Note

Inhibitory Effect on the \( \alpha \)-Glucosidase Reaction by the Aggregated State of Sulfoquinovosyldiacylglycerol

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The inhibitory activity of aggregated SQDG toward the \( \alpha \)-glucosidase reaction decreased with increasing turbidity of the SQDG suspension. Dideacylated derivatives of SQDG, sulfoquinovosyldiacylglycerol and sulfoquinovose, showed no inhibition toward the reaction. The expression of inhibition by the SQDG family would require a hydrophobic acyl group(s) to produce an aggregate and/or interact with a hydrophobic site in the enzyme.

Key words: SQDG; inhibition; \( \alpha \)-glucosidase; aggregation

Sulfoquinovosyldiacylglycerol (SQDG, 1) has been obtained from the edible brown alga, *Hizikia fusiformis*, as an inhibitor of the yeast \( \alpha \)-glucosidase reaction.\(^{11}\) SQDG showed competitive inhibition and had a \( K_i \) value of 2.9 \( \mu \text{M} \).\(^{11}\) Its dideacylated derivative, sulfoquinovosyldiacylglycerol (SQMG, 2), which was also obtained from *H. fusiformis*, inhibited the reaction more potently than SQDG.\(^{11}\)

![Diagram of SQDG, SQMG, and other compounds](image)

Digalactosyldiacylglycerol (DGDG, 3) from *H. fusiformis* has shown a suppressing effect on the inhibitory activity of coexisting SQDG toward the \( \alpha \)-glucosidase reaction, while DGDG alone showed no inhibition.\(^{11}\) The reason for this suppressing effect was suggested to be an apparent decrease in the SQDG concentration, because both amphiphilic glycolipids formed an aggregated state in aqueous suspension.\(^{11}\)

In the present paper, we disclose that large-scale aggregation of SQDG decreased its inhibitory activity toward the \( \alpha \)-glucosidase reaction and that the dideacylated derivatives of SQDG, sulfoquinovosyldiacylglycerol (SQG, 4) and sulfoquinovose (SQ, 5), showed no inhibitory activity.

SQDG (from Lipid Products, Surrey, UK) in chloroform-methanol was evaporated under reduced pressure to dryness and suspended at 250 \( \mu \text{M} \) in a 10 mm phosphate buffer (pH 7.0) with a mixer. Aliquots of the SQDG suspension were put into test tubes and sonicated for between 30 s and 10 min. The turbidity of each resulting SQDG suspension was measured by its absorbance at 600 nm with a Hitachi U-2000 spectrophotometer.

The spectrophotometric method\(^{21}\) was employed as an assay to evaluate the inhibitory effect against the \( \alpha \)-glucosidase reaction. Using 0.2 mm or 0.4 mm \( \beta \)-nitrophenyl \( \alpha \)-d-glucopyranoside (Tokyo Kasei Kogyo) as a substrate, aliquots of each turbidity-controlled SQDG suspension (0.1 ml) and a 10 mm phosphate buffer (pH 7.0, 2.2 ml) were put into a test tube. The tubes were preincubated at 37°C for 5 min before adding 0.56 unit/ml of yeast \( \alpha \)-glucosidase (from *Saccharomyces* sp., Wako Pure Chemicals Industries), and the mixture was incubated at 37°C for 2 min. The reaction was stopped by adding 0.25 mm sodium carbonate (1.5 ml). \( \beta \)-Nitrophenol produced was determined by measuring the absorbance at 400 nm.

The 50% inhibitory concentration (IC\(_{50}\)) value of each turbidity-controlled SQDG suspension toward the \( \alpha \)-glucosidase reaction is shown in Fig. 1. The greater the turbidity of the SQDG suspension, the more increased were the IC\(_{50}\) values toward the \( \alpha \)-glucosidase reaction. These results show that the extent of aggregation affected the apparent inhibitory activity.

Figure 2 shows the reciprocal of the reaction velocity vs. the reciprocal of the substrate concentration for each turbidity value. These plots for the turbidity-controlled SQDG suspensions show competitive inhibition like the plots for a concentration-controlled SQDG solution.\(^{21}\)

Sulfoquinovosyldiacylglycerol (SQG, 4) was obtained by a mild alkaline methanolation, SQDG (25.9 mg) being dissolved in 0.03 \( \mu \text{M} \) methanolic NaOH and left at room temperature for 2 h. The reaction mixture was then neutralized, evaporated, and dissolved in water and diethyl ether. The aqueous layer was evaporated.

