Uptake of Heterocyclic Aromatic Amine by Insoluble Dietary Fiber in Artificial Gastric and Intestinal Juice

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Abstract

Objectives: The purpose of the present *in vitro* study was to predict to what extent dietary fiber (DF) takes up heterocyclic aromatic amine (HAA) and how DF acts to intercept HAAs *in vivo*.

Methods: The sorption isotherms of 2-amino-3-methyl-3H-imidazo[4,5-f]quinoline (IQ), 2-amino-3,4-dimethyl-3H-imidazo[4,5-f]quinoline (MeIQ), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole acetate (Trp-P-1), and 3-amino-1-methyl-5H-pyrido[4,3-b]indole acetate (Trp-P-2) for DF were measured in artificial intestinal juice (AIJ) and artificial gastric juice (AGJ) at 37°C. The desorption of HAA from DF was measured in AGJ and AIJ.

Results: The sorption isotherms were statistically classified into four types. The percentage of Trp-Ps taken up by carboxymethylated cellulose (CMC) and agar in AGJ (pH, 1.2) was 52–56% and 58–78%, respectively. On the other hand, the percentage of Trp-Ps taken up by CMC and agar in AIJ (pH, 6.8) was 97–98% and 87–89%, respectively. The percentage of IQ and MeIQ sorbed by CMC was 21–27% in AGJ and 100% in AIJ. Collagen and chitin did not remove any HAAs in AGJ, but removed 4–69% in AIJ. In the four-component solution, the percentage of HAA taken up by DF was almost the same or significantly increased, with a few exceptions, as compared with that in the one-component solution.

Conclusion: The results indicated that MeIQ is mainly held on the surface of CMC in AIJ, and that Trp-P-1 and Trp-P-2 are mainly held in the interior of agar in AGJ and AIJ. The results on sorption-desorption *in vitro* indicate that sorbed HAAs in the stomach are held more firmly by agar than by CMC while DF passes through the human intestinal tract. CMC and agar would be expected to be more useful than collagen and chitin as agents intercepting HAAs.

Key words: dietary fiber, heterocyclic aromatic amine, type of sorption isotherm, sorption mechanism, amount sorbed

Introduction

The sorption of mutagen by DF (dietary fiber) has been suggested as one mechanism by which DF protects against colorectal cancer. The ability of chlorophyllin-chitosan to sorb heterocyclic aromatic amine (HAA) (1), the role of cell walls in protection against carcinogens (2-3), the higher sorption power of burdock, dropwort, and bamboo for IQ (2-amino-3-methyl-3H-imidazo[4,5-f]quinoline), Trp-P-1 (3-amino-1,4-dimethyl-

5H-pyrido[4,3-b]indole acetate) and Trp-P-2 (3-amino-1-methyl-5H-pyrido[4,3-b]indole acetate) (4), and the binding of IQ, MeIQ (2-amino-3,4-dimethyl-3H-imidazo[4,5-f]quinoline) and MeIQx (2-amino-3,8-dimethyl-3H-imidazo[4,5-f]quinoline) by DF (5) have been reported. No qualitative observation, however, on the sorption isotherms of DF for HAA has been made. A qualitative analysis of the relationship between DF and HAA or solvent (artificial juice) based on sorption isotherms would provide valuable information on the strength of the binding of HAA to DF and on how HAAs are taken up by DF in the human gastrointestinal tract.

As mutagens, IQ and MeIQ, known to occur in fried or broiled food, and Trp-P-1 and Trp-P-2, formed by pyrolysis of tryptophan, were used. As insoluble DF, CMC (carboxymethylated cellulose, cellulose from plant cell walls), agar (polysaccharide from red algae), collagen (fibrous protein from the skin

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and bone of fish and cows), and chitin (polysaccharide from the carapace of crustacea) were tested.

In this paper, the classification of sorption isotherms, the percentage of HAA taken up by DF in AGJ (artificial gastric juice) and AIJ (artificial intestinal juice), and the sorption-desorption of HAA in AGJ and AIJ are discussed. The purpose of the present *in vitro* study was to predict to what extent DF takes up HAA and how DF acts to intercept HAAs *in vivo*.

