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Purification and Characterization of Farnesyl Diphosphate/Geranylgeranyl Diphosphate Synthase

A THERMOSTABLE BIFUNCTIONAL ENZYME FROM *METHANOBACTERIUM THERMOAUTOTROPHICUM**

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Farnesyl diphosphate (FPP)/geranylgeranyl diphosphate (GGPP) synthase, a bifunctional enzyme that synthesizes C_{15} and C_{20} isoprenoid diphosphates from isopentenyl diphosphate and dimethylallyl diphosphate, was purified to homogeneity from the archaebacterium *Methanobacterium thermoautotrophicum*. The only activities detected from synthesis of FPP and GGPP copurified through $(NH_4)_2SO_4$ precipitation and four chromatographic steps. The pure enzyme was a 79-kDa homodimer that catalyzed the sequential addition of isopentenyl diphosphate to dimethylallyl diphosphate, geranyl diphosphate, and FPP by a nonprocessive mechanism which allowed substantial amounts of FPP to accumulate during turnover, creating a pool for further elongation to GGPP or for synthesis of squalene. The bifunctional enzyme required Mg^{2+} or Mn^{2+} and was optimally active at 65 °C. Catalysis of chain elongation in *M. thermoautotrophicum* differs from related reactions in eubacteria and eukaryotes, where distinct FPP synthases and GGPP synthases are found.

Archaebacteria are unique organisms that diverged from other forms of life at the earliest stages of evolution (1, 2). They inhabit unusual, often extreme, environments and are classified on that basis as methanogens (strict anaerobes), halophiles (high salt), thermophiles (high temperature), and thermoacidophiles (high temperature and low pH). Several biochemical features set archaebacteria apart from eubacteria and eukaryotes. These include differences in the architecture of their cell walls (3), highly diverged ribosomal RNAs (2), unique cofactors (4), unique membrane lipids (5), differences in tRNAs (6, 7), and differences in translational and transcriptional machinery (8, 9).

Archaebacterial membranes contain a core lipid consisting of saturated C_{20} isopranyl glyceryl ethers (5, 10) instead of the fatty acid ester lipids found in other organisms. This feature is unique to archaebacteria and places a substantial commitment on the isoprene pathway for synthesis of diterpenes. Zhang *et al.* (11) studied the biosynthesis of archaebacterial membrane lipids in cell-free preparations from *Methanobacterium thermoautotrophicum* and discovered that the diterpene units are derived from geranylgeranyl diphosphate (GGPP).[1] The hydrocarbon moieties in GGPP are transferred to (*S*)-glyceryl phosphate (GP) in two steps. The first is catalyzed by geranylgeranylglyceryl phosphate (GGGP) synthase, an enzyme which attaches the geranylgeranyl moiety to the C(3) glyceryl hydroxyl in GP, and the second by digeranylgeranylglyceryl phosphate synthase, which alkylates the C(2) hydroxyl. The isoprenoid double bonds are reduced, and polar head groups are attached in subsequent reactions. Some of the hydrocarbon chains in the core membrane lipids of thermophiles are joined by a novel 4′-4 linkage at the end of the diterpene chain to give a rugged membrane stabilized by bipolar molecules that span the bilayer (12).

In addition to C_{20} isoprenoids found in the core membrane lipids, archaebacteria contain a variety of C_{15}, C_{20}, and higher isoprenoids, including squalene and partially saturated derivatives of squalene (13), carotenoids (14), isoprenoid quinones (15), and prenylated proteins (16). The isoprene chains are assembled by a 1′-4 coupling of IPP to allylic diphosphates. These reactions are typically catalyzed by a family of prenyltransferases, each with its own selectivity for the ultimate length of the hydrocarbon chain, the stereochemistry of the double bonds in the allylic substrate, and the stereochemistry of the newly formed double bond (17). For example, in eukaryotes distinct prenyltransferases synthesize polyprenyl diphosphates needed for various branches of the isoprenoid pathway. These include FPP synthase for steroids (18–23), GGPP synthase for diterpenes and carotenoids (24–26), a long chain trans polyprenyl diphosphate synthase for ubiquinones (27), and a long chain cis polyprenyl diphosphate synthase for dolichols (28), as illustrated in Scheme I. A similar motif is found in eubacteria.

As part of our study of isoprenoid biosynthesis in archaebacteria, we began an investigation of the 1′-4 condensing prenyltransferases in *M. thermoautotrophicum*, a strict anaerobe and moderate thermophile, which uses H_2 and CO_2 as sole energy and carbon sources to produce methane (29), by tracking FPP synthase and GGPP synthase activities during purification. To our surprise, we discovered that the two activities copurified and subsequently were able to demonstrate that a single, bifunctional enzyme produces FPP and GGPP in *M. thermoautotrophicum*. We now report the purification and properties of the prenyltransferase.

