

Uptake of Polycyclic Aromatic Hydrocarbons by Insoluble Dietary Fiber

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The sorption isotherms of polycyclic aromatic hydrocarbons (PAHs) by insoluble dietary fiber (IDF) were measured in artificial gastric juice (AGJ) and artificial intestinal juice (AIJ) at 37°C and then the sorption behavior of IDF was examined. Benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and benzo[*a*]pyrene were used. It was suggested that the uptake of PAHs by carboxymethyl cellulose (CMC), agar, and IDF from powdered young barley leaves (YBL) involved not single sorption behavior, but complex sorption behavior. YBL had an extraordinarily high sorption capacity (60.7–147.3 nmol/g in AGJ and 76.7–162.7 nmol/g in AIJ), as compared with CMC (1.5–3.6 nmol/g in AGJ and 1.1–2.7 nmol/g in AIJ) and agar (0.1–0.4 nmol/g in AGJ and 0.3–0.7 nmol/g in AIJ) at a residual concentration of 5.0 nM. The sorption of PAHs in AGJ and the desorption of PAHs from IDF in both AGJ and AIJ indicate that the sorbed PAH molecules are held firmly by IDF while it passes through the digestive tract *in vivo*. From the results on sorption capacity and the removability of PAHs from IDF, YBL would be expected to be more useful than CMC and agar.

Key words — polycyclic aromatic hydrocarbon, insoluble dietary fiber, sorption capacity, sorption mechanism

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are present in the incomplete combustion products of organic materials. Some of these PAHs have mutagenicity¹⁾ or carcinogenicity, especially benzo[*a*]pyrene (BaP), which is also regarded as an endocrine-disrupting chemical. The concentration of mutagenic PAHs in cigarette smoke is 61.0 ng/cigarette.²⁾ The amounts of BaP in the smoke from 10 commercial brands of cigarette was 11.9–19.3 ng/cigarette.³⁾ Atmospheric PAH concentrations in Seoul, Republic of Korea, ranged from 11 to 350 ng/m³ in 1998–1999.⁴⁾ The average concentration of mutagenic PAHs was higher during the winter (1.95–1.87 ng/m³) than during the summer (0.34–0.88 ng/m³) in Kawasaki city, Japan, in 2000.⁵⁾ The BaP levels of 27 smoked fish samples from traditional kilns ranged from 0.2 to 4.1 µg/kg, with a mean value of 1.2 µg/kg.⁶⁾ The concentra-

tions of carcinogenic PAHs in 20 seafood samples were in the range of 9 to 123 ng/kg wet weight.⁷⁾ The average total amount of PAHs in 18 coffee samples was 10.12 µg/kg, while mate tea showed a lower level of contamination (0.70 µg/kg).⁸⁾ The average concentrations of PAHs in 25 crude sunflower oils and in 10 refined sunflower oils were 11.05–11.15 and 6.9 µg/kg, respectively.⁹⁾ The mutagenic PAH level in olive pomace oil was 74.5–92.1 µg/kg.¹⁰⁾ Soot, which is formed as a consequence of incomplete combustion of coal and biomass, contains high levels of PAHs, being the most important source of PAHs in the environment.¹¹⁾ High concentrations of PAHs in river sediment suggested that hydrophobic PAHs in water might easily accumulate in river sediment after adsorbing to suspended solids.¹⁾ Therefore human exposure to PAHs occurs by direct inhalation of polluted air^{4,5,11)} and cigarette smoke,^{2,3)} dietary intake of foodstuffs,^{6–8)} polluted water,¹⁾ and edible oils,^{9,10)} and dermal contact with soot¹¹⁾ and polluted soil. The results of the above studies^{1–11)} suggest that mutagenic PAHs are taken into the human body gradually through various routes.

The sorption of mutagens has been suggested

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as one mechanism by which dietary fiber (DF) protects against colorectal cancer. DF can potentially adsorb BaP.¹²⁾ The absorption of BaP from the intestinal tract is markedly affected by dietary components.¹³⁾ The ability of BaP to adsorb *in vitro* to α -cellulose was strongly related to the hydrophobicity of BaP.¹⁴⁾ No sorption mechanism of PAHs by DF, however, based on their sorption isotherms has been elucidated. The sorption process generally consists of the penetration of PAHs into insoluble DF (IDF) and the adsorption of PAHs by IDF. In the sorbing system, PAHs penetrate the swelled IDF.

