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Genistein, a soy isoflavone, is a potent α-glucosidase inhibitor

Dong-Sun Lee[a], Sang-Han Lee[b,*]

[a] *Biophysical Chemistry Laboratory, The Institute of Physical and Chemical Research, RIKEN, Harima Institute, Hyogo 679-5148, Japan*
[b] *Biopotency Evaluation Laboratory, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Rm. 201, 209-11, Shinsung-Dong, Yusung-Gu, Taejon 305-333, South Korea*

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Abstract Genistein is an isoflavone that is known to be contained in soybean. It was proved that genistein plays a pivotal role in homeostasis in the human body. In the course of screening for useful α-glucosidase inhibitors, we isolated and identified genistein as a candidate for α-glucosidase inhibitor from fermentation broths of a *Streptomyces* sp. Genistein was shown to be a reversible, slow-binding, non-competitive inhibitor of yeast α-glucosidase with a K_i value of 5.7×10^{-8} M when the enzyme mixture was pretreated with genistein. These results show a possibility that genistein could be a useful tool for metabolic disorders.

Key words: Genistein; α-Glucosidase inhibitor; Enzyme kinetics

1. Introduction

Glycosidases are not only essential to carbohydrate digestion, but also vital for the processing of glycoproteins and glycolipids. Glycosidases are also involved in a variety of metabolic disorders and other diseases such as diabetes [1], viral attachment [2] and cancer formation [3]. Because of their importance, glycosidase inhibitors can be important tools for studying their mechanisms of action and are also prospective therapeutic agents for some degenerative diseases [4]. Glucosidases are located in the brush-border surface membrane of intestinal cells [5], and are the key enzymes of carbohydrate digestion [6]. Some researchers have reported that oral administration of specific α-glucosidase inhibitors could effectively improve hyperglycemia as well as diabetic complications [7,8]. Additionally, α-glucosidase inhibitors such as nojirimycin, *N*-butyldeoxynojirimycin, and castanospermine are potent inhibitors of human immunodeficiency virus (HIV) replication and HIV-mediated syncytium formation in vitro [9–11]. Recently, we found that genistein, isolated from *Streptomyces* sp., slightly inhibited the action of DNA topoisomerase II (data not shown). Interestingly, in the course of our search for α-glucosidase inhibitors, genistein was isolated and identified as a potent α-glucosidase inhibitor (Fig. 1). In this study, we describe the inhibitory mode of action of genistein against α-glucosidase.

2. Materials and methods

2.1. Reagents

p-Nitrophenyl (PNP) glycosides used in this study were purchased from Sigma (St. Louis, MO, USA). α-Glucosidase (from baker's yeast, Sigma), β-glucosidase (from almond, Sigma), α-mannosidase (jack beans, Sigma), and β-mannosidase (from snail acetone powder, Sigma) were commercially available. Genistein was isolated and purified from a *Streptomyces* sp. by various analytical procedures or was commercially available (Sigma).

2.2. Enzyme assays

To evaluate whether genistein inhibits various commercially available glycosidases, the assay for glycosidases was done as described previously [12,13]. In brief, α-glucosidase and the other glycosidases were assayed using 50 mM phosphate buffer at pH 6.7, and the appropriate PNP glycosides (at 1 mM) were used as substrates. The concentration of the enzymes is specified in each experiment. Genistein at the designated concentration was added to the enzyme solution in the buffer and incubated at 30°C for 1 h, and the substrate was then added to initiate the enzyme reaction. When pretreatment is not specified, mixtures of substrate and genistein at designated concentrations were prepared beforehand and added to the enzyme solution. The enzyme reaction was carried out at 30°C for 30 min, and then three volumes of 1 M Na_2CO_3 were added to terminate the reaction. Enzymatic activity was quantified by measuring the absorbance at 405 nm. One unit of α-glucosidase and other glycosidases is defined as the amount of enzyme liberating 1.0 μmol of PNP per minute under the assay conditions specified.

