Enzymatic Formation of Dehydrodolichal and Dolichal, New Products Related to Yeast Dolichol Biosynthesis*

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Two new polyprenyl products in addition to dehydrodolichol and dolichol were detected by two-plate silicic gel thin layer chromatography of nonpolar products formed from [1-14C]isopentenyl diphosphate and farnesyldiphosphate in the reaction with a crude 1,000 × g supernatant of yeast homogenates in the presence of NADPH. The new products were indistinguishable from authentic dehydrodolichol and dolichol. Analyses of the time-dependent and pH-dependent formation of the four products including dehydrodolichol and dolichol suggested that the biosynthetic pathway from dehydrodolichol leading to dolichol is different from that to dolichol. In double-labeled experiments with a combination of [1-14C]isopentenyl diphosphate and a [4B-3H]NADPH-generating system, the ratio of 14C- and 3H-derived radioactivities found in dolichol was six times higher than that in dolichol. A small amount of 3H-labeled dehydrodolichol was also detected. Considering the fact that dolichol is synthesized from dehydrodolichol (Sagami, H., Kurisaki, A., and Ogura, K. (1993) J. Biol. Chem. 268, 10109–10113), we propose that dehydrodolichol is a common branch point intermediate in the biosynthetic pathways leading to dolichol and dolichol and that dehydrodolichal is an intermediate in the pathway from dehydrodolichol to dolichol.

Since the discovery of the involvement of the long-chain polyisoprenyl-P, dolichyl-P, in the pathway of assembly of the oligosaccharide chain of N-linked glycoproteins (I, 2), a great deal of progress has been made in the understanding of the biosynthetic pathway of dolichol (3–9). The initial steps from mevalonate to farnesyl-P2 are identical with those of the biosynthetic pathway of dolichol (3–9). The initial steps from mevalonate to farnesyl-P2 are identical with those of the biosynthetic pathway of dolichol (3–9). The initial steps from mevalonate to farnesyl-P2 are identical with those of the biosynthetic pathway of dolichol (3–9).

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Corey and Schmidt (16). The reaction mixture was centrifuged at 10,000 × g for 10 min to remove brown pellets. The dichloromethane extracts containing dolichol were dried under vacuum, dissolved in toluene, and purified by similar chromatography to that of dehydrolipid in toluene. Both aldehydes were quantitatively obtained.

Enzyme Preparations and Assay—Yeast cells were grown in YPD medium (2% Bacto-peptone, 1% Bacto-yeast extract, 2% glucose, 0.003% adenosine sulfate) at 23 °C to late logarithmic phase, collected, washed, suspended in 100 mM Tris-HCl (pH 7.5) containing 1 mM dithiothreitol, and incubated at 23 °C for 5 min. The cells were collected, resuspended in a buffer containing 10 mM Tris-HCl (pH 7.5), 1.2 mM sorbitol, 0.75% Bacto-yeast extract, 1.5% Bacto-peptone, 0.5% glucose, and 0.2 mg/ml Zymolyase 100T, and then incubated at 23 °C for 30 min. Spheroplasted cells were collected, washed with 1.2 mM sorbitol, resuspended in a preparation buffer containing 20 mM Hepes-KOH (pH 7.5), 1 mM potassium acetate, 2 mM magnesium acetate, 1 mM dithiothreitol, 1 mM EDTA, 1 mM EGTA, 0.6 mg/ml protease inhibitors (leupeptin, antipain, chymostatin, pepstatin A, aprotinin), and 1 mM phenylmethylsulfonyl fluoride, and homogenized with 30 strokes in a Teflon glass homogenizer. The homogenates were centrifuged at 1,000 × g for 30 min, and the resulting supernatant was used as crude enzyme. Protein was determined with DC protein assay reagents (Bio-Rad). Mutant crude enzymes were prepared as described above except that cells were grown at 23 °C to mid-logarithmic phase and then divided into two equal aliquots; one aliquot was incubated at 36 °C for 90 min and the other remained at 23 °C.

