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Substrate specificities of farnesyl diphosphate synthases of *Bacillus stearothermophilus* and porcine liver with allylic substrate homologs having vinyl or ethynyl group

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ABSTRACT

To investigate substrate specificities of farnesyl diphosphate synthases from porcine liver and *Bacillus stearothermophilus*, we have examined the reactivities of vinyldimethylallyl and ethynyldimethylallyl diphosphates as allylic substrate homologs.

The reaction of vinyldimethylallyl diphosphate with isopentenyl diphosphate by farnesyl diphosphate synthase of porcine liver gave vinylgeranyl and vinylfarnesyl diphosphates, which shows that the reaction stopped at the single or double condensation of isopentenyl diphosphate, respectively. However, the similar reaction by the use of wild-type farnesyl diphosphate synthase of *B. stearothermophilus* gave vinylfarnesyl diphosphate, exclusively.

On the other hand, the reaction of *Z*-ethynyldimethylallyl diphosphates with isopentenyl diphosphate by the use of wild-type of farnesyl diphosphate synthase of *B. stearothermophilus* gave *Z*-ethynylfarnesyl diphosphate, as the sole product. Moreover, a mutated farnesyl diphosphate synthase (Y81D) reaction of *Z*-ethynyldimethylallyl diphosphates with isopentenyl diphosphate gave three kinds of products: ethynylgeranyl, ethynylfarnesyl, and ethynylgeranylgeranyl diphosphates. Using wild-type of farnesyl diphosphate synthase of *B. stearothermophilus*, the reaction of *E*-ethynyldimethylallyl diphosphate with isopentenyl diphosphate gave only *E*-ethynylfarnesyl diphosphate as double condensation product.

1. Introduction

More than 30,000 structurally different isoprenoids, such as steroids, carotenoids, prenyl side-chain of quinones, prenyl proteins, and natural rubber, exist in nature. In a biosynthetic pathway, linear prenyl diphosphates are common structures as a precursor of isoprenoids, as shown in Scheme 1 [1–4].

Prenyltransferases catalyze the sequential condensation of isopentenyl diphosphate (IPP) with an allylic diphosphate such as dimethylallyl diphosphate (DMAPP) to give linear prenyl diphosphates. There are many prenyl diphosphate synthases which have different specificities with respect to the chain length and the geometry of the double bonds of the isoprene unit added in the products.

Prenyltransferases can be classified into two major families, *E*- and *Z*-prenyl chain elongating enzymes. Furthermore, they can be subdivided into three kinds of groups, short chain—(I), medium chain—(II), and long chain-elongating enzymes—(III), as shown in Table 1 [5–7].

E-farnesyl diphosphate synthase [EC 2.5.1.10] is included in a group of *E*-short chain elongating enzymes, which catalyze the condensation of isopentenyl diphosphate with dimethylallyl diphosphate or geranyl diphosphate (GPP) to give (all-*E*)-farnesyl diphosphate (FPP) as the ultimate product, as shown in Scheme 2.

We have recently reported the reactivities of some allylic substrate homologs such as 8-hydroxygeranyl-, 8-methoxygeranyl-, 8-propoxygeranyl-, and 4-methoxymethoxydimethylallyl diphosphates by the use of wild and mutated types of *Bacillus stearothermophilus* farnesyl diphosphate synthases [8,9].

When modified FPP synthase (Y81D or Y81S) in which tyrosine-81 (Y81) was replaced with such a smaller, hydrophilic amino acid residues were incubated with aspartate (D) or serine (S), the longer prenylchain product was obtained. We observed that the FPP synthase acted like a geranylgeranyl diphosphate synthase. Therefore, we imagined that some non-natural products, which could be converted into some useful synthons, might be obtained by the

