Kor. J. Microbiol. Biotechnol.
Vol. 34, No. 4, 368–372 (2006)

Inhibition of α-Glucosidase Activity by Quercetin

Lee, Dong-Sun[1,3], Jong Guk Kim[1], and Sang-Han Lee[2,*]
Departments of [1]Microbiology, and [2]Food Science & Technology, Kyungpook National University, Daegu 702-701, Korea
[3]Department of Biochemistry & Molecular Biology, University of Texas Medical School, Houston, TX 77030, U.S.A.

Quercetin is a flavonoid molecule that is known to be in various sources of natural products such as vegetables and fruits. It has been proven that quercetin plays a crucial role in the prevention of colon cancer as well as homeostasis as radical scavenger in human body. It is also well-known that glycosidases, including α-glucosidase, are involved in a variety of degenerative metabolic disorders. In the course of screening useful α-glucosidase inhibitors, we screened out quercetin as a α-glucosidase inhibitor from chemical libraries. Quercetin was shown to be a reversible, slow-binding, and noncompetitive inhibitor of yeast α-glucosidase with a K_i value of 6.3×10^{-8} M when it was included with an enzyme mixture. Together, these results show that quercetin has potential in treating disorders including diabetes, although the further mechanistic study is needed.

Key words: Quercetin, α-glucosidase inhibitor, reversible, enzyme kinetics

Glycosidases are not only essential to carbohydrate digestion, but also they are vital in the processing of glycoproteins and glycolipids. Glycosidases are involved in a variety of metabolic disorders and other diseases such as cancer [2], diabetes [4], and viral attachments [8]. Due to their importance, glycosidase inhibitors are vital because of their mechanisms of action and because they can act as prospective therapeutic agents for some degenerative diseases [3]. Glucosidases are located in the brush-border surface membrane of intestinal cells, and are the key enzymes of carbohydrate digestion [1]. Some researchers have reported that the oral administration of specific α-glucosidase inhibitors could effectively improve hyperglycemia as well as diabetic complications [7, 12]. In addition, it has been proven that α-glucosidase inhibitors, such as castanospermine, nojirimycin, and N-butyldeoxynojirimycin, have potential in blocking human immunodeficiency virus (HIV) and HIV-mediated syncytium formations *in vitro* [5, 6, 17]. Recently, we found that quercetin slightly inhibited the action of DNA topoisomerase II (data not shown). It is interesting to note that, in the course of searching for α-glucosidase inhibitors, quercetin was screened as having potential (Fig. 1). In this study, we selected out quercetin as α-glucosidase inhibitor. We examined its inhibitory mode with regard to α-glucosidase inhibitor, and suggested that it could be a curative candidate for such metabolic diseases.

p-Nitrophenyl (PNP) glycosides and quercetin used in this study were purchased from Sigma Chemical Co. (St. Louis, MO). α-Glucosidase (from baker's yeast, Sigma Chemical Co.), β-glucosidase (from almond, Sigma Chemical Co.), α-mannosidase (Jack Beans, Sigma Chemical Co.), and b-mannosidase (from snail acetone powder, Sigma Chemical Co.) were commercially available.

To examine whether quercetin inhibits various glycosidases, the assay for glycosidases was carried out as described previously [10, 16]. Briefly, α-glucosidase and other glycosidases were assayed using a 50 mM phosphate buffer at a pH level of 6.7 and a 50 mM sodium citrate buffer at a pH level of 4.5, respectively, and 1 mM of *p*-nitrophenyl glycoside was used as a substrate. The unit of each enzyme is specified in the experiments. Quercetin was added to the enzyme solution in a buffer and incubated at 30°C for 1 h. The substrate was added to initiate the enzyme reaction. When pretreatment was not specified, mixtures of substrate and quercetin were prepared 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 of sodium bicarbonate were added to terminate the reaction. Enzymatic activity was quantified by measuring absorbency at 405 nm. One

*Corresponding author
Tel: 82-53-950-7754, Fax: 82-53-950-6772
E-mail: sang@knu.ac.kr

unit of α-glucosidase activity is defined as the enzyme amount that can liberate 1.0 mmole of *p*-nitrophenol per min. A half milliliter of α-glucosidase (100 U/ml) and quercetin (10 mM), which was treated for the appropriate amount of time at 30°C, was dialyzed against a phosphate buffer (5 mM, pH 6.7) at 4°C for 24 h, by exchanging the buffer every 12 h. Another 0.5 ml set was kept at 4°C for 24 h without dialysis. The contents of the dialysis tubes were calculated for residual enzyme activity, as described in the above-mentioned enzyme assays. The enzyme reaction was performed according to the above-mentioned conditions with inhibitors of various concentration levels. The inhibition types were determined by a Michaelis-Menten equation [14].

