBF materials were treated with a mixture of 0.2 M phosphate buffer (pH 7.2) and 30 ml of amylase AH (Amano Seiyaku) for 1 h at 90°C. After cooling to 45°C, the materials were adjusted to pH 8.0 and treated with a mixture of 0.2 M phosphate buffer (pH 8.0) and 30 g of pancreatic F (Amano Seiyaku) for 3 h at 45°C. Subsequently, the materials were washed with water and suspended in 5 L of water. After adjusting to pH 1.5 with 0.4 N HCl, RBF was washed with water. The RBF was dried at 80°C overnight and placed in a sonicator for 5 h with acetone and hexane to defat it. Finally, RBF was heated overnight at 80°C until dry. Purification of other DFs, spinach, burdock, cabbage, soybean, carrot, corn and cellulose fibers was performed by a modified method of RBF purification.

Rice bran fiber was composed of 24.0% for noncellulose polysaccharide, 10.3% for cellulose, 12.8% for protein, 10.7% for lignin, 30.0% for ash, 0.1% for fat and 4.7% for water. Fractionated NDF was composed of cellulose (29.3%), hemicellulose (47.5%), lignin (16.3%), lipid (0%) and ash (1.4%), and ADF was made up of cellulose (60.2%), hemicellulose (5.0%), lignin (33.4%), lipid (0%) and ash (3.7%). Dietary fiber was chemically analyzed based on the Southgate method [24] and divided into fractions of cellulose, hemicellulose and lignin.

2.3. Determination of unbound chemical to RBF and HPLC

A mixture of chemical (10 μg/ml) and RBF (10 mg/ml) was treated and centrifuged for 30 min at 5000 rpm. The supernatant was analyzed on a Dupont Zorbax ODS column (0.46 cm i.d.×25 cm) and eluted with acetonitrile–water (80:20, v/v) at a flow rate of 0.9 ml/min for high-performance liquid chromatography (HPLC) (Shimazu LC 4A).

2.4. Extraction from bound mutagens in urine

Urine samples of 10 subjects, both smokers and nonsmokers, were collected at a volume of 100–200 ml. A mixture of each urine sample (100 ml) with 1 g of RBF (0.4 g for blue rayon) was gently shaken for 30 min at 37°C in a flask. Rice bran fiber was extracted by centrifugation for 20 min at 3000 rpm, washed with water and dried at 37°C. The same procedure was repeated three times using a fresh RBF (or blue rayon) each time. Various bound mutagens were eluted with ethyl acetate–ammonia (50:1, v/v) for reextraction from RBF and with methanol-concentrated ammonia (50:1) for that from blue rayon [25]. Rice bran fiber was removed by centrifugation for 20 min at 3000 rpm, and the supernatant was evaporated until dry under reduced pressure.

2.5. Influence of chemicals bound to RBF in bacterial culture

Bacterial strains used were *Escherichia coli* IFO 3806, *Bacillus cereus* BC 1233, *Salmonella typhimurium*

FE 3600, *Salmonella paratyphi* B wild strain, *Staphylococcus aureus* 209P, *Vibrio cholerae* ATCC 3363, *Aeromonas caviae* ATCC 11486, *Vibrio parahaemolyticus* wild, *Vibrio fluvialis* VDV aerobic bacteria, and *Peptostreptococcus magnus* GAI 0663, *Peptostreptococcus anaerobius* ATCC 27337, *Clostridium perfringens* ATCC 15703, *Eubacterium limosum* ATCC 8480, *Fusobacterium nucleatum* F-1, *Bacteroides gingivalis* 381, *Bacteroides thetaiotaomicron* 5482, *Bacteroides uniformis* 0061 and *Bacteroides fragilis* YCH 46 anaerobic bacteria.

2.6. Mutagenicity test

Mutagenicity test for *S. typhimurium* his$^-$ strain TA98 with the S9 mix was carried out by a plate-incorporation test applied β-glucuronidase (Wako Industries) as described previously [19,26].

2.7. Statistical analysis

Data of mutagenicity in smokers' and nonsmokers' urine were treated statistically with Student's t test ($P<.05$).

