Vol. 116, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 31, 1983 Pages 500-506

DECAPRENYL PYROPHOSPHATE SYNTHETASE FROM MITOCHONDRIA OF PIG LIVER

Koichi Ishii, Hiroshi Sagami, and Kyozo Ogura

Chemical Research Institute of Non-Aqueous Solutions,
Tohoku University, Katahira, Sendai 980, Japan

Received September 13, 1983

SUMMARY: Decaprenyl pyrophosphate synthetase which catalyzes the synthesis of all-trans-decaprenyl pyrophosphate from isopentenyl pyrophosphate and either farnesyl pyrophosphate or geranylgeranyl pyrophosphate has been partially purified from mitochondria of pig liver. This enzyme lacks dimethylallyl-transferring and geranyl-transferring activities.

Prenyltransferases which catalyze the fundamental chain elongation steps of isoprenoid biosynthesis in animal cells produce precursors such as farnesyl-PP (PP stands for pyrophosphate) for steroid biosynthesis, all-trans-polyprenyl-PP for ubiquinone biosynthesis, and cis,trans-mixed polyprenyl-PP leading to dolichyl phosphate which is a sugar carrier lipid in glycoprotein biosynthesis. From the cytosol of animal cells two kinds of prenyltransferase have so far been purified; they are farnesyl-PP synthetase (1-3) and geranylgeranyl-PP synthetase (4). Wong and Lennarz have shown that dolichol is synthesized from farnesyl-PP by successive addition of isopentenyl-PP in microsomes of rat liver (5). Using pig liver preparations, we have revealed that farnesyl-PP synthetase is located in the microsomes as well as in the cytosol fraction (6). Momose and Rudney have shown that mevalonate and isopentenyl-PP are incorporated into the side chain of ubiquinone in mitochondria of rat liver and guinea pig liver (7). Daleo et al., have also reported that mitochondria of rabbit and chicken liver are capable of converting mevalonate and isopentenyl-PP into dolichyl phosphate (8). These observations suggest that the mitochondrion has its own prenyltransferase system independent of the cytosol and microsomal prenyltransferases. However, no report has appeared on the separation of mitochondrial prenyltransferase. This paper reports the

0006-291X/83 $1.50
Copyright © 1983 by Academic Press, Inc.
All rights of reproduction in any form reserved.

separation and partial purification from pig liver mitochondria of three prenyltransferases including a new enzyme which catalyzes the synthesis of all-trans-decaprenyl-PP.

MATERIALS AND METHODS

Materials: Fresh pig liver was purchased from a meat market. [1-^{14}C]-Isopentenyl-PP (specific activity, 53 Ci/mol) was a product of Amersham, Non-labeled allylic pyrophosphates were prepared as described previously (9).

Enzyme assay: The enzyme activity was measured as described previously (10) by determination of the amount of [1-^{14}C]isopentenyl-PP incorporated into acid labile materials. In a standard experiment, the incubation mixture contained, in a final volume of 1.0 ml, 50 mM potassium phosphate buffer (pH 6.7), 10 mM iodoacetamide, 6 mM potassium fluoride, 50 μM allylic pyrophosphate, 25 μM [1-^{14}C]isopentenyl-PP (1 Ci/mol) or 10 μM [1-^{14}C]isopentenyl-PP (53 Ci/mol), and a suitable amount of enzyme protein. After the mixture was kept at 37°C for 3 h, 0.2 ml of 6 M HCl was added to the reaction mixture to terminate the enzymatic reaction. To complete the hydrolysis of the products the mixture was kept at 65°C for 30 min. and the hydrolysates were extracted with hexane after addition of 0.4 ml of 6 M NaOH. The hexane layer was washed with water and an aliquot of the extract was counted for radioactivity. The enzyme activity was expressed as the radioactivity in the hexane extract.