![Graph of IC\(_{50}\) Values for Turbidity-controlled SQDG Suspensions toward the \( \alpha \)-Glucosidase Reaction](image)
under reduced pressure, and the resulting concentrate was subjected to CM-Cellulose C-500 column chromatography (H⁺-type, 3.0 × 30 cm, Seikagaku Corporation) by eluting with water. The eluate was evaporated under reduced pressure to obtain SQG (13.4 mg). SQG (4) was identified by instrumental analyses. The HR negative FAB MS data for 4 gave a pseudo-molecular ion (M−H)⁻ at m/z 317.0540 (calculated: 317.0543 for C₁₃H₁₁O₅S), while the NMR data for 4 (Table) show a similar pattern to that described by Johns et al.⁴¹ to support its structure.

Sulfoquinovose (SQ, 5) was obtained by an acidic hydrolysis, SQDG (26.7 mg) being dissolved in 1 N HCl and refluxed for 2 h. The reaction mixture was then cooled and dissolved in water and diethyl ether. The aqueous layer was evaporated and subjected to Sephadex LH-20 column chromatography (2.0 × 30 cm, Pharmacia Fine Chemicals) by eluting with water. The eluate was evaporated under reduced pressure to obtain SQ (5; 6.3 mg). SQ (5) was identified by instrumental analyses. The HR negative FAB MS data for 5 gave a pseudo-molecular ion (M−H)⁻ at m/z 243.0212 (calculated: 243.0175 for C₁₀H₁₅O₅S), while the NMR data for 5 (Table) indicated two sets of signals characterizing a mixture of α- and β- anomers. The 13C-NMR data for 5 were identical with the data for SQ.⁴¹

Neither SQG (4) nor SQ (5) had any inhibitory activity toward the α-glycosidase reaction at any concentration. SQDG is thought to exist in an aggregated state in a buffer because of its amphiphilic character. In general, an increase in turbidity is attributed to the increasing size or number of aggregates.⁵¹ Lineweaver-Burk plots (Fig. 2) for a turbidity-controlled SQDG suspension at a constant concentration show competitive inhibition toward the α-glycosidase reaction. These results suggest that the "effective" SQDG concentration decreased with increasing turbidity. The most potent inhibitory activity of SQDG is exhibited by aggregates of small size. In our previous experiments,⁵¹ DGDG suppressed the inhibitory activity of coexisting SQDG toward the α-glycosidase reaction because of the formation of mixed aggregates. This supports the proposition that SQDG in a buffer exists in an aggregated form.

Glycosidase reactions proceed through an oxocarbonium ion intermediate derived from glycosides.⁶⁰ The cationic intermediate is stabilized between carboxylate groups at the active site of the enzyme.⁶⁰ Amino-sugars⁷ such as 1-deoxyribojirimycin (dNM) inhibit the glycosidase reaction by mimicking the cationic intermediate.⁶⁰

N-Alkylated dNMs inhibit the α-glycosidase reaction more potently than dNM,⁶¹ the N-alkyl chain being considered to interact with a hydrophobic pocket at the active site of the enzyme.⁶⁰ The hydrophobic acyl groups of SQDG and SQMG would be concerned not only with making aggregates but also interacting with a hydrophobic pocket. The more potent inhibition by SQMG than SQDG⁵¹ can be ascribed to forming a smaller aggregate and/or better fitting the hydrophobic pocket.

Neither SQG nor SQ, the deacylated derivatives, inhibited the α-glycosidase reaction. They exist in a dissolved state due to the lack of hydrophobic acyl groups. SQG and SQ, having a sulfonate group, are negatively charged in the solution. They would not have access to the active site of the enzyme by electrostatic repulsion due to having carboxylate groups.

The reason why aggregates of SQDG or SQMG, having negative charges on the surface, can approach the active site of the enzyme has been described by Dickinson and Stainsby,⁵¹ in that the counter-ions were strongly adsorbed to the surface of ionic surfactants after forming a micelle. This reduced the net charge of the surface. We speculate that aggregates of SQDG or SQMG adsorbing counter-ions could easily approach the active site.

The physical properties, e.g., size definition, of SQDG and SQMG aggregates and the interaction between yeast α-glycosidase and the SQDG family are now being investigated.

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References