Effect of Flavonoids on α-Glucosidase and β-Fructosidase from Yeast

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Received December 9, 1983

The influence of 16 flavonoids on yeast α-glucosidase and β-fructosidase was investigated. Either p-nitrophenyl-α-D-glucopyranoside or maltose was used as substrate for α-glucosidase, and sucrose for β-fructosidase. Quercetin, myricetin, fisetin, and quercitrin strongly inhibited α-glucosidase at 0.1 mM. The concentrations which gave 50% inhibition were 4, 8, 8, 20, and 20 μM for myricetin, quercetin, fisetin, kaempferol, and quercitrin, respectively. The mode of inhibition was found to be mixed type, close to non-competitive type with quercetin and quercitrin. Fisetin, naringenin, and morin showed mixed type inhibition. Albumin from bovine serum slightly affected the anti-α-glucosidase activity of quercetin. The flavonoids tested exhibited little effect on β-fructosidase activity at 0.1 mM.

Flavonoids have attracted interest as food components. They show bitterness in taste, anti-vitamin B1 activity, antioxidative action, mutagenicity17 reaction with proteins,2 and so forth. They have been studied from the food chemical point of view. On the other hand, their biochemical, physiological, and pharmacological effects have also been investigated and various activities were found.3 Some investigators consider them to be semi-essential nutrients.

We have reported that flavonoids inhibit alkaline phosphatase4 and glyoxalase I,5 as part of our study of biochemical effect of flavonoids.

The present report describes inhibition of yeast α-glucosidase (α-D-glucoside glucohydrolase EC 3.2.1.20), but weak or no inhibition of β-fructosidase (β-D-fructofuranoside fructohydrolase EC 3.2.1.26) by flavonoids, the mode of inhibition, and the effect of albumin on the inhibitory activity. Some structure-activity relationships are also discussed.

MATERIALS AND METHODS

Materials. Yeast α-glucosidase, β-fructosidase, hexokinase, glucose-6-phosphate dehydrogenase, ATP sodium salt, and NADP sodium salt were obtained from Boehringer. Other chemicals were also purchased from the commercial sources indicated. Maltose, quercetin, morin, quercitin, and rutin (Wako); naringenin, and naringin (Tokyo Kasei); fisetin, chrysin, and myricetin (Aldrich); hesperidin (Nakarai); kaempferol (Sigma); p-nitrophenyl-α-D-glucopyranoside (Koch-Light). Apiin, herbacitrin, myricitrin, robinin, and gossypitrin were obtained through the courtesy of Professor Toshio Nakabayashi of Shizuoka University.

Enzyme activity assay.

(1) α-Glucosidase with p-nitrophenyl-α-D-glucopyranoside (NPG). The reaction was performed in a cuvette which was kept at 37°C in a temperature-controlled cuvette holder in a Shimadzu spectrophotometer UV 240. The reaction mixture contained 2.96 ml of 0.2 mM NPG in 0.1 M phosphate buffer, pH 6.0, 20 μl of 15 mM flavonoid in dimethyl sulfoxide (DMSO), and 20 μl of enzyme solution. Absorbance at 402 nm was recorded every 5 sec for the initial 30 sec. The enzyme amount giving an increase of about 0.07/30 sec in absorbance was used. The degree of inhibition (%) was expressed as (1 - ΔA_total/ ΔA_control) × 100, where ΔA indicates the absorbance increase in 30 sec.

(2) α-Glucosidase with maltose. Glucose was determined in terms of NADPH produced by the action of hexokinase and glucose-6-phosphate dehydrogenase in the presence of ATP and NADP.6 The reaction mixture contained 1.43 ml of 0.1 M acetate buffer, pH 6.0, 0.50 ml of 0.58 M maltose, 20 μl of 10 mM flavonoid in DMSO, and
50 μl of α-glucosidase solution. The amount of enzyme per reaction mixture was one which liberated 3.3 μmol of glucose per minute. The reaction was initiated by adding the enzyme solution and the mixture was kept at 37°C for 4 min. The reaction was terminated by putting the mixture into a boiling bath for 3 min. An aliquot (10 μl) was taken to determine the amount of glucose liberated, by the method described in ref. 6.