Assay Conditions and Products Analysis—The standard assay mixture contained, in a final volume of 0.5 ml, Tris-HCl buffer (pH 8.0), 1 mM dithiothreitol, 100 mM potassium fluoride, 50 mM [1-14C]sopentenyl-P2, 40 mM farnesyl-P2, 1 mM NADPH, and 2.5 mg/ml crude enzymes. The mixture was incubated at 23 °C for 30 min. The enzymatic products were extracted with butanol saturated with water. The butanol extracts were treated with acid phosphatase according to the method of Fujii et al. (17). Liberated products were extracted with hexane, and the hexane extracts were passed through RP-18 Sep-Pak in methanol. Nonpolar products were eluted with hexane and analyzed by two-plate thin layer chromatography as described in a previous report (18).

Preparation of Phospholipid Vesicles—Small unilamellar vesicles (SUVs) were prepared by a sonication method similar to that of Van Dessel and Lagrou (19). Phosphatidylcholine (3 mmol) and [1-14C]dehydrodolichol (10,000 cpm) were added to 100 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, 1% (v/v) 3-(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid. The mixture was evaporated under N2, dried under vacuum for 3 h, and then suspended in 1 ml of a preparation buffer containing 50 mM Tris-HCl (pH 8.0), 0.25 mM suropeptide, 1 mM EDTA, and 0.02% NaN3. The suspension was then sonicated for 6 min (Branson Sonifier 200) on ice and centrifuged at 20,000 × g for 20 min. Quantitative encapsulation of [1-14C]dehydrodolichol in SUVs was confirmed by liquid scintillation counting of an aliquote (50 μl) of the supernatant. The supernatant (650 ml) was used as SUVs source. The liposome and 1,000 × g supernatant of yeast homogenates were incubated as described under "Assay Conditions and Products Analysis," except that the assay mixture contained 0.25 mM sucrose.

Double-labeled Experiments—The application of [4B-3H]NADPH generating system in double-labeled experiments was performed by the modified method of Moran et al. (20). A mixture containing, in a final volume of 0.2 ml, 50 mM Tris-HCl buffer (pH 7.8), 0.03 mM [1-14C]glucose (1 mCi), 1 mM ATP, 10 mM MgCl2, and 0.23 unit of hexokinase, was incubated at 25 °C for 2 h. The reaction products were passed through a Dowex 50W-X8 column (0.5 ml) in water to remove Mg2++. [1-14C]glucose-6-phosphate in the run-through fraction was incubated with 0.5 mM of NADP- and 0.5 unit of glucose-6-phosphate dehydrogenase, in a total volume of 0.3 ml, at 23 °C for 5 min. Then, the preincubated mixture was added to the standard assay mixture.

RESULTS

Establishment of the in Vitro Dolichol Assay in Yeast—To confirm whether the formation of dolichol by yeast enzyme preparations can be detected, we first applied the assay system established with crude enzyme preparations of rat liver to the yeast system. Fig. 1A shows growth of yeast and synthesis of dolichol with a 10,000 × g supernatant of yeast homogenates. The activity for dolichol synthesis was the highest in the late-logarithmic phase of growth. Therefore, in the following experiments we harvested the cells before the stationary phase (A600 nm, 1.0). Next we prepared 1,000, 3,000, 5,000, 10,000, and 100,000 × g supernatants of homogenates and assayed their activities for synthesis of dehydrodolichyl compounds and dolichol (Fig. 1B). The 1,000 × g supernatant had an activity for dolichol synthesis about 10 times as high as that of the 10,000 × g supernatant. The 100,000 × g supernatant had no activity for dolichol synthesis, although it had a considerable activity for the synthesis of dehydrodolichyl compounds. Therefore, we used the 1,000 × g supernatant to examine the assay conditions concerning optimum pH, time, and NADPH dependence. We found that there was no remarkable improvement of the dolichol assay conditions earlier established for the 10,000 × g supernatant of rat liver homogenates (13). We also examined the effects of various detergents such as Triton X-100, CHAPS, octyl glucopyranoside, deoxycholate, and Tween 80 on the activity of dolichol synthesis. As shown in Fig. 2, all the detergents except Tween 80 completely at their critical micellar concentrations inhibited the activity of dolichol synthesis, whereas the activity for dehydrodolichol synthesis was less affected. Since the specific activity of the yeast enzyme system was higher than that of the rat liver enzyme system, in these studies a protein concentration of 2.5 mg/ml was used in the following experiments. Analysis of products derived from [1-14C]sopentenyl-P2 and farnesyl-P2 with or without acid phosphatase treatment revealed the absence of dolichyl diphasphate and dolichyl phosphate in the enzymatic products and no difference in the chain length distribution between dehydrodolichyl products and dolichol (data not shown).