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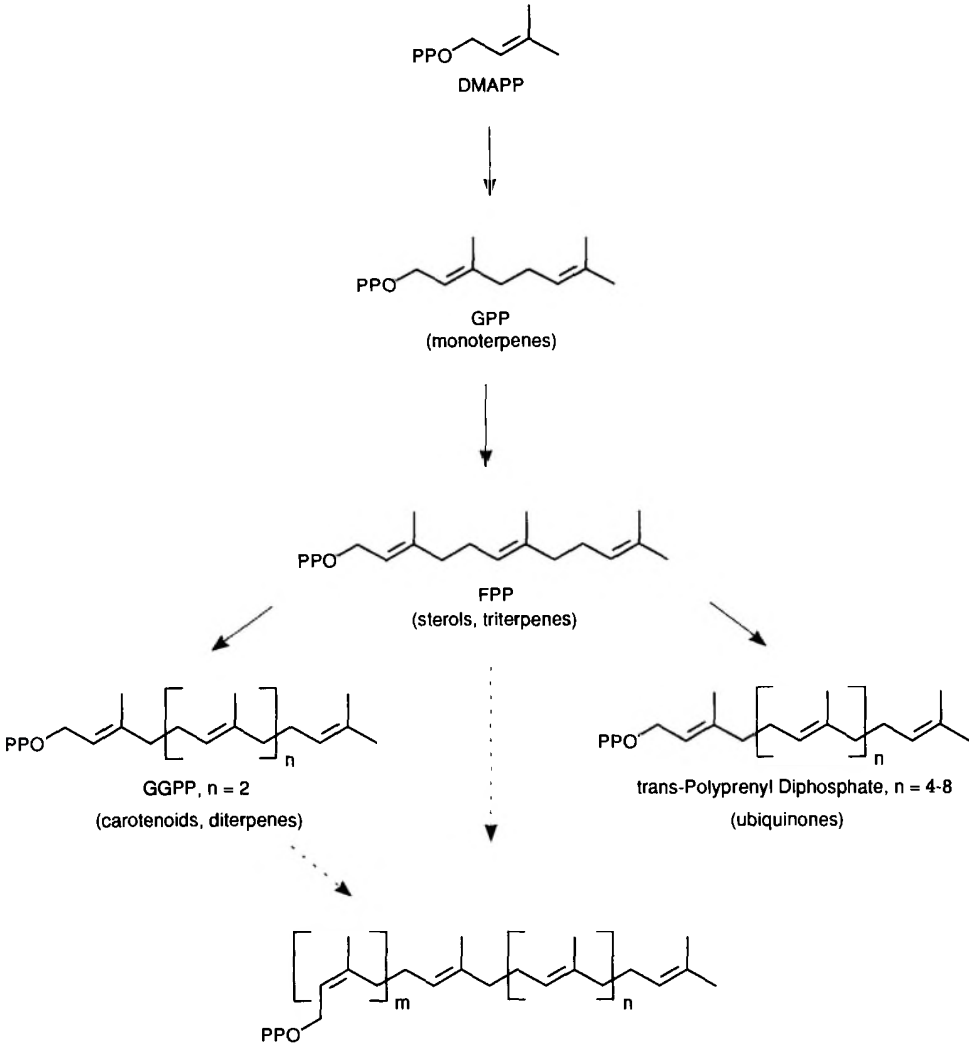
[1] The abbreviations used are: GGPP, geranylgeranyl diphosphate; BHDA, bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride; BME, β-mercaptoethanol; CAPS, 3-(cyclohexylamino)propanesulfonic acid; DMAPP, dimethylallyl diphosphate; FPP, farnesyl diphosphate; GC, gas chromatography; GCMS, gas chromatography mass spectrometry; GGGP, geranylgeranylglyceryl phosphate; GP, glyceryl phosphate; GPP, geranyl diphosphate; IPP, isopentenyl diphosphate; MOPS, 3-(*N*-morpholino)propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).



SCHEME I

EXPERIMENTAL PROCEDURES

Materials—Frozen cells of *M. thermoautotrophicum*, Marburg, were obtained from Professor Lacy Daniels (University of Iowa) and stored at −70 °C until needed. [1-^{14}C]IPP was from Du Pont-New England Nuclear. IPP, DMAPP, GPP, FPP, and GGPP were synthesized by the method of Davisson *et al.* (30). Bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic anhydride was from Pfaultz & Bauer. Other materials, where not specified, were from Sigma.

General Procedures—Protein concentrations were determined by the procedure of Bradford (31). Dialysis was for 4 h in 6,000–8,000 molecular weight cut-off tubing (Spectrum) and 2 h in 12,000–14,000 molecular weight cut-off (Spectrum). Centrifugations were at 15,000 revolutions/minute (JA-20 rotor) or 10,000 revolutions/minute (JA-14 rotor) in a Beckman TJ-6 centrifuge. Chromatographies were run on a Pharmacia fast protein liquid chromatography system operating at 4 °C. Protein samples were filtered through a 0.45-μm cellulose acetate filter prior to loading, and detection was at 280 nm. Fractions were collected in plastic tubes, and selected samples were assayed for prenyltransferase activity. Active fractions were pooled and analyzed by 12% stacking SDS-PAGE on a Bio-Rad Mini-Protein II system. Protein bands were stained with Coomassie Blue R. Isoelectric focusing gel electrophoresis was run on an LKB 2217 Ultraphor Electrofocusing Unit using LKB Ampholine PAGplates, pH 3.5–9.5, and pI standards.