In this paper, the sorption isotherms of four mutagenic PAHs and nonmutagenic chrysene¹⁾ by three types of IDF were measured in artificial gastric juice (AGJ) and artificial intestinal juice (AIJ). The five types of PAH (Fig. 1) are basically common mutagenic or injurious substances found in cigarette smoke,^{2,3)} polluted air^{4,5,11)} foodstuffs,^{6–8)} edible oils,^{9,10)} and polluted water.¹⁾ The classification of sorption isotherms of PAHs by IDF, the amounts of PAHs sorbed by IDF, and the sorption-desorption of PAHs in AGJ and AIJ were investigated to elucidate the sorption mechanism of PAHs by IDF, the most suitable IDF to take up PAHs, and how PAHs are taken up by IDF in the human gastrointestinal tract. The purpose of the present *in vitro* study was to predict to what extent IDF takes up PAHs and how IDF acts to intercept PAHs *in vivo*.

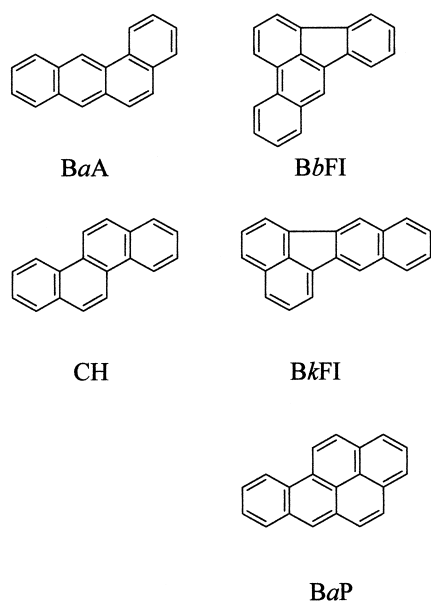


Fig. 1. Structure of PAHs Used

BaA, benz[a]anthracene; CH, chrysene; BbFl, benzo[b]fluoranthene; BkFl, benzo[k]fluoranthene; BaP, benzo[a]pyrene.

MATERIALS AND METHODS

Materials — PAHs and IDF were purchased as follows: benz[a]anthracene (BaA), chrysene (CH), benzo[b]fluoranthene (BbFl), benzo[k]fluoranthene (BkFl), BaP, pyrene as an internal standard, and carboxymethyl cellulose for column chromatography (CMC) from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); and agar (powder) from Sigma (St. Louis, MO, U.S.A.). BaA, CH, BbFl, and BkFl were of the highest grade for environmental analysis, and BaP and pyrene were of the highest grade. IDF from the powder of young barley leaves (YBL) (carbohydrate, 12.6%; protein, 29.7%; lipid, 6.8%; dietary fiber, 47.2%; Yamamoto Kanpō Seiyaku Inc., Komaki, Japan), which is commercially available, was prepared as follows. After the powder of young barley leaves was gelatinized for 10 min in a boiling water bath, the saccharides in the tissue were decomposed using isoamylase from *Pseudomonas* in a citric buffer solution (pH 3.5) and then using α -amylase from porcine pancreas in a phosphate buffer solution (pH 6.9), respectively, for 20 hr at 40°C. The suspension was incubated by adding sodium dodecyl sulfate, and the supernatant (protein) was removed. The deproteinizing procedure was repeated five times. The soluble vitamins and components decomposed from saccharides were removed by washing the residue in distilled water a few times. Lipid and oil-soluble vitamins were removed by extracting the residue in diethyl ether five times, and then the water-insoluble residue was dried for 24 hr at 100°C. It was confirmed that no soluble saccharide and protein were retained in YBL using the phenol-sulfuric acid method and the semimicro-Kjeldahl method, respectively. DF from the powder of young barley leaves was used because it contains more IDF (approximately 95% by weight) than DF from starch, guar gum, and cereals.

