Effects of pH on the in Vitro Sorption of Mutagens to Dietary Fibers

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Dietary fibers, alginate and defatted corn fiber, sorbed food mutagens, Trp-P-1 and Glu-P-1, which are heterocyclic amines formed in the cooking process. The sorption behavior of the heterocyclic amines to defatted corn fiber and alginates was analyzed under pH-controlled conditions. Glu-P-1 and alginic acid had $pK_a$ values of 4.2 and 3.6, respectively, whereas Trp-P-1, which showed alkaline in solution, possessed two $pK_a$ values of 4.8 and 7.7. Defatted corn fiber, which was mainly composed of cellulose and hemicellulose, did not show a significant $pK_a$ value. The amount of sorbed Trp-P-1 to the alginates increased as the pH value of the buffer was elevated, and was much more than that sorbed to defatted corn fiber at each pH condition. These results suggest that the alginates held the amino group of Trp-P-1 or Glu-P-1 on their carboxyl group as a cation exchanger.

The desmutagenic effects of dietary fiber on food mutagens have been shown by epidemiological analyses, 11 and in vitro and in vivo experiments. 3–10 Most of these positive effects of dietary fibers on colon cancer have been assumed to be caused by the sorption of mutagenic and/or carcinogenic compounds to these fibers. 11

For an exact understanding of the desmutagenic effect of dietary fibers, it is necessary to reveal the mechanism for the sorption of mutagens to dietary fibers. In our previous study, 11 we analyzed the sorption of three mutagens, Trp-P-1, Glu-P-1, and dimethylnitrosamine, to dietary fibers (defatted corn fiber, alganic acid, calcium alginate, pectic acid, and chitosan) in distilled water. We found that the alginates and pectic acid had higher binding capacity for the mutagens than defatted corn fiber, and that this binding was irreversible in distilled water, which seemed favorable for the desmutagenicity of dietary fibers. However, the effects of dietary fibers on the sorption of the mutagens observed in distilled water may not always be reproducible with the daily diet, because dietary fibers and mutagens encounter very acidic conditions in the stomach before reaching the intestine where the pH is neutral. To analyze the sorption mechanism for mutagens in vitro in detail, we need to know the effect of pH on the sorption behavior of mutagens.

In this paper, we report the in vitro sorption behavior of heterocyclic amines (Trp-P-1 and Glu-P-1) to defatted corn fiber, alganic acid powder, and calcium alginate gel under pH-controlled conditions (pH 1.5–9.0).

Materials and Methods

Materials. Calcium alginate was purchased from Wako Pure Chemical Industries, and sodium alginate (the ratio of D-mannuronic acid (M) to L-guluronic acid (G) was unknown) was obtained from Kanto Chemical Co. Two kinds of sodium alginates (M/G=2.4 and M/G=0.53), and alganic acid powder were obtained from Kimitsu Chemical Industry Co. Corn fiber was obtained from Nihon Shokuhin Kako Co. Trp-P-1 acetate and Glu-P-1 hydrochloride were purchased from Wako Pure Chemical Industries.

Preparation of spherical alginate gel beads. A 1% (w/v) sodium alginate solution in distilled water was dropped into a 1% (w/v) CaCl$_2$ solution and left for at least one night with stirring. The formed beads were then rinsed thoroughly with distilled water and used as described next.

Preparation of the defatted corn fiber. To prepare the defatted corn fiber, 100 g of corn fiber was suspended in 500 ml of a chloroform–methanol (2:1) mixture for about 10 min. After centrifugation, the supernatant was removed by aspiration, and the precipitate was resuspended in the chloroform–methanol mixture. After the second centrifugation, the precipitate was suspended in 2 liters of distilled water and restored for two days at room temperature with stirring. The suspension was then kept without stirring until the corn fiber precipitated naturally. After removing the supernatant by aspiration, the corn fiber was completely dried under reduced pressure. Hereafter, the sample thus prepared is referred to as defatted corn fiber.

Sorption isotherm. A mutagen at various concentrations was mixed with dietary fiber (10 mg of defatted corn fiber or 5 mg alginate) in 10 ml of a 0.05 M KCl–HCl buffer (pH 1.5), 0.05 M Tris–HCl buffer (pH 7.0), or 0.025 M KCl–0.05 M H$_2$BO$_3$ buffer (adjusted with NaOH at pH 8.0 or 9.0) in a 15-ml centrifuge tube. Each mixture was incubated with stirring at 0°C. Alganic acid became soluble in the buffer at a higher pH values, whereas defatted corn fiber and calcium alginate were insoluble in every buffer. However, by adding Trp-P-1, alganic acid was rapidly precipitated as insoluble material. Therefore, the sorption isotherm of each mutagen could be easily obtained by measuring the concentration of the mutagen in the supernatant when a constant concentration (equilibrium concentration) of the mutagen had been reached. The concentrations of Trp-P-1 and Glu-P-1 were measured by a UV spectrophotometer (Hitachi U-2000) at 261 and 250 nm, respectively.

