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Carbohydrate Research 307 (1998) 347–350

CARBOHYDRATE RESEARCH

Note

The identification of glycerol-3-yl 6-deoxy-6-*C*-sulfo-α-D-glucopyranoside (glyceryl α-sulfoquinovoside) as a metabolite in *Rhizobium*, a non-photosynthetic organism

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Received 21 August 1997; accepted in revised form 5 November 1997

Abstract

We have isolated and characterized glycerol-3-yl 6-deoxy-6-*C*-sulfo-α-D-glucopyranoside (glyceryl α-sulfoquinovoside), a key metabolite involved in sulfoquinovosyl diacylglycerol (SQDG) biosynthesis in photosynthetic organisms, from the cellular metabolic pools of *Rhizobium*. The compound has not been found in any other non-photosynthetic organisms. This result suggests some commonalities between the biosynthesis of SQDG in *Rhizobium* and in plant organelles and photosynthetic organisms and further adds to the evolving picture that there is considerable gene overlap between *Rhizobium* and photosynthetic systems, including plants. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: Glycerol-3-yl 6-deoxy-6-*C*-sulfo-α-D-glucopyranoside; Glyceryl sulfoquinovoside; Sulfoquinovosyl diacylglycerol; Sulfolipids; *Rhizobium*; Gene transfer

1,2-Di-*O*-acylglycerol-3-yl 6-deoxy-6-*C*-sulfo-α-D-glucopyranoside (sulfoquinovosyl diacylglycerol, SQDG, **1**) is a major lipid in the membranes of organelles involved in carbon dioxide fixation in plants. It is also found in photosynthetic bacteria which fix carbon dioxide [1]. It is a glycolipid in which the 6-position of glucose is transformed to a sulfonic acid function, and the resulting 6-deoxy-6-*C*-sulfo-α-D-glucopyranose (sulfoquinovose) is glycosidically linked via an α-linkage to diacylglycerol. The only report of this lipid being present in organisms outside of these photosynthetic systems is a recent one where it was isolated and characterized from nitrogen-fixing bacteria of the genus *Rhizobium* [2]. These bacteria fix nitrogen symbiotically with plants. The presence of this lipid in *Rhizobium* raises the question of whether the biosynthetic route in this organism is the same as (or at least related to) the one found in plants and photosynthetic organisms.

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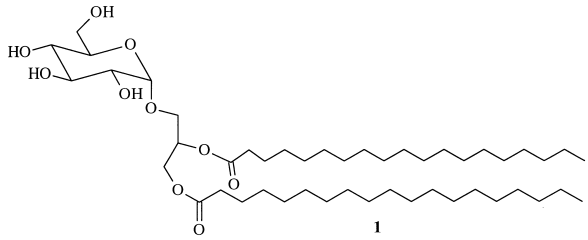
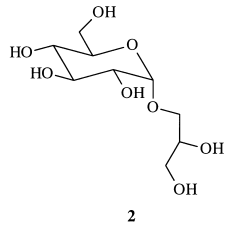
PII S0008-6215(97)10078-7

A key strategy for answering the questions raised above is to characterize the intermediates involved in SQDG biosynthesis in *Rhizobium* and determine whether they are also part of the pathway to SQDG in plants or other photosynthetic organisms. This strategy is aided by the fact that sulfur is not found in many biomolecules (comparatively speaking), and one can therefore target molecules that are radiolabeled if the bacteria are cultured in a medium containing ^{35}S as the sole sulfur source. We describe here the isolation and characterization of glycerol-3-yl 6-deoxy-6-*C*-sulfo-α-D-glucopyranoside (**2**) from the metabolic pools of *Rhizobium*. It was first isolated from *Chlorella* and *Scenedesmus*, two photosynthetic organisms [3]. We have recently identified digalactosyl diacylglycerol (a typical plant lipid) to be the predominant lipid in *in-planta* bacteroid forms of *Bradyrhizobium* [4]. We have also demonstrated that the corresponding *gluco* lipid is present in the cultures of bacteroid-like forms of the same organism [5]. This remarkable propensity for *Rhizobium* to synthesize these characteristic plant lipids suggests some very important development ties between these bacteria and plants.





