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Purification and Characterization of Geranylgeranyl Diphosphate Synthase from *Methanobacterium thermoformicicum* SF-4

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Geranylgeranyl diphosphate (GGPP) synthase [EC 2.5.1.29] was purified to homogeneity from *Methanobacterium thermoformicicum* SF-4. The enzyme was a dimeric protein consisting of two identical subunits ($M_r = 39{,}000$) and catalyzed prenyl transfer reactions using isopentenyl diphosphate ($K_m = 30.8\ \mu M$) and either dimethylallyl diphosphate ($K_m = 16.8\ \mu M$), geranyl diphosphate ($K_m = 12.6\ \mu M$), or farnesyl diphosphate ($K_m = 14.7\ \mu M$) as allylic partners. During a sequential elongation, $C_5 \rightarrow C_{10} \rightarrow C_{15} \rightarrow C_{20}$, intermediates were accumulated with various ratios to the final product GGPP. In the presence of 0.8 M KCl, GGPP synthease activity was greatly enhanced, stabilized to heat treatment at 65°C for 30 min, and protected from inhibition by *p*-chloromercuribenzoic acid. No other prenyltransferase synthesizing C_{20} or shorter prenyl diphosphate was observed in *M. thermoformicicum* SF-4. These suggest that GGPP synthase alone is important in the biosynthetic pathways to squalene and membrane polar lipids at a chain elongation stage in this strain.

Membrane lipids of *Archaea*, which forms one of the three domains of living organisms,[1] are alkyl ether glycerolipids, in contrast to the fatty acid ester glycerolipids found in *Bacteria* (eubacteria) and *Eucarya* (eukaryotes).[2,3] The most common structures of alkyl ether glycerolipids are 2,3-di-*O*-phytanyl-*sn*-glycerol (diether lipid) and 2,3-di-*O*-biphytanyl-*sn*-diglycerol (tetraether lipid).[2] In a methanogen, the ether linkages of diether lipids have been reported to be formed from geranylgeranyl diphosphate (GGPP) and glycerol-1-phosphate by two prenyl transfer reactions.[4] Most of *Archaea* including methanogens also have squalene and hydrosqualenes which are major components of isoprenoid neutral lipids and account for 5–10% of total lipids.[5] Squalene is known to be synthesized *via* farnesyl diphosphate (FPP).

A formate-utilizing thermophilic methanogen has been isolated and identified as *Methanobacterium thermoformicicum* SF-4.[6] Aspartate aminotransferase of the methanogen was characterized by some different substrate specificity and subunit composition from that of other organisms.[7] Also, F_{420}-reducing hydrogenase of the methanogen had two different binding sites for deazaflavin and methylviologen.[8] In this paper, we report the purification and characterization of a thermostable GGPP synthase [EC 2.5.1.29] from *M. thermoformicicum* SF-4.

Materials and Methods

Materials. [4-^{14}C]Isopentenyl diphosphate (IPP) (2.2 GBq/mmol) was purchased from Du Pont–New England Nuclear. Isopentenol, dimethylallyl alcohol, geraniol, all-*trans*-farnesol, and all-*trans*-geranylgeraniol were gifts from Kuraray Co., Ltd. (Tokyo, Japan) and their diphosphates were prepared by phosphorylation of the corresponding prenols by the procedure of Cornforth *et al.*[9] Phenyl-Sepharose CL-4B, Sephacryl S-300HR, PBE94, and activated CH-Sepharose 4B were obtained from Pharmacia LKB Biotechnology (Uppsala, Sweden). High performance thin layer chromatography (HPTLC) RP-8 plates were from Merck (Darmstadt, F.R.G.). Cellulose powder (100–200 mesh), ultrafiltrator UHP-62, molecular weight standards, and alkaline phosphatase were obtained from Toyo Roshi Co. (Tokyo, Japan), Advantec Toyo Co. (Tokyo, Japan), Boehringer Mannheim GmbH (Mannheim, F.R.G.), and Sigma Chemical Co. (St. Louis, U.S.A.), respectively. All other chemicals used in this experiment were of reagent grade.

