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Substrate specificity of thermostable farnesyl diphosphate synthase with respect to 4-alkyl group homologs of isopentenyl diphosphate

Masahiko Nagaki [a,*], Hiroto Yamamoto [a], Ayumi Takahashi [a], Yuji Maki [b], Junji Ishibashi [b], Tokuzo Nishino [c], Tanetoshi Koyama [d]

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

[b] *Department of Biological and Material Chemistry, Faculty of Science, Yamagata University, Koshirakawa-cho, Yamagata 990-8560, Japan*

[c] *Department of Biochemistry and Engineering, Faculty of Engineering, Tohoku University, Aoba, Aoba-ku, Sendai 980-8579, Japan*

[d] *Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Katahira, Aoba-ku, Sendai 980-8577, Japan*

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Abstract

In order to investigate substrate spcificity of *Bacillus stearothermophilus* farnesyl diphosphate synthase (FPS), we examined the reactivity of 4-alkyl group homologs of isopentenyl diphosphate (IPP).

The enzymatic reactions of the 4-methyl homologs, (*E*)-3-methylpent-3-enyl diphophates (**1a**) and (*Z*)-3-methylpent-3-enyl diphophates (**1b**) with geranyl diphosphate (GPP) gave 4-methylfarnesyl diphosphates (**2a** and **2b**), respectively. The stereochemistry of each aldehyde derived from **2a** or **2b** was determined by CD spectrometry to be (*S*)-4-methylfarnesal or (*R*)-4-methylfarnesal, respectively. Similarly, **1a** reacted with dimethylallyl diphosphate (DMAPP) to give a mixture of (4*S*)-4-methylgeranyl diphosphates (**3a**) and (4*S*,8*S*)-4,8-dimethylfarnesyl diphosphates (**4a**). The (*Z*)-isomer **1b** also reacted with DMAPP to give the corresponding enantiomers with (4*R*)- and (4*R*,8*R*)-configurations.

On the other hand, reactions of the 4-ethyl homologs, (*E*)-3-methylhex-3-enyl diphosphates (**1c**) and (*Z*)-3-methylhex-3-enyl diphosphates (**1d**) with GPP gave two types of 4-ethylfarnesyl diphosphates. Reactions of **1c** or **1d** with DMAPP also gave two types of 4-ethylgeranyl- and 4,8-diethylfarnesyl diphosphates.

Meanwhile, reaction of the 4-propyl homologs, (*E*)-3-methylhept-3-enyl diphosphates (**1e**) and (*Z*)-3-methylhept-3-enyl diphosphates (**1f**) with GPP gave two types of 4-propylfarnesyl diphosphates. Reactions of **1e** or **1f** with DMAPP gave only two types of the 4-propyl GPPs.

However, neither (*E*)-3-methyloct-3-enyl diphosphates (**1g**) or (*Z*)-3-methyloct-3-enyl diphosphates (**1h**), nor (*E*)-4-bromo-3-methylbut-3-enyl diphosphate (**1i**) or (*Z*)-4-bromo-3-methylbut-3-enyl diphosphate (**1j**) was acceptable as a substrate for the thermophilic FPS at all. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Thermostable farnesyl diphosphate synthase; Substrate specificity; Homologs of isopentenyl diphosphate; Prenyltransferase; Chiral compound

1. Introduction

Prenyltransferase reaction proceeds with the condensation of an allylic prenyl diphosphate with isopentenyl diphosphate (IPP) stereospecifically and

* Corresponding author. Tel.: +81-172-39-3947; fax: +81-172-39-3947.
E-mail address: nagaki@cc.hirosaki-u.ac.jp (M. Nagaki).

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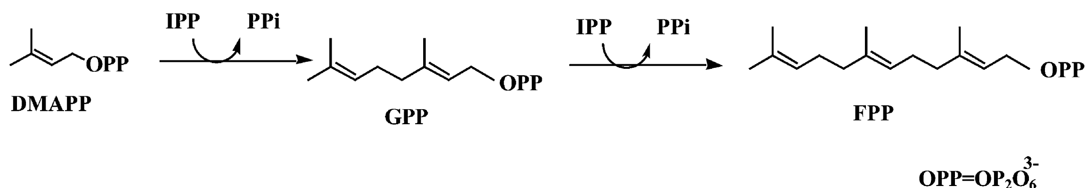


Scheme 1. FPP synthase reaction.

the condensation terminates precisely until the elongation of prenyl chain reaches certain lengths depending on the specificities of the enzyme [1–3].

