Hexaprenyl Pyrophosphate Synthetase from Micrococcus luteus B-P 26

SEPARATION OF TWO ESSENTIAL COMPONENTS*  
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Hexaprenyl pyrophosphate synthetase was detected in extracts of Micrococcus luteus B-P 26. During the course of purification the enzyme was resolved into two components, each of which had no catalytic activity but restored the hexaprenyl pyrophosphate synthetase activity when combined with each other. Both fractions, designated components A and B in the order of their elution from hydroxyapatite, were purified free of farnesyl pyrophosphate synthetase co-occurring in the same bacterium. They appeared to be proteins of molecular weights of approximately 20,000 (component A) and 60,000 (component B). Component A was more stable as compared with component B which was easily destroyed by relatively mild heat treatment. The hexaprenyl pyrophosphate synthetase reconstituted of these two components catalyzed the synthesis of all-trans-hexaprenyl pyrophosphate from isopentenyl pyrophosphate and all-trans-farnesyl or all-trans-geranylgeranyl pyrophosphate, but it did not catalyze a reaction between isopentenyl pyrophosphate and either dimethylallyl or geranyl pyrophosphate.

Prenyltransferases catalyze the head-to-tail condensation between isopentenyl pyrophosphate and allylic prenyl pyrophosphate to produce various prenyl pyrophosphates which are used to such diverse products as steroids, carotenoids, glycosyl carrier lipids, and the side chains of quinones (1). The mechanism which regulates the polymerization of isoprene units in such a way that it stops at a certain chain length has been of particular interest. At least six distinct prenyltransferases have so far been highly or partially purified from various organisms and well characterized. They are the synthetases of geranyl (2), farnesyl (3-11), geranylgeranyl (7, 12-15), heptaprenyl (16), nonaprenyl (solanesyl) (17), and undecaprenyl pyrophosphates (18-20).

In order to isolate a new enzyme with a different chain length specificity, we have studied prenyltransferases from a strain of Micrococcus luteus which produces menaquinone-C26 exclusively (21). As a result, hexaprenyl pyrophosphate synthetase was found in a crude extract of this bacterium, but a further purification resulted in a complete loss of the activity. Investigation of the cause of this inactivation of enzyme

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RESULTS AND DISCUSSION

Prenyltransferase activities were detected in a 0-80% ammonium sulfate fraction obtained from *M. luteus* B-P 26 extract when assayed with \[^{14}C\]isopentenyl pyrophosphate and either of geranyl, farnesyl, or geranylgeranyl pyrophosphates, indicating the presence of an enzyme or enzymes catalyzing the formation of polypropenyl pyrophosphate with a chain length no shorter than C_{20}. Isopentenyl pyrophosphate isomerase activity, as assayed with \[^{14}C\]isopentenyl pyrophosphate alone, was not appreciable. Product analysis actually indicated that the final product derived from \[^{14}C\]isopentenyl pyrophosphate and farnesyl pyrophosphate or geranylgeranyl pyrophosphate was all-trans-hexaprenyl pyrophosphate. However, when the crude enzyme was chromatographed on hydroxyapatite, only farnesyl pyrophosphate synthetase activity was recovered, and the hexaprenyl pyrophosphate synthetase activity was not detected in any fraction. A prenyltransferase activity peak appeared with the maximum at fraction number 54 when assayed with \[^{14}C\]isopentenyl pyrophosphate and geranyl pyrophosphate, but no fraction was active when assayed with \[^{14}C\]isopentenyl pyrophosphate and farnesyl pyrophosphate (Fig. 1). The disappearance of the hexaprenyl pyrophosphate synthetase activity after hydroxyapatite chromatography seemed too marked to be attributed only to denaturation of the enzyme, suggesting that the enzyme activity might depend on an unknown cofactor which had been removed from the enzyme during the course of chromatography. Therefore, we examined whether the hexaprenyl pyrophosphate synthetase activity could be restored by recombining the chromatographically separated fractions.

Taking it as a working hypothesis that there might be a factor which modulates the catalytic function of a prenyltransferase in such two modes that the enzyme acts as hexaprenyl pyrophosphate synthetase in the presence of the factor and acts as farnesyl pyrophosphate synthetase in its absence, we first assayed all the chromatographic fractions for polypropenyl pyrophosphate synthetase activity with a supplement of a farnesyl pyrophosphate synthetase fraction. As shown by the curve with open circles in Fig. 1, a remarkable peak for polypropenyl pyrophosphate synthetase activity appeared in a region after fraction number 76 when the fractions were assayed with \[^{14}C\]isopentenyl pyrophosphate and farnesyl pyrophosphate in the presence of fraction 54. The product of this reconstitutive incubation was found again to be hexaprenyl pyrophosphate. Consequently, it appeared as if the peak centered at fraction number 82 corresponded to the factor which we had initially hypothesized. However, the possibility that the farnesyl pyrophosphate synthetase might be involved in this restorative activity was eliminated as follows.

