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Farnesyl Diphosphate Synthase Reactions of Geranyl Diphosphate Analogues Having Oxygen Atoms in Their Alkyl Chains

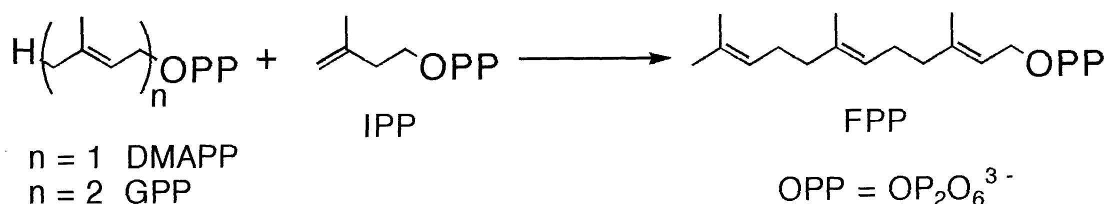
Yuji MAKI,* Hikaru SATOH, Masayo KURIHARA, Takae ENDO, Gotaro WATANABE, and Kyozo OGURA†

Department of Chemistry, Faculty of Science, Yamagata University, Yamagata 990

†Institute for Chemical Reaction Science, Tohoku University, Sendai 980

Seven geranyl diphosphate analogues having oxygen atoms in their alkyl chains were synthesized and examined for their reactivities as substrates in the reaction catalyzed by pig liver farnesyl diphosphate synthase. All of these compounds acted as substrates to give farnesyl diphosphate analogues. It was suggested that the enzyme cavity for the geranyl moiety is tolerant enough to accommodate alkyl moieties containing oxygen atoms.

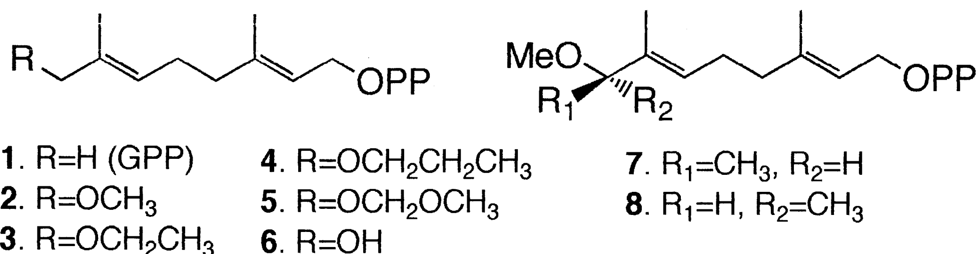
Prenyltransferases are unique and attractive from both mechanistic and synthetic viewpoints in that they catalyze the stereospecific polymerization of isoprene units to give products with certain chain lengths depending on the specificities of the individual enzymes. Farnesyl diphosphate synthase (FPP synthase) [2.5.1.10], the most important and best characterized prenyltransferase, catalyzes the condensations of isopentenyl diphosphate (IPP) with dimethylallyl diphosphate (DMAPP) and with geranyl diphosphate (GPP) to



give *E,E*-farnesyl diphosphate (FPP) as the ultimate product.[1,2] It has been reported that a number of DMAPP analogues having various hydrocarbon chains are accepted as substrates by FPP synthase and that

their reactivities are dependent on the alkyl chain lengths.[3–6] These authors have shown that an analogue which has the same chain length as GPP is as reactive as GPP and that analogues with chains either shorter or longer than that of GPP had lower reactivities than GPP. These results suggest that the binding site for the prenyl moiety has the most suitable size and shape for GPP. FPP synthase reactions with substrate analogues have also been shown to provide unique methods of synthesis of natural products.[7, 8] However, little study has been conducted with analogues having hetero atoms, which might be useful not only as probes for the cavity of the binding site but also as artificial substrates of synthetic interest.[9] These circumstances prompted us to investigate the substrate specificity of this enzyme by using various GPP analogues having oxygen atoms in their alkyl chains.

GPP analogues (**2–8**) were synthesized and subjected to the reaction catalyzed by pig liver FPP synthase.[12] The compounds studied here were prepared from the corresponding alcohols *via* their chlorides by the method of Davisson *et al.*[10] 8–Hydroxy–GPP was synthesized from 8–hydroxygeranyl chloride obtained by selective chlorination of 8–hydroxygeraniol prepared by SeO_2 oxidation of geranyl acetate followed by hydrolysis.[11] The structures of the compounds synthesized were confirmed on the basis of physicochemical data including MS, IR and NMR spectra. The reactivities of these compounds were assayed by a



conventional method based on the acid lability of the product of FPP synthase reaction.[4] The standard incubation mixture for enzymatic reaction contained, in a final volume of 1 ml, 20 μmol of Tris–HCl, pH 7.4, 5 μmol of $MgCl_2$, 25 nmol of [1–^{14}C] IPP, 25 nmol of a compound to be examined (**1–8**), and 0.22 mg of pig liver FPP synthase. After incubation at 37 °C for 30 min, the mixture was treated with diluted hydrochloric acid, and the product was extracted with hexane. The amount of product was determined by counting the radioactivity of the extract.[12] All these compounds (**2–8**) were found to act as substrates with relative activities shown in Table 1. The Km and relative Vmax values are also shown in the same table.

