Tetrahedron Letters,Vol.29,No.31,pp 3807-3810,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

NOVEL BEHAVIOR OF UNDECAPRENYL DIPHOSPHATE SYNTHASE TOWARD AN ARTIFICIAL SUBSTRATE. FORMATION OF THE (S)-4-METHYL DERIVATIVE OF Z,E,E,-GERANYLGERANYL DIPHOSPHATE

Tanetoshi Koyama, Michio Ito, Shin-ichi Ohnuma, and Kyozo Ogura*

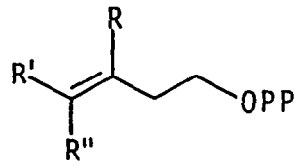
Chemical Research Institute of Non-Aqueous Solutions
Tohoku University, Sendai, 980, Japan

Summary: E-3-Methyl-3-pentenyl diphosphate acted as an artificial substrate in the reaction with E,E-farnesyl diphosphate catalyzed by undecaprenyl diphosphate synthase of Bacillus subtilis. The synthase reaction with this substrate proceeded in the same stereochemical manner as that with the natural substrate, 3-methyl-3-butenyl diphosphate, but it had a full stop at the stage of one step condensation, thereby forming a chiral compound, (Z,E,E)-(S)-4-methylgeranylgeranyl diphosphate.

From mechanistic and synthetic viewpoints, prenyltransferase reactions are unique and attractive in that the polymerization of isoprene units proceeds stereospecifically and terminates precisely when the chains reach certain lengths. For example, farnesyl diphosphate synthase (FPP synthase) [EC 2.5.1.10] catalyzes the consecutive condensation of two molecules of isopentenyl diphosphate (IPP) with dimethylallyl diphosphate (DMAPP) to give E,E-farnesyl diphosphate (FPP), whereas undecaprenyl diphosphate synthase (UPP synthase) catalyzes the polymerization of eight molecules of IPP with FPP as the primer to give E,E-farnesyl-all-Z-octaprenyl diphosphate (undecaprenyl diphosphate, UPP) [EC 2.5.1.31].

Previous studies using a number of artificial substrates have shown that the size of the hydrocarbon moiety of product is decisive in the termination of the chain elongation by FPP synthase.[1-7] This paper, however, reports that the introduction of a methyl group at C-4 of IPP causes the UPP synthase reaction to have a full stop at the stage where a single condensation of the C_6 homolog with the FPP primer is completed to form a chiral C_{21} compound.

First, the substrate specificity of UPP synthase was examined with the following homologs of IPP, E-3-methyl-3-pentenyl diphosphate (1), Z-3-methyl-3-pentenyl diphosphate (2), and 3-ethyl-3-butenyl diphosphate (3), all known to act as substrates for FPP synthase. Only 1, however, was found to act as a substrate for UPP synthase in the reaction with FPP. The incubation mixture contained, in a final volume of 1 ml, 1 μmol of a homolog to be examined, 0.3 nmol of [1-^{14}C]FPP, 100 μmol of Tris-HCl buffer (pH 8.5), 0.5 μmol of $MgCl_2$, 5 mg of Triton X-100, and 4.8 mg protein of UPP synthase. The enzyme fraction was prepared from Bacillus subtilis cells[8] and was free from any other prenyltransferases. After incubation at 37 °C for 24 h the mixture was extracted with 1-butanol, and the extract was treated with acid phosphatase.[9] The hydrolysate was extracted with pentane and chromatographed on silica gel TLC with benzene/AcOEt (9/1). The scanning of the developed plate for the product of 1 showed two radioactivity peaks attributed to a new product and the farnesol recovered from the primer. The Rf value of the product was larger than that of all-E-geranylgeraniol but smaller than that of all-E-decaprenol. The mass spectrum of this alcohol exhibited peaks at m/z 304(M, $C_{21}H_{36}O$), 286(M-18), 217(M-18-69), 149(M-18-69-68), 81, and 69, indicating that the alcohol was a 4-methyl derivative of geranylgeraniol. The alcohol was further cochromatographed on TLC with authentic (Z,E,E)-4-methyl geranylgeraniol,[10] which migrated faster than its all-E isomer. For determination of the absolute structure, 425 μg of the alcohol obtained by an 800 ml incubation was subjected to ozonolysis. The 3-methyllevulinic acid (5) thus obtained showed a CD spectrum with $[\theta]_{280}$= -3600 °± 700 °. For configurational correlation, both (S)- and (R)-3-methyllevulinic acids were prepared by respective ozonolysis of (S)- and (R)-4-methylfarnesol synthesized by the FPP synthase method,[7] and the S isomer showed a CD spectrum with $[\theta]_{280}$= -3200 °± 200 °.[11] Thus, the product of the UPP synthase reaction of 1 with FPP was proved to be (S)-(Z,E,E)-4-methylgeranylgeranyl diphosphate (4) with an enantiomeric excess of 100% within experimental error. The yield of 4 based on the substrate, [1-^{14}C]FPP was 11.4%. No product other than 4 was detected.