The harmful effects of oxidative processes in living organisms, in addition to chemical and biochemical media, can be reduced by antioxidants. The efficacy of an antioxidant depends on its reduction potential and its kinetics in eliminating diverse-free radicals. Reactive oxygen species decisively contribute to many diseases. Flavonoids are benzo-g-pyrone derivatives of a plant origin found in various fruits and vegetables as well as in tea and red wine. Quercetin is the most ubiquitous flavonoid molecule in nature. Quercetin can effectively protect cells and tissue against the deleterious effects of reactive oxygen species. This antioxidant activity results from the scavenging of free radicals and other oxidizing intermediates; from the chelation of iron or copper ions and from the inhibition of oxidases [8]. For free radical-scavenging properties, the scavenging of lipid- and protein-derived radicals is presumably of special importance. A number of actions show a potential anti-cancer agent, including cell-cycle regulation, interaction with Type II estrogen binding sites, and tyrosine kinase inhibition [11]. Quercetin appears to be associated with little toxicity when administered orally or intravenously. In vitro and some preliminary animal and human data indicate that quercetin inhibits tumor growth [13].

In the course of searching for α-glucosidase inhibitors from natural products, quercetin was found as an agent for α-glucosidase inhibitors (Fig. 1). In this study, it can be demonstrated that quercetin is a potent and selective α-glucosidase inhibitor. This suggests that it could be useful in treating metabolic disorders including metastasis, and AIDS.

We examined whether quercetin inhibits α-glucosidase.

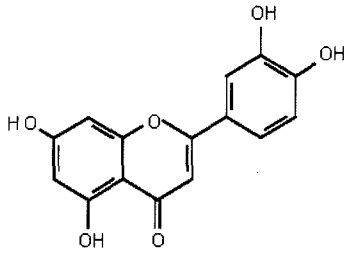

Fig. 1. The structure of quercetin.

As shown in Fig. 2, α-glucosidase was the most sensitive to quercetin, and the concentration that was required for 50% inhibition (IC_{50}) was 5.1×10^{-8} M. At higher concentration levels, quercetin inhibited the activity of α-mannosidase, β-mannosidase, and β-glucosidase with sensitivity decreasing in that order. The IC_{50} values were 4.1×10^{-7} M, 4.2×10^{-6} M and 1.0×10^{-5} M, respectively. The activity of α-glucosidase was reduced by quercetin in a dose-responsive manner (Fig. 2).

Next, we examined the effects of quercetin on the inhibition of α-glucosidase (Fig. 3). We assumed that quercetin acted as an analog of glucose or bound to the glucose-binding site of α-glucosidase. The α-glucosidase inhibitory activity of quercetin increased due to pre-incubation of the inhibitor with the enzyme (Fig. 3B). When the substrate and quercetin were added simultaneously, the IC_{50} was ca. 4.0×10^{-6} M. This value decreased about 74-fold (ca. 5.0×10^{-8} M) when α-glucosidase was treated

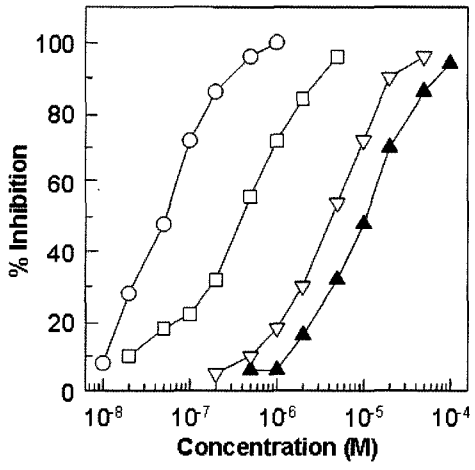

Fig. 2. Inhibition by quercetin against various glucosidases. Enzyme solutions were treated with various concentration levels of quercetin. The amount of enzymes was as follows: 1 U/ml α-glucosidase (white circles), 0.5 U/ml b-glucosidase (white reverse-triangles), 0.5 U/ml α-mannosidase (white squares), 0.1 U/ml β-mannosidase (black triangles). The enzyme and quercetin mixtures were kept at room temperature for 1 h.

with quercetin at 30°C for 1 h before the initiation of the enzyme reaction. We concluded that quercetin may bind to α-glucosidase and that α-glucosidase inhibitory activity of quercetin increased when the inhibitors were pre-incubated with the enzyme (Fig. 3). Because the time required reaching the binding equilibrium varies with the enzyme concentration level (Fig. 2 and 3), the inhibition mode seems to have slow-binding capability.