Materials and Methods

Materials

CMC for column chromatography, agar (powder) and chitin of analytical grade were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Collagen from the bovine Achilles tendon, shredded, was purchased from Funakoshi Inc. (Tokyo, Japan). IQ, MeIQ, Trp-P-1, and Trp-P-2 of biochemical grade were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals used were of analytical grade.

Preparation of HAA solution

AIJ (pH, 6.8) and AGJ (pH, 1.2) were prepared according to the methods of Japanese Pharmacopoeia (6). One-component solutions of IQ, MeIQ, Trp-P-1, and Trp-P-2 and mixed solutions of the four HAAs were prepared using AIJ and AGJ. The initial concentrations of each HAA solution were 18, 37, 75, 150, and 300 $\mu\text{mol/l}$.

Procedure for determining sorption isotherms

DF (100 mg) was added to a hermetic glass container (25 ml) containing the solution (3 ml), and the slurry was incubated in a water bath shaker at 37°C. The time to reach sorption equilibrium was 10 min. After the incubation, the suspension was centrifuged for 10 min at 3000 rpm. The supernatant of AIJ was used for the determination without adjusting the pH. The supernatant of AGJ was adjusted to pH 3.5 with a 0.08 mol/l sodium hydroxide solution to increase the separation factor in the four HAA chromatograms. The residual concentration of HAA in the supernatant was determined by HPLC (high performance liquid chromatography). The amount sorbed was estimated using the calibration curve of HAA. It was confirmed that no HAA was adsorbed on the inner surface of the glass container. Each experiment was performed in duplicate or triplicate. The sorption isotherm was obtained by plotting the average of the amount sorbed ($\text{CV} < 0.2\text{--}1.5\%$) against the residual concentration. The line was smoothed using the fittest equation yielding the lowest AIC value (Table 1).

Amount of HAA taken up by DF

The amount of each HAA taken up by DF (100 mg) was calculated using the equation, percent sorption (%) = $(C_i - C) / C_i \times 100$, where C_i is the initial concentration ($\mu\text{mol/l}$) and C is the residual concentration ($\mu\text{mol/l}$). The percent sorption (%) was taken as an average of the values obtained using HAA solutions with five different initial concentrations from 18 to 300 $\mu\text{mol/l}$. The value was expressed in terms of percentage, because this made it easy to know the degree to which DF can

take up HAAs from solutions of various initial concentrations.

Desorption of HAA from DF

After the sorption of HAA by DF in AGJ, the suspension was centrifuged (3000 rpm, 10 min) and the supernatant was removed. The precipitate was transferred to AGJ or AIJ (3 ml) in another glass container and the HAAs were eluted from the precipitate with AGJ or AIJ for 10 min in a water bath shaker at 37°C. The amount of HAA sorbed by the DF was calculated using the residual concentration found in the supernatant obtained upon centrifugation of the suspension.

HPLC

IQ, MeIQ, Trp-P-1, and Trp-P-2 in AIJ as supernatant were measured by the method of Egoshi et al. (4). A Hitachi HPLC system, model L-6200 (Hitachi Co., Ltd., Tokyo, Japan), equipped with a Hitachi L-4200 UV-VIS detector was used. A 250 $\times$ 4.6 mm column of Inertsil ODS-2 (GL Sciences Inc., Tokyo, Japan) and a mobile phase of acetonitrile/20 mmol/l potassium dihydrogen phosphate (26:74, v/v) delivered at a flow rate of 0.7 ml/min were used to achieve the separation of IQ, MeIQ, Trp-P-1, and Trp-P-2. A peak (sensitivity, 2.5 AUFS) was detected at 262 nm. The injection volume was 5 μl . The column temperature was room temperature. The concentration of HAA in AGJ as supernatant was determined using a mobile phase of acetonitrile/20 mmol/l sodium dihydrogen phosphate (20:80, v/v). A peak (sensitivity, 3.0 AUFS) was detected at 272 nm.

The calibration curves for the HAAs in the solutions of pH 3.5 and 6.8 were established over a range 0.65 to 80 $\mu\text{g/ml}$.

