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Dynamic Interaction between Components of Hexaprenyl Diphosphate Synthase from *Micrococcus luteus* BP-26†

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ABSTRACT: Hexaprenyl diphosphate synthase from *Micrococcus luteus* BP-26, which has been known to be dissociated into two essential components, designated as components A and B, during hydroxyapatite chromatography [Fujii, H., Koyama, T., & Ogura, K. (1982) *J. Biol. Chem. 257*, 14610–14612], was also resolved similarly by Sephadex G-100 or DEAE ion-exchange chromatography. Each component takes various self-aggregated forms. The apparent molecular mass of component B estimated by gel filtration on Superose 12 varied depending on its concentration, ranging from ~18 to 49 kilodaltons (kDa). On the other hand, the apparent molecular mass of component A varied depending on not only its concentration but also the ionic strength of the medium, ranging from ~13 to 24 kDa. When a mixture of components A and B preincubated in the presence of Mg^{2+} but in the absence of substrate was subjected to Superose 12 gel filtration, they were eluted at positions identical with those observed when they were chromatographed individually. In contrast, when a mixture of components A and B incubated in the presence of Mg^{2+} and substrates was filtrated on Superose 12, the elution positions were markedly changed, showing that an ~24-kDa aggregate of component A (designated as *A*) and an ~27-kDa aggregate of component B (designated as *B*) were the major species. Evidence was also obtained to show that farnesyl diphosphate (FPP) binds to the components to form an aggregate, *A*–*B*–FPP–Mg^{2+}, which probably represents an intermediary state of enzyme catalysis.

Prenyltransferases catalyze the head-to-tail condensation between isopentenyl diphosphate (IPP)[1] and an allylic diphosphate to generate various prenyl diphosphates. The early investigations on the biosynthesis of bacterial isoprenoid compounds have led to the conclusion that the prenyl diphosphates are intermediates of bacterial isoprenoid compounds such as prenyl side chains of the respiratory quinones and sugar-carrier lipids (Poulter & Rilling, 1981).

Seven prenyltransferases that catalyze the condensation of isoprene units to give long-chain prenyl diphosphates whose chain lengths are longer than C_{20} have so far been separated from various bacteria. They can be classified into two groups according to the reactions they catalyze. One is the group of enzymes that catalyze the formation of (*Z*)-prenyl chains [(*Z*)-polyprenyltransferase], and the other group catalyzes the formation of (*E*)-prenyl chains [(*E*)-polyprenyltransferase]. Undecaprenyl diphosphate synthase (Kurokawa et al., 1971; Keen & Allen, 1974; Baba & Allen, 1978, 1980; Takahashi & Ogura, 1982; Muth & Allen, 1984) is representative of the former, producing undecaprenyl diphosphate which is the precursor of well-known bacterial sugar-carrier lipid for cell wall biosynthesis. (*Z*)-Nonaprenyl diphosphate synthase (Ishii et al., 1986) is also one of the (*Z*)-polyprenyltransferases. (*E*)-Polyprenyltransferases include hexaprenyl diphosphate (C_{30}) (Fujii et al., 1982), heptaprenyl diphosphate (C_{35}) (Takahashi et al., 1980), octaprenyl diphosphate (C_{40}) (Fujisaki et al., 1986), solanesyl diphosphate (C_{45}) (Sagami et al.,

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[1] Abbreviations: FPP, (2*E*,6*E*)-farnesyl diphosphate; IPP, isopentenyl diphosphate; DTT, dithiothreitol; FPLC, fast protein liquid chromatography; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate.

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1977), and decaprenyl diphosphate (C_{50}) synthases (Ishii et al., 1985), and they are responsible for the biosynthesis of the side chains of prenylquinones such as menaquinones and ubiquinones. Most of these long-chain prenyltransferases have been found to require detergent or phospholipid for catalytic activity in vitro or to be activated markedly by them as observed generally for membrane-associated enzymes. Hexaprenyl diphosphate and heptaprenyl diphosphate synthases, however, have been shown not to require detergent or phospholipid for their catalytic activities (Takahashi et al., 1980; Fujii et al., 1982). Some detergents act on these enzymes as inhibitors rather than activators.