To examine whether the inhibition of α-glucosidase of quercetin is reversible, the inhibitor (10 mM) was added to α-glucosidase (100 U/ml) before dialysis. A half-milliliter of α-glucosidase (100 U/ml) and quercetin (10 mM), treated for 2 h at 30°C, was dialyzed against a phosphate buffer (5 mM, pH 6.7) at 4°C for 24 h. Another 0.5 ml set was kept at 4°C for 24 h without dialysis. The contents in the dialysis tubes were examined for residual enzyme activity. When quercetin alone was dialyzed with the buffer completely without enzyme, no inhibitory activity was detected even at a dilution of 10^5-fold (data not shown). The enzyme activity in the dialysis membrane was similar regardless of dialysis (Fig. 4, compare white squares with white circles), while the activity of α-glucosidase, pre-incubated with quercetin, was not calculated before dialysis (Fig. 4, black reverse-triangles), but the level of activity did recover after dialysis (Fig. 4, white triangles). These results show that inhibition of quercetin against α-glucosidase is reversible.

A comparison of the plots indicates that the enzyme, which was treated with the inhibitor followed by dialysis (Fig. 4, plot of white triangles), required a concentration of about 20-fold higher than the control (dialyzed enzyme without inhibitor, Fig. 4, plot of white circles) in order to attain the same level of enzyme activity. In the very least, the dialysis experiment showed that the inhibition of α-glucosidase of quercetin might be reversible.

Double-reciprocal plots of α-glucosidase kinetics with quercetin are shown in Fig. 5. Noncompetitive inhibition was partially observed when quercetin and substrate were added simultaneously, showing the K_i value as 4.4×10^{-7} M (Fig. 5A). Enzyme activity was also inhibited noncompetitively when the enzyme was pretreated with quercetin for 1 h. At this point, the K_i value was 6.3×10^{-8} M (Fig. 5B). This K_i value was calculated using the values of V_{max} obtained with 0 and 0.8 mM of quercetin. In the present study, the data suggest that quercetin has potential to be a successful α-glucosidase inhibitor. Moreover, recently, it is reported that the various flavonoids including anthocyanidin, isoflavone, and flanonol groups have potential to inhibit α-glucosidase as well as α-amylase [15]. This is meaningful because it could be examined clinically as an anti-cancer or anti-HIV agent without having any serious toxicity in humans as well as lab animals.

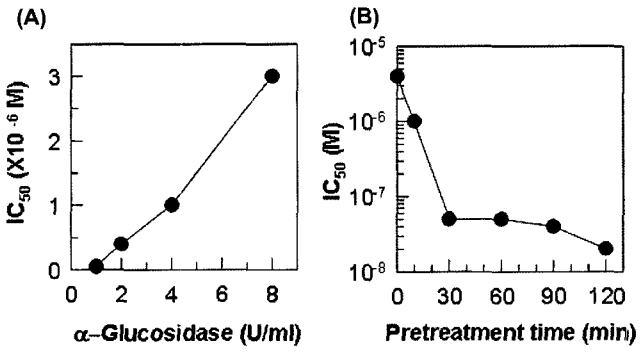

Fig. 3. The effects of enzyme amount and pretreatment time on the inhibition of α-glucosidase. The IC_{50} value for the inhibition of α-glucosidase varies depending on the amount of α-glucosidase (A) and on the time of pretreatment (B) with quercetin. In panel A, different amounts of α-glucosidase were treated with quercetin for 1 h. In panel B, α-glucosidase (1 U/ml) was pretreated with quercetin for 0-120 min in a phosphate buffer (50 mM, pH 6.7) at 30°C. After pretreatment of α-glucosidase with quercetin, PNP-α-glucopyranoside was added to the mixture to initiate the reaction. The IC_{50} values were determined by quantifying the amount of PNP liberated.