Statistical analysis

Statistical analyses on the sorption isotherms were performed using the nonlinear least-squares regression program, WinNonlin (Pharsight Co., Mountain View, CA, USA), to compare the applicability of four types of equations to the sorption data. AIC (Akaike's information criterion) 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 (7). Since the number of experimental data was 5, the sorption equations with parameters of two or less (8) 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

type 4, $V = a + bC$, ($a < 0$), empirical linear equation

where V is the amount sorbed; C is the residual concentration; and a , b , and n are the parameters. P values of less than 0.05 were considered statistically significant.

Results

Qualitative analysis of sorption isotherms

The sorption isotherms are affected by the competition between HAA as sorbate or artificial juice as solvent and DF. The classification of an isotherm can offer valuable information on the state in which HAAs are taken up by DF in AIJ or AGJ. The classification should be based on the initial slope rather

than the shape at higher concentrations (9). The shapes of the isotherms depended remarkably on the kinds of DF and HAA and on the pH of the artificial juice (Figs. 1 and 2). The applicability of the four types of equations to the sorption data was compared using AIC values evaluated from a simultaneous curve (Table 1). Each of the four types of equations was tested. Although the lowest AIC value was elucidated to be the optimum (7), it was clear that the AIC values of two types, three types, or four types resemble each other very closely, that there is a large difference in AIC values though the equations are statistically significant ($p < 0.05$), and that modeling is unsuccessful (Table 1). The isotherm of MeIQ for CMC in AGJ and that of IQ for collagen in AIJ were classified into type 1 and type 2, respectively. The isotherms of MeIQ for CMC in AIJ, of Trp-P-1 and Trp-P-2 for CMC in AGJ, and of MeIQ for agar in AGJ were also classified into two types, type 1 and type 2. The isotherms of IQ for CMC in AIJ, of IQ and MeIQ for chitin in AIJ, and of IQ for agar in AGJ were classified into types 1, 2, and 3. The isotherms of Trp-P-1 and Trp-P-2 for agar in AIJ and of Trp-P-1 for chitin in AIJ were classified into types 1, 2, 3, and 4. There were some isotherms for which modeling was unsuccessful.

Percentage of HAAs taken up by DF in AGJ and in AIJ

The percentage of each HAA taken up by CMC and agar in AGJ was obtained to elucidate the amount of one-component taken up and the effect of mixed components on one-component (Table 2). Collagen and chitin could not take up any HAAs in AGJ. The data in Table 2 can be used to predict the removability of HAA by DF while both HAA and DF pass through the stomach. The proportion of Trp-P-1 and Trp-P-2 taken up by CMC and agar in AGJ was 52–56% and 58–78% in the one-component solution, respectively. That of IQ and MeIQ taken up by CMC and agar was 21–27% and 14–35%, respectively. In the four-component solution, with a few exceptions, the percentage of each HAA taken up by CMC and agar was almost the same as that in the one-component solution. A significant increase in the percentage of IQ and Trp-P-2 taken up by agar and a significant decrease in that of IQ and MeIQ taken up by CMC were observed in the four-component solution.

The percent sorption of the four HAAs taken up by CMC was 97–100% in the one-component AIJ solution (Table 3). The proportion of Trp-P-1 and Trp-P-2 taken up by agar and chitin was 87–89% and 51–69% in the one-component solution, respectively. That of the four HAAs taken up by collagen was 4–30%. In the four-component solution, a significant increase in the percentage of IQ taken up by agar and collagen, MeIQ taken

Fig. 1 Sorption isotherms of dietary fibers for a one-component heterocyclic aromatic amine in artificial intestinal juice. black circles, IQ; white circles, MeIQ; black triangles, Trp-P-1; white triangles, Trp-P-2.

The sorption isotherms of agar for IQ and MeIQ are represented by the upper-horizontal axis and right-hand axis. The line was smoothed using the optimum equation yielding the lowest AIC value in Table 1.

