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Inhibitory Activity of Cyanidin-3-rutinoside on α-Glucosidase

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Cyanidin-3-rutinoside, a natural anthocyanin, inhibited α-glucosidase from baker's yeast in dose-responsive manner. The IC_{50} value was 19.7 μM ± 0.24 μM, compared with the IC_{50} value of voglibose (IC_{50} = 23.4 ± 0.30 μM). Cyanidin-3-rutinoside was found to be a non-competitive inhibitor for yeast α-glucosidase with a K_i value in the range of 1.31–1.56 × 10^{-5} M. These results indicated that cyanidin-3-rutinoside could be classed as a new α-glucosidase inhibitor.

Keywords: Cyanidin-3-rutinoside; α-Glucosidase inhibition; Enzyme kinetics

INTRODUCTION

α-Glucosidase is an enzyme that catalyzes the final step in the digestion of carbohydrates. It is located in the brush-border surface membrane of intestinal cells. Inhibitors of this enzyme delay digestion of complex carbohydrates and disaccharides to absorbable monosaccharides. This leads to a reduction in glucose absorption and, subsequently, the rise of postprandial hyperglycemia is attenuated. Inhibition of α-glucosidase has considerable potential for treatment of diabetes, obesity and hyperlipidemia.[1–3] Additionally, an α-glucosidase inhibitor such as nojirimycin is also a potential inhibitor of human immunodeficiency virus (HIV) replication and HIV-mediated syncytium formation *in vitro*.[4–6] Interestingly, α-glucosidase inhibitors have been used for treatment of B- and C-type viral hepatitis.[7,8]

Anthocyanins are flavonoid phenolic compounds that occur in the red, purple, and blue colors of many kinds of fruit. Recently, a great deal of renewed interest in anthocyanins has emerged because of their potential health benefits as antioxidant, anticarcinogenic and anti-inflammatory agents and for their anti-hyperglycemic effect.[9–12] Interestingly, the anthocyanin extracts from various plants have been reported to cause α-glucosidase inhibition.[13] Cyanidin-3-rutinoside (Figure 1), 3-O-β-D-(6‴-O-α-L-(6‴-deoxy-mannosyl))glucopyranosyl-cyanidin, was first extracted from *Antirrhinum majus*.[14] It has also been found in litchi,[15] black current,[16] capulin[17] and sweet cherry.[18] Apart from a recent report, cyanidin-3-rutinoside from sweet cherry exhibited cyclooxygenase I and II inhibitory activites,[19] little is known about the biological activity of cyanidin-3-rutinoside. The synthesis of cyanidin-3-rutinoside from quercetin-3-rutinoside (Figure 2) was described in 1995[20] and this method requires a short reaction time and a simple procedure for purification. Hence, the aim of this study was to investigate the inhibitory activity of synthetic cyanidin-3-rutinoside against α-glucosidase activity by comparing its activity with quercetin-3-rutinoside and voglibose (a well known α-glucosidase inhibitor).

MATERIALS AND METHODS

Reagents

α-Glucosidase (EC 3.2.1.20) from baker's yeast (type I), *p*-nitrophenyl-α-D-glucopyranoside (PNP-G) and quercetin-3-rutinoside were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO). Voglibose was

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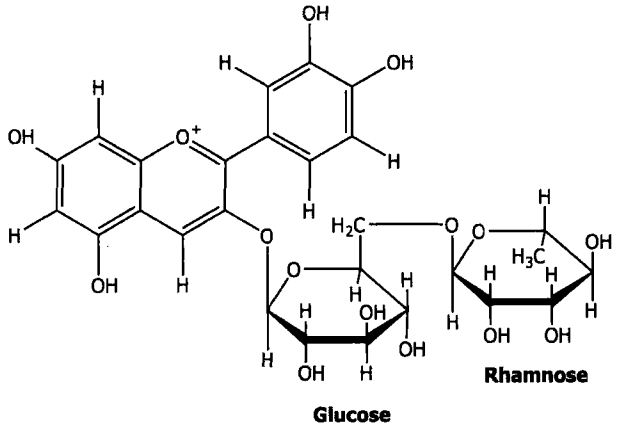

FIGURE 1 Chemical structure of cyanidin-3-rutinoside.

obtained from Takeda Medical Co. Ltd. (Osaka, Japan). All others chemicals used were of analytical grade.