Preparation of AGJ, AIJ, and PAH Solution — AGJ (pH 1.2) and AIJ (pH 6.8) were prepared according to the method of the *Japanese Pharmacopoeia*.¹⁵⁾ Initial concentrations of the single PAH alcohol solution were 7.5, 15, 30, and 60 ng/ml. Linearity of the calibration curve of single PAH standards was established over a range of 7.5 to 60 ng/ml ($R = 0.9930\text{--}0.9967$, $n = 4$). The alcohol solutions of the five mixed PAHs with appropriate concentrations from 50 to 180 nmol/l were prepared for determining the sorption isotherm.

Procedure for Determining Sorption Isotherm — The quantity of IDF used to obtain the amount

Table 1. Comparison of AIC^{a)} Values in Different Sorption Equations Applied to Sorption Isotherms of PAHs on Insoluble Dietary Fiber in AGJ and AIJ

Insoluble dietary fiber in juice	PAH	Type 1 ^{b)}	Type 2 ^{b)}	Type 3 ^{b)}
CMC in AGJ	BaA	155	152	155
	CH	153	145	163
	BbFl	175	174	173
	BkFl	182	175	184
	BaP	182	178	181
Agar in AGJ	BaA	97	92	94
	CH	79	79	77
	BbFl	112	110	110
	BkFl	89	88	88
	BaP	95	95	93
YBL in AGJ	BaA	78	77	76
	CH	143	142	141
	BbFl	145	145	143
	BkFl	137	137	135
	BaP	133	164	135
CMC in AIJ	BaA	131	128	137
	CH	129	127	148
	BbFl	140	140	138
	BkFl	146	144	153
	BaP	106	102	134
Agar in AIJ	BaA	26	24	51
	CH	25	20	45
	BbFl	39	39	37
	BkFl	53	50	55
	BaP	45	49	76
YBL in AIJ	BaA	34	34	32
	CH	66	66	64
	BbFl	61	62	63
	BkFl	62	77	63
	BaP	60	61	62

a) Akaike's information criterion, $AIC = n \ln SS + 2m$; n , number of experimental data; SS , sum of residual squares; m , number of parameters in sorption equation. b) Sorption equation, type 1, $V = abC/(1 + aC)$, Langmuir equation; type 2, $V = aC^{1/n}$, Freundlich equation; type 3, $V = aC$, Henry equation; V , amount sorbed; C , residual concentration; a , b , and n , constants. No significant differences were observed between the AIC value groups of types 1, 2, and 3.

of PAH sorbed was determined from the relation between the quantity of IDF and the amount sorbed to be 20 mg. The time to reach sorption equilibrium was 5 min, indicating that PAHs diffuse rapidly to reach the sorption space of IDF. IDF (20 mg) was added to a hermetic glass container (25 ml) containing the five mixed PAHs in alcohol (0.2 ml) and AGJ or AIJ (1.8 ml), and the slurry was incubated in a water bath shaker at 37°C. After incubation, the suspension was centrifuged for 5 min at 3000 rpm. An internal standard (0.025 µg/ml pyrene alcohol solution, 50 µl) was added to the supernatant (1 ml).

The residual concentration of PAH in the supernatant was determined using HPLC. It was confirmed that no PAH was adsorbed on the inner surface of the glass container. The amount sorbed was estimated using the calibration curve of PAH. The sorption isotherm was obtained by plotting the amount sorbed against the residual concentration. The line was smoothed using the equation yielding the lowest Akaike's information criterion (AIC) value (Table 1).

Desorption of PAH from IDF in AGJ and AIJ — After the sorption of PAH by IDF in AGJ,

the suspension was filtered. The precipitate was transferred to AGJ or AIJ (2.0 ml) in another glass container and the PAHs were eluted from the precipitate in AGJ or AIJ for 5 min at 37°C. The elution time was set to compare the amount sorbed with the amount desorbed during the same elapsed time. The amount of PAH sorbed by IDF was calculated using the residual concentration found in the supernatant obtained upon centrifugation (3000 rpm, 5 min) of the suspension.

