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Short-Chain Prenyl Diphosphate Synthase That Condenses Isopentenyl Diphosphate with Dimethylallyl Diphosphate in *ispA* Null *Escherichia coli* Strain Lacking Farnesyl Diphosphate Synthase

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A short-chain prenyl diphosphate synthase in an *Escherichia coli* mutant that lacked the gene coding for farnesyl diphosphate synthase, *ispA*, was separated from other prenyl diphosphate synthases by DEAE-Toyopearl column chromatography. The purified enzyme catalyzed the condensation of isopentenyl diphosphate with dimethylallyl diphosphate to form farnesyl diphosphate and geranylgeranyl diphosphate.

[**Key words:** *Escherichia coli*, isoprenoid, isopentenyl diphosphate, dimethylallyl diphosphate, farnesyl diphosphate, prenyl diphosphate synthase]

Isoprenoids are natural compounds synthesized from isopentenyl diphosphate (IPP) via allylic diphosphates with various numbers of carbons (1). There are three genes coding for prenyl diphosphate synthases in the chromosome of *Escherichia coli*: *ispA* for farnesyl diphosphate (FPP) synthase (2), *ispB* for octaprenyl diphosphate synthase (3), and *rth* (*uppS*) for undecaprenyl diphosphate synthase (4, 5). FPP synthase catalyzes the sequential condensation of IPP with dimethylallyl diphosphate (DMAPP) and geranyl diphosphate (GPP) to form all-*E*-FPP, which is the substrate for both octaprenyl diphosphate synthase and undecaprenyl diphosphate synthase. Both *ispB* (6) and *rth* (5) are essential for growth. However, the *ispA*-null mutant is viable, although its growth rate is lower than that of the wild-type strain (7). There are low enzyme activities in the cell of the mutant for the condensation of IPP with DMAPP. We studied this activity to find enzymes that substitute for FPP synthase in the mutant.

The *E. coli ispA*-null mutant SF7 (7) and the wild-type strain W3110 were cultured in 2×LB medium (8) at 37°C. The resulting cells were collected by centrifugation at 6000×*g* for 20 min at 4°C and suspended in 10 mM potassium phosphate buffer (pH 7.4) containing 1 mM 2-mercaptoethanol, 5 mM EDTA, 2 mM phenylmethanesulfonyl fluoride, and 10 mM octyl-β-thioglucopyranoside. The suspension was sonicated 10 times for 10 s, and centrifuged at 18,000×*g* for 40 min. The activity for the condensation of IPP with DMAPP in the supernatant of SF7 was 5% that of W3110 (7). To identify the enzymes responsible for this residual activity in the mutant, the cell-free homogenate of SF7 was subjected to DEAE-Toyopearl column chromatography, and the prenyl diphosphate synthase activity of each fraction was determined (Fig. 1). Between the two large peaks of undecaprenyl diphosphate synthase (peak A) and octaprenyl diphosphate synthase (peak B), there was a small peak of enzyme activity (peak N: fractions 38–44). These fractions were pooled and applied to a DEAE-Toyopearl 650 M column again. Three peaks of enzyme activity (N1, N2, and N3) appeared in the second DEAE Toyopearl column chromatography (Fig. 2).

We characterized the enzyme corresponding to N1. FPP was the most effective substrate as allylic diphosphate, and the activities with GPP and DMAPP were 50% and 11% that with FPP, respectively. The analysis of the prenols resulting from the treatment of prenyl diphosphates with acid phosphatase (9) revealed geranylgeraniol in the reaction with FPP, and farnesol in the reaction with GPP (Fig. 3). Thus, the reaction products of the enzyme corresponding to N1 were geranylgeranyl diphosphate (GGPP) in the reaction of IPP with FPP, and FPP in the reaction of IPP with GPP. Both GGPP and FPP were produced in the reaction of IPP with DMAPP. This enzyme required magnesium ion for its activity, and its highest activity was observed at 1 mM Mg^{2+}. No detergent was necessary for the enzyme activity; however, the addition of 0.05% (w/v) Triton X-100 activated the enzyme by 1.5-fold. The addition of 0.1% (w/v) or a higher concentration of Triton X-100 was rather inhibitory to the enzyme reaction. These results revealed that the enzyme corresponding to N1 was a short-chain prenyl diphosphate synthase, the reaction product of which is FPP or GGPP. This enzyme was further purified by Butyl-Toyopearl, Mono-Q, and Superose-12 column chromatographies. The specific activity of the fraction from the Superose-12 col-

