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Subcellular Fractionation of Polyprenyl Diphosphate Synthase Activities Responsible for the Syntheses of Polyprenols and Dolichols in Spinach Leaves

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Polyisoprenoid alcohols occurring in spinach leaves were analyzed by a two-plate TLC method. *Z,E*-mixed polyprenols (C_{55-60}), glycinoprenols (C_{50-55}), and solanesol (C_{45}) were mainly found in chloroplasts, whereas dolichols (C_{70-80}) were mainly found in microsomes. Analysis of enzymatic products derived from [1-^{14}C]isopentenyl diphosphate and farnesyl diphosphate (FPP) with subcellular fractions revealed that chloroplasts and microsomes had the ability to synthesize *Z,E*-mixed polyprenyl (C_{50-65}) and all *E*-polyprenyl (C_{45-50}) diphosphates, and *Z,E*-mixed polyprenyl (C_{70-85}) diphosphates, respectively. FPP and geranylgeranyl diphosphate (GGPP) were both accepted for these enzymatic reactions, the former being a better substrate than the latter. NMR analysis of naturally occurring spinach *Z,E*-mixed polyprenol (C_{55}) and dolichol (C_{75}) revealed that the number of internal *trans* isoprene residues in the former was three in comparison with two internal *trans* residues found for the latter. These results indicate that two kinds of polyprenyl diphosphate synthases occur in spinach: One is the chloroplast enzyme involved in the synthesis of the shorter-chain (C_{50-65}) *Z,E*-mixed polyprenols and the other is the microsomal enzyme involved in the synthesis of longer-chain (C_{70-85}) *Z,E*-mixed polyprenols, which is converted to dolichols.

Key words: chloroplast, dolichol, microsomes, polyprenol, prenyltransferase, spinach.

Two types of *Z,E*-mixed polyprenols with different stereochemistries for their chemical structures have been reported in various plants. One is betulaprenol-type polyprenol with an *E,E*-farnesyl residue at the ω-end of its prenyl chain and the other is ficaprenol-type polyprenol with an *E,E,E*-geranylgeranyl residue at that of its prenyl chain. Swiezewska *et al.* (*1*) have analyzed various plant leaves in a search for polyprenols, and reported that ficaprenol-type polyprenols with a predominant C_{55} isoprenologue occur in many angiosperm plants and that betulaprenol-type polyprenols with much longer-chain isoprenologues occur in gymnosperm plants. Many plants have been reported to accumulate *Z,E*-mixed polyprenols in tissues and to vary in quantity depending on their age or the season. The role of these *Z,E*-mixed polyprenols occurring in leaves has not been clarified yet. On the other hand, the occurrence of dolichols, which are different from *Z,E*-mixed polyprenols in that the α-isoprene residue is saturated, has also been reported recently (*1*, *2*). The dolichols play roles as sugar carrier lipids in glycoprotein biosynthesis. In order to determine the relation between *Z,E*-mixed polyprenols and dolichols with respect to their carbon chain length, it is necessary to analyze their lengths in the same tissue. Recently, we analyzed several tissues of rubber as an angiosperm and of ginkgo as a gymnosperm (*3*) using a two-plate thin-layer chromatography (TLC) method (*4*). In young rubber leaves, the C_{50-60} and C_{75-85} *Z,E*-mixed polyprenol families and C_{75-105} dolichols were found. In old leaves large amounts of the shorter-chain *Z,E*-mixed polyprenols were found. In young ginkgo leaves, the C_{60-65} and C_{80-85} *Z,E*-mixed polyprenol families and C_{70-90} dolichols were found. In old leaves extremely large amounts of the longer-chain *Z,E*-mixed polyprenols were found. It is expected that the dolichols in both cases are derived from the longer-chain *Z,E*-mixed polyprenols through the action of NADPH-dependent *Z,E*-mixed polyprenol reductase (*5*). Therefore, these facts imply the occurrence of two different biosynthetic pathways leading to the formation of these shorter and longer-chain *Z,E*-mixed polyprenols in both angiosperms and gymnosperms.