Dolichol-synthesizing Activities of Temperature-sensitive Yeast Mutants—The mutant strain nos. 64, 149, 283, 279, and 358 used in this study are phase 1 mutants thought to be defective in the biosynthesis reactions responsible for the
source of dolichyl-P (21). We prepared a 1,000 × g supernatant of each mutant pretreated at the permissive temperature (23 °C) or the restrictive temperature (37 °C), incubated the supernatant with [1-14C]isopentenyl-P₂ and farnesyl-P₂, and analyzed the products by two-plate thin layer chromatography. Dolichol was formed in all of these mutants pretreated at the restrictive temperature. A mutant (no. 283), which has been already identified to be allelic to sec59, which is a temperature-sensitive mutant defective in dolichol kinase activity, was almost equal to the wild type strain in the capability of synthesizing dolichol. However, surprisingly, the abilities to synthesize dehydrodolichal compounds and dolichol by the mutant nos. 64, 149, 279, and 358 were higher than that of the wild type strain. Remarkably, these mutants seem to have a mutation which causes an increase in vitro activity involved in the formation of dehydrodolichal compounds and dolichol. We chose to use the mutant, #149 in the following experiments.

**Enzymatic Synthesis of Dehydrodolichal from Dehydrodolichol**—To see whether the hydride of NADPH is in fact incorporated into dolichol and whether the hydride is derived from 4B- or 4A-hydrogen of NADPH, we tried to prepare stereospecifically labeled [4B-3H]NADPH and [4A-3H]NADPH according to the method of Moran et al. (20). However, we had difficulty in preparing and purifying labeled NADPH in amounts enough for the minimum concentration of 0.1 mM in the standard assay mixture for dolichol formation. We took another approach using an NADPH-generating system to assay the incorporation of the hydrogen of NADPH into dolichol, because this approach was expected to provide in situ even such high concentrations of labeled NADPH as 1.0 mM. Preliminary experiments with a crude 1,000 × g supernatant showed that NADPH⁺ rather than NADPH had a stimulatory effect on the activity for dolichol synthesis, suggesting the occurrence of an endogenous system of synthesizing NADPH from NADPH⁺. Extended dialysis of the 1,000 × g supernatant considerably reduced the effects of the endogenous system on dolichol formation. Fig. 3 shows the effects of the concentration of glucose 6-phosphate or NADPH on the formations of dolichol and squalene in NADPH-generating systems. In the case of squalene (Fig. 3B) synthesis, the formation was saturated with the addition of 0.1 mM glucose 6-phosphate in the presence of 1 mM NADPH⁺ or with 0.5 mM NADP⁺ in the presence of 1 mM glucose 6-phosphate. The formation of dolichol (Fig. 3A) was increased with increasing concentrations of NADPH⁺ in the presence of 1 mM glucose 6-phosphate, whereas it was saturated at a concentration of 0.1 mM glucose 6-phosphate in the presence of 1 mM NADP⁺ and then decreased with the increase of the concentration of glucose 6-phosphate. The amount of formation of dolichol with the NADPH-generating system was larger than that with 1 mM NADPH alone. We next examined the incorporation of tritium of [4B-3H]NADPH formed from NADPH⁺ and [1-3H]glucose 6-phosphate by the NADPH-generating system into dolichol. Significant incorporation of radioactivity into the dolichol fraction (903 dpm) was observed. To our surprise, the dehydrodolichol fraction also contained radioactivity (1,345 dpm). This implies that dehydrodolichol and dehydrodolichal are interconvertible by the action of NADPH⁺/NADPH-dependent dehydrogenases.

