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Unusual Farnesyl Pyrophosphate Synthetase Reaction of an Artificial Substrate with Ni^{2+}

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Farnesyl pyrophosphate (FPP) synthetase catalyzed the condensation of geranyl pyrophosphate (GPP) with 4-methyl-4-pentenyl pyrophosphate in the presence of Ni^{2+} to give an unexpected product, homofarnesyl pyrophosphate having an exomethylene group, together with Z-homofarnesyl pyrophosphate.

Farnesyl pyrophosphate (FPP) synthetase (EC 2.5.1.10) catalyzes the conversion of dimethylallyl-PP (DMAPP) to geranyl-PP (GPP) and ultimately to E,E-farnesyl-PP by electrophilic condensation which links C-1 in the allylic substrate with C-4 in isopentenyl-PP (IPP) with concomitant formation of E double bond.[1] Among many artificial substrates so far examined, 4-methyl-4-pentenyl-PP (1a) is of particular interest because it acts as an artificial substrate for this enzyme, reacting with GPP to give a nonallylic Z-homofarnesyl-PP (2a).[2] Mg^{2+} is required for the synthetase reaction. Recently, however, it was reported that Zn^{2+} was even more effective in activating the enzyme than Mg^{2+}.[3] This fact stimulated us to study the enzyme reaction with the artificial substrate in the presence of metal ions other than Mg^{2+}.

This paper is concerned with the finding that FPP synthetase catalyzes the condensation of GPP with 1a in the presence of Ni^{2+} in such a way that a new product having an exomethylene moiety is formed together with the Z-isomer (2a) of homofarnesyl-PP.

The enzymatic reaction of 1a was carried out in a large scale as follows: The incubation mixture contained, in a final volume of 200 ml, 5 mmol of Tris-HCl buffer (pH 7.5), 1 mmol of $NiCl_2$, 5 mol of GPP, 5 mol of 1a and 17.5 mg of FPP synthetase purified from pig liver.[4,5] The mixture was kept at 37 °C for 5 h and then treated with alkaline phosphatase as usual. The product extracted with pentane was purified by HPLC and subjected to GC-MS analysis. Surprisingly, the product derived from GPP and 1a was found

to consist of two components in an approximate ratio of 5 : 1 as monitored by selected ion monitoring at m/z 69. The component corresponding to one of the peaks was identified with authentic Z-homofarnesol (2b) on the basis of the retention time and the mass spectrum. The other component which emerged slower than 2b showed the molecular ion peak at m/z 236 ($C_{16}H_{28}O$) and fragment ions similar to those of 2b except for some peaks, suggesting that this product would be an isomer of homofarnesol (Fig. 1). Since the retention time of this compound is different from that of the E-isomer of 2b, it is reasonable from mechanistic consideration to assume that the enzyme reaction might have proceeded in such an aberrant manner that it formed a double bond positional isomer of homofarnesol as well as 2b.

