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Substrate specificity of thermostable farnesyl diphosphate synthase with alkyl group homologs of isopentenyl diphosphate

Masahiko Nagaki,* Hiroki Kannari,† Junji Ishibashi,† Yuji Maki,† Tokuzo Nishino,†† Kyozo Ogura,††† and Tanetoshi Koyama†††

*Department of Materials Science and Technology, Faculty of Science and Technology, Hirosaki University Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan

†Department of Biological and Material Chemistry, Faculty of Science, Yamagata University Koshirakawa-cho, Yamagata 990-0021, Japan

††Department of Biochemistry and Engineering, Faculty of Engineering, Tohoku University Aobaku, Sendai, Miyagi 980-8579, Japan

†††Institute for Chemical Reaction Science, Tohoku University Aobaku, Sendai, Miyagi 980-8577, Japan

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Abstract

3-Alkyl group homologs of isopentenyl diphosphate were examined for the reactivity as substrates of the thermostable farnesyl diphosphate (FPP) synthase of *Bacillus stearothermophilus*. Even 3-*n*-propyl- and 3-*n*-butyl-but-3-enyl diphosphates, which are hardly acceptable by animal FPP synthases, are accepted by this bacterial enzyme as substrates to react with dimethylallyl- and geranyl diphosphates, yielding 7-methyl-3-*n*-propylocta-2,6-dienyl- and 7,11-dimethyl-3-*n*-propyldodeca-2,6,10-trienyl diphosphate, respectively.

Keywords :Enzymes and enzyme reactions: Terpenes and terpenoids; Stereospecificity: Substituent effects

Farnesyl diphosphate (FPP) synthase [EC 2. 5. 1. 10] is one of the prenyltransferases, which catalyze consecutive 1'-4 condensations of isopentenyl diphosphate (IPP, **2**, R = CH_3) with dimethylallyl diphosphate (DMAPP, **1**) or with geranyl diphosphate (GPP, **3**, R = CH_3) to give *E,E*-FPP (**4**, n = 1, m = 2, R = CH_3) as shown in Scheme 1 [1]. The substrate specificity of FPP synthase from porcine liver has been studied extensively with respect to a number of allylic and homoallylic substrate homologs [2-7]. Some of the

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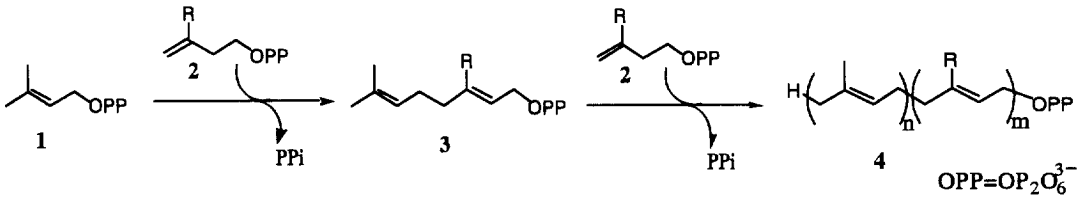
artificial substrates have been effectively applied for stereospecific syntheses of biologically active compounds [8-10]. The substrate specificity of porcine liver FPP synthase has been shown to be considerably tolerant toward allylic diphosphates, DMAPP or GPP homologs, but stringent toward homoallylic diphosphates, IPP homologs [5, 6].



DMAPP 1
IPP 2, R = CH_3
GPP 3, R = CH_3
FPP 4, R = CH_3

2a, **3a**, **4a**, and **4a'** : R = H
2b, **3b**, **4b**, and **4b'** : R = CH_2CH_3
2c, **3c**, **4c**, and **4c'** : R = $CH_2CH_2CH_3$
2d, **3d**, **4d**, and **4d'** : R = $CH_2CH_2CH_2CH_3$

n = 1, m = 2 : **4a**, **4b**, **4c**, and **4d**
n = 2, m = 1 : **4a'**, **4b'**, **4c'**, and **4d'**

Scheme 1.