During a series of our studies to characterize these prenyltransferases, an interesting observation was made about hexaprenyl diphosphate synthase separated from *Micrococcus luteus* BP-26. This enzyme is dissociated into two dissimilar protein fractions, designated component A and component B, during hydroxyapatite chromatography. Although neither component has prenyltransferase activity alone, enzyme activity is restored when they are recombined (Fujii et al., 1982). Subsequently, heptaprenyl diphosphate synthase from *Bacillus subtilis* was also found to be dissociated into two essential components (components I and II) by DEAE-Sephadex chromatography (Fujii et al., 1983). Studies on the properties of these components have shown that there is a characteristic common to these enzymes, namely, one component (component A or component I) is more heat stable than the other component (component B or component II). These facts suggest that a novel mechanism must be involved in the catalysis of these particular prenyltransferases in contrast to the catalytic mechanism of the other polyprenyl diphosphate synthases, which require detergent for activity. In order to obtain insight into the mechanism, we improved the purification of components A and B and examined their properties, focusing our attention on the interaction of the two components during enzyme catalysis. This paper describes these results.

Materials and Methods

Materials. Cells of *M. luteus* BP-26 were grown by the method described previously (Fujii et al., 1982). [1-^{14}C]IPP (sp act. 56 Ci/mol) was purchased from Amersham/Searle Corp. and diluted with nonlabeled IPP to the described specific activity. Nonlabeled IPP and farnesyl diphosphate (FPP) were the same preparations as used previously (Fujii et al., 1980). [1,5-^{14}C]FPP was synthesized from [1-^{14}C]IPP and dimethylallyl diphosphate by the action of pig liver farnesyl diphosphate synthase. Dithiothreitol (DTT) was purchased from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan).

Enzyme Assay. The enzyme activity was measured by determination of the amount of [1-^{14}C]IPP incorporated into butanol-extractable polyprenyl products. Unless otherwise stated, the standard assay solution contained, in a final volume of 1 mL, 100 μmol of Tris-HCl buffer, pH 7.0, 1 μmol of $MgCl_2$, 25 nmol of FPP, 25 nmol of [1-^{14}C]IPP (sp act. 1 Ci/mol), and a suitable amount of enzyme. After incubation at 37 °C for 1 h, the reaction mixture was chilled with an ice bath. After addition of 3 mL of butanol and 1 mL of saturated NaCl solution, the mixture was shaken vigorously to extract the reaction products, and an aliquot of the organic layer was counted for radioactivity. After hexaprenyl diphosphate synthase was resolved into two components (components A and B), the synthase activity was assayed with a supplement of a fraction containing the counterpart component. One unit represents 1 nmol of IPP incorporated into product per minute. Protein concentration was determined by the Bio-Rad protein assay with bovine γ-globin (Bio-Rad) as a standard.