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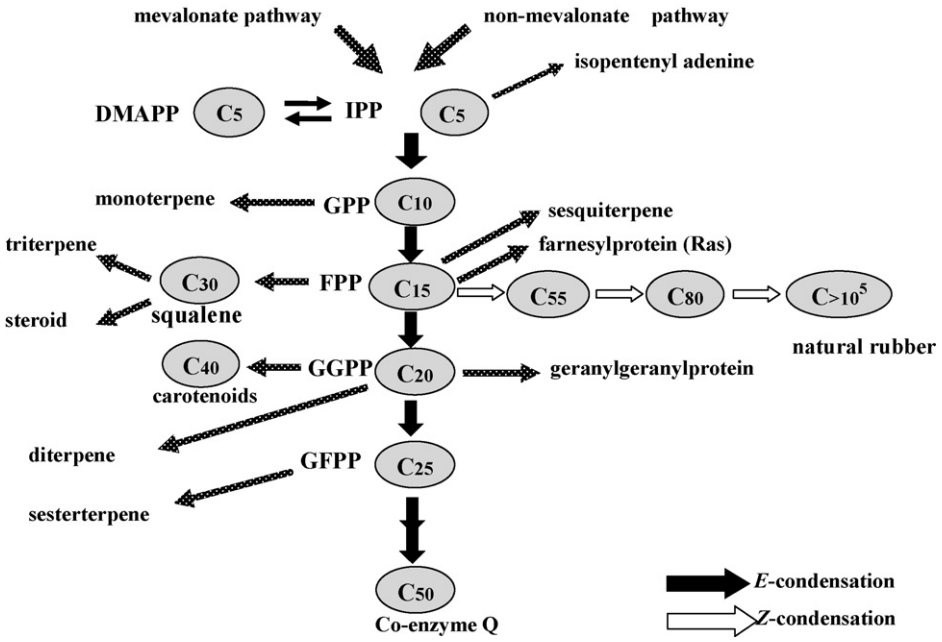
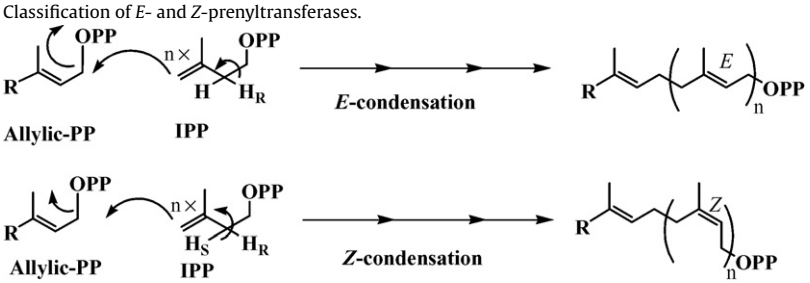
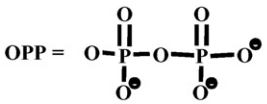


Scheme 1. The biosynthesis of isoprenoids.

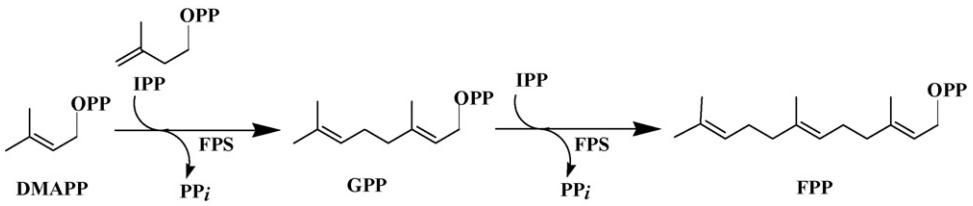
Table 1
Classification of *E*- and *Z*-prenyltransferases.





Group	P-transferase	Product	Structure	Activated factor
E-prenyl chain elongation				
E-I	GPS, FPS	C_{10}, C_{15}	Homodimer	
E-II	HexPS, HepPS	C_{30}, C_{35}	Heterodimer	
E-III	OctPS, SPS	C_{40}, C_{45}	Homodimer	Carrier protein
Z-prenyl chain elongation				
Z-I	FPS	C_{15}	Homodimer	
Z-II	DPS, UPS	C_{50}, C_{55}	Homodimer	Lipid (Triton X-100)
Z-III	DeDolPS, rubberS	C_{75-120}, $C_{>5000}$	Homodimer	

GPS, geranyl diphosphate synthase; FPS, farnesyl diphosphate synthase; HexPS, hexaprenyl diphosphate synthase; HepPS, heptaprenyl diphosphate synthase; OctPS, octaprenyl diphosphate synthase; SPS, solanesyl diphosphate synthase; DPS, decaprenyl diphosphate synthase; UPS, undecaprenyl diphosphate synthase; DeDolPS, dehydrodolichyl diphosphate synthase; rubberS, natural rubber synthase.



Scheme 2. Farnesyl diphosphate synthase (FPS) reactions of dimethylallyl diphosphate with isopentenyl diphosphate.

mutated enzymatic reaction with some artificial homologs having multiple bonds.