Fig. 2 Sorption isotherms of dietary fibers for a one-component heterocyclic aromatic amine in artificial gastric juice. black circles, IQ; white circles, MeIQ; black triangles, Trp-P-1; white triangles, Trp-P-2.

The sorption isotherms of CMC for IQ and MeIQ are represented by the lower-horizontal axis and right-hand axis. The sorption isotherms of agar for IQ and MeIQ are represented by the upper-horizontal axis and right-hand axis. The line was smoothed using the optimum equation yielding the lowest AIC value in Table 1.

up by agar, and Trp-P-1 taken up by collagen and a significant decrease in that of MeIQ taken up by collagen were observed. The finding that the percentage of HAA taken up by DF in the four-component solution was almost the same or significantly increased as compared with that in the one-component solution indicates an increase in mutual affinity among the HAAs in the solution.

Sorption and desorption of HAAs in AGJ and in AIJ

The sorption and desorption of HAAs in AGJ and AIJ were measured to estimate the removability of HAAs from DF

Table 1 Comparison of AIC^{a)} values of different sorption equations applied to the sorption isotherms of HAA on DF in AIJ and AGJ

Dietary fiber	HAA	type 1 ^{b)}	type 2 ^{b)}	type 3 ^{b)}	type 4 ^{b)}
CMC in AIJ	IQ	25.6*	25.6**	28.5*	— ^{c)}
	MeIQ	6.4**	9.6*	22.9***	— ^{c)}
	Trp-P-1	— ^{c)}	1.0*	— ^{c)}	1.9**
	Trp-P-2	15.7	8.3**	— ^{c)}	7.6**
Agar in AIJ	IQ	-11.6	-13.5***	-13.8***	— ^{c)}
	MeIQ	9.1***	— ^{c)}	6.9***	7.0***
	Trp-P-1	18.3***	15.6***	15.9***	14.3***
	Trp-P-2	13.5**	11.5***	11.1***	9.9***
Collagen in AIJ	IQ	-11.1	-9.7***	-10.0	— ^{c)}
	MeIQ	-7.3	1.1***	-9.5***	-7.8***
	Trp-P-1	-14.4*	-9.6***	-6.1**	— ^{c)}
	Trp-P-2	-1.6	— ^{c)}	-3.7***	-2.8***
Chitin in AIJ	IQ	-14.3**	-13.7***	-15.6**	— ^{c)}
	MeIQ	-16.7*	-18.7**	-16.0*	— ^{c)}
	Trp-P-1	3.2**	2.8***	0.8**	2.5**
	Trp-P-2	-0.4*	-5.3**	-3.1*	-2.4*
CMC in AGJ	IQ	— ^{c)}	— ^{c)}	— ^{c)}	— ^{c)}
	MeIQ	-51.2***	-42.7	— ^{c)}	— ^{c)}
	Trp-P-1	-15.0*	-20.6*	-10.5**	— ^{c)}
	Trp-P-2	-14.9*	-18.2*	-13.1**	— ^{c)}
Agar in AGJ	IQ	-28.9***	-29.0*	-30.9***	— ^{c)}
	MeIQ	-41.3***	-45.3**	-34.1**	— ^{c)}
	Trp-P-1	4.4*	-6.7**	2.3*	-20.4*
	Trp-P-2	3.0**	— ^{c)}	0.5**	-4.8**

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; type 4, $V = a + bC$, ($a < 0$), empirical linear equation; V , amount sorbed; C , residual concentration; a , b , and n , constant.

c) Modeling unsuccessful.

*, $p < 0.001$; **, $p < 0.01$; ***, $p < 0.05$

(Table 4). The desorption from DF was measured *in vitro* to predict whether the sorbed molecules are held firmly by DF while it passes through the digestive tract *in vivo*. The amounts of Trp-P-1 and Trp-P-2 taken up by CMC in AGJ were 1.54 and 1.56 $\mu\text{mol/g}$. The amounts of Trp-Ps desorbed from CMC in AGJ were 0.70 to 0.77 $\mu\text{mol/g}$, being approximately 50% of the amount sorbed. On the other hand, 2.82 to 2.71 $\mu\text{mol/g}$ of Trp-Ps was taken up by agar in AGJ while 0.17 to 0.36 $\mu\text{mol/g}$, approximately 6 to 13%, was desorbed. The percent desorption from agar was four to eight times smaller than that from CMC in AGJ.

