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α-Glucosidase Inhibitor Isolated from Coffee

Shin-Duk Kim*

Department of Chemical and Biological Engineering, Seokyeong University, Seoul 136-704, Republic of Korea

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**Corresponding author*
Phone: +82-2- 940-7171;
Fax: +82-2-919-0345;
E-mail: sdkim@skuniv.ac.kr

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A potent α-glucosidase inhibitor (compound I) was isolated from coffee brews by activity-based fractionation and identified as a β-carboline alkaloid norharman (9H-pyrido[3.4-*b*]indole) on the basis of mass spectroscopy and nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR, and COSY). The norharman showed potent inhibition against α-glucosidase enzyme in a concentration-dependent manner, with an IC_{50} value of 0.27 mM for maltase and 0.41 mM for sucrase. A Lineweaver-Burk plot revealed that norharman inhibited α-glucosidase enzyme uncompetitively, with a K_i value of 0.13 mM.

Keywords: α-Glucosidase inhibitor, β-carboline, norharman, coffee, uncompetitive inhibitor

Introduction

Coffee is the most commonly consumed beverage around the world and the health benefits of coffee consumption have been extensively studied [10]: coffee has strong antioxidant activities *in vivo* [16, 18] and also reduces the risk of Alzheimer's [4] and Parkinson's diseases [11]. Recent studies have demonstrated that habitual coffee consumption significantly reduces the risk of type II diabetes [17, 19], but it remains unclear what mechanisms and what coffee components are responsible for this observation. Animal as well as *in vitro* studies have implicated several possible mechanisms for a beneficial effect of coffee consumption on glucose metabolism: improving insulin sensitivity [14], inhibition of glucose 6-phosphatase [2], increase of glucagon-like peptide I concentration [15], and decrease of the rate of intestinal absorption of glucose [12].

α-Glucosidase is necessary for carbohydrate digestion. Carbohydrates must be hydrolyzed by α-glucosidase in order to be absorbed in the small intestine. The inhibition of α-glucosidase slows down the process of carbohydrate digestion and avoids postprandial hyperglycemia, which is a major cause of chronic diabetes-associated complication [8]. Thus, many efforts have been made to develop an α-glucosidase inhibitor as a therapeutic agent for the suppression of metabolic abnormality, like hyperglycemia, obesity, and non-insulin-dependent type II diabetes mellitus [3].

This work was intended to evaluate the α-glucosidase inhibitory effect of coffee, previously reported as hypoglycemic, and to characterize the active principle isolated from coffee.

Materials and Methods

General

p-Nitrophenyl (PNP)-α-D-glucopyranoside, PNP-α-D-mannopyranoside, PNP-β-D-glucopyranoside, and PNP-β-D-galactopyranoside were purchased from Sigma-Aldrich (St. Louis, MO, USA). Yeast α-glucosidase, almond β-glucosidase, *E. coli* β-galactosidase, jack beans α-mannosidase, rat intestinal acetone powders, and norharman were also obtained from Sigma-Aldrich. Unless stated otherwise, all other chemicals were purchased from Sigma-Aldrich. All the reagents were of analytical grade.

The ultraviolet absorption spectrum was obtained with a Shimadzu model UV-160 instrument. High-resolution fast atom bombardment MS spectra were recorded on a JEOL model JMS-AX505 HA instrument. ^{1}H and ^{13}C-NMR spectroscopic data were gained through a Brucker AV 500 instrument operating at 500 and 125 MHz, respectively. $(CD_3)_2CO$ was used as the deuterated NMR solvent.

Enzyme Inhibition Assay

The inhibitory activity of intestinal α-glucosidase was determined as described previously, with a minor modification [5]. The suspension of rat intestinal acetone powder in 100 mM sodium phosphate buffer (pH 7.0) was centrifuged at 12,000 rpm for 15 min.

The resultant supernatant was used as the crude small intestinal α-glucosidase solution. For the assay of inhibitory activities of maltase and sucrase, the reaction mixture contained the crude enzyme solution, 20 mM maltose or 200 mM sucrose, 100 mM sodium phosphate buffer (pH 7.0), and a certain amount of inhibitor (50% dimethyl sulfoxide solution) in a total volume of 0.5 ml. The reaction mixture was incubated for 15 min at 37°C, and then boiled for 5 min to stop the reaction. After the interfering phenolic compounds were removed from the reaction mixture with a basic alumina column (1 × 3 cm), α-glucosidase enzyme activity was estimated by measuring the free glucose with the glucose oxidase method. Acarbose was used as the positive control.

The activities of the various glycosidase enzymes were evaluated spectrophotometrically by measuring the *p*-nitrophenol released from the relevant substrate, *p*-nitrophenol glycoside [13]. The assay mixtures and the test samples in 50% dimethyl sulfoxide solution were incubated in a 96-well plate as follows: 20 µl of 0.1 M phosphate buffer (pH 7.0), 20 µl of test sample, 10 µl of enzyme (1 U/ml), 10 µl of 25 mM substrate, and 40 µl of methanol. After incubation at 37°C for 15 min, 300 µl of 1 N NH_4OH solution was added to stop the reaction and basify the assay mixture, and 4-nitrophenol released was quantified colorimetrically at 405 nm with a microplate reader (Bio-Rad Model 550; CA, USA).