3. Results

3.1. Binding effects of 1-NP and Trp-p-1 to RBF

Each chemical, 1-NP, Trp-P-1 suspended in methanol and PCB in nonane at a concentration of 10 μg, was, respectively, incubated with RBF (10 mg/ml) suspended in methanol–water (1:1, v/v) for different times at 37°C, and the mixture was centrifuged for 20 min at 3000 rpm. Unbound chemical in the supernatant was analyzed by means of HPLC and gas chromatography (GC). It was found that each chemical was bound to RBF within 10 min under this condition (Fig. 1a). To determine the binding effects at different pH and

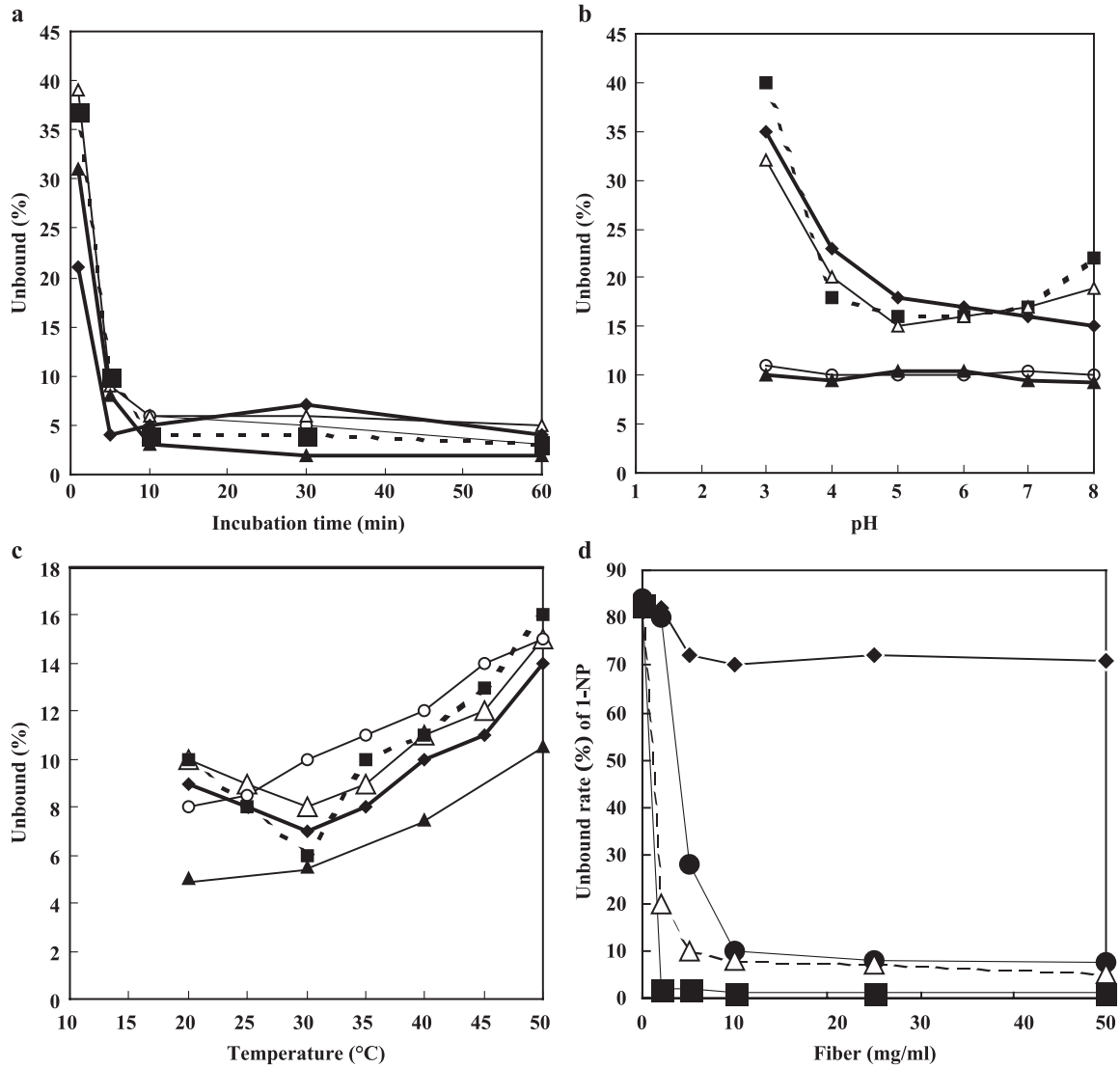

Fig. 1. Influence of incubation time (a), pH (b), temperature (c) and various fibers (d) on binding chemicals to rice fiber (RBF). Means±S.D. of three independent experiments. (a–c) 1-NP (○), PCB (▲), Trp-p-1 (△), Trp-p-2 (◆), IQ (■). (d) Cellulose (◆), RBF (●), lignin (△), active charcoal (■).