**Chemical Synthesis of Dehydrodolichal and Dolichal**—To see the chemical behavior of dehydrodolichal on silica gel thin layer chromatography, we chemically synthesized an authentic specimen of dehydrodolichal by treating dehydrodolichol with MnO₂. We also synthesized dolichol by treatment of dolichol with pyridinium dichromate for comparison. As shown in Fig. 4A, the dehydrodolichal compound (lane 2) migrated much slower than the dolichal compound (lane 6) on a silica gel thin layer plate, although dehydrodolichal (lane 1) had a little greater mobility than dolichol (lane 5). To confirm that these compounds are aldehydes, they were treated with NaBH₄ or LiAlH₄. Both compounds were reduced to dehydrodolichol (lanes 3 and 4) and dolichol (lanes 7 and 8). These authentic compounds on a silica gel plate also turned blue-violet on addition of a Schiff's reagent, indicating that the two compounds obtained by the treatment with MnO₂ and pyridinium dichromate are in fact dehydrodolichal and dolichal, respectively. To see their separation dependent on the carbon chain length, we compared these aldehydes with each other on a...
reverse-phase silica gel plate (Fig. 4B). Dehydrodolichol (lane 3) migrated with almost the same R value as dehydrodolichal (lane 4). A similar observation was made with dolichol (lane 5) and dolichal (lane 6). When dehydrodolichol and its corresponding dolichol are compared, dehydrodolichol (lane 1) moved faster than dolichol (lane 2). A similar relation was also observed in the case of dehydrodolichal (lane 7) and dolichal (lane 8).

Two-plate Chromatography—In order to search for dehydrodolichal, which is expected to occur in enzymatic products, the portion of the first silica gel plate on which radioactive nonpolar products had been developed was subjected to the second reverse-phase chromatography. As shown in Fig. 5, two new polyprenyl families indicated by arrows 2 and 3 were observed in addition to dehydrodolichol and dolichol families (arrows 4 and 5), respectively. The faint radioactivity family spots (arrow 3) were in good agreement to those of the authentic dehydrodolichal family. Further, the radioactivity family spots (arrow 2) comigrated with authentic dolichal. Their migrations were also confirmed by another two-plate silica gel chromatography using a different solvent system, toluene:ethyl acetate (9:1). These results indicate that not only dehydrodolichal but also dolichal is formed in considerable amounts in these in vitro experiments.

Effect of NADP⁺ and NADPH on the Formation of Dehydrodolichol, Dehydrodolichal, Dolichal, and Dolichol—We examined the time-dependent formation of dehydrodolichol, dehydrodolichal, dolichal, dolichol, and squalene in the in vitro system (Table I). In the case of assays with NADP⁺, the formation of dehydrodolichal and dolichal preceded that of dolichol. It is suggested that the formation of squalene as well as dolichol is dependent on NADPH synthesized from exogenous NADP⁺ by the action of an endogenous NADPH-generating system, although dialyzed enzyme preparations were used. In the case of assays with NADPH, dolichal and dolichol were predominantly synthesized over dehydrodolichal.

Effect of pH on the Formation of Dehydrodolichol, Dehydrodolichal, Dolichal, and Dolichol in the Presence of NADP⁺ and NADPH—Since NADP⁺/NADPH-dependent oxidoreduction seemed to be involved in the biosynthetic pathway from dehydrodolichol to dehydrodolichal, dolichal, and dolichol, we examined the effects of pH on the formation of these products.
and squalene in the presence of both NADP⁺ and NADPH (Table II). While the synthesis of dolichol was optimal at pH 7–8, that of dolichal was optimal at pH 8–9.

Incorporation of the 4B-Hydride of NADPH into Dehydrodolichol, Dehydrodolichal, Dolichal, Dolichol, and Squalene—To establish whether dolichol is formed from dehydrodolichol directly or via intermediates such as dehydrodolichal and dolichal, we performed double-labeled experiments with [1-14C] isopentenyl-P₂ as a single precursor in the presence of the [4B-3H]NADPH-generating system. Table III shows the radio-activity of ³H and ¹⁴C in dehydrodolichol, dehydrodolichal, dolichal, dolichol, and squalene fractions on the two-plate thin layer chromatogram. All the detectable compounds contained ³H-derived radioactivity. Assuming that one hydride from NADPH is incorporated into one molecule of dolichal or dolichol, the ratio of ³H- to ¹⁴C-derived radioactivity is calculated to be 18 for each compound. Based on this value, the specific activities of NADPH utilized in the syntheses of dolichal and dolichol were estimated to be about 2- and 12-fold diluted, respectively. The dilution of NADPH in the case of dolichol synthesis was in good agreement to that in the case of squalene synthesis. These results indicate that the site for biosynthesis of dolichal is different from that of dolichol and that there is no direct interconversion between dolichal and dolichol.