All of the experiments were carried out in triplicate. The IC_{50} value, the concentration of the compound that is required to achieve 50% enzyme inhibition, was assessed from a nonlinear regression curve of % inhibition versus the concentration of the compound. The type of enzyme inhibition was determined from graphical analysis of the data with a double- reciprocal Lineweaver-Burk plot.

Isolation of Inhibitory Compound from Coffee

Filtered brewed coffee was made using a household coffee maker: 75 g of ground roast coffee of Columbian Supremo (Arabica variety) and 500 ml of water to give a brewed coffee. Commercial instant coffee (Tasters' choice, Nestle) was prepared by dissolving 75 g of instant coffee in 300 ml of hot water. The filtered brewed coffee and instant coffee solutions were separately centrifuged at 12,000 rpm and room temperature for 15 min, and used for isolation of the α-glucosidase inhibitor. The supernatant was adjusted to pH 9 with 1 N NaOH and extracted with ethyl acetate. The ethyl acetate layer was re-extracted with 0.1 N HCl solution. This acidic solution was then adjusted to pH 10 with 1 N NH_4OH and extracted again with ethyl acetate. The ethyl acetate layer containing basic components was subsequently evaporated *in vacuo*. Forty batches of the above ethyl acetate extracts (total 3 kg each of ground coffee and instant coffee) were concentrated and applied to silica gel column chromatography using chloroform-acetone (70:30) as the eluting solvent system. Fractions containing the active compound (F3–F6) were pooled, evaporated, and applied to a Sephadex LH-20 column chromatography (3 × 35 cm) with MeOH as an eluent. Fraction numbers 10–12, which showed a high inhibition and a similar TLC profile (silica gel 60 F_{254}, Merck; chloroform:acetone = 1:1; rf 0.2), were combined and further purified. As a final step for purification of the compound, semipreparative HPLC (Waters µBondapak C_{18} reversed-phase column) was conducted using 75% MeOH as a solvent system and the compound was detected through absorption at 254 nm. The retention time was 14.5 min. After removing the HPLC solvent in a rotary evaporator, the active compound was precipitated as a white powder from cold acetone.

Results and Discussion

Both instant coffee and ground brewed coffee solutions inhibited the α-glucosidase enzyme activity. Instant coffee showed a slightly higher degree of inhibition than brewed coffee (data not shown). The activity-based fractionation of coffee solutions by solvent extractions and chromatography techniques yielded an active compound I (2.24 µg/g of roasted ground coffee; 3.85 µg/g of instant coffee) that exhibited a strong inhibitory activity against α-glucosidase.

The isolated compound I was shown to be chromatographically pure by TLC and HPLC with various solvent systems. The compound I was considered as a nitrogen-containing compound because it reacted with Dragendorff's spray reagent and produced an orange red spot on the TLC plate. The UV spectrum of compound I showed λ_{max} in methanol at 230, 285, and 348 nm. The molecular formula of compound I was elucidated as $C_{11}H_8N_2$ (M^+ *m/z* 168.0736; calcd. 168.0688) by high-resolution mass analysis. The ^1H NMR spectrum of compound I revealed seven peaks of aromatic protons (δ7.2–8.9 ppm) and one free proton peak (δ10.63 ppm). The ^{13}C NMR spectrum showed 11 carbon peaks in the range of 110 to 145 ppm (Table 1). Taken together, the structure of compound I was deduced as β-carboline, norharman (9H-pyrido[3.4-*b*]indole; Fig. 1) from the ^1H NMR, ^{13}C NMR, and ^1H-^1H COSY spectra and confirmed by comparison of its physical data with those of the authentic specimen.

Compound I exhibited a strong concentration-dependent inhibition against α-glucosidase enzyme, but it did not show any significant inhibitory effects against β-glucosidase, α-mannosidase, and β-galactosidase when tested at a concentration of 10 mM (Table 2). The inhibitory profile

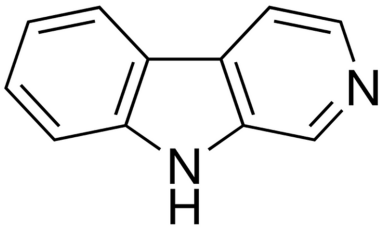

Fig. 1. Structure of compound I (β-carboline alkaloid norharman).

Table 1. ^{1}H and ^{13}C NMR data for compound I in $(CD_3)_2CO$ (δ in ppm and *J* in Hz).