The ^1H NMR spectrum (Fig. 1) of a purified sulfur-containing metabolite of *Rhizobium*, isolated as described in Section 2 "Materials and Methods", contained signals that were immediately attributable to α-linked sulfoquinovose [2]. There was a pair of doublet of doublets between 2.8 and 3.3 ppm attributable to the protons at the 6-position of sulfoquinovose. The first doublet of doublets (J 10 + 15 Hz) appeared at 2.93 ppm and

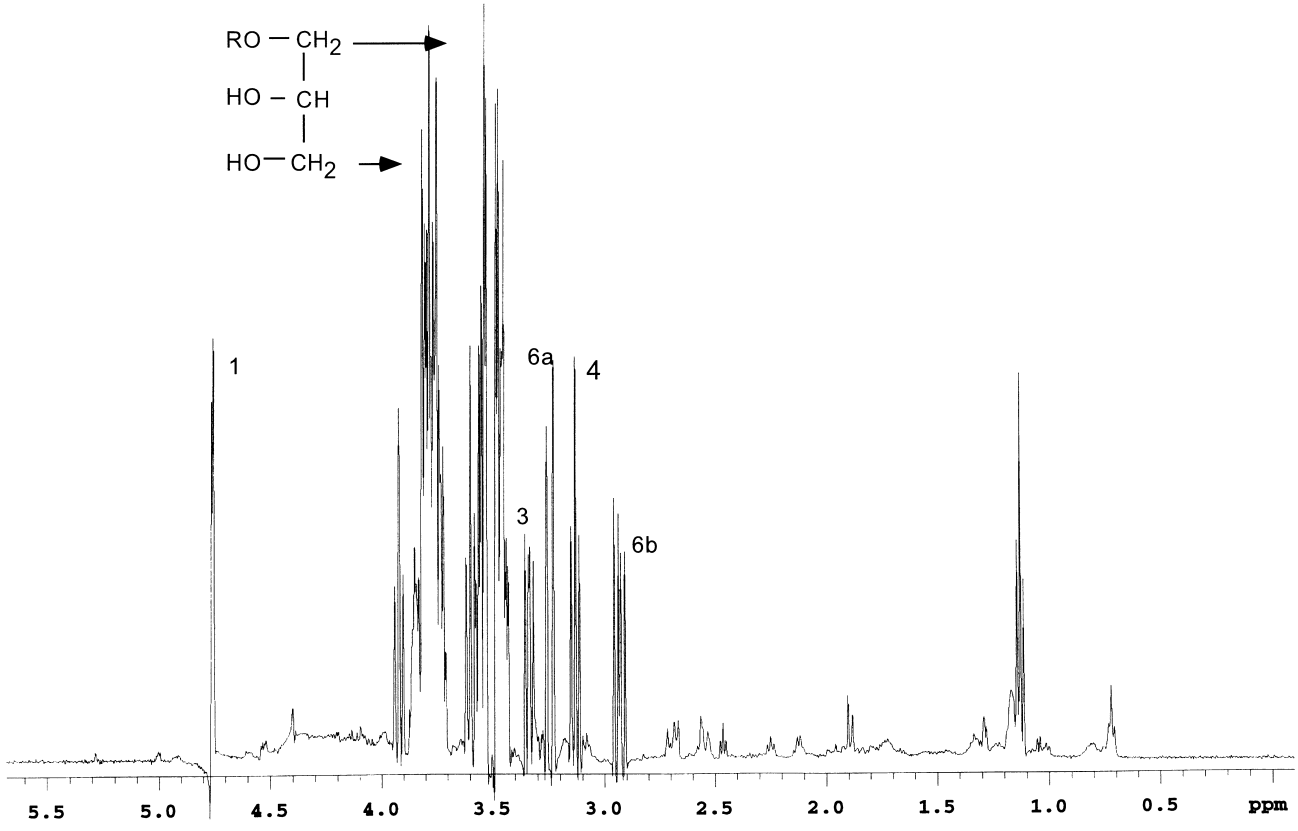


Fig. 1. The ^1H NMR spectrum of the sulfur-containing metabolite. The signals at ~1.1 ppm are from an impurity.

second one (J 0.1 + 15 Hz) appeared at 3.24 ppm. The larger splitting was due to geminal coupling. The anomeric proton signal appeared at 4.76 ppm as a narrow doublet (J 3.7 Hz) indicating that the ring was in the α-D-pyranosyl configuration. The signals for H-3 [a triplet (J 9 Hz) at 3.34 ppm)] and H-4 (a triplet with 9.4 Hz coupling at 3.13 ppm) were also readily assignable. Signals with large geminal couplings between 3.4 and 3.9 ppm, indicative of two different sets of methylene groups, meant that the glycerol moiety was linked through a primary hydroxyl group as the aglycon. The glycosidic linkage was confirmed by hydrolyzing the metabolite and passing the hydrolysis products through an anion-exchange column to trap the free 6-*C*-sulfonic acid. Glycerol was identified as the sole component of the column flow-through by ^{1}H NMR spectroscopy. No determination was made regarding the enantiomeric compostition of the glycerol. The molecular weight of the intact glycoside was confirmed by negative-ion electrospray ionization mass spectrometry which yielded a peak at m/z 317 for the [M − H] ion.