In the present study, several vegetables were used as dicotyledonous and monocotyledonous plants to analyze the occurrence of polyisoprenoid alcohols. Also, spinach was used to analyze the subcellular localization of polyisoprenoid alcohols and the *in vitro* prenyltransferase activities. This is the first report concerning the occurrence of independent biosynthetic pathways for two different *Z,E*-mixed polyprenol families occurring in plants.

EXPERIMENTAL PROCEDURE

Materials—[1-^{14}C]Isopentenyl diphosphate (IPP) was purchased from Amersham Pharmacia Biotech. *Z,E*-mixed polyprenols (C_{50-60}) from silkworm feces and *Z,E,E*-geranylgeraniol were donated by Takasago Perfumery Com-

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Abbreviations: IPP, isopentenyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate.

pany and Kuraray Corporation, respectively. *E,E*-Farnesyl diphosphate (FPP), *E,E,E*-geranylgeranyl diphosphate (GGPP), and *Z,E,E*-GGPP were prepared according to the method of Davisson *et al.* (*6*). Potato acid phosphatase (Type II) was obtained from Sigma. Spinach (*Spinacia oleracea* L.), parsley (*Petroseilinium crispum*), perilla (*Perilla frutescens*), yukina (*Brassica campestris*), and leek (*Allium tuberosum*) were locally purchased. All other chemicals were of reagent grade.

NMR spectra were recorded in $CDCl_3$ with a JEOL LAMBDA 400 instrument. C_{18} Sep-Pak columns were obtained from Waters Associates. Silica-gel 60 and LKC-18 silica-gel thin-layer plates were purchased from Merck and Whatman, respectively.

Extraction of Polyisoprenoid Alcohols from Plants—The procedures for the extraction of tissue polyisoprenoid alcohols were basically the same as described in a previous paper (*7*). Leaves were thoroughly washed with water, dried in an oven at 60°C for one day and then immersed in acetone: hexane (1:1) for three days. The extracts were each suspended in a mixture of 20% ethanol, 20% KOH, and 0.25% pyrogallol, and then saponified at 85°C for 1 h. The mixture was neutralized with conc. HCl and then extracted with hexane. The hexane soluble extracts were dried, dissolved in a small volume of methanol and then applied to a C_{18} Sep-Pak column. The polyisoprenoid alcohols were eluted with hexane and analyzed by a two-plate TLC method (*4*).

Purification of Z,E-Mixed Polyprenol and Dolichol—To prepare *Z,E*-mixed polyprenol (C_{55-65}) and dolichol (C_{65-80}) occurring in spinach leaves as NMR samples, leaves (1.5 kg) were extracted with acetone:hexane (1:1) and then saponified as described earlier, and the non-saponifiable crude polyisoprenoid alcohols were applied to a silica-gel 60 (Merck) column (3 × 20 cm) equilibrated with a solvent system of toluene:ethyl acetate (9:1). The fractions corresponding to *Z,E*-mixed polyprenol (C_{55}) and dolichol (C_{75}) were separately combined and further purified on a reverse-phase C_{18} silica-gel (Merck) column (2 × 15 cm) equilibrated with a solvent system of acetone:methanol (9:1). The amounts of purified *Z,E*-mixed polyprenol (C_{55}) and dolichol (C_{75}) were 6.0 and 1.3 mg, respectively. The purity of these compounds was confirmed by TLC.

Subcellular Fractionation of Spinach Leaves—The procedures used for subcellular fractionation of spinach leaves were basically the same as described in a previous report (*7*). Leaves (20 g) were homogenized with a juicer in 100 ml of 50 mM Tricine buffer (pH 7.8). The homogenate was filtered through a gauze and then centrifuged at 300 ×*g* for 5 min. The supernatant was centrifuged at 2,600 ×*g* for 10 min. The precipitates were resuspended in the same buffer and then centrifuged similarly. The washed precipitates (130 mg) were used as the chloroplast fraction. The 2,600 ×*g* supernatant was further centrifuged at 20,000 ×*g* for 10 min. The precipitates were resuspended in the Tricine buffer and then centrifuged similarly. The obtained precipitates (50 mg) were used as the mitochondrial fraction. Furthermore, the 20,000 ×*g* supernatant was centrifuged at 106,000 ×*g* for 90 min. The precipitates were resuspended in the same Tricine buffer and then centrifuged similarly. The washed precipitates (90 mg) were used as the microsomal fraction. The chloroplast fraction was confirmed under a Zeiss microscope.