We have reported [11] recently that but-3-enyl diphosphate (**2a**), which is hardly acceptable for the liver enzyme, is accepted as substrate by the thermostable FPP synthase of *Bacillus stearothermophilus*. Furthermore, Maki *et al.* have reported that this enzyme shows different substrate specificities toward allylic substrate homologs than does the liver enzyme [12]. These findings suggested that further studies on the substrate specificity of this thermostable enzyme would be interesting from not only mechanistic but also synthetic viewpoints. This paper describes comparative reactivities of alkyl group homologs of IPP in the reaction catalyzed by FPP synthases of *B. stearothermophilus* and animal livers.

3-Alkyl group homologs of IPP(**2b** to **2d**) were synthesized according to our method reported previously [5, 13] except that the diphosphorylation of the corresponding alcohols was carried out by the method of Davisson *et al.* [14]. FPP synthases were purified from *B. stearothermophilus* [15], porcine liver [16], and avian liver [17] according to the methods described in the respective literatures. The incubation mixture for the thermostable FPP synthase reaction contained, in a final volume of 5 ml, 500 μmol Tris-HCl buffer (pH 8.5), 50 μmol $MgCl_2$, 250 μmol β-mercaptoethanol, 250 μmol NH_4Cl, 25 μmol KCl, 2.5 μmol IPP homologs (**2a** - **2d**), 2.5 μmol an allylic diphosphate (DMAPP or GPP), and 102 μg the recombinant FPP synthase of *B.*

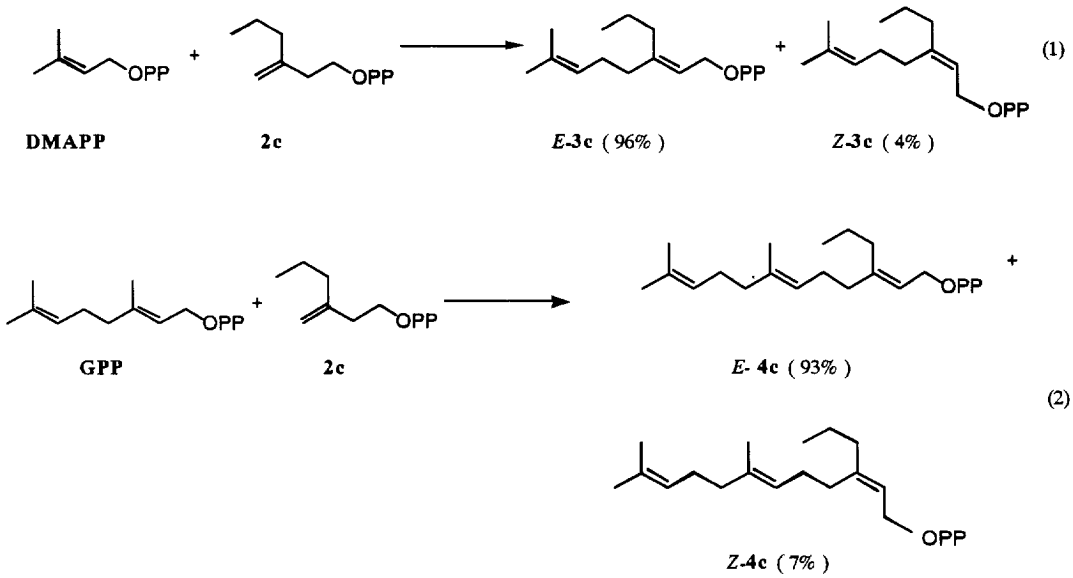
stearothermophilus [15]. The mixture was incubated at 55°C for 2 h, and the reaction mixture was treated with alkaline phosphatase, and then the homologs of prenyl alcohols, derived from the reaction products were extracted with *n*-pentane. The extracts were passed through a mini column (LiChrolut, Si 200 mg, Merck) and subjected to GC[1] or HPLC[2] analyze.