(3) β-Fructosidase. The reaction mixture contained 2.50 ml of 2 M sucrose in 0.2 M phosphate buffer, pH 6.8, and 25 μl of 10 M flavonoid in DMSO. The reaction was initiated by adding 0.10 ml of enzyme solution, which produced 0.46 μmol of glucose and fructose in 1 min, and the mixture was kept at 37°C for 20 min. An aliquot (100 μl) was used to determine the reducing power due to glucose and fructose liberated by the modification of Somogyi and Nelson's method described by Hatanaka and Kobara.

RESULTS AND DISCUSSION

Effect of flavonoids on α-glucosidase activity

The effect is summarized in Table I when NPG was used as substrate. Quercetin, myricetin, fisetin, quercitrin, and kaempferol showed strong inhibition, whereas myricitrin, morin, naringenin, gossypitrin, herbacirin, and rutin exhibited medium to weak inhibition at 0.1 mM. Figure 1 shows inhibition (%) of the activity as a function of quercetin concentration. The quercetin concentration giving 50% inhibition (I50) was determined to be 8 μM from this result. Other flavonoids had I50 values listed in Table II. Myricetin, quercetin, and fisetin were found to be the most potent in inhibitory action. When maltose was employed as substrate, quercetin and fisetin showed considerable inhibition, too (Table III). The degree of inhibition was reduced to some extent, probably because the substrate concentration was much higher.

![Figure 1. Inhibition of α-Glucosidase Activity as a Function of Concentration.](image)

**TABLE I. EFFECTS OF FLAVONOIDS ON α-GLUCOSIDASE ACTIVITY (SUBSTRATE = p-NITROPHENYL-α-D-GLUCOPYRANOSE)***

The enzyme activity was estimated by measuring p-nitrophenol liberated. Experimental details are described in the text.

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>Inhibition (%)</th>
<th>Flavonoid</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>96</td>
<td>Gossypitin</td>
<td>48</td>
</tr>
<tr>
<td>Myricetin</td>
<td>94</td>
<td>Herbacirin</td>
<td>38</td>
</tr>
<tr>
<td>Fisetin</td>
<td>89</td>
<td>Rutin</td>
<td>27</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>87</td>
<td>Robinin</td>
<td>7</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>78</td>
<td>Hesperidin</td>
<td>2</td>
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<tr>
<td>Myricitrin</td>
<td>69</td>
<td>Apiin</td>
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</tr>
<tr>
<td>Morin</td>
<td>68</td>
<td>Naringin</td>
<td>0</td>
</tr>
<tr>
<td>Naringenin</td>
<td>66</td>
<td>Chrysin^a</td>
<td>0</td>
</tr>
</tbody>
</table>

^a The concentration was 0.01 mM owing to low solubility.

**TABLE II. I50 VALUES OF FLAVONOIDS INHIBITORY TO α-GLUCOSIDASE***

The reaction condition was the same as that described in the legend to Table I except that concentrations of quercetin were varied. Each point indicates the average of quadruplicate measurements.

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>I50 (μM)</th>
<th>Flavonoid</th>
<th>I50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myricetin</td>
<td>4</td>
<td>Kaempferol</td>
<td>20</td>
</tr>
<tr>
<td>Quercetin</td>
<td>8</td>
<td>Myricitrin</td>
<td>30</td>
</tr>
<tr>
<td>Fisetin</td>
<td>8</td>
<td>Morin</td>
<td>40</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>20</td>
<td>Naringenin</td>
<td>50</td>
</tr>
</tbody>
</table>

**TABLE III. EFFECTS OF QUERCETIN AND FISETIN ON α-GLUCOSIDASE ACTIVITY (SUBSTRATE = MALTOSE)***

The enzyme activity was estimated by measuring glucose formed with enzymatic assay method. For the details, see MATERIALS AND METHODS.

