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(S)-Geranylgeranylglyceryl Phosphate Synthase

PURIFICATION AND CHARACTERIZATION OF THE FIRST PATHWAY-SPECIFIC ENZYME IN ARCHAEBACTERIAL MEMBRANE LIPID BIOSYNTHESIS*

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The first pathway-specific step in the biosynthesis of the core membrane diether lipids in archaebacteria is the alkylation of the primary hydroxyl group in (*S*)-glyceryl phosphate by geranylgeranyl diphosphate. The reaction is catalyzed by (*S*)-3-*O*-geranylgeranylglyceryl phosphate ((*S*)-GGGP) synthase. The cytosolic enzyme was purified to homogeneity from the moderately thermophilic archaebacterium *Methanobacterium thermoautotrophicum* by a combination of ammonium sulfate precipitation, four chromatographic steps (DE52, Q-Sepharose, phenyl-Superose, and Protein Pak), and native polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis of gel-purified GGGP synthase gave a single band at 29 kDa. The enzyme requires Mg^{2+} for optimal activity, although prenyltransfer is also seen in buffers containing Mn^{2+} or Zn^{2+}. A well defined pH optimum occurs between 6.0 and 7.5. Maximal activity is seen at 50–65 °C. The Michaelis constants for GGGP synthase are V_{max} = 4.1 ± 0.5 μmol min^{-1} mg^{-1}, K_M^{GGPP} = 4.1 ± 1.1 μM, and K_M^{GP} = 41 ± 5 μM.

Archaebacteria are sufficiently different from other forms of life to be classified as a distinct kingdom (1). These unusual organisms normally inhabit hostile environments characterized by high concentrations of salt, high temperatures, low pH, or the complete lack of oxygen and can resemble eubacteria, eukaryotes, or neither, depending on which phenotypic characteristics are considered (2). Archaebacteria have distinctive biochemical features as well, including highly diverged ribosomal RNAs, unique metabolic cofactors, and novel membrane lipids (2).

Archaebacterial membranes contain lipids consisting of saturated isoprenoid chains attached to glycerol by ether linkages (3). The core membrane diethers contain two phytane moieties and form bilayers in much the same way as fatty acid esters form bilayers in other organisms. In addition, thermophilic archaebacteria contain unique cyclic tetraethers where the ends of the phytane chains of two diethers are joined covalently (3). This modification stabilizes the bilayer by creating membrane-spanning lipids with polar head groups at the inner and outer surfaces. It has been shown that the degree of cyclization increases with increasing growth temperature (4).

Archaebacteria contain a variety of isoprenoid compounds in addition to the phytanyl chains in the core membrane lipids. These include farnesylated derivatives of glycerol (5, 6), squalene and partially hydrogenated derivatives of squalene (7), isoprenoid quinones (5), and prenylated proteins (8). Labeling studies indicate that isoprenoids are assembled from acetate by a mevalonate pathway similar to that found in eukaryotes (9).

Only a few studies have been reported for specific enzymes in the pathway. Cabrera *et al.* (10) found activity for a soluble hydroxymethylglutaryl-CoA reductase in cell-free preparations of *Halobacterium halobium* and reported that inhibition with mevinolin, a potent inhibitor of the enzyme, also reduces lipid synthesis. Zhang and Poulter found activity for isopentenyl diphosphate isomerase in *Methanobacterium thermoautotrophicum* (11). We recently discovered that the methanogen has a bifunctional short chain prenyltransferase which catalyzes elongation of dimethylallyl diphosphate by isopentenyl diphosphate and synthesizes both C_{15} (FPP) and C_{20} (GGPP)[1] isoprenoid diphosphates for different branches in the pathway (12).