Synthesis of Cyanidin-3-rutinoside

Cyanidin-3-rutinoside was synthesized from quercetin-3-rutinoside following the Elhabiri method.[20] After purification, the structure of this compound was confirmed by ^{1}H NMR, ^{13}C-NMR and mass spectrometry data.

α-Glucosidase Activity

Assessment of the α-glucosidase inhibitory activity of cyanidin-3-rutinoside was carried out according to reported methods.[21–23] Briefly, α-glucosidase from baker's yeast was assayed using 0.1 M phosphate buffer (pH 6.9) with 1 mM *p*-nitrophenyl-α-D-glucopyranoside (PNP-G) as substrate. The concentration of the enzyme was specified between 1–4 U/ml in each experiment. 40 μl of α-glucosidase was incubated in the absence or presence of various concentrations of cyanidin-3-rutinoside (10 μl) at 37°C. The preincubation time was specified between 0–60 min and PNP-G (950 μl) was then added to the mixture. The reaction was carried out at 37°C for 20 min, and then 1 ml of 1 M Na_2CO_3 was added to terminate the reaction. Enzymatic activity was quantified by measuring the absorbance at 405 nm. One unit of α-glucosidase is defined as the amount of enzyme liberating 1.0 μmol of PNP per minute under the conditions specified.

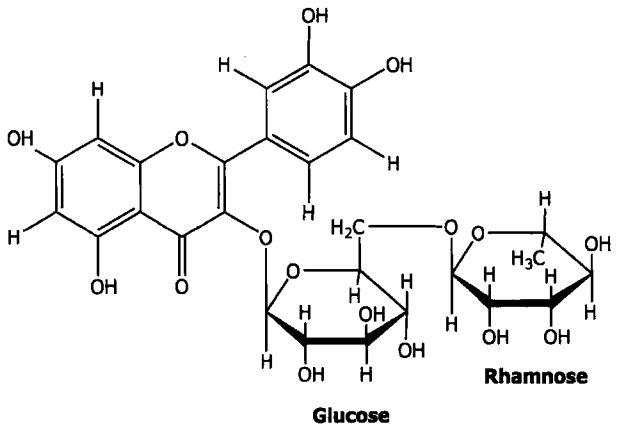

FIGURE 2 Chemical structure of quercetin-3-rutinoside.

Kinetics of Enzyme Inhibition

In order to evaluate the type of inhibition using the Lineweaver-Burk plot, the enzyme reaction was performed according to the above reaction but including various concentrations of cyanidin-3-rutinoside.

Statistical Analysis

The experiment for α-glucosidase inhibition was assayed in duplicate, 5 assays per test activity. The results are expressed as the mean ± SE. IC_{50} values were determined from plots of concentration vs percent inhibition curves using a Sigma Plot.

RESULTS

Primarily, cyanidin-3-rutinoside was synthesized from quercetin-3-rutinoside and obtained as a red powder. The structure of cyanidin-3-rutinoside was confirmed by ^{1}H-NMR, ^{13}C-NMR and MS (data not shown) and is in good agreement with a previous report.[20] α-Glucosidase inhibition by cyanidin-3-rutinoside occurred in a dose-dependent manner (Figure 3). The IC_{50} value of cyanidin-3-rutinoside was 19.7 ± 0.24 μM, whereas the IC_{50} value of voglibose was 23.4 ± 0.30 μM. However, 1 mM quercetin-3-rutinoside showed only 36.56% inhibition which was very low when compared with cyanidin-3-rutinoside and voglibose. Figure 4 shows the IC_{50} values under treatment with various enzyme concentrations following a specific preincubation time at 10 min. The IC_{50} value increased from 19.7 ± 0.24 μM to 135.0 ± 0.87 μM when the amount of α-glucosidase in the mixture was increased from 1.0 to 4.0 U/ml. The IC_{50} value was also determined under different preincubation conditions as shown in Figure 5. When PNP-G (1 mM) and cyanidin-3-rutinoside were added simultaneously, the IC_{50} was 8.34 ± 0.45 mM which was higher than that for 10 min preincubation by about 423-fold. This result shows that the IC_{50} value decreased with increasing preincubation time and it gave a minimal value for 30 min preincubation (8.90 ± 0.34 μM). Double-reciprocal plots of α-glucosidase kinetics with cyanidin-3-rutinoside are shown in Figure 6. The graph demonstrated that baker's yeast α-glucosidase was non-competitively inhibited with a K_i value in