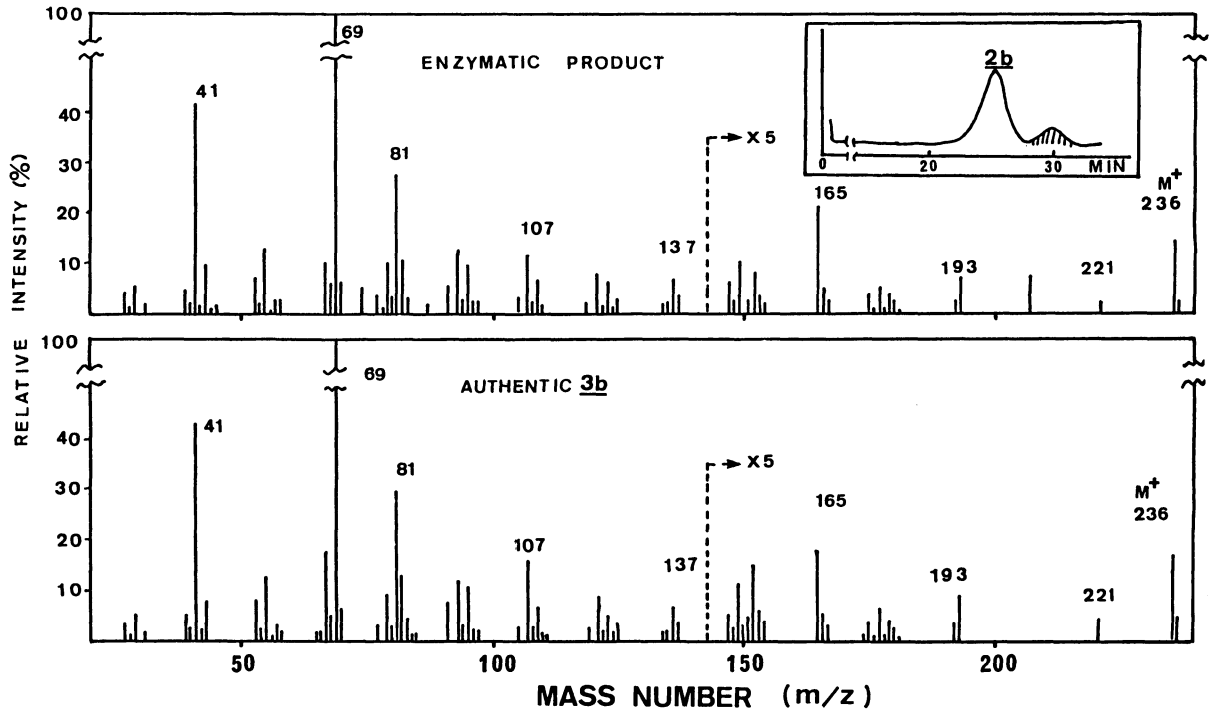
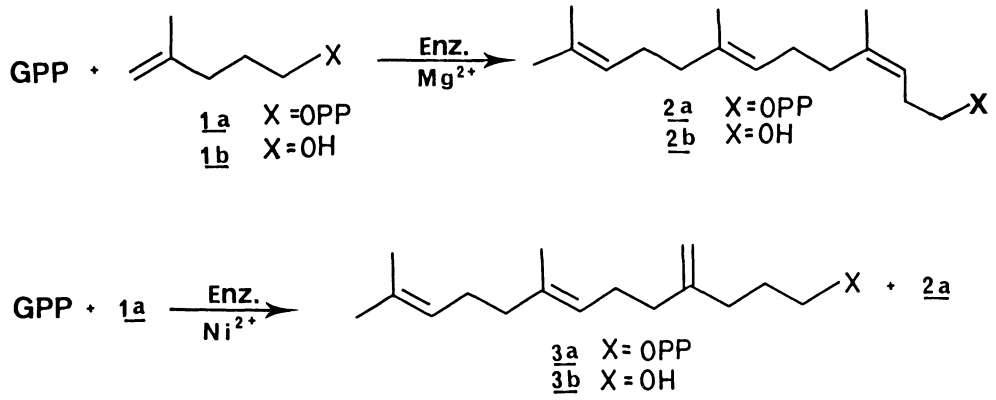


Fig. 1. Mass spectra of the enzymatic product obtained from GPP with 1a in the presence of Ni (upper) and of authentic 3b (lower). The samples were analyzed by Shimadzu-LKB gas chromatograph - mass spectrometer Type 9000. The gas chromatography was carried out at 180 °C on a 1 m column of 20% Carbowax 20M with He gas at a flow rate of 30 ml/min. The mass spectrum was taken at an ionizing potential of 70 eV. The inset shows the GLC chromatogram of the enzymatic products monitored by selected ion monitoring at m/z 69. Only the spectrum for the peak at 30 min is shown.

The reactivity of 1a with GPP in the presence of Ni^{2+} is 0.41 relative to that in the presence of Mg^{2+}. Since the amount of the enzymatic product was slight, the most possible isomer was chemically synthesized and compared with the enzymatic reaction product. Geranyl bromide was treated with 4-methyl-4-pentenol in the presence of BuLi and TMEDA in dry THF at -70 °C to give a mixture of 2b, 3b, and 4,[6] which were separated from each other by preparative GLC and characterized on the basis of their physical data.[7] Both the retention time and the mass spectrum of the enzymatic product were identical with those of 3b as shown in Fig. 1.