During their studies on the biosynthesis of archaebacterial lipids, Zhang and Poulter (13) found that two prenyltransferases are required to synthesize core membrane diethers in *M. thermoautotrophicum*. One is cytosolic and catalyzes alkylation of the primary hydroxyl in (*S*)-glyceryl phosphate (GP) by GGPP. The other is microsomal and catalyzes alkylation of the remaining hydroxyl moiety in the monoether by a second GGPP, as shown in Scheme 1. A similar pathway was found in *H. halobium* (11). The alkylation of GP by GGPP is a prenyltransfer reaction where a hydroxyl group is the nucleophile in the acceptor substrate. Except for the biosynthesis of archaebacterial lipids, there are only a few examples of prenyl transfer reactions to oxygen-containing acceptors (14, 15), and none of the enzymes responsible for these reactions has been characterized. We now report the purification and catalytic properties of (*S*)-3-*O*-geranylgeranylglyceryl phosphate (GGGP) synthase, the enzyme that catalyzes the first pathway-specific step in the biosynthesis of archaebacterial core membrane lipids.

EXPERIMENTAL PROCEDURES

Materials—Frozen cells of *M. thermoautotrophicum* Marburg were provided by Dr. Lacy Daniels. GGGP, (*S*)-GP, and (*S*)-[1-^{3}H]GP were available from an earlier study (13). BHDA was from Pfaultz & Bauer. SDS was from Bio-Rad. Ultrapure acrylamide and $(NH_4)_2SO_4$ were from ICN Biochemicals. Other materials were from Sigma unless specified otherwise.

General Procedures—Dialyses were for 4 h in M_r 6,000–8,000 cut-off tubing or 2 h in M_r 12,000–14,000 cut-off tubing (Spectrum). Centrifugations were performed at 20,000 × *g* (JA-20 rotor) or 10,000 × *g* (JA-14 rotor) in a Beckman TJ-6 centrifuge. A Pharmacia fast protein liquid chromatograph was used for chromatography. Protein samples were

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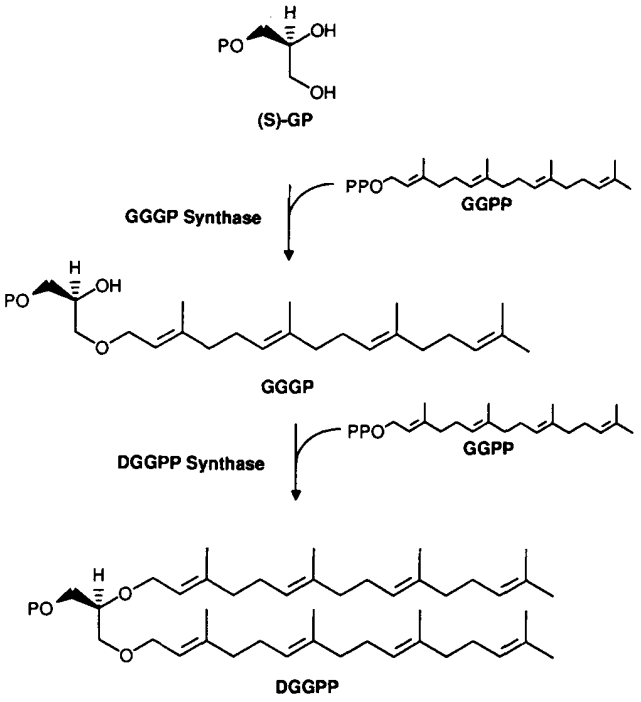
[1] The abbreviations used are: GGPP, geranylgeranyl diphosphate; BHDA, bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride; BisTris, bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane; 2-ME, 2-mercaptoethanol; CAPS, 3-(cyclohexylamino)propanesulfonic acid; GGGP, 3-geranylgeranylglyceryl phosphate; GP, 1-glyceryl phosphate; PAGE, polyacrylamide gel electrophoresis; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).



SCHEME I.

filtered through 0.45-μm cellulose acetate and loaded through a 50-ml superloop. Proteins were detected at 280 nm and collected in plastic tubes. All runs were at 4 °C. Fractions containing GGGP synthase activity were pooled and analyzed by 12% stacking SDS-PAGE (16) using a Bio-Rad Mini-PROTEAN II electrophoresis system. Protein bands were stained by Coomassie Blue R. Protein concentrations were determined by the method of Bradford (17) using bovine serum albumin as a standard.