For desorption, after the mutagen had been sufficiently sorbed to the dietary fiber, the supernatant was removed, and the dietary fiber was resuspended in another buffer of different pH at 0°C. The concentration of mutagen was then measured with the UV spectrophotometer until a constant concentration was reached.

Determination of $pK_a$. Algamic acid or defatted corn fiber was suspended in distilled water. Each time a specified volume of 0.1 M HCl or NaOH had been added to the suspension, the pH value of the solution was measured by a pH meter. $pK_a$ values for the dietary fibers were determined from the inflexion points obtained by plotting pH value versus volume of the HCl or NaOH added. Similarly, $pK_a$ values for Trp-P-1 or Glu-P-1 were determined by titrating 20 ml of the 2.5 mM mutagen solution with 0.01 M HCl or 0.01 M NaOH.

Results

Sorption behavior of Trp-P-1 under pH-controlled conditions

Figure 1 shows the sorption isotherms of Trp-P-1 to algamic acid powder at pH 1.5, 7.0, 8.0, and 9.0. At pH 7.0, 8.0, and 9.0, 1 mol or more of Trp-P-1 was sorbed per 1 mol

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of uronic acid in alginic acid, while little sorption was observed at pH 1.5. When a similar analysis was carried out with calcium alginate gel, which was insoluble at every pH in contrast to alginic acid powder, about 1 mol of Trp-P-1 was sorbed under high pH conditions (Fig. 2). On the other hand, as shown in Fig. 3, the amount of Trp-P-1 sorbed to defatted corn fiber increased as the pH value of the solution was increased.

**Desorption of sorbed Trp-P-1 and Glu-P-1**

Once Trp-P-1 and Glu-P-1 had been sorbed by the alginates or defatted corn fiber, they could hardly be released from the dietary fiber in distilled water.\(^\text{12}\) The desorption isotherm of Trp-P-1 from defatted corn fiber by lowering pH from 7.0 to 1.5 is shown in Fig. 4. Most of the sorbed Trp-P-1 was released from the defatted corn fiber, the amount of Trp-P-1 still sorbed being nearly equal to that of the sorbed Trp-P-1 in a pH 1.5 solution. We obtained similar results when alginic acid was used, as shown in Fig. 5.

Desorption isotherms of Glu-P-1 from the alginates is shown in Fig. 6. In distilled water, all of the molecules sorbed to alginic acid, calcium alginate gel, and defatted
Fig. 6. Sorption and Desorption Isotherms of Glu-P-1 for Alginates at 0°C.

Sorption isotherms: O, to alginic acid in distilled water; △, to calcium alginate gel (M/G = 2.4) in distilled water; ◊, to calcium alginate gel (M/G = 0.53) in distilled water.

Desorption isotherms: ●, from alginic acid by reducing pH to 1.5; ▲, from calcium alginate gel (M/G = 2.4) by reducing pH to 1.5; ▼, from calcium alginate gel (M/G = 0.53) by reducing pH to 1.5.

Table 1. pKₐ Values for Dietary Fibers and Heterocyclic Amines

<table>
<thead>
<tr>
<th>Dietary fiber</th>
<th>pKₐ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginic acid</td>
<td>3.6</td>
</tr>
<tr>
<td>Defatted corn fiber</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Heterocyclic amine</th>
<th>pKₐ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu-P-1 acetate</td>
<td>4.8, 7.7</td>
</tr>
<tr>
<td>Glu-P-1 hydrochloride</td>
<td>4.2</td>
</tr>
</tbody>
</table>

N.D., not determined.

pKₐ values for dietary fibers and heterocyclic amines

The values for pKₐ determined by pH titration are shown in Table 1. The pKₐ value for alginic acid was 3.6, which is nearly equal to that for pectic acid powder composed of uronic acid,13 while defatted corn fiber did not show a clear pKₐ value in its titration curve. Trp-P-1 acetate had two pKₐ values of 4.8 and 7.7, and Glu-P-1 hydrochloride, which was insoluble in a pH range above 4.2, had a single pKₐ value at 4.2.

