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A NEW PRENYLTRANSFERASE FROM MICROCOCCUS LYSODEIKTICUS

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SUMMARY A new prenyltransferase which catalyzes the synthesis of geranyl pyrophosphate as the only product from dimethylallyl pyrophosphate and isopentenyl pyrophosphate has been separated from other known prenyltransferases from Micrococcus lysodeikticus. This enzyme fraction is also capable of synthesizing all-trans geranylgeranyl pyrophosphate from farnesyl pyrophosphate and isopentenyl pyrophosphate though it lacks ability to synthesize farnesyl pyrophosphate.

Prenyltransferases catalyzing the fundamental carbon chain elongation in isoprenoid biosynthesis have been purified from various organisms (1). They all catalyze the sequential condensation of isopentenyl pyrophosphate with allylic prenyl pyrophosphates to give the final product depending upon the specificity of the enzyme. Farnesyl pyrophosphate synthetase which is known to occur widely in eucaryotes catalyzes the condensation of isopentenyl pyrophosphate with either dimethylallyl pyrophosphate (C_5) or geranyl pyrophosphate (C_{10}) with equal facility (2-7). Geranylgeranyl pyrophosphate synthetase which has been partially purified from M. lysodeikticus or higher plants catalyzes all the three condensations of $C_5 \rightarrow C_{10} \rightarrow C_{15} \rightarrow C_{20}$ without accumulation of any intermediates (8-10). However, the simplest prenyltransferase that would catalyze a single reaction between dimethylallyl pyrophosphate and isopentenyl pyrophosphate to give geranyl pyrophosphate has not been found in any organisms.

A recent work from this laboratory has shown that solanesyl pyrophosphate synthetase from M. lysodeikticus catalyzes the formation of all-trans C_{40} and C_{45} prenyl pyrophosphate from geranyl and isopentenyl pyrophosphates

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and that this enzyme, unlike farnesyl pyrophosphate synthetase or geranyl-geranyl pyrophosphate synthetase, could not utilize dimethylallyl pyrophosphate as a starting substrate (11). This fact led us to expect that there might be involved as yet unknown enzyme responsible for the supply of geranyl pyrophosphate. We report here the isolation from this bacterium a new type of prenyltransferase which catalyzes discontinuous condensation of isopentenyl pyrophosphate with dimethylallyl pyrophosphate or with farnesyl pyrophosphate to afford geranyl pyrophosphate or geranylgeranyl pyrophosphate, respectively.

MATERIALS AND METHODS

Spray-dried cells of M. lysodeikticus were purchased from Worthington Biochemical Co. Essentially the same result was also obtained when freshly harvested cells were used. Hydroxylapatite was prepared according to the method of Bernardi (12). [^{14}C]isopentenyl pyrophosphate and non-labeled allylic pyrophosphates were the same preparations as used in the previous study (11).

Enzyme assay and product analysis: The enzyme activity was measured by determining the amount of incorporation of [^{14}C]isopentenyl pyrophosphate into acid-labile material as described previously (11). Unless otherwise stated, the incubation mixture contained, in a final volume of 1 ml, 75 μmoles of tris-(hydroxymethyl)aminomethane-HCl buffer (pH 7.4), 5 μmoles of $MgCl_2$, 20 μmoles of potassium fluoride, 15 μmoles of iodoacetamide, 50 nmoles of allylic pyrophosphate, 25 nmoles of [^{14}C]isopentenyl pyrophosphate (specific activity, 1.2 Ci per mole) and enzyme. After incubation at 37°C for 40 min., 0.2 ml of 6 N HCl was added, and the mixture was kept at 65°C for 20 min. to complete the hydrolysis of the products, and was extracted with hexane. The enzyme activity was expressed as the radioactivity in the hexane extract. Identification of the products was carried out on both pyrophosphate esters and their free prenols by co-chromatography with authentic samples. Silica gel thin layer chromatography was performed with propanol-ammonia-water (6:3:1, v/v) for the pyrophosphates and with hexane-ethyl acetate (4:1, v/v) for the prenols which were obtained as usual by alkaline phosphatase treatment. High speed liquid chromatography and gaschromatography were also used for the identification of geraniol and all-trans-geranylgeraniol.

