Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 12, pp. 1241-1244, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00196-4

SULFOQUINOVOSYLDIACYLGLYCEROL AS AN α-GLUCOSIDASE INHIBITOR

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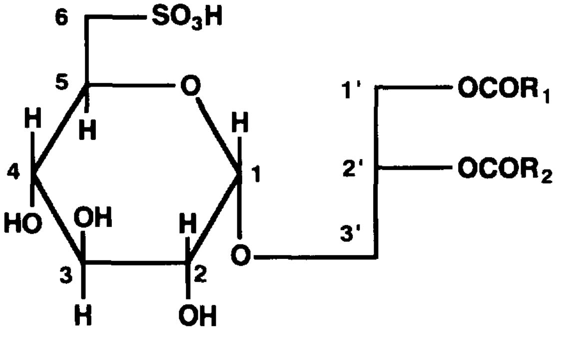
Abstract: Sulfoquinovosyldiacylglycerol isolated from an edible brown alga *Hizikia fusiforme* inhibited yeast α-glucosidase.

α-Glucosidases are nutritionally and biochemically important enzymes on dietary carbohydrate digestion and post-translational processing of glycoproteins.[1] Small intestinal α-glucosidase, namely maltase, hydrolyzes maltose to glucose which is absorbed through the gut wall.[2] Since absorption of glucose elevates the blood glucose level to cause postprandial hyperglycemia, it is necessary for diabetics to decrease the hydrolytic rate of maltose. Puls, *et al.*[3] revealed that small intestinal α-glucosidase inhibitors reduced the postprandial increment of blood glucose. Endplasmic reticular α-glucosidases I and II process asparagine-linked glycans of glycoproteins.[4] This processing deletes three glucose residues and is followed by other glycosidases processing to afford the high mannose-, the hybrid-, and the complex-type of glycoproteins. Inhibition of α-glucosidases causes abnormal functionality of glycoproteins because of incomplete modification of glycans. Suppression of this processing is to be expected for antiviral activity and decreasing of growth rate of tumors.[1]

α-Glucosidase inhibitors have so far been elucidated by many groups. The inhibitors are divided into three types, polyhydroxylated *N*-substituted heterocyclic compounds,[1] polyhydroxylated cycloalkenes,[5] and oligomers of pseudo-sugars.[6] Most of them inhibit α-glucosidases by mimicking the pyranosyl moiety of the α-glucosides. Known α-glucosidase inhibitors, acarbose,[6] miglitol,[7] and AO-128,[8] have been attempted to control diabetes. Deoxynojirimycin was used for functional elucidation of glycan of glycoproteins as a biochemical tool.[1] Some inhibitors of processing α-glucosidases I and II decrease the infectivity of the human immunodeficiency virus.[1]

In the course of our search for α-glucosidase inhibitory activity of algal extracts,[9] organic solvent extracts of *Hizikia fusiforme*, an edible brown alga, had inhibitory activity against yeast α-glucosidase. In this communication, we disclose that sulfoquinovosyldiacylglycerol (**1**, SQDG) isolated from *Hizikia fusiforme* is a potent α-glucosidase inhibitor.



1, $R_1=R_2$=Alkyl

Dried *Hizikia fusiforme* purchased from the market was extracted with methanol-water (4:1). With the guidance of the α-glucosidase inhibition assay,[10] the extract was fractionated by a series of solvent partitions into an *n*-butanol soluble fraction. This fraction was chromatographed on silica gel and Sephadex LH-20, and finally purified using TLC to afford the inhibitor (**1**) as a pale yellow waxy solid ($[\alpha]_D$ +33° , c 0.045 in methanol).

The inhibitor (**1**) was positive in orcinol-sulfuric acid[11] and Azure A[12] tests which revealed presence of a sugar moiety and sulfur atom in its structure. The IR spectrum[13] of **1** showed absorption peaks characteristic of carbonyl and sulfonic groups. Negative FAB MS[13] displayed several pseudo-molecular ion peaks. The ion peaks were characterized molecular species of lipids.[14] GC[15] of the methanolysates of **1** gave fatty acid methyl ester peaks. The ^{1}H NMR and ^{13}C NMR spectra[13] of the inhibitor (**1**) showed characteristic signals (δ 0.89-2.81, and 5.36 ppm for ^{1}H; δ 14.5-35.2, 130.8, 130.9, 174.9, and 175.1 ppm for ^{13}C) of fatty acid residues, and three methylene and six methine signals attached to hetero atoms. Hexose and glycerol moieties were revealed from connectivities elucidated from 2D NMR experiments. The doublet proton (δ 4.76 ppm, J =3.7 Hz) and the carbon (δ 100.0 ppm) were assigned from their chemical shifts and coupling constant to the α anomer. The ^{1}H and ^{13}C NMR signals at 6-position of the sugar moiety appeared at a higher field than those of glucose. These results indicated the sugar moiety to be a 6-sulfoquinovose residue. From a further consideration of 1D and 2D NMR spectra and by comparison with data in the literature,[16] the inhibitor **1** was identified SQDG.

Inhibitory activity of SQDG (**1**) against yeast α-glucosidase was determined by a spectrophotometric method[17] using *p*-nitrophenyl-α-D-glucopyranoside as a substrate. Mean molecular weight (M_n 814) of SQDG (**1**) was calculated by fatty acid composition determined by GC. Kinetic evaluation (Figures 1 and 2) discloses SQDG (**1**) has competitive inhibition and a K_i value of 2.9±0.3 μM against yeast α-glucosidase.