Enzyme assay. The enzyme activity was measured by the incorporation of [^{14}C]IPP into acid-labile allylic diphosphate. The standard assay mixture contained 7.5 μmol of Tris–HCl buffer (pH 7.6), 0.3 μmol of $MgCl_2$, 80 μmol of KCl, 4 nmol of [^{14}C]IPP (66,000 dpm), 10 nmol of each of allylic diphosphate (dimethylallyl diphosphate (DMAPP)), geranyl diphosphate (GPP), and FPP), and a suitable amount of enzyme in a final volume of 100 μl. After incubation at 60°C for 5 min, the reaction mixture was mixed with 200 μl of conc. HCl–methanol mixture (1 : 4). The mixture was kept at 60°C for 5 min to complete the hydrolysis of enzyme reaction products. The hydrolysates were extracted with 800 μl of ligroin, and a 640-μl portion of the extract was counted for radioactivity with a scintillation counter (Tri-Carb 460, Packard). Protein was measured by Bradford's method[10] using bovine serum albumin as a standard.

Product analysis. For identification of the reaction products, the prenols obtained by alkaline phosphatase treatment[4] of products were chromatographed on reversed phase TLC (HPTLC RP-8) with the solvent system of methanol–water (9 : 1). The positions of authentic standards were made visible with iodine vapor. Iodine-positive spots on TLC plate were scraped up and counted for radioactivity with a liquid scintillation counter.

Coupling procedure. Coupling for affinity chromatography was done by the method of Bartlett *et al.*[11] with a slight modification as described below. 3-Aminopropyl phosphonate (100 mg) was dissolved in 2 ml of water at 9°C, and to the solution was added 100 μl of ethyl trifluorothiol acetate dissolved in 500 μl of acetone. The reaction mixture was kept below 4°C in an ice bath and adjusted periodically to pH 9.5 with 5 N LiOH. After 20–30 min, the mixture was adjusted to pH 5 with CF_3COOH, concentrated from water 4 or 5 times to remove residual reagents, and then dried under reduced pressure over KOH for 24 h. The product, *N*-trifluoroacetyl 3-aminopropyl phosphonate dissolved in 2 ml of anhydrous dimethylformamide, was treated with 60 mg of 1,1′-carbonyldiimidazole. The reaction mixture was kept at 20°C for 6 h under anhydrous conditions, and 0.7 mmol farnesyl monophosphate monotributylammonium salt in 2 ml of anhydrous dimethylformamide was added. The mixture was stored in a desiccator over $CaSO_4$ at 20°C for

Abbreviations: GGPP, geranylgeranyl diphosphate; FPP, farnesyl diphosphate; GPP, geranyl diphosphate; DMAPP, dimethylallyl diphosphate; IPP, isopentenyl diphosphate; HPTLC, high performance thin layer chromatography; PCMB, *p*-chloromercuribenzoic acid.

24 h. The desired product, *P*-(3-*N*-trifluoroacetyl-amino-1-propyl)-*O*-farnesyl phosphonophosphate, was purified by silica gel flash chromatography and hydrolyzed by stirring with 0.1 N KOH at 20°C for 5 h. Normal phase silica gel TLC analysis of the hydrolysates with the solvent system of 40% aqueous CH_3CN containing 1% of 28% ammonia water showed only one compound, *P*-(3-amino-1-propyl)-*O*-farnesyl phosphonophosphate (R_f 0.62) which was ninhydrin- and molybdate reagent (Dittmer reagent[12])-positive. The compound was suspended in ethylene glycol–0.1 M $NaHCO_3$ (1 : 1) and coupled with activated CH-Sepharose 4B (Fig. 1).