2.3. Dialysis experiment

α-Glucosidase (0.5 ml, 100 U/ml) and genistein (10 μM) treated for appropriate times at 30°C were dialyzed against phosphate buffer (5 mM, pH 6.7) at 4°C for 24 h, changing the buffer every 12 h. Another 0.5 ml aliquot was kept at 4°C for 24 h without dialysis. The contents in dialysis tubes were determined for residual enzyme activity as described in Section 2.2.

2.4. Kinetics of enzyme inhibition

The enzyme reaction was performed according to the above reaction conditions with inhibitors of various concentrations. Inhibition types for the inhibitors were determined by Dixon plot and its replot of slope versus the reciprocal of the substrate concentration [14].

3. Results

A potent α-glucosidase inhibitor was isolated and purified from a *Streptomyces* sp. by various analytical procedures (data not shown). We screened out one of 2160 fermentation broths, and confirmed that the purified compound was genistein (Fig. 1), which was identical to authentic genistein (Sigma) (data not shown). Thereafter, we used genistein that is commercially available (Sigma).

*Corresponding author. Fax: (82)-42-860 4605.
E-mail: sang@mail.kribb.re.kr

Abbreviations: PNP, *p*-nitrophenyl; HIV, human immunodeficiency virus

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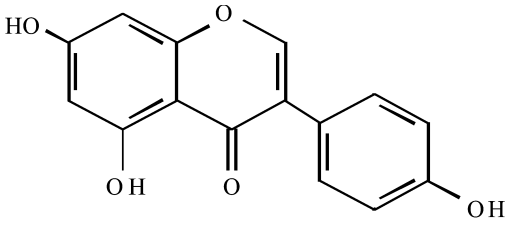

Fig. 1. The structure of genistein.

As shown in Fig. 2, α-glucosidase was the most sensitive to genistein, and the concentration required for 50% inhibition (IC_{50}) was 50 nM. At higher concentrations, genistein inhibited the activities of α-mannosidase, β-mannosidase, and β-glucosidase with sensitivities decreasing in that order. The IC_{50} values were 840 nM, 4.5 μM and 18 μM, respectively. This result shows that the activity of α-glucosidase is reduced by genistein in a dose-responsive manner.

We first examined the effects of genistein on inhibition of α-glucosidase (Fig. 3). When we added 2 mM glucose to the reaction mixture, the IC_{50} value was increased more than five-fold. Therefore, we assumed that genistein at least partially acts as an analog of glucose or binds to the glucose-binding site of α-glucosidase. In Fig. 3A, we investigated whether the IC_{50} value increased from 50 nM to 1.6 μM when the amount of α-glucosidase in the reaction mixture was raised from 1.0 to 8.0 U/ml. The results partially suggest that the inhibitor might bind to the glucose-binding site of α-glucosidase, although the binding has not yet been investigated. The α-glucosidase inhibitory activity by genistein was increased by preincubation of the inhibitor with the enzyme (Fig. 3B). When the substrate (1 mM) and genistein (10 μM) were added simultaneously, the IC_{50} was ca. 8.0 μM. This value was decreased 154-fold (ca. 50 nM) when α-glucosidase was treated with genistein at 30°C for 1 h before the initiation of enzyme reaction.

To examine whether the inhibition of α-glucosidase by genistein is reversible or not, the inhibitor (10 μM) was added to α-glucosidase (100 U/ml) before dialysis. α-Glucosidase (0.5 ml, 100 U/ml) and genistein (10 μM) treated for 2 h at 30°C were dialyzed against phosphate buffer (5 mM, pH 6.7) at 4°C for 24 h, changing the buffer every 12 h. Another 0.5 ml aliquot was kept at 4°C for 24 h without dialysis. The contents in dialysis tubes were determined for residual enzyme activity. When genistein alone was dialyzed with the buffer completely, no inhibitory activity was detected even at 10^5-fold dilution (data not shown). The enzyme activity in the dialysis membrane was similar with and without dialysis (Fig. 4, compare □ with ○), while the activity of α-glucosidase preincubated with genistein was not detected before dialysis (Fig. 4, ▽), but the activity was recovered after dialysis

