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SUBSTRATE SPECIFICITY OF FARNESYL DIPHOSPHATE SYNTHASE FROM *Bacillus stearothermophilus*

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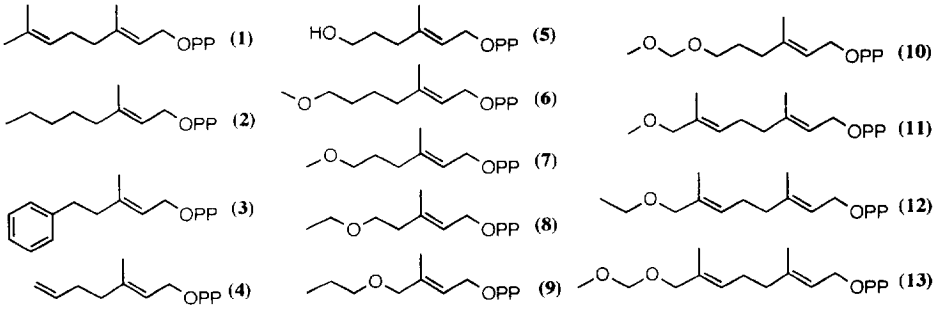
Abstract: The substrate specificity of farnesyl diphosphate synthase from *Bacillus stearothermophilus* was studied and compared with that of the pig liver counterpart. Allylic diphosphate analogs having hydrocarbon chains were accepted as good substrates by both enzymes. However, the bacterial enzyme hardly accepted analogs having oxygen atoms in their chains, which are well accepted by the pig liver enzyme, indicating that the former has more stringent specificity than the latter.

Farnesyl diphosphate (FPP) synthase [EC 2.5.1.10] catalyzes the condensation of isopentenyl diphosphate (IPP) with dimethylallyl diphosphate (DMAPP) and with geranyl diphosphate (GPP) to give *E,E*-FPP as the final product. It has been reported that FPP synthase from pig liver accepts as substrate a number of GPP- and DMAPP analogs having various alkyl chains.[1-4] Recently we have found that the pig liver enzyme accepts analogs even having oxygen atoms in their alkyl chains.[5,6] Based on the reactivities of these analogs, we have assumed that the prenyl recognition site for the allylic substrates might be spacious and surrounded by both hydrophobic and hydrophilic walls.[6] Koyama *et al.* have recently succeeded in an efficient overproduction of thermostable FPP synthase from *Bacillus stearothermophilus* in *E. coli*.[7] This enzyme is extremely interesting from both mechanistic and synthetic viewpoints[8,9] because of its thermostability and sufficient availability. It has also been shown that this enzyme shows only a 22% sequence similarity to the corresponding enzymes from mammalian sources.[7,10] The marked difference in primary structure and thermostability between the bacterial and the mammalian liver enzymes led us to investigate the substrate specificity of FPP synthase of *B. stearothermophilus* with respect to artificial substrates. We were also interested in comparative specificities of the bacterial and mammalian enzymes from a standpoint of synthetic application of enzymes.

The substrate analogs (**2-13**) were synthesized as reported previously (Fig.1).[5,6] The enzymes from pig liver[11] and *B. stearothermophilus*[7] were obtained by the methods as reported. The incubation mixture for

the thermostable enzyme reaction contained, in a final volume of 1 ml, 20 μmol of Tris-HCl, pH 8.5, 50 μmol of ammonium chloride, 5 μmol of $MgCl_2$, 25 nmol of [1-^{14}C]IPP (specific activity, 37 MBq/mol), 25 nmol of a compound to be examined (**1-13**) and 9.6 μg of the enzyme protein. After incubation at 55 °C for 15 min, the mixture was treated with diluted hydrochloric acid and the product was extracted with hexane. The amount of products was determined by counting the radioactivity of the extract. The reaction with the pig liver enzyme was examined in the same way as described above for the bacterial enzyme reaction, except that the incubation temperature, time and pH were 37 °C, 30 min and 7.4, respectively and that ammonium chloride was omitted. The reactivities of these compounds (**1-13**) are shown in Fig.2. These values are referred to as relative velocities of the incorporation of IPP into the products under the conditions described above.



