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Biosynthesis of Isoprenoids in *Escherichia coli*: Stereochemistry of the Reaction Catalyzed by Farnesyl Diphosphate Synthase

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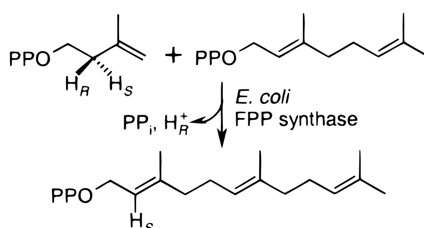
ABSTRACT

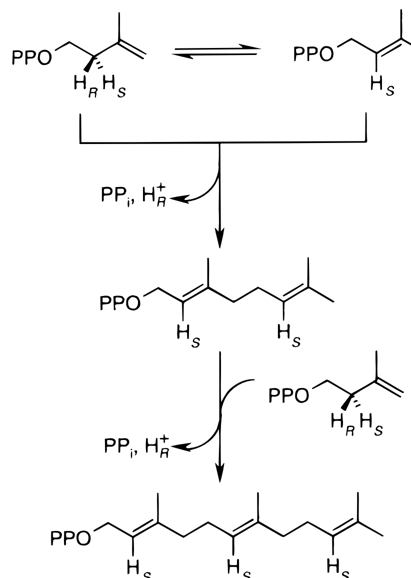


Farnesyl diphosphate (FPP) synthase from *Escherichia coli* catalyzes the condensation of isopentenyl diphosphate (IPP) and geranyl diphosphate (GPP) with selective removal of the *pro-R* hydrogen at C2 of IPP, the same stereochemistry observed for the pig liver, yeast, and avian enzymes.

The reversible interconversion of isopentenyl diphosphate (IPP)[1] and dimethylallyl diphosphate (DMAPP), and the chain elongation reactions leading to geranyl diphosphate (GPP) and farnesyl diphosphate (FPP), are crucial steps in the biosynthesis of most isoprenoids[2] (Scheme 1). These reactions are catalyzed by the enzymes IPP:DMAPP isomerase and FPP synthase, respectively. In yeast and higher eukaryotes, the isomerization and chain elongation reactions proceed with selective removal of the *pro-R* hydrogen at C2 of IPP.[2]

Studies in *E. coli*, however, suggested that in bacteria one of the two enzymes might have the opposite stereochemistry.[3] The stereospecificity of the *E. coli* isomerase was recently determined using recombinant enzyme and was shown to be the same as that of its eukaryotic relatives—the *pro-R*

Scheme 1



(1) Abbreviations used: BHDA = bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid; DMAPP = dimethylallyl diphosphate; FPP = farnesyl diphosphate; GPP = geranyl diphosphate; IPP = isopentenyl diphosphate; IPTG = isopropyl-β-D-galactopyranoside; MEP = methylerythrose phosphate; TPP = thiamine diphosphate.

(2) For a review, see: Poulter, C. D.; Rilling, H. C. In *Biosynthesis of Isoprenoid Compounds*; Porter, J. W.; Porter, S. L., Eds.; Wiley: New York, 1981.

(3) Giner, J.-L.; Jaun, B.; Arigoni, D. *Chem. Commun.* **1998**, 1857.

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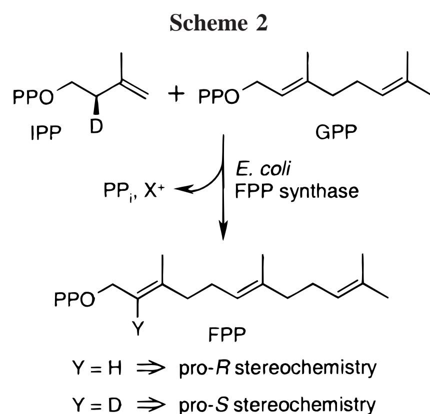
hydrogen at C2 of IPP is removed in the IPP → DMAPP isomerization.[4] We now report that FPP synthase from *E. coli*, like the eukaryotic enzymes, also catalyzes the condensation of IPP with GPP by selectively removing the *pro-R* hydrogen at C2 of IPP.

Recombinant *E. coli* FPP synthase was obtained following standard molecular biology techniques. The *ispA* gene which encodes for FPP synthase was amplified from *E. coli* genomic DNA by "sticky-end" PCR[5] and ligated into a pET11a expression vector[6] utilizing *Nde*I and *BamH*I restriction sites. The resulting plasmid was purified and transformed into BL21(DE3) competent cells. Expression studies in small scale cultures showed no difference in the total amount of overexpressed protein and enzyme activity when the cells were grown up in LB, superbroth, or minimal (M9) media.[7] Preparative-scale cultures in LB were grown at 37 °C, and plasmid-directed protein synthesis was induced by addition of IPTG. Following an additional incubation period, cells were harvested and disrupted. Overexpressed *E. coli* FPP synthase was purified from cell extracts in two steps as described previously for the avian enzyme.[8]