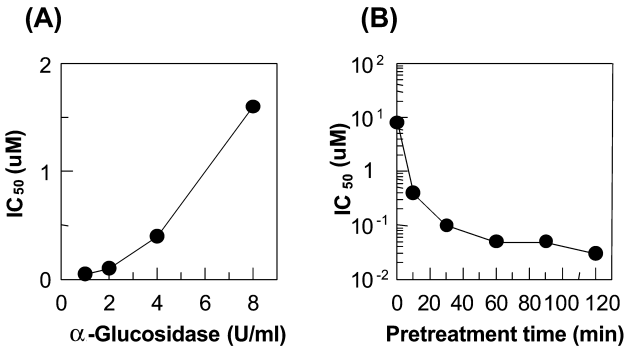

Fig. 3. The IC_{50} value for the inhibition of α-glucosidase varies depending on the amount of α-glucosidase (A) and on the time of pretreatment with genistein (B). A: Different amounts (1, 2, 4, and 8 U/ml) of α-glucosidase were treated with genistein (10 μM) for 1 h. B: α-Glucosidase (1 U/ml) was pretreated with genistein (10 μM) for 0–120 min in phosphate buffer (50 mM, pH 6.7) at 30°C. After pretreatment of α-glucosidase with genistein, PNP-α-glucopyranoside was added to the mixture to initiate the reaction. The IC_{50} values were determined by quantifying the PNP liberated.

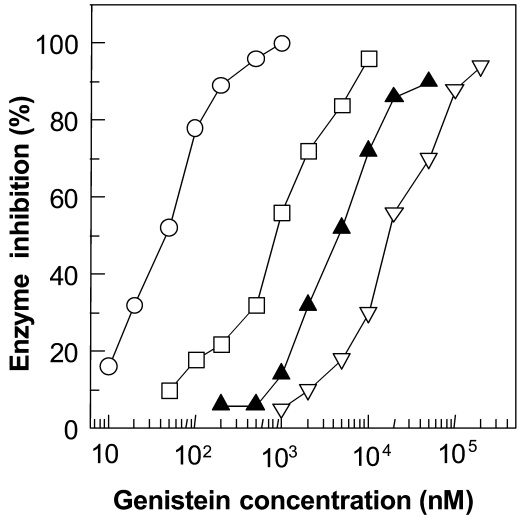

Fig. 2. Inhibition by genistein against various glucosidases. Enzyme solutions were treated with designated concentrations of genistein. The amount of enzymes were as follows: 1 U/ml α-glucosidase (○), 0.5 U/ml β-glucosidase (▽), 0.5 U/ml α-mannosidase (□), 0.1 U/ml β-mannosidase (▲). The mixtures of enzyme and genistein were kept at room temperature for 1 h.

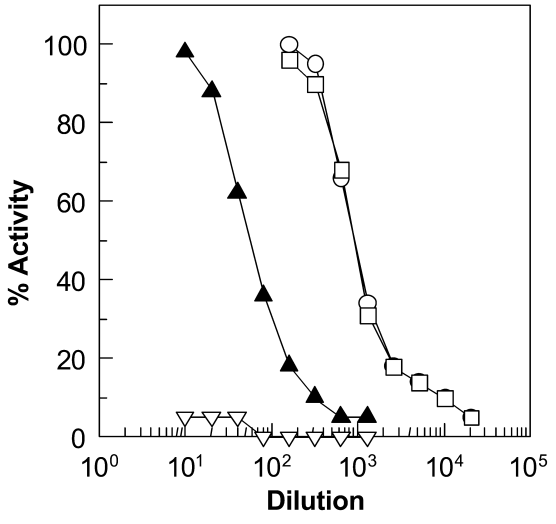

Fig. 4. The recovery of inhibitory action of genistein against α-glucosidase after dialysis. The inhibitor (10 μM) was added to α-glucosidase (100 U/ml) before dialysis. α-Glucosidase (0.5 ml, 100 U/ml) and genistein (10 μM) treated for 2 h at 30°C were dialyzed against phosphate buffer (5 mM, pH 6.7) at 4°C for 24 h, changing the buffer every 12 h. Another 0.5 ml aliquot was kept at 4°C for 24 h without dialysis. α-Glucosidase alone (□,○) and α-glucosidase/genistein (▽,▲) were dialyzed against 5 mM phosphate buffer (pH 7.0) at 4°C (○,▲) or were kept at 4°C (□,▽) for 24 h.