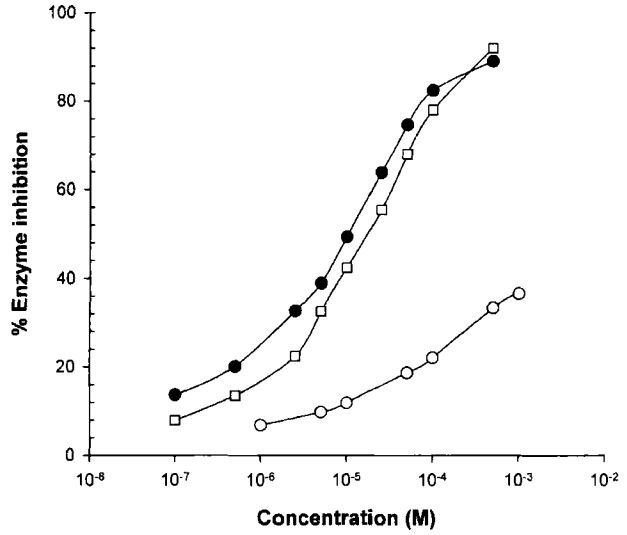

FIGURE 3 Inhibition of α-glucosidase by (●) cyanidin-3-rutinoside, (○) quercetin-3-rutinoside and (□) voglibose. 1 U/ml α-glucosidase was treated with various concentrations of inhibitors for 10 min, 37°C before adding 1 mM PNP-G. The reaction was carried out at 37°C for 20 min, and then 1 ml of 1 M Na_2CO_3 was added to terminate the reaction. Enzymatic activity was quantified by measuring the absorbance at 400 nm. The IC_{50} value was calculated from plots of concentration vs percentage inhibition curves.

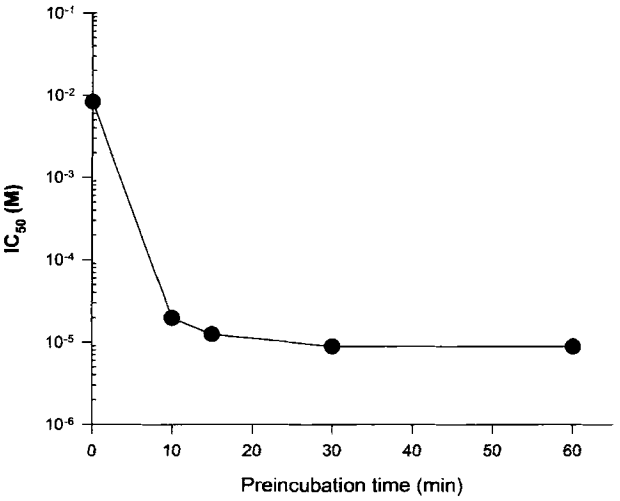

FIGURE 5 The IC_{50} value for the inhibition of α-glucosidase varied with the preincubation time. 1 U/ml α-glucosidase was treated with various concentrations of cyanidin-3-rutinoside for 0–60 min in phosphate buffer (pH 6.9) at 37°C. After the preincubation period, 1 mM PNP-G was added to the mixture to initiate the reaction.

the range of $13.1-15.6 \times 10^{-6}$ M. The, K_i value was calculated using the values of V_{max} obtained at 0 and 50 μM.

DISCUSSION

Much attention has been paid to the biological functions of dietary flavonoids. Indeed, anthocyanins, natural pigments of the flavonoid family, have been shown to have multiple biological effects.[24] Bioavailability of cyanidin-3-rutinoside, which is directly absorbed and distributed to the blood in rats and humans, has recently been reported.[25] We first examined whether cyanidin-3-rutinoside inhibited α-glucosidase. The activity of α-glucosidase was reduced by cyanidin-3-rutinoside in a dose-dependent manner. We showed in the present study that cyandin-3-rutinoside is a potent α-glucosidase inhibitor as well as voglibose, which has been

