Biosynthesis of isoprenoids in *Escherichia coli*: stereochemistry of the reaction catalyzed by isopentenyl diphosphate : dimethylallyl diphosphate isomerase

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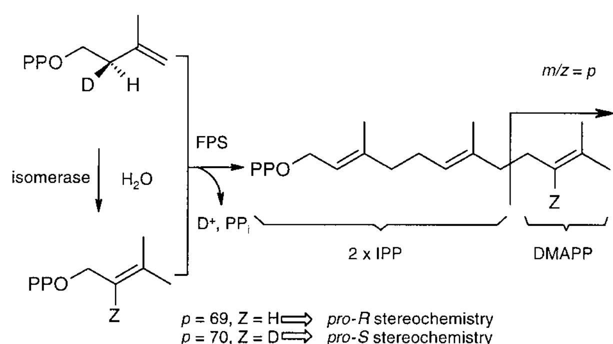
The interconversion of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) catalyzed by *E. coli* and *S. pombe* IPP isomerase proceeds with removal of the *pro-R* proton at C2 of IPP and addition of a water-derived proton to the *re* face of the C2–C3 double bond in DMAPP; this is the same stereochemistry observed for *S. cerevisiae* and rat liver enzymes.

The isomerization of isopentenyl diphosphate (IPP)† to dimethylallyl diphosphate (DMAPP) is a key activation step in the biosynthesis of isoprenoid compounds by the mevalonate pathway.[1] In eukaryotes and yeast, IPP isomerase catalyzes an antarafacial [1.3] transposition of hydrogen by a proton addition–elimination mechanism. For IPP→DMAPP, the *pro-R* proton at C2 of IPP is removed, while for DMAPP→IPP a proton from water is delivered to the *re* face of the C2–C3 double bond.

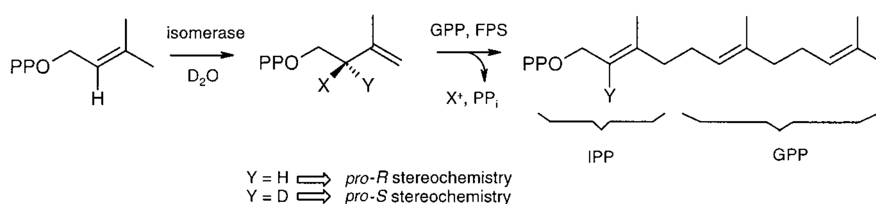
Many bacteria, including *E. coli*, and plant chloroplasts synthesize isoprenoids from 1-deoxy-D-xylulose by a non-mevalonate pathway.[2] In experiments where 3-deutero-1-deoxy-D-xylulose was fed to *E. coli* cultures, deuterium was found exclusively at the C2 position of the ω-isoprene unit of the ubiquinone-8 side chain.[3] These results are in contrast to the biosynthesis of isoprenoids in yeast and rat liver by the mevalonate pathway, where the *pro-R* proton is removed both in the isomerization and chain elongation reactions.[1] In an attempt to reconcile these observations we have determined the stereochemistry at C2 for IPP→DMAPP and DMAPP→IPP using recombinant *E. coli* enzyme.[4]

The experiment to determine the stereochemistry for IPP→DMAPP is outlined in Scheme 1. A 2 mg (6.7 μmol) sample of (*R*)-2-deuteroisopentenyl diphosphate {(*R*)-[2-^{2}H]-IPP} was incubated with 0.02 units of recombinant *E. coli* IPP isomerase and 1 unit of avian FPP synthase (FPS)[5] in a 20 mM BHDA buffer (pH 7.0) containing 1 mM $MgCl_2$, 50 mM KCl, and 0.5 mM DTT, at 37 °C. After 5 h, diethanolamine buffer (0.5 M), pH 10.5, 0.5 mM $ZnCl_2$, and 100 units of calf intestinal alkaline phosphatase were added. Incubation was continued for 8 h, after which time the samples were extracted with *tert*-butyl methyl ether. Farnesol, synthesized enzymatically from (*R*)-[2-^{2}H]-IPP, was analyzed by GC/MS. A parallel set of reactions was run using recombinant *Schizosaccharomyces pombe* IPP isomerase[6] as a control. Electron impact mass spectra for the enzymatically synthesized farnesol samples were similar to that from an authentic sample of unlabeled alcohol. An intense peak was seen at *m/z* 69 (100%) for the $C_5H_9^+$ fragment from the ω-isoprene unit. Thus, little of the deuterium originally at C2 in [2-^{2}H]-IPP was incorporated into the portion of farnesol arising from DMAPP. Chemical ionization (CH_4) mass spectra for enzymatic and unlabeled farnesol gave peaks at *m/z* 223 (1–3%, [M + 1]$^+$) and 205 (18%, [M +1−H_2O]$^+$). In addition, the enzymatic samples had small peaks at *m/z* 224 ([224]/[223] = 0.3) and 206 ([206]/[205] = 0.4), consistent with a small amount (~10%) of (*S*)-[2-^{2}H]-IPP in the sample. As shown in Scheme 1, the lack of deuterium incorporation in the ω-isoprene unit of farnesol indicates that the *pro-R* methylene hydrogen is preferentially removed from IPP by both *E. coli* and *S. pombe* IPP isomerases during isomerization to DMAPP.



