Inhibitory Effect of Sulfoquinovosyl Diacylglycerol on Prokaryotic DNA Polymerase I Activity and Cell Growth of Escherichia coli

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Abstract: We isolated the glycolipids fraction from spinach (Spinacia oleracea L.) and found that the fraction inhibited the activities of prokaryotic DNA polymerase I from Escherichia coli (E. coli) and cell growth of E. coli. The fraction contained mainly three glycolipids, monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG), and purified SQDG inhibited these activities, however, purified MGDG and DGDG had no influence. In the tested strains of E. coli, SQDG inhibited the cell proliferation of the JM109 strain. It could be considered that a SQDG-containing thylakoid membrane in plant chloroplasts might have anti-bacterial activity.

Key words: glycolipid, sulfoquinovosyl diacylglycerol, DNA polymerase inhibitor, E. coli cell growth inhibitor, spinach

1 INTRODUCTION

We have established an assay method to detect DNA polymerase (pol) inhibitors, and we have found more than 10 compounds of new pol inhibitors over the past 10 years from natural materials1). Of these, sulfo-glycolipids in the class sulfoquinovosyl diacylglycerol (SQDG) from a fern and an alga are particularly potent inhibitors of eukaryotic pols from mammals2,3). SQDG is a major glycolipid of the chloroplast membrane in plants4). We have widely screened for the glycolipids fraction containing SQDG from common vegetables that show such inhibitory activity, and found that spinach (Spinacia oleracea L.) had the largest amount of SQDG and was the strongest mammalian pol inhibitor in the tested vegetables5). In this report, we report that purified SQDG and the glycolipids fraction from spinach can inhibit the activity of prokaryotic pol such as Escherichia coli (E. coli) pol I and the cell growth activity of E. coli, and discuss whether the glycolipids fraction could be an antibacterial compound.

2 EXPERIMENTAL

2.1 Materials

Dried spinach (Spinacia oleracea L.) was obtained from Shinyu Co. Ltd. (Hiroshima, Japan). Pol I from E. coli was
purchased from Takara (Tokyo, Japan). The *E. coli* strains (JM109, DH5α, TOP10, TOP10F') were obtained from Invitrogen (Carlsbad, USA) or TOYOBO (Tokyo, Japan). All other reagents were of analytical grade and purchased from Nakarai Tesque, Ltd. (Kyoto, Japan).

2.2 Isolation of the glycolipids fraction from spinach

The glycolipids fraction was prepared using hydrophobic column chromatography as described previously. Three major compounds were analyzed by TLC, and no other compounds were detected (data not shown). Each of these compounds was completely purified by silica gel column chromatography, and their chemical structures were determined by 1H-, 13C- and DEPT (Distortionless Enhancement by Polarization Transfer) NMR spectroscopic analyses. These compounds were glycolipids such as MGDG (monogalactosyl diacylglycerol), DGDG (digalactosyl diacylglycerol) and SQDG and no other glycolipids were detected. From fatty acid analysis by gas chromatography, the major fatty acids in MGDG were stearic acid (18:0) and oleic acid (18:1), DGDG was mostly palmitic acid (16:0) and oleic acid (18:1), and SQDG was mostly palmitic acid (16:0) and linolenic acid (18:3).

2.3 *E. coli* DNA polymerase I inhibitory assay

The reaction mixture for *prokaryotic* pol I (0.05 units) was described previously. The substrate of the pol used was poly(dA)/oligo(dT)12-18 and dTTP (2'-deoxythymidine 5'-triphosphate) as the DNA template-primer and dNTP substrate, respectively. One unit of pol activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol of dNTP into synthetic DNA template-primers during 60 min at 37°C under normal reaction conditions for each enzyme.

2.4 *E. coli* cell growth inhibitory assay

Each strain of *E. coli* (JM109, DH5α, TOP10, TOP10F') was cultured in liquid LB medium at 30°C for 16 h. For the cell growth inhibitory assay, 20 μL aliquot of the overnight culture of each *E. coli* strain was inoculated into 2 mL of fresh liquid LB medium containing the glycolipids. Each glycolipid was dissolved in DMSO to a final concentration of 20 mg/mL as stock solutions. After incubation at 30°C for 16 h with shaking at 100 rpm, the inhibition efficiency of proliferation was determined by OD600 using Genequant pro (Biochrom, Cambridge, UK).