Farnesyl diphosphate synthase (FPS) [EC 2.5.1.10], catalyzes the consecutive condensation of IPP with dimethylallyl diphosphate (DMAPP) or with geranyl diphosphate (GPP) to give (all-(*E*))-farnesyl diphosphate (FPP) as the ultimate product (Scheme 1). FPS has been shown to be useful for the chiral syntheses of some biologically active compounds. Koyama et al. have reported the application of this enzyme from porcine liver to the syntheses of a trail pheromone of Pharaoh's ant, faranal [4,5] and of an insect juvenile hormone, 4-MeJH-I [6]. The principle of these stereospecific synthesis by use of FPS is the application of the latent stereochemistry which can be visualized only when the 4-alkyl homologs of IPP are used as the homoallylic substrates [7].

As the FPS gene for *Bacillus stearothermophilus* was cloned and an efficient overproduction system of the enzyme in *Escherichia coli* cells has been constructed [8], this paper describes the substrate specificity of the thermostable FPS with respect to 4-alkyl group homologs of the homoallylic substrate IPP to develop or explore the extent of the stereospecific reaction to produce several chiral synthons.

2. Experimental

2.1. Analysis

The prenyl alcohols which obtained by alkaline phosphatase treatment of the enzymatic reaction products were measured by HPLC. The conditions of HPLC (Hitachi type: L-6200) were similar to those previously reported [9,10].

^{1}H- and ^{13}C NMR spectra were recorded on a JEOL JNM-GX 270 FT NMR spectrometer using tetramethylsilane as an internal standard in $CDCl_3$.

The relative yields were calculated on the basis of the yield of FPP afforded from the reaction with IPP and DMAPP in the similar method as previously reported [9,10].

Identification of the prenyl alcohols derived from the enzymatic reaction, was carried out using GC–MS (JMS-AM II 50 type GCG Mass spectrometer and HP 5890 series II Gas chromatograph). The conditions of GC–MS were similar as previously reported [11].

In order to measure CD spectra, the prenyl alcohols derived from large scale incubations were oxidized by active MnO_2 to give the corresponding aldehydes according to the method previously reported [12,13]. CD spectra were measured in hexane with 2 mm light path on Jasco CD spectrograph, type J-400X.

2.2. Chemicals

2.2.1. Syntheses of IPP-homologs (*1a–j*)

3-Methylpent-3-enyl diphosphates, (*E*)-3-methylpent-3-enyl diphophates (**1a**), (*Z*)-3-methylpent-3-enyl diphophates (**1b**) were synthesized according to the method reported previously [14–17].

3-Methylhex-3-enyl diphosphates, (*E*)-3-methylhex-3-enyl diphosphates (**1c**), (*Z*)-3-methylhex-3-enyl diphosphates (**1d**) were synthesized by the Wittig reaction [18–20]. The phosphorus ylide was prepared from propyltriphenylphosphonium bromide with butyllithium. 3-Ketobutyl-*t*-butyldimethylsilyl ether, which was derived from hydroxy-2-butanone after protection of the hydroxy group, was reacted with the Wittig reagent to give a mixture of (*E*)- and (*Z*)-3-methylhex-3-enyl-*t*-butyldimethylsilyl ether quantitatively at the 2:1 ratio. The (*E*)- and (*Z*)-ethers were separated by silica gel column chromatography. ^{1}H NMR of (*E*)-3-methylhex-3-enyl-*t*-butylsilyl

ether (270 MHz, $CDCl_3$, TMS, *J*/Hz) δ: 0.05 (6H, s), 0.89 (9H, s), 0.94 (3H, t, $J = 7$), 1.61 (3H, s), 2.0 (2H, m, $J = 7$), 2.19 (2H, t, $J = 7$), 3.66 (2H, t, $J = 7$), and 5.16 (1H, t, $J = 8$). ^{13}C NMR (70 MHz, $CDCl_3$, TMS) δ: −5.3, 15.2, 18.0, 18.4, 22.7, 26.0, 31.7, 65.9, 128.5, and 131.5. GC–MS: *m/z* 228 $[M]^+$ (rel. int. 0.01%), 171 $[M-57]^+$ (49.6), 141 $[M-57-30]^+$ (8.3), and 75 (base peak). The retention time of this (*E*)-ether on GC was 5.55 min. ^{1}H NMR of the (*Z*)-isomer δ: 0.05 (6H, s), 0.89 (9H, s), 0.93 (3H, t, $J = 7$), 1.69 (3H, s), 2.0 (2H, m, $J = 7$), 2.26 (2H, t, $J = 7$), 3.63 (2H, t, $J = 7$), and 5.20 (1H, t, $J = 7$). ^{13}C NMR δ: −5.3, 15.4, 18.0, 18.4, 21.2, 26.0, 35.7, 65.9, 128.9, and 131.4. GC–MS: *m/z* 228 $[M]^+$ (rel. int. 0.01%), 171 $[M-57]^+$ (45.7), 141 $[M-57-30]^+$ (9.1), and 75 (base peak). The retention time of this ether on GC was 5.49 min.