HPLC—Each PAH was measured using the method of Karl and Leinemann.⁶⁾ A Hitachi HPLC system, model L-6200 (Hitachi Co., Ltd., Tokyo, Japan), equipped with a Hitachi F-1500 fluorescence spectrophotometer was used. A 30 × 2.0 mm i.d. guard column and a 250 × 4.6 mm i.d. column of Wakosil-PAHs (Wako Pure Chemical Industries, Ltd.) were used. The excitation wavelength was set at 280 nm and emission at 395 nm, with column temperature, 40°C; injection volume, 20 μl; and flow rate, 1.0 ml/min. Mobile phase A was 80% methanol + 20% water and mobile phase B was 100% acetonitrile. The gradient was 45% A + 55% B for 5 min, increasing linearly up to 90% A + 10% B for 12 min, and then the same for 12–22 min. The equilibration time until the next analysis was 20 min at 45% A + 55% B.

Statistical Analysis—Statistical analyses of the sorption isotherms were performed using the non-linear least-squares regression program WinNonlin (Pharsight Co., Mountain View, CA, U.S.A.), to compare the applicability of three types of equation to the sorption data. The AIC value was used to decide the sorption type that provided the best fit of the amount sorbed-residual concentration curve. The lowest AIC value was considered to be the optimum of several possible equations.¹⁶⁾ The sorption equations were defined as:

type 1, $V = abC/(1 + aC)$, Langmuir equation

type 2, $V = aC^{1/n}$, Freundlich equation

type 3, $V = aC$, Henry equation

where V is the amount sorbed; C is the residual concentration; and a , b , and n are the parameters.

The significance level in the AIC value group was determined with multiple comparisons using Scheffe's method in the SuperANOVA program (Abacus Concepts, Inc., Berkeley, CA, U.S.A.). Differences of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Type of Sorption Isotherm

The shapes of the sorption isotherms depended on the type of IDF and on the pH of the artificial juices, as shown in Figs. 2–7. The applicability of the isotherm equation to sorption data was investigated to elucidate the sorption behavior of IDF in AGJ and AIJ. The applicability of the sorption equation was compared using AIC values taken from a simultaneous curve. Three sorption equations for which the number of parameters was 2 or less were selected empirically. Each of the three, that is, the type 1 Langmuir equation, the type 2 Freundlich equation, and the type 3 Henry equation, was tested. Table 1 gives a comparison of AIC values. Although the lowest AIC value was elucidated to be the optimum,¹⁶⁾ the AIC values for type 2 or type 3 resembled each other very closely.

No significant differences were observed between the AIC value groups of types 1, 2, and 3 for the sorption of PAHs by CMC, agar, and YBL. The isotherm of type 1 is known as the Langmuir equation, which anticipates that sorbed molecule layers are in general only one molecule in thickness. The

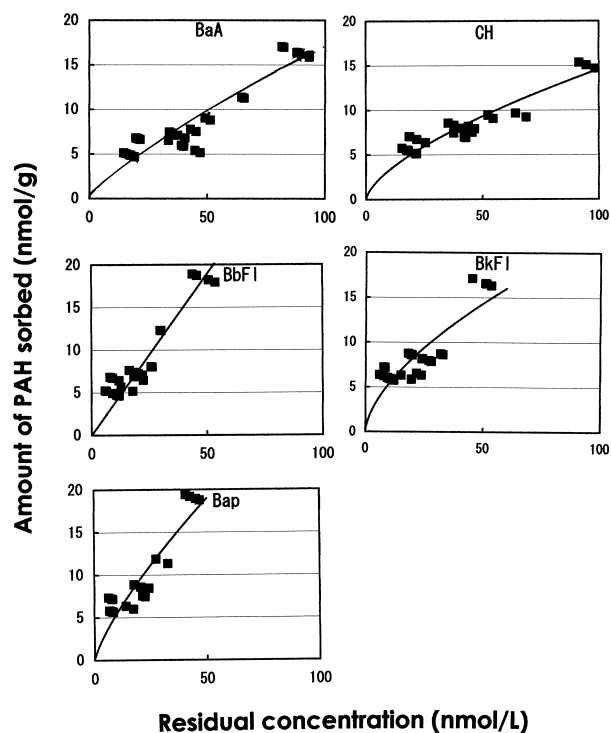


Fig. 2. Sorption Isotherms of CMC for PAHs in Artificial Gastric Juice
BaA, benz[a]anthracene; CH, chrysene; BbFl, benzo[b]fluoranthene; BkFl, benzo[k]fluoranthene; BaP, benzo[a]pyrene; CMC, carboxymethyl cellulose.