Thus, it is evidenced that the enzymatic reaction of GPP with 1a in the presence of Ni^{2+} results in the formation of not only Z-homofarnesyl-PP (2a) but also its isomer (3a). This is the first example showing that FPP synthetase gives an exomethylene product. The enzymatic reaction in the presence of Co^{2+} instead of Ni^{2+} also gave 2a and 3a. Furthermore, it is interesting that E-homofarnesyl-PP is not formed at all in these enzymatic reactions.



The formation of the Z double bond in the enzyme reaction of 1a with GPP in the presence of Mg^{2+} is explained with the model proposed by Ogura et al.[2] The results of the present study would be explained with this model as follows: The metal ions required for this enzyme reaction form metal-substrate complexes, which are accommodated in the active site of the enzyme. In the case of 1a-Mg^{2+} complex, both methyl and pyrophosphate groups can fit to M-site and P-site when the pro-R hydrogen at C-3 of 1a is located near to the base of the enzyme. Although Ni^{2+} forms a similar complex, its shape is different from the Mg^{2+} complex and cannot fit exactly to the binding site. Probably the pro-R hydrogen at C-3 is located a little far from the base and instead the methyl group approaches to it. As a result, both the pro-R hydrogen at C-3 and a hydrogen of the methyl group of 1a are eliminated to give 2a and 3a (Fig. 2).

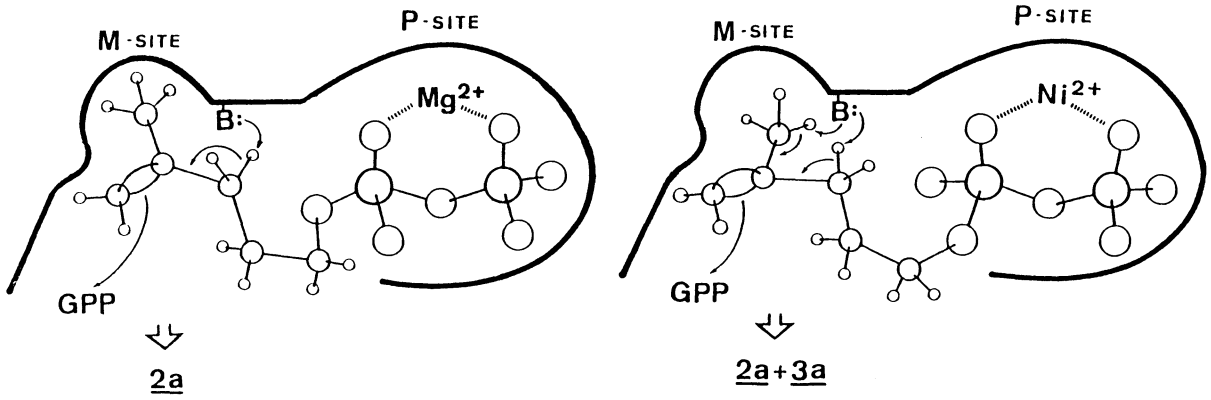


Fig.2. Proposed models of 1a-Mg^{2+} (left) and 1a-Ni^{2+} (right) bound to the IPP binding site of FPP synthetase.

FPP synthetase reaction of 1a with DMAPP in the presence of Ni^{2+} also gave homoneryl-PP and its isomer possessing an exomethylene group.

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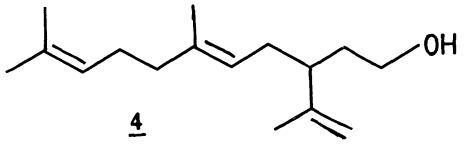
7) The compound 3b, NMR ($CDCl_3$), δ 1.60-1.70 (m, 11H, CH_3-C=, C-C$\underline{H}_2$-CH_2-OH), 2.00 (m, 10H, -CH_2-C=), 3.67 (t, 2H, -CH_2-C$\underline{H}_2$-OH), 4.76 (s, 2H, CH_2=C-C), and 5.10 (t, 2H, C=C$\underline{H}$-CH_2-).
The compound 4, NMR ($CDCl_3$), δ 1.60-1.67 (m, 14H, CH_3-C=, -C$\underline{H}_2$-CH_2-OH), 2.00 (m, 7H, C=C-CH_2- and -C$\underline{H}$-C=C), 3.60 (t, 2H, -CH_2-C$\underline{H}_2$-OH), 4.80 (s, 2H, CH_2=C) and 5.10 (t, 2H, C=C$\underline{H}$-CH_2).



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