Product analysis: Polyprenyl products were extracted with 1-butanol from the reaction mixture and chromatographed on a silica gel plate with 1-propanol/ammonia/water (6:3:1, v/v). Enzymatic hydrolysis of the products with potato acid phosphatase was carried out by the method of Fujii et al. (11). The free polyprenols were analyzed by reversed-phase thin-layer chromatography (TLC) and radio high pressure liquid chromatography (HPLC). The reversed-phase TLC was carried out on a HPTLC plate (RP-18, Merck) with acetone/water (19:1, v/v) and the radio HPLC was performed with a 50 cm column of Hitachi porous gel 3011 as described in a previous paper (12), except for the flow rate, 0.6 ml/min.

Preparation of polyprenyltransferase: Fresh pig liver (1 kg) was cut into small pieces and homogenized with a Potter Elvehjem homogenizer in 10 mM Tris-HCl buffer (pH 7.3) containing 0.28 M sucrose and 0.1 mM EDTA. The homogenate was centrifuged according to the procedure of Parsons and Williams (13), as modified by Nishino and Rudney (14). The mitochondrial pellet was resuspended in 50 mM potassium phosphate buffer (pH 7.3) containing 0.02 % bovine serum albumin. Mitochondrial enzymes were solubilized by freezing and thawing as follows. The mitochondrial suspension was frozen at -80°C for one hour and then thawed at 37°C for 5 min. After the treatment was repeated twice, the mixture was centrifuged at 37,000 g for 30 min. The protein fraction precipitating between 0 and 50 % saturation of ammonium sulfate from the supernatant was chromatographed on a Sephadex G-100 column (3.2 x 90 cm) equilibrated with 50 mM potassium phosphate buffer (pH 7.0) containing 50 mM KCl. The fractions having prenyltransferase activity were combined and further chromatographed on a hydroxylapatite column (1.6 x 22 cm) or on a DEAE-cellulose column (1.6 x 32 cm) equilibrated with 10 mM potassium phosphate buffer (pH 7.0) containing 1 mM $MgCl_2$ and 1 mM β-mercaptoethanol. The hydroxylapatite column was developed with a linear gradient of phosphate buffer from 10 mM to 300 mM, and finally with 400 mM phosphate buffer (pH 7.0). The elution from the DEAE-cellulose was performed with a linear gradient of 0-0.35 M KCl in phosphate buffer (pH 7.0).

RESULTS

When a 0-50 % ammonium sulfate fraction from 37,000 g supernatant obtained from the pig liver mitochondrial fraction after freezing and thawing was filtered through Sephadex G-100, two peaks for enzyme activity were observed (Figure 1). The peak emerging later corresponded to isopentenyl-PP isomerase. Analysis of the products of the incubation with [1-^{14}C]isopentenyl-PP (1 Ci/mol) and dimethylally-PP showed that the peak emerging earlier corresponded to farnesyl-PP synthetase. However, the same fraction was also active when assayed with a combination of [1-^{14}C]-isopentenyl-PP of higher specific activity (53 Ci/mol) and farnesyl-PP, suggesting that the fraction contained another prenyltransferase which catalyzed the formation of polyprenyl pyrophosphate with a chain length longer than C_{15}. Therefore, this fraction was further chromatographed on