Purification of GGPP synthase. Methanobacterium thermoformicicum SF-4 (DSM 6457) was grown and cell-free extracts were prepared by sonication as described previously.[7] The extracts were put on a PBE94 column (3.2 × 17 cm) equilibrated with 50 mM Tris–HCl buffer, pH 7.6, (Tris buffer) containing 0.3 M KCl. The column was washed with the same buffer, and eluted with Tris buffer containing 0.7 M KCl. Active fractions were combined and concentrated with an ultrafiltrator UHP-62 fitted with a UP-20 membrane (cut-off, 20,000). To the concentrated solution was added $(NH_4)_2SO_4$ up to 1.0 M, and the solution was centrifuged to remove insoluble materials. The supernatant was absorbed onto a phenyl-Sepharose column (1.5 × 30 cm) equilibrated with Tris buffer containing 1.0 M $(NH_4)_2SO_4$. Elution was done with a linear gradient of 1.0–0 M $(NH_4)_2SO_4$ in Tris buffer. Active fractions, which were eluted with Tris buffer containing about 0.5 M $(NH_4)_2SO_4$, were combined and $(NH_4)_2SO_4$ was added to the combined solution up to 2.0 M. The solution was put on a cellulose column (3.2 × 12.5 cm). The column was fully washed with Tris buffer containing 2.0 M $(NH_4)_2SO_4$ and then active fractions were eluted with about 1.6 M $(NH_4)_2SO_4$ during a linear gradient of 2.0–1.5 M $(NH_4)_2SO_4$ in Tris buffer. The fractions were combined and concentrated by ultrafiltration as described above. The concentrated solution was put on a column of Sephacryl S-300HR (2 × 100 cm) equilibrated with Tris buffer containing 0.15 M KCl. GGPP synthase activity was eluted at around the 78 kDa position which was marked with molecular mass markers. The active fractions were concentrated, dialyzed against 10 mM Tris–HCl buffer (pH 7.6) containing 1 mM $MgCl_2$ (Tris–Mg buffer), and slowly put onto an FPP analog-CH-Sepharose 4B affinity column (0.6 × 5.0 cm) at a rate of 5 ml/h. The column was washed with Tris–Mg buffer containing 3 mM KCl and GGPP synthase was eluted with Tris–Mg buffer containing 5 mM KCl.

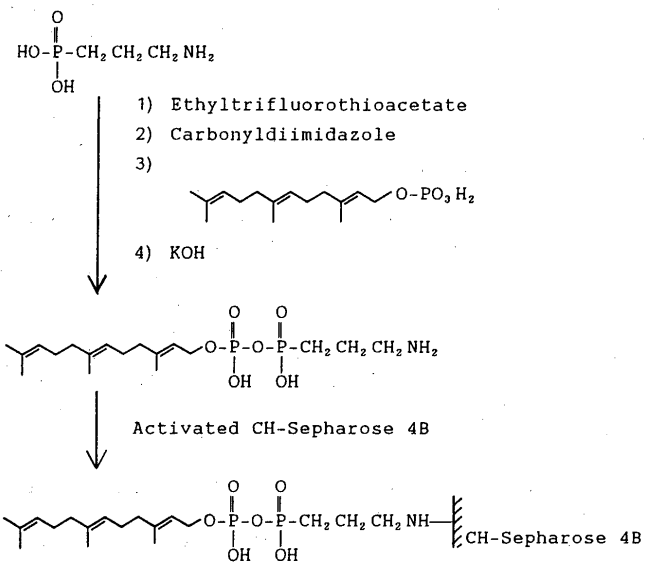

Fig. 1. Coupling Procedure and Structure of FPP Analog-Coupled CH-Sepharose 4B.

Detailed Procedure is described in Materials and Methods.

Results

Purification and general properties of GGPP synthase

GGPP synthase was purified from the methanogen *Methanobacterium thermoformicicum* SF-4. The purification process is summarized in Table I. The enzyme was purified over 158-fold to apparent homogeneity on nondenatured gradient-PAGE, and GGPP synthase activity was recovered in gel slices corresponding to the protein band (Fig. 2). No other prenyltransferase synthesizing C_{10}, C_{15}, and/or C_{20} using IPP and allylic partners such as DMAPP, GPP, and FPP, *i.e.*, GPP synthase, FPP synthase, and other GGPP synthases, was found during the purification procedures. The specific activities of the purified enzyme were 140 and 289 nmol min^{-1} mg^{-1} when 40 μM IPP and either 100 μM FPP or 100 μM GPP were used as substrates, respectively.