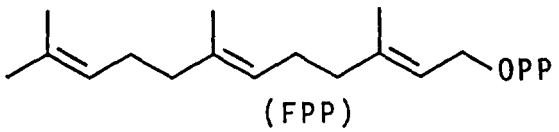
IPP : $R = CH_3$, $R' = R'' = H$

1 : $R = R' = CH_3$, $R'' = H$

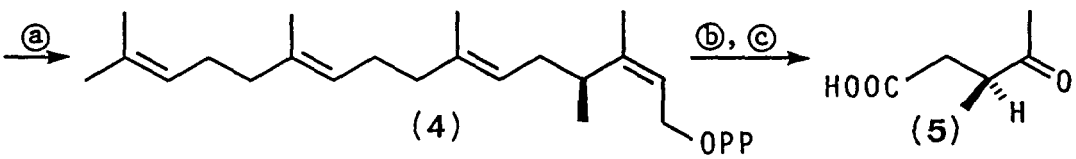
2 : $R = R'' = CH_3$, $R' = H$

3 : $R = C_2H_5$, $R' = R'' = H$

$OPP = OP_2O_6H_3$



(FPP) + (1)



ⓐ UPP synthase ⓑ Acid phosphatase ⓒ O_3

The formation of **4** indicates that the reaction of **1** occurs in the same stereochemical manner as that of IPP but in a different manner in that the reaction of **1** is limited to a single condensation. This catalysis may be synthetically useful like the case of FPP synthase as demonstrated previously.[12,13] The stringent specificity of this enzyme, taken in conjunction with the full stop at C_{21}, is of great interest, because it contrasts with the case of FPP synthase, which accepts both **1** and **2** and gives products which are even longer than the normal product.

Acknowledgment: This work was supported by the Asahi Glass Foundation for the contribution to industrial technology, special coordination funds of the Science and Technology Agency, and Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

References and Notes

(1) Popják, G.; Rabinowitz, J. L.; Baron, J. M. Biochem. J. **1966**, 113, 861-868

(2) Popják, G,; Holloway, P.W.; Baron, J. M. Biochem. J. **1969**, 111, 325-332.

(3) Ogura, K.; Nishino, T.; Koyama, T.; Seto, S. J. Am. Chem. Soc. **1970**, 92, 6036-6041.

(4) Nishino, T.; Ogura, K.; Seto, S. J. Am. Chem. Soc. **1971**, 93, 794-795.

(5) Nishino, T.; Ogura, K.; Seto, S. J. Am. Chem. Soc. **1972**, 94, 6849-6853.

(6) Koyama, T.; Ogura, K.; Seto, S. Chem. Lett. **1973**, 401-404.

(7) Koyama, T.; Saito, A.; Ogura, K.; Seto, S. J. Am. Chem. Soc. **1980**, 102, 3614-3618.

(8) Takahashi, I.; Ogura, K. J. Biochem. (Tokyo) **1982**, 92, 1527-1537.

(9) Koyama, T.; Fujii, H.; Ogura, K. Methods Enzymol. **1985**, 110, 153-155.

(10) The Z,E,E- and E,E,E- isomers of 4-methylgeranylgeraniol were synthesized from 1-methyl-1-E,E-farnesyl-2-propanone obtained by condensation of E,E-farnesyl chloride and ethyl 2-methylacetoacetate. The isomers were separated by HPLC and the structures were confirmed by NMR. The detailed synthesis will be reported elsewhere.

(11) Both (S)- and (R)-4-methylfarnesols synthesized by farnesyl diphosphate synthase reaction were demonstrated to be of 100% enantiomeric purity: Ohnuma, S.; Koyama, T.; Ogura, K. Abstr. of the 31st. Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics. **1987**, 292-294.

(12) Kobayashi, M.; Koyama, T.; Ogura, K.; Seto, S.; Ritter, F. J.; Brüggemann-Rotgans, I. E. M. J. Am. Chem. Soc. **1980**, 102, 6602-6604.

(13) Koyama, T.; Ogura, K.; Baker, F. C.; Jamieson, G. C.; Schooley, D. A. J. Am. Chem. Soc. **1987**, 109, 2853-2854.

(Received in Japan 24 November 1987)