Assay for GGGP Synthase—A 190-μl sample of 50 mM BHDA, 20 μM GGPP, 100 μM (*S*)-[1-^{3}H]GP (3 μCi/μmol), 10 mM 2-ME, and 3 mM $MgCl_2$, pH 7.0, was equilibrated at 60 °C. A 10-μl sample containing protein was added, and the mixture was incubated at 60 °C for 10 min. The reaction was terminated by addition of 100 μl of 0.5 M EDTA. Saturated NaCl (0.5 ml) was added, followed by 2 ml of *n*-butyl alcohol. The sample was agitated vigorously on a vortex mixer. After standing for 10 min, 1 ml of the *n*-butyl alcohol layer was mixed with 10 ml of Packard Opti-Fluor and counted by liquid scintillation spectrometry. GGGP synthase activity was calculated from radioactivity in the *n*-butyl alcohol layer.

Purification of GGGP Synthase—Frozen cells of *M. thermoautotrophicum* Marburg (20 g) were divided into four portions, and each was suspended in 25 ml of extraction buffer (50 mM Tris-Cl, 10 mM 2-ME, 1 mM EDTA, and 50 mM NaCl, pH 7.5). Protease inhibitors phenylmethylsulfonyl fluoride and pepstatin A were added to 1 mM and 1 μg/ml, respectively, and the cells were disrupted by sonication at 0 °C. The homogenate was centrifuged for 30 min. The pellet was resuspended, sonicated, and centrifuged as described above. The supernatants were combined and treated with $(NH_4)_2SO_4$. Protein precipitating between 50 and 80% $(NH_4)_2SO_4$ was dissolved in 50 ml of extraction buffer containing 1 mM phenylmethylsulfonyl fluoride and dialyzed twice against 2 liters of 25 mM BisTris-Cl buffer containing 5 mM 2-ME, pH 7.0.

The dialyzed sample was loaded onto a 2.5 × 14-cm DE52 column (Whatman) equilibrated with 500 ml of starting buffer (25 mM BisTris-Cl, 5 mM 2-ME, and 2% ethylene glycol, pH 7.0). The column was washed with 200 ml of starting buffer containing 250 mM NaCl and eluted with 500 ml of a 250–500 mM NaCl linear gradient in starting buffer at a flow rate of 2 ml/min. Active fractions were pooled, dialyzed against 2 × 2 liters of 10 mM phosphate and 5 mM 2-ME, pH 7.0, and loaded onto a Q-Sepharose 16/10 HP column (Pharmacia LKB Biotechnology Inc.) equilibrated with starting buffer (10 mM phosphate and 5 mM 2-ME, pH 7.0). The column was eluted with 350 ml of a 0–1 M linear gradient of $(NH_4)_2SO_4$ in starting buffer at 2 ml/min. Active fractions were pooled, and 1.0 M potassium phosphate, pH 7.0, was added to a final concentration of 50 mM. Powdered $(NH_4)_2SO_4$ was added to a final concentration of 1 M, and the sample was loaded onto a phenyl-Superose HR 10/10 column (Pharmacia) equilibrated with 100 ml of starting buffer (50 mM phosphate, 5 mM 2-ME, and 1.0 M ammonium sulfate, pH 7.0). The column was washed with starting buffer and eluted with a 160-ml linear gradient of 1–0 M $(NH_4)_2SO_4$ at 1 ml/min. The active fractions were pooled, dialyzed against 2 × 2 liters of 25 mM BisTris acetate and 5 mM 2-ME, pH 7.0, and loaded onto a 0.75 × 7.5-cm Protein Pak column (Waters) equilibrated with 50 ml of starting buffer (25 mM BisTris acetate and 5 mM 2-ME, pH 7.0). The column was eluted with 100 ml of a linear gradient of 0–1 M sodium acetate in starting buffer at a flow rate of 0.5 ml/min. The active fractions were pooled.