In this paper, we report substrate specificities of porcine liver FPP synthase and the wild and mutated type of *B. stearothermophilus* FPP synthases with respect to three kinds of allylic substrates, vinyldimethylallyl diphosphate (vinylDMAPP) and *Z*- (or *E*)-ethynyldimethylallyl diphosphate (*Z*- (or *E*)-ethynylDMAPP).

2. Experimental

2.1. Analysis

The prenyl alcohols produced by alkaline phosphatase treatment of the products from enzymatic reactions were measured by HPLC. The HPLC conditions using a Hitachi type L-6000, equipped with Hitachi L-7420 (LaChrom) type UV–vis detector with a ChromatoDAQ II (ULVAC) and with a LichroCART (Merck) column with the solvent mixture of hexane:2-propanol (80:1 (A) and 40:1 (B)), were similar to those previously reported [8–11].

Identification of the reaction products derived from enzymatic reactions was carried out using GC–MS, a JMS-AM II 50 type GCG mass spectrometer connected with HP 5890 series II gas chromatograph equipped with Ultra-alloy-1 (S). The column temperature was programmed from 90 °C to 280 °C with a linear gradient of temperature increase at a rate of 15 °C/min and then held at 280 °C for 3 min. The yields of FPP synthase reactions were determined as relative to those of FPP derived from IPP and GPP. Identification of the products derived from reactions was carried out using LC–MS (Hitachi NanoFrontier LD). The HPLC analyses were performed on a Hitachi ELITE LaChrom HPLC system equipped with a pumping system (L-2100), a column oven (L-2300), and a UV–vis detector coupled with EZChromElite Windows XP software. The samples were separated on an ODS column (GL Science Inertsil ODS-3, 33 mm × 2.1 mm) as eluent A (0.1% formic acid in acetonitrile) and B (0.1% formic acid in distilled water) at a flow rate of 0.2 mL/min. The gradient elution started with 70% acetonitrile (30% water) and reached 100% acetonitrile in 12 min. The LC effluent was introduced into the ESI source. The mass spectra were acquired using the Hitachi NanoFrontier LD spectrometer with an ESI source. Nitrogen was used as the sheath and auxiliary gas and helium was used as the collision gas. The ESI MS spectra were acquired in positive ion modes. For full scan MS analysis, the spectra were recorded in the range of *m*/*z* 100–2000.

The IR spectra were taken using a Hitachi 260-10 and a BIO-RAD FTS-30. The NMR spectra were recorded on a JEOL JNMGX 270 FT NMR and JEOL JNM-ECA 500 FT NMR spectrometers using TMS as an internal standard in $CDCl_3$.

2.2. Chemicals

2.2.1. Syntheses of (2Z)-3-methyl-2,4-pentadienyl diphosphate

A solution of (*Z*)-3-methyl-2-penten-4-yn-1-ol (*Z*-ethynylDMAOH, 0.30 g, 3.1 mmol) in methanol was hydrogenated on Lindlar catalyst (wet ca. 0.2 g) with a few drops of quinoline at room temperature. After 3 h the catalyst was filtrated and purified by small silica gel column (3 cm × 40 cm) chromatography (eluent: pentane/ether = 3/1), and gave (*Z*)-3-methyl-2,4-pentadien-1-ol (vinylDMAOH, 0.28 g, 2.8 mmol, yield: 94%). The mass spectrum of the alcohol with a retention time on GC at 2.18 min, showed a molecular ion at *m*/*z* 98 (rel. int. 20.8%), corresponding to $C_6H_{10}O$, together with fragment ions at *m*/*z* 80 (base peak) and 65 (66). ^{1}H NMR ($CDCl_3$, TMS) of it was as follows: δ 1.86 (3H, s), 4.28 (2H, d J = 6.0 Hz), 5.15 (1H, d J = 11.0 Hz), 5.27 (1H, d J = 11.0 Hz), 5.60 (1H, t J = 7.2 Hz), and 6.75 (1H, d J = 11.0 Hz). ^{13}C NMR (DEPT) was as follows: δ 58.0 (CH_2), 129.1 (CH), 134.9 (C), 133.2 (CH), 115.2 (CH), and 19.7 (CH_3). The IR spectrum (ν_{max}/cm^{-1}) showed characteristic absorptions at 3400 (ν_{O-H}), and 1680–1625 ($\nu_{C=C}$).