As we pointed out earlier, the occurrence of these characteristic plant lipids and their biosynthetic intermediates in *Rhizobium* is rather remarkable and deserves some special comment. It is clear that this is not a typical trait passed down through the phyla by vertical gene transfer since it appears to be a very special property of *Rhizobium* and is not observed in other related non-photosynthetic bacteria phylogenetically placed above and below *Rhizobium*. From where did these traits appear? One possibility is through lateral gene transfer from the plant to *Rhizobium* at some stage of their co-development. This possibility was raised to explain why *Rhizobium* contains a glutamate synthase gene that is similar to plant glutamate synthases but not to other bacterial genes [6]. The possibility of lateral gene transfer is supported by the findings that there is a link between the rhizobial genome and that of *Agrobacterium*, an organism that is noted for its capacity to transfer genetic material back and forth between itself and the plant host.

Recently, the complete nucleotide sequence of the symbiotic plasmid from *Rhizobium* sp NGR234 was determined, and it was found that several regions, especially the conjugal transfer loci of the plasmid, was highly similar to that of the mobile *Agrobacterium* Ti plasmid [7]. This suggests a common origin for these genetic elements. It has also been demonstrated that *Rhizobium* efficiently transfers genes involved in symbiosis laterally to other rhizosphere organisms including those not belonging to the family *Rhizobiaceae* [8,9]. In one of these studies [9], it was shown that when a strain of *Rhizobium loti* was introduced into an area that had no indigenous strains, within seven years only 19% of the bacteria that grew at the same rate as the inoculant strain had the same *Spe* I genomic fingerprint as the inoculant. The other 81% were different, but they, nevertheless, contained the symbiotic DNA region of the *Rhizobium loti* strain. This indicated that the diverse strains arose by transfer of chromosomal symbiotic genes from *Rhizobium loti* to non-symbiotic rhizobia. Phenomena such as these can be easily explained by the presence of the transposable elements known to be associated with the symbiotic genes of *Rhizobium*. The presence of such elements also affords an easy mechanism whereby plant genes can be picked up by the bacterium after rearrangements, duplications or the like, and integrated into its genome. This would also explain the chromosomal location of symbiotic genes in *Bradyrhizobium japonicum* and *Rhizobium loti* [10,11].

1. Materials and methods

Bacteria were cultured in liquid modified BIII medium and labeled with ^{35}S added as sulfate as previously described [12]. ^{1}H NMR spectroscopy was carried out on a Varian VXR 500 instrument operating at 500 MHz for protons. All spectra were obtained in deuterium oxide, and the residual water line was suppressed by selective inversion-recovery. Spectra were recorded at 50 °C. Mass spectra were obtained in the negative-ion mode on a Fisons Platform electrospray instrument. The sample was infused in pure water. Cells from 2 liters of culture were harvested by centrifugation at 8000 g for 20 min, and total metabolites were extracted by treating the cell pellet with a mixture of 1:1 ethanol–water. The aqueous ethanol extract was subjected to chromatography on a silica-gel column (1.5 cm × 2 cm) using 60:40:10 l-propanol–concentrated ammonia–water. Fractions (~0.5 mL) were collected and analyzed for radioactivity, and those corresponding to peaks containing radioactive material were pooled and concentrated. The early eluting pool was passed through a C_{18} reversed-phase column in pure water, and the forerun that contained most of the radioactivity

was collected. The forerun was then subjected to gel-filtration chromatography on a Biogel P-2 column (1.5 cm×90 cm) using pure water as the eluant. The major peak containing radioactivity was subjected to anion-exchange column chromatography (0.5 cm×2 cm, DEAE-cellulose). The column was washed with pure water and then eluted sequentially with 0.5 M and 1.0 M formic acid (5 mL each). The compound of interest eluted in the first of these fractions. It was concentrated and analyzed by ^{1}H NMR spectroscopy. Approximately one-third of the sample containing the signals of interest was hydrolyzed by heating in 2 M trifluoroacetic acid at 120 °C for 1 h. The hydrolysate was concentrated to dryness and passed through a short column of anion-exchange resin (0.2 cm×0.4 cm) in pure water, and the flow-through was collected. It was concentrated to dryness and analyzed by ^{1}H NMR spectroscopy.

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