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>58</td>
</tr>
<tr>
<td>Fisetin</td>
<td>46</td>
</tr>
</tbody>
</table>
Flavonoids Inhibit α-Glucosidase

**Table IV. Kinetic Constants of α-Glucosidase Inhibition by Flavonoids**

The reaction conditions were the same as those described in the legend to Table I except that concentrations of NPG were varied. Each set of $K_i$ and $K_i'$ were determined from slopes and intercepts in double-reciprocal plots.

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>$K_i$ (μM)</th>
<th>$K_i'$</th>
<th>Mode of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>6.2</td>
<td>8.2</td>
<td>Mixed type, close to non-competitive</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>13</td>
<td>22</td>
<td>Mixed type, close to non-competitive</td>
</tr>
<tr>
<td>Fisetin</td>
<td>3.2</td>
<td>24</td>
<td>Mixed type</td>
</tr>
<tr>
<td>Naringenin</td>
<td>41</td>
<td>120</td>
<td>Mixed type</td>
</tr>
<tr>
<td>Morin</td>
<td>47</td>
<td>230</td>
<td>Mixed type</td>
</tr>
</tbody>
</table>

The presence and absence of quercetin, indicating that the mode was mixed type very close to non-competitive type. The results of kinetic studies with a number of flavonoids are summarized in Table IV. In all the cases studied, both slopes and intercepts were changed by the presence of each flavonoid tested. This suggests that flavonoids can bind both free enzyme and enzyme-substrate complex. Quercitrin appears to inhibit in the enzyme in a similar manner as quercetin, although the $K_i$ and $K_i'$ for quercitrin were one order larger than those for quercetin. The $K_i$ and $K_i'$ are dissociation constants of the enzyme-inhibitor complex and enzyme-substrate-inhibitor complex, respectively, as shown in Chart 1. Two possibilities have been considered from the structure of flavonoids. One is that some flavonoids like rutin and quercitrin have glycosyl substituents, which may cause competitive inhibition of α-glucosidase through the glycosyl groups. The other is that the product, $p$-nitrophenol has, at least some structural resemblance to aglycons, which may cause binding of the aglycons to the substrate binding site of the enzyme. But the result suggests that flavonoids can bind both free enzyme and enzyme-substrate complex. This means that flavonoids are able to combine with the enzyme at site(s) other than the substrate-binding site.

**Effect of albumin on inhibition by quercetin of α-glucosidase**

Inhibition of α-glucosidase caused by flavonoids may seem non-specific in nature, because polyphenol compounds often bind to protein non-specifically and eventually deactivate enzymes by denaturation. To examine the specificity of quercetin inhibition to α-glucosidase, bovine serum albumin was added to the reaction mixture. The amount of albumin added ranged from 1-fold to 100-fold the concent-
tration of protein originally present in the enzyme preparation. The presence of added albumin did not much affect the inhibitory action of quercetin, indicating specificity of the flavonoid compound to \( \alpha \)-glucosidase (Fig. 3).

**Relationship of the influence of flavonoids on \( \alpha \)-glucosidase and glyoxalase I**

Comparison was made between the inhibition by flavonoids of \( \alpha \)-glucosidase and glyoxalase I. Fairly good correlation can be seen in Fig. 4, the correlation coefficient being 0.86. Therefore flavonoids which inhibit \( \alpha \)-glucosidase activity also usually hinder the action of glyoxalase I. Nevertheless the modes of inhibition by quercetin were different in the two enzyme reactions. The mode of \( \alpha \)-glucosidase inhibition by quercetin was mixed type, while it inhibited glyoxalase I competitively.

**Fig. 3. Effects of Albumin on \( \alpha \)-Glucosidase Inhibition by Quercetin.**

The reaction conditions were the same as those described in the legend to Table I except that various amount of albumin was added to the reaction mixture. Each point indicates the average of quadruplicate measurements. The arrow indicates the concentration of albumin equal to that of protein contained in the enzyme solution.

**Fig. 4. Relationship of the Influence of Flavonoids on \( \alpha \)-Glucosidase and Glyoxalase I.**

The plotted values were taken from Table I and ref. 5.