The (*Z*)-methyl-2,4-pentadienyl chloride (vinylDMACl, 2.1 mmol) was prepared by chlorination with N-chlorosuccinimide (11 mmol), dimethyl sulfide and vinylDMAOH essentially by the method of Corey et al. [12]. Then, the chloride was converted to the corresponding diphosphate by Davisson's method [13].

2.2.2. Synthesis of (Z)- or (E)-3-methyl-2-penten-4-yl diphosphate

The (*Z*)- and (*E*)-3-methyl-2-penten-4-yn-1-ol were purchased from Fluka (Buchs SG). These alcohols were also converted to the corresponding diphosphate esters according to the above-mentioned method [13].

2.3. Purification of FPP synthases of B. stearothermophilus and porcine liver

We purified wild-type FPP synthase cloned from *B. stearothermophilus* ATCC 10149, and the site-directed mutated type FPP synthases (Y81D and T81S) overexpressed in *Escherichia coli* DH5a cells, according to the method described previously [8–11,14].

Porcine liver FPP synthase was purified according to the method reported previously [10,15].

2.4. Conditions of the enzymatic reaction

The incubation mixture for *B. stearothermophilus* FPP synthase reaction contained, in a total volume of 1 mL, 200 μmol of Tris–HCl buffer (pH 8.5), 10 μmol of $MgCl_2$, 50 μmol of β-mercaptoethanol, 50 μmol of NH_4Cl, 5 μmol of KCl, 0.5 μmol of an allylic substrate (vinyl or ethynylDMAPP) to be examined, 0.5 μmol of IPP, and wild and mutated FPP synthases (ca. 25 μg). 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. In order to get enough product for NMR spectroscopic analysis, the reaction volume was increased 30 times the total volume mentioned above, after purification by HPLC, the products were analysed.

The porcine liver FPP synthase reaction was examined under similar conditions to those described above for the thermophilic bacterial enzyme except that the incubation temperature and pH were 37 °C and 7.0, respectively.

3. Results and discussion

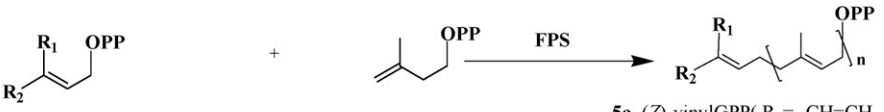
In order to investigate the reactivities of DMAPP homologs with vinyl or ethynyl group, we examined substrate specificities of thermostable FPP synthases of *B. stearothermophilus* and porcine liver FPP synthase as shown in Scheme 3 and Table 2.

3.1. Reaction of vinylDMAPP (**2**) with IPP (**1**) using FPP synthase

3.1.1. Reactions of vinylDMAPP (**2**) with IPP (**1**) using FPP synthase from B. stearothermophilus and porcine liver

The alcohol derived from alkaline phosphatase treatment of the reaction products of vinylDMAPP (**2**) with IPP (**1**) by wild-type FPP synthase from *B. stearothermophilus* gave a peak at 19.1 min (29.9%) on HPLC (eluent A), which was subjected to LC–MS and GC–MS analyses. The LC–mass spectrum of the alcohol showed a main peak at *m*/*z* 276.1938 $[M+H+CH_3CN]^+$ (calcd. for $C_{18}H_{30}NO$: 276.1984). The GC–mass spectrum of the alcohol showed a molecular ion at *m*/*z* 234 (rel. int. 0.1%), corresponding to $C_{16}H_{26}O$ with fragment ions at *m*/*z* 216 $[M-18]^+$ (28.1), 201 $[M-18-15]^+$ (34.1), 135 $[M-81]^+$ (14.4), and 79 (base peak).