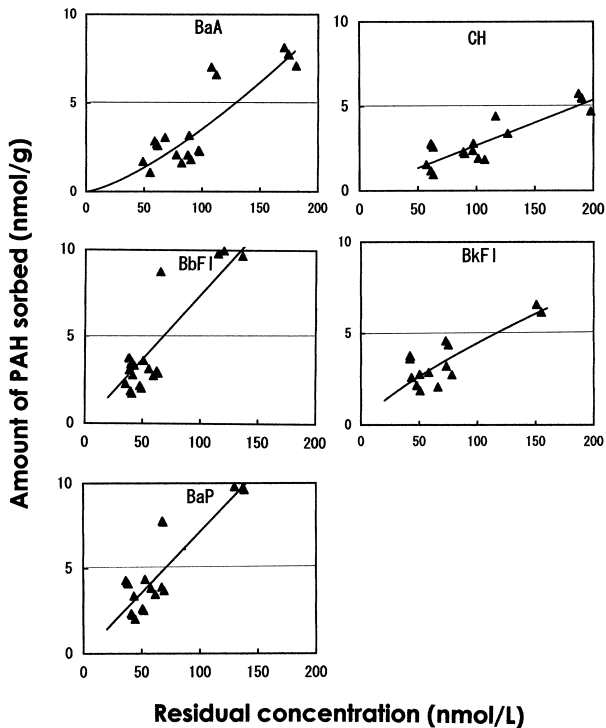


Fig. 3. Sorption Isotherms of Agar for PAHs in Artificial Gastric Juice
 BaA, benz[a]anthracene; CH, chrysene; BbFl, benzo[b]fluoranthene; BkFl, benzo[k]fluoranthene; BaP, benzo[a]pyrene.

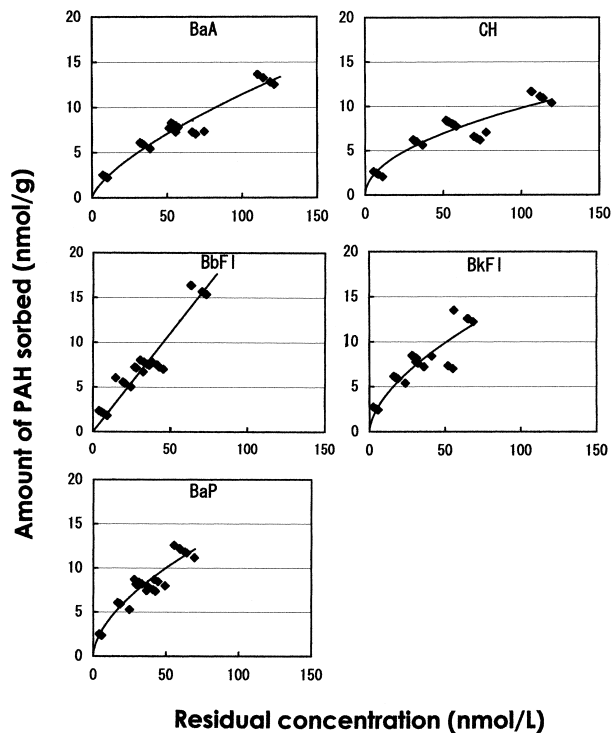


Fig. 5. Sorption Isotherms of CMC for PAHs in Artificial Intestinal Juice
 BaA, benz[a]anthracene; CH, chrysene; BbFl, benzo[b]fluoranthene; BkFl, benzo[k]fluoranthene; BaP, benzo[a]pyrene; CMC, carboxymethyl cellulose.