On nondenatured gradient-PAGE, the molecular weight

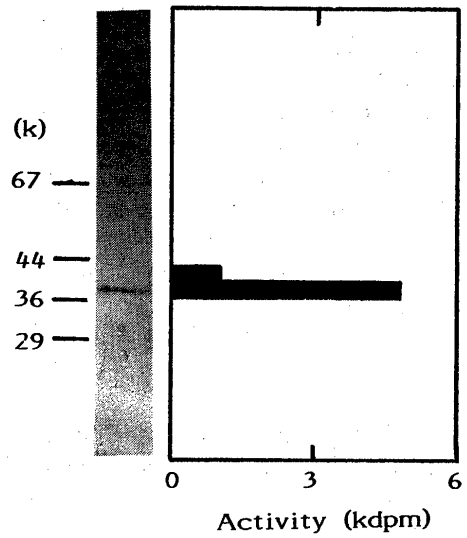

Fig. 2. Nondenatured Gradient-PAGE of Purified GGPP Synthase from *M. thermoformicicum* SF-4.

Closed bars indicate GGPP activity detected in gel slices of nondenatured gradient-gel.

Table I. Purification of GGPP Synthase from *M. thermoformicicum* SF-4

Step	Protein (mg)	Specific activity[a] (nmol min^{-1} mg^{-1})		Purification[b] (-fold)	Yield[b] (%)	Activity ratio G/F[c]
		IPP + GPP	IPP + FPP			
Crude extracts	2185	2.31	0.888	1	100	2.6
PBE94	1224	2.49	0.999	1.1	63.0	2.5
Phenyl-Sepharose CL-4B	133	25.8	9.50	10.7	65.2	2.7
Cellulose	21.6	67.1	24.1	27.1	26.8	2.8
Sephacryl S-300HR	9.07	121	41.9	47.2	19.6	2.9
Affinity chromatography	3.74	289	140.1	158	10.1	2.1

[a] IPP + GPP, 40 μM IPP and 100 μM GPP were used as substrates. IPP + FPP, 40 μM IPP and 100 μM FPP were used as substrates.
[b] Based on assay using 40 μM IPP and 100 μM FPP as substrates.
[c] Ratio of IPP + GPP activity to IPP + FPP activity.

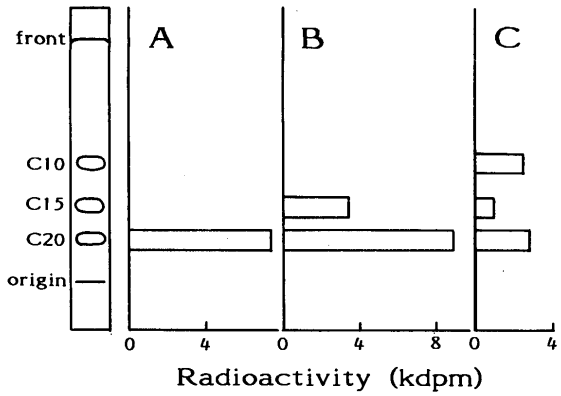

Fig. 3. Product Analysis of GGPP Synthase Purified from *M. thermoformicicum* SF-4.

After a 5-min enzyme reaction using 200 μM IPP and either (A) 40 μM FPP, (B) 40 μM GPP, or (C) 40 μM DMAPP as substrates, respectively, prenols were obtained by alkaline phosphatase treatment. When IPP at 100 μM was used, similar results were obtained.

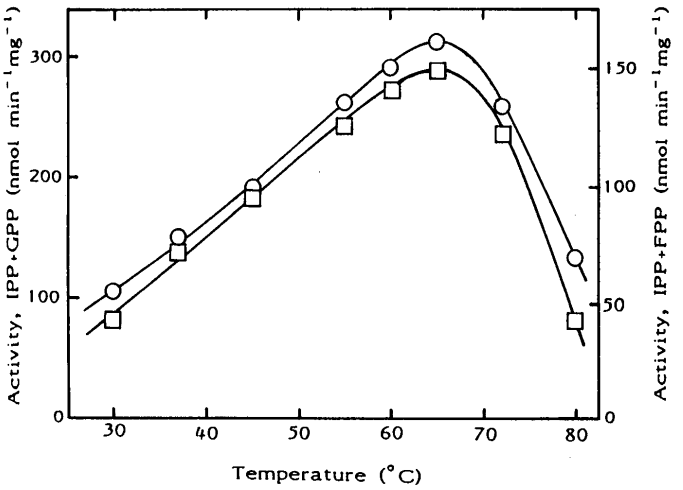

Fig. 4. Temperature Profile of GGPP Synthase of *M. thermoformicicum* SF-4.