The alcohol obtained from the large scaled incubation of the enzymatic reaction of **2** and **1**, gave a ^{1}H NMR ($CDCl_3$, TMS) spectrum: δ 1.60 (3H, s), 1.68 (3H, s), 1.81 (3H, d J = 1.2 Hz), 2.03 (2H, t J = 5.9 Hz), 2.04 (2H, t J = 6.4 Hz), 2.12 (2H, dt J = 7.2, 7.4 Hz), 2.26 (2H,



2, (*Z*)-vinylDMAPP (R_1= -CH=CH_2, R_2=-CH_3)
3, (*Z*)-ethynylDMAPP (R_1= -C≡CH, R_2=-CH_3)
4, (*E*)-ethynylDMAPP (R_1= -CH_3, R_2=-C≡CH)

1, IPP

5a, (*Z*)-vinylGPP(R_1= -CH=CH_2, R_2=-CH_3, n=1)
5b, (*Z*)-vinylFPP(R_1= -CH=CH_2, R_2=-CH_3, n=2)
5c, (*Z*)-vinylGGPP(R_1= -CH=CH_2, R_2=-CH_3, n=3)
6a, (*Z*)-ethynylGPP (R_1= -C≡CH, R_2=-CH_3, n=1)
6b, (*Z*)-ethynylFPP (R_1= -C≡CH, R_2=-CH_3, n=2)
6c, (*Z*)-ethynylGGPP (R_1= -C≡CH, R_2=-CH_3, n=3)
7a, (*E*)-ethynylGPP (R_1= -CH_3, R_2= -C≡CH, n=1)
7b, (*E*)-ethynylFPP (R_1= -CH_3, R_2= -C≡CH, n=2)

porcine liver FPS	**2** + **1**	→	**5a** + **5b**
B. stearothermophilus FPS (wild-type)	**2** + **1**	→	**5b**
(mutated Y81S)	**2** + **1**	→	**5c**
porcine liver FPS	**3** + **1**	→	**6b**
B. stearothermophilus FPS (wild-type)	**3** + **1**	→	**6b**
(mutated Y81D)	**3** + **1**	→	**6a** + **6b** + **6c**
porcine liver FPS	**4** + **1**	→	**7b**
B. stearothermophilus FPS (wild-type)	**4** + **1**	→	**7b**

Scheme 3. Farnesyl diphosphate synthase reactions of vinyl- or ethynylDMAPP with IPP.

dt *J* = 7.4, 7.5 Hz), 4.16 (2H, t *J* = 5.6 Hz), 5.08 (1H, dd *J* = 1.7, 10.9 Hz), 5.42 (1H, dt *J* = 1.2, 6.9 Hz), and 6.77 (1H, dd *J* = 11.0, 17.0 Hz), suggesting (2*E*, 6*E*, 10*Z*)-3,7,11-trimethyltrideca-2,6,10,12-tetraen-1-ol (**5b-OH**) structure.

The porcine liver FPP synthase-catalyzed reaction of **2** with **1** afforded two products, which were then hydrolyzed with phosphatase to the corresponding alcohols. The retention times of the alcohols on HPLC (eluent A) were 19.0 (23.3% yield) and 21.5 min (14.3% yield) respectively. The former was found to be the similar product, **5b-OH**, to that above-mentioned bacterial enzyme reaction of **2** with **1**, suggesting that the chain elongation stopped after the condensation of two molecules of **1**.

The mass spectrum of the latter showed a molecular ion at *m*/*z* 166 (rel. int. 1.8%), corresponding to $C_{16}H_{26}O$, together with major fragment ions at *m*/*z* 148 $[M-18]^+$ (33.0), 133 $[M-18-15]^+$ (37.8), 67 $[M-18-15]^+$ (22.6), 81(52.6), and 79 (base peak), indicating that the alcohol has a (2*E*, 6*Z*)-3,7-dimethylnona-2,6,8-trien-1-ol (vinylGOH) structure. It is reasonable to assign the product is 8-vinylGPP (**5a**) by considering the manner of the enzymatic reaction.

From the wild-type *B. stearothermophilus* FPP synthase reaction of **2** with **1**, **5b-OH** was also obtained, but no **5a-OH** was detectable.

3.1.2. Reaction of **2** with **1** using mutated FPP synthase (Y81S) from *B. stearothermophilus*

The prenyl alcohol derived from the reaction of **2** with **1** by the mutant enzyme (Y81S FPP synthase) showed a peak on GC at 13.2 min. The mass spectrum of the product gave a molecular ion $[M]^+$ at *m*/*z* 302 (rel. int. 3.1%), corresponding to $C_{21}H_{34}O$, with other fragment ions were observed at *m*/*z* 284 $[M-18]^+$ (12.6), 269 $[M-18-15]^+$ (12.3), 203 $[M-18-81]^+$ (8.3), 135 $[M-18-81-68]^+$ (5.1), and 81 (base peak), indicating that the product has a (2*E*, 6*E*, 10*E*, 14*Z*)-3,7,11,15-tetramethylheptadeca-2,6,10,14,16-pentaen-1-ol (16-vinylgeranylgeraniol, *Z*-vinylGGOH) structure. It is reasonable to assign the product to *Z*-vinylGGPP (**5c**) according to the mechanism of FPP synthase reaction [16]. These results suggest that the mutated FPP synthase, Y81S reaction with **2** and **1** stops at the stage of triple condensation of **1**. The relative yield of the Y81S reaction with **2** and **1** was 4.0% based on the product derived from a wild-type FPP synthase reaction between natural substrates DMAPP and **1**.