Similarly, 3-methyhept-3-en-1-ol (**1e-OH** and **1f-OH**) and 3-methyloct-3-en-1-ol (**1g-OH** and **1h-OH**) were also synthesized by the Wittig reaction. ^{1}H NMR of **1e-OH** δ: 0.90 (3H, t, $J = 7$), 1.33 (2H, m), 1.63 (3H, s), 2.00 (2H, dd, $J = 7$), 2.26 (2H, t, $J = 6$), 3.65 (2H, ddd, $J = 1.3, 2.3, 6$), and 5.42 (1H, ddd, $J = 1.9, 5.4, 9$). ^{13}C NMR δ: 15.4, 18.0, 22.9, 30.2, 42.8, 63.9, 128.1, and 131.3. GC–MS: *m/z* 128 $[M]^+$ (rel. int. 6.9%), 110 $[M-18]^+$ (15.1), 95 $[M-18-15]^+$ (70.2), and 81 (base peak). The retention time of this alcohol on GC was 9.34 min.

^{1}H NMR of **1f-OH** (270 MHz, $CDCl_3$, TMS, *J*/Hz) δ: 0.90 (3H, t, $J = 7$), 1.32 (2H, m), 1.72 (3H, dd, $J = 1$ long range coupling), 2.00 (2H, q, $J = 7$), 2.33 (2H, t, $J = 7$), 3.67 (2H, t, $J = 7$), and 5.33 (1H, t, $J = 7$). ^{13}C NMR δ: 13.8, 18.0, 23.2, 30.1, 35.2, 63.9, 128.7, and 131.1. GC–MS: *m/z* 128 $[M]^+$ (rel. int. 6.6%), 110 $[M-18]^+$ (15.7), 95 $[M-18-15]^+$ (74.9), and 81 (base peak). The retention time on GC was 9.30 min.

^{1}H NMR of **1g-OH** (270 MHz, $CDCl_3$, TMS, *J*/Hz) δ: 0.88 (3H, t, $J = 7$), 1.24–1.32 (4H, m), 1.63 (3H, s), 2.2 (2H, m), 2.24 (2H, t, $J = 6$), 3.63 (2H, t, $J = 7$), and 5.42 (1H, dd, $J = 2, 6$). ^{13}C NMR δ: 15.2, 20.4, 22.6, 26.9, 30.5, 31.5, 63.9, 128.8, and 132.4. GC–MS: *m/z* 142 $[M]^+$ (rel. int. 3.6%), 124 $[M-18]^+$ (5.0), 109 $[M-18-15]^+$ (12.2), 95, and 81 (base peak). GC retention time, 4.23 min.

^{1}H NMR of **1h-OH** (270 MHz, $CDCl_3$, TMS, *J*/Hz) δ: 0.89(3H, t, $J = 7$), 1.27–1.33 (4H, m), 1.71 (3H, d, $J = 2$ long range), 2.03 (2H, m), 2.33 (2H, t, $J = 7$), 3.67 (2H, t, $J = 7$), and 5.33 (1H, t, $J = 7$). ^{13}C NMR (70 MHz, $CDCl_3$, TMS) δ: 14.0, 23.3, 22.7, 27.8, 31.7, 35.3, 60.8, 128.8, and 130.9. GC–MS: *m/z* 142 $[M]^+$ (rel. int. 3.3%), 124 $[M-18]^+$ (4.5), 109 $[M-18-15]^+$ (12.3), 95, and 81 (base peak). GC retention time, 4.21 min.

In the GC of these homologs, the (*Z*)-isomers have a shorter retention time than those of the corresponding (*E*)-isomers [14]. The geometry of the homologs was determined according to the general rule of the chemical shifts [14]. The chemical shift of the methyl group of the (*Z*)-isomer which combines with the double bond was shown in high magnetic field than that of the (*E*)-isomers.

The tosylates which were derived from the alcohols were converted to the corresponding diphosphates by Davisson's method [21].