Further Product Analyses—To determine whether enzyme preparations of a wild type yeast as well as mutant no. 149 have also the ability to synthesize dehydrodolichal and dolichal in addition to dehydrodolichol and dolichol, we analyzed the enzymatic products. Dolichal, dehydrodolichol, and dolichol were formed. The formation of dehydrodolichal was too small to detect. The radioactive dolichal fraction was converted 89% to dolichol by treatment of LiAlH₄. No chemical oxidation of dehydrodolichol or dolichol into dehydrodolichal or dolichal was observed in experiments (23 or 50 °C, pH 9 or 10, overnight incubation). Further, we also analyzed naturally occurring nonpolar polypropenyl compounds of a wild type yeast. Dolichal was easily detected, but dehydrodolichal, dehydrodolichal, or dolichal were not detected. We have found unknown compounds which migrated faster than dolichal on normal-phase silica gel thin layer chromatography and which were separated into a family with respect to carbon chain length on reverse-phase C₁₈ silica gel thin layer chromatography. This compound was resistant to mild alkaline hydrolysis.

**TABLE I**

| Time  | (A) Dedolichol | (B) Dedolichal | (C) Dolichal | (D) Dolichol | A + B + C + D | Squalene |
|-------|---------------|---------------|-------------|--------------|---------------|---------|
| min   | dpm           | dpm           | dpm         | dpm          | dpm           | dpm     |
| Radioactivity in the presence of NADP⁺  |
| 5     | 2,451 (21.0)  | 16 (0.1)      | 79 (0.7)    | 0 (0.0)      | 2,564 (21.8)  | 0       |
| 15    | 6,663 (57.2)  | 290 (2.5)     | 301 (2.6)   | 0 (0.0)      | 7,254 (62.2)  | 0       |
| 30    | 10,427 (89.5) | 634 (5.4)     | 461 (4.0)   | 133 (1.1)    | 11,655 (100)  | 197     |
| Radioactivity in the presence of NADPH  |
| 5     | 1,938 (21.9)  | 76 (0.9)      | 84 (1.0)    | 100 (1.1)    | 2,188 (24.8)  | 10      |
| 15    | 3,605 (40.9)  | 107 (1.2)     | 271 (3.1)   | 658 (7.5)    | 4,641 (52.7)  | 34      |
| 30    | 7,226 (82.0)  | 230 (2.6)     | 574 (6.5)   | 782 (8.9)    | 8,812 (100)   | 316     |

**TABLE II**

| pH   | (A) Dedolichol | (B) Dedolichal | (C) Dolichal | (D) Dolichol | A + B + C + D | Squalene |
|------|---------------|---------------|-------------|--------------|---------------|---------|
|      | dpm           | dpm           | dpm         | dpm          | dpm           | dpm     |
| Radioactivity at pH 7.0, 8.0, and 9.0  |
| 7.0  | 5,543 (84.7)  | 93 (1.4)      | 340 (5.2)   | 567 (8.7)    | 6,543 (100)   | 629     |
| 8.0  | 15,061 (87.2) | 259 (1.5)     | 1,068 (6.2) | 891 (5.2)    | 17,299 (100)  | 1,050   |
| 9.0  | 14,577 (88.4) | 472 (2.9)     | 1,157 (7.0) | 278 (1.7)    | 16,481 (100)  | 699     |
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The enzymatic assays were conducted as described under “Experimental Procedures” except that treatment with acid phosphatase was omitted and that the incubation was carried out overnight. A 1,000 × g supernatant of mutant no. 149 was used for the assay. The theoretical ratios of $^{3}H$-to $^{14}C$-radioactivities were calculated on the basis of the assumption that the number of average isoprene units constituting the carbon chain of dolichol or dolichal are 16. Dedolichol, dehydrodolichol, dolichal, and dehydrodolichal.

| Compound     | $^{3}H$ | $^{14}C$ | $^{3}H/^{14}C (A)$ (Theoretical) | $^{3}H/^{14}C (B)$ (Experimental) | Dilution (A/B) |
|--------------|---------|---------|---------------------------------|----------------------------------|----------------|
| Dedolichol   | 1,208   | 10,421  | 0                               | 0.12                             |                |
| Dedolichal   | 90      | 94      | 0                               | 0.85                             |                |
| Dolichal     | 15,002  | 1,618   | 18                              | 9.27                             | 1.94           |
| Dolichol     | 3,320   | 2,191   | 18                              | 1.52                             | 11.84          |
| Squalene     | 37,777  | 9,140   | 48                              | 4.13                             | 11.62          |