Purification of Component A. All steps were performed at 0–4 °C. Cells (wet weight 100 g) were suspended in 200 mL of 100 mM Tris-HCl buffer (pH 7.0) containing 0.3 mM EDTA and 2 mM DTT (buffer A) and disrupted 10 times by sonication at 20 kHz for 1 min with 5-min intervals in an ice bath with a Branson Sonifier. The resulting mixture was centrifuged at 10000*g* for 10 min to recover undisrupted cells as precipitate. The supernatant was further centrifuged at 108000*g* for 60 min. This procedure was repeated more than twice with the recovered cells. The precipitate obtained from the 108000*g* supernatant by 70% ammonium sulfate saturation was dissolved in 150 mL of buffer A containing 21 g of ammonium sulfate. The solution was applied to a column (7.0 × 12 cm) of butyl-Toyopearl previously equilibrated with buffer A containing ammonium sulfate at 25% saturation. Elution was carried out with 700 mL of the same buffer and then with 700 mL of buffer A containing ammonium sulfate at 15% saturation. Component A was eluted with buffer A containing 7% ethanol (v/v). Fractions that had the component A activity were pooled and concentrated to 30 mL by ultrafiltration with a YM 5 membrane (Amicon Co. Ltd.) under nitrogen atmosphere. The concentrated solution was applied to a column (3.8 × 98 cm) of Sephadex G-100 previously equilibrated with buffer A. The column was eluted with the same buffer at a flow rate of 0.41 mL/min, and 13.5-mL fractions were collected. Fractions that had component A activity were combined and concentrated to 20 mL by ultrafiltration. The solution was dialyzed and applied to a hydroxyapatite column (3.2 × 30 cm) equilibrated with 10 mM potassium phosphate buffer (pH 7.0) containing 2 mM DTT (buffer B). The column was washed with the same buffer and then eluted with a 1400-mL linear gradient of 10–250 mM potassium phosphate buffer (pH 7.0) that contained 2 mM DTT. Component A was eluted at concentrations ranging from 50 to 100 mM potassium phosphate buffer. Fractions that contained component A were combined and concentrated to 20 mL by ultrafiltration. The concentrate was dialyzed and applied to a column (2.9 × 36 cm) of DEAE-Toyopearl equilibrated with 20 mM potassium phosphate buffer (pH 7.0) containing 2 mM DTT and 0.3 mM EDTA. Elution was started with the same buffer followed by a 1300-mL linear gradient of KCl from 0 to 0.2 M in the same buffer system. Component A was eluted at a conductivity around 900 μS. Fractions containing component A were concentrated to 5 mL by ultrafiltration. The resulting solution (5 mg of protein/mL) can be kept frozen at −80 °C for several months without loss of the activity as the component.

Purification of Component B. The protein fraction precipitated between 0% and 50% of saturation with ammonium sulfate from the 108000*g* supernatant was divided into four equal portions. Each portion was dissolved in 25 mL of buffer A and applied to a column (3.8 × 98 cm) of Sephadex G-100 equilibrated with buffer A. The column was eluted at a flow rate of 0.41 mL/min, and 13.5-mL fractions were collected. Fractions that had component B activity were combined and brought to 70% saturation with ammonium sulfate. The ammonium sulfate fraction was dissolved in 50 mL of buffer B, and the solution was dialyzed against buffer B. Half of the dialysate was applied to a column (3.2 × 35 cm) of hydroxyapatite. The hydroxyapatite chromatography was performed as described in the purification of component A. Component B was eluted at concentrations ranging from 150 to 230 mM phosphate buffer. Fractions that contained component B were combined and concentrated to 20 mL by ultrafiltration. The concentrate was dialyzed and applied to a column (1.9 × 30

Table I: Purification of Component A

step	protein (mg)	act. (units)	recovery (%)	sp act. (units/mg)	purity (x-fold)
108000g supernatant	8984	1437	100	0.160	(1)
0–70% $(NH_4)_2SO_4$ precipitation	8333	1275	88.7	0.153	0.96
butyl-Toyopearl	1186	832.6	57.9	0.702	4.39
Sephadex G-100	401.2	433.3	30.2	1.08	6.75
hydroxyapatite	94.6	296.0	20.6	3.13	19.6
DEAE-Toyopearl	5.5	220.9	15.4	40.2	251

Table II: Purification of Component B

step	protein (mg)	act. (units)	recovery (%)	sp act. (units/mg)	purity (x-fold)
108000g supernatant	7397	1183	100	0.160	(1)
0–55% $(NH_4)_2SO_4$ precipitation	3237	957	80.9	0.200	1.25
Sephadex G-100	724.8	775.5	65.6	1.07	6.69
hydroxyapatite	84.0	513.2	43.4	6.11	38.1
DEAE-Toyopearl	40.1	283.3	23.9	7.07	44.2

cm) of DEAE-Toyopearl equilibrated with 50 mM potassium phosphate buffer (pH 7.0) containing 2 mM DTT and 0.3 mM EDTA. The column was washed with the same buffer and eluted with a 700-mL linear gradient of KCl from 0 to 0.3 M in the same buffer system. Component B was eluted at a conductivity around 2400 μS. Fractions containing component B were collected and concentrated to 4 mL by ultrafiltration. The concentrate (10 mg of protein/mL) can be kept frozen at −80 °C for several months without loss of the activity as the component.