Prenyltransferase Assay—Assay buffer was 50 mM BHDA, 10 mM BME, and 3 mM $MgCl_2$, pH 7.0. A standard assay mixture contained 200 μM DMAPP, GPP, or FPP and 20 μM [1-^{14}C]IPP (10 μCi/μmol) in a total volume of 190 μl, preequilibrated to 60 °C. The reaction was initiated by addition of 10 μl of enzyme in assay buffer. After incubation for 5 min at 60 °C, 0.25 ml of CH_3OH-HCl (4:1) was added, and incubation was continued for 10 min. One milliliter of ligroin was added, and the contents of the tube were vigorously agitated on a vortex mixer. One-half milliliter of the ligroin layer was mixed with 10 ml of Packard Opti-Fluor, and radioactivity was determined by liquid scintillation spectrometry on a Packard Tri-Carb 4530 Liquid Scintillation Spectrometer.

Purification of Prenyltransferase—Sixty g of frozen cells of *M. thermoautotrophicum*, Marburg, were divided into six equal portions, and each was suspended in 25 ml of extraction buffer (50 mM Tris-Cl, 10 mM BME, 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, immediately before sonication. Cells were cooled in ice-water and disrupted by a Branson Sonic Power Sonifier equipped with a 1/2-inch horn. The suspension was spun at 15,000 × *g* for 30 min, and the resuspended pellet was sonicated and centrifuged as described above. The supernatants were combined and clarified by treatment with 1.5% streptomycin sulfate, pH 7.0. $(NH_4)_2SO_4$ (ICN Biochemicals, ultrapure) was added to 55% saturation. The suspension was spun at 9,000 × *g* for 30 min, and the supernatant was brought to 80% saturation with $(NH_4)_2SO_4$ before a second spin. The 55–80% $(NH_4)_2SO_4$ pellet was dissolved in 10 mM phosphate, 5 mM BME, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml pepstatin A, pH 7.0, and dialyzed against 2 × 2 liters of the same buffer without inhibitors.

Dialyzed protein was loaded onto a 2.5 × 14-cm hydroxyapatite column (Bio-Rad) equilibrated with 700 ml of starting buffer (10 mM phosphate, 5 mM BME, pH 7.0). The column was washed with 50 ml of the loading buffer followed by 70 ml of 50 mM phosphate, 5 mM BME, pH 7.0, and then eluted with a 500-ml linear gradient of 50–200 mM phosphate, 5 mM BME, pH 7.0, at a flow rate of 2 ml/min. The active fractions were pooled and loaded directly onto a Pharmacia Q Sepharose 16/10 HP column equilibrated with 10 mM phosphate, 5 mM BME, pH 7.0. The column was eluted with a 400-ml linear gradient of 0–1 M $(NH_4)_2SO_4$ in the same buffer at a flow rate of 2 ml/min. The active fractions were combined, the phosphate concentration was increased to 50 mM, and $(NH_4)_2SO_4$ was added to a final concentration of 1.3 M. The sample was applied to a Pharmacia Phenyl-Superose HR 10/10 column equilibrated with 50 mM phosphate, 10 mM BME, 1.3 M $(NH_4)_2SO_4$, pH 7.0, and eluted with a 130-ml 1.3–0 M linear gradient of $(NH_4)_2SO_4$ in the same buffer at a flow rate of 1.0 ml/min. The active fractions were combined and dialyzed against 2 × 2 L of 50 mM phosphate, 10 mM BME, pH 7.0, and applied to a Pharmacia Mono Q 5/5 column equilibrated with the dialysis buffer. The column was eluted with 60 ml of a 0.2–0.52 M linear gradient of $(NH_4)_2SO_4$ in starting buffer at a flow rate of 0.5 ml/min. The active fractions were analyzed on SDS-PAGE and those showing one major protein band were combined (0.17 mg/ml).

Assignment of Bands on Polyacrylamide Gels—Denaturing polyacrylamide gels were prepared by the procedure of Garfin (32). Native gels were prepared as described for denaturing gels except SDS and BME were omitted in all buffers, and the samples were not boiled. Native gels were preelectrophoresed for 20 min, protein purified through the Mono Q step loaded, and the sample was electrophoresed for about 40 min at 30 mA. The left-most lane was cut from the gel and lightly stained for 10 min with a solution of 40% methanol and 10% acetic acid containing 0.1% (w/v) Coomassie Blue R. The stained section was aligned with the remainder of the gel, and the gel was cut into five segments, with one segment encompassing the position of the stained band. The segments were chopped into small pieces and incubated for 4 h in 0.5 ml of 10 mM phosphate, 10 mM BME, 3 mM $MgCl_2$, pH 7.0, at 37 °C. The extracts from each segment were assayed for prenyltransferase activity. The most active fraction was analyzed by SDS-PAGE.