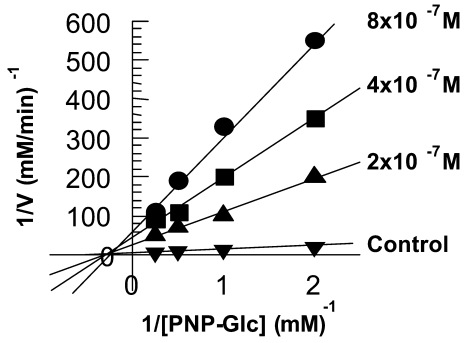

Fig. 5. Double-reciprocal plots of the inhibition kinetics of yeast α-glucosidase by genistein. α-Glucosidase (50 μl, 10 U/ml) that was treated first with genistein for 1 h at 30°C was treated with a mixture of 50 μl of each designed concentration of PNP-α-glucopyranoside to initiate the enzyme reaction.

(Fig. 4, ▲). These results show that inhibition mode of genistein against α-glucosidase is reversible.

Double-reciprocal plots of α-glucosidase kinetics with genistein are shown in Fig. 5. Non-competitive inhibition was partially observed when genistein and substrate were added simultaneously, showing a K_i value of 500 nM (data not shown). The enzyme activity was also inhibited non-competitively when the enzyme was pretreated with genistein (10 μM) for 1 h. At this point, the K_i value was 57 μM (Fig. 5). This K_i value was calculated using the values of V_{max} obtained at 0 and 0.8 μM of genistein.

4. Discussion

There is much evidence suggesting that compounds present in soybeans can prevent cancer in many different organ systems [15], although the evidence for specific soybean-derived compounds having a suppressive effect on carcinogenesis in animal model systems is limited [16]. It is well known that soy isoflavone genistein attenuates growth factor- and cytokine-stimulated proliferation in cancer cells [17]. Genistein is also a potent and specific inhibitor of tyrosine autophosphorylation of the epidermal growth factor receptor. Additionally, its mode of action includes inhibition of DNA topoisomerase II activity, regulation of cell cycle checkpoints, and antiangiogenic and antioxidant activities [15,16]. In the course of development of α-glucosidase inhibitors from microbial sources, we found genistein, a soy isoflavone, as a candidate for α-glucosidase inhibitors (Fig. 1). We show in this study that genistein is a potent α-glucosidase inhibitor suggesting that genistein could be a useful tool for metabolic disorders.

We first examined whether genistein specifically inhibits α-glucosidase. The activity of α-glucosidase is reduced by genistein in a dose-responsive manner (Fig. 2). We concluded that genistein may bind to α-glucosidase, and α-glucosidase inhibitory activity by genistein was increased by preincubation of the inhibitor with the enzyme (Fig. 3). Because the time required to reach the binding equilibrium varies with the enzyme concentration (Figs. 2 and 3), the inhibition mode seems to be a slow-binding capability. A comparison of the plots indicates that the enzyme treated with the inhibitor followed by dialysis (Fig. 4, ▲) required a concentration about 20-fold higher than the control (dialyzed enzyme without inhibitor, Fig. 4, ○) to attain the same enzyme activity. At least, the dialysis experiment showed that the inhibition of α-glucosidase by genistein might be reversible. Double-reciprocal plots of α-glucosidase with genistein revealed that the mode of enzyme inhibition was non-competitive when the enzyme was pretreated with genistein for 1 h. In present study, the data partially suggest that genistein offers a possibility of being developed as successful α-glucosidase inhibitor.

In conclusion, the results suggest that genistein could be used for mechanism studies on the glycosylation of some metabolic diseases. The inhibition studies provide useful information for the design of new potent inhibitors for glycosidases. To date, the yeast α-glucosidase is known to be very different from mammalian digestive enzymes, suggesting that ongoing experiments should be focused on the inhibitory activity of genistein against mammalian intestinal α-glucosidases. Further studies on the elucidation of molecular mechanisms of trimming and the inhibition mode of action of more potent genistein derivatives against α-glucosidase could also be rewarding.

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