Extraction of Polyisoprenoid Alcohols from Subcellular Fractions—Each subcellular fraction was suspended in a mixture of 20% ethanol, 20% KOH, and 0.25% pyrogallol, and then saponified at 85°C for 1 h. The following procedures were the same as described earlier.

Enzyme Assay—Prenyltransferase activity was assayed according to the procedure in (*7*) with some modification. The standard assay mixture contained, in a final volume of 0.5 ml, 50 mM Tris-HCl buffer (pH 8.5), 2 mM dithiothreitol, 50 mM KF, 1 mM $MgCl_2$, 10 μM [1-^{14}C]IPP, 100 μM allylic diphosphate, and an appropriate amount of enzyme fraction. The mixture was incubated at 30°C for 4 h. The reaction products were extracted with 1 ml of 1-butanol saturated with water, and the butanol extracts were washed twice with 0.5 ml of saturated NaCl. The washed extracts were treated with potato acid phosphatase (44 units) according to the method of Fujii *et al.* (*8*). After incubation at 37°C for 12 h, the hydrolysates were extracted with hexane. The hexane extracts were suspended in a mixture of 20% EtOH, 20% KOH, and 0.25% pyrogallol, and then saponified at 85°C for 1 h. The mixture was extracted with hexane, and the extracts were analyzed as described earlier. The positions of authentic standards were visualized with iodine vapor. The radioactivity of polyisoprenoid alcohols developed on TLC was determined with a Fuji Bioimage Analyzer BAS 1000 using *Z,E*-mixed polyprenol standards (1,000 dpm) as references.

RESULTS

In a preliminary experiment we analyzed the polyisoprenoid alcohols in several commercially available vegetables (dicotyledons such as perilla, parsley, spinach, and yukina,

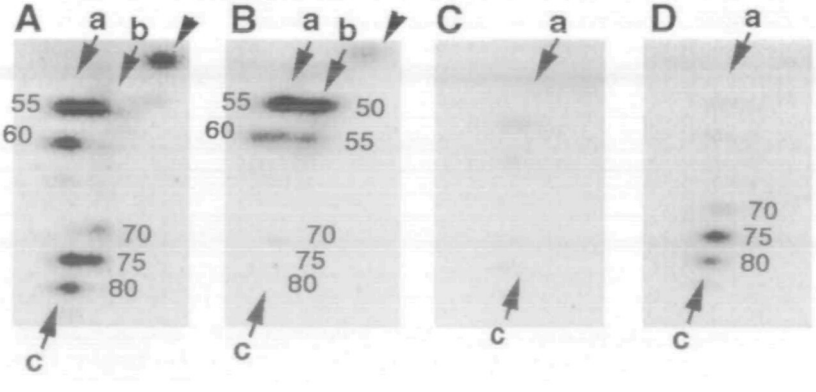

Fig. 1. **Analysis of polyisoprenoid alcohols extracted from subcellular fractions of spinach leaves by two-plate TLC.** Polyisoprenoid alcohols extracted from a homogenate of leaves (A), chloroplast fraction (B), mitochondrial fraction (C), and microsomal fraction (D) were applied to TLC plates. The positions of polyisoprenoid alcohols were visualized with iodine vapour. The arrowheads indicate solanesol, and the arrows indicate the ficaprenol (a), glycinoprenol (b), and dolichol (c) families. The numbers refer to the carbon chain lengths of polyisoprenoid alcohols.

and monocotyledons such as leek). The dicotyledonous plant leaves contained shorter-chain *Z,E*-mixed polyprenols (C_{55-60}) and longer-chain dolichols (C_{75-85}), whereas the monocotyledonous leek leaves contained shorter-chain *Z,E*-mixed polyprenols (C_{50-55}) but no longer-chain dolichols (C_{75-85}). In order to determine the relation between shorter-chain *Z,E*-mixed polyprenols and longer-chain dolichols with respect to their biosyntheses, we mainly chose in the present study spinach because spinach is commercially available all the year round in comparison with the other vegetables.

