Inhibitory Effect of Vegetables, Fruits and Herbs on \( \alpha \)-Glucosidase in an Immobilized Enzyme Assay System

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A system using immobilized enzyme according to Oki et al., [Biol. Pharm. Bull. 23(9) 1084–1087 (2000)], which mimics the small intestinal membrane, was applied to the screening of several kinds of vegetables, fruits and herbs in terms of their inhibitory effects on \( \alpha \)-glucosidase (AGH).

AGH was partially purified from hog intestinal membrane by salting out, followed by immobilization on CNBr-activated Sepharose 4B as a matrix. As substrate, p-nitrophenyl-\( \alpha \)-d-glucopyranoside (PNPG) was employed, producing p-nitrophenol (p-NP) by enzymic hydrolysis. Seven vegetables (Japanese radish, cabbage, onion, Chinese cabbage, tomato, cucumber, and carrot), 5 fruits (mandarin, apple, watermelon, melon, and grapefruit) and 4 herbs (Italian parsley, rucoela, mache, and dill) were subjected to evaluations of the inhibitory effect on AGH.

All of the test samples exerted an inhibitory effect on immobilized AGH (iAGH). Raw Chinese cabbage and boiled tomato among vegetables, mandarin among fruits and dill among herbs exerted the strongest inhibitory effect on iAGH.

Keywords: Immobilized enzyme, \( \alpha \)-glucosidase, Inhibition, Vegetable, Fruit, Herb

Introduction

Type 2 diabetes is caused by the decreased secretion of insulin from pancreatic Langerhans \( \beta \) cells, or an increased resistance to the hormone (Peters K and Richards F.M., 1977). Delaying glucose absorption is known to be effective for controlling postprandial blood glucose level of this type of diabetes (Bischoff H., 1994; Toeller M., 1994).

Postprandial blood glucose levels are known to increase quickly after consumption of starch in case of diabetics whose \( \alpha \)-glucosidase (AGH, EC 3.2.1.20) has a higher activity than ordinary subjects. Sufferers’ capability to secrete insulin is considered to be exhausted prematurely by the frequent and rapid increase in postprandial blood glucose.

AGH inhibitors were confirmed to be useful as therapeutic drugs to prevent hyperglycemia, which effectively delay absorption of glucose (Bischoff H., 1994; Toeller M., 1994). In addition, some synthetic AGH inhibitors such as acarbose and voglibose have been developed for treatment of type 2 diabetes (Toyota T., 1995; Goda T., 1994). However, these drugs are associated with harmful side effects including abdominal distention and fulminant hepatitis. Therefore, controlling blood glucose through diet is suggested to be more desirable for patients. Thus, the screening of foods in terms of AGH inhibitory activity has attracted considerable attention (Matsumoto K., 2002). Some AGH inhibitors in foods have been reported, including acylated anthocyanin pigments from purple sweet potato and red morning glory (Matsui T., 2003; Matsui T., [1] [2], 2001), polyphenol constituents (epicatechin) from *Salacia reticulate* (Yoshikawa M., 2001), and ellagittannins, casuarictin, and eugenii from cloves (Toda M., 2000). In order to measure the inhibitory effect of food components on AGH, p-nitrophenyl-\( \alpha \)-d-glucopyranoside (PNPG) has been employed as a substrate, which is hydrolyzed into p-nitrophenol (p-NP) (Matsui T., 1996). However, Odaka et al. (1992) reported that the in vitro inhibitory effect of pharmaceuticals did not agree with those obtained by in vivo experiments, probably due to the discrepancy in the environment of AGH in the mammalian intestine and in the in vitro system. And Oki et al. (2000) reported on a pseudo-in vivo assay system using rat intestinal AGH immobilized on Sepharose, which mimics the environment of the mammalian small intestine.

In this study, the assay system developed by Oki et al., (2000) was applied to the partially purified AGH from hog small intestine. By using the system, the inhibitory effects of some vegetables, fruits and herbs on immobilized AGH (iAGH) were evaluated.

Materials and Methods

Reagents Cyanogen bromide-activated Sepharose 4B was obtained from Amersham Biosciences Corp (Uppsala, Sweden). Krebs-Henseleit buffer and PNPG were purchased from Sigma (USA). Voglibose (BASEN, 0.2 mg/tablet) was obtained from Takeda (Osaka, Japan) and acarbose (Glucobay, 50 mg/tablet) from Bayer Medical (Osaka, Japan). Fresh hog small intestine was purchased...
from Tokyo Shibaura (Tokyo, Japan).