temperature during incubation, 1-NP and PCB for environmental mutagens and Trp-p-1, Trp-p-2 and IQ for different food-derived carcinogens were similarly tested as shown in Fig. 1b,c. The binding effects of food-derived carcinogens, Trp-p-1 and 2, were not sufficient under acidic conditions of pH 3–5 (Fig. 1b), but they were exerted at pH 5–7. It was found that 1-NP and PCB was steadily bound to RBF regardless of the various pH conditions. The results suggest that food-derived carcinogens were slightly reversible under acidic conditions because of the dissociation of the amino substituent (NH_2) in heterocyclic amine. On the other hand, the temperature for incubation only slightly influenced binding to RBF (Fig. 1c). Approximately 90% of 1-NP, Trp-P-1 and PCB was bound to RBF at 20–30°C, but the binding effects tended to decrease at higher temperatures from 40°C to 50°C. On the basis of the results, it is possible that a small amount of mutagens and carcinogens were bound to RBF within 10 min.

Chemicals bound to RBF were effectively recovered at a ratio of 81.3% for ethyl acetate–ammonia solvents (1:1, v/v) and of 71.6% for methanol–ammonia (1:1, v/v) while they were not for methanol, ethyl acetate, water, acetonitrile, chloroform or sulfuric acid. Chemicals used in this study, therefore, might either have a physical involvement with the chemical structure of RBF or have affinity with a hydrophobic substituent.

3.2. Chemical components of RBF

To determine the binding target of RBF, the fiber was partially purified and divided into two fractions, NDF and ADF, as described in Materials and Methods. Table 1 summarizes the ratio of 1-NP, Trp-P-1 and PCB bound to total dietary fiber (TDF), NDF and ADF. The binding effect to ADF was much higher than that to NDF and TDF, and 1-NP, Trp-p-1 and PCB were bound at a rate of over 97% to ADF. These results indicate that the unbound ratio of chemicals appeared to be associated with the contents of lignin in ADF rather than in NDF. In fact, 1-NP was steadily bound to lignin, RBF and activated charcoal as a control, but not to cellulose when only 1-NP was treated for binding test (Fig. 1d).

3.3. Interaction between lignin and PCB to various DFs

The binding effects of PCB on 13 different dietary fibers (NDF and ADF) were investigated on the bases of the results of lignin content (Fig. 2). The unbound ratio (%) of

Table 1
Binding of 1-NP, Trp-P-1 and PCB to TDF, NDF and ADF

Fraction	Lignin contents (%)	Percentage unbound (%±S.D.)		
		1-NP	Trp-P-1	PCB
TDF	10.7	10.9±0.1	11.0±0.2	7.3±0.6
NDF	15.1	7.9±0.1	8.2±0.1	4.9±1.7
ADF	56.1	3.6±0.1	3.9±0.1	2.1±0.2

TDF, total dietary fiber (RBF). Means (%) ±S.D. of three independent experiments.