Enzyme reactions were done for 5 min at various temperature using 40 μM IPP and either 100 μM FPP (—□—) or 100 μM GPP (—○—) as substrates, respectively.

of GGPP synthase was estimated to be 39,000. However, the molecular weight was calculated to be 78,000 by gel filtration on Sephacryl S-300HR. Analysis by SDS–PAGE gave the same value (39,000) as in the case of nondenatured gradient-PAGE, suggesting that GGPP synthase of this methanogen has a dimeric structure with a tendency to dissociate to monomers under these electrophoretic conditions.

The GGPP synthase catalyzed the synthesis of GGPP using IPP and either DMAPP, GPP, or FPP. Apparent K_m values for DMAPP, GPP, and FPP in the reaction using 40 μM IPP were 16.8, 12.6, and 14.7 μM, respectively, found by Lineweaver–Burk plots. In the reaction using FPP at 40 μM, the apparent K_m value for IPP was estimated to be 30.8 μM. When IPP and either DMAPP or GPP were used as substrates, intermediates, C_{10} and C_{15}, and C_{15}, were produced, respectively (Fig. 3). Substrate inhibition was observed at high concentrations of IPP (over 200 μM) and allylic diphosphates, over 400 μM DMAPP, 400 μM GPP, and 200 μM FPP (data not shown).

The optimum pH was 8.2 as assayed with Tris–HCl buffer. A linear relation between product formation and incubation time was maintained for at least 15 min.

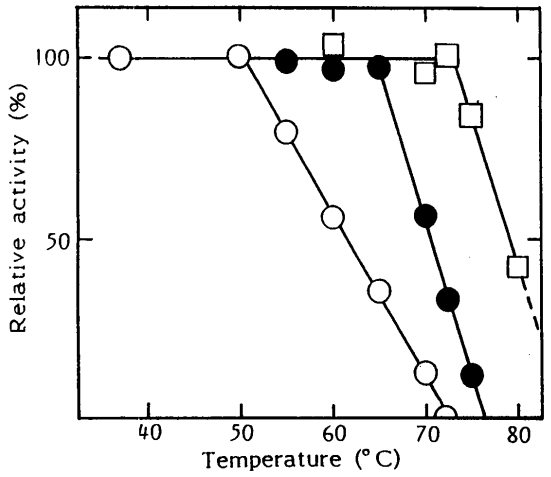

Fig. 5 Effects of KCl on the Thermal Stability of GGPP Synthase of *M. thermoformicicum* SF-4.

After heat treatment with no addition of KCl (—○—), with 0.8 M KCl (—●—), or with 3.5 M KCl (—□—) at various temperature for 30 min, enzyme activity was measured using 40 μM IPP and 100 μM FPP as substrates in the standard assay mixture. When 40 μM IPP and 100 μM GPP were used, the same result was obtained. Note that the standard assay mixture contained at a final concentration of 0.8 M KCl.

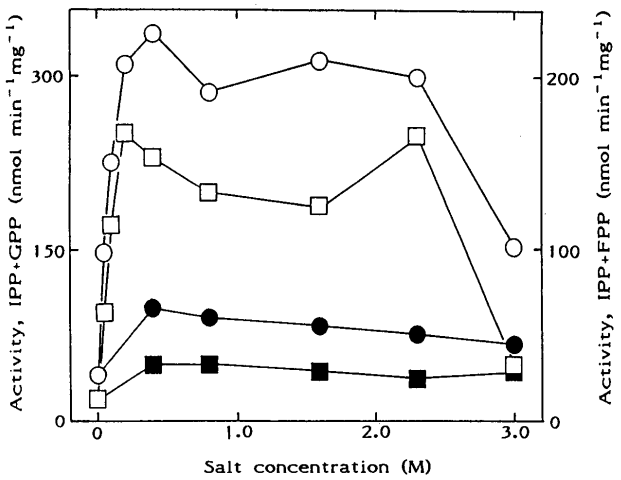

Fig. 6. Effects of KCl and NaCl on the Activity of GGPP Synthase of *M. thermoformicicum* SF-4.