Product Analysis—A total of 0.4 ml of a mixture containing 200 μM IPP and 200 μM DMAPP, GPP, or FPP, 3.4–8.5 μg of enzyme in 50 mM BHDA buffer, pH 7.6, containing 10 mM BME, 10 mM $MgCl_2$, and 0.1 mg/ml BSA was incubated for 4–6 h at 60 °C. One milliliter of 100 mM glycine buffer, pH 10.4, containing 1 mM $MgCl_2$, 1 mM $ZnCl_2$, and 20 μl of alkaline phosphatase (Sigma VII-S, 10 units/μl) were added, and incubation was continued for 24 h at 37 °C. Saturated NaCl (0.1 ml) was added, and the mixture was extracted three times with 2 ml of hexane. The combined hexane extracts were concentrated with a gentle flow of N_2 and analyzed by GC on a DB-5 capillary column followed by GCMS analysis using a 25 M BP-5 column (SGE). Authentic samples of dimethylallyl alcohol, geraniol, *E,E*-farnesol, and *E,E,E*-geranylgeraniol were used as standards.

Native Molecular Weight Determination—Samples were chromatographed on a Pharmacia Superdex 75 HR 10/10 column at a flow rate of 0.2 ml/min using 150 mM NH_4OAc, buffer pH 7.0. The column was calibrated with alcohol dehydrogenase (150 kDa), bovine serum albumin (68 kDa), pepsin (34.7 kDa), and cytochrome *c* (12.4 kDa). Prenyltransferase activity was determined by the acid lability assay, as previously described.

Metal Ion, pH, and Temperature Dependence of Prenyltransferase Activity—The standard assay buffer was used with the following changes. $MnCl_2$, $ZnCl_2$, and $CaCl_2$ replaced $MgCl_2$ in the metal ion dependence studies. Buffers for pH dependence were 50 mM sodium acetate (pK_a = 4.76), PIPES (pK_a = 6.76), MOPS (pK_a = 7.2), triethanolamine (pK_a = 7.76), ethanolamine (pK_a = 9.5), and CAPS (pK_a = 10.4). The pH of each was determined at the assay temperature.

RESULTS

Purification—The purification summarized in Table I is typical. The 55–80% $(NH_4)_2SO_4$ fraction of the clarified cell-free homogenate gave a 3-fold purification with greater than 70% recovery of activity. The pellet was resuspended in buffer, dialyzed, and chromatographed on hydroxyapatite. Activity eluted at about 100 mM phosphate (Fig. 1), and the combined active fractions were loaded directly onto Q Sepharose. Enzyme eluted between 400–500 mM $(NH_4)_2SO_4$ with a 25–30-fold purification (Fig. 2). The active fractions were pooled, brought to 1.3 M with $(NH_4)_2SO_4$, and loaded onto Phenyl-Superose.

TABLE I
Purification of FPP/GGPP Synthase
Based on 60 g of wet cells.

Steps	Protein	Units	Recovery	Specific activity[a]	Purification
	mg	*nmol/min*	%	*nmol/min·mg*	*-fold*
Cell-free extracts[b]	3838	822	100	0.21	1
55–80% $(NH_4)_2SO_4$	977	660	80	0.68	3.2
Hydroxyapatite	218	447	54	2.1	10
Q Sepharose	20	1132	138	58	275
Phenyl-Superose	1.5	259	32	177	845
Mono Q	0.5	163	20	329	1567

[a] Assay was done in 50 mM BHDA, 10 mM BME, and 3 mM $MgCl_2$, pH 7.0, buffer at 60 °C using 200 μM GPP and 20 μM IPP.
[b] Clarified by precipitation with 1.5% streptomycin sulfate.

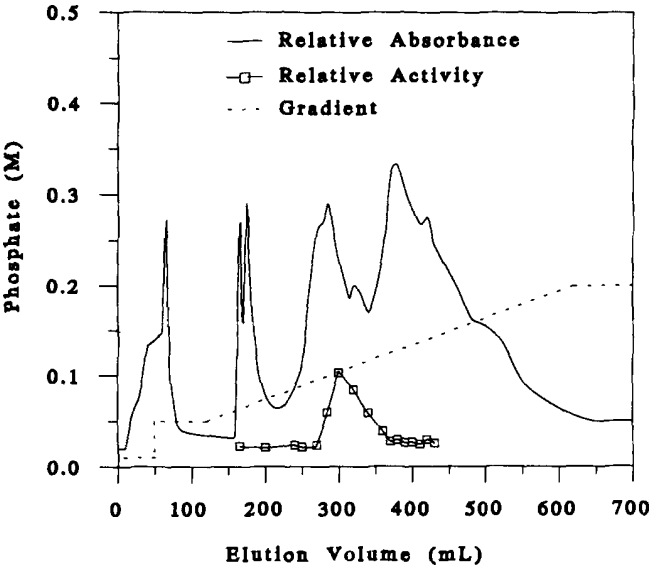


FIG. 1. **Chromatography on hydroxyapatite.** Buffer A: 10 mM phosphate, 5 mM BME, pH 7.0. Buffer B: 500 mM phosphate, 5 mM BME, pH 7.0. Elution: 70 ml of 50 mM phosphate followed by a 500-ml linear gradient from 50 to 200 mM phosphate at 2 ml/min. FPP was the allylic substrate for assay.