Fig,1 GPP and Substrate Analogs -OPP = $-OP_2O_6^{3-}$

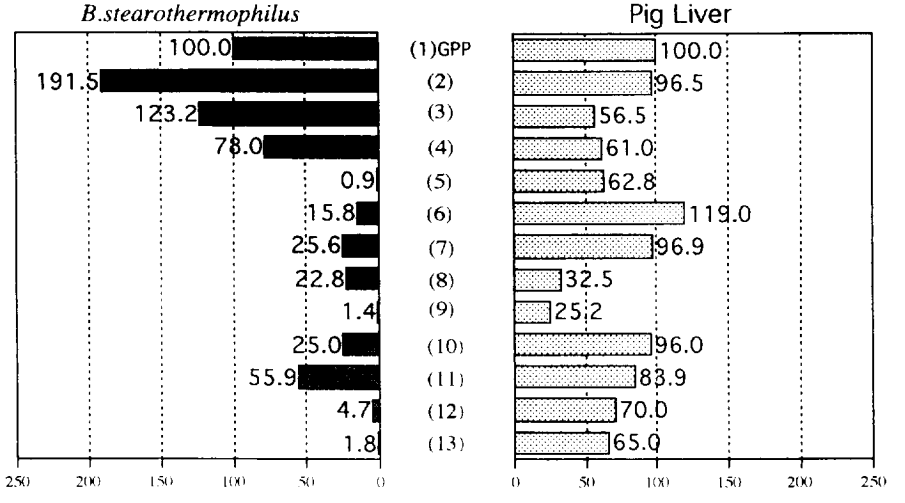


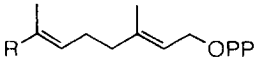
Fig.2 Relative reactivities of GPP and Substrate Analogs

Compounds **2**, **3** and **4**, which are analogs having hydrocarbon moieties without oxygens, are accepted

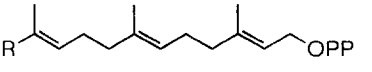
as substrates more efficiently by the bacterial enzyme than by the liver enzyme. Surprisingly, compound **2**, which is a desmethyl-dihydro derivative of GPP, and compound **3**, which has a phenyl group at the edge of its chain, were accepted by the bacterial enzyme as even better substrates than GPP. The apparent *Km* values of **2** were estimated to be 11.5 μM for the bacterial enzyme and 17.5 μM for the liver enzyme, while those of GPP are 8.4 μM and 4.4 μM, respectively.[5,7]

In contrast, compounds **5-13**, which are analogs having oxygen atoms in their chains, were accepted as substrates by the pig liver enzyme, some of them showing almost similar reactivities to that of GPP, whereas the bacterial enzyme hardly accepted these oxygen-containing analogs except **11**. It is noteworthy that compound **5** is a good substrate for the liver enzyme but an almost inactive substrate for the bacterial enzyme. Compound **7**, which is the 7-oxa analog of **2**, was accepted by both enzymes, but the reactivity with the bacterial enzyme was about one fourth that of GPP, while this compound showed almost the same reactivity as GPP in the reaction catalyzed by the liver enzyme. The apparent *Km* values of **7** were estimated to be 205 μM for the bacterial enzyme and 15.6 μM for the liver enzyme, suggesting that the poor reactivity of **7** is attributable to a decrease in the affinity of binding caused by insertion of an oxygen into the alkyl chains. Compound **11** is an exception, because it acts as substrate for both the liver and bacterial enzymes with the activities being as much as 84% and 56% that of GPP, respectively. The activity of **12**, which is longer than **11** by one methylene, was as high as 70% that of GPP for the liver enzyme, but it was only 4.7 percent that of GPP for the bacterial enzyme. Compound **13**, which is longer than GPP by two methylenes and two oxygen atoms, was also accepted as a good substrate only by the liver enzyme.