Native PAGE was performed on a 10% gel prepared as described for stacking SDS-PAGE except that SDS and 2-ME were omitted in all buffers, and samples were not boiled. The gel was pre-run for 20 min. Samples were dialyzed against 10 mM NH_4HCO_3 and concentrated on a SpeedVac. After electrophoresis at a constant current (30 mA) for 40 min, the gel was rinsed with water and soaked in 0.3 M $CuCl_2$ for 5 min (18). The gel was rinsed with water again, and protein bands were visualized on a black background. Those regions containing protein were sliced from the gel, soaked in 25 mM Tris-Cl and 10 mM EDTA, pH 9.0, for 10 min, and then cut into small pieces. Proteins in individual fractions were electroeluted into 40 mM Tris acetate, pH 8.2, using a Schleicher & Schuell Elutrap and assayed for GGGP synthase activity.

Native Molecular Mass—A Superdex 75 HR 10/10 column (Pharmacia) was eluted at 0.2 ml/min with 50 mM phosphate, 5 mM 2-ME, and 150 mM NaCl, pH 7.0. Standards were aldehyde dehydrogenase (150 kDa), bovine serum albumin (68 kDa), pepsin (34.7 kDa), and cytochrome *c* (12.4 kDa). A 0.9 × 80-cm Sephacryl S-200 HR column (Pharmacia) was eluted at 0.1 ml/min with 100 mM phosphate and 10 mM 2-ME, pH 7.0. Mass standards were β-amylase (200 kDa), phosphorylase (97.4 kDa), pepsin (34.7 kDa), and cytochrome *c* (12.4 kDa). Fractions were collected and assayed for GGGP synthase activity.

Metal Ion, pH, and Temperature Dependence of GGGP Synthase—All assays contained 20 μM GGPP, 100 μM GP, and 17.5 ng of GGGP synthase that had been purified through the Protein Pak step. The samples were incubated for 5 min. For studies of the effect of metal ions, the enzyme was first dialyzed against 50 mM phosphate, 10 mM 2-ME, and 5 mM EDTA, pH 7.0, and then against the same buffer without EDTA. The pH studies were in 50 mM buffer containing 10 mM 2-ME and 3 mM $MgCl_2$. The pH was measured at the reaction temperature. For temperature dependence studies, the enzyme was assayed in 50 mM BHDA, 10 mM 2-ME, 3 mM $MgCl_2$, pH 7.0.

Kinetic Constants—Initial velocities were measured for varied concentration of one substrate in the presence of a saturating concentration of the other in 50 mM BHDA, 10 mM 2-ME, 3 mM $MgCl_2$, and 0.1 mg/ml bovine serum albumin, pH 7.0. Assay mixtures were preequilibrated at 60 °C, and the reaction was initiated by addition of GGGP synthase. K_m and V_{max} were calculated using Enzfitter™.

RESULTS

Purification of GGGP Synthase—The purification of GGGP synthase is summarized in Table I. The 50–80% $(NH_4)_2SO_4$ fraction from cell-free homogenates normally gave a 1.5-fold purification with 70% recovery of activity. Chromatography on DE52 (Fig. 1) gave a broad peak, and attempts to improve resolution were unsuccessful. However, combination and dialysis of the active fractions gave an excellent recovery of units with an almost 10-fold purification. The sample was chromatographed on Q-Sepharose, as shown in Fig. 2, using a linear

TABLE I
Purification of GGGP Synthase

Results are based on 20 g of wet cells.