Purification of enzyme: Crude extracts of M. lysodeikticus were prepared as described previously (11). The protein fraction precipitating between 30 and 50% saturation of ammonium sulfate was chromatographed on a DEAE Sephadex A-50 column (2.8 x 38 cm) equilibrated with 25 mM phosphate buffer (pH 6.8) containing 0.05 M NaCl. The elution was carried out with a linear gradient of NaCl from 0.05 to 0.85 M. The fractions corresponding to the first peak were pooled and further chromatographed on a hydroxylapatite column (1.2 x 20 cm) equilibrated with 1 mM phosphate buffer (pH 6.4). The protein was eluted with a linear gradient of 1 mM - 100 mM phosphate buffer (pH 6.4)

RESULTS

When a 30-50% ammonium sulfate fraction obtained from M. lysodeikticus was chromatographed on DEAE Sephadex, two peaks of prenyltransferase activity were observed, the second peak corresponding to solanesyl pyrophosphate

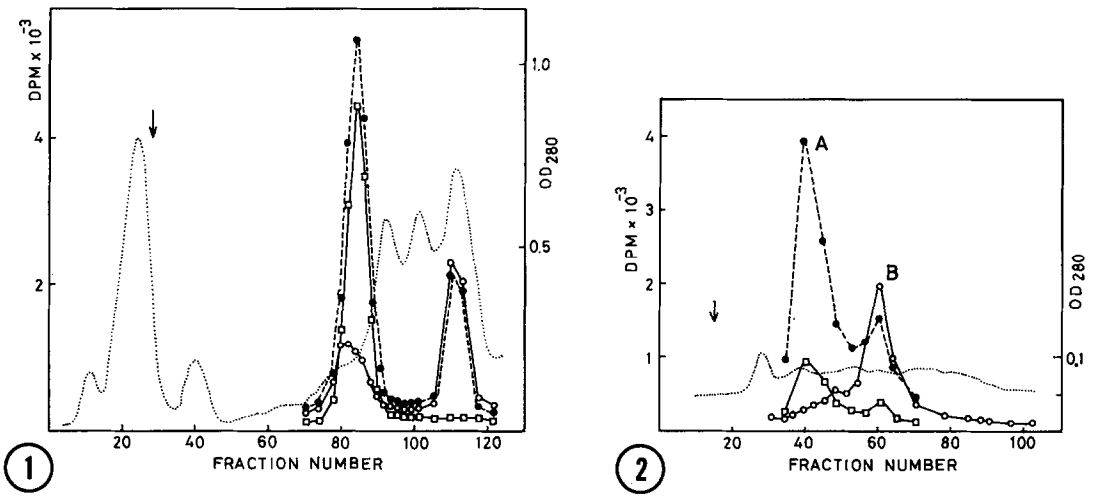

Figure 1. DEAE Sephadex A-50 chromatography. Fractions of 14 ml were collected and assayed for optical density at 280 nm (·····), and enzyme activity. The allylic substrates used were dimethylallyl (□—□), geranyl (○—○), and farnesyl pyrophosphate (●---●). The arrow indicates the starting point of linear gradient. Aliquots of 0.2 ml each were used for the enzyme assay.

Figure 2. Hydroxylapatite chromatography. Fractions of 6.0 ml were collected and assayed for optical density at 280 nm (·····), and enzyme activity with dimethylallyl (□—□), geranyl (○—○), and farnesyl pyrophosphate (●---●). The arrow indicates the starting point of linear gradient. Aliquots of 0.3 ml each were used for the enzyme assay.

synthetase activity reported previously (11)(Fig. 1). The first emerging fraction was similar to geranylgeranyl pyrophosphate synthetase reported first by Kandutsch et al. (8) in that geranylgeranyl pyrophosphate was formed when it was incubated with isopentenyl pyrophosphate and geranyl or farnesyl pyrophosphate but different in that both geranyl and geranylgeranyl pyrophosphates were formed when incubated with isopentenyl pyrophosphate and dimethylallyl pyrophosphate. Since this fact suggested that this fraction might contain geranyl pyrophosphate synthetase as well as geranylgeranyl pyrophosphate synthetase, it was further purified. As shown in Fig. 2, the hydroxylapatite chromatography resolved it into two fractions having prenyltransferase activity. One (fraction B) was active with all the three allylic substrates as expected for the known geranylgeranyl pyrophosphate synthetase, and the other (fraction A) had dimethylallyl-transferring activity and no geranyl-