3-*n*-Propyl-but-3-enyl diphosphate (**2c**) was found to be acceptable as substrate for the FPP synthase as well as the desmethyl- (**2a**) and 3-ethyl-derivatives (**2b**). The alcohols derived from the reaction of **2c** with DMAPP eluted on GC as two peaks, a major (16.25 min) and a minor peak (16.03 min) in a relative ratio of 96 and 4%, respectively. These products were then subjected to GC-MS analysis. Both products gave similar spectra showing a molecular ion at m/z 182, corresponding to $C_{12}H_{22}O$, with fragment ions at m/z 164 $[M - 18]^+$, 149 $[C_{11}H_7]^+$, 121 $[C_9H_{13}]^+$, 93 $[C_7H_9]^+$, 79 $[C_6H_7]^+$, and 69 $[C_5H_9]^+$ (base peak), though the relative intensities of the fragment ions were different, suggesting that these products are geometrical isomers of 7-methyl-3-*n*-propylocta-2,6-dien-1-ol(**3c**-OH).

In order to determine the geometry of the double bond at the 2-position of the products, 2*E*- and 2*Z*-(**3c**-OH) were synthesized from 8-methyl-7-nonen-4-one *via* the corresponding ethyl 7-methyl-3-*n*-propylocta-2,6-dienoate by the Horner-Emmons reaction[3]. 2*E*- and 2*Z*-(**3c**-OH) had retention times on HPLC at 14.03 and 14.95 min, respectively, which were coincided with those of the major and minor products. Consequently, it was proved that the thermostable FPP synthase catalyzed the condensation of **2c** with DMAPP to produce 2*E*- and 2*Z*-7-methyl-3-*n*-propylocta-2,6-dienyl diphosphates (**3c**) as shown in Scheme 2-(1). No further condensation yielding farnesyl derivatives was detected.

Similarly, the alcohols derived from the reaction of **2c** with GPP gave two peaks on

[1] The GC (Hitachi G 5000) was carried out at a linear programmed temperature at a rate of 5 °C/min from 90 to 210 °C on a "Quadorex" column 25 m x 0.25 mm (Tokyo Kasei). [2] The conditions of HPLC were the same as described [11]. [3] The Horner-Emmons reactions of 8-methyl-7-nonen-4-one, which was obtained by the reaction of ethyl 3-oxohexanoate with dimethylallyl chloride followed by decarboxylation, with diethyl ethoxycarbonylmethylphosphonate gave ethyl 7-methyl-3-*n*-propylocta-2,6-dienoate. Then, the ester was reduced with DIBAL to give *E*- and *Z*-7-methyl-3-*n*-propylocta-2,6-dien-1-ol, which were separated by flash silicagel chromatography. ^{1}H NMR of *E*-7-methyl-3-*n*-propylocta-2,6-dien-1-ol (400 MHz,$CDCl_3$,TMS, J/Hz) δ; 0.86, 3H, t. J= 7.3, 1.40, 2H, sextet J=7.6, 1.47, 1H, br.s., 1.61, 3H, s., 2.01, 2H, t. J=7.4, 2.05, 2H, m., 2.10, 2H, m., 4.14, 2H, t. J=6.4, 5.11, 1H, ddd. J=1.2,1.5, 5.5, and 5.42, 1H, t. J=6.8. ^{1}H NMR of *Z*-isomer δ: 0.90, 3H, t. J=7.3, 1.34,1H, br.s., 1.44, 2H, sextet J=7.5, 1.60, 3H, s., 2.01, 2H, t. J=7.4, 2.06, 4H, m., 4.12, 2H, d. J=7.1, 5.11, 1H, ddd. J=1.2,1.5, 5.9, and 5.42, 1H, t. J=7.1. ^{13}C NMR of *E*-7-methyl-3-*n*-propylocta-2,6-dien-1-ol(100 MHz, $CDCl_3$, TMS) δ: 13.98, 17.63, 21.83, 25.61, 26.60, 32.44, 36.67, 59.10, 123.66, 123.91, 131.63, and 143.61. ^{13}C NMR of *Z*-isomer; δ: 13.87, 17.60, 21.02, 25.60, 27.03, 30.30, 38.79, 59.02, 123.83. 123.90, 132.27, and 143.53.