Discussion

We have reported that alginates, pectic acid, and defatted corn fiber sorbed Trp-P-1 and Glu-P-1 in distilled water, and that almost no molecules of Trp-P-1 and Glu-P-1 were desorbed from these dietary fibers in distilled water.12 Therefore, the dietary fibers were expected to decrease the occurrence of cancer or tumors induced by such mutagens. However, the environment, especially pH, in gastrointestinal tract is different from that in distilled water, and furthermore, the environment varies along the tract. In this study, we examined the sorption behavior of dietary fibers for food mutagens Trp-P-1 and Glu-P-1 in buffers of various pH value. Alginates and defatted corn fiber sorbed Trp-P-1 and Glu-P-1. We observed that a larger amount of Trp-P-1 was sorbed to alginates under neutral or alkaline conditions than under acidic conditions, and that more Glu-P-1 was sorbed to alginates in distilled water with a pH of about 4 than in the solution of pH 1.5. However, the sorbed Trp-P-1 and Glu-P-1 P-1 were released from these dietary fibers by lowering the pH to 1.5. In the case of pectic acid consisting of similar uronic acid, the sorption isotherms were similar to those of alginic acid under the pH conditions studied (data not shown). We also observed that the amount of Trp-P-1 sorbed to the alginates was much larger than that to the defatted corn fiber at each pH value in this experiment.

On the basis of these results and the pKₐ value of alginates, we assume that alginates and pectic acid bound the amino group of Trp-P-1 or Glu-P-1 at its carboxyl group. In the case of chitosan, which is a polymer of glucosamine and is known for its anion-exchange capacity, neither Trp-P-1 nor Glu-P-1 was sorbed under any pH conditions. In addition, DMN was not sorbed to any dietary fibers (data not shown). These results also support the idea that the potent ability of the dietary fibers for sorption of the mutagens could be ascribed to the ionic binding between the dietary fibers and the mutagens. Previously, we have found that the amount of sorbed Trp-P-1 molecules was much larger than the amount of Glu-P-1 in distilled water.12 The reason may be explained by the observations that the pH of the Glu-P-1 solution was about 4.0, which is nearly equal to the pKₐ of Glu-P-1, while the Trp-P-1 solution was alkaline. At about pH 4, which is also nearly equal to the pKₐ value for uronic acid, the number of dissociated carboxyl groups of uronic acid varies depending on the M/G ratio of the alginates. We suggest that the different form of the sorption isotherms of Glu-P-1 to alginates might reflect the difference of dissociation level of the alginates.

When defatted corn fiber was used, which is mainly composed of hemicellulose and cellulose, it sorbed more Trp-P-1 in the buffer at higher pH values. The sorbed Trp-P-1 on defatted corn fiber was desorbed by lowering the pH from 7.0 to 1.5, like alginates. However, since defatted corn fiber did not show a definite pKₐ value on its titration, the sorption of Trp-P-1 to defatted corn fiber may not have occurred by the mechanism observed in the Trp-P-1-alginates sorption system. In defatted corn fiber, the hydroxyl group is the most probable candidate responsible for the sorption of Trp-P-1. Therefore, its sorption must be mediated by hydrogen bonding which is weaker than ion bonding. We assume that this weaker bonding might result in the lower sorption capacity of defatted corn fiber than that of alginates. However, it is noteworthy that the defatted corn fiber, which does not have a dissociated group in its molecule, could sorb more mutagens under alkaline conditions than under neutral conditions. This observation may suggest that some conformational change suitable for the sorption of Trp-P-1 occurred under the alkaline conditions.

In this study, the sorption behavior was analyzed at 0°C to decrease the deterioration of the dietary fibers and mutagens. We also observed that the sorption of these dietary fibers for the heterocyclic amines at 37°C was very similar to that at 0°C (data not shown). This observation may suggest that this sorption system would be effective.
even in vivo. The heterocyclic amines used in this study are indirect mutagens which are metabolically activated by cytochrome P-450 of microsomes in the liver.\textsuperscript{14} Therefore, to decrease the occurrence of cancer or tumors, it is necessary for the fibers to bind the mutagens before absorption in the intestinal tract. The strong sorption capacity of dietary fibers, especially of alginates and pectic acid, for heterocyclic amines at neutral pH may decrease the occurrence of cancer or tumors in the intestine, even if the mutagens might be released in the stomach.

References
1) D. P. Burkitt, Cancer, 28, 3—13 (1971).