In order to examine whether farnesyl pyrophosphate synthetase itself or some other substance present in fraction 54 was responsible for this dramatic restoration of hexaprenyl pyrophosphate synthetase activity, we assayed each fraction of the same chromatography in turn with \[^{14}C\]isopentenyl pyrophosphate and farnesyl pyrophosphate with a supplement of fraction 82 to follow the exact elution profile of the substance responsible for the restored activity. As indicated by the closed triangles in Fig. 1, the hexaprenyl pyrophosphate synthetase activity assayed by this reconstitution was associated not with farnesyl pyrophosphate synthetase fractions but with fractions eluted a little earlier than farnesyl pyrophosphate synthetase. Furthermore, the fraction, designated component A, having the restorative activity was separated from farnesyl pyrophosphate synthetase by Sephadex G-200 chromatography (Fig. 2). Rechromatography on Sephadex G-200 yielded component A completely free of farnesyl pyrophosphate synthetase. Thus, farnesyl pyrophosphate synthetase was proved not to be involved in this restoration of hexaprenyl pyrophosphate synthetase activity.

The fraction in the hydroxyapatite chromatography (Fig. 1), designated component B, having component A-dependent hexaprenyl pyrophosphate synthetase activity was also further purified by rechromatography on hydroxyapatite and Sephadex G-200.

Since either component A or B itself has no activity as prenyltransferase but restores hexaprenyl pyrophosphate synthetase activity when combined with each other, it is reasonable at this stage to assume that these two fractions correspond to an enzyme and its essential factor or to two dissociable subunits of an enzyme complex.

Component A was more stable against heat treatment than component B. The former retained 75% of its original activity for restoration after a 5-min treatment at 50 °C while the latter lost 75% of its activity. Component A preincubated with trypsin was not able to restore the hexaprenyl pyrophosphate activity.

![Fig. 1. Chromatography of a 0-80% ammonium sulfate fraction on hydroxyapatite.](image1)

![Fig. 2. Sephadex G-200 chromatography of component A.](image2)
Synthetic activity with a supplement of component B in the presence of trypsin inhibitor which had no effect on the synthetase activity. Component B was also inactivated similarly by the treatment with trypsin. However, these components were not affected by RNAse or DNAse. From gel filtration on Sephadex G-200, the molecular weights of components A and B were estimated to be approximately 20,000 and 60,000, respectively.

The dependency of hexaprenyl pyrophosphate synthetase activity on the concentration of component A showed a sigmoid curve as shown in Fig. 3. The products of enzymatic reaction were hydrolyzed with acid phosphatase, and the hydrolysates were analyzed by chromatography followed by mass spectrometry. The high pressure liquid chromatography resolved the hydrolysates into two radioactive polyprenols in a ratio of 1:5.

Both showed typical mass spectra for polyprenols exhibiting fragments derived by loss of water (M - H2O)1 and subsequent elimination of terminal units in a regular manner yielding (C15H27O2)n. The major and minor products showed a molecular ion at m/z 426 corresponding to hexaprenol, C30H44O2, and m/z 358 corresponding to pentaprenol, C25H38O2, respectively.

We have previously observed that the distribution of the polyprenyl pyrophosphates synthesized by the action of non-aprenyl pyrophosphate synthase is changed depending on the concentration of Mg2+ used in the incubation (22). Similar tendency was also observed in this hexaprenyl pyrophosphate synthase reaction; the amount of C6 product relative to C6 product increased as Mg2+ concentration was elevated. However, the chain length never exceeded C30 even at 20 mM of Mg2+.

As shown in Table I, dimethylallyl pyrophosphate or geranyl pyrophosphate was not accepted as a primer by this enzyme but all-trans-farnesyl and all-trans-geranylglyceranyl pyrophosphates were accepted. There is no reason to doubt that farnesy1 pyrophosphate is the natural primer for this enzyme since farnesyl pyrophosphate synthetase has concomitantly been isolated from the same bacterium. It is noteworthy that prenyltransferase responsible for the synthesis of all-trans-polyprenyl pyrophosphates with chain length longer than C30 commonly lack an ability to catalyze the initial step of condensation starting with dimethylallyl pyrophosphate.

It would be interesting to examine whether similar component B are also involved in heptaprenyl pyrophosphate (16) and nonaprenyl pyrophosphate synthetases which have often been difficult to obtain in highly pure form due to their apparent liability in chromatography.

Further purification of these components and the mechanistic studies are now in progress.

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