Steps	Protein[a]	Units	Recovery	Specific activity[a]	Purification
	mg	*nmol/min*	*%*	*nmol/min mg*	*-fold*
Extracts	1318	486	100	0.37	1
50–80% $(NH_4)_2SO_4$	661	335	69	0.51	1.8
DE52	80	496	102	6.2	17
Q-Sepharose	7.7	262	54	34	92
Phenyl-Superose	0.28	172	35	614	1659
Protein Pak	0.084	97	20	1155	3122

[a] Assays were done in 50 mM BHDA buffer, pH 7.0, containing 10 mM 2-ME, 3 mM $MgCl_2$, 20 μM GGPP, and 100 μM (*S*)-GP at 60 °C.

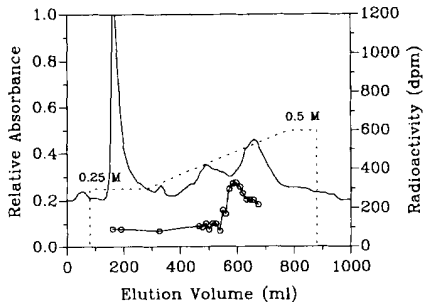


FIG. 1. **Chromatography on DE52.** Relative absorbance at 280 nm (——), radioactivity incorporated into GGGP, dpm (○), and NaCl concentration in elution buffer (- - -). Buffer A consisted of 25 mM BisTris-Cl, 5 mM 2-ME, and 2% ethylene glycol, pH 7. Buffer B consisted of 1 M NaCl in buffer A. Elution was accomplished with a 200 ml of 0.25 M NaCl followed by a 500-ml linear gradient of 0.25–0.5 M NaCl at 2 ml/min.

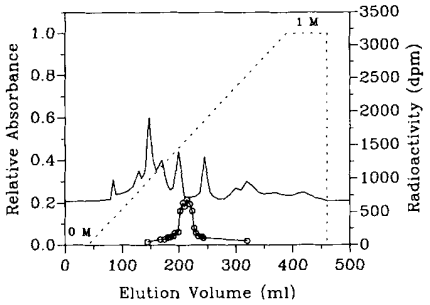


FIG. 2. **Chromatography on Q-Sepharose.** Relative absorbance at 280 nm (——), radioactivity incorporated into GGGP, dpm (○), and $(NH_4)_2SO_4$ concentration in elution buffer (- - -). Buffer A consisted of 10 mM phosphate and 5 mM 2-ME, pH 7. Buffer B consisted of 1 M $(NH_4)_2SO_4$ in buffer A, pH 7.0. Elution was accomplished with a 350-ml linear gradient of 0–1 M $(NH_4)_2SO_4$ at 2 ml/min.

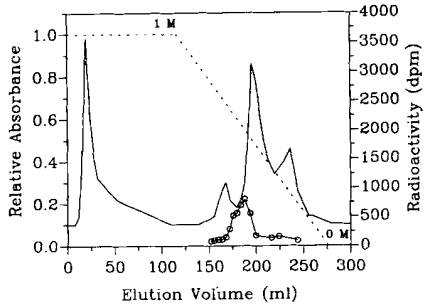


FIG. 3. **Chromatography on phenyl-Superose.** Relative absorbance at 280 nm (——), radioactivity incorporated into GGGP, dpm (○), and $(NH_4)_2SO_4$ concentration in elution buffer (- - -). Buffer A consisted of 1 M $(NH_4)_2SO_4$ in buffer B, pH 7. Buffer B consisted of 50 mM phosphate and 5 mM 2-ME, pH 7.0. Elution was accomplished with a 160 ml of 1–0 M linear gradient of $(NH_4)_2SO_4$ at 1 ml/min.