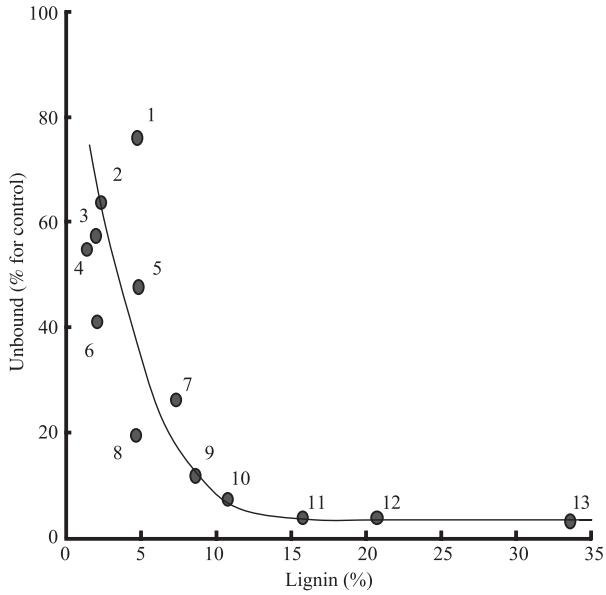

Fig. 2. Correlation between lignin and PCB (KC600) binding to RBF. 1, Burdock fiber; 2, corn fiber; 3, Okara fiber; 4, mush room fiber; 5, Hijiki fiber; 6, sweet potato fiber; 7, wheat bran fiber; 8, spinach fiber; 9 and 10, RBF including noncellular polysaccharide, cellulose and lignin; 11, NDF; 12, ADF; 13, rice fiber containing lignin and cellulose.

PCB increased with decreasing lignin content; PCB binding to NDF and ADF with high lignin content was more effective than other DFs such as burdock, corn, mushroom, sweet potato, wheat bran and spinach fibers when unbound PCB was measured (Fig. 2). In addition to PCB, binding of PCDFs and TCDDs to RBF was analyzed to compare it with active carbon (Table 2). Isomers of PCDF and TCDD were completely bound to active carbon in this condition, and substantially bound to RBF at a ratio of over 90% for tetra-, and hexaCBs, TCDFs and TCDD, except these of PCB (KC500), and 3,4,3′ ,4′ -tetraCB.

Twenty-two chemicals in corn fiber, RBF, cellulose and pulp-lignin were investigated to determine unbound ratios, all of which were treated with these fibers in a methanol–water mixture for 30 min at 37°C (Table 3). The unbound ratio of heterocyclic amines, nitrated aromatic compounds and three typical carcinogens to corn fiber and cellulose was higher than RBF and pulp-lignin; the interaction between RBF (and pulp lignin) and lignin showed a powerful affinity at a ratio of 80–92%. This suggests that the bulk of inorganic chemicals possess the ability to bind to RBF.

3.4. Binding effects of polycyclic aromatic compounds (PAHs) and PCBs

To determine the interaction between PAHs having a multiple ring structure and binding effects, nine PAHs at various concentrations were treated for 30 min. The unbound ratios of benzene, naphthalene and coumarin were at a higher level than the threshold level for detection. In contrast, PAH compounds with 3–5 aromatic rings were effectively bound to RBF at a small amount of 1 μg/ml (Fig. 3). It was found, therefore, that the binding ability of

Table 2
Binding of polychlorinated compounds to rice fiber (10 mg/ml)

Compound		Concentration (ppm)	Unbound (%)	
			Rice fiber	Active carbon (10 mg)
PCDFs	–	(0.5)	4.8	0
PCB (KC600)	–	(1.0)	6.9	0
PCB (KC500)	–	(1.0)	15.5	0
3,4,3′,4′ -	TetraCB	(0.2)	12.9	0
2,4,5,2′,4′,5′ -	TetraCB	(0.2)	6.2	0
2,3,4,5,3′,4′ -	HexaCB	(0.2)	4.7	0
2,3,4,5,2′,4′,5′ -	HexaCB	(0.2)	3.2	0
2,3,7,8-	TetraCDF	(0.2)	6.5	0
1,3,6,8-	TetraCDF	(0.2)	7.3	0
2,3,4,7,8-	PentaCDF	(0.2)	5.3	0
1,2,4,6,7-	PentaCDF	(0.2)	6.2	0
1,2,3,4-	TetraCDD	(0.2)	9.2	0
2,3,7,8-	TetraCDD	(0.2)	6.1	0

PAHs to RBF was associated with the number of aromatic rings, suggesting hydrophobic bonding of the lignin structure, although this might be reversible by physical factors such as pH and temperature as shown in Fig. 1b,c.

3.5. Inhibitory effect of inorganic and organic compounds on PCB binding to RBF

It is well known that cell types with walls containing hydrophobic polymers occur in food plant. It also was considered that lignin protects these walls from degradation under various chemical condition. Then, inhibitory effects of PCB (KC600) bound to RBF were studied at low- and high-dose levels of 2.5 and 250 mM, respectively, for nine inorganic compounds, and 1 and 10 μg/ml, respectively for six organic compounds (Fig. 4). Inhibition of binding of chemicals, except starch, cholesterol and oleic acid, was observed at low doses of inorganic and organic compounds, while it was not observed at high doses of these compounds; that is, binding was at a high-dose level of cholesterol and oleic acid in organic compounds, and of $CaCl_2$, $(NH_4)_2PO_4$ and NaH_2PO_4 in inorganic compounds, while the unbound ratio to RBF was elevated. Binding to RBF was slightly inhibited.