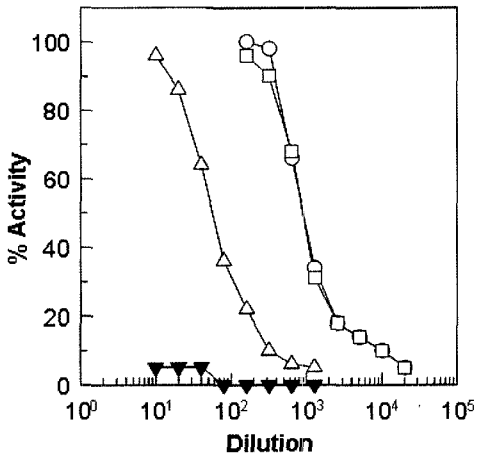

Fig. 4. Recovery of quercetin inhibitory action against α-glucosidase after dialysis. The inhibitor (10 mM) was added to α-glucosidase (100 U/ml) before dialysis. A half milliliter of α-glucosidase (100 U/ml) and quercetin (10 mM), treated for 2 h at 30°C, was dialyzed against a phosphate buffer (5 mM, pH 6.7) at 4°C for 24 h. Another 0.5 ml set was kept at 4°C for 24 h without dialysis. α-Glucosidase alone (white squares, white circles) and α-glucosidase/quercetin (black reverse-triangles, white triangles) were dialyzed against 5 mM of phosphate buffer (pH 7.0) at 4°C (white circles, white triangles) or were kept at 4°C (white squares, black reverse-triangles) for 24 h.

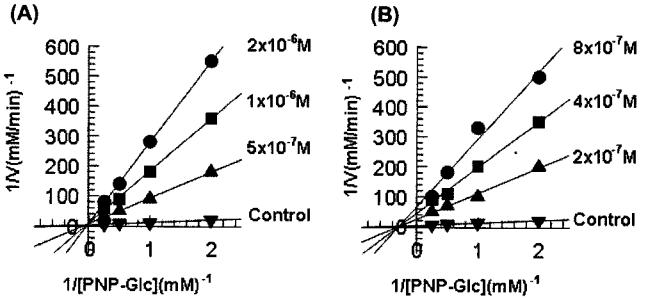

Fig. 5. Double reciprocal plots of the inhibition of yeast α-glucosidase by quercetin. In (A), 50 ml of α-glucosidase (10 U/ml) were treated with a mixture of a designed concentration of PNP-α-glucopyranoside and quercetin (50 ml). In (B), the same amount of enzymes, substrates, and inhibitors were used, but the enzymes were treated first with quercetin for 1 h at 30°C and then, 50 ml of the substrate were added to initiate the enzyme reaction.

In sum, the results suggest that quercetin could be used as a mechanism to study 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 of α-glucosidase is known to be very different from mammalian digestive enzymes, suggesting that ongoing experiments should examine on the inhibitory activity of quercetin against mammalian intestinal α-glucosidases. Further studies on the elucidation of molecular mechanisms in terms of trimming and the inhibition of more potent quercetin derivatives against α-glucosidase could prove to be useful. It is believed that quercetin can be used as an anti-cancer or anti-diabetic agent without any harmful side effects.

Acknowledgment

This work was partly supported by a Grant (20050301-034-474-006) from the BioGreen 21 Program, RDA, Korea.

초 록

Quercetin에 의한 α-glucosidase 활성 저해

이동선[1,3] · 김종국[1] · 이상한[2,*]
경북대학교 [1]미생물학과, [2]식품공학과, [3]텍사스의대 생화학분자생물학과

Quercetin은 flavonoid 계통의 물질로서 자연계에서 채소나 과일에 매우 흔하다. 또한 인체에서는 radical scavenger로서 신체항상성 뿐만 아니라 직장암의 예방에도 중요한 역할을 한다. Glucosidase를 포함한 glycosidase는 많은 종류의 대사성 질환에 관련이 있다. 유용한 α-glucosidase 저해제를 스크리닝 하던 중 chemical library로부터 quercetin을 선별하였다. Quercetin은 α-glucosidase에 가역적으로, 느리게 결합하며, 비경쟁적인 저해제인데, 6.3×10^{-8} M의 Ki 값을 가진다. 이러한 결과는 비록 기전 연구가 더 필요하지만, quercetin이 당뇨병과 같은 퇴행성 질환을 치료할 수 있다는 잠재성을 보여주는 것이다.