The enzyme eluted at approximately 800 mM $(NH_4)_2SO_4$ with a linear descending salt gradient (Fig. 3). Active fractions were combined, dialyzed, and loaded onto Mono Q. Elution with $(NH_4)_2SO_4$ (Fig. 4) gave enzyme that was >95% pure, as judged by SDS-PAGE. Samples from each step in the purification are shown in Fig. 5, *part A*. Purified enzyme was also analyzed by native PAGE. A slice of the gel was stained (Fig. 5, *part B*), and the remainder was sectioned as indicated. Individual sections were assayed for activity, and protein from the active section was analyzed by SDS-PAGE. Samples from the Mono Q step and after native PAGE both ran at 38 kDa. The maximum specific activity of purified enzyme was 3.9 ± 0.1 μmol min^{-1} mg^{-1} with GPP as the allylic substrate. A typical purification gave a 1600–3200-fold increase in specific activity, depending on the activity of the crude homogenate.

Native Molecular Mass—The native molecular mass of purified enzyme was 79 kDa, as measured on a calibrated Superdex 75 column (data not shown). SDS-PAGE of the same sample gave a single band at 38 kDa. Thus, we conclude that the enzyme is a homodimer.

Coelution of FPP and GGPP Synthase Activities—Prenyl-

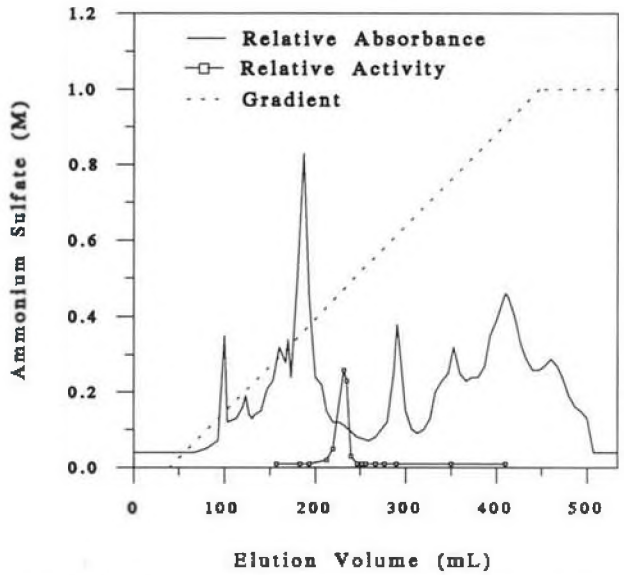


FIG. 2. **Chromatography on Q Sepharose.** Buffer A: 10 mM phosphate, 5 mM BME, pH 7.0. Buffer B: 1.0 M $(NH_4)_2SO_4$ in Buffer A. Elution: a 400-ml linear gradient from 0 to 1.0 M $(NH_4)_2SO_4$ at 2 ml/min. FPP was the allylic substrate for assays.

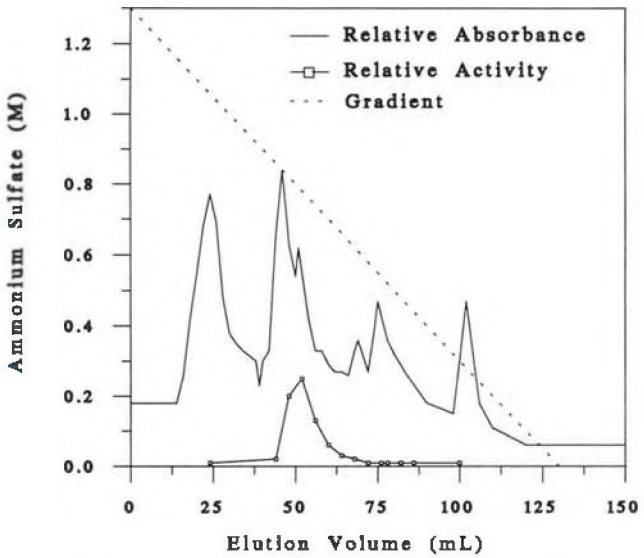


FIG. 3. **Chromatography on Phenyl-Superose.** Buffer A: 1.3 M $(NH_4)_2SO_4$ in Buffer B. Buffer B: 50 mM phosphate, 10 mM BME, pH 7.0. Elution: A 130-ml linear gradient from 1.3 to 0 M $(NH_4)_2SO_4$ at 1 ml/min. FPP was the allylic substrate for assay.