3.6. Influence of aerobic and anaerobic bacteria on binding of 1-NP and PCB to RBF

It is possible that chemicals bound to RBF are practically released by enteric bacteria in the gastrointestinal tract. Thus, 1-NP and PCB bound to RBF were tested to see whether or not they were released by enteric bacteria; the bacterial cultures used pathogenic bacteria including *E. coli*, *B. cereus*, *S. typhimurium*, *S. paratyphi*, *S. aureus*, *V. cholerae* non-O1, *Vibrio mimicus*, *Aeromonas caviae*, *V. parahaemolyticus*, *V. fluvialis* for aerobic bacteria, and *P. magnus*, *P. anaerobius*, *C. perfringens*, *Bacteroides adolescentis*, *E. limosum*, *F. nucleatum*, *B. gingivalis*, *B. thetaiotaomicron*, *B. uniformis* and *B. fragilis* for anaerobic bacteria. To elucidate the

Table 3
Binding of mutagens/carcinogens to DFs

Chemical		Unbound (%)			
		Corn fiber	Rice fiber	Cellulose	Lignin
Typical carcinogen	4-NQO	77.2	16.1	–	–
	B(a)P	78.4	10.5	78.4	–
	AF2	68.3	12.6	–	–
Nitroarene	1-NP	71.6	10.8	82.3	9.3
	1,3-DNP	68.4	7.3	74.9	3.8
	1,6- and 1,8-DNP	75.9	8.8	89.3	7.4
	3-NF	68.5	16.5	92.7	9.2
	3,7-DNF	71.5	11.7	94.6	9.5
	3,9-DNF	70.9	10.5	98.3	9.6
Heterocyclic amine	Trp-P-1	70.5	11.0	68.7	8.3
	Trp-P-2	65.7	11.3	71.7	8.2
	Glu-P-1	58.8	13.9	96.6	8.1
	Glu-P-2	61.5	18.3	97.7	9.3
	IQ	66.4	12.4	97.5	9.1
	MeIQ	67.2	15.2	96.4	8.8
	MeIQx	68.4	12.1	96.3	7.4
	AαC	66.5	14.4	94.9	7.5
	MeAαC	65.3	10.7	94.6	8.3
Flavonoid	Quercetin	–	10.2	–	–
	Rhamnetin	–	10.9	–	–
Others	Formaldehyde	–	100.0	–	–
	Ascorbic acid	–	97.7	–	–

–, Not tested.

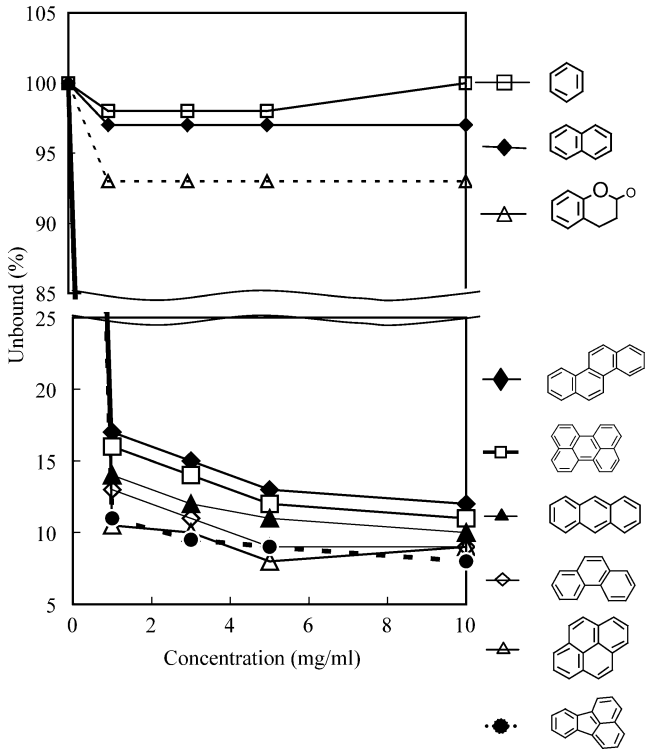

Fig. 3. Binding of polycyclic aromatic compounds to RBF.