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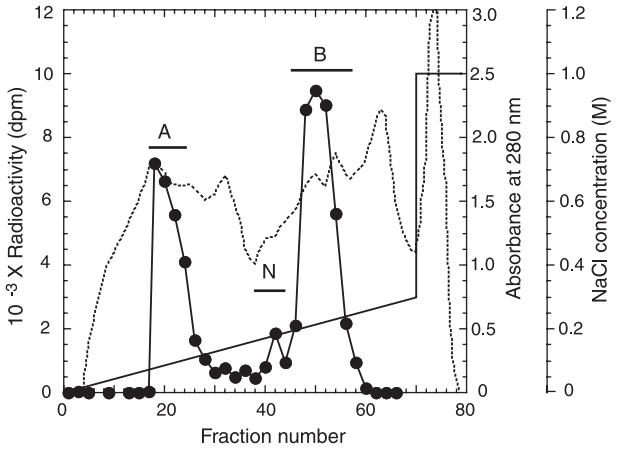

FIG. 1. DEAE-Toyopearl 650 M chromatography of the ammonium sulfate fraction of SF7. The cell-free homogenate of SF7 was adjusted to 65% saturation with ammonium sulfate and stirred for 30 min. The resulting precipitate was collected and dissolved in 10 mM potassium phosphate buffer (pH 7.4) containing 1 mM 2-mercaptoethanol (buffer A) and dialyzed extensively against the same buffer. The resulting solution was applied to a DEAE-Toyopearl column (0.8×50 cm) equilibrated with buffer A. Elution was carried out with a linear gradient of NaCl from 0 to 0.3 M in buffer A, as shown in this figure, and 20-ml fractions were collected. Each fraction was assayed for prenyl diphosphate synthase activity. The reaction mixture contained, in a final volume of 200 µl, 0.4 µmol of $MgCl_2$, 2 µmol of 2-mercaptoethanol, 0.1 mg of Triton X-100, 10 µmol of potassium phosphate buffer (pH 7.4), 0.48 nmol of [1-^{14}C]IPP (specific activity, 2.0 TBq/mol), 6 nmol of FPP, and 20 µl of each fraction. After incubation for 1 h at 37°C, 200 µl of saturated NaCl solution saturated with 1-butanol was added. The products were extracted with 1-butanol saturated with NaCl solution, and the radioactivity of the extract was measured. Peak A included undecaprenyl diphosphate synthase, and peak B included octaprenyl diphosphate synthase. Symbol and lines: closed circles, enzyme activity; broken line, absorbance at 280 nm; solid line, NaCl concentration.

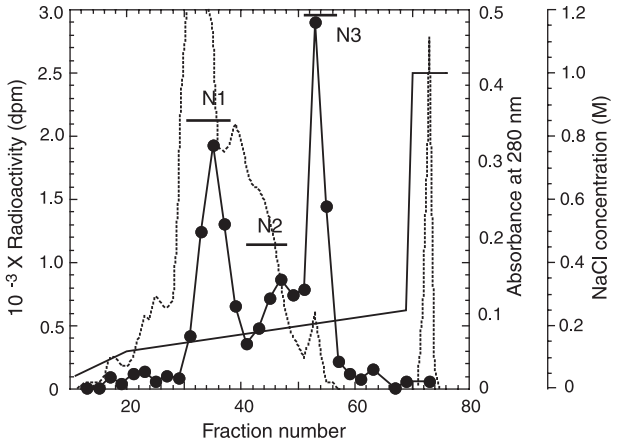

FIG. 2. DEAE-Toyopearl 650 M chromatography of N peak fraction. The N peak fraction (fractions 38–44) in the first DEAE-Toyopearl 650 M chromatography was collected and dialyzed against buffer A. The resulting solution was applied to a DEAE-Toyopearl 650 M column (0.5×23 cm) equilibrated with buffer A. Elution was carried out at a flow rate of 2 ml/min with a gradient of NaCl as shown in this figure. Ten ml fractions were collected. Each fraction (150 µl) was used for the assay of prenyl diphosphate synthase activity. Symbols and line: closed circles, enzyme activity; broken line, absorbance at 280 nm; solid line, NaCl concentration.