As shown in Fig. 1A, spinach leaves were found to contain *Z,E*-mixed polyprenols (C_{55-60}), glycinoprenols (C_{50-55}) (*9*), dolichols (C_{70-80}), and solanesol (C_{45}). The distributions of these polyisoprenoid alcohols were quite similar to those in soybean leaves (*7*, *9*). We prepared chloroplast, mitochondrial, and microsomal fractions by differential centrifugation of the crude homogenate and determined the content of polyisoprenoid alcohols in each fraction. The chloroplast fraction (Fig. 1B) was found to contain *Z,E*-mixed polyprenols (C_{55-60}), glycinoprenols (C_{50-55}), and solanesol (C_{45}). Trace amounts of shorter-chain *Z,E*-mixed polyprenols were found in the mitochondrial fraction (Fig. 1C). As shown in Fig. 1D, the microsomal fraction was found to contain dolichols (C_{70-80}).

It is expected that there are at least four kinds of biosynthetic pathways responsible for the formation of *Z,E*-mixed polyprenols (C_{55-60}), glycinoprenols (C_{50-55}), *Z,E*-mixed dolichols (C_{70-80}), and solanesol (C_{45}). To clarify these pathways, we prepared 106,000 ×*g* supernatant and precipitate fractions, and assayed these fractions for prenyltransferase activity. As shown in Fig. 2A, the supernatant fraction was found to have the ability to synthesize FPP and GGPP as the major products. On the other hand, the 106,000 ×*g* precipitate fraction (Fig. 2B) was found to have the ability to synthesize FPP, GGPP, *Z,E*-mixed-polyprenyl-PPs (C_{25-30}, C_{50-65}, and C_{70-75}), and all-*E*-polyprenyl-PPs (C_{45-50}). The crude homogenate was further fractionated by differential centrifugation into chloroplast, mitochondrial and microsomal fractions, and each fraction was assayed for prenyltransferase activity with a combination of [1-^{14}C]IPP and FPP. Each fraction had the ability to synthesize FPP and GGPP, as shown in Fig. 3. The chloroplast fraction (Fig. 3A) was also found to have the ability to synthesize *Z,E*-mixed polyprenyl-PPs (C_{25-30} and C_{55-65}) and all *E*-polyprenyl-PPs (C_{45-50}). In the case of the mitochondrial fraction (Fig. 3B), *Z,E*-mixed polyprenyl-PP (C_{20-30})-synthesizing activities were also observed. On the other hand, the microsomal fraction had the ability to synthesize *Z,E*-mixed polyprenyl PPs (C_{65-80}), the major chain lengths being C_{70-75} (Fig. 3C). The formation of an unknown compound with a chain length of C_{30} was observed with this fraction, suggesting that this might be presqualene alcohol.

Having established that chloroplasts and microsomes contain polyprenyl diphosphate synthase activities responsible for the formation of naturally occurring shorter-chain *Z,E*-mixed polyprenols (C_{50-60}) and longer-chain dolichols (C_{75-80}), we next examined the allylic substrate specificities of the two polyprenyl diphosphate synthases. As shown in Fig. 4, *E,E*-FPP and *E,E,E*-GGPP were both accepted as allylic substrates by the chloroplast enzyme, the former being a better substrate than the latter. *Z,E,E*-GGPP was not accepted by the enzyme. In the case of the microsomal polyprenyl diphosphate synthase (Fig. 5A), a similar allylic substrate specificity was observed. However, detectable amounts of polyprenyl products were observed, as assayed with [1-^{14}C]IPP alone, suggesting the occurrence of an IPP isomerase activity in the microsomal fraction. We chemically synthesized *N,N*-dimethylaminoethyl diphosphate, which inhibits IPP isomerase activity (*10*) but not polyprenyl diphosphate synthase activity (unpublished data), and assayed the microsomal fraction in the presence of this inhibitor (Fig. 5B). FPP was actually a better allylic substrate than GGPP. These results indicate that two spinach