From the results on desorption in AIJ, it was found that the

Table 2 Percentage of HAA taken up by DF in AGJ

Dietary fiber	Percentage of HAA (%)							
	One-component				Four-component			
	IQ	MeIQ	Trp-P-1	Trp-P-2	IQ	MeIQ	Trp-P-1	Trp-P-2
CMC	21 $\pm$ 1	27 $\pm$ 1	56 $\pm$ 2	52 $\pm$ 2	0*	16 $\pm$ 2*	54 $\pm$ 6	49 $\pm$ 6
Agar	14 $\pm$ 1	35 $\pm$ 1	78 $\pm$ 5	58 $\pm$ 3	26 $\pm$ 3*	35 $\pm$ 2	83 $\pm$ 2	70 $\pm$ 3*

The amounts of HAA taken up by collagen and chitin were almost zero. The percentage of HAA shows the value (mean $\pm$ SD) calculated over the five HAA concentrations used. $n=5$; *, $p < 0.05$: one-component vs. four-component.

Table 3 Percentage of HAA taken up by DF in AIJ

Dietary fiber	Percentage of HAA (%)							
	One-component				Four-component			
	IQ	MeIQ	Trp-P-1	Trp-P-2	IQ	MeIQ	Trp-P-1	Trp-P-2
CMC	100±0.6	100±0.1	98±1	97±1	100±0.3	100±0.1	98±1	99±1
Agar	11±2	36±4	89±9	87±6	39±3*	60±7*	93±1	90±3
Collagen	4±0.4	30±2	27±4	22±2	8±2*	21±4*	32±1*	21±2
Chitin	20±2	28±3	69±9	51±5	25±4	32±4	67±2	58±5

The percentage of HAA shows the value (mean±SD) calculated over the five HAA concentrations used. n=5; *, p<0.05: one-component vs. four-component.

Table 4 Sorption and desorption of HAA in AGJ and AIJ

Dietary fiber		CMC				Agar			
		Amount of HAA (μmol/g)				Amount of HAA (μmol/g)			
		IQ	MeIQ	Trp-P-1	Trp-P-2	IQ	MeIQ	Trp-P-1	Trp-P-2
AGJ	Sorption	0.0	0.02	1.54	1.56	1.17	1.43	2.82	2.71
	Desorption	0.0	0.01	0.70	0.77	0.40	0.26	0.17	0.36
AIJ	Desorption	0.0	0.00	0.02	0.03	0.22	0.14	0.11	0.27
Final amount		0.0	0.01	0.82	0.76	0.55	1.03	2.54	2.08

The sorption was measured using the four-component solution. Each initial concentration of HAA was 150 μmol/l.

Trp-Ps in CMC and agar were elutable with AIJ. The percent desorption, however, was small, 2.4 to 3.8% and 4.2 to 9.5%, respectively (Table 4). The final percent sorption of Trp-Ps taken up by CMC and agar was 48.7–53.2% and 76.8–90.1%, respectively.

Discussion

The sorption isotherms of HAAs for DF in AGJ and AIJ were classified into four types, 1–4 (Table 1). The isotherm of MeIQ for CMC in AGJ (Fig. 2) reveals that the amount sorbed approaches a limiting quantity, corresponding to type 1, the Langmuir type. Since the type 1 curve occurs when there is high affinity between the sorbate and the surface of the sorbent (9), the strength of interaction between DF and HAA or artificial juice is expressed as an inequality, DF-HAA>>DF-juice. The isotherm of IQ for collagen in AIJ (Fig. 1) is of the so-called Freundlich type, corresponding to type 2 (Table 1). In this case, there is no strong competition from the solvent for sorption sites of DF (9). Type 2 is expressed as DF-HAA>DF-juice. From the results for isotherms classified into types 1 and 2, it seems that MeIQ and IQ are mainly held on the surface of CMC and collagen, respectively, and that the attractive force of MeIQ-CMC is stronger than that of IQ-collagen.