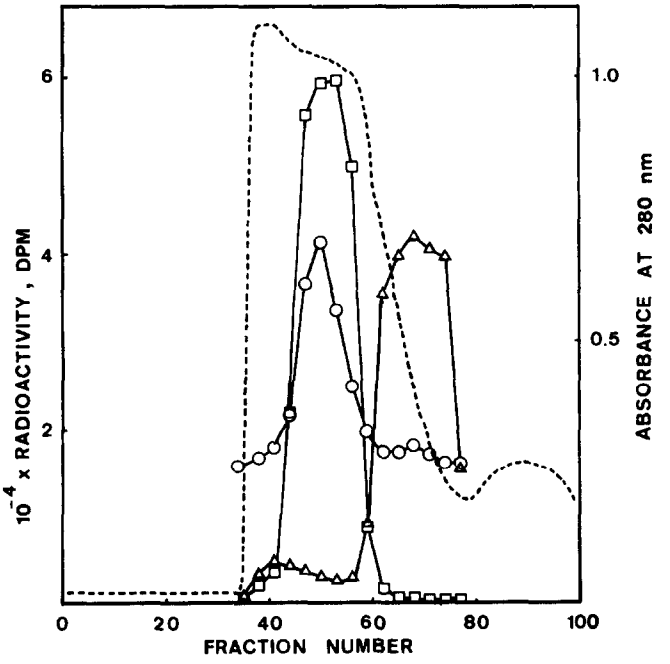

Figure 1. Chromatography of a 0-50 % ammonium sulfate fraction on Sephadex G-100.
Enzyme activity was assayed as described under "Materials and Methods". □, enzyme activity as assayed with [1-^{14}C]isopentenyl-PP (1 Ci/mol) and dimethylallyl-PP; ○, enzyme activity as assayed with [1-^{14}C]isopentenyl-PP (53 Ci/mol) and farnesyl-PP; △, enzyme activity as assayed with [1-^{14}C]isopentenyl-PP (1 Ci/mol) alone in the absence of iodoacetamide; ----, absorbance at 280 nm.

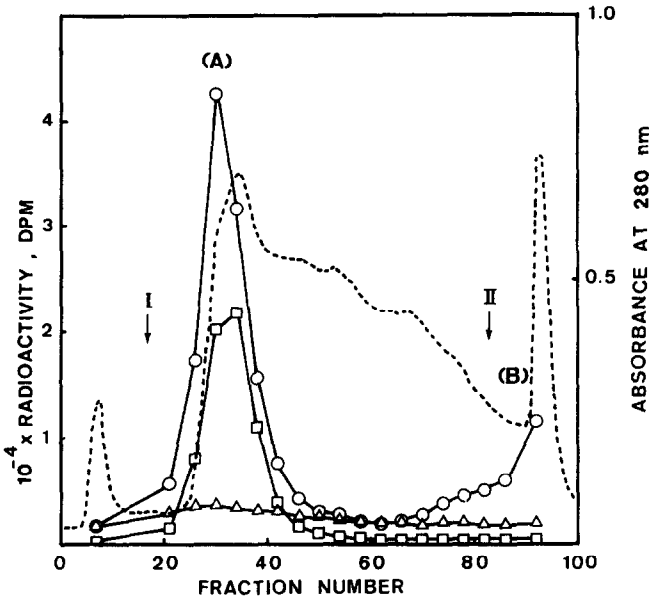

Figure 2. Hydroxyapatite chromatography of Sephadex G-100 purified enzyme.
□, enzyme activity as assayed with [1-^{14}C]isopentenyl-PP (1 Ci/mol) and dimethylallyl-PP; ○, enzyme activity assayed with [1-^{14}C]isopentenyl-PP (53 Ci/mol) amd farnesyl-PP; △, enzyme activity as assayed with [1-^{14}C]isopentenyl-PP (53 Ci/mol) alone. ----- , absorbance at 280 nm. Arrow I and II indicate the starting points of linear gradient and of 0.4 M phosphate buffer elution, respectively.

hydroxylapatite. When the eluted fractions were assayed with [1-^{14}C]-isopentenyl-PP (1 Ci/mol) and dimethylallyl-PP, a single peak for dimethylallyl-transferring activity was observed in fractions eluted by phosphate buffer of relatively low concentrations as shown by the curve with square symbols in Figure 2. The reaction product with these fractions was all-trans-farnesyl-PP. However, when the fractions were assayed with a combination of [1-^{14}C]isopentenyl-PP of higher specific activity and farnesyl-PP, two peaks for enzyme activity were observed; one is associated with the farnesyl-PP synthetase fraction (A) and the other with fractions (B) eluted more later. Figure 3-A shows the reversed-phase TLC of the hydrolysates obtained by acid phosphatase treatment of the product derived from [1-^{14}C]isopentenyl-PP and farnesyl-PP with fraction B. The major peak (R_f 0.14) and the minor peak (R_f 0.59) coincided with the spots of all-trans-decaprenol and all-trans-geranylgeraniol, respectively.