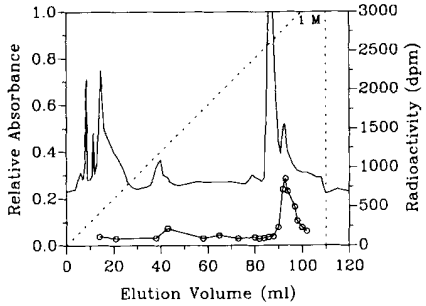


FIG. 4. **Chromatography on Protein Pak.** Relative absorbance at 280 nm (——), radioactivity incorporated into GGGP, dpm (○), and sodium acetate concentration in elution buffer (- - -). Buffer A consisted of 25 mM BisTris acetate and 5 mM 2-ME, pH 7.0. Buffer B consisted of 1 M sodium acetate in buffer A. Elution was accomplished with a 100-ml linear gradient of 0–1 M sodium acetate at 0.5 ml/min.

gradient of 0–1 M $(NH_4)_2SO_4$. The enzyme in 1 M $(NH_4)_2SO_4$ was loaded directly onto a phenyl-Superose column and eluted with a 1–0 M decreasing gradient of $(NH_4)_2SO_4$ (see Fig. 3). Upon chromatography on Protein Pak (Fig. 4), activity for GGGP synthase eluted with a small peak near the end of a 0–1 M linear gradient of sodium acetate.

SDS-PAGE of a sample gave a single major band at 29 kDa and several minor bands (>30 kDa), as shown in Fig. 5*A*. At this point we estimated that GGGP synthase was 85% pure.

Samples of the enzyme from two different chromatographies were purified to homogeneity by native PAGE as shown in Fig. 5*B*. The proteins were visualized by reversible copper staining (18), individual bands were removed, and the separated proteins were recovered by electroelution. In both cases, only the major band had GGGP synthase activity. PAGE-purified enzyme gave a single band at 29 kDa upon SDS-PAGE. This band corresponded to the major 29-kDa band in protein samples after the Protein Pak step.

Native Molecular Mass—The native molecular mass of GGGP synthase was measured on calibrated Superdex 75 and Sephacryl S-200 columns. Values of 150 and 159 kDa, respectively, were obtained. Since SDS-PAGE gave a single band at 29 kDa, the native enzyme appears to be a homopolymer, perhaps a pentamer.

General Properties of GGGP Synthase—Purified archaebacterial GGGP synthase transferred a single geranylgeranyl moiety to the primary hydroxyl group in (*S*)-GP. The enzyme was inactive when assayed in metal-free buffer after dialysis in the presence of EDTA. Activity was restored by addition of divalent metal. Substantial activity was seen with Mg^{2+}, Mn^{2+}, and Zn^{2+}. However, the enzyme was inactive when Ca^{2+} was added. As illustrated in Fig. 6, the specific activity of GGGP synthase increased with increasing Mg^{2+} concentrations up to a saturating value of 4 mM. At low concentrations, Mn^{2+} was more effective than Mg^{2+}, but higher concentrations of Mn^{2+} were inhibitory.

Maximal activity of GGGP synthase was seen between 50 and 65 °C (see Fig. 7) at temperatures in the normal range for optimal growth of *M. thermoautotrophicum*. At higher temperatures, the enzyme is irreversibly denatured. The pH-rate profile shown in Fig. 8 is a bell-shaped curve with a maximum between 6.0 and 7.5. Kinetic constants were determined at 60 °C in 50 mM BHDA, 10 mM 2-ME, and 3 mM $MgCl_2$, pH 7.0. Under these conditions, K_M^{GGPP} = 4.3 ± 1.1 μM, K_M^{GP} = 41 ± 5 μM, and V_{max} = 4.1 ± 0.5 μmol min^{-1} mg^{-1}.

DISCUSSION

Prenyltransferases catalyze the electrophilic alkylation of electron-rich acceptors by allylic isoprenoid diphosphates with concomitant expulsion of a proton and PP_i (19). These reactions constitute the major building steps in the isoprenoid biosynthetic pathway. Although prenyl transfers to heteroatoms such as nitrogen, oxygen, or sulfur in acceptor substrates are not uncommon, most work has focussed on the enzymes that catalyze alkylation of unsaturated hydrocarbon moieties (20). Representative reactions of this type are the basic chain elongation catalyzed by farnesyl diphosphate synthase (19), the synthesis of squalene catalyzed by squalene synthase during the first committed step in cholesterol biosynthesis (21), and alkylation of the aromatic ring in tryptophan by dimethylallyl-tryptophan synthase (22).