Superose 12 Gel Filtration of Component B. Samples (200 μL) containing suitable amounts of component B in 50 mM phosphate buffer (pH 7.0) containing 150 mM KCl and 2 mM DTT (buffer C) were incubated at 37 °C for 25 min. Each solution was chromatographed with a Pharmacia FPLC on a Superose 12 column (1 × 30 cm) equilibrated with buffer C. The column was eluted at 22 °C with buffer C at a flow rate of 0.4 mL/min. Fractions (0.2 mL) were collected, and 0.1-mL aliquots were assayed for component B activity in the same way as described before except that the specific activity of [1-^{14}C]IPP was 10 Ci/mol.

FPLC Analysis of the Interaction between Components A and B. A mixture (200 μL) of components A (2.2 units) and B (2.2 units) in 100 mM Tris-HCl buffer (pH 7.0) containing 1 mM $MgCl_2$ and 2 mM DTT (buffer D) was incubated at 37 °C for 25 min in the presence of 10 nmol of FPP, or in the presence of 10 nmol of FPP and 10 nmol of IPP, or in the absence of both substrates. The mixture was applied to a Superose 12 column (1 × 30 cm) equilibrated with buffer D. The column was eluted at 22 °C with buffer D at a flow rate of 0.4 mL/min. Fractions (0.4 mL) were collected, and 0.1-mL aliquots were assayed for component A and B activities.

Results

Purification of Components. Component A and component B were purified 250-fold and 44-fold from the crude extract of *M. luteus* BP-26, respectively (Tables I and II). Though neither of the purified fractions is homogeneous in terms of protein, they were free from any other prenyltransferases which were present in the crude homogenates.

During purification of the components, they were separated not only by hydroxyapatite chromatography as reported previously (Fujii et al., 1982) but also by gel filtration or ion-exchange chromatography with DEAE-Toyopearl or DE-52.

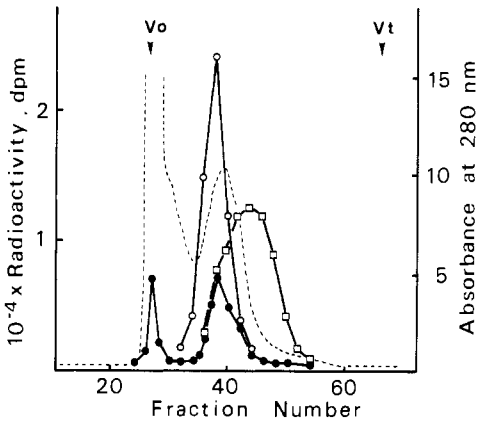

FIGURE 1: Sephadex G-100 chromatography of 0–50% ammonium sulfate fraction. Enzyme activity was assayed as described under Materials and Methods in the absence of components (●) and in the presence of component A (○) or component B (□). Protein was measured by absorbance at 280 nm (---). The arrows indicate excluded (V_0) and included (V_t) volumes.

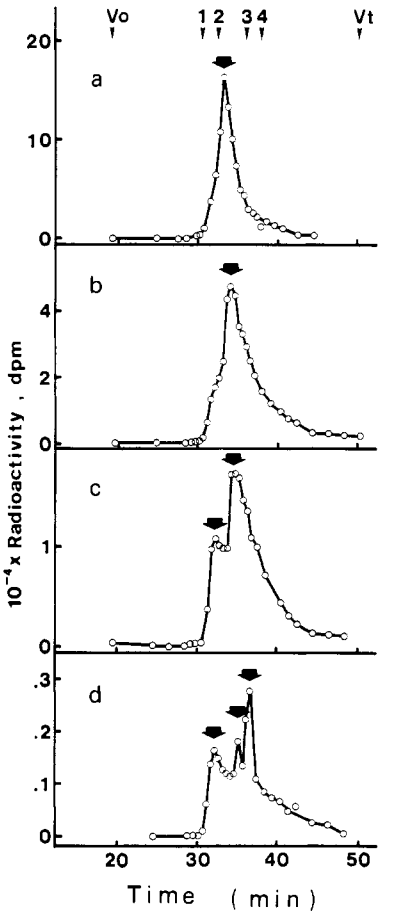

FIGURE 2: Superose 12 gel filtration of component B. A solution (200 μL) containing 7.6 units (panel a), 2.5 units (panel b), 1.2 units (panel c), or 0.7 unit (panel d) of component B was chromatographed on a Superose 12 column as described under Materials and Methods. Molecular mass standards were blue dextran (V_0), bovine serum albumin (1), ovalbumin (2), chymotrypsinogen A (3), ribonuclease A (4), and acetone (V_t).