The isotherms of IQ and Trp-P-1 for chitin in AIJ (Fig. 1) were found to be a straight line that coincides with the origin, corresponding to the optimum type 3, the Henry type (Table 1). Since the type 3 curve indicates a constant partition of HAAs between DF and artificial juice (9), the relationship is expressed as DF-HAA ≈ DF-juice. It appeared with chitin, into which IQ and Trp-P-1 penetrated further as their concentrations in AIJ increased. The parameter, “ α ”, slope of the Henry equation (type 3) for the sorption of IQ and MeIQ for CMC in AIJ, was 2.60 and 2.68, respectively, and that for the other sorptions was 0.003–0.618. The former and the latter differed remarkably from each other in the partition coefficient of HAAs between

DF and artificial juice. It was considered that the partition of HAAs into DF was greater in the case of the former and smaller in the case of the latter and that the sorption behavior was fundamentally the same, because the amount of HAA distributed into each DF was taken up by the DF. Since the other types of IQ and Trp-P-1 for chitin in AIJ, however, resemble each other in AIC value (Table 1), it was considered that their sorption behavior was mainly of type 3 and also was affected by the other types.

The isotherms of Trp-P-1 and Trp-P-2 for agar in AGJ (Fig. 2) show that no amount of HAA is sorbed until it increases linearly with the residual concentration, corresponding to type 4. Since the sorption sites of DF are mainly occupied by artificial juice in dilute solution, the relationship is expressed as DF-HAA<DF-juice. The results for the type 4 sorption suggest that Trp-P-1, Trp-P-2, and AGJ penetrate agar and that their molecules, accompanied by AGJ, are firmly held in the interior of the agar. It was considered that this sorption was also affected by the sorption of other types.

After the qualitative analysis on sorption isotherms, it is important to evaluate quantitatively the sorption of HAA by DF in AGJ and AIJ. The sorption capacity of DF depends upon the lyophilic property of HAA. Trp-P-1 and Trp-P-2 are of the non-IQ type, having high hydrophobicity (3). IQ and MeIQ are of the IQ type, having low hydrophobicity (3). The percentages of HAAs taken up by DF in AGJ (Table 2) indicate that CMC and agar markedly take up HAAs of the non-IQ type with high hydrophobicity compared to those of the IQ type. The fact that Trp-Ps were taken up mostly by agar rather than by CMC in AGJ suggests that the penetration by the Trp-Ps of the agar (type 4 isotherm) is quantitatively greater than the sorption onto the surface of CMC (type 2 isotherm) in an acidic solution.

The result that more of each of the four HAAs was taken up by DF in AIJ (Table 3) than in AGJ (Table 2) indicates that more HAA was taken up in the neutral (pH, 6.8) than in the acidic pH range (pH, 1.2). The results of IQ-type sorption by

DF in AGJ and AIJ were consistent with those of Sjödin et al. (5). It may be predicted that the residual HAAs which are not taken up in the stomach will be caught while passing through the intestinal tract with DF. CMC and agar were more suitable than collagen and chitin over the pH range found in the human gastrointestinal tract.

Sorption-desorption was measured to estimate the removability of HAAs from DF. The results of the sorption-desorption in AGJ (Table 4) indicate that the sorbed HAAs are firmly held by agar rather than by CMC. The findings confirmed the explanation that Trp-Ps are weakly sorbed on the surface of CMC in AGJ (type 2 isotherm) and firmly held within agar in AGJ (type 4 isotherm). The results of the desorption from DF in AGJ and AIJ indicate that Trp-Ps held by DF in AGJ were desorbed less

by a neutral buffer (pH, 6.8) than by an acidic buffer (pH, 1.2). The results on final sorption amount (Table 4) and type of sorption isotherm (Table 1) indicate that the sorbed Trp-Ps in the stomach are firmly held in the interior of the agar rather than by the surface of CMC while the DF passes through the intestinal tract *in vivo*.

From the sorption isotherms, the removability and sorption-desorption, CMC and agar would be expected to be more useful than collagen and chitin as agents to intercept HAAs.

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