**Partial purification of AGH** AGH was partially purified from hog small intestine according to the method of Cogoli et al. (1972). The mucous membrane of the fresh small intestine (1 kg) was sampled using the edge of a glass slide collected in plastic plates. These samples were then homogenized with sea sand in a china bowl, with slow addition of 10 mM of Krebs-Henseleit buffer containing 600 mg L-cysteine, 0.1375 g EDTA, and 600 mg papain (0.5 unit/g) per liter. After filtration, the homogenate was centrifuged at 50,000 × g for two hours. The supernatant was adjusted to 40% saturation of ammonium sulfate. After removing the precipitate by centrifugation (10,000 × g, 30 min), the supernatant was adjusted to 60% saturation of ammonium sulfate, and the resulting precipitate was collected by ultracentrifugation (55,000 × g, one hr). The precipitate was dissolved in 10 mM potassium phosphate buffer (pH 6.8) and dialyzed against the same buffer at 4°C overnight. The dialyze was ultrafiltered using an ultrafilter (Q2000 076E, MW: 200,000, φ76 mm, Advantec, Tokyo, Japan) to remove the fraction with a molecular weight of over 200,000 the filtrate was lyophilized and stored at −4°C until use. The resulting AGH was immobilized by the method of Oki et al. (2000).

**Samples subjected to experiment** Vegetables (Japanese radish, cabbage, onion, Chinese cabbage, tomato, cucumber, and carrot) and fruits (mandarin, apple, watermelon, melon, and grapefruit) were obtained from the local market in Sakado City, Saitama prefecture, in 2004. Herbs (Italian parsley, rucola, mache, and dill) were kindly provided by SB Co. Ltd., Japan.

**Preparation of sample solution** An edible portion of each sample was weighed and placed in a blender, followed by the addition of distilled water to reduce the concentration by 50% (w/v). After blending for 3 min in a mixer, the suspension was placed into a polyethylene container and homogenized for 3 min. The homogenate was frozen at −40°C overnight, and centrifuged at 10,000 rpm for 10 min at 4°C, thawed, and then filtered. Distilled water was added to the filtrate and diluted twice before mincing. Thus, whole water-soluble components of the plant material were regarded to have been captured in solution, which was designated the original sample solution, and stored at −40°C until use. A portion of this solution was boiled for 10 min as a boiled sample. Thirty mL of the original sample solution was adjusted to pH 6.8 to make a stock solution of 60 mL with 0.1 M phosphate buffer. This was diluted to the varying concentrations before the test.

**Assay of enzyme activity** A half of mL of 20 mM PNPG as substrate and an equivolume of the test solution consisting of five different concentrations, were added to an open-tip mini-column charged with 50 mg iAGH wet gel, being end-capped. After incubation on a rotating cultivator (4 rpm, RT-5, TAITEC, Saitama, Japan) in an incubator at 37°C for 90 min, the reaction was terminated by separating the solution from the iAGH column by pressed filtration. One hundred μL of the filtrate was placed into a well on a microtiter plate to which 100 μL of 1 M sodium carbonate was then added and allowed to stand at room temperature for 3 min on a microplate mixer before colorimetry measurements at 415 nm. All measurements were carried out in triplicate and the average value was calculated. The IC50, which indicated the concentration required to reduce enzyme activity by 50%, was calculated on the basis of the relationship between the initial production rate of p-NP from PNPG and the concentration (mg/mL) of test substances in reaction solution. Acarbose (Glucofay 100, acarbose 100 mg/tablet, Bayer Co., Ltd.), an AGH inhibitor, was used as a standard inhibitor.

**Results**

**Stability of iAGH system** The reaction rate of iAGH was observed to be kept constant until 90 min while that of the mobile AGH began to decrease after 30 min, suggesting that the immobilized sample was more stable than the mobile. The reaction rate was determined based on the amount of p-NP liberated from PNPG during the incubation at 90 min. On the other hand, the immobilized system remained relatively stable after repeated two-times.

Inhibitory effect of vegetables, fruits and herbs on iAGH

Table 1 shows the concentrations of vegetables required to reduce iAGH activity by 50%. All of the raw vegetables (Japanese radish, cabbage, onion, Chinese cabbage, tomato, cucumber, and carrot) exerted inhibitory effects on iAGH. Chinese cabbage showed the strongest effects of the raw vegetables. The effect of boiled vegetables on iAGH activity was also examined. All of the boiled vegetables also had inhibitory effect on iAGH, with that of tomato fruit exhibiting the strongest effect among the boiled samples.