When the 0–55% ammonium sulfate fraction was chromatographed on Sephadex G-100, a peak of component B appeared with a smaller elution volume than that of component A (Figure 1). The prenyltransferase fraction eluted in the V_0 region was assigned to undecaprenyl diphosphate synthase.

Gel Filtration of Components B and A. To determine the molecular mass of component B, gel filtration was carried out on a Superose 12 column in buffer C. Figure 2 illustrates gel

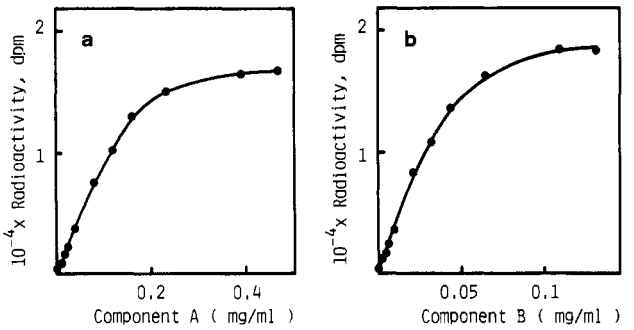

FIGURE 3: Effect of concentration of components on enzyme activity. Component B (22 μg) was incubated as described under Materials and Methods in the presence of various concentrations of component A (panel a). Component A (56 μg) was incubated similarly in the presence of various concentrations of component B (panel b).

filtration profiles for component B applied at various concentrations.

When a sample containing 0.68 unit of component B was filtrated, three fractions with apparent molecular masses of about 49, 25, and 18 kDa were observed (panel d). When a sample containing 1.2 units of component B was applied, two different peaks appeared at positions corresponding to molecular masses of 49 and 29 kDa (panel c). When a sample containing 2.5 units was applied, an elution peak emerged at a position coresponding to a molecular mass of 32 kDa with a shoulder around 50 kDa (panel b). However, when a sample containing 7.6 units of component B was applied, the protein eluted as a single peak with a molecular mass of 38 kDa (panel a). These results show that component B has a tendency to take some different aggregated forms depending upon its concentration.

On the other hand, experiments carried out to estimate the molecular mass of component A under the same conditions showed that the component gave a single symmetrical peak with an apparent molecular mass of 15 kDa without dependence on its concentration (data not shown). When component A was chromatographed in buffer D, whose ionic strength was half of that of buffer C, however, two peaks were observed, one corresponding to molecular mass of 24 kDa, the other to 13 kDa (data not shown). The 24-kDa peak predominated at high concentration of the component or under low ionic strength.

Effect of Concentration of Components on Hexaprenyl Diphosphate Synthase Activity. The hexaprenyl diphosphate synthase activity was examined as a function of the concentration of component A or B. These two components behaved like an enzyme and its substrate for each other, showing typical saturation curves (Figure 3).

We previously reported that the dependence of the synthase activity on the concentration of component A showed a sigmoidal curve (Fujii et al., 1982). Subsequently, however, the sigmoidal nature was found to be attributable to the lability of the component at low concentration.

Interaction between Components. It appeared of interest to investigate what kind of aggregate of components A and B exists during the enzyme reaction. Figure 4 shows chromatographic profiles of mixtures of components A and B preincubated in the presence and absence of substrate. In the absence of substrate, component B eluted as a peak at a position corresponding to a molecular mass of about 42 kDa with a shoulder around 54 kDa, and component A eluted as a peak at a position corresponding to a molecular mass of about 24 kDa with a shoulder around 15 kDa (panel a). The elution positions of these components were identical with those observed when they were applied individually, indicating that no association occurred between the two components under these conditions. In contrast, in the presence of FPP and IPP, major peaks of components A and B appeared at 24- and 27-kDa positions, respectively (panel c).