GGGP synthase catalyzes the first committed step in the biosynthesis of archaebacterial diether core membrane lipids.

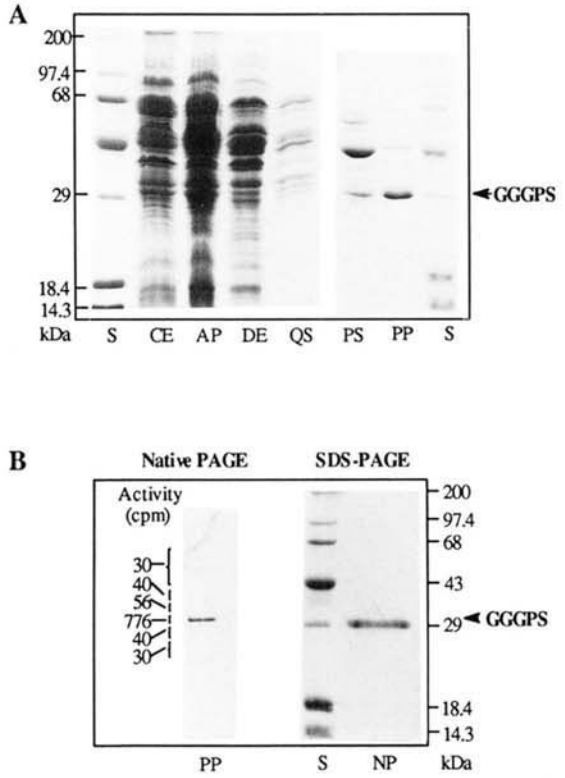


FIG. 5. **PAGE analysis of GGGP synthase.** *Panel A*, 12% SDS-PAGE of proteins from each step in the purification. *Panel B*, native PAGE of GGGP synthase. *CE*, crude extracts; *AP*, $(NH_4)_2SO_4$ precipitate; *DE*, DE52; *QS*, Q-Sepharose; *PS*, phenyl-Superose; *PP*, Protein Pak; *NP*, native PAGE-purified; *S*, protein standards; *GGGPS*, GGGP synthase. Protein standards were as follows: myosin (H chain), 200 kDa; phosphorylase *b*, 97.4 kDa; bovine serum albumin, 68 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 29 kDa; β-lactoglobin, 18.4 kDa; lysozyme, 14.3 kDa.

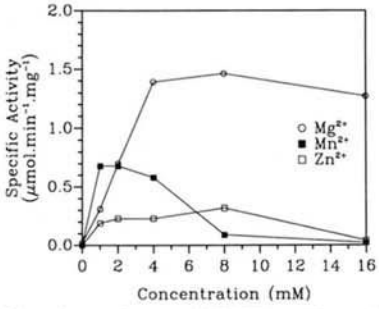


FIG. 6. **Metal ion dependence of GGGP synthase.** Measured in 50 mM BHDA buffer, pH 7.0, containing 10 mM 2-ME, 20 μM GGPP, and 100 μM GP. GGGP synthase was dialyzed against buffer containing EDTA and then against metal-free buffer without EDTA before use in the assays.

As illustrated in Scheme 1, the substrates for the prenyltransferase are (*S*)-GP and GGPP (13). Thus, the glyceryl unit in archaebacterial membrane lipids has the opposite absolute stereochemistry of the glyceryl moiety in the ester lipids from eubacteria and eukaryotes. Biosynthetic studies indicate that GGPP is synthesized from acetyl coenzyme A by the normal mevalonate pathway (9). However, labeling experiments uncovered two different pathways for biosynthesis of the glycerol moiety. In *Sulfolbus acidocaldarius*, glycerol was incorporated intact (23), whereas glycerol labeled with deuterium at C-2 lost the label when the molecule was incorporated into the membranes of *H. halobium* (24). Zhang and Poulter (11) studied the substrate selectivity for GGGP synthase in cell-free extracts