Table 4
Influence of bacteria on 1-NP or PCB bound to RBF

Bacterial culture		Binding effect (%)	
		1-NP	PCB
Control (broth culture+1-NP or Trp-P-1)		100	100
Aerobes	*E. coli* IFO 3806	79.3	102.1
	B. cereus BC 1233	86.3	86.5
	S. typhimurium FE 3600	78.2	88.9
	S. paratyphi B	84.6	86.6
	S. aureus 209P	79.1	84.9
	V. cholerae non-O1	87.9	76.1
	V. mimicus ATCC 3365	87.8	95.8
	A. caviae ATCC 11486	84.5	89
	V. parahaemolyticus	88.7	88
	V. fluvialis CDC	85.5	80.1
Anaerobes	*P. magnus* GAI 0663	86.1	94.2
	P. anaerobius ATCC 27337	79.4	100
	C. perfringens GAI 0668	78.3	96.6
	B. adolescentis ATCC 15703	83.5	94.2
	E. limosum ATCC 8480	80.2	93.1
	F. nucleatum F-1	79.6	94.2
	B. gingivalis 381	82.7	91.4
	B. thetaiotaomicron 5482	75.2	95.6
	B. uniformis 0061	83.4	95.2
	B. fragilis YCH 46	77.3	97.4

1-NP and PCB of 10 μg/ml each bound to RBF (19 mg) was incubated in a separate nutrient broth prepared with 2×10^8 cells/ml.

influence of enteric bacteria on binding, 1-NP and PCB of 10 μg/ml each bound to RBF (10 mg) was incubated in a separate nutrient broth prepared to 2×10^8 cells /ml, and only in the broth culture as a control for 4 h at 37°C. After the incubation, the bacterial broth culture was centrifuged for 30 min at 5000 rpm, and the supernatant was analyzed by HPLC to determine free unbound chemicals. Binding of 1-NP and PCB was not affected in any broth cultures (Table 4). These data suggest that PAHs and chlorinated compounds were easily bound to RBF without inhibition by enteric bacteria, suggesting that chemicals bound to RBF were stable in the intestinal tract and would be excreted in stool without being fermented.

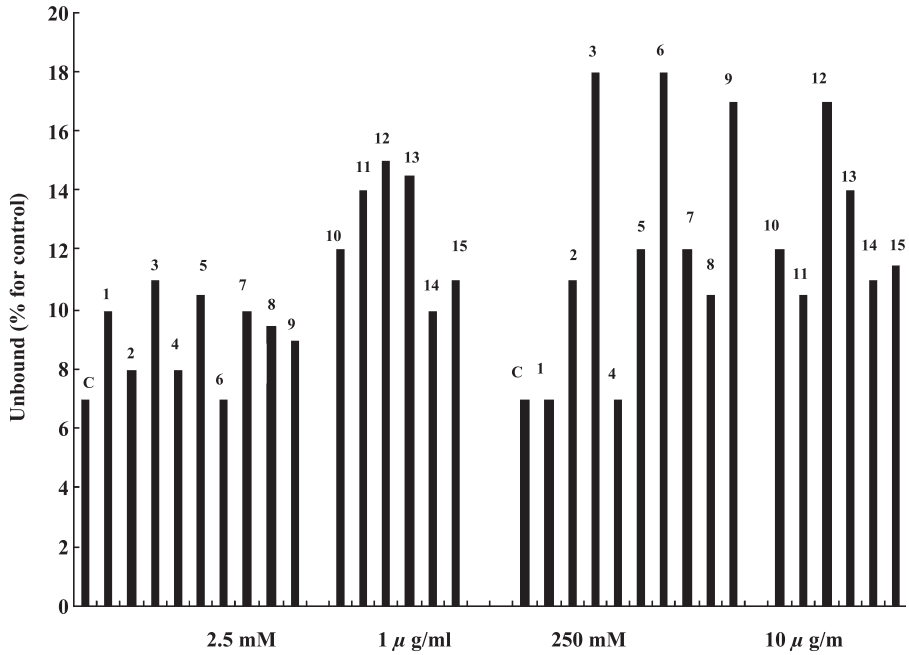

Fig. 4. Inhibitory effects of metal and organic compounds on binding of PCB (KC600) to RBF. 1, NaCl; 2, KCl; 3, $CaCl_2$; 4, $MgCl_2$; 5, $NaNO_3$; 6, $(NH_4)_2PO_4$; 7, CH_3COONa; 8, NH_4Cl; 9, NaH_2PO_4; 10, glucose; 11, starch; 12, cholesterol; 13, oleic acid; 14, bile salts; 15, NH_3; c, distilled water.