Activity was measured for 5 min at 60°C using 40 μM IPP and 100 μM GPP with KCl (—○—) or NaCl (—●—), or using 40 μM IPP and 100 μM FPP with KCl (—□—) or NaCl (—■—) at various salt concentration as indicated.

Temperature profile and effects of KCl on thermal stability and activity

M. thermoformicicum SF-4 is moderately thermophilic (optimum temperature for growth: 60°C[6]) and the enzymes of this organism are expected to be thermophilic and thermostable up to 60°C or more. The highest activity of GGPP synthase was observed at 65°C (Fig. 4). Over 80% of the maximum activity was still observed at 72°C.

Because the intracellular K^+ content of the genus *Methanobacterium* has been reported to be 0.4–0.8 M,[13] the addition of 0.8 M KCl to the enzyme solution was attempted in the case of heat treatment for 30 min. The enzyme activity was completely stable up to 65°C (Fig. 5). However, the heat treatment at 60°C without KCl reduced the enzyme activity to about a half, suggesting the necessity of KCl for thermostability. Furthermore, in the presence of 3.5 M KCl, the enzyme activity was stable up to around 72°C.

An addition effect of KCl was observed in the assay mixture as well as in the enzyme solution. The enzyme

Table II. Effects of Various Substances on the Activity of GGPP Synthase of *M. thermoformicicum* SF-4

Incubation[a] with	Concentration (mM)	Activity[b] (%)
None	—	100
Pyrophosphate	1	92.6
	10	3.3
GGPP	1	23.7
PCMB	1	10.2
PCMB+KCl	1+800	72.0
PCMB+NaCl	1+800	56.6
PCMB+β-Mercaptoethanol	1+5	100.4
β-Mercaptoethanol	5	107.5
Spermidine	1	102.3
Putrescine	1	100.2
Choline	1	98.7
SDS	1%	3.3
Triton X-100	1%	108.9

[a] Incubation of enzyme with various substances was done in 20 μl of 50 mM Tris buffer (pH 7.6) at 37°C for 1 h, and then 80 μl of reaction mixture was added. Note that final concentration of these substances in enzyme reaction mixture was a fifth of the value indicated.
[b] Activity was measured using 40 μM IPP and 100 μM GPP as substrates.

Table III. Enzyme Reaction Products in Various Assay Mixtures

Assay mixture	Radioactivity (dpm)		Ratio F/GG[g]
	Farnesol[f]	Geranylgeraniol[f]	
Control[e]	6591	5574	1.18
Substrate inhibition[b]	5741	2112	2.72
Product inhibition[c]	5412	2440	2.22
Without KCl[d]	1141	549	2.08
0.8 M NaCl[e]	2680	1112	2.41

[a] The standard assay mixture contained 40 μM IPP and 100 μM GPP as substrates. Assay was done for 5 min at 60°C.
[b] GPP at 800 μM.
[c] GGPP at 200 μM.
[d] Removal of 0.8 M KCl from the standard assay mixture.
[e] Substitution of 0.8 M NaCl for 0.8 M KCl.
[f] Farnesol and geranylgeraniol obtained by alkaline phosphatase treatment were separated by reversed phase TLC as described in Materials and Methods.
[g] Ratio of the radioactivity of farnesol to that of geranylgeraniol.

activity was enhanced to 10 times by the addition of 0.2–2.3 M KCl (Fig. 6). On the other hand, the addition of NaCl enhanced the activity by only 2 times.

Effects of various substances on activity

After incubation of enzyme with various substances at 37°C for 1 h, the residual enzyme activities were measured (Table II). Pyrophosphate at 10 mM strongly inhibited the activity. In the presence of GGPP at the concentration of 1 mM, the enzyme activity was also reduced to be below 24% of full activity. Both GGPP and pyrophosphate were enzyme reaction products, indicating typical product inhibition. *p*-Chloromercuribenzoic acid (PCMB) was a potent inhibitor of the enzyme. Interestingly, when 0.8 M KCl was combined with 1 mM PCMB, the inhibitory effect of PCMB was suppressed. The enzyme activity remained up to 72%. In the presence of NaCl, the enzyme activity was also protected from the inhibition by PCMB to a lesser extent. The addition of polyamines (spermidine and putrescine) and choline was not effective. The enzyme activity was inhibited by SDS, but not by Triton X-100.