The experiment to determine the stereospecificity of the condensation of IPP and GPP by *E. coli* FPP synthase is outlined in Scheme 2. GPP (12 mg, 32.2 μmol) and

Scheme 2



recombinant *E. coli* FPP synthase (0.57 unit)[9] were preincubated at 37 °C for 2 min in a 20 mM BHDA buffer, pH 7.0, containing 25 mM $MgCl_2$, 50 mM KCl, and 0.5 mM DTT. A 5 mg (16.6 mmol) sample of (*R*)-[2-^{2}H]isopentenyl diphosphate[10] was then added in three portions over the course of 45 min (final reaction volume was 2.0 mL), and incubation was continued for 8 h. At the end of the incubation, the voluminous white precipitate that had formed was redissolved by gradual addition of 3 equiv of EDTA with vigorous agitation. FPP from the reaction was purified by preparative reverse phase HPLC on a C18 Shodex Asahipack (Phenomenex) column[11] and analyzed by ^{1}H NMR spectroscopy. An identical experiment was run in parallel with chicken liver FPP synthase as control.

Figure 1 shows the low-field region of the spectrum of purified FPP synthesized by the *E. coli* FPP synthase. Signals at δ 4.52, 5.24, and 5.52 correspond to protons at C1, C6/C10, and C2, respectively. The ratios of areas under the peaks at δ 4.52 and 5.52 (4.52/5.52 = 2.2) and peaks at δ 5.24 and 5.52 (5.24/5.52 = 2.1) clearly show that little if any of the deuterium initially present in the *pro-R* position at C2 of IPP was transferred to the product FPP. This result, which is identical to that obtained with the control enzyme, demonstrates that *E. coli* FPP synthase selectively removes the *pro-R* hydrogen at C2 of IPP during the condensation reaction.

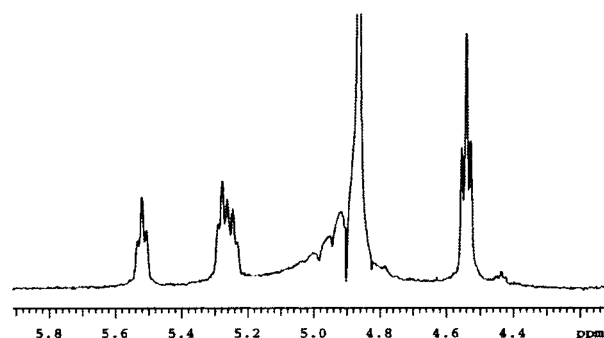


Figure 1. 500 MHz ^{1}H NMR spectrum of enzymatically produced FPP, low-field region. Signal at δ 5.52 (vinylic proton at C2) demonstrates deuterium was not incorporated into FPP. Broad signal centered at δ 4.9 is the residual absorption of the solvent after presaturation.

Isoprenoid biosynthesis is known to occur in *E. coli* by a recently discovered methylerythrose phosphate (MEP)-dependent pathway.[12] The carbon atoms in the isoprene unit come from glyceraldehyde and pyruvate. The two three-carbon precursors are condensed with loss of CO_2 to give 1-deoxy-D-xylulose phosphate **1**[13,14] (Scheme 3). The phosphate **1** is then the substrate for a reductoisomerase[15] that catalyzes a skeletal rearrangement and reduction to produce

(4) Leyes, A. E.; Baker, J. A.; Hahn, F. M.; Poulter, C. D. *Chem. Commun.* **1999**, 717.

(5) Zeng, G. *Biotechniques* **1998**, *25*, 206.

(6) Studier, F. W.; Rosenberg. A. H.; Dunn, J. J.; Dubendorff, J. W. *Methods Enzymol.* **1990**, *185*, 60. Plasmid commercially available from Novagen Inc., 601 Science Dr., Madison, WI 53711.

(7) For a description of culture media, see: Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning: A Laboratory Manual;* Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1982.

(8) Tarshis, L. C.; Yan, M.; Poulter, C. D.; Sacchettini, J. C. *Biochemistry* **1994**, *33*, 10871.

(9) One unit is the amount of protein (mg) that can produce 1 μmol of product in 1 min.

(10) Leyes, A. E.; Poulter, C. D. *Org. Lett.* **1999**, *1*, 1067−1070.

(11) Elution conditions: 5 mL min^{-1}, isocratic 20% MeCN−80% 25 mM NH_4HCO_3 for 5 min and then linear gradient to 100% MeCN in 25 min.