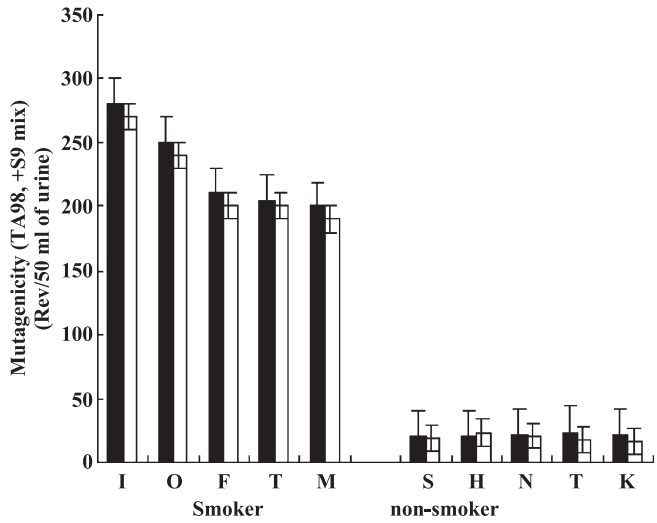

Fig. 5. Comparison of mutagenicity in smokers' and nonsmokers' urine using blue rayon and RBF. Means±S.D. of three independent experiments. Blue rayon (■), RBF (□).

3.7. Extraction of mutagens in urine using RBF and blue rayon

To compare the extraction of blue rayon and RBF, 10 urine samples (five smokers and five nonsmokers) were collected, and the effect of extraction of mutagens on both materials was determined (Fig. 5). A mixture of blue rayon (0.5 g) and RBF (1 g) with each urine sample (100 ml) in a separate tube was treated with the sample (100 ml) for 30 min at 37°C. Then, blue rayon and RBF were collected by centrifugation for 20 min at 3000 rpm, and mutagens bound to blue rayon and RBF were extracted with ethyl acetate–ammonia for RBF, and with methanol-concentrated ammonia for blue rayon, as described in Materials and Methods. Urinary mutagens extracted were bioassayed for the *Salmonella* tester strain TA98 in the presence of S9 mix. Urinary mutagens contain a variety of conjugates such as glucuronides and sulfates, and so the test was carried out in the presence of β-glucuronidase. This showed that when mutagens in smokers' urine were extracted, the yield was higher than that from nonsmokers' urine using blue rayon or RBF, and the difference was significant ($P<.05$). The ability to adsorb mutagens by RBF corresponded to that of blue rayon, suggesting that RBF was sufficiently adsorbent to recover urine mutagens. In urine samples, various metabolites were present normally in the form of glucuronized and sulfate conjugates. In this experiment, metabolites were shown to be adsorbed or bound to RBF, similar to the results obtained with blue rayon. In another experiment, RBF was available for extraction from cooked beef extracts, and similar results in urine were obtained (data not shown).

4. Discussion

Dietary components express a wide range of activities that can affect carcinogenesis. In these components, dietary desmutagens, antimutagenic agents acting outside the cells, function as chemical inactivators, enzymatic inducers, scavengers or antioxidants. Dietary fiber is widely considered to protect against cancer, especially colorectal cancer [2,3,5], although a large prospective epidemiological study has shown no apparent effect of DF intake on the development of colorectal cancer. The initial aim of using RBF was to promote the fecal excretion of polychlorinated compounds such as PCBs, PCDFs and polychlorinated-*p*-dioxines (PCDDs) remaining in Yusho patients in Japan. We first reported that oral administration of RBF was given to Japanese Yusho patients and Yu-Cheng patients in Taiwan [15], and the fecal excretion of these compounds was achieved; it was found that following the combined use of RBF and cholestyramine, an anion exchange resin that is not absorbed and metabolized, polychlorinated compounds in Yusho patients were partly excreted in their stool [15].