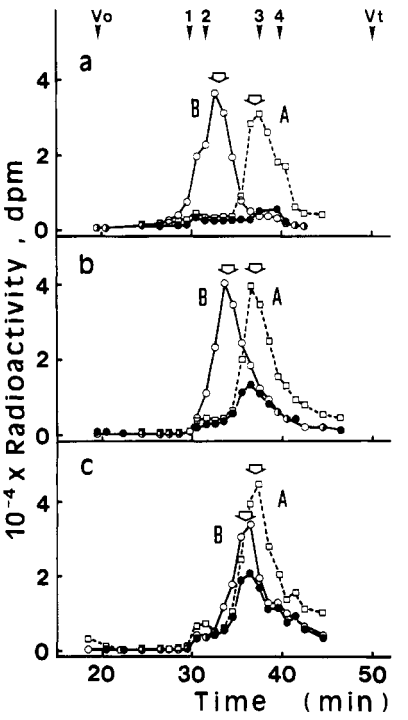

FIGURE 4: FPLC analysis of interaction between components A and B. A solution (200 μL) containing components A and B was incubated alone (panel a), with FPP (panel b), or with FPP and IPP (panel c) at 37 °C for 25 min and then applied to a Superose 12 column as described under Materials and Methods. Fractions containing 0.4 mL were collected, and 0.1-mL aliquots were assayed for enzyme activity in the absence of components (●) and in the presence of component A (○) or component B (□). Molecular mass standards were the same as described in the legend to Figure 2.

These results reveal that various aggregates of components A and B are converted, by adding the substrates to start the enzyme reaction, into an aggregate of component A with a molecular mass of 24 kDa and that of component B with a molecular mass of 27 kDa, respectively. As shown in Figure 4, panel b, when the components were incubated in the presence of FPP and Mg^{2+} but in the absence of IPP, the shoulder peaks of components A and B observed in panel a disappeared, and the elution position of componet B was shifted to a lower molecular mass region. These results suggest that FPP participates in the interaction between the two components.

Furthermore, as shown in panel c, a small peak was detected at an elution time of 30–32 min, which corresponded to a molecular mass position of about 50 kDa. This value is in approximate accord with the sum of the molecular mass values corresponding to the major peaks of components A and B under these conditions. This small peak seems to be attributable to an associated state which is active as hexaprenyl diphosphate synthase. No similar peak was observed in panel a, but a small peak could be detected at about the 50-kDa position in panel b. These results suggest that the substrate(s) is (are) essential for the formation of the complex consisting of components A and B and that components A and B exist as a 24-kDa aggregate of component A, a 27-kDa aggregate of component B, and a 50-kDa complex formed between the two aggregates when components A and B cooperatively catalyze the enzyme reaction.