Scheme 2. *B. Stearothermophilus* FPP synthase reaction with **2c**.

GC, showing major and minor ones at 27.81(93%) and 27.55 min (7%), respectively. The MS spectrum of the major material showed a molecular ion at m/z 250, corresponding to $C_{17}H_{30}O$, and fragment ions at m/z 232 $[M - 18]^+$, 189 $[C_{14}H_{21}]^+$, 147 $[C_{11}H_{15}]^+$, 121 $[C_9H_{13}]^+$, 93 $[C_7H_9]^+$, and 69 $[C_5H_9]^+$ (base peak), indicating that the alcohol has the 7,11-dimethyl-3-*n*-propyldodeca-2,5,10-trien-1-ol (**4c**-OH) structure. The amount of the minor product was too small for GC-MS analysis. To determine the geometry of the double bond at the 2-position of these products, authentic 2*E*- and 2*Z*-**4c**-OH were synthesized from 8,12-dimethyltrideca-7,11-dien-4-one by a similar method described above. The synthesized *E*- and *Z*-isomers gave retention times on GC at 27.77 and 27.50 min, respectively, which were coincided with those of the major and minor products. Thus, it was proved that the bacterial FPP synthase catalyzes the condensation of **2c** with GPP to produce *E*- and *Z*-**4c**. The reactivity was three times smaller than that with DMAPP [Scheme 2-(2)].

3-*n*-Butylbut-3-enyl diphosphate (**2d**) also reacted with DMAPP to give 2*E*-3-*n*-butyl-7-methylocta-2,6-dienyl diphosphate, but the yield was poor. No product was detected in the reaction between **2d** and GPP.

In order to compare the substrate specificity of this enzyme with those of animal FPP synthase, we examined the reactions of the 3-alkyl-IPP homologs (**2a - 2d**) catalyzed by the FPP synthases from avian and porcine liver under optimal conditions for the liver enzymes. As a result, the IPP homologs showed much higher reactivities with the thermostable

enzyme than the liver enzymes, as shown in Table 1.

Table 1
Relative yield of the products derived from IPP homologs (**2b-2d**) with DMAPP or GPP in the enzymatic reaction catalyzed by FPP synthases from *B. stearothermophilus*, avian liver, and porcine liver.

IPP homologs	Products[a]	Production (%) *B. stearothermophilus* FPP synthase	avian liver FPP synthase	porcine liver FPP synthase
reaction with GPP				
IPP	FPP	100	100	100
2b	**4b'**	48	25	27
2c	**4c**	10[b]	n. d.	1.8
2d	**4d**	n. d.	n. d.	n. d.
reaction with DMAPP				
IPP	FPP	105	112	101
2b	**3b**	68	1	n. d.
	4b	40	6.7	10
2c	**3c**	29[c]	1	1.4
2d	**3b**	4	n. d.	n. d.

a. The products were enzymatically converted to the corresponding alcohols and analyzed by GC.
b. Total yield of the *E*- and *Z*-isomers, which were produced in the ratio of 93: 7.
c. Total yield of the *E*- and *Z*-isomers, which were produced in the ratio of 96: 4.
n. d., not detected.
Each value represents the mean of at least three determinations.

It is interesting that the bacterial enzyme catalyzes the formation of both 2*E*- and 2*Z*-isomers of GPP- and FPP homologs, when (**2c**) is employed in place of IPP. It seems possible that **2c** binds to the IPP binding site of the enzyme with distortion because of the bulky *n*-propyl group so that the subtraction of the proton at the 2-position results in the formation of the 2*Z*-isomer as well as the 2*E*-isomer. This is reminiscent of the FPP synthase reaction with 4-methylpent-4-enyl diphosphate, which produces exclusively *Z*-isomers of homofarnesol by the action of porcine liver FPP synthase [18].

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