Enzyme reaction products in various assay mixtures

As shown in Table III, after the enzyme reaction using 40 μM IPP and 100 μM GPP as substrates, the ratio of the radioactivity of farnesol to that of geranylgeraniol was 1.18 (control). When to the assay mixture was added 800 μM GPP, which causes substrate inhibition or 200 μM GGPP, which causes product inhibition, the radioactivity of farnesol after the enzyme reaction was above 80% in comparison with control, but that of geranylgeraniol was further reduced to about 40%. Consequently, the ratio of the radioactivity of farnesol to geranylgeraniol increased to more than two under both conditions of substrate inhibition and product inhibition. Without 0.8 M KCl and with 0.8 M NaCl substituted for 0.8 M KCl, the ratio also increased to 2.08 and 2.41, respectively. These results indicate that much FPP was accumulated during a sequential elongation, $C_{10} \rightarrow C_{15} \rightarrow C_{20}$, under the above conditions.

Discussion

Archaeal isoprenoid biosynthesis is very active, because membrane polar and neutral lipids consist of isoprenoid hydrocarbons. GGPP and FPP are direct precursors for the biosynthesis of the diether polar lipids of methanogens[4] and the neutral lipid squalene,[5] respectively. Farnesylated protein has been found in a strain of methanogen.[14] As shown in Table I, a very high activity of prenyltransferase (geranyl-transferring activity: 2.3 nmol min^{-1} mg^{-1}) was observed in the crude extracts of *M. thermoformicicum* SF-4 as well as animal liver[15] and yeast.[16]

Purification of prenyltransferase from *M. thermoformicicum* showed that only one enzyme, GGPP synthase, is present. This enzyme seems not to be a membrane-associated protein because of weak binding to a hydrophobic interaction column, as compared to tight binding of F_{420}-reducing hydrogenase, which is a membrane-bound protein requiring Tris buffer containing organic solvent such as 25% dimethyl sulfoxide for elution from the column.[8] The GGPP synthase of *M. thermoformicicum* SF-4 is a dimer with two identical subunits, M_r 39,000, and that is similar to FPP synthases from animal liver[17,18] and yeast.[16] GGPP synthases purified from plant[19] and fungus[20] are M_r 74,000 (37,000 per subunit) and 60,000 (30,000 per subunit), respectively. *Neurospora* GGPP synthase[21] is slightly larger (47,876 per subunit) than methanogen's. This is the first report of the purification of a GGPP synthase from the methanogen to electrophoretic homogeneity, and with a possible subunit structure.

A fairly high intracellular K^+ concentration (0.4–0.8 M) has been found in *Methanobacterium*[13] and *Methanothermus*.[22] Some enzyme activities of *Methanothermus* and the extreme halophile *Halobacterium* are stimulated and/or thermostabilized by salt.[22,23] The GGPP synthase activity of *M. thermoformicicum* SF-4 was also greatly stimulated and thermostabilized in the presence of KCl. In addition, KCl protected against the inhibition by a sulfhydryl-binding agent, PCMB. These indicate that the addition of KCl may cause some conformational changes

which are responsible for the increasing activity and thermostability. At least, the intracellular K^+ concentration in *M. thermoformicicum* SF-4 seems to be needed for some thermophilic properties (optimum temperature for growth: 60°C).

The GGPP synthase of *M. thermoformicicum* SF-4 accumulated intermediates, GPP and FPP, besides the final product GGPP during a sequential elongation, $C_5 \rightarrow C_{10} \rightarrow C_{15} \rightarrow C_{20}$, in contrast to GGPP synthase from *Capsicum* and FPP synthases from various sources, which accumulated their respective final products alone. Only GGPP synthase of *Bacillus subtilis* has been reported to accumulate FPP besides GGPP.[24] The accumulation of intermediates by GGPP synthase of *M. thermoformicicum* SF-4 may be due to a low affinity for GPP and FPP. The K_m values for both substrates were ten times or more the values of GGPP synthase from a plant[19] or FPP synthases from animals[17] and yeast.[16] Usually, animals, plants, and eubacteria have two kinds of specific prenyltransferases synthesizing FPP and GGPP as final products,[25,26] except for *Micrococcus luteus* having GGPP synthase, but not FPP synthase.[27] *M. thermoformicicum* SF-4 has only one prenyltransferase. This strain as well as other methanogens produces polar lipids, squalene, and hydrosqualenes. Therefore, GGPP and FPP are needed as precusors for those. That is, the GGPP synthase from *M. thermoformicicum* SF-4 is an intermediate-accumulating enzyme which may be important in the regulation of the biosynthesis of polar lipids and squalene in this methanogen.