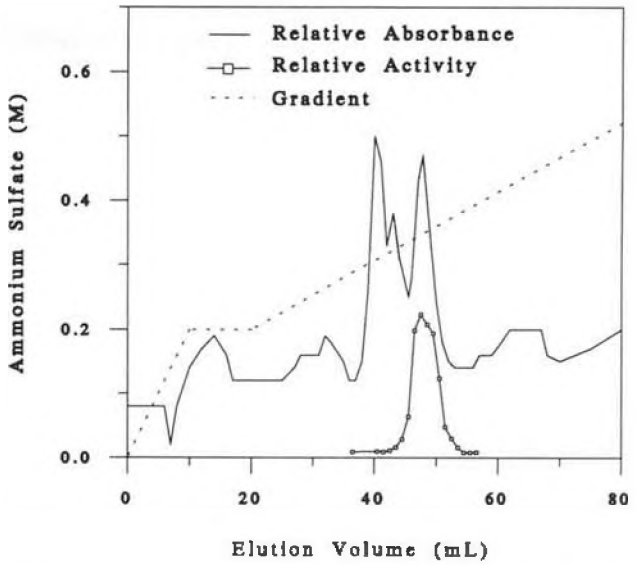


FIG. 4. **Chromatography on Mono Q.** Buffer A: 50 mM phosphate, 10 mM BME, pH 7.0. Buffer B: 1.3 M $(NH_4)_2SO_4$ in Buffer A. Elution: A 60-ml linear gradient from 0.2 to 0.52 M $(NH_4)_2SO_4$ at 0.5 ml/min. FPP was the allylic substrate for assays.

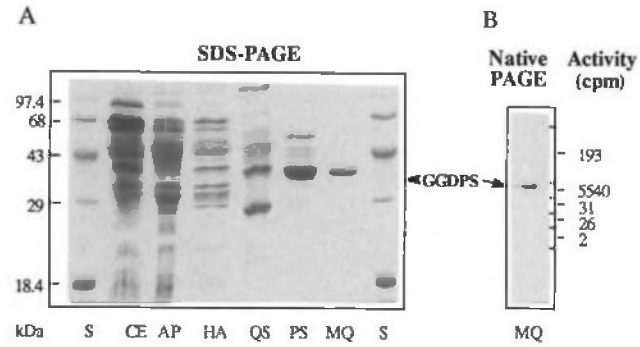


FIG. 5. **SDS-PAGE analysis during purification of FPP/GGPP synthase.** *Part A*, 12% SDS-PAGE of proteins from each step in the purification. *Part B*, 12% native PAGE of proteins after the Mono Q step and prenyltransferase activity (counts/minute) in indicated slices of the gel. *GGDPS*, GGPP synthase; *CE*, cell-free extract; *AP*, 55–80% $(NH_4)_2SO_4$ fraction; *HA*, hydroxyapatite; *QS*, Q Sepharose; *PS*, Phenyl-Superose; *MQ*, Mono Q; *S*, protein standards: 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.

transferase activity was evaluated in crude homogenates and for protein after the Mono Q step using DMAPP, GPP, and FPP as the allylic substrates. The respective purification factors were 2960, 3130, and 2600, through the $(NH_4)_2SO_4$ precipitation and four chromatographic steps. In addition, individual fractions from the chromatographic separations shown in Figs. 1–4 were assayed for FPP synthase and GGPP synthase activity. In each instance, the assays with GPP (FPP synthase activity) and FPP (GGPP synthase activity) as allylic substrates gave superimposable peaks. No other peaks were seen that had FPP or GGPP synthase activity. These results provide strong evidence that the purified 79-kDa homodimer is a bifunctional enzyme which synthesizes both FPP and GGPP in *M. thermoautotrophicum*.

General Properties of FPP/GGPP Synthase—The purified archaebacterial FPP/GGPP synthase uses DMAPP, GPP, or FPP as its allylic substrate. Under the assay conditions, the ratio of activities was DMAPP/GPP/FPP = 4.1:5.9:1. Kinetic constants were determined at 60 °C in 50 mM BHDA, 10 mM BME, 10 mM Mg_2Cl, pH 7.6, where the enzyme showed maximal velocity at saturating levels of substrate (see below). $K_M^{GPP} = 105 \pm 19$ μM, $K_M^{IPP} = 167 \pm 14$ μM, and $k_{cat}^{GPP} = 2.5$ s^{-1} for the reaction of IPP and GPP. $K_M^{FPP} = 41 \pm 7$ μM, $K_M^{IPP} = 95 \pm 12$ μM, and $k_{cat}^{FPP} = 0.4$ s^{-1} for the reaction of IPP and FPP. The activity of FPP/GGPP synthase drops abruptly for chain elongation beyond C_{20}. Incubations with IPP and GGPP gave little activity for synthesis of higher polyprenyl diphosphates. We estimate that GGPP is at least 150-fold less reactive than FPP as an allylic substrate.

The distribution of products for condensation of IPP with each of the allylic substrates was measured by capillary GC analysis of the alcohols obtained from the allylic diphosphate products after hydrolysis. In a typical experiment, DMAPP, GPP, or FPP was incubated with IPP and enzyme. The reaction was terminated by addition of glycine buffer, pH

10.4, and the diphosphate moieties were hydrolyzed by addition of alkaline phosphatase. The hexane-soluble extracts were analyzed by capillary GC and GCMS. A distribution of products was seen whose composition depended on which allylic diphosphate was used as the initial substrate and how much IPP was consumed. DMAPP gave a mixture of GPP, FPP, and GGPP, while GPP yielded FPP and GGPP, and FPP yielded only GGPP. Higher amounts of enzyme or longer reaction times shifted the distribution of products toward GGPP in each case. These results are consistent with a stepwise or partially processive mechanism for chain elongation.