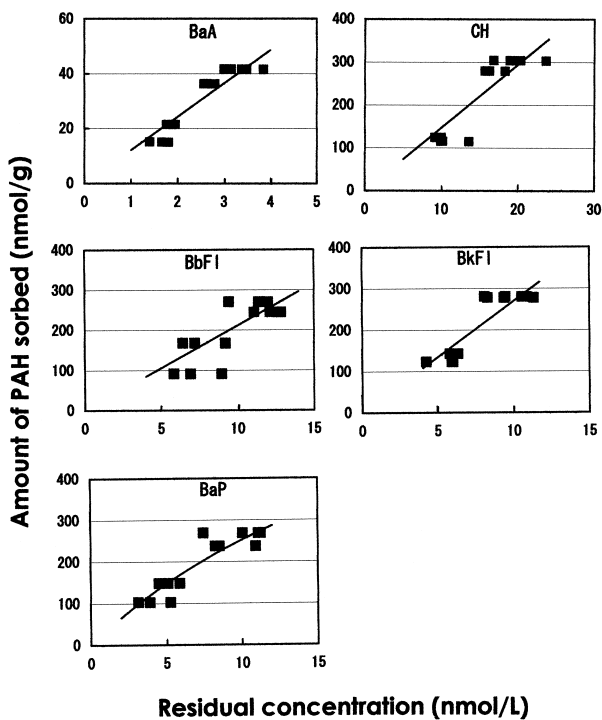


Fig. 4. Sorption Isotherms of YBL for PAHs in Artificial Gastric Juice
 BaA, benz[a]anthracene; CH, chrysene; BbFl, benzo[b]fluoranthene; BkFl, benzo[k]fluoranthene; BaP, benzo[a]pyrene; YBL, insoluble dietary fiber prepared from the powder of young barley leaves.

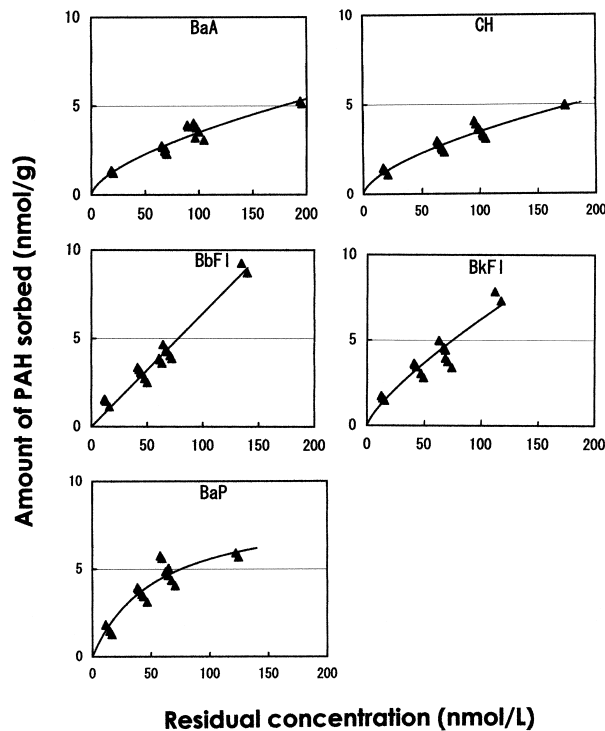


Fig. 6. Sorption Isotherms of Agar for PAHs in Artificial Intestinal Juice
 BaA, benz[a]anthracene; CH, chrysene; BbFl, benzo[b]fluoranthene; BkFl, benzo[k]fluoranthene; BaP, benzo[a]pyrene.

Table 2. Amount of PAHs Sorbed at a Residual Concentration of 5.0 nmol/l

Insoluble dietary fiber in juice	Amount sorbed (nmol/g)				
	BaA	CH	BbFl	BkFl	BaP
CMC in AGJ	1.46 ^{a)}	2.11 ^{a)}	1.91 ^{a)}	3.55 ^{a)}	3.25 ^{a)}
Agar in AGJ	0.06 ^{a)}	0.14 ^{a)}	0.37 ^{a)}	0.33 ^{a)}	0.36 ^{a)}
YBL in AGJ	60.72 ^{a)}	73.57 ^{a)}	105.61	134.88	147.26
CMC in AIJ	1.44	2.22	1.11	2.56	2.67
Agar in AIJ	0.55	0.55	0.32	0.63	0.73
YBL in AIJ	76.67 ^{a)}	83.06 ^{a)}	151.25	162.72	156.93

a) The values were calculated using the most appropriate equation.