SQDG is known as one of the major glyceroglycolipids of cell membranes in plants, but its functionality has been only elucidated feeding stimulation activity to Gastropods[18] and antiviral activity against the human immunodeficiency virus (HIV).[19] This is the first report of inhibitory activity of SQDG against enzymes. SQDG could have a biologically important roll, such as an enzyme inhibitor, as a constitutive lipid of the biological membranes since it has a unique sulfonic moiety. Inhibitory activity of SQDG (**1**) *in vivo* and the hydrolyzates derived from **1** is now in congress.

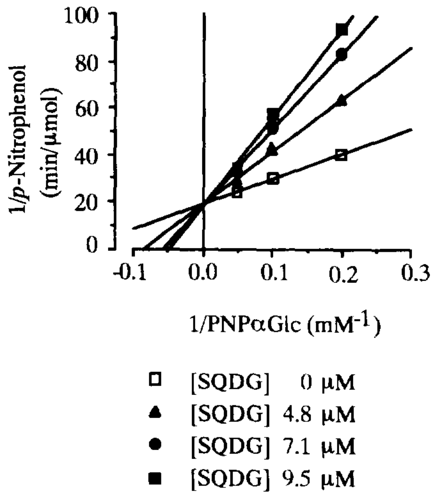


Figure 1. Lineweaver-Burk plot of inhibition of SQDG (**1**) against yeast α-glucosidase. SQDG, sulfoquinovosyldiacylglycerol; PNPαGlc, *p*-nitrophenyl-α-D-glucopyranoside.

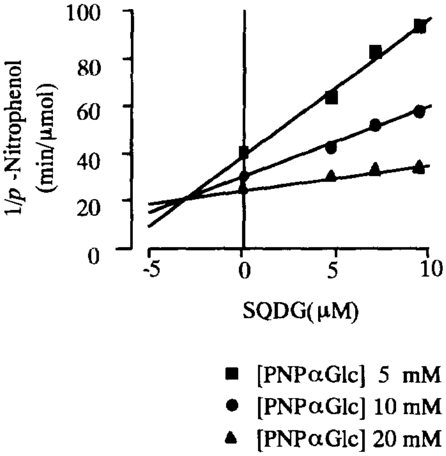


Figure 2. Dixon plot of inhibition of SQDG (**1**) against yeast α-glucosidase. SQDG, sulfoquinovosyldiacylglycerol; PNPαGlc, *p*-nitrophenyl-α-D-glucopyranoside.

Acknowledgments: We thank Mrs. Eri Fukushi, Faculty of Agriculture, Hokkaido University, for MS measurement. Part of this work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (to H. K.).

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10. α-Glucosidase inhibition assay was carried out using two methods. One is a Kurihara's agar plate method[9] and the other is a spectrophotometric method using *p*-nitrophenyl α-D-glucopyranoside as a substrate.
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13. Spectral data of **1**: IR λ_{max} cm^{-1} (KBr) 3362, 2925, 2854, 2366, 1735, 1720, 1458, 1174, 1035; FAB MS (negative mode) *m/z*: 765, 791, 815, 819, 846, 875. ^{1}H NMR (500 MHz, methanol-d_4) and ^{13}C NMR (125 MHz, methanol-d_4) data were assigned by HH COSY, HMQC, and HMBC experiments. ^{1}H NMR δ (multiplicity, *J* in Hz, position): 5.36 (m, acyl), 5.33 (m, 2'-H), 4.76 (d, 3.7, 1-H), 4.49 (dd, 12.1, 2.4, 1'-Ha), 4.19 (dd, 12.1, 7.0, 1'-Hb), 4.09 (dd, 10.8, 5.2, 3'-Ha), 4.06 (dt, 9.3, 2.0, 5-H), 3.63 (t, 9.3, 3-H), 3.57 (dd, 10.8, 6.3, 3'-Hb), 3.41 (dd, 3.7, 9.3, 2-H), 3.33 (dd, 14.3, 2.0, 6-Ha), 3.09 (t, 9.3, 4-H), 2.92 (dd, 14.3, 9.3, 6-Hb), 2.81 (m, acyl), 2.33 (m, acyl), 2.02 (m, acyl), 1.59 (m, acyl), 1.28 (br. s, acyl), 0.89 (t, 6.8, acyl); ^{13}C NMR δ (position): 175.1 (acyl), 174.9 (acyl), 130.9 (acyl), 130.8 (acyl), 100.0 (1-C), 74.9 (4-C), 74.8 (3-C), 73.4 (2-C), 71.7 (2'-C), 69.8 (5-C), 67.1 (3'-C), 64.3 (1'-C), 54.2 (6-C), 35.2-14.5 (acyl).
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15. Each peak of GC was identified comparing with retention times of standard fatty acid methyl esters. Fatty acid composition of **1**: $C_{16:0}$, 56.6%; $C_{18:1}$, 9.6%; $C_{18:3}$, 6.9%; $C_{22:0}$, 5.2%; $C_{16:1}$, 4.7%, $C_{14:0}$, 4.0%, $C_{20:1}$, 3.8%.
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17. Inhibition assay condition is as follows. Buffer used was 10 mM phosphate buffer (pH 7.0). Five to 20 mM *p*-nitrophenyl-α-D-glucopyranoside (0.1 mL), 0 to 0.20 mg/mL SQDG (0.1 mL), and buffer (2.2 mL) were added into a test tube. They were preincubated at 37 °C for 5 min, added 5 μg/mL yeast α-glucosidase (0.1 mL), and incubated at 37 °C for 2 min. The reaction was stopped by adding 0.25 M sodium carbonate (1.5 mL). The reaction mixture was measured absorbance at 400 nm.
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(*Received in Japan* 16 *March* 1995; *accepted* 22 *April* 1995)