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References

1) C. R. Woese, O. Kandler, and M. L. Wheelis, *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 4576–4579 (1990).
2) M. De Rosa, A. Gambacorta, and A. Gliozzi, *Microbiol. Rev.*, **50**, 70–80 (1986).
3) T. G. Tornabene, R. S. Wolfe, W. E. Balch, G. Holzer, G. E. Fox, and J. Oro, *J. Mol. Evol.*, **11**, 259–266 (1978).
4) D.-L. Zhang, L. Daniels, and C. D. Poulter, *J. Am. Chem. Soc.*, **112**, 1264–1265 (1990).
5) T. G. Tornabene, T. A. Langworthy, G. Holzer, and J. Oro, *J. Mol. Evol.*, **13**, 73–83 (1979).
6) K. Yamamoto, A. Tachibana, G. Dhavises, T. Tanaka, M. Taniguchi, and S. Oi, *Agric. Biol. Chem.*, **53**, 533–534 (1989).
7) T. Tanaka, T. Tokuda, A. Tachibana, M. Taniguchi, and S. Oi, *Agric. Biol. Chem.*, **54**, 625–631 (1990).
8) A. Tachibana, T. Tanaka, M. Taniguchi, and S. Oi, *Biosci. Biotech. Biochem.*, **57**, 156–157 (1993).
9) R. H. Cornforth and G. Popják, *Methods Enzymol.*, **15**, 359–390 (1968).
10) M. M. Bradford, *Anal. Biochem.*, **74**, 248–254 (1976).
11) D. L. Bartlett, C. H. R. King, and C. D. Poulter, *Methods Enzymol.*, **110**, 171–184 (1985).
12) J. C. Dittmer and R. L. Lester, *J. Lipid Res.*, **5**, 126–127 (1964).
13) G. D. Sprott and K. F. Jarrell, *Can. J. Microbiol.*, **27**, 444–451 (1981).
14) W. W. Epstein, D. Lever, L. M. Leining, E. Bruenger, and H. C. Rilling, *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 9668–9670 (1991).
15) B. C. Reed and H. C. Rilling, *Biochemistry*, **14**, 50–54 (1975).
16) M. S. Anderson, J. G. Yarger, C. L. Burck, and C. D. Poulter, *J. Biol. Chem.*, **264**, 19176–19184 (1989).
17) G. F. Barnard and G. Popják, *Biochim. Biophys. Acta*, **661**, 87–99 (1981).
18) N. L. Eberhardt and H. C. Rilling, *J. Biol. Chem.*, **250**, 863–866 (1975).
19) O. Dogbo and B. Camara, *Biochim. Biophys. Acta*, **920**, 140–148 (1987).
20) F. L. Brinkhaus and H. C. Rilling, *Arch. Biochem. Biophys.*, **266**, 607–612 (1988).
21) A. Carattoli, N. Romano, P. Ballario, G. Morelli, and G. Macino, *J. Biol. Chem.*, **266**, 5854–5859 (1991).
22) R. Hensel and H. König, *FEMS Microbiol. Lett.*, **49**, 75–79 (1988).
23) D. J. Kushner, in "Archaebacteria", ed. by C. R. Woese and R. S. Wolfe, Academic Press, Orlando, Florida, 1985, pp. 171–214.
24) I. Takahashi and K. Ogura, *J. Biochem.*, **92**, 1527–1537 (1982).
25) K. Ogura, T. Shinka, and S. Seto, *J. Biochem.*, **72**, 1101–1108 (1972).
26) T. Korenaga, H. Sagami, and K. Ogura, Abstracts of Papers, the Annual Meeting of the Japanese Biochemical Society, Fukuoka, October, 1992, p. 812.
27) H. Sagami and K. Ogura, *J. Biochem.*, **89**, 1573–1580 (1981).