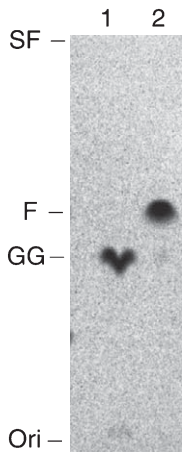

FIG. 3. Autoradiograms of TLC of prenyl alcohols obtained by enzymatic hydrolysis. The reaction of the enzyme corresponding to N1 was carried out with [1-^{14}C]IPP and FPP (lane 1) or [1-^{14}C]IPP and GPP (lane 2). The products were extracted with 1-butanol and treated with acid phosphatase (9). The resulting polyprenols were extracted with hexane and analyzed by reversed-phase thin-layer chromatography on an LKC-18 plate with acetone-water (9:1 v/v). Radioactivity on the plate was detected using a BAS-2000 system. Lines indicate the positions of authentic prenols as follows: F, (all-*E*)-farnesol; GG, (all-*E*)-geranylgeraniol; Ori, origin; SF, solvent front.

umn was 13.1-fold that of the fraction from the DEAE-Toyopearl column (Table 1). The molecular mass of the enzyme was 65±8 kDa, as estimated by Superose-12 chromatography.

Octaprenyl diphosphate was produced in the reaction of IPP with FPP by both the enzymes corresponding to N2 and N3. The enzyme corresponding to N3 is considered to be same as that corresponding to B in Fig. 1 from its elution profile in the chromatography, and to be octaprenyl diphosphate synthase that was described previously (10). The enzyme corresponding to N2 is also considered to be octaprenyl diphosphate synthase, the isoform of that corresponding to N3. The Mono-Q chromatography of the enzyme corresponding to N2 gave a single peak, and the chromatography of the enzyme corresponding to N3 also gave another peak. Thus, no interconversion between the enzymes corresponding to N2 and N3 was observed, unlike that observed for the isoforms of mammalian FPP synthases that are interconvertible with each other during column chromatography (11, 12).

The enzymes corresponding to N1 and N2 were described for the first time in this study. Either one or both of these enzymes are considered to also occur in the wild-type strain, because a corresponding peak was observed in the DEAE-Toyopearl column chromatogram of cell-free homogenate of W3110. The enzyme corresponding to N1 was the only enzyme that catalyzed the synthesis of FPP from IPP and DMAPP in the *ispA*-null mutant. This enzyme is considered to be mainly responsible for the condensation of IPP with DMAPP in the homogenate of the mutant (7), although both octaprenyl diphosphate synthase and undecaprenyl diphosphate synthase have a weak activity for the condensation of IPP with DMAPP.

The only gene of *E. coli* that is significantly homologous

TABLE 1. Purification of short-chain prenyl diphosphate synthase

Step	Total protein (mg)	Total activity[a] (pmol/min)	Specific activity (pmol/min/mg)	Purification (fold)	Recovery (%)
DEAE-Toyopearl	93.9	28.8	0.307	1	100.0
Butyl-Toyopearl	11.4	4.60	0.404	1.31	16.0
Mono-Q	1.90	7.26	3.82	12.4	25.1
Superose-12	0.93	3.74	4.02	13.1	13.0

[a] Activity was expressed as pmol of IPP incorporated into products per minute.

with *ispA* is *ispB* (13). It is unlikely that the newly found enzyme contains part of the polypeptide derived from *ispA* because the mutant used in this study lacked more than two thirds of *ispA* (7). The molecular mass of the enzyme corresponding to N1, 65±8 kDa, is similar to those of octaprenyl diphosphate synthase, which is a dimer of a 35,217-Da polypeptide encoded by *ispB* (14), and of undecaprenyl diphosphate synthase, which is a dimer of a 28,444-Da polypeptide encoded by *rth* (15). The identification of the polypeptide component of this enzyme will clarify whether a component of this enzyme is derived from *ispB*, *rth*, or another gene that have not been characterized yet.

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