FPP/GGPP synthase requires a divalent metal for activity. The enzyme was inactive in metal-free buffers. Addition of Mg^{2+} or Mn^{2+} restored activity in a concentration-dependent manner, as shown in Fig. 6. Increasing concentrations of Mg^{2+} gave corresponding increases in activity up to a maximal value of 3.5 μmol min^{-1} mg^{-1} at ~10 mM, typical of saturation behavior. Although Mn^{2+} was more effective than Mg^{2+} at concentrations below 2.5 mM, higher concentrations of Mn^{2+} were inhibitory. Enzyme activity returned to background levels at Mn^{2+} concentrations above 10 mM. Zn^{2+}, which stimulated activity in avian liver FPP synthase at low (~0.5–1.0 mM) concentrations (33), was not effective for archaebacterial FPP/GGPP synthase, nor was the enzyme active with Ca^{2+} as the divalent metal.

The temperature dependence for activity of FPP/GGPP synthase is shown in Fig. 7. The enzyme is the most robust of the prenyltransferases reported to date. It has maximal activity between 55–70 °C, within the temperature range for optimal growth of *M. thermoautotrophicum* (29). The archaebacterial enzyme can be handled at room temperature and stored for prolonged periods at 4 °C in 50 mM phosphate buffer, pH 7.0, containing 10 mM BME. The pH-rate profile of FPP/GGPP synthase was "bell-shaped" with an optimum at pH 7.0–8.5. The isoelectric point for the enzyme was 4.7.

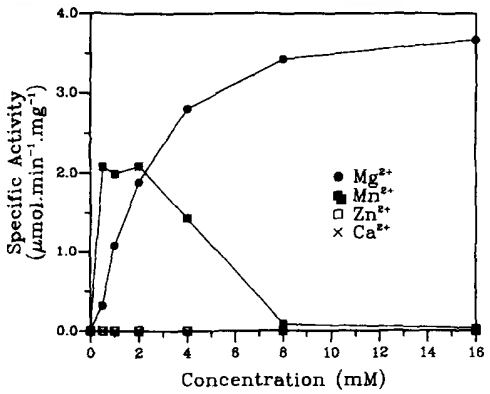


FIG. 6. **Metal ion dependence of FPP/GGPP synthase.** Measured in 50 mM BHDA and 10 mM BME, pH 7.0, at 55 °C. 200 μM GPP and 200 μM IPP were substrates.

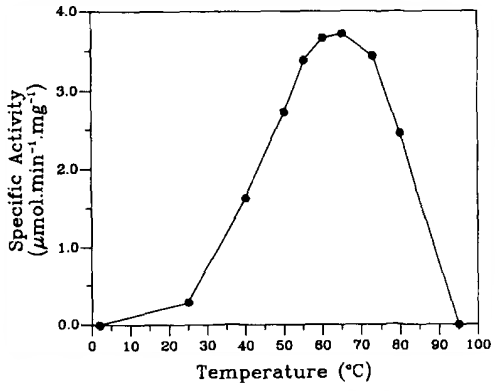


FIG. 7. **Temperature dependence of V_{max} for FPP/GGPP synthase.** Measured in 50 mM BHDA, 3 mM $MgCl_2$, and 10 mM BME, pH 7.5. 200 μM GPP and 100 μM IPP were substrates.

FPP/GGPP synthase lost activity in buffers that did not contain a disulfide-reducing agent. When purified enzyme in 50 mM phosphate buffer containing BME was dialyzed against 10 mM NH_4HCO_3 without BME, pH 7.9, for several hours at 4 °C, analysis by native PAGE showed a new band that migrated more rapidly than for enzyme stored in phosphate buffer containing BME (see Fig. 8, *part A*).

In a separate run, the bands corresponding to the two enzyme forms were excised and assayed for activity. Both catalyzed synthesis of GGPP from IPP and FPP, although the faster running band was substantially less active. Further analysis by SDS-PAGE showed that FPP/GGPP synthase stored in phosphate buffer with BME gave a single 38 kDa band under reducing and nonreducing electrophoresis conditions (Fig. 8, *part B, left*). However, enzyme stored in NH_4HCO_3 without BME gave a single 38 kDa band when BME was present and bands at 38 and 32 kDa when BME was absent in the electrophoresis sample buffer (Fig. 8, *part B, right*). Clearly FPP/GGPP synthase is susceptible to oxidation in the absence of reducing agents. Its behavior during SDS-PAGE is consistent with the formation of an intersubunit disulfide in the absence of BME. However, the presence of a faster moving band on native gels is suggestive of further oxidation. It should be noted that *M. thermoautotrophicum* is a strict anaerobe. Our data indicate that the native enzyme is fully reduced and that oxidation is an artifact of handling the protein in the absence of a reducing agent. Related oxidations of the native reduced form of FPP synthase from porcine (20, 35) and avian[2] sources have been observed.

DISCUSSION

The prenyltransferases that catalyze chain elongation are highly selective for the chain length of their products and the stereochemistry of the newly formed double bonds. In most organisms, the short chain prenyltransferases GPP synthase (37, 38), FPP synthase (18–23), and GGPP synthase (24–26) elongate DMAPP to C_{10}, C_{15}, and C_{20} products, respectively. The C_{15} and C_{20} products serve, in turn, as substrates for longer chain prenyltransferases in the ubiquinone (27) and dolichol (28) biosynthetic pathways.