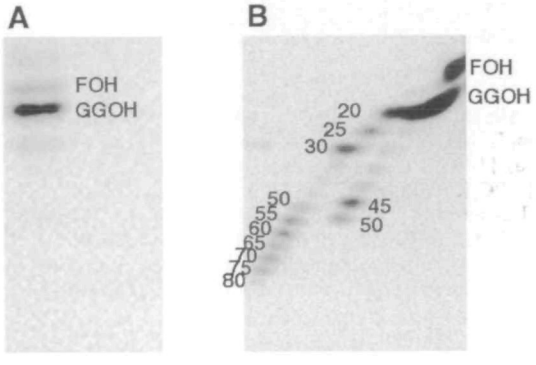

Fig. 2. **Enzymatic formation of polyprenyl compounds by the 106,000 ×*g* supernatant (A) and precipitate (B) fractions.** The assay was carried out with a combination of [1-^{14}C]IPP (10 μM) and FPP (100 μM) as described under "EXPERIMENTAL PROCEDURE." Autoradiography of the radioactive products after reverse-phase LKC-18 TLC (A) and two-plate TLC (B) are shown. The numbers refer to the carbon chain lengths of polyisoprenoid alcohols. The authentic compounds used in B were solanesol (C_{45}), and *Z,E*-mixed polyprenols (C_{55-95}) from *Ginkgo biloba* and those from silkworm feces (C_{55-80}). FOH, farnesol; GGOH, geranylgeraniol.

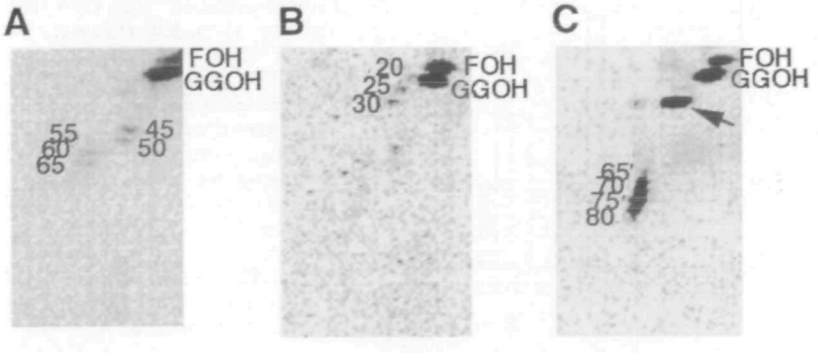

Fig. 3. **Enzymatic formation of polyisoprenoid compounds by the chloroplast (A), mitochondrial (B), and microsomal (C) fractions.** The assay was the same as described in the legend to Fig. 2. The autoradiography of radioactive products after two-plate TLC is shown. The numbers refer to the carbon chain lengths of polyisoprenoid alcohols. The authentic compounds used were solanesol (C_{45}), and *Z,E*-mixed polyprenols (C_{55-95}) from *Ginkgo biloba* and those from silkworm feces (C_{55-80}). The arrow in C indicates an unknown enzymatic product. FOH, farnesol; GGOH, geranylgeraniol.

polyprenyl diphosphate synthases have tolerant substrate specificities, although FPP is preferred to GGPP as an allylic substrate by these enzymes. We also tried to examine the allylic substrate specificity of a polyprenyl diphosphate synthase from another source (leek). As shown in Fig. 6A, leeks contained relatively large amounts of shorter-chain *Z,E*-mixed polyprenols (C_{50-80}). The crude homogenate was shown to have the ability to synthesize polyprenyl-PPs (C_{50-65}) from FPP or GGPP, FPP being a better substrate than GGPP (Fig. 6B).