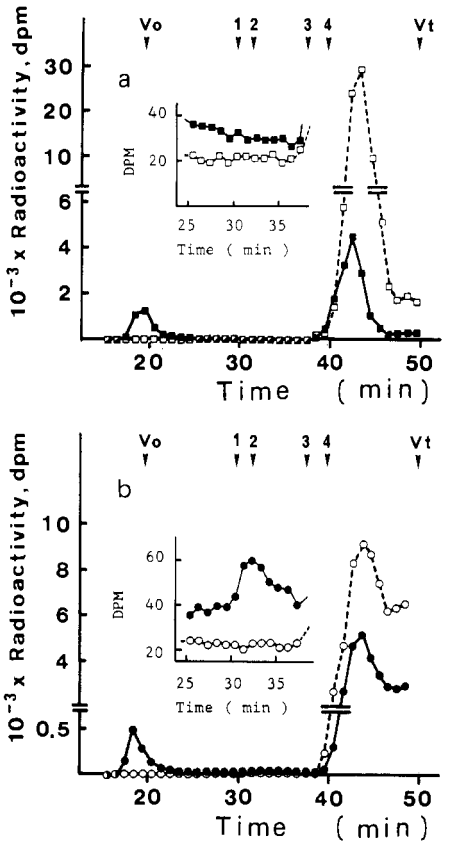

FIGURE 5: Formation of the complex between components and substrates. (a) A sample (200 μL) containing 10 nmol of [1-^{14}C]IPP (sp act. 5 Ci/mol) and 10 nmol of nonlabeled FPP in buffer D was incubated at 37 °C for 25 min in the absence (□) or presence of 2.2 units each of components A and B (■) and applied to a Superose 12 column (1 × 30 cm) equilibrated with buffer D. The column was eluted with the same buffer at a flow rate of 0.4 mL/min. Fractions (0.4 mL) were collected and counted for radioactivity. The inset shows a magnified illustration of the elution profile of radioactivity ranging from 25 to 37 min. (b) A sample (200 μL) containing 10 nmol of nonlabeled IPP and 10 nmol of [^{14}C]FPP (2.5 Ci/mol) in buffer D was incubated in the absence (○) or presence of 2.2 units each of components A and B (●) and was chromatographed as described above. Fractions (0.4 mL) were collected and counted for radioactivity. Molecular mass standards were the same as described in the legend to Figure 2.

Formation of a Complex of Components and Substrates. Panel a in Figure 5 shows the chromatogram on Superose 12 of the two components preincubated in the presence of FPP, [1-^{14}C]IPP, and Mg^{2+}. The peak of radioactivity in the V_0 region and that at 41–48 min are attributable to the enzyme reaction product and recovered [1-^{14}C]IPP, respectively. No radioactivity peak was detected in other regions.

When the mixture preincubated in the presence of ^{14}C-labeled FPP, nonlabeled IPP, and Mg^{2+} was chromatographed, however, a small but significant peak of radioactivity was observed at an elution time of 30–33 min, in which the 50-kDa complex was eluted (panel b). These results suggest that component A, component B, and FPP–Mg^{2+} form a ternary complex and that neither IPP nor the product, hexaprenyl diphosphate, is included in this complex.

Discussion

Hexaprenyl diphosphate synthase was found to be separated into two components not only by hydroxyapatite chromatography as previously reported (Fujii et al., 1982) but also by Sephadex G-100 chromatography. This result contradicts our assumption that components A and B would associate to form a complex under physiological conditions by such ionic and/or hydrophobic interaction as could maintain the complex during gel filtration. Farnesyl diphosphate synthase from chicken liver or yeast has been described as a dimer of a subunit with a molecular mass of about 43 kDa (Reed & Rilling, 1975; Eberhardt & Rilling, 1975), and that from human or pig liver has also been reported to consist of two subunits of about 38 kDa (Barnard & Popják, 1981). Furthermore, undecaprenyl diphosphate synthase from *Lactobacillus plantarum* has been reported to be a dimer of an ~30-kDa subunit (Muth & Allen, 1984). However, each dimer of these prenyltransferases is identified as a single entity and is never dissociated during gel filtration. The monomeric forms of these enzymes are confirmed only with denatured specimens by SDS–polyacrylamide gel electrophoresis. The present study has revealed that the interaction between components A and B is very weak in comparison with those of the subunits of the other prenyltransferases.

Component B loses its activity by heat treatment at 50 °C as easily as do farnesyl diphosphate synthase and undecaprenyl diphosphate synthase of this bacterium, whereas component A is resistant against heat treatment (Fujii et al., 1982). Our recent studies show that this enzyme has an SH group (or groups) essential for the catalytic activity and that this group resides on component B (unpublished data). These results suggest that component B holds the active site responsible for catalytic activity and that component A might have an auxiliary function to remove the hydrophobic product from the active site so that the catalytic reaction can turn around. In order to know whether some detergent could substitute for component A, we examined the effects of various kinds of detergent such as Triton X-100, Tween 80, Brij 58, sodium deoxycholate, and sodium lauryl sulfate. None of them, however, acted in place of component A to activate component B at all (data not shown).

Components A and B are similar in that each tends to associate with itself to form various aggregates depending on its concentration. The aggregation of component A was also dependent on the ionic strength of the medium. Such properties have not been observed in other prenyltransferases that catalyze the synthesis of prenyl products with chain lengths shorter than C_{25} or longer than C_{35}. Examination of the elution peaks of the components by Superose 12 chromatography reveals that components A and B form at least two and three aggregates, respectively.