Thus, it was expected that the mutated synthase has a function like a GGPP synthase. On the other hand, Tarshis et al. has reported that the mutated avian FPP synthase, F112A and/or F113S give some multiple condensation products of C_{20} or C_{25} [17,18].

3.2. Reaction of (*Z*)-ethynylDMAPP (**3**) with **1** using FPP synthase

3.2.1. Reactions of (*Z*)-ethynylDMAPP (**3**) with **1** using FPP synthase from *B. stearothermophilus* and porcine liver

The alcohol derived from the product of the wild-type FPP synthase of *B. stearothermophilus* catalyzed reaction of (*Z*)-ethynylDMAPP (**3**) with **1** gave a peak with a retention time of 21.4 min on HPLC (eluent B), which was subjected to LC–MS and

Table 2
Relative yield (%) of the product derived from farnesyl diphosphate synthase reaction.

Allylic substrate	Product	Porcine liver FPP synthase	Wild-type FPP synthase	Mutated FPP synthase Y81D	Mutated FPP synthase Y81S
Reaction with IPP					
DMAPP	FOH	100	100		
2	5a	14.3	n.d.		n.d.
	5b	23.3	29.9		n.d.
	5c				4.0[a]
3	6a	n.d.	n.d.	2.2[a]	
	6b	21.9	88.8	0.4[a]	
	6c	n.d.	n.d.	15.5[a]	
4	7a	n.d.	n.d.		
	7b	1.4	0.8		

The relative yield of product alcohols derived from reaction FPP synthase were relative to the yields of FOH derived from the corresponding enzymatic reaction with DMAPP of IPP.

[a] The products were converted to the corresponding alcohols and analyzed by GC.

GC–MS analyses. The LC–mass spectrum of the alcohol showed a molecular ion at *m*/*z* 274.2198 $[M+H+CH_3CN]^+$ (calcd. for $C_{18}H_{28}NO$: 274.2171), which suggested the formula $C_{16}H_{24}O$. The GC–mass spectrum of the alcohol showed a molecular ion at *m*/*z* 232 (rel. int. 1.3%), corresponding to $C_{16}H_{24}O$ with fragment ions at *m*/*z* 214 $[M-18]^+$ (42.1), 199 $[M-18-15]^+$ (70.6), 135 $[M-18-79]^+$ (5.1), 131 $[199-68]^+$ (39.4), and 77 (base peak).

The alcohol which obtained by the incubation mixture of 30 times scales gave a ^1H NMR ($CDCl_3$, TMS) spectrum of the alcohol: δ 1.61 (3H, s), 1.68 (3H, s), 1.84 (3H, d J = 1.5 Hz), 2.04 (4H, t J = 7.3 Hz), 2.12 (2H, dt J = 7.1, 7.3 Hz), 3.09 (1H, s) 4.16 (2H, d J = 7.0 Hz), 5.12 (1H, dd J = 1.3, 6.9 Hz), 5.42 (1H, dt J = 1.3, 7.0 Hz), and 5.71 (1H, t J = 7.1 Hz), indicating that the product has a (2*E*, 6*E*, 10*Z*)-3,7,11-trimethyltrideca-2,6,10-trien-12-yn-1-ol (*Z*-ethynylfarnesol, ethynylFOH, **6b**-**OH**) structure. The relative yield was 88.8% based on the product derived from the reaction between the natural substrates **DMAPP** and **1**.

When the porcine liver FPP synthase reaction of **3** with **1** was examined, the similar product, **6b**-**OH** was obtained with a yield of 21.9%.

In any of the reactions with porcine or the bacterial enzyme, only **6b**-**OH** was detected as a sole product.

