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Total Synthesis of Geranylgeranylglyceryl Phosphate Enantiomers: Substrates for Characterization of 2,3-O-Digeranylgeranylglyceryl Phosphate Synthase

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Abstract

In order to determine the enantioselectivity of (*S*)-2,3-di-*O*-geranylgeranylglyceryl phosphate synthase (DGGGPS) from the thermoacidophilic archaeon *Sulfolobus solfataricus*, we developed an efficient enantioselective route to the enantiomeric geranylgeranylglyceryl phosphates (*R*)-GGGP and (*S*)-GGGP. Previous routes to these substrates involved enzymatic conversions, due to the lability of the polyprenyl chains towards common phosphorylation reaction conditions. The synthesis described herein employs a mild trimethyl phosphite/carbon tetrabromide oxidative phosphorylation to circumvent this problem. In contrast to previous results suggesting that only (*S*)-GGGP can act as the prenyl-acceptor substrate, both (*R*)-GGGP and (*S*)-GGGP were found to be substrates for DGGGPS.

All living organisms have been classified into three primary kingdoms: archaebacteria, eubacteria, and eukaryotes.¹ While the membrane lipids in eubacteria and eukaryotes are composed of glyceryl esters of fatty acids, archaebacterial membrane lipids contain isoprenyl glyceryl ethers.^{2,3} The core structures of archaeal membrane lipids, including diether and bipolar tetraether lipids, contain fully-reduced C₂₀ or C₂₅ prenyl groups.^{4,5} Biosynthesis of the isoprene moieties in the core lipids follows the mevalonate pathway used also by eubacteria and eukaryotes.^{6–8} The prenyl transfer reactions responsible for building the isoprenoid diphosphates, e.g., geranylgeranyl diphosphate (GGPP), are catalyzed by a family of prenyltransferases.^{9–12} As shown in Figure 1, the biogenesis of the core-structure of the archaeal membrane lipids starts with the prenyl transfer reaction catalyzed by (*S*)-3-*O*-geranylgeranylglyceryl phosphate [(*S*)-GGGP] synthase, which selectively uses (*S*)-glyceryl phosphate as the prenyl acceptor (Fig. 1). Then, the product is utilized as the presumed acceptor substrate for the biosynthesis of (*S*)-2,3-Di-*O*-geranylgeranylglyceryl phosphate (DGGGP), an advanced intermediate of archaeal membrane lipids.¹³

To date, only an enzyme-assisted synthesis of (*S*)-GGGP has been reported.¹¹ An enantiospecific chemical synthesis of both individual enantiomers of GGGP was required to validate this biosynthetic hypothesis, due in part to the acid-sensitive geranylgeranyl group of GGGP. In order to fully characterize the substrate selectivity of DGGGP synthase (DGGGPS), we developed a mild and effective route to the two GGGP enantiomers. The instability of

GGGP indeed posed significant challenges, and the biological results with the enantiomers were unexpected.

The synthesis of (*S*)-GGGP (**8**) is summarized in Scheme 1. Treatment of the (2*E*,6*E*,10*E*) - geranylgeraniol **1** with Ph₃P/CBr₄ at 25 °C afforded geranylgeranyl bromide **2**.¹⁴ Next, (*S*)- solketal was alkylated with geranylgeranyl bromide by using KH as base, to give ether **3** in 73% yield.¹¹ The reported HCl/THF method to remove the acetonide¹³ resulted in a complex mixture containing the desired product in low yield. The desired diol **4** was thus prepared in 75% yield using *p*-TsOH in methanol.^{15,16} In order to obtain selective phosphorylation of the primary hydroxyl, we first tried a strategy involving protection of the secondary hydroxyl as a silyl ether. Thus, both hydroxyl groups of diol **4** were protected as TBS ethers (TBSCl, imidazole, anhydrous DMF).^{15,16} Subsequently, the more labile primary TBS ether was selectively removed at rt by using HF·Py (HF·Py/Py/THF=1:2:5). Unfortunately, phosphorylation of the primary alcohol under standard conditions (dimethylphosphoryl chloride, *t*-BuOK, CH₂Cl₂)^{15,16} failed to give the desired product due to the lability of the polyene system.

A second strategy proved more successful. The use of the trimethyl phosphite/carbon tetrabromide oxidative phosphorylation method¹⁷ was deemed sufficiently mild to permit phosphorylation without damage to the geranylgeranyl moiety. Treatment of diol **4** with 1.1 eq of CBr₄ and 1.2 eq of P(OMe)₃ gave selective phosphorylation of the primary alcohol to give the protected phosphate **7**. Essentially no bisphosphate product was detected.