2.2.2. Chemical synthesis of 4-ethylfarnesol

The Horner–Emmons reactions of (*E*)-2-oxo-3-ethyl-6,10-dimethylundeca-5,9-diene, which was obtained from the reaction of ethyl 2-ethyl-3-oxobutanoate with geranyl chloride, with diethyl-ethoxycabonylmethylphosphonate gave ethyl (2*E*,6*E*)-4-ethyl-3,7,11-trimethyldodeca-2,6,10-trienoate. The 2(*Z*)-isomer was not obtained. Then, the ester was treated with DIBAL to give (2*E*,6*E*)-4-ethyl-3,7,11-trimethyldodeca-2,6,10-trien-1-ol (4-ethylfarnesol), which was purified by silica gel column chromatography. ^{1}H NMR of 4-ethyl-3,7,11-trimethyldodeca-2,6,10-trien-1-ol δ: 0.80 (3H, t, $J = 7.4$), 1.35 (3H, m), 1.56 (3H, s), 1.58 (3H, s), 1.60 (3H, s), 1.68 (3H, d, $J = 0.8$), 2.03 (6H, m), 4.16 (2H, d, $J = 6.9$), 5.07 (3H, m), and 5.41 (1H, dd, $J = 6.8, 0.7$). ^{13}C NMR δ: 12.1, 12.6, 16.2, 17.7, 25.4, 25.7, 26.8, 32.0, 39.8, 50.9, 59.4, 123.1, 124.8, 124.9, 131.3, 135.4, and 141.7. GC–MS: *m/z* 250 $[M]^+$ (rel. int. 1.8%), 232 $[M-18]^+$ (14.2), 203 $[M-18-29]^+$ (10.2), 163 $[M-18-69]^+$ (8.9), 95 $[M-18-69-68]^+$ (64.2), and 69 $[C_5H_9]^+$ (base peak).

2.3. Purification of the thermostable FPS

According to our reported method [8], the crude cell-free extracts of *E. coli* JM109 cells harboring the expression plasmid, pEX 11 for the FPS gene from *B. stearothermophilus* ATCC10149, were heat-treated,

and then, the thermostable FPS was purified by two column chromatographic procedures.

2.4. Conditions of the enzymatic reaction

The standard reaction mixture contained, in a final volume of 2.0 ml, 200 mmol of Tris–HCl buffer (pH 8.5), 20 μmol of $MgCl_2$, 10 μmol of KCl, 100 μmol β-mercaptoethanol, 1.0 μmol of an allylic substrates (DMAPP or GPP), 1.0 μmol of an IPP-homolog to be examined, and 25 μg of the FPS of *B. stearothermophilus*.

After incubation at 55 °C for 3 h, the reaction mixture was treated with alkaline phosphatase for 5 h, and extracted with pentane and analyzed by HPLC and GC–MS [9].

Large scale incubation were carried out in 250-fold of the standard method, in order to prepare enough amount of the samples for CD spectra.

3. Results and discussion

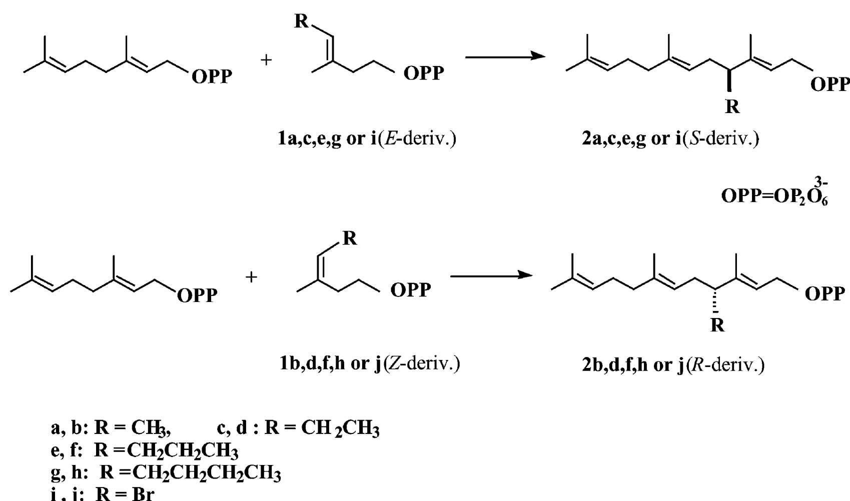
In order to explore the extent of the reactivity of artificial homoallylic substrates, we examined substrate specificity of the thermostable FPS of *B. stearothermophilus* with respect to the alkyl groups at 4-position of IPP with GPP or DMAPP as an allylic substrate.

3.1. Reaction of (*E*)- or (*Z*)-3-methylpent-3-enyl diphosphate (**1a** or **1b**) with GPP (**3**)

The reaction of (*E*)-3-methylpent-3-enyl diphosphate (**1a**) with GPP gave a product with a yield of 70.4%, which was then hydrolyzed with alkaline phosphatase to the corresponding alcohol (Scheme 2). The MS spectrum of the alcohol with a retention time on HPLC at 16.5 min, showed a molecular ion at *m/z* 236 (rel. int. 0.8%), corresponding to $C_{16}H_{28}O$, and other fragment ions were observed at *m/z* 218 $[M-18]^+$ (2.6), 149 $[M-18-69]^+$ (6.8), 81 $[M-18-69-68]^+$ (68.5), and 69 $[C_5H_9]^+$ (base peak), indicating that the product has a 4-methylfarnesol (**2a-OH**) structure [14,15].