It is of interest to learn how many molecules of IPP are incorporated into the final product, because it should reflect the capacity of the binding site of enzyme. The products from these substrates were identified by comparing their *Rf* values on reversed TLC as reported before.[4] Compound **2** gave a mixture of a product of type A and a product of type B, which result from the incorporation of one and two molecules of IPP, respectively. The formation ratios of the former and the latter were 12:1 for the liver enzyme and 20:1 for the



(A)



(B)

bacterial enzyme. Similarly, compound **4** also gave a mixture of type A and type B with the ratios being 2:1 and 6:1 for the liver and bacterial enzymes, respectively. In the case of **3**, **6** and **10**, the reaction of either enzyme gave a corresponding product of type A exclusively. In the case of **7**, **8** and **9**, the reaction by the bacterial enzyme stopped after a single condensation with IPP, while the reaction by the liver enzyme gave a mixture of products of type A and type B.[6] The reaction of compound **5** by the liver enzyme gave only a product of type B. These results indicate that the capacity of the binding site of the liver enzyme is greater than that of the bacterial counterpart.

As described above, FPP synthase catalyzes the condensation of DMAPP with two molecules of IPP to give *E,E*-FPP. There is no difference in catalytic ability between the enzymes from *B. stearothermophilus* and

pig liver, as far as their abilities are examined with the natural substrates. However, these two enzymes were shown to have such a marked difference in the substrate specificity with respect to artificially modified substrates. Both enzymes accept the artificial substrates having hydrophobic chains as good substrates. The apparent *Km* values of **2** for the two enzymes are almost the same. In contrast, most of the GPP and DMAPP analogs having oxygen atoms in their chains acted as much better substrates for the liver enzyme than for the bacterial enzyme. Compound **11** is an exceptionally good oxygen containing substrate for the bacterial enzyme.

In conclusion, the bacterial enzyme is not so tolerant as the liver enzyme with respect to oxygen-containing artificial substrates. The liver enzyme may have a bigger binding cavity for the prenyl groups of substrates than the bacterial counterpart. The cavity of the bacterial enzyme may be more hydrophilic than that of the liver enzyme, which is assumed to be surrounded by hydrophobic and hydrophilic walls.[6] Further studies are under way to examine not only detailed kinetic properties of these artificial substrates but also their behavior toward mutated enzymes[12] in which some of the amino acids constituting the putative binding site for DMAPP and GPP are replaced by other amino acids.

References and Notes

1. Popják, G.; Holloway, P.W.; Baron, J. M. *Biochem. J.*, **1969**, *111*, 333.
2. Popják, G.; Rabinowitz, J. L.; Baron, J. M. *Biochem. J.*, **1969**. *113*, 861.
3. Nishino,T.; Ogura, K.; Seto, S. *Biochim. Biophys. Acta*, **1971**, *235*, 322.
4. Nishino, T.; Ogura, K.; Seto, S. *J. Am. Chem. Soc.*, **1971**, *93*, 794.
5. Maki, Y.; Satoh, H.; Kurihara, M.; Endo, T.; Watanabe, G.; Ogura, K. *Chem. Lett.*, **1994**, 1841.
6. Maki, Y.; Kurihara, M.; Endo,T.; Abiko, M.; Saito, K.; Watanabe, G.; Ogura, K. *Chem. Lett.*, **1995** (in press).
7. Koyama, T.; Obata, S.; Osabe, M.; Takeshita, A.; Yokoyama, K.; Uchida, M.; Nishino, T.; Ogura, K. *J. Biochem. (Tokyo)*, **1993**, *113*, 355.
8. Kobayashi, M.; Koyama, T.; Ogura, K.; Seto, S.; Ritter, F. J.; Brüggemann-Rotgans, I. E. M. *J. Am. Chem. Soc.*, **1980**, *102*, 6602.
9. Koyama, T.; Ogura, K.; Baker, F. C.; Jamieson, G. C.; Schooley, D. A. *J. Am. Chem. Soc.*, **1987**, *109*, 2853.
10. Chen, A.; Kroon, P. A.; Poulter, C. D. *Protein Science*, **1994**, *3*, 57.
11. Holloway, P. W.; Popják, G. *Biochem. J.*, **1967**, *104*, 57.
12. Koyama, T. ; Obata, S.; Saito, K.; Takeshita-Koike, A.; Ogura, K. *Biochemistry*, **1994**, *33*, 2644.

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