

SUBSTRATE SPECIFICITY OF GERANYLGERANYL PYROPHOSPHATE SYNTHETASE

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The *trans* isomers of 3-methyl-2-octenyl, 3-methyl-2-nonenyl, 3-methyl-2-decenyl, 3-methyl-2-undecenyl, and 3-methyl-2-dodecenyl pyrophosphates were found to act as artificial substrates for geranylgeranyl pyrophosphate synthetase of *Micrococcus lysodeikticus*.

The substrate specificity of farnesyl pyrophosphate synthetase has been studied extensively, and a number of artificial substrates have been found. We have shown that the products having the longest carbon chain formed by the enzymatic reaction of 3-methyl-2-alkenyl pyrophosphate with isopentenyl pyrophosphate (1) are trishomo-farnesyl pyrophosphate (*trans,trans,trans*-3,7,11-trimethylpentadeca-2,6,10-trien-1-yl pyrophosphate) and its 10-*cis* isomer, which result from the condensation of 3-methyl-2-heptenyl pyrophosphate with two molecules of 1, and octakishomogeranyl pyrophosphate (*trans,trans*-3,7-dimethylhexadeca-2,6-dien-1-yl pyrophosphate) which is derived from *trans*-3-methyl-2-dodecenyl pyrophosphate and one molecule of 1.^{1,2}

These results have stimulated us to examine the substrate specificity of geranylgeranyl pyrophosphate synthetase which is responsible for the synthesis of the one C₅ unit longer prenyl pyrophosphate. In this paper we report the substrate specificity of this enzyme with respect to 3-methyl-2-alkenyl pyrophosphates.

3-Methyl-2-alkenyl pyrophosphates were prepared by the method as reported previously.^{1,2} The incubation mixture for the enzymatic reaction contained, in a final volume of 1.0 ml, 50 μ mol of Tris-HCl buffer pH 7.4, 5 μ mol of MgCl₂, 5 μ mol of iodoacetamide, 25 nmol of ¹⁴C-isopentenyl pyrophosphate (1.2 nCi/nmol), 25 nmol of 3-methyl-2-alkenyl pyrophosphate to be examined, and 0.5 mg of geranylgeranyl pyrophosphate synthetase obtained from *Micrococcus lysodeikticus* essentially by the method of Kandutsch *et al.*³, except that DEAE Sephadex was used instead of hydroxylapatite for the purification. The enzymatic reaction was assayed as usual by determining radioactivity in acid-labile allylic pyrophosphate into which ¹⁴C-isopentenyl pyrophosphate was incorporated by the condensation.

Five compounds out of thirteen 3-methyl-2-alkenyl pyrophosphates were found to act as artificial substrates. The reactivities of these substrates are given in Table I. Neither *cis* nor *trans* isomers of 3-methyl-2-pentenyl, 3-methyl-2-hexenyl, 3-methyl-2-heptenyl pyrophosphate (n = 2, 3, and 4 in gross structure 2) were active. The reactivity of *trans*-3-methyl-2-tetradecenyl pyrophosphate (n = 11 in 2) was also as low as negligible.

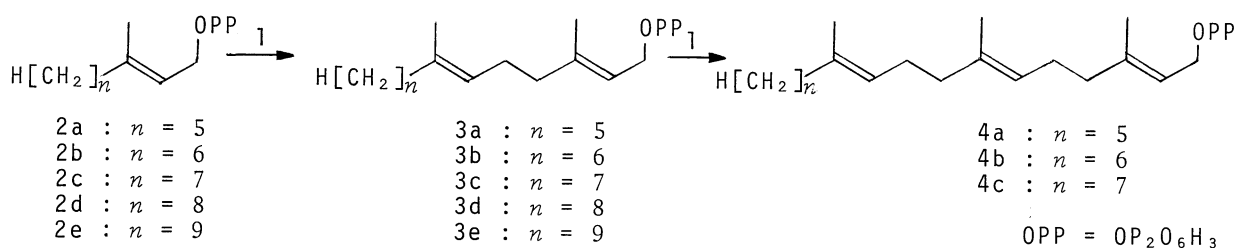
For analysis of the product, the radioactive materials liberated by alkaline phosphatase hydrolysis were subjected to a reverse phase TLC (on Avicel impregnated with paraffin oil with a system of acetone/water, 65/35), and the distribution of

Table I. Reactivity of 3-methyl-2-alkenyl pyrophosphate in the enzymatic reaction with isopentenyl pyrophosphate

| Compound | Reactivity* | |
|--|-------------|---|
| 3-methyl-2-butenyl PP** | 1 | |
| <i>trans</i> -3-methyl-2-octenyl PP (2a) | 0.29 | * Reactivity is expressed as the relative amount of conversion of ^{14}C -isopentenyl pyrophosphate into allylic pyrophosphate. ** PP stands for pyrophosphate. |
| <i>trans</i> -3-methyl-2-nonenyl PP (2b) | 0.47 | |
| <i>trans</i> -3-methyl-2-decenyl PP (2c) | 0.30 | |
| <i>trans</i> -3-methyl-2-undecenyl PP (2d) | 0.55 | |
| <i>trans</i> -3-methyl-2-dodecenyl PP (2e) | 0.46 | |

radioactivity was determined. The material derived from the C_9 compound (2a) showed a radioactivity peak at R_f 0.33, whereas that derived from the C_{10} compound (2b) showed a major peak at R_f 0.28 and a minor peak at R_f 0.57. Two peaks were also observed in the chromatogram of the product from the C_{11} compound (2c), the major peak being at R_f 0.22 and the minor one at 0.49. On the other hand, the products from the C_{12} compound (2d) and C_{13} compound (2e) showed a single peak at R_f 0.35 and 0.28, respectively. Comparison of these chromatograms suggests that the former three react with two molecules of 1 and that the latter two react with one molecule of 1. This was confirmed by GC-MS analysis. The alcohols obtained from 2a, 2b, 2c, 2d, and 2e showed peaks at m/e 278 (M^+), 260 (M^+-18); 292 (M^+), 274 (M^+-18); 306 (M^+), 288 (M^+-18); 252 (M^+), 234 (M^+-18); and 266 (M^+), 248 (M^+-18), respectively.

These results indicate that the reactions of these artificial substrates with 1 proceed as shown below.



The substrate specificity of geranylgeranyl pyrophosphate synthetase is much more stringent than expected by analogy with that of farnesyl pyrophosphate synthetase. The longest carbon chain product that can be formed by geranylgeranyl pyrophosphate synthetase is the C_{21} compound, hexakishomofarnesyl pyrophosphate (4c) derived from the C_{11} substrate (2c) and two molecules of 1. Neither homogeranylgeranyl pyrophosphate nor geranyl pyrophosphate homolog having a carbon chain longer than C_{19} can not be synthesized.

References

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