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Note

A novel α-glucosidase inhibitor from pine bark

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Abstract—Inhibitors of carbohydrate-hydrolysing enzymes play an important role for the treatment of diabetes. To our knowledge, a number of inhibitors such as, 1-deoxynojirimycin, acarbose and voglibose have been identified as a result of screening of secondary metabolites up to now. In this note, we report the inhibitory effect on carbohydrate hydrolysis of ethanol extracts from more than 1400 species of plants with the aim of identifying a potential antihyperglycemic drug. *Pinus densiflora* bark extracts showed the highest inhibition activity against several carbohydrate-hydrolysing enzymes.

Keywords: α-Glucosidase inhibitor; *Pinus* sp.; Postprandial hyperglycemia

Diabetes mellitus is one of the most serious, chronic diseases that is developing with an increase in obesity and ageing in the general population.[1] Some drugs have been developed for diabetes, and the best way to control postprandial plasma glucose level is with a medication in combination with dietary restriction and an exercise programme.[2,3] One of the therapeutic approaches for decreasing of postprandial hyperglycemia is to retard absorption of glucose by the inhibition of carbohydrate-hydrolysing enzymes, for example α-amylase and α-glucosidase, in the digestive organs.[4–7] There are reports of established α-glucosidase inhibitors such as acarbose[8] and voglibose[9] from microorganisms, and nojirimycin, and 1-deoxynojirimycin[10–12] from plants and their effects on blood glucose levels after food uptake.[13–15]

We therefore investigated the inhibitory effects of ethanol extracts from more than 1400 species of native plants in Korea on α-amylase. In this study, pine extracts showed the highest inhibition activity against several carbohydrate-hydrolysing enzymes, including α-amylases. We further investigated the inhibition activity from the different Korean *Pinus* species based on this lead (Table 1).

Needle and bark extracts from *Pinus densiflora* showed high inhibition activity against α-amylase from saliva, but needle extract exhibited a little higher inhibition activity than bark extract in *Pinus thunbergii*. However, *Pinus koraiensis* showed the opposite result, in that the bark extract had higher inhibitory activity. Furthermore, *Pinus rigida* and *Taxus cuspidate* showed very little inhibitory effect against α-amylase. From these results, we investigated the possibility of producing an α-amylase inhibitor from the bark of *P. densiflora*.

In side-by-side experiments, we also investigated the inhibitory activity of pine bark ethanol extracts, acarbose and voglibose against α-amylase from human salivary and porcine pancreatin because of their mammalian origins, from barley as an example of a plant species, and from *Bacillus* sp. and *Aspergillus oryzae* due to their microbial origins (Table 2). Both pine bark extracts and acarbose showed similar inhibitory activity against α-amylase from mammalian sources and no inhibitory activity against *A. oryzae* α-amylase. However, the pine bark extract showed higher inhibitory activity on *Bacillus* sp. than on mammalian α-amylase, and acarbose showed 10 times less inhibition activity. These different inhibitory activities may reflect the structural differences of the enzymes from different species. Research on these aspects is particularly important in the discovery, design and synthesis of new

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Table 1. The inhibition of human salivary α-amylase by needles and bark extracts of different *Pinus* species

Species	Inhibition (%)	
	Bark[a]	Needles[a]
Pinus densiflora	89.6 ± 5.2	72.7 ± 4.9
Pinus thunbergii	17.6 ± 2.3	82.4 ± 5.3
Pinus rigida	18.6 ± 4.3	23.4 ± 1.6
Pinus koraiensis	79.2 ± 8.9	8.6 ± 2.3
Taxus cuspidata	17.9 ± 2.7	0.6 ± 0.4

[a] Extracts = 100 μL. Lyophilised needles and bark each tissue was extracted 10 times with 70% ethanol.

Table 2. Inhibitory effect of pine bark extract, acarbose and voglibose for α-amylase and α-glucosidase of various origins

Origins	IC_{50}[a] (μg/mL)		
	Extract	Acarbose	Voglibose
α-Amylase			
Human salivary	1.70	2.57	NT[b]
Porcine pancreatin	1.69	1.75	NT
Bacillus sp.	0.57	19.49	NT
Aspergillus oryzae	NI[c]	NI	NT
Barley	4.04	NI	NT
α-Glucosidase			
Porcine small intestine	155.00	35.00	0.035
Saccharomyces cerevisiae	0.025	NI[c]	NI
Bacillus stearothermophilus	0.033	NI	NI

[a] The IC_{50} value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions.
[b] NT: not tested.
[c] NI: no inhibition.

inhibitors that can effectively be used to study inhibitory mechanisms.

In order to investigate the inhibitory activity of pine bark extract on α-glucosidase, we used α-glucosidase from a yeast, *Saccharomyces cerevisiae*, and a bacterium, *Bacillus stearothermophilus*, as examples of microbial origin, and from porcine small intestine as an example of an enzyme of mammalian origin. Using the enzyme from porcine small intestine, the pine bark extract showed less inhibitory activity on α-glucosidase than either acarbose or voglibose (Table 2). However, the inhibitory effects on the α-glucosidase from microbial origins were contradictory with one another. Compared to pine bark extract, which showed very high inhibition activity on α-glucosidase from *S. cerevisiae* and *B. stearothermophilus*, both acarbose and voglibose showed very low inhibitory activity.