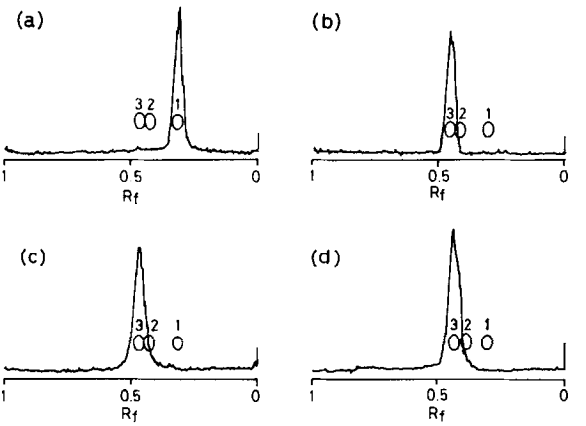

Figure 3. Thin layer radiochromatograms of prenols liberated by the phosphatase treatment of the products of enzymatic reaction. The products were derived from [^{14}C]isopentenyl and dimethylallyl pyrophosphates with fraction A (a), [^{14}C]isopentenyl and farnesyl pyrophosphates with fraction A (b), [^{14}C]isopentenyl and dimethylallyl pyrophosphates with fraction B (c), and [^{14}C]isopentenyl and geranyl pyrophosphates with fraction B (d). The incubation time was 3 hr. The circles indicate the spots of reference prenols: 1, geraniol; 2, all-trans-farnesol; 3, all-trans-geranylgeraniol.

transferring activity, but unexpectedly it had also farnesyl-transferring activity. Isopentenyl pyrophosphate isomerase, though not shown in Fig. 2, was eluted earlier than fraction A.

The product analysis clearly indicated that fraction B always synthesized geranylgeranyl pyrophosphate irrespective of the allylic substrate used. On the other hand, in the reaction with fraction A geranyl pyrophosphate was exclusively formed when dimethylallyl pyrophosphate was the substrate, but the product was geranylgeranyl pyrophosphate when the substrate was farnesyl pyrophosphate. Figure 3 shows typical results of the radio-thin layer chromatography. Analysis by high speed liquid chromatography and gaschromatography also revealed that geranylgeraniol obtained in either case was all-trans isomer. Although the reactivity of geranyl pyrophosphate as substrate for fraction A was extremely low, a large scale incubation made it possible to confirm that the product was also geranylgeranyl pyrophosphate.

Table I. Enzyme specificity for allylic substrates

Substrate	Enzyme Activity, dpm			
	Fraction A		Fraction B	
None	285		276	
Dimethylallyl-PP	4,022	(0.70)	1,248	(0.14)
Geranyl-PP	736	(0.04)	2,953	(0.59)
Farnesyl-PP	5,643	(1.00)	2,533	(1.00)

PP stands for pyrophosphate. The enzyme activity was measured under the same conditions as described in Materials and Methods section except that the concentration of iodoacetamide was 5 mM. The figures in parentheses indicate the relative velocities for $C_5 \rightarrow C_{10}$, $C_{10} \rightarrow C_{15}$, and $C_{15} \rightarrow C_{20}$ steps of reaction calculated based on that the specific activities of geranylgeranyl-PP formed from [^{14}C]isopentenyl-PP with dimethylallyl-PP, geranyl-PP, and farnesyl-PP relative to that of geranyl-PP formed from [^{14}C]isopentenyl-PP with dimethylallyl-PP should be 3, 2, and 1, respectively.

Table I shows the comparative effectiveness of the three allylic pyrophosphate substrates. The relative velocity for each step of reaction accounts for the fact that the reaction with fraction A stops at the C_{10} stage whereas there is no accumulation of intermediates in the reaction catalyzed by fraction B.