Scheme 1

Scheme 2 outlines a complementary set of experiments to determine the stereochemistry at C2 in the DMAPP→IPP direction. DMAPP (6 mg, 20 μmol) was incubated for 4 h with 0.6 units of IPP isomerase in a deuterated buffer[7] (pD 7.0).‡ The isomerization was rendered irreversible by adding geranyl diphosphate (GPP) (18 mg, 42 μmol) and FPP synthase (6.4 units) to the mixture so that newly formed [2-^{2}H]-IPP was immediately converted to FPP. FPP synthesized from the coupled reactions was purified by reversed phase HPLC on a C18 Magellan column§ and analyzed by ^{1}H NMR spectroscopy. As illustrated in Fig. 1, the low field region of the spectrum showed peaks at δ 4.52, 5.24 and 5.52 corresponding to protons at C1, C6/C10 and C2, respectively. The ratio of the intensities for the resonances at δ 4.52 and 5.52 (5.52/4.52 = 2.1) demonstrates that deuterium from the buffer was not incorporated at C2 of FPP. A portion of the sample was hydrolyzed with alkaline phosphatase as described previously, and analyzed by GC/MS (CI, CH_4). Samples of enzymatic and authentic unlabeled farnesol gave identical spectra. Eukaryotic FPP synthases remove the *pro-R* hydrogen from IPP during



Scheme 2

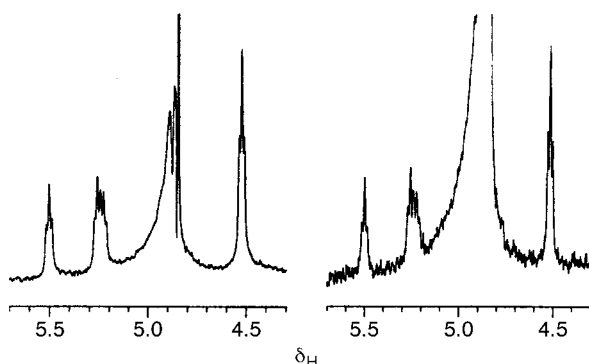


Fig. 1 500 MHz ^{1}H NMR spectra of enzymatically produced FPP in D_2O, low field region. Left: *S. pombe* isomerase reaction. Right: *E. coli* isomerase reaction. Signal at δ 5.52 corresponds to vinylic proton at C2. Solvent peak (δ 4.85) was presaturated before pulsing.

chain elongation. The absence of label in FPP therefore requires synthesis of (*R*)-2-deuteroisopentenyl diphosphate by addition of a deuterium to the *re* face of DMAPP and subsequent loss of deuterium during chain elongation.

Our data show that the stereochemistry at C2 for the reactions catalyzed by *E. coli* IPP isomerase is the same as the eukaryotic isomerases—the *pro-R* proton is removed during IPP→DMAPP and a proton is added to the *re* face of the double bond during DMAPP→IPP. These findings have interesting implications in light of the recently reported labeling studies in *E. coli* with deoxy-D-xylulose.[3] If (*S*)-[2-^{2}H]-IPP is produced from 3-deutero-1-deoxy-D-xylulose, the stereochemistry of chain elongation by *E. coli* FPP synthase must be different than its eukaryotic relatives. However, if the (*R*)-enantiomer of [2-^{2}H]-IPP is formed, the molecule of DMAPP incorporated into the ω-position of the ubiquinone side chain cannot be synthesized from IPP by the action of the *E. coli* isomerase we have characterized. Perhaps *E. coli* has another as yet unidentified IPP isomerase with the opposite stereospecificity. Alternatively, there is no *a priori* requirement that IPP be synthesized before DMAPP by the non-mevalonate pathway, and both diphosphates might be synthesized simultaneously from a common precursor. In this scenario, the *E. coli* isomerase would not be a required enzyme for isoprenoid biosynthesis but it would allow the cell to balance the concentrations of IPP and DMAPP during periods of active metabolism to optimize their utilization.

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Notes and references

† Abbreviations used: BHDA = bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid; DMAPP = dimethylallyl diphosphate; FPP = farnesyl diphosphate; FPS = farnesyl diphosphate synthase; GPP = geranyl diphosphate; [2-^{2}H]-IPP = 2-deuteroisopentenyl diphosphate; IPP = isopentenyl diphosphate.

‡ Reaction conditions were as described for IPP→DMAPP, except that the buffer was deuterated.

§ Elution conditions: 0.7 ml min^{-1}, isocratic 20% MeCN-80% 25 mM NH_4HCO_3 for 7 min, then linear gradient to 100% MeCN in 20 min.

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