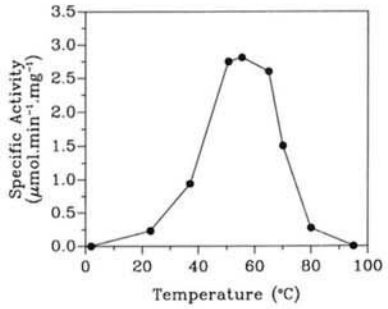


FIG. 7. **Temperature dependence of specific activity for GGGP synthase.** Measured in 50 μM BHDA buffer, pH 7.0, containing 10 μM 2-ME, 3 μM $MgCl_2$, 20 μM GGPP, and 100 μM (*S*)-GP.

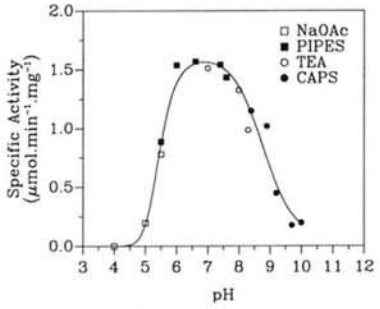


FIG. 8. **pH-specific activity profile for GGGP synthase.** Buffers used were sodium acetate (□), PIPES (■), triethanolamine (○), and CAPS (●).

from *H. halobium* and found that (*S*)-GP is the preferred prenyl acceptor. Thus, the differences detected by the labeling experiments must occur before the prenyl transfer step catalyzed by GGGP synthase.

Cytosolic GGGP synthase from *M. thermoautotrophicum* was purified to >85% homogeneity by a combination of ion exchange and hydrophobic chromatographies. A highly purified sample was obtained by native PAGE. Active GGGP synthase was identified by visualizing the gel with $CuCl_2$, according to the procedure described by Lee *et al.* (18), without destroying enzymatic activity. This procedure allowed us to detect protein bands, and gel slices containing individual protein bands were assayed to determine which contained GGGP synthase. Comparisons of molecular masses determined by SDS-PAGE and gel filtration suggest that GGGP synthase is a homopolymer with four or more subunits. Although a variety of structural motifs, including monomers (25), homodimers (21, 26), and heterodimers (27), have been reported for prenyltransferases, GGGP synthase may be the first example of a higher order aggregate.

Like other prenyltransferases, GGGP synthase required a divalent metal ion for activity (18). Although Mg^{2+} was preferred, prenyltransferase activity was also seen with Mn^{2+} and Zn^{2+}. The metal ion dependence for GGGP synthase was similar to that reported for farnesyl diphosphate synthase (28). In the latter case, Laskovics *et al.* (29) concluded that Mg^{2+} was not bound directly to the enzyme but was sequestered along with the substrates as a magnesium salt of the diphosphate moieties.

The mechanism for alkylation of (*S*)-GP by GGPP appears to be related to the electrophilic reactions seen for farnesyl diphosphate synthase (18) and dimethylallyl tryptophan synthase (21). These enzymes catalyze rupture of the carbon-oxygen bond in the diphosphate-isoprene linkage to generate highly electrophilic allylic carbocations that subsequently alkylate the prenyl acceptors. Formation of the electrophilic intermediates depends on having an allylic double bond in the

prenyl donor to provide the reactivity required to generate the carbocation. Zhang and Poulter (13) found that cytosolic preparations from *M. thermoautotrophicum* catalyze alkylation of (*S*)-GP by phytyl diphosphate, a derivative of GGPP with a single double bond at C-2, at a slower but significant rate. However, the fully saturated derivative, phytanyl diphosphate, is inactive. Thus, the mechanism for prenyl transfer of a geranylgeranyl group from GGPP to the hydroxyl group in GP appears to be an electrophilic substitution similar to those that have been established for other prenyltransferases.

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