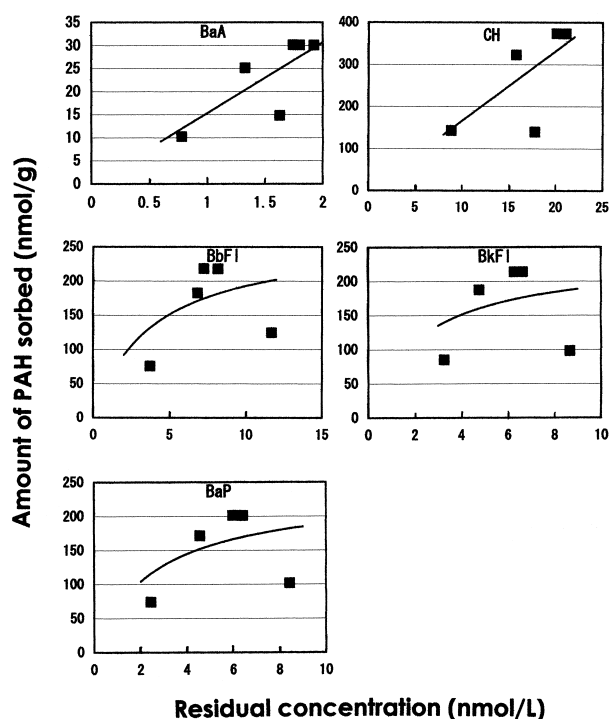


Fig. 7. Sorption Isotherms of YBL for PAHs in Artificial Intestinal Juice

BaA, benz[*a*]anthracene; CH, chrysene; BbFl, benzo[*b*]fluoranthene; BkFl, benzo[*k*]fluoranthene; BaP, benzo[*a*]pyrene; YBL, insoluble dietary fiber prepared from the powder of young barley leaves.

isotherm of type 2 has been explained by postulating the formation of layers several molecules thick. The isotherm of type 3, generally known as the Henry equation, predicts a linear relation between the amount sorbed and sorbate concentration. The uptake of PAHs by CMC, agar, and YBL in AGJ and AIJ is suggested to involve not a single sorption behavior, but a complex sorption behavior of types 1–3. It is not possible to conclude from the results given above that PAHs are taken up chemically by IDF.

Amount of PAH Sorbed by IDF

The amounts of PAHs sorbed by CMC, agar,

and YBL were measured to determine a suitable IDF to take up PAHs and then compare the sorption capacity of this IDF in AGJ (pH 1.2) with that in AIJ (pH 6.8). Since the sorption capacity of various types of IDF should be evaluated by comparing the amount sorbed at the same residual concentration, the amounts sorbed were obtained at 5.0 nmol/l, which was common to the sorption isotherms in Figs. 2–7. The results are shown in Table 2. The amounts of PAHs sorbed by CMC were greater in AGJ (1.46–3.55 nmol/g) than in AIJ (1.44–2.67 nmol/g). Those sorbed by agar were greater in AIJ (0.32–0.73 nmol/g) than in AGJ (0.06–36 nmol/g). Those sorbed by YBL were greater in AIJ (76.67–162.72 nmol/g) than in AGJ (60.72–147.26 nmol/g). The results of the amounts sorbed indicate that YBL had an extraordinarily high sorption capacity as compared with CMC and agar and would be expected to be the most suitable IDF to take up PAHs in AGJ and AIJ. The results that more of the five PAHs was sorbed by YBL in AIJ than in AGJ indicate that more of the PAHs was taken up in the neutral (pH 6.8) than in the acidic pH range (pH 1.2).

The result that more BbFl, BkFl, and BaP than BaA and CH was sorbed by YBL agreed with the result of the amount of hydrophobic BaP sorbed by α -cellulose.¹⁴⁾ It is therefore confirmed that the higher the hydrophobicity of PAHs, the more sorbed by YBL, because the logarithms of the octanol-water partition coefficient ($\log P$) of BaA and CH (a five-membered ring) and BaP (a six-membered ring) are 5.91, 5.73, and 6.20, respectively.¹⁷⁾ YBL is composed of cellulose (55%), arabinoglucuronoxylan (30%), xyloglucan (3%), rhamnogalacturonan (5%), and other polysaccharides (7%).¹⁸⁾ YBL, composed of polymers of glucose, glucuronic acid, xylose, and rhamnose, has more hydrophilic groups than CMC and agar. If the sorption of PAHs by YBL is affected by the number of hydrophilic groups, more BaA and CH would be taken up by YBL than