*3.2.2. Reaction of **3** with **1** using mutated B. stearothermophilus FPP synthase (Y81D)*

The prenyl alcohol derived from the reaction of **3** with **1** by the mutant enzyme (Y81D) followed by the phosphatase treatment showed three peaks on GC at 6.2, 10.0, and 12.6 min, each of which were subjected to GC–MS. The mass spectrum of the first alcohol (retention time: 6.2 min, 2.2% yield by GC) showed a molecular ion at *m*/*z* 164 (rel. int. 1.0%), corresponding to $C_{11}H_{16}O$ with other fragment ions at *m*/*z* 146 $[M-18]^+$ (26.7), 131 $[M-18-15]^+$ (base peak), and 67 $[M-18-79]^+$ (17.0) indicating that a 3,8-dimethylnona-2,6-dien-8-yl-1-ol (ethynylGOH) structure. The alcohol can be assigned to 6*Z*-ethynylGOH, **6a**-**OH**, according to the reaction mechanism of the FPP synthase [16].

The second alcohol (retention time on GC: 10.0 min, 0.4% yield) was assigned to **6b**-**OH**, by coincidence with the retention time of the above-mentioned product (**6b**-**OH**) derived from the reaction using wild-type enzyme.

It is expected that the product, **5b**-**OH** or **6b**-**OH** is useful to convert to some biologically active substances such as insect juvenile hormones.

The mass spectrum of the third alcohol (retention time: 12.6 min, 19.5% yield by GC) showed a molecular ion at *m*/*z* 300 (rel. int. 0.3%), corresponding to $C_{21}H_{32}O$ together with other fragment ions at *m*/*z* 282 $[M-18]^+$ (10.5), 267 $[M-18-15]^+$ (11.4), 203 $[M-18-79]^+$ (8.4), 135 $[203-68]^+$ (9.2), and 79 (base peak), indicating that the alcohol had a 3,7,11,15-tetramethylheptadeca-2,6,10,14-tetraen-16-yl-1-ol (ethynylGGOH) structure. It is also reasonable to assign the alcohol to 14*Z*-ethynylGGOH, **6c**-**OH**, suggesting that chain elongation stopped after the condensation of three molecules of **1**.

*3.3. Reactions of (E)-ethynylDMAPP (**4**) with **1** using FPP synthases from porcine liver and B. stearothermophilus*

The hydrolysate derived from the alkaline phosphatase treatment of the product obtained from a *B. stearothermophilus* FPP synthase reaction of (*E*)-ethynylDMAPP (**4**) with **1** which showed a retention time on HPLC (eluent B) at 21.1 min (relative yield: 0.8%), was subjected to GC–MS analysis. In the mass spectrum of the alcohol, the molecular ion was observed at *m*/*z* 232 (rel. int. 7.3%), corresponding to $C_{16}H_{24}O$, with fragment ions at *m*/*z* 214 $[M-18]^+$ (39.7), 199 $[M-18-15]^+$ (72.8), 135 $[M-18-79]^+$ (6.9), 131 $[199-68]^+$ (38.8), and 77 (base peak), indicating that the product has a 3,7,11-trimethyltrideca-2,6,10-trien-12-yl-1-ol (ethynylFOH) structure. It is reasonable to assign to 10*E*-ethynylFOH (**7b**-**OH**), suggesting that the chain elongation stopped after the condensation of two molecules of **1**.

Furthermore, we assigned the formation of a similar product, **7b**-**OH** (relative yield: 1.4%) by the action of porcine liver FPP with **4** and **1**.

4. Conclusion

Scheme 3 illustrates the prenyl chain elongating reactions with DMAPP homologs having a vinyl or an ethynyl group. As the results, the reaction of **2** with **1** by FPP synthase of porcine liver gave **5a** and **5b** which stopped at the first stage and second stage of the sequential condensation of IPP, respectively. However, the similar reaction by the use of wild-type FPP synthase of *B. stearothermophilus* gave **5b**, exclusively. **5b**-**OH** or **6b**-**OH** might be useful as synthons for the biologically active substance, such as juvenile hormone.

On the other hand, the reaction of **3** with **1** by use of wild-type of FPP synthase of *B. stearothermophilus* gave **6b**, solely. Moreover, a mutated FPP synthase (Y81D) reaction of **3** with **1** gave three kinds of products: **6a** (2.2%), **6b** (0.4%), and **6c** (19.5%).

Using wild-type FPP synthase of the thermostable bacteria, the reaction of **4** with **1** gave only **7b** as double condensation product.

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