3 RESULTS AND DISCUSSION

We tested the effects of purified MGDG, DGDG and SQDG (Fig. 1) and the glycolipids fraction from spinach on prokaryotic pol I from *E. coli*. The relative activity of the enzyme (0.05 units) at a set concentration (100 μg/mL) of the compounds is shown in Fig. 2A. SQDG inhibited pol I activity, but MGDG and DGDG did not influence the activity. The mixtures of the two or three purified glycolipids were also investigated. The mixture of 50 μg/mL of DGDG and 50 μg/mL of SQDG (i.e., “MGDG+SQDG” in Fig. 2) was a stronger inhibitor than “MGDG+DGDG” and “MGDG+SQDG”. This result suggested that MGDG might weaken the inhibitory effect of pol I activity. The mixture of 33.3 μg/mL of MGDG, 33.3 μg/mL of DGDG and 33.3 μg/mL of SQDG (i.e., “MGDG+DGDG+SQDG”) had the same inhibitory effect as the glycolipids fraction from spinach. The weight percents of MGDG, DGDG and SQDG in the glycolipids fraction were 48.9%, 21.5%, and 30.6%, respectively; therefore, these results suggested that SQDG in the glycolipids fraction must be important for the inhibition of *E. coli* pol I. When the cell extract of *E. coli* strains was used as the total pol (i.e., pols I to IV) in the cells, the inhibitory effect of pol activity by these compounds did not change (data not shown).

Glycolipids that inhibit prokaryotic pol activity might be suitable anti-bacterial agents; therefore, we investigated...
their influence on an *E. coli* strain, JM109. Fig. 2B shows the inhibition of 100 μg/mL compound on cell growth. In the purified glycolipids, SQDG was the strongest inhibitor, and DGDG showed moderate inhibition. On the other hand, MGDG did not influence cell growth. The inhibitory effect on *E. coli* cell proliferation showed almost the same tendency as that on pol I from *E. coli*; therefore, SQDG, which inhibited pol I activity, might directly affect cell proliferation in *E. coli* cells.

Next, the cell growth inhibitory effect on the strains of *E. coli* were investigated. As shown in Table 1, 100 μg/mL of purified SQDG and 100 μg/mL of the glycolipids fraction from spinach significantly inhibited the cell proliferation of the JM109 strain, and the inhibitory effect on cell growth was almost the same concentration as that on pol I activity. Therefore, the inhibition of pol activity might directly affect cell proliferation in the *E. coli* JM109 strain; howev-

![Figure 2](image-url)
er, the other *E. coli* strains tested (i.e., DH5α, TOP10 and TOP10F') did not influence cell growth. The biochemical difference among the four strains is not clear, but it is considered that the components and structure of the cell membrane may be different. It was suggested that SQDG in the glycolipids fraction was able to interact and penetrate into the cell membrane of some *E. coli* strains such as JM109 and reach the nucleus, inhibiting prokaryotic pol activity, although, the compound could not penetrate other *E. coli* strains such as DH5α, TOP10 and TOP10F'. The interaction of SQDG and the outer surface of the *E. coli* cell membrane will be addressed in further studies.

The lipid composition of thylakoid membranes is highly conserved among higher plants such as spinach (*Spinacia oleracea* L.), algae, and cyanobacteria, comprised mainly of the following three glycolipids, MGDG, DGDG, and SQDG (50). MGDG and DGDG are noncharged lipids, whereas SQDG possesses a negatively charged head group. Thylakoid membranes of *hf-2* with SQDG showed PSII activity 14); therefore, these results concluded that SQDG has the specific function of maintaining PSII activities.

### Table 1

<table>
<thead>
<tr>
<th><em>E. coli</em> strain</th>
<th>% of control activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SQDG</td>
</tr>
<tr>
<td>JM109</td>
<td>59.8 ± 17.9</td>
</tr>
<tr>
<td>DH5α</td>
<td>98.6 ± 7.4</td>
</tr>
<tr>
<td>TOP10</td>
<td>100.4 ± 11.6</td>
</tr>
<tr>
<td>TOP10F'</td>
<td>101.9 ± 2.1</td>
</tr>
</tbody>
</table>

SQDG or spinach glycolipids fraction (at a concentration of 100 μg/mL each) was incubated with *E. coli* strains. Cell growth activity in the absence of glycolipid was taken as 100%. Data are expressed as the mean ± SD; n = 4.

**ACKNOWLEDGMENTS**

This work was supported in part by a Grant-in-aid for Kobe-Gakuen University Joint Research (A), and "Academic Frontier" Project for Private Universities; matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology), 2006-2010, (H. Y. and Y. M.). Y. M. acknowledges Grants-in-aid from the Mochida Memorial Foundation for Medical and Pharmaceutical Research (Japan), and the Nakashima Foundation (Japan).

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