Among the analogues, substrate **2**, which is larger than GPP because one oxygen atom is inserted in the alkyl chain, was the most reactive under the standard conditions. It was almost as reactive as GPP, but its Km value was three times as large as that of GPP. In order to identify the product from **2**, the reaction

mixture was treated with alkaline phosphatase to cleave the diphosphates. The hydrolysate was extracted with pentane and then subjected to reversed phase TLC (LKC–18 plate, Whatman, acetone : water = 9:1). TLC analyses showed a single radioactive spot with an Rf value of 0.50, which was smaller than that of 8–methoxygeraniol (0.62) and the same as that of chemically synthesized 12–methoxyfarnesol. The GC–mass spectrum of the hydrolysate was also identical with that of 12–methoxyfarnesol. These results indicate that the product was an FPP analogue yielded by the condensation of **2** with one molecule of IPP. Similarly, the reaction products of all other artificial substrates were also identified as the corresponding FPP analogues. It is surprising that even compound **6**, which has a free hydroxyl group, was accepted as a good substrate in terms of both Vmax and Km values, because the recognition site for the geranyl group had been assumed to be hydrophobic.[13] Substrate **6** was accepted by the enzyme with an affinity similar to that of the natural substrate (GPP), while all the other analogues showed lower affinifies than GPP. A tendency for the enzyme to favor oxygen insertion into an allylic substrate was also observed in the reactivities of **4** and **5**. It has been shown that pig liver FPP synthase cannot accept as a substrate *E,E,*–3,7–dimethyl–2,6–dodecadienyl diphosphate, tetrakishomo–GPP (C_{14} compound)[5], which corresponds to **4** or **5** in terms of chain length. In addition, compound **5**, which has two oxygen atoms in its alkyl chain, was a better substrate in terms of both affinity and veloicity than **4**, which has the same chain length but only one oxygen atom.

Table.1 Relative reactivities and Km and Vmax values of GPP and its analogues

Substrates	Reactivity[a) %]	Km μM	Vmax(relative)
1	100	4.40 ±1.1	1.00
2	83.2	13.9 ±5.0	0.83
3	70.0	9.90 ±4.5	0.80
4	22.1	23.2 ±5.8	0.17
5	51.2	9.50 ±1.4	0.65
6	77.7	5.41 ±0.7	0.73
7	29.0	11.4 ±3.6	0.30
8	25.8	22.6 ±5.7	0.38

a) Relative amounts of the products formed under the standard conditions.

The sufficient reactivities with **2**, **3**, **4**, **5**, and **6** indicate that the enzyme cavity for the prenyl moiety is more tolerant than expected and that there may be a residue or residues in the cavity which interact with the oxygens inserted in these substrates to hold the substrates in a position in favor of catalysis. In order to examine the possibility of such an interaction, chiral substrates (**7** and **8**) were prepared and subjected to the enzyme reaction with the expectation that one of the two enantiomers would be preferred.[14] As shown in

Table 1, **7** had a somewhat better affinity than **8**, with the Km value of the former being half that of the latter. Koyama *et al.* have previously demonstrated that FPP synthase catalyzes the condensation of IPP with (*RS*)-6,7-epoxygeranyl diphosphate, showing a preference of the *S* enantiomer.[15] Coupled together, these results suggest that there might be interactions between the oxygen atoms of these artificial substrates and a group(s) in the cavity. It is also noteworthy that **7** and **8** are far less reactive than **2** and **3**, since **7** and **8** differ from **2** and **3** only in having branched structures at the 8-position.

In conclusion, every oxygen-containing GPP analogue examined in this study reacted with one molecule of IPP to give the corresponding FPP analougue. Thus the enzyme cavity seems to be tolerant enough to accommodate not only fully hydrophobic but also partially hydrophilic moieties containing oxygen atoms. In view of the above described effect of oxygen insersion into the alkyl chain of unreactive tetrakishomo-GPP, the interaction between the oxygen atom inserted in a substrate and a hydrophilic site in the cavity might enhance the formation of the substrate-enzyme complex in a productive manner leading to the enzymatic reaction.

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