Table 3. Sorption and Desorption of PAHs in AGJ and AIJ

Insoluble dietary fiber	Juice	Procedure	Percent of PAH				
			BaA	CH	BbFl	BkFl	BaP
CMC	in AGJ	Sorp.	82.1 ± 1.1	84.9 ± 2.5	91.3 ± 2.2	92.7 ± 1.6	93.5 ± 0.8
		Desorp.	3.2 ± 1.3	1.0 ± 1.8	1.2 ± 2.1	1.3 ± 1.5	1.1 ± 1.7
	in AIJ	Desorp.	1.8 ± 1.8	0.9 ± 1.5	3.8 ± 2.1	3.5 ± 2.2	3.8 ± 3.1
		Final sorp.	77.1	83.1	86.3	87.9	88.6
Agar	in AGJ	Sorp.	41.6 ± 1.9	41.1 ± 4.7	56.2 ± 3.5	55.3 ± 7.1	60.3 ± 5.1
		Desorp.	3.7 ± 2.6	0	0	3.2 ± 1.8	1.7 ± 3.0
	in AIJ	Desorp.	1.6 ± 2.2	0	0	4.7 ± 1.6	3.2 ± 2.8
		Final sorp.	36.3	41.1	56.2	47.4	55.4
YBL	in AGJ	Sorp.	98.4 ± 0.2	98.7 ± 0.2	99.2 ± 0.1	99.3 ± 0.1	99.2 ± 0.1
		Desorp.	0.7 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
	in AIJ	Desorp.	0.8 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.1
		Final sorp.	96.9	97.7	97.7	98.2	98.2

Each initial concentration of the five mixed PAHs alcohol solution (1 ml) was as follows: BaA, 2.10; CH, 1.60; BbFl, 1.35; BkFl, 1.45; BaP, 1.35 nmol. IDF (20 mg) was added to a glass container containing the five mixed PAH alcohol solution (0.2 ml) and AGJ (1.8 ml). After incubation, the amount of each PAH taken up by IDF was calculated using the equation: percent equation (%) = $(C_i - C_r) \div C_i \times 100$, where C_i is the initial concentration (nmol/ml) and C_r is the residual concentration (nmol/ml). Values are mean ± S.D. of 3–4 experiments.

BaP. Since the assumption is contrary to the experimental results, it is considered that the sorption of PAHs by YBL is not affected by hydrophilic groups. The results presented here agree with those¹⁴⁾ showing that the sorption ability of BaP to cellulose was related to the hydrophobicity of BaP. It was reported that the sorption capacity of a hydrophilic polymer acidified with hydrogen sulfide was enlarged by the neutralization with base, owing to the swelling of polymer.¹⁹⁾ From the result that more PAH was taken up in the neutral than acidic pH range, it is suggested that the sorption spaces of YBL are enlarged in the neutral rather than acidic range. The results can be well explained by assuming that the sorbed PAHs are physically immobilized in the microfibrils of cellulose fiber and that the microspacings of YBL are enlarged after swelling in AIJ rather than in AGJ.

Sorption-Desorption of PAH in AGJ and AIJ

The sorption and desorption of PAHs in AGJ and AIJ were measured to estimate the removability of PAHs from IDF, as shown in Table 3. The value of sorption and desorption was expressed in percentage terms, because this made it easy to determine the degree to which IDF can take up PAHs from the solution. The percent desorption of PAHs from IDF was measured *in vitro* to predict whether the sorbed PAH molecules are held firmly by IDF while it passes through the digestive tract *in vivo*.

The percent sorption of PAHs by CMC, agar, and YBL in AGJ was 82.1–93.5%, 41.1–60.3%, and 98.4–99.3%, respectively. The percent desorption of PAHs from CMC, agar, and YBL in both AGJ and AIJ was 1.9–5.0%, 0–7.9%, and 1.0–1.5%, respectively. The final percent sorption of PAHs by CMC, agar, and YBL was 77.1–88.6%, 36.3–56.2%, and 96.9–98.2%, respectively. From the results of sorption and desorption, it was elucidated that PAHs are taken up irreversibly by IDF and that the sorbed PAHs are more firmly held by YBL than by CMC and agar. The results on the amount sorbed (Table 2) and the percent sorption-desorption (Table 3) suggest that YBL would be more useful than CMC and agar as an agent to intercept PAHs *in vivo*.

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