Similarly, the prenyl alcohol derived from the reaction of the (*Z*)-isomer **1b** with GPP showed a peak on HPLC at 16.3 min (87.7% yield). The MS spectrum of the product showed a molecular ion at *m/z* 236 (rel. int. 0.9%), corresponding to $C_{16}H_{28}O$, together with fragment ions at *m/z* 218 $[M-18]^+$ (3.7), 149 $[M-18-69]^+$ (8.6), 81 $[M-18-69-68]^+$ (58.4), and 69 $[C_5H_9]^+$ (base peak), indicating that the alcohol has the 4-methylfarnesol (**2b-OH**) structure [14,15].

In order to determine the stereochemistry of the products (**2a-OH** and **2b-OH**), we oxidized the alcohols with active MnO_2 to give the corresponding



Scheme 2. Thermostable FPP synthase reaction between GPP and IPP homologs.

aldehydes, which were purified by HPLC. The 4-methylfarnesal (**2a-CHO**) which was derived from the reaction product of **1a** and GPP showed CD-spectral data of $\Delta\varepsilon_{(\lambda=247)} = +0.71 \pm 0.31$. This value indicates the stereochemistry of the 4-position to be (*S*)-configuration [12]. Thus, the enzymatic product is reasonably assigned to (4*S*)-4-methylfarnesyl diphosphate (**2a**). On the other hand, the 4-methylfarnesal derived from the product of the reaction between **1b** and GPP, followed by MnO_2 oxidation showed $\Delta\varepsilon_{(\lambda=247)} = -0.77 \pm 0.25$, demonstrating the (*R*)-configuration. Thus, the enzymatic product is assigned to be (4*R*)-4-methylfarnesyl diphosphate (**2b**). Previously, Ohnuma reported that the aldehyde from the reaction between **1a** and GPP by use of porcine liver FPS showed $\Delta\varepsilon_{(\lambda=247)} = +0.90 \pm 0.29$, and the aldehyde from **1b** and GPP using porcine liver FPS showed $\Delta\varepsilon_{(\lambda=247)} = -0.71 \pm 0.31$ [13]. Then, they showed that the enantiomeric excesses of the products by use of the liver FPS were both almost 100% [12]. Judging from the coincidence within experimental errors of the figures of the 4-methylfarnesals derived from the bacterial enzymatic product with the counterpart aldehydes from the products by the porcine liver enzyme, it is reasonable to assign that the enantiomeric excess of the 4-methylfarnesyl diphosphate synthesized by use of *B. stearothermophilus* FPS is almost 100%.

These results indicate that the (*S*)- or (*R*)-configuration can also be prepared selectively by employing **1a** or **1b** as a homoallylic substrate, respectively, in the thermostable FPP synthase reaction with GPP.

*3.2. Reaction of (E)- or (Z)-3-methylpent-3-enyl diphosphate (**1a** or **1b**) with DMAPP (**2**)*

The alcohols derived from the reaction of **1a** with DMAPP showed two peaks on HPLC at retention times of 17.4 (66.7% yield) and 15.1 min (19.3% yield), which were subjected to GC–MS analysis. The MS spectrum of the former alcohol showed a molecular ion at *m/z* 168 (rel. int. 1.7%), corresponding to $C_{11}H_{20}O$, with fragment ions at *m/z* 150 $[M-18]^+$ (0.7), 137 $[M-31]^+$ (20.6), 81 $[M-18-69]^+$ (34.9), and 69 $[C_5H_9]^+$ (base peak). Thus the alcohol has a 4-methylgeraniol structure [7]. The MS spectrum of the latter alcohol showed a molecular ion at *m/z* 250 (rel. int. 0.2%), corresponding to $C_{17}H_{30}O$, with fragment ions at *m/z* 232 $[M-18]^+$ (0.6), 219 $[M-31]^+$ (0.6), 189 $[M-18-43]^+$ (2.5), 163 $[M-18-69]^+$ (7.2), 95 $[M-18-69-68]^+$ (25.5), and 69 $[C_5H_9]^+$ (base peak), indicating the alcohol has a 4,8-dimethylfarnesol structure [7]. These results are similar to those from the reactions with porcine liver FPS [7]. Although we have not determined the configurations of the two asymmetric centers constructed by the bacterial FPS reaction, it is reasonable to assign that they were (4*S*)-4-methylgeraniol (**3a-OH**) and (4*S*,8*S*)-4,8-dimethylfarnesol (**4a-OH**), respectively, because the reaction between **1a** or **1b** and GPP was proved to proceed, in Section 3.1, in the same stereochemical manner to that of the porcine liver FPS (Scheme 3).