There are reports that voglibose and acarbose have high inhibitory effects on mammalian α-glucosidase, but no inhibitory activity for yeast *S. cerevisiae*.[17,20] A similar result reported that glucono-1,5-lactone, identified as an inhibitor of rabbit α-glucosidase,[18] inhibited only mammalian α-glucosidases. Various α-glucosidase inhibitors for mammalian species have been reported, and most of these inhibitors showed either a low effect or no effect on α-glucosidase from microbial origins. On the contrary, (+)-catechin, an inhibitor of yeast *S. cerevisiae* α-glucosidase, did not show any inhibitory effect on enzymes from mammalian species. In the case of the pine bark extract, we show that it is a very powerful inhibitor of both *S. cerevisiae* and *B. stearothermophilus* enzymes, and we find a moderate inhibitory effect on α-glucosidase from mammalian tissues (porcine small intestine). As reported previously, α-glucosidase broadly consists of type I from yeast *S. cerevisiae*, and type II from the mammalian source, and there are structural differences between the two types.[19]

As we showed above, the ethanol extract of pine bark is an effective inhibitor of both α-amylase and α-glucosidase from animals and may function as an antihyperglycemic. To understand the inhibitory mechanisms more clearly and to develop an antihyperglycemic drug, we must purify the active components from the extract. This work is currently underway in our research laboratory.

1. Experimental

1.1. Inhibition assay for α-amylase activity

The samples were dried and then extracted with 10 times their weight of 70% EtOH for 12 h at room temperature. The extracts were centrifuged and stored at −80 °C prior to the assay of α-amylase inhibition. α-Amylase was premixed with extract at various concentrations (0.1–5.0 μg/mL) and starch as a substrate was added as a 0.5% starch solution in phosphate buffer to start the reaction. The reaction was carried out at 37 °C for 5 min and terminated by addition of 2 mL of the DNS reagent (1% 3,5-dinitrosalicylic acid, 12% sodium potassium tartrate in 0.4 M NaOH).[16] The reaction mixture was heated for 15 min at 100 °C and then diluted with 10 mL of distilled water in an ice bath. α-Amylase activity was determined by measuring spectrum at 540 nm. The IC_{50} value was defined as the concentration of α-amylase inhibitor to inhibit 50% of its activity under the assay conditions.

1.2. Inhibition assay for α-glucosidase activity

α-Glucosidase (0.075 unit) was premixed with the extract at various concentrations (0.01–200 μg/mL). 3 mM *p*-nitrophenyl glucopyranoside (pNPG) as a substrate in phosphate buffer was added to the mixture to start the reaction. The reaction was incubated at 37 °C for 30 min and stopped by adding 2 mL of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the *p*-nitrophenol release from pNPG at 400 nm. The IC_{50}

value was defined as the concentration of α-glucosidase inhibitor to inhibit 50% of its activity under the assay conditions.

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References

1. King, H.; Aubert, R. E.; Herman, W. H. *Diabetes Care* **1998**, *21*, 1414–1431.
2. Yki-Jarvinen, H. *Diabetologia* **1990**, *33*, 579–585.
3. Goke, B.; Herrmann-Rinke, C. *Diabetes Metab. Rev.* **1998**, *14*(suppl.), 1S31–1S38.
4. Toeller, M. *Eur. J. Clin. Invest.* **1994**, *24*(suppl. 3), 31–35.
5. Clissold, S. P.; Edwards, C. *Drugs* **1988**, *35*, 214–243.
6. Saito, N.; Sakai, H.; Sekihara, H.; Yajima, Y. *J. Int. Med. Res.* **1998**, *26*, 219–232.
7. Rury, R. H.; Robert, C. T.; Carole, A. C. *Diabetes Care* **1999**, *22*, 960–964.
8. Schmidit, D. D.; Frommer, W.; Junge, B.; Muller, L.; Wingender, W.; Truscheit, E.; Schäfer, D. *Naturwissenschaften* **1977**, *64*, 535–536.
9. Matsuo, T.; Odaka, H.; Ikeda, H. *Am. J. Clin. Nutr.* **1992**, *55*, 314s–317s.
10. Asano, N.; Tomioka, E.; Kizu, H.; Matsui, K. *Carbohydr. Res.* **1994**, *253*, 235–245.
11. Asano, N.; Kizu, H.; Oseki, K.; Tomioka, E.; Matsui, K. *J. Med. Chem.* **1995**, *38*, 2349–2356.
12. Chen, F. J.; Nakashima, N.; Kimura, I.; Kimura, M. *Yakugaku Zasshi* **1995**, *115*, 476–482.
13. Maeda, K.; Kakabayashi, S.; Matsubara, H. *Biochem. Biophys. Acta* **1985**, *828*, 213–221.
14. Watanabe, J.; Kawabata, J.; Kurihara, H.; Niki, R. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 177–178.
15. Hansawasdi, H.; Kawabata, J.; Kasai, T. *Biosci., Biotechnol., Biochem.* **2000**, *64*, 1041–1043.
16. Miller, G. L. *Anal. Chem.* **1959**, *31*, 426–428.
17. Matsui, T.; Oki, T.; Osakima, Y. *Z. Naturforsch.* **1999**, *54*, 259–263.
18. Cogoli, A.; Semenza, G. *J. Biol. Chem.* **1975**, *250*, 7802–7809.
19. Chiba, S. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1233–1239.
20. Matsui, T.; Yoshimoto, C.; Osajima, K.; Oki, T.; Osajima, Y. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 2019–2022.