TABLE III
Assays with [4B-3H]NADPH-generating system

Mevalonic Acid

\[
\begin{align*}
E,E \text{-Farnesyl-PP} & \quad \downarrow \\
Z,E,E \text{-Geranylgeranyl-PP} & \quad \downarrow \\
\text{Dehydrodolichol-PP} & \quad \downarrow \\
\text{Dehydrodolichol} & \quad \rightarrow \\
\text{Dolichal} & \quad \rightarrow \\
\text{Dolichol} & \quad \rightarrow
\end{align*}
\]

FIG. 6. Possible biosynthetic pathways of dolichol and dolichal.

To elucidate the stereochemistry of the hydride transfer from NADPH in dolichol biosynthesis, we prepared [4B-3H]NADPH and [4A-3H]NADPH. However, the yields of both radioactive forms of NADPH thus obtained were too low to satisfy even the minimum concentration of NADPH established for the yeast enzyme assay. Therefore, we employed an NADPH-generating system for elucidation of the stereochemistry of NADPH reduction. The crude enzyme preparation was dialyzed sufficiently to remove at least free glucose 6-phosphate, because it was found that dolichol was formed with crude nondialyzed enzymes without addition of exogenous glucose 6-phosphate. In the reaction with the dialyzed enzymes, the formation of dolichol was increased with increasing concentration of exogenous glucose 6-phosphate. The activity with the NADPH-generating system containing NADP$^{+}$, glucose 6-phosphate, and glucose-6-phosphate dehydrogenase was much higher than that with NADPH alone. These puzzling results were readily reproducible. This observation raised the possibility that dolichol is not synthetized directly from dehydrodolichol by the action of NADPH-dependent reductase. If NADP$^{+}$ and NADPH are both necessary to form dolichol from dehydrodolichol, it could be postulated that dehydrodolichol is first oxidized to dehydrodolichal by an NADP$^{+}$-dependent dehydrogenase, and then the dehydrodolichal is reduced to dolichal by an NADPH-dependent reductase. In a preliminary doubly-labeled experiment, we found that the dolichol fraction contained $^{3}H$-derived radioactivity from [4B-3H]NADPH. To our surprise, the dehydrodolichal fraction also contained a small but significant amount of $^{3}H$-derived radioactivity. This finding led us to search for a new polyprenyl compound such as dehydrodolichal.

As shown in Fig. 5, nonpolar products were separated into dehydrodolichol, dehydrodolichal, dolichal, and dolichol in addition to squalene. We assumed the biosynthetic pathway of these polyprenyl products as follows, dehydrodolichal $\rightarrow$ dehydrodolichal [arrow] dolichal [arrow] dolichol. The involvement of these intermediates in the biosynthetic pathway to dolichol from dehydrodolichol was attractive, because the hydride transfer onto the $\beta$-carbon of $\alpha,\beta$-unsaturated carbonyl groups is well known. However, experiments concerning time-dependent and $pH$-dependent formation of these compounds revealed that these new products and dolichol differed from each other in the biosynthetic pathway from dehydrodolichol. Double-labeled experiments also showed a different biosynthetic pathway to dolichol from to dolichal. On the basis that one 4B-hydride of NADPH is incorporated into one molecule of
squalene, the numbers of 4B-hydride incorporated into dolichol and dolichal are estimated to be one and six, respectively. However, it is difficult to explain the large difference in hydride incorporation between dolichol and dolichal. One possible reason is that the biosynthetic site for dolichal formation might be more accessible to exogenous NADPH than that for dolichol formation. Therefore, at present we propose that dolichal is synthesized from dehydrodolichol via dehydrodolichol and that dolichol is formed directly from dehydrodolichol (Fig. 6). However, we do not exclude the possibility that dolichol is formed from dehydrodolichol, because in a preliminary study the addition of NADP⁺ to an assay mixture containing NADPH enhanced the activity of dolichol synthesis.

Steen et al. (22) have reported the identification of dolichyl dolichoate in bovine thyroid. The formation of dehydrodolichol is well understood as being involved in the biosynthesis of the dolichoate. Thus, the dolichoate pathway might be different from the dolichol pathway, which is responsible for the formation of dolichyl-P, a sugar carrier lipid in N-linked glycoproteins and glycosyl phosphatidylinositol-anchored proteins.

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