Although further purification of fraction A was attempted with an expectation that the dimethylallyl-transferring and farnesyl-transferring activities might be separated from each other, such separation has not been successful. These two activities were associated with the same fraction on Sephadex G-100 filtration. Isoelectric focusing chromatography and polyacrylamide gel electrophoresis resolved fraction A into several protein fractions, but the recovery of the enzyme activity was not satisfactory in either case.

No difference in stability was observed between these two activities, and the enzyme lost about 20% of the activity in terms of both dimethylallyl and farnesyl transfers when stored frozen at -20°C for 17 days.

DISCUSSION

The new prenyltransferase separated from the known geranylgeranyl pyrophosphate synthetase catalyzes the condensation of isopentenyl pyrophosphate and dimethylallyl pyrophosphate to give only geranyl pyrophosphate. In this

context, this enzyme should be called geranyl pyrophosphate synthetase, and probably the biological significance is to provide the starting substrate of solanesyl pyrophosphate synthetase. In other words, this enzyme is responsible for the initial step of chain elongation in menaquinone biosynthesis. However, the significance of the concomitant capability of this enzyme fraction to synthesize geranylgeranyl pyrophosphate from farnesyl pyrophosphate is difficult to understand. It remains uncertain whether these two catalytic activities are the properties of a single enzyme or a mixture of geranyl pyrophosphate synthetase and geranylgeranyl pyrophosphate synthetase the specificity of which differs from that of the known geranylgeranyl pyrophosphate synthetase. M. lysodeikticus, like other bacteria, produces C_{55} polyprenol having cis,trans-mixed stereochemistry in such a way that it could be derived from all-trans-farnesyl pyrophosphate or cis,trans,trans isomer of geranylgeranyl pyrophosphate (13-15). Consequently, if this enzyme synthesized this isomer of geranylgeranyl pyrophosphate, it would be responsible for the synthesis of a precursor of the C_{55} polyprenol. However, this is not the case since the enzymatic product was identified to be the all-trans isomer.

It is reasonable to assume that farnesyl pyrophosphate must be provided not only as the starting substrate for the chain elongation giving rise to the C_{55} polyprenol but also as the substrate for this enzyme. However, farnesyl pyrophosphate synthetase has not been found in the extract of this bacterium. The mechanism of supply of farnesyl pyrophosphate will be also a problem to be solved.

REFERENCES

1. Beytía, E. D. and Porter, J. W. (1976) Ann. Rev. Biochem., 45, 113-142.
2. Dorsey, J. K., Dorsey, J. W., and Porter, J. W. (1966) J. Biol. Chem., 241, 5353-5360.
3. Holloway, P. W. and Popják, G. (1967) Biochem. J., 104, 57-70.
4. Ogura, K., Nishino, T., and Seto, S. (1968) J. Biochem. (Tokyo), 64, 197-203.
5. Green, T. R. and West, C. A. (1974) Biochemistry, 13, 4720-4729.
6. Eberhardt, N. L. and Rilling, H. C. (1975) J. Biol. Chem., 250, 863-866.
7. Reed, B. C. and Rilling, H. C. (1975) Biochemistry, 14, 50-54.

8. Kandutsch, A. A., Paulus, H., Levin, E., and Bloch, K. (1964) J. Biol. Chem., 239, 2507-2515.
9. Porter, J. W. (1969) Pure Appl. Chem., 20, 449-481.
10. Ogura, K., Shinka, T., and Seto, S. (1972) J. Biochem. (Tokyo), 72, 1101-1108.
11. Sagami, H., Ogura, K., and Seto, S. (1977) Biochemistry, 16, 4616-4622.
12. Bernardi, G. (1971) Methods Enzymol., 22, 325-339.
13. Scher, M., Lennarz, W. J., and Sweeley, C. C. (1968) Proc. Natn. Acad. Sci. U. S. A., 59, 1313-1320.
14. Allen, C. M., Keenan, M. V., and Sack, J. (1976) Arch. Biochem. Biophys., 175, 236-248.
15. Kurokawa, T., Ogura, K., and Seto, S. (1971) Biochem. Biophys. Res. Commun., 45, 251-257.