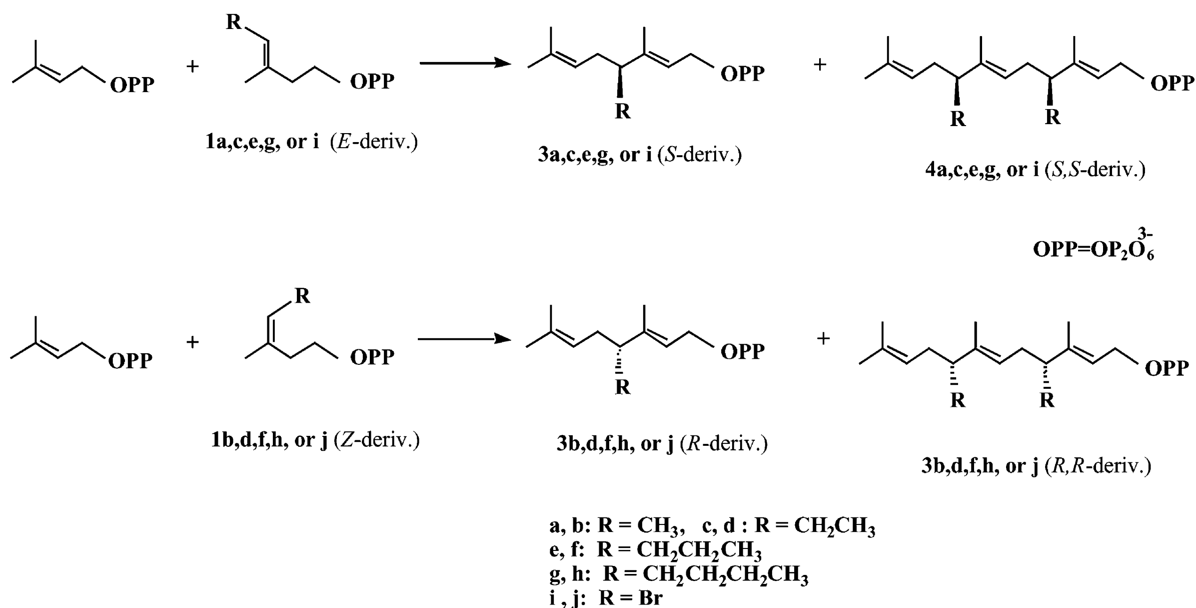
In the similar manner, the two alcohols derived from the reaction products of **1b** with DMAPP eluted in two peaks at 17.3 min (35.3% yield) and 15.0 min (17.6% yield) on HPLC.

The MS spectrum of the major product showed a molecular ion at *m/z* 168 (rel. int. 1.29%), corresponding to $C_{11}H_{20}O$, together with fragment ions at *m/z* 150 $[M-18]^+$ (2.1), 81 $[M-18-69]^+$ (36.4), and 69 $[C_5H_9]^+$ (base peak). Thus the prenyl alcohol has a 4-methylgeraniol structure [7]. The MS spectrum of the minor product showed a molecular ion at *m/z* 250 (rel. int. 0.1%), corresponding to $C_{17}H_{30}O$, with fragment ions at *m/z* 232 $[M-18]^+$ (0.6), 163 $[M-18-69]^+$ (8.3), 95 $[M-18-69-68]^+$ (17.1), and 69 $[C_5H_9]^+$ (base peak), indicating a 4,8-dimethylfarnesol structure [7]. These prenyl alcohols were also reasonably assigned to (4*R*)-4-methylgeraniol (**3b-OH**) and (4*R*,8*R*)-4,8-dimethylfarnesol (**4b-OH**), respectively [7].

*3.3. The reaction of (E)- or (Z)-3-methylhex-3-enyl diphosphate (**1c** or **1d**) with GPP*

Both of the 4-ethyl homolog of IPP, **1c** and **1d** were found to be also acceptable as substrates for the thermophilic FPS in the reaction with GPP.

The prenyl alcohol derived from the reaction of **1c** with GPP showed a peak at 13.5 min on HPLC. The MS spectrum of the prenyl alcohol showed a molecular ion at *m/z* 250 (rel. int. 0.7%), corresponding to $C_{17}H_{30}O$, with fragment ions at *m/z* 232 $[M-18]^+$ (17.2), 203 $[M-18-29]^+$ (9.3), 163 $[M-18-$



Scheme 3. Thermostable FPP synthase reaction between DMAPP and IPP homologs.

69]$^+$ (4.7), and 69 $[C_5H_9]^+$ (base peak), indicating the 4-ethylfarnesol structure (**2c-OH**). These results showed good correspondence with those of the authentic sample, (*R*,*S*)-4-ethylfarnesol synthesized chemically as well as the 4-ethylfarnesol derived from the similar reaction by the liver enzyme [15].

We have not yet determined the absolute structures of the ethylfarnesol. If the stereochemistry of the enzymatic reaction with 4-ethyl homologs of IPP **1c** is similar to that with the 4-methyl homologs of IPP **1a**, this product can be assigned to (4*S*)-4-ethylfarnesyl diphosphate (**2c**).