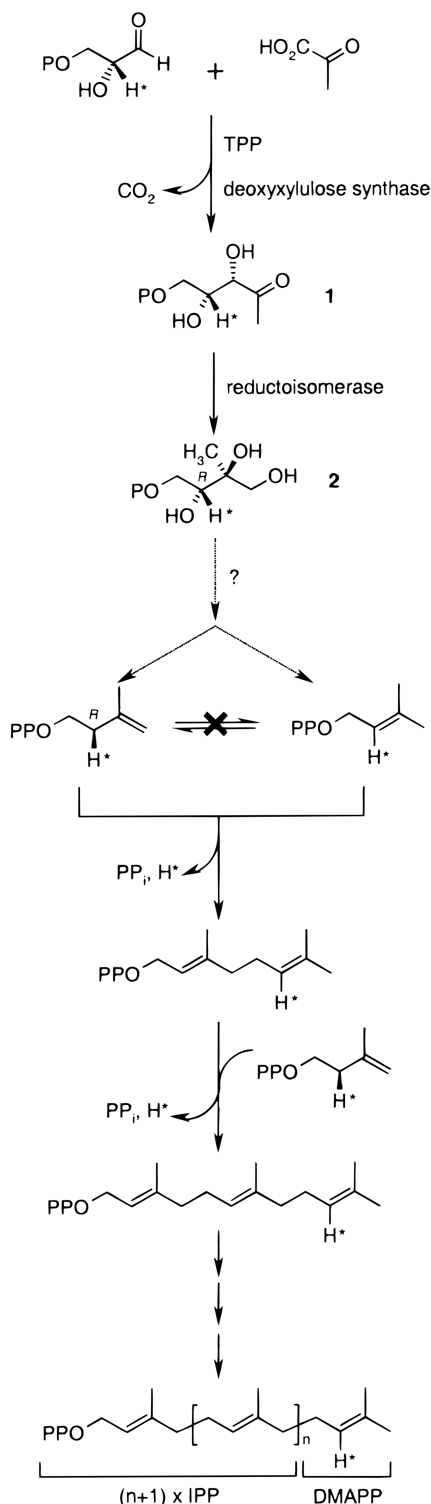
(12) The MEP nomenclature is parallel to the MVA (mevalonate) pathway.

(13) Rohmer, M.; Seeman, M.; Horbach, S.; Bringer-Meyer, S.; Sahm, H. *J. Am. Chem. Soc.* **1996**, *118*, 2564.

(14) Sprenger, G.; Schörken, U.; Wiegert, T.; de Graaf, A. A.; Taylor, S. V.; Begley, T. P.; Bringer-Meyer, S.; Sahm, H. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 12857.

(15) Takahashi, S.; Kuzuyama, T.; Watanabe, H.; Seto, H. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9879.

Scheme 3



2-*C*-methylerythritol phosphate (**2**),[16] in the first pathway-specific step in the pathway. Current knowledge of this biosynthetic route is limited to the first two steps, and the sequence of events leading to the synthesis of IPP from **2** is poorly understood. However, additional information is now available from the observed stereospecificity of the *E. coli* FPP synthase.

Studies[3] where [4-^{2}H]-1-deoxy-D-xylulose (**1**) was fed to *E. coli* cultures showed that the label was incorporated exclusively at the C2 position of the ω isoprenoid unit in the side chain of ubiquinone-8, which arises from DMAPP (Scheme 3). Since the *E. coli* FPP synthase selectively removes the *pro-R* hydrogen at C2 of IPP during chain elongation, the absence of label at C2, C6, C10, C14, C18, C22, and C26[17] in the side chain requires in vivo synthesis of (*R*)-[2-^{2}H]isopentenyl diphosphate, with subsequent loss of the label during chain elongation (Scheme 3). This conclusion is consistent with the reconstructed stereochemical course of the biosynthesis of isoprenoids in *Catharanthus roseus*.[18] The labeling patterns of lutein and phytol synthesized in vivo from [2-^{13}C,3-^{2}H]-1-deoxy-D-xylulose were rationalized by the intermediacy of (*R*)-[2-^{2}H]isopentenyl diphoshate.

The inferred *R* stereochemistry of the IPP synthesized by *E. coli* from [4-^{2}H]-1-deoxy-D-xylulose has important implications in regard to the biosynthetic origin of DMAPP. While isomerization of (*R*)-[2-^{2}H]isopentenyl diphosphate leads to loss of the label at C2 of DMAPP,[4] the label was in fact found exclusively at that position in ubiquinone-8.[3] This result would rule out IPP as the immediate precursor of DMAPP in *E. coli* and suggests that IPP and DMAPP may be synthesized independently from an as yet unidentified intermediate.

Alternatively, one cannot exclude at this point the existence of another isomerase in *E. coli* which removes the pro-*S* hydrogen at C2 of IPP during isomerization to DMAPP. This possibility, however, seems less attractive. The high rate of deuterium incorporation in the experiment reported by Giner *et al.*[3] would require that the DMAPP incorporated into ubiquinone-8 be synthesized almost exclusively by the action of this putative isomerase, with little or no intervention of the isomerase we identified in *E. coli*.[4,19]

The stereochemistry of IPP biosynthesized from [4-^{2}H]-1-deoxy-D-xylulose in *E. coli* also imposes a stereochemical constraint to the steps leading to the synthesis of [2-^{2}H]-isopentenyl diphosphate from (*R*)-[3-^{2}H]-2-*C*-methylerythritol (**2**) (Scheme 3). Since the deuterium atom in **2** is in the *R* locus, it is concluded that the reduction of C3 of **2** must proceed with retention of configuration. Subsequent proposals about structure of intermediates and reaction mechanism must account for this observation.

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