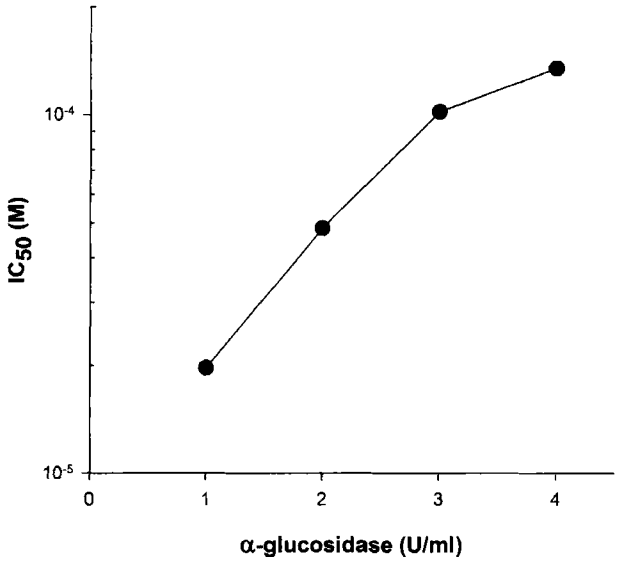

FIGURE 4 The IC_{50} value for the inhibition of α-glucosidase varied depending on the amount of α-glucosidase. Different amounts (1,2,3 and 4 U/ml) of α-glucosidase was treated with various concentration of cyanidin-3-rutinoside at 37°C for 10 min. 1 mM PNP-G was added to the mixture to initiate the reaction.

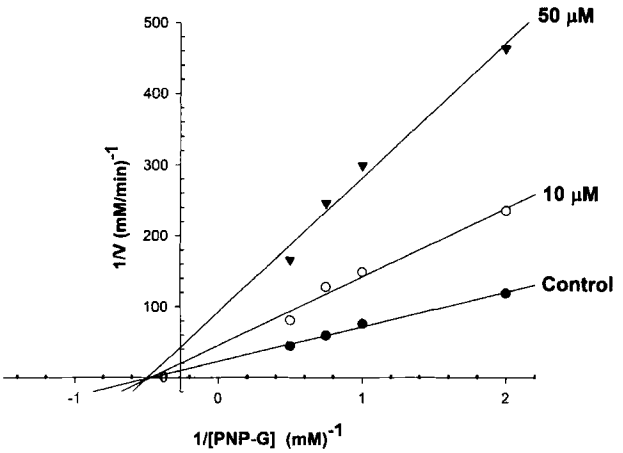

FIGURE 6 Double-reciprocal plots of the inhibition kinetics of yeast α-glucosidase by cyanidin-3-rutinoside. 1 U/ml α-glucosidase was treated with various concentration of cyanidin-3-rutinoside for 10 min in phosphate buffer (pH 6.9) at 37°C. After the preincubation period, various concentrations of PNP-G were added to the mixture to initiate the reaction.

previously used as a positive inhibitor of yeast α-glucosidase.[21] Quercetin-3-rutinoside is a flavonol glycoside, and has little effect as an inhibitor of α-glucosidase. The difference at C-4 of quercetin-3-rutinoside and cyanidin-3-rutinoside may correlate to α-glucosidase inhibitory activity. We observed that the IC_{50} value increased when the enzyme concentration was raised. Interestingly, α-Glucosidase inhibitory activity was also increased by increasing the preincubation time between cyanidin-3-rutinoside and the enzyme. This result suggested that the time required to reach the binding equilibrium varies with the enzyme concentration, the inhibition mode seems to be of a slow-binding type. The mode of inhibition of the studied compound was non-competitive when the enzyme was pretreated with cyanidin-3-rutinoside for 10 min. In conclusion, cyanidin-3-rutinoside should be further evaluated for development as a new potent α-glucosidase inhibitor for the potential treatment of various diseases, including diabetes, obesity, and AIDS. However, the yeast α-glucosidase is known to be very different from the mammalian digestive enzymes, suggesting that future and ongoing experiments should be focused on the inhibitory activity of cyanidin-3-rutinoside against mammalian intestinal α-glucosidases. Additional studies on α-glucosidase inhibitory effects of cyanidin-3-rutinoside using crystallography, evaluation of the activity profile of other compounds in the anthocyanin family as inhibitor of α-glucosidase, and so forth, are in progress.

Acknowledgements

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