On the other hand, the reaction product of **1d** with GPP showed a similar retention time on HPLC to that of the authentic 4-ethylfarnesol. Furthermore, the MS spectrum of the product also showed similar cleavage patterns to those of the authentic sample. If the stereochemical manner of the enzymatic reaction of GPP with **1d** is similar to that with **1b**, this product may be assigned to (4*R*)-4-ethylfarnesol (**2d-OH**).

It is noteworthy that the production yields of the reaction with 4-ethyl-IPP homologs were quite different between the **1c** and **1d**. The (*E*)-isomer gave the product **2c** more than 40-times as that of the (*Z*)-isomer did. Recently, we have reported that the (*E*)-homologs of IPP, **1a** and **1c** were moderately accepted as substrates for *Micrococcus luteus* B-P 26 undecaprenyl diphosphate synthase, but the (*Z*)-isomers, **1b** and **1d** were not accepted at all [22]. It is very interesting to examine the difference in susceptibilities of these prenyl chain elongating enzymes with respect to the geometric difference of substrates.

3.4. The reaction of (E)- or (Z)-3-methylhex-3-enyl diphosphate (1c or 1d) with DMAPP

The FPS reaction of **1c** with DMAPP gave two condensation products, whose alcohols obtained by alkaline phosphatase treatment showed retention times on HPLC at 9.2 and 15.0 min, respectively.

The MS spectrum of the former showed a distinct dehydration ion $[M-18]^+$ at *m/z* 260 (rel. int. 2.2%), corresponding to $C_{19}H_{22}$, although the molecular ion was obscure. The other fragment ions at *m/z* 191 $[M-18-69]^+$ (1.8), and 69 $[C_5H_9]^+$ (base peak), clearly indicated that the alcohol has a 4,8-diethylfarnesol (**4c-OH**) structure. The MS spectrum of the latter showed a molecular ion at *m/z* 182 (rel. int. 0.2%), corresponding to $C_{12}H_{22}O$, and other fragment ions were observed at *m/z* 164 $[M-18]^+$ (7.5), 135

$[M-18-29]^+$ (8.6), 95 $[M-18-29-69]^+$ (19.8), and 69 $[C_5H_9]^+$ (base peak), indicating that the alcohol has a 4-ethylgeraniol (**3c-OH**) structure. Thus, the FPS reaction of **1c** with DMAPP was found to give condensation products with one or two molecules of **1c**.

These products may be assigned to (4*S*,8*S*)-(all-(*E*)) -4,8-diethyl-3,7,11-trimethyldodeca-2,6,10-trien-1-ol and (4*S*)-(all-(*E*))-4-ethyl-3,7-dimethylocta-2,6-dien-1-ol, if the similar stereochemical manner of the enzymatic reaction could be still maintained with the IPP homolog having 4-ethyl group.

On the other hand, the condensation alcohols derived from the enzymatic reaction of **1d** with DMAPP showed a similar elution pattern on HPLC to that of **3c**- and **4c-OH**. The MS spectrum of the former showed a molecular ion at *m/z* 278 (rel. int. 0.3%), corresponding to $C_{19}H_{34}O$, with other fragment ions at *m/z* 260 $[M-18]^+$ (0.1), 191 $[M-18-69]^+$ (1.7), and 69 $[C_5H_9]^+$ (base peak), indicating that the alcohol has a 4,8-diethylfarnesol (**4d-OH**) structure. Meanwhile, the MS spectrum of the latter showed a molecular ion at *m/z* 182 (rel. int. 0.5%), corresponding to $C_{12}H_{22}O$, together with other fragment ions at *m/z* 164 $[M-18]^+$ (1.2), 135 $[M-18-29]^+$ (5.8), and 69 $[C_5H_9]^+$ (base peak), indicating a 4-ethylgeraniol (**3d-OH**) structure. These products may be also assigned to (4*R*,8*R*)-(all-(*E*))-4,8-diethyl-3,7,11-trimethyldodeca-2,6,10-trien-1-ol and (4*R*)-(all-(*E*))-4-ethyl-3,7-dimethylocta-2,6-dien-1-ol, respectively, if the similar stereochemical manner of the enzymatic reaction maintained.

*3.5. The reaction of (E)- or (Z)-3-methylhept-3-enyl diphosphate (**1e** or **1f**) with GPP or DMAPP*

The thermostable FPS reaction of (*E*)- or (*Z*)-3-methylhex-3-enyl diphosphate (**1e** or **1f**) as a substrate with GPP was found to proceed, however, the yield was less than 4% as shown in Table 1.