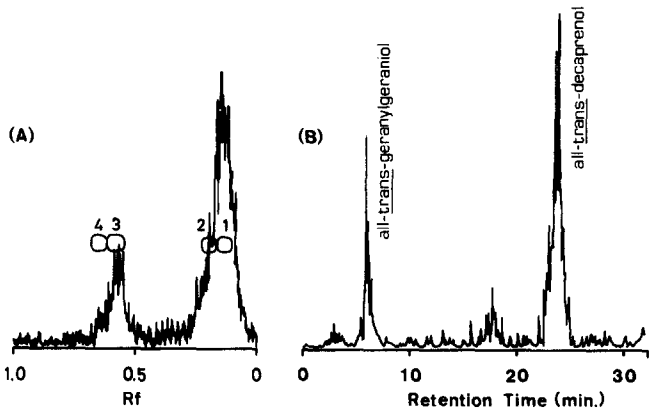

Figure 3. (A) Reversed-phase TLC of prenols liberated by the phosphatase treatment of the enzyme reaction products. The products were derived from [1-^{14}C]isopentenyl-PP (53 Ci/mol) and farnesyl-PP with fraction B of hydroxyapatite chromatography. The circles indicate the spots of reference alcohols: 1, all-trans-decaprenol; 2, all-trans-nonaprenol (solanesol); 3, all-trans-geranylgeraniol; 4, all-trans-farnesol. (B) Radio HPLC of the same prenols as described above. The elution was performed with methanol-hexane (4:1, v/v) as described under "Materials and Methods".

The same hydrolysates were also identified by radio HPLC with authentic specimens of these prenols. The reaction product of fraction B was extractable with 1-butanol and the major radioactive material migrated in silica TLC with R_f 0.37 which corresponded to that expected for decaprenyl-PP. These results indicated that fraction B contained decaprenyl-PP synthetase catalyzing the chain elongation up to C_{50} from C_{15} and geranylgeranyl-PP synthetase. As for the farnesyl-transferring activity observed when fraction A was assayed with isopentenyl-PP of higher specific activity (Figure 2), the product was identified with geranylgeranyl-PP. Probably the formation of geranylgeranyl-PP with this fraction is due to the action of farnesyl-PP synthetase rather than geranylgeranyl-PP synthetase, since crystalline preparation of farnesyl-PP synthetase is known to involve a weak activity of geranylgeranyl-PP synthesis (3).

Applying the Sephadex G-100 purified fraction to DEAE-cellulose chromatography, we also obtained a partially purified decaprenyl-PP synthetase fraction free of geranylgeranyl-PP synthetase but contaminated with farnesyl-PP synthetase. The product analysis indicated that the DEAE-cellulose purified enzyme catalyzed the synthesis of all-trans-

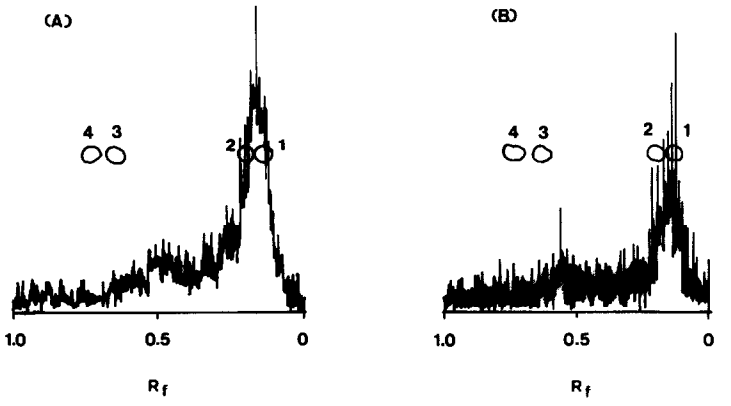

Figure 4. Reversed-phase TLC of prenols liberated by the phosphate treatment of the enzyme reaction products with DEAE-cellulose purified enzyme. The products were derived from [1-^{14}C]isopnentenyl-PP (53 Ci/mol) and farnesyl-PP (A), or geranylgeranyl-PP (B). The circles indicate the spots of reference alcohols: 1, all-trans-decaprenol; 2, solanesol; 3, all-trans-geranylgeraniol; 4, all-trans-farnesol.

decaprenyl-PP from isopentenyl-PP and either farnesyl-PP or geranylgeranyl-PP (Figure 4). The product derived from isopentenyl-PP and dimethylallyl-PP or geranyl-PP was farnesyl-PP only.