Table 2 shows the effect of raw fruits on iAGH activity. All fruit extracts (mandarin, apple, watermelon, melon, and grapefruit) inhibited iAGH. Mandarin had the strongest inhibitory potential.

Table 3 shows the effect of raw herbs on iAGH activity. Similarly, the herbs (Italian parsley, rucola, mache, and dill) exerted inhibitory effect on iAGH, with dill possessing the strongest activity.

**Table 1. Inhibitory effect of raw and boiled vegetables on α-glucosidase acarbose equivalents in terms of IC50 (mg/mL).**

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>Raw IC50 (mg/mL)</th>
<th>Boiled IC50 (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese radish</td>
<td>153</td>
<td>332</td>
</tr>
<tr>
<td>Cabbage</td>
<td>92</td>
<td>103</td>
</tr>
<tr>
<td>Onion</td>
<td>66</td>
<td>82</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>25</td>
<td>261</td>
</tr>
<tr>
<td>Tomato</td>
<td>140</td>
<td>74</td>
</tr>
<tr>
<td>Cucumber</td>
<td>326</td>
<td>347</td>
</tr>
<tr>
<td>Carrot</td>
<td>112</td>
<td>319</td>
</tr>
</tbody>
</table>
Table 2. Inhibitory effect of fruits on α-glucosidase in terms of IC₅₀ (mg/mL).

<table>
<thead>
<tr>
<th>Fruits</th>
<th>IC₅₀ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandarin</td>
<td>12</td>
</tr>
<tr>
<td>Apple</td>
<td>204</td>
</tr>
<tr>
<td>Watermelon</td>
<td>198</td>
</tr>
<tr>
<td>Melon</td>
<td>122</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>74</td>
</tr>
</tbody>
</table>

Table 3. Inhibitory effect of herbs on α-glucosidase in terms of IC₅₀ (mg/mL).

<table>
<thead>
<tr>
<th>Herbs</th>
<th>IC₅₀ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italian parsley</td>
<td>115</td>
</tr>
<tr>
<td>Ruecola</td>
<td>73</td>
</tr>
<tr>
<td>Mache</td>
<td>71</td>
</tr>
<tr>
<td>Dill</td>
<td>28</td>
</tr>
</tbody>
</table>

Discussion

The digestion process of disaccharides occurs on the cell membranes by microvilli on the surface of the intestinal wall and is the final stage of the degradation of carbohydrates into their monomeric units. The surface of the microvilli is well known to be covered by the glycocalyx made up of branched mucopolysaccharide, which is considered to contribute to protecting the small intestine against the action of proteases and the invasion of various toxins. The AGH molecule incorporated with the glycocalyx is considered to differ in its performance concerning to enzymic reaction from the enzyme freely dissolved in solution.

A pseudo-in vivo assay system using AGH immobilized on a matrix such as Sepharose 4B might be relevant to the evaluation of activity of a membrane enzyme. (Oki T., 2000). Oki et al. suggested that the affinity of iAGH with maltose as a substrate was lower than that of mobile AGH.

In this experiment, a synthetic substrate of PNPG was used instead of maltose, which produced p-glucose and may have confounded measurements, while p-NP from PNPG was found to be unrelated to the natural components in samples. All of the samples tested in this study inhibited iAGH, although the inhibitory potency varied.

Except for the tomato, raw vegetables tested were found to exhibit a greater inhibitory effect on AGH activity than boiled samples. The mean IC₅₀ ratios for the immobilized system relative to the mobile system according to Nasu et al. (2005) were 0.717 and 0.934 for raw and boiled samples (unpublished).

Conversely, the mean IC₅₀ values for herbs obtained from the immobilized system was considerably greater than those of mobile system (Nasu R., 2005), suggesting that the inhibitory effect of herbs in the immobilized system less effective than the mobile one system. Fruits showed a similar tendency to that observed in herbs.

Conversely, the IC₅₀ of Acarbose was found to be 0.036 mg/mL, being approximately ten thousands times greater in inhibitory effect than those of the plants tested in this experiment. Although the vegetables and the fruits tested were not as effective in inhibiting AGH as drugs such as Acarbose, ordinary consumption should be considered in controlling postprandial blood glucose as measured with glycemic index.

References