**Table V. Substituent Groups of Flavonoids Tested**

<table>
<thead>
<tr>
<th>Name</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>8</th>
<th>2'</th>
<th>3'</th>
<th>4'</th>
<th>5'</th>
</tr>
</thead>
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<tr>
<td><strong>Flavonol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaempferol</td>
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<td>OH</td>
<td>OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>Robinin</td>
<td>O-Glya</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>Quercetin</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>O-Glya</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>Rutin</td>
<td>O-Glyb</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>Myricetin</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>Myricitrin</td>
<td>O-Glya</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>Morin</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td>OH</td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>Fisetin</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>Herbacitrin</td>
<td>OH</td>
<td>OH</td>
<td>O-Glya</td>
<td>OH</td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>Gossypitrin</td>
<td>OH</td>
<td>OH</td>
<td>O-Glya</td>
<td>OH</td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td><strong>Flavone</strong></td>
<td></td>
<td></td>
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<tr>
<td>Apiin</td>
<td>OH</td>
<td>OH</td>
<td>O-Glyd</td>
<td></td>
<td></td>
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<td></td>
<td>OH</td>
</tr>
<tr>
<td>Chrysin</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Flavanone</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Naringenin</td>
<td>OH</td>
<td>OH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>Naringin</td>
<td>OH</td>
<td>OH</td>
<td>O-Glya</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>Hesperidin</td>
<td>OH</td>
<td>OH</td>
<td>O-Glya</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
</tbody>
</table>

\* Rhamnosyl. \* Rhamnoglucosyl. \* Glucosyl. \* Glucoapiosyl. \* Glucorhamnosyl.
Flavonoids Inhibit α-Glucosidase

Table VI. Effects of Flavonoids on β-Fructosidase Activity

The enzyme activity was estimated by measuring glucose and fructose with a modification of Somogyi and Nelson's method. Experimental details are described in the text.

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>Inhibition (%)</th>
<th>Flavonoid</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naringin</td>
<td>7</td>
<td>Morin</td>
<td>0</td>
</tr>
<tr>
<td>Quercetin</td>
<td>4</td>
<td>Naringenin</td>
<td>0</td>
</tr>
<tr>
<td>Rutin</td>
<td>4</td>
<td>Hesperidin</td>
<td>0</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Structure–activity relationship

Table V summarizes substituent groups on the phenylbenzopyrone ring of the flavonoids tested. A requisite for the inhibitory action seems to be the presence of a certain number of hydroxyl groups. Those flavonoids whose number of hydroxyl groups are less than three (e.g. naringin and hesperidin) have no inhibitory activity. The idea that the inactivity of naringin and hesperidin results from their saturated bond between C2 and C3 is reversed by the fact that fairly strong inhibition was observed with naringenin which has the same structure at this position. A hydroxyl group at the 5-position does not seem essential, because fisetin showed very strong inhibition in spite of the absence of a 5-OH. Glycosylation of hydroxyl groups decreases the inhibitory activity; kaempferol to robinin, quercetin to quercitrin and to rutin, naringenin to naringin, myricetin to myricitrin, although the degree of the regression varies from flavonoid to flavonoid.

Effects of flavonoids on β-fructosidase activity

As can be seen from Table VI, the flavonoids tested showed only weak or no inhibition to β-fructosidase in contrast to α-glucosidase. The result would indirectly support the idea of specific action of flavonoids on α-glucosidase conversely.

Flavonoids are known to inhibit various kinds of enzymes, including cyclic nucleotide phosphodiesterase, protein kinase, glyoxalase I, and lipoxygenase. Thus flavonoids show physiological effects like anti-inflammatory and anti-allergic action, anti-promotor activity in carcinogenesis, and suppression of platelet aggregation through inhibition of the enzymes mentioned above. The authors aim at investigating influence of flavonoids on the digestion, absorption, and metabolism of nutrients. The yeast enzymes were used as preliminary tests for ones from mammalian sources. Experiment with disaccharide-hydrolyzing enzymes from porcine intestinal mucosa are now in progress.

Acknowledgment. We express our thanks to Professor Toshio Nakabayashi of Shizuoka University for his generous gift of certain flavonoids.

REFERENCES