The prenyl alcohol derived from the reaction of **1c** with GPP showed a peak on HPLC at 13.4 min, which was then subjected to GC–MS analysis. Though the molecular ion was ambiguous, the dehydration ion, $[M-18]^+$ was observed distinctly at *m/z* 246 (rel. int. 2.7%), corresponding to $C_{18}H_{30}$, and fragment ions at 203 $[M-18-43]^+$ (7.9), 177 $[M-69]^+$ (5.8), 134 $[M-18-43-69]^+$ (10.3), and 69 $[C_5H_9]^+$ (base peak), indicating a 4-propylfarnesol structure (**2e-OH**). If the stereochemistry of the enzymatic condensation with 4-propyl homologs of IPP **1e** is similar to that with 4-methyl homologs of IPP **1a**, this product could be assigned to (4*S*)-4-propylfarnesyl diphosphate (**2e**).

Table 1
Relative yield of the products derived from IPP homologs with allylic substrates in the enzymatic reaction catalyzed by FPP synthase

IPP homologs	Products[a]	Production (%)[b] FPS from *B. stearothermophilus*
Reaction with GPP		
IPP	FPP	100
1a	**2a**	82.6
1b	**2b**	77.9
1c	**2c**	25.4
1d	**2d**	0.6
1e	**2e**	4.0
1f	**2f**	1.6
1g	**2g**	n.d.
1h	**2h**	n.d.
1I	**2I**	n.d.
1j	**2j**	n.d.
Reaction with DMAPP		
IPP	FPP	100
1a	**3a**	66.7
	4a	19.3
1b	**3b**	35.3
	4b	17.6
1c	**3c**	19.7
	4c	2.3
1d	**3d**	0.7
	4d	2.3
1e	**3e**	0.6
	4e	n.d.
1f	**3f**	0.3
	4f	n.d.
1g	**3g**	n.d.
	4g	n.d.
1h	**3h**	n.d.
	4h	n.d.
1I	**3i**	n.d.
	4i	n.d.
1j	**3j**	n.d.
	4j	n.d.

n.d.: not detected.
[a] The products were enzymatically converted to the corresponding alcohols and analyzed by HPLC.
[b] Each value represents the mean of at least three determinations.

Similarly, the reaction product of **1f** with GPP showed a similar retention time on HPLC. Furthermore, the MS spectrum of the product showed similar cleavage pattern, at *m/z* 246 (5.0%), 203 (10.6), 177 (4.1), 134 (5.1), and 69 (base peak). Then the product could be also assigned to (4*R*)-4-propylfarnesyl diphosphate (**2f**).

On the other hand, the thermophilic FPS reaction of (*E*)- or (*Z*)-3-methylhex-3-enyl diphosphate with DMAPP was found to proceed the elongation stopped only with one molecule of the IPP homolog. The alcohol derived from the product of FPS reaction of **1e** with DMAPP gave a peak on HPLC at 14.2 min. The MS spectrum of the product showed a molecular ion at *m/z* 196 (rel. int. 2.1%), corresponding to $C_{13}H_{24}O$, and other fragment ions were observed at *m/z* 178 $[M-18]^+$ (8.9), 153 $[M-43]^+$ (4.4), 109 $[M-18-69]^+$ (4.4), 84 $[M-43-69]^+$ (23.2), and 69 $[C_5H_9]^+$ (base peak), indicating that the alcohol has a 4-propylgeraniol (**3e-OH**) structure. It may be assigned to (4*R*)-3,7-dimethyl-4-propylocta-2,6-dien-1-ol.

*3.6. The reaction of (E)- or (Z)-3-methyloct-3-enyl diphosphate (**1g** or **1h**), or of (E)- or (Z)-4-bromo-3-methylbut-3-enyl diphosphate (**1i** or **1j**) with GPP or DMAPP*

Neither (*E*)- or (*Z*)-3-methyloct-3-enyl diphosphate (**1g** or **1h**), nor (*E*)- or (*Z*)-4-bromo-3-methylbut-3-enyl diphosphate (**1i** or **1j**) was acceptable as a substrate for thermophilic FPS at all.

Table 1 shows the relative yields of the products derived from IPP homolog (**1a** or **1b**) with DMAPP or GPP in the thermostable FPS reaction, showing approximately equal yields to that with the natural substrate, IPP. However, the yields of the products derived from **1c–f** with DMAPP or GPP were extremely less than those from **1a** or **1b**.

The thermostable FPS seems more useful for the syntheses of farnesol homologs having a chiral center at 4-position than the porcine liver enzyme [15].

4. Conclusions

Farnesol homologs having an alkyl groups at 4-position with (*S*)- or (*R*)-configuration can be prepared selectively by employing **1a** (**1c** or **1e**) or **1b** (**1d** or **1f**) as a homoallylic substrate, respectively, in the enzymatic reaction by thermostable FPS. It is very interesting that **1a** or **1b** showed high relative yields, however, homologs having large 4-alkyl group (**1c–f**) showed low yields.

From a viewpoint of synthetic application of enzymatic reaction, these products as chiral synthons seem to have an advantage over syntheses of biologically active substances such as pheromones and hormones.

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