Regulation of the flow of metabolites into various chain elongation pathways is not well understood. Two distinct enzymes catalyze chain elongation of DMAPP to FPP (18–23, 39, 40) or GGPP (24–26, 41) in eubacteria and eukaryotes. Typically, DMAPP and GPP are substrates for FPP synthase, while DMAPP, GPP, and FPP are substrates for GGPP synthase. In addition GGPP synthetase competes with squalene synthase (42, 43), *trans*-polyprenyl diphosphate synthase (27), and *cis*-polyprenyl diphosphate synthase (28) for available FPP. In eubacteria and many eukaryotes, all of these activities are located in the cytoplasm or on cytoplasmic-accessible surfaces and compete for a common cytoplasmic pool of GPP and FPP. Regulation must occur through modulation of the activities at individual branch point enzyme. In higher plants, portions of the pathway are physically segregated by compartmentation. For example, biosynthesis of carotenoids is localized in plastids, perhaps as early as IPP (44). Some plants also possess highly specialized secretory

[2] M.-J. Yang and C. D. Poulter, unpublished results.

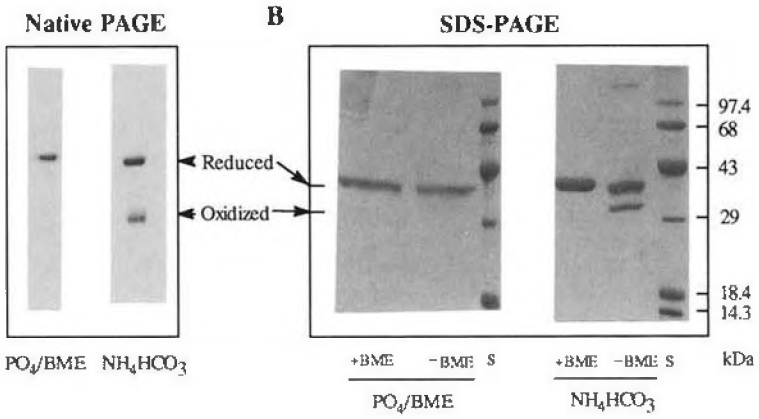


FIG. 8. **Analysis of oxidized and reduced forms of FPP/GGPP synthase.** *Part A*, native PAGE and *part B*, SDS-PAGE of FPP/GGPP synthase in 50 mM phosphate, 10 mM BME, pH 7.0, and after dialysis against 10 mM NH_4HCO_3. In SDS-PAGE, BME was present (+*BME*) or absent (−*BME*) in the electrophoresis buffer. Protein standards are the same as in Fig. 5.

structures for a monoterpene biosynthesis that effectively segregate C_{10} metabolism from other branches of the pathway (45).

The architecture of the central part of the isoprene pathway is apparently different in *M. thermoautotrophicum*. During isolation of short chain prenyltransferases, the activities for synthesis of FPP and GGPP copurified. No other activities for synthesis of FPP or GGPP that could be attributed to another enzyme were detected. However, at shorter reaction times, substantial amounts of GPP and FPP were formed from DMAPP, but the distribution shifted toward GGPP at longer times. Clearly, the archaebacterial enzyme is not highly processive. *M. thermoautotrophicum* is not known to synthesize monoterpenes, and presumably the only fate for GPP is elongation to FPP. The catalytic efficiency of FPP/GGPP synthase toward FPP or GGPP can be approximated by $k_{cat}^{Allylic\text{-}PP}/K_M^{IPP} \cdot K_M^{Allylic\text{-}PP}$, assuming $K_M^{Allylic\text{-}PP} \sim K_D^{Allylic\text{-}PP}$. Based on the steady-state kinetic constants we measured for the bifunctional archaebacterial enzyme, the relative rates of conversion should be similar at subsaturating levels of IPP and allylic substrate. Thus, under steady-state conditions, a pool of FPP is available for squalene synthase, long chain prenyltransferases, and the final step catalyzed by FPP/GGPP synthase.

At the enzyme level, the bifunctional prenyltransferase from *M. thermoautotrophicum* is remarkably similar to FPP and GGPP synthases from other organisms. Typically, these enzymes are homodimers comprised of 39–43-kDa subunits for FPP synthase (18–23) and 30–38-kDa subunits for GGPP synthase (24–26), and all require a divalent metal ion, normally Mg^{2+}, for activity. The Michaelis constants for the archaebacterial enzyme are at the high end of the normal range for 1′-4 condensing prenyltransferases (36) and may reflect substantially higher basal levels of isoprenoid intermediates in *M. thermoautotrophicum*.

In summary, the bifunctional FPP/GGPP synthase in *M. thermoautotrophicum* shows many of the biochemical properties of individual FPP and GGPP synthases from eubacteria and eukaryotes. However, the archaebacterium has a single enzyme that provides both FPP and GGPP, whereas other organisms typically contain distinct FPP and GGPP synthases.

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