Farnesyl Diphosphate Synthase Reactions with Dimethylallyl Diphosphate Analogues Having Oxygen Atoms in Their Chains

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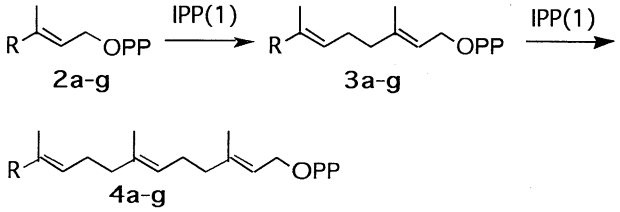
Six dimethylallyl diphosphate analogues having oxygen atoms in their alkyl chains were found to act as substrate in the reaction catalyzed by pig liver farnesyl diphosphate synthase to give geranyl diphosphate analogues or farnesyl diphosphate analogues. The number of IPP incorporated into the product depended on not only the length of the chain but also its hydrophobicity.

Farnesyl diphosphate (FPP, **4a**) synthase[2.5.1.10] is one of the first members of prenyl transferases in the biosynthetic pathway leading to a variety of isoprenoid compounds.[1] It catalyzes the consecutive 1-4' condensation of dimethyl diphosphate (DMAPP, **2a**) with two molecules of isopentenyl diphosphate (IPP, **1**) to ultimately give *E,E*-farnesyl diphosphate (scheme 1).[2-4] It has been reported that DMAPP analogues having various hydrocarbon chains are accepted and that the reactivity of an artificial substrate and the number of IPP that reacts with it, are dependent on the chain length of its alkyl group.[5-8] For example, 3-methyl-(*2E*)-octenyl diphosphate (**2b**), which has the same chain length as GPP, is as reactive as GPP, reacting with one molecule of IPP to give a C_{14} compound, while 3-methyl-(*2E*)-heptenyl diphosphate (**2g**), the chain of which is shorter by one methylene, is less reactive than GPP, reacting with one or two molecules of IPP to yield a mixture of C_{13} and C_{18} compounds. Meanwhile, our recent finding that geranyl diphosphate (GPP, **3a**) analogues containing oxygen atoms in their chains can be good substrates[9-10] led us to be interested in the effect of insertion of oxygen into the chains of DMAPP analogues on the number of IPP that reacts with them. This paper describes the finding that the number of IPP that reacts with a DMAPP analogue depends not only on the chain length but also on the hydrophobicity of the chain.

Oxygen-containing DMAPP analogues (**2c-2f**) were synthesized and examined as substrates for the reaction catalyzed by pig liver FPP synthase.[11] These compounds were prepared from the corresponding alcohols via their chlorides by the method of Davisson et al.[12] Their structures were confirmed on the basis of physicochemical data, including MS, IR, and NMR spectra. The reactivities were assayed by a conventional method based on the acid lability of the products 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 studied (**2b-2f**), and 0.22 mg of FPP synthase partially purified from pig liver.[11] After incubation at 37 °C for 30 min, the mixture was treated with diluted hydrochloric acid, the product was extracted with hexane, and the amount of IPP incorporated into product was determined by counting the radioactivity of the hexane extract.

As a result, all these compounds were found to act as substrates with kinetic properties as shown in Table 1. The products are also shown in the same table.



a. R=CH_3-
b. R=$CH_3(CH_2)_4$-
c. R=$CH_3O(CH_2)_3$-
d. R=$CH_3OCH_2O(CH_2)_3$-
e. R=$HO(CH_2)_3$-
f. R=$CH_3OCH_2OCH_2$-
g. R=$CH_3(CH_2)_3$-

Scheme 1.

Table1. Relative reactivities, Km and Vmax values, and products

Substrates	Reactivity %[a]	Km (μM)	Vmax(relative)	Product
3a	100	4.4	1.00	
2b	96.9	17.5	4.5	**3b**
2c	101.7	15.6	2.6	**3c+4c**
2d	117.3	3.1	0.96	**3d**
2e	62.8	7.3	0.70	**4e**
2f	7.3			**4f**

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

Since compound **2b**, the main chain of which is as long as that of GPP, is known to react with one molecule of IPP,[8] it was taken as a standard of DMAPP analogues without oxygen insertion. Compound **2c**, the 7-oxa analogue of **2b**, reacted with one or two IPP molecules to give a mixture of **3c** and **4c**. Interestingly, compound **2d**, the 8-methoxy-7-oxa analogue of **2b**, was also as reactive as GPP. In order to identify the product from **2b** and **2d**, each reaction mixture was treated with alkaline phosphatase, and the hydrolysate was extracted with pentane and subjected to reverse phase TLC (LKC-18, Whatmann, acetone : water = 9:1). The hydrolysate derived from **2b** and **2d** showed radioactive spots with *Rf* values of 0.37 and 0.54, respectively. GC-mass analysis showed that the product alcohol from **2b** was 3,7-dimethyl-(*2E,6E*)-dodecadien-1-ol(**3b**, alcohol), which is a product resulting from the incorporation of one molecule of IPP. The hydrolysate of the product from **2d** was identified by comparing its GC-mass spectrum with that of chemically synthesized 11,13-dioxa-3,7-dimethyl-(*2E,6E*)-tetradecadien-1-ol(**3d**, alcohol). This product can be easily converted into 3,7-dimethyl-(*2E,6E*-)-decadien-1,10-diol, which is hair pencil pheromone of butterfly,[13] by treating it with acid to remove the MOMO-group. Compound **2c** reacted with one or two molecules of IPP, yielding a mixture of

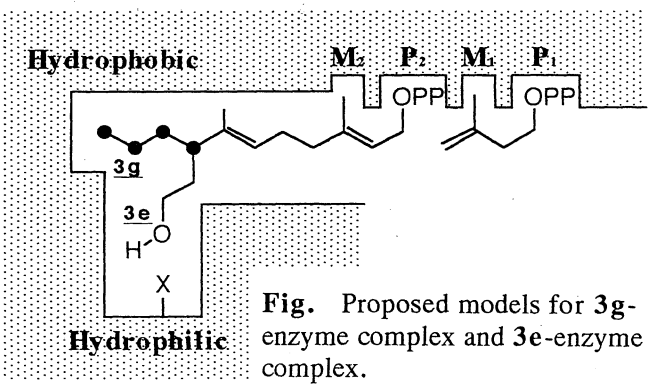


Fig. Proposed models for **3g**-enzyme complex and **3e**-enzyme complex.