The data for the interaction between components A and B demonstrate that hexaprenyl diphosphate synthase exists, in the presence of substrates and Mg^{2+}, in three forms: an aggregate of component A with molecular mass of about 24 kDa (designated as *A*), an aggregate of component B with a molecular mass of about 27 kDa (designated as *B*), and their associated complex with a molecular mass of about 50 kDa. We presume that aggregate *A* and aggregate *B* represent the aggregates formed when the components are in a functional state. In the presence of FPP and Mg^{2+} but in the absence of IPP, the associated complex of *A* and *B* was also observed. This observation, together with the results of the substrate-binding studies (Figure 5, panel b), reveals that the complex is composed of *A*, *B*, and FPP–Mg^{2+}. These results and the fact that no association occurred between the two components in the absence of substrates indicate that FPP–Mg^{2+} is required for the association between *A* and *B*. From the observation that both component A and component B exist, even in the presence of substrate and Mg^{2+}, in favor of their aggregated forms, *A* and *B*, rather than the complex *A*–*B*–FPP–Mg^{2+}, it seems likely that the association and dissociation between

A and B are rapid unless FPP–Mg^{2+} binds the associate complex of A–B. It is reasonable to assume that A–B–FPP–Mg^{2+} is an intermediary state upon which the condensation with IPP occurs and proceeds until hexaprenyl diphosphate ($C_{30}PP$) is produced. The results of gel filtration of the preincubated mixture containing component A, component B, Mg^{2+}, FPP, and [1-^{14}C]IPP (Figure 5, panel a) show that $C_{30}PP$ is not bound to any form of the components. This implies that the complex A–B–$C_{30}PP$–Mg^{2+} must be dissociated into A–B and $C_{30}PP$–Mg^{2+} every time upon turnover of catalysis. Probably, A–B is rapidly or simultaneously dissociated into A and B which are ready to associate again with FPP to give A–B–FPP–Mg^{2+}. The dissociation of A–B might be essential to release $C_{30}PP$–Mg^{2+} from A–B–$C_{30}PP$–Mg^{2+}. The $C_{30}PP$–Mg^{2+} dissociated from the enzyme will form large micellar aggregates, as indicated by the elution volume by Superose 12 (Figure 5). This must be a crucial point of the reaction mechanism by which the catalytic reaction yielding insoluble material can turn over without aid of detergent.

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Bovine Brain Contains Two Types of Phosphatidylinositol Kinase†

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ABSTRACT: Two phosphatidylinositol (PI) kinases from bovine brain were separated by rate zonal sucrose gradient centrifugation of detergent-solubilized membranes. Of the total PI kinase activity, 43% migrates on sucrose gradients with a size of approximately 55 kilodaltons (kDa); this kinase has properties similar to one of two PI kinase activities characterized in fibroblasts [Whitman, M., Kaplan, D. R., Roberts, T., & Cantley, L. (1987) *Biochem. J.* (in press)] and has been termed type 2. The remainder of the activity migrates in a second peak with a size of approximately 230 kDa. This enzyme possesses properties which are unlike both fibroblast PI kinase activities and has been termed type 3. The type 2 and type 3 enzymes have very different affinities for adenine nucleotides and are readily distinguishable by their sensitivities to inhibition by adenosine. The K_Ms of types 2 and 3 kinases for ATP are 54 and 742 μM, and the K_is for adenosine are 18 and 1520 μM, respectively. The two enzymes also differ in their affinities for PI, phosphatidylinositol 4-phosphate, and Mg^{2+} as well as in stimulation and inhibition by other phospholipids. When PI kinase from erythrocyte ghosts is fractionated by sucrose gradient centrifugation, only one peak of activity is observed which is indistinguishable from brain type 2 PI kinase.

The stimulated metabolism of membrane inositol phospholipids is among the first responses of cells to a variety of neurotransmitters, growth factors, and hormones (Berridge, 1984). It is thought that the initial response to ligand/receptor interaction is the activation of a specific phospholipase C which hydrolyzes phosphatidylinositol 4-phosphate (PIP)[1] and PIP_2

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[1] Abbreviations: PI, phosphatidylinositol; PIP, phosphatidylinositol 4-phosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; kDa, kilodalton(s); Con A, concanavalin A; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; TLC, thin-layer chromatography.

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