The next challenge in this synthesis was liberation of the free phosphate monoester from the protected triester. We first tried TMSBr, a standard deprotecting reagent for removal of methyl and ethyl groups in the synthesis of acyl migration-prone lysophosphatidic acid derivatives.^{15,16} However, GGGP did not survive this strong Lewis acid. By using a solution of TMSBr in 2,4,6-trimethylpyridine (*sym*-collidine),^{18–20} we obtained the desired monophosphate in the acidic form. Titration with 1 N aq NaOH afforded (*S*)-GGGP (**8**) as the stabilized sodium salt.

In order to determine the enantioselectivity of DGGGPS, both the enantiomers (*S*)-GGGP (**8**) and (*R*)-GGGP (**12**), were required. Starting with (*R*)-solketal, (*R*)-GGGP (**12**) was synthesized using the successful route as summarized in Scheme 2.

DGGGPS is a member of the UbiA prenyltransferase family that can catalyze the transfer of a prenyl group to its biological acceptor substrate (*S*)-GGGP (**8**). With the enantiomeric substrates (*R*)-GGGP (**12**) and (*S*)-GGGP (**8**) in hand, we determined the activity of DGGGPS toward each of these substrates. From the results of radio-HPLC analysis (Fig. 2) and reversed-phase TLC analysis (Fig. 3), we found the DGGGP and presumably its enantiomer were formed in the reactions using (*S*)-GGGP (**8**) and (*R*)-GGGP (**12**), respectively. In these reactions, the starting reagent [¹⁴C]-GGPP was formed first from [¹⁴C]isopentenyl diphosphate and (*E,E*)-farnesyl diphosphate by the activity of GGPS. Then, [¹⁴C]-GGPP was used as the prenyl donor substrate for DGGGPS. Thus, the results demonstrated that the C₂₀-prenyl group of GGPP could be transferred to either of the two GGGP enantiomers by the action of DGGGPS. (*S*)-GGGP seems to be marginally preferred in Fig. 2, while the (*R*)-enantiomer appeared to be preferred in Fig. 3. These results are reproducible but qualitative; the GGGPs produced are quite labile. Nevertheless, to our surprise, both (*R*)-GGGP (**12**) and (*S*)-GGGP (**8**) were accepted at a comparable extent as substrates for DGGGPS. During the biosynthesis of archaeal membrane lipids, GGPS catalyzes the transfer of prenyl groups from GGPP to (*S*)-glyceryl phosphate in formation of (*S*)-GGGP (**8**), and both the ether linkage between (*S*)-GGGP (**8**) and another geranylgeranyl group are formed under the control of DGGGPS. GGGPSs are known to have strict substrate preferences: (*R*)-glyceryl phosphate is a very poor substrate.

13, 21 Thus, our results strongly suggest that the chirality of archaeal membrane lipid is determined by GGGPS, not by DGGGPS. However, (*R*)-GGGP (**12**) and (*S*)-GGGP (**8**) will be important tools for more detailed analysis of the specific activity and enantioselectivity of DGGGPS in future studies.

In conclusion, (*S*)-GGGP and (*R*)-GGGP were each synthesized by a five-step procedure starting from the (2*E*,6*E*,10*E*) –geranylgeraniol and the appropriate enantiomer of solketal. A regioselective phosphorylation of diol **4** was achieved using CBr₄/P(OMe)₃, and the instability problem of geranylgeranyl group was circumvented by judicious selection of mild reaction conditions. The LKC18 reversed phase-TLC analysis and radio-HPLC analysis have shown that the DGGGPS can catalyze the transfer of prenyl group to the secondary hydroxy groups of both (*R*)-GGGP and (*S*)-GGGP.

Supplementary Material

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Acknowledgements

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Figure 1. Biosynthesis of DGGGP, a key intermediate for archaeal membrane lipids. Key: G-1-P, (S)-glyceryl phosphate; GGGPS, (S)-GGGP synthase; DGGGPS, DGGGP synthase

Figure 2.

Radio-HPLC analysis of 1-butanol-extracts from the enzyme reaction with [^{14}C]-GGPP synthase and DGGGPS, using 0.5 nmol [^{14}C]-isopentenyl diphosphate, 0.5 nmol (*E,E*) farnesyl diphosphate, 0.4 μmol (*S*)-GGGP (A) or (*R*)-GGGP (B) as substrates. GGOH, geranylgeraniol; DGGGOH, digeranylgeranylglycerol.

Figure 3.

Autoradiogram of TLC, from left to right: 1. (*S*)-GGGP; 2. (*R*)-GGGP; 3. without an acceptor substrate. GGGOH, geranylgeranylgerol; GGOH, geranylgeraniol; DGGGOH, digeranylgeranylgerol; Ori, origin; S.f., solvent front.

Scheme 1.
Synthesis of (S)-GGGP (**8**).

Scheme 2.
Synthesis of (R)-GGGP (**12**).