15-oxa-3,7,11-trimethyl-(*2E,6E,10E*)-hexadecatrienyl diphosphate (**4c**), and 11-oxa-3,7-dimethyl-(*2E,6E*)-tetradecadienyl diphosphate (**3c**). The structures of the products were confirmed on the basis of the GC-mass spectra and mobilities on TLC of their hydrolysates. The *Rf* values for the longer and the shorter products were 0.43 and 0.58, respectively. The ratio of the former to the later was 1:12. Even compound **2e**, which has a free hydroxyl group, was also accepted as a good substrate, reacting with two molecules of IPP to afford a hydroxyl derivative of FPP (**4e**). This product was hydrolyzed to 3,7,11-trimethyl-(*2E,6E,10E*)-tetradecatriene-1,14-diol, which was identified with that of an authentic sample synthesized chemically from geranylgeranyl acetate.[14] Compound **2f**, the 5,7-dioxa analogue of **2b**, also acted as substrate. However, its reactivity was much lower than those of **2b** and any other analogues studied here. TLC analysis of the hydrolysate of the product from **2f** showed a single radioactive spot, which coincided with that of an authentic sample of 13, 15-dioxa-3, 7, 11 - trimethyl - (*2E,6E,10E*) - hexadecatrien -1- ol(**4f**, alcohol), indicating that **2f** reacted with two molecules of IPP.[5]

Both compounds **2b** and **2d** react with only one molecule of IPP, as does GPP (**3a**).[8] However, compound **2c** reacts with one or two molecules of IPP, affording a mixture of **3c** and **4c**. Substrate **2e**, which is shorter than GPP, always reacts with two molecules of IPP. The number of IPP incorporated into the product was unchanged even if the incubation time was varied. Substrate **2f**, the 5,7-dioxa analogue of **2b**, also reacts with two molecules of IPP, though **2b** reacts with only one molecule of IPP. It should depend on the reactivity of the GPP analogues produced from a DMAPP analogue whether the DMAPP analogue reacts with one or two molecules of IPP. Coupled with our previous findings [5], the present findings lead us to propose a hypothetical model in which the binding cavity for allylic diphosphate is surrounded by a hydrophobic wall on one side and a somewhat hydrophilic wall on the other side (Figure). GPP analogues having hydrocarbon chains are bound along the former wall, which allows them to make productive binding if the chains are as long as or shorter than "trishomogeranyl"("n-propylgeranyl") (**3g**). On the other hand, oxygen-containing analogues are bound along the other wall, which is so tolerant as to allow a productive binding even for **3e**, the chain of which is as long as "tetrakishomogeranyl". It is also interesting that all the oxygen-containing analogues except **2f** are highly reactive. Some interaction between the oxygen atom(s) of the analogue and a hydrophilic residue(s) might contribute to a highly productive binding. The low reactivity of **2f** might be due to the hydrophobic nature of M_2-site, which recognizes the methyl group of the allylic diphosphate substrates, and its vicinity, which tends to avoid the ethereal oxygen at 5-position.

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References and Notes

1 S. Ohnuma, T. Koyama, and K. Ogura, *J. Biol. Chem.*, **266**, 23076 (1991).
2 C. D. Poulter and H. C. Rilling, "*Biosynthesis of Ioprenoid Compounds*", ed by J. W. Porter & S. L. Spurgeon, **1**, 224 (1981)
3 G. Popák, P. W. Holloway, R. R. M. Bond, and M. Roberts, *Biochem. J.*, **111**, 333 (1969).
4 K. Ogura, T. Nishino, T. Shinka, and S. Seto, *Methods in Enzymol.*, **110**, 167 (1985).
5 Y. Maki, H. Satoh, M. Kurihara, T. Endo, G. Watanabe, and K. Ogura, *Chem. Lett.*, **1994**,1841.
6 T. Koyama, H. Inoue, S. Ohnuma, and K. Ogura, *Tetrahedron Lett.*, **31**, 11189 (1990).
7 G. Popják, J. L. Rabinowitz, and J. M. Baron, *Biochem. J.*, **113**, 861 (1969).
8 K. Ogura, T. Nishino, T. Koyama, and S. Seto, *J. Am Chem. Soc.*, **92**, 6036 (1970).
9 T. Nishino, K. Ogura, and S. Seto, *Biochem. Biophys. Acta*, **235**, 322 (1971).
10 T. Nishino, K. Ogura, and S. Seto, *J. Am. Chem.Soc.*, **94**, 6849 (1972).
11 R. W. Holloway and G. Popják, *Biochem. J.*, **104**, 57 (1967).
12 V. J. Davisson, A. B. Woodside, and C. D. Poulter, *Methods in Enzymol.*, **110**, 139 (1985).
13 J. Meinwald, Y. C. Meinwald, and P. H. Mazzochi, *Science*, **164**, 1174 (1969).
14 The authentic sample was prepared from geranylgeranyl acetate by a synthetic method similar to that of hair pencil pheromone described in ref. 13.