Rice bran fiber largely consists of noncellulose polysaccharide (24%), cellulose (10.3%) and lignin (10.7%). Lignin contents were measured at 18.7% for RBF, 15.3% for burdock fiber and 9.1% for spinach fiber; the lignin contents in RBF were characteristically much higher than those in other DFs [18]. In this experiment, binding of food-derived carcinogens, and 1-NP and PCB, was much more effective for acid detergent fibers than for neutral detergent fibers. Reinhold et al. [9] reported that binding of iron to wheat and maize fiber was minimal below pH 4.0, while it rapidly rose above pH 5.0–7.0. In the case of RBF, the binding effect of mutagens and carcinogens was similarly pH dependent. Food-derived mutagens and carcinogens strongly interacted with lignin, but the binding was reversible because of the possible dissociation of the amino substituent (NH_2) under acidic conditions. These results suggest hydrogen bonding between lignin and chemicals with a triple ring chemical structure. In addition, chemicals were recovered from RBF at a rate of 81.3% by extraction with a mixture of ethyl

acetate and ammonia, but not with a solvent alone: solvents included methanol, ethyl acetate, water, acetonitrile, chloroform and sulfuric acid.

The binding effect to RBF might be liked to lignin polymer formation. Accordingly, it is possible that binding would be reduced if polymerization was necessary in the physical and chemical states; that is, binding to RBF was variable with pH and temperature, and in the presence of organic and inorganic chemicals, as described above. In a similar result, the cellulose-binding domain was reported to be temperature sensitive, showing an increased affinity at lower temperature [26]. In another experiment in rats, we found that the binding effect of Trp-p-1 and 1-NP was reduced in the stomach, but not in the small intestine, cecum or colon (unpublished data), while there was also a report that binding between cellulose-treated lignin and MeIQx was reversible [12]. Regarding binding of 1-NP and PCB to RBF, the results were not influenced by enteric bacterial culture, demonstrating that their release is *inhibited* in the gastrointestinal tract. As a conclusion of the in vivo experiments, it was suggested that the fecal excretion of PCB and food-derived carcinogens was accelerated by administration of RBF in rats, but the other DFs that had lower lignin contents, such as corn and burdock fibers, reduced fecal excretion.

Normally, lignin originates from the cell walls of food plants, parenchyma cells that are extensively degraded by intestinal bacteria in colon, and hydrophobic polymers, as well as suberin and cutin [13]. In various DFs, lignin has been reported to exert a significant effect on the excretion of pesticides such as chlorpropham and chlorothalonil [10], and on uterine mammary carcinogenesis induced by *N*-methyl-*N*-nitrosourea [27]. It was predicted, therefore, that they might form covalent binding with RBF or modify the potential for hydrogen bonding with lignin. Dietary fiber is widely considered to protect against colorectal cancer. However, it was reported that although some DFs are insoluble materials able to protect against the cancer, others appear to enhance carcinogenesis [13]. Lignin and the other polymers might be involved to the protection.

Human urinary carcinogens were investigated as biomarkers of tobacco-induced carcinogenesis, and food mutagens [28], and for the assessment of oxidative DNA damage [29]. In general, urinary mutagens were extracted and concentrated with XAD-2 resin and blue cotton [30]. The blue cotton (or blue rayon) extraction proved to be effective in isolating mutagenic compounds from cooked beef extract [25,30]. Similarly, RBF was found to be effective for extraction of mutagens and conjugates such as glucuronides and sulfates that are not split by the enzymes in *Salmonella* or in the mammalian liver as in the S9 mix. For urine samples obtained from smokers, the extraction of mutagens by RBF corresponded to that of the blue rayon used as control [25,29]. These results suggest that RBF has binding ability for conjugates containing metabolites in urine although the extraction of urinary components was performed using 10 urine samples.

Among the Japanese, the dietary habits for the past 50 years have changed from a diet consisting mostly of rice, fish and vegetables to a high-fat diet, including excessive intake of synthetically manufactured hybrid foods; therefore, various cancers such as colon and prostate cancer have been increasing since 1960 [31]. It is necessary to focus on the significance of RBF, originally common in the rice-based diet, in Japan.

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