REFERENCES

1. Armada, L. J., A. D. Mackey, and J. F. Gregory III. 2002. Intestinal brush border membrane catalyzes hydrolysis of pyridoxine-5'-beta-D-glucoside and exhibits parallel developmental changes of hydrolytic activities toward pyridoxine-5'-beta-D-glucoside and lactose in rats. *J. Nutr.* **132**: 2695-2699.
2. Doucet J. 2005. Use of antidiabetic drugs in elderly patients. *Diabetes Metab.* **2**: 977-5S98-5S104.
3. Casey, R. C., T. R. Jr. Oegema, K. M. Skubitz, S. E. Pambuccian, S. M. Grindle, and A. P. Skubitz. 2003. Cell membrane glycosylation mediates the adhesion, migration, and invasion of ovarian carcinoma cells. *Clin. Exp. Metastasis* **20**: 143-152.
4. Dove, A. 2002. Seeking sweet relief for diabetes. *Nat. Biotechnol.* **20**: 977-981.
5. Fischer, P. B., G. B. Karlsson, T. D. Butters, R. A. Dwek, and F. M. Platt. 1996. N-butyldeoxynojirimycin-mediated inhibition of human immunodeficiency virus entry correlates with changes in antibody recognition of the V1/V2 region of gp120. *J. Virol.* **70**: 7143-7152.
6. Fischer, P. B., G. B. Karlsson, R. A. Dwek, and F. M. Platt. 1996. N-butyldeoxynojirimycin-mediated inhibition of human immunodeficiency virus entry correlates with impaired gp120 shedding and gp41 exposure. *J. Virol.* **70**: 7153-7160.
7. Gogas, H., F. Shapiro, C. Aghajanian, D. Fennelly, L. Almadrones, W. J. Hoskins, and D. R. Spriggs. 1996. The impact of diabetes mellitus on the toxicity of therapy for advanced ovarian cancer. *Gynecol. Oncol.* **61**: 22-26.
8. Jenkins, D. J., R. H. Taylor, D. V. Goff, H. Fielden, J. J. Mis-

iewicz, D. L. Sarson, S. R. Bloom, and K. G. Alberti. 1998. The anti-herpes simplex virus activity of n-docosanol includes inhibition of the viral entry process. *Antiviral. Res.* **40**: 85-94.

9. Lee, D. S. and S. H. Lee. 2001. Genistein, a soy isoflavone, is a potent alpha-glucosidase inhibitor. *FEBS Lett.* **501**: 84-86.
10. Peterson, G. 1995. Evaluation of the biochemical targets of quercetin in tumor cells. *J. Nutr.* **125**(3 Suppl): 784S-789S.
11. Rosenstock, J., and P. Raskin. 1988. Hypertension in diabetes mellitus. *Cardiol. Clin.* **6**: 547-560.
12. Santell, R. C., N. Kieu, and W. G. Helferich. 2000. Quercetin inhibits growth of estrogen-independent human breast cancer cells in culture but not in athymic mice. *J. Nutr.* **130**: 1665-1669.
14. Segel, I. H. 1975. *Enzyme kinetics: behavior and analysis of rapid equilibrium and steady-state enzyme system.* Wiley, New York, p100-202.
15. Tadera K, Y. Minami, K. Takamatsu, and T. Matsuoka. 2006. Inhibition of alpha-glucosidase and alpha-amylase by flavonoids. *J. Nutr. Sci. Vitaminol. (Tokyo).* **52**: 149-153.
16. Tsujii, E., M. Muroi, N. Shiragami, and A. Takatsuki. 1996. Nectrisine is a potent inhibitor of alpha-glucosidases, demonstrating activities similarly at enzyme and cellular levels. *Biochem. Biophys. Res. Commun.* **220**: 459-466.
17. Walker, B. D., M. Kowalski, W. C. Goh, K. Kozarsky, M. Krieger, C. Rosen, L. Rohrschneider, W. A. Haseltine, and J. Sodroski. 1987. Inhibition of human immunodeficiency virus syncytium formation and virus replication by castanospermine. *Proc. Natl. Acad. Sci. U.S.A.* **84**: 8120-8124.
18. de Groot H, and U. Rauen. 1998. Tissue injury by reactive oxygen species and the protective effects of flavonoids. *Fundam. Clin. Pharmacol.* **12**: 249-255.

(Received Oct. 4, 2006/Accepted Nov. 30, 2006)