Position	δ_C	δ_H	Multiplicity, *J*
1	134.8	8.90	(1H, br, s)
3	139.5	8.33	(1H, d) *J* = 5.5
4	115.1	8.00	(1H, d) *J* = 5.5
5	122.3	8.28	(1H, d) *J* = 8.0
6	120.1	7.21	(1H, ddd) *J* = 8.0, 7.0, 1.0
7	128.9	7.53	(1H, ddd) *J* = 7.5, 7.5, 1.0
8	112.4	7.60	(1H, dd) *J* = 8.2, 1.0
9 NH		10.63	(1H, br. s)
10	122.1	-	
11	112.6	-	
12	137.2	-	
13	141.6	-	

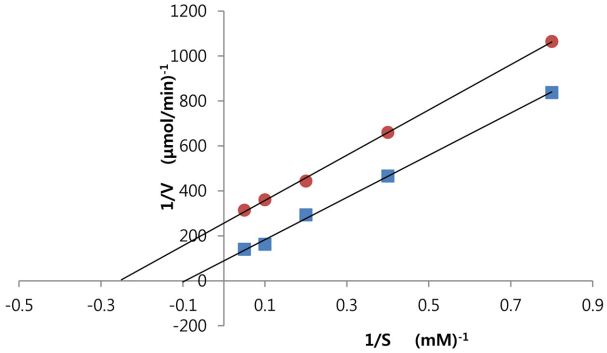

Fig. 2. A Lineweaver-Burk plot analysis of rat intestine α-glucosidase inhibition by compound I.
4-Nitrophenol-α-D-glucopyranoside was used as a substrate. The concentration of compound I was 0 mM (■) or 0.25 mM (●). The values are expressed as means of triplicate reactions.

indicated that compound I was more active against maltase than sucrase (IC_{50} values: 0.27 mM for maltase and 0.41 mM for sucrase). The efficacy of α-glucosidase inhibition was lower than that of the antidiabetic drug acarbose (IC_{50} value: 0.18 mM for maltase and 0.02 mM for sucrase). Nonetheless, this observation implied indeed the potential of compound I as an α-glucosidase inhibitor. Pre-incubation of compound I with the enzyme prior to the addition of substrate resulted in an increase in enzyme inhibition, suggesting that this compound responded to the enzyme slowly. When the quantity of compound I that could inhibit enzyme activity up to 90% was incubated with the enzyme solution for 10 min and then the compound I was removed using a PD 10 desalting column (Pharmacia, Piscataway, NJ, USA), the α-glucosidase activity was totally recovered. From this result, it was concluded that compound I was a reversible inhibitor. A double-reciprocal Lineweaver-Burk curve plotted under various amounts of compound I revealed the parallel lines intercepting on the 1/V axis with the increasing concentration of compound I, decreasing the V_{max} and K_m values. The kinetic data indicated that compound I inhibited the enzyme uncompetitively and the K_i value was 0.13 mM (Fig. 2). As a result, compound I, a reversible uncompetitive inhibitor of α-glucosidase, was isolated from coffee and identified as an active principle. When given in combination with a high carbohydrate diet orally, compound I significantly reduced the postprandial plasma glucose levels in non-diabetic rats (unpublished data).

Table 2. Inhibitory effects of compound I against various glycosidases.

Enzyme	IC_{50} (μM)
α-Glucosidase (yeast)	180 ± 3.2
Maltase (rat intestine)	270 ± 4.5
Sucrase (rat intestine)	410 ± 11.3
β-Glucosidase (almond)	$>1.0 \times 10^4$
α-Mannosidase (jack bean)	$>1.0 \times 10^4$
β-Galactosidase (*E. coli*)	$>1.0 \times 10^4$

Values are expressed as the means of triplicate reactions ± standard deviation.

Compound I, a tricyclic indole β-carboline alkaloid norharman, is distributed widely in biological systems and exhibits a broad spectrum of pharmacological and neurological effects: antidepressant and antianxiety effects in rats [7], inhibitory activities of monoamine oxidase and nitric oxide synthase [9], as well as an increase of insulin secretion of 2- to 3-fold from isolated human islets of Langerhans [6]. However, the α-glucosidase inhibitory activity of norharman has not been previously reported. Coffee has been noted as the main exogenous source of norharman. A high variability in β-carboline content of coffee samples was observed between coffee species (arabica, robusta) and also depended on the roast degree and instant coffee production process. Drinking an average of three cups of coffee per day could consume up to 72 μg of norharman [1], although this will depend on the coffee strength.

Coffee contains numerous substances. However, the effects of individual components on glucose metabolism were not clearly determined as yet. A cohort study has supported that the most prominent coffee compound caffeine is irrelevant to the risk of type II diabetes [20]. Chlorogenic acid, a major polyphenol in coffee, was reported to decrease glucose concentrations in rats, caused by increasing insulin

sensitivity as well as decreasing hepatic glucose output due to the inhibition of glucose 6-phosphatase [12]. Without excluding any other possible mechanism, this report observes α-glucosidase inhibitory activity as a possible mechanism of the hypoglycemic effect of coffee and assigns β-carboline alkaloid norharman as one of the active principles in coffee. Coffee appears to contain active principles other than norharman, as evidenced by several active peaks in chromatography systems. It may be possible that various active constituents in coffee act synergistically against α-glucosidase activity. Characterization of the other active principles is in progress.

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