DISCUSSIONS

Three prenyltransferases, decaprenyl-PP synthetase, farnesyl-PP synthetase, geranylgeranyl-PP synthetase, were detected and partially purified from pig liver mitochondria. The decaprenyl-PP synthetase is a new enzyme which catalyzes the synthesis of all-trans-decaprenyl-PP (C_{50}) from farnesyl-PP (C_{15}) but not from dimethylallyl-PP (C_5) or geranyl-PP (C_{10}). The biological role of this enzyme must be to supply the side chain of ubiquinone-10 which is known to occur in pig liver. The mitochondrial farnesyl-PP synthetase principally catalyzed the $C_5 \rightarrow C_{10} \rightarrow C_{15}$ reactions and the geranylgeranyl-PP synthetase catalyzed at least the $C_{15} \rightarrow C_{20}$ reaction. These short chain prenyl pyrophosphate synthetases seem to be implicated in the biosynthetic pathway to ubiquinone to make a connection between isopentenyl-PP isomerase and decaprenyl-PP synthetase.

In bacteria, a set of two prenyltransferases is implicated in the chain elongation from C_5 to a certain all-trans-polyprenyl pyrophosphate. Geranyl-PP synthetase ($C_5 \rightarrow C_{10}$) and solanesyl pyrophosphate synthetase

($C_{10} \rightarrow C_{40}$, C_{45}) (15), geranylgeranyl-PP synthetase ($C_5 \rightarrow C_{20}$) and heptaprenyl pyrophosphate synthetase ($C_{20} \rightarrow C_{35}$) (16), and farnesyl-PP synthetase ($C_5 \rightarrow C_{15}$) and hexaprenyl pyrophosphate synthetase ($C_{15} \rightarrow C_{30}$) (12) have so far been purified from several bacteria. It is noteworthy that similar is the case of the chain elongation leading to the side chain of ubiquinone-10 in pig liver mitochondria. However, the activity of decaprenyl-PP synthetase is much lower as compared with that of the mitochondrial farnesyl-PP synthetase in contrast to that of bacterial polyprenyl-PP synthetases which are almost equally active to their short chain prenyl-PP synthetases. This may reflect the ability or inability of mammals or bacteria to synthesize steroids.

REFERENCES

1. Dorsey, J. K., Dorsey, J. A., and Porter, J. W. (1966) J. Biol. Chem. 241, 5353-5360
2. Holloway, P. W. and Popjak, G. (1967) Biochem. J. 104, 57-70
3. Reed, B. C. and Rilling, H. C. (1975) Biochemistry 14, 50-54
4. Sagami, H., Ishii, K., and Ogura, K. (1981) Biochem. Int. 3, 669-675
5. Wong, T. K. and Lennarz, W. J. (1982) J. Biol. Chem. 257, 6619-6624
6. Ishii, K., Sagami, H., and Ogura, K. (1983) J. Biochem. (Tokyo) 93, 1635-1639
7. Momose, K., and Rudney, H. (1972) J. Biol. Chem. 247, 3930-3940
8. Daleo, G. R., Hopp, H. E., Romero, P. A., and Pont Lezica, R. (1977) FEBS Lett, 81, 411-414
9. Shinka, T., Ogura, K., and Seto, S. (1977) J. Biochem. (Tokyo) 78, 1177-1181
10. Koyama, T., Saito, Y., Ogura, K., and Seto, S. (1977) J. Biochem. (Tokyo) 82, 1585-1590
11. Fujii, H., Koyama, T., and Ogura, K. (1982) Biochim. Biophys. Acta 712, 716-718
12. Fujii. H., Koyama, T., and Ogura, K. (1982) J. Biol. Chem. 257, 14610-14612
13. Parsons, D. F. and Williams, G. R. (1967) Methods Enzymol. 10, 443-448
14. Nishino, T. and Rudney, H. (1977) Biochemistry 16, 605-609
15. Sagami, H., Ogura, K., and Seto, S. (1977) Biochemistry 16, 4616-4622
16. Takahashi, I., Ogura, K., and Seto, S. (1980) J. Biol. Chem. 255, 4539-4543