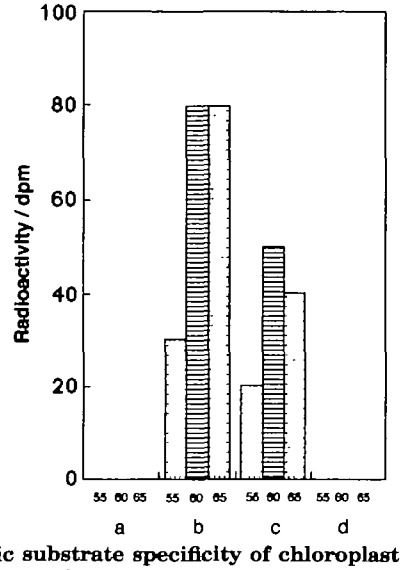

Fig. 4. **Allylic substrate specificity of chloroplast polyprenyl diphosphate synthase.** The assay was carried out with [1-^{14}C]IPP alone (a), [1-^{14}C]IPP plus *E,E*-FPP (b), [1-^{14}C]IPP plus *E,E,E*-GGPP (c), or [1-^{14}C]IPP plus *Z,E,E*-GGPP (d). The enzymatic products were treated as described under "EXPERIMENTAL PROCEDURE" and then subjected to two-plate TLC, and the spots of radioactivity corresponding to the polyisoprenoid alcohols were quantitatively analyzed. The numbers (55, 60, and 65) refer to the carbon chain lengths of polyprenols.

Both the 106,000 ×*g* supernatant and precipitate fractions had the ability to produce FPP and GGPP, as shown in Fig. 2. Also, two polyprenyl diphosphate synthases located mainly in chloroplasts and microsomes, respectively, were able to accept FPP and GGPP as allylic substrates. Since the formation of FPP and GGPP was observed with every subcellular fraction, naturally occurring *Z,E*-mixed polyprenols derived from products produced through catalysis by the polyprenyl diphosphate synthases might be a mixture of two polyprenols with different stereochemistries such as betulaprenol-type polyprenols with an *E,E*-farnesyl residue and ficaprenol-type polyprenols with an *E,E,E*-geranylgeranyl residue at the ω-end of the carbon chain. We tried to purify two kinds of polyisoprenoid alcohols corresponding to *Z,E*-mixed polyprenol (C_{55}) and dolichol (C_{75}) by silica-gel column chromatography of crude lipid fractions, followed by reverse-phase silica gel column chromatography. The compounds, *Z,E*-mixed polyprenol (C_{55}) and dolichol (C_{75}), gave NMR spectra characteristic of a polyisoprenoid alcohol. Although several signals could not be assigned, it was possible to determine the numbers of *cis* and *trans* isoprene residues by measurement of the relative areas of the peaks corresponding to methyl groups *cis* and *trans* as to olefinic protons (*11*). In the case of *Z,E*-mixed polyprenol (C_{55}), the theoretical area ratios of the *cis* peak to the *trans* peak were calculated to be 2.67:1 and 1.75:1 for betulaprenol-type and ficaprenol-type polyprenols, respectively. Analysis of the spinach *Z,E*-mixed polyprenol (C_{55}) revealed that the ratio was 1.57:1 (the *cis* peak at 1.682 ppm and the *trans* peak at 1.599 ppm), suggesting the stereochemistry of a ficaprenol-type polyprenol. In the case of dolichol (C_{75}), the theoretical area ratios of the *cis* peak to the *trans* peak were calculated to be 4.00:1 and 2.75:1 for betulaprenol-type and ficaprenol-type dolichols, respectively. The spinach dolichol (C_{75}) was determined to have a ratio of 3.75:1 (the *cis* peak at 1.679 ppm and the *trans* peak at 1.598 ppm), suggesting the stereochemistry of a betulaprenol-type dolichol. These results indicate that spinach *Z,E*-mixed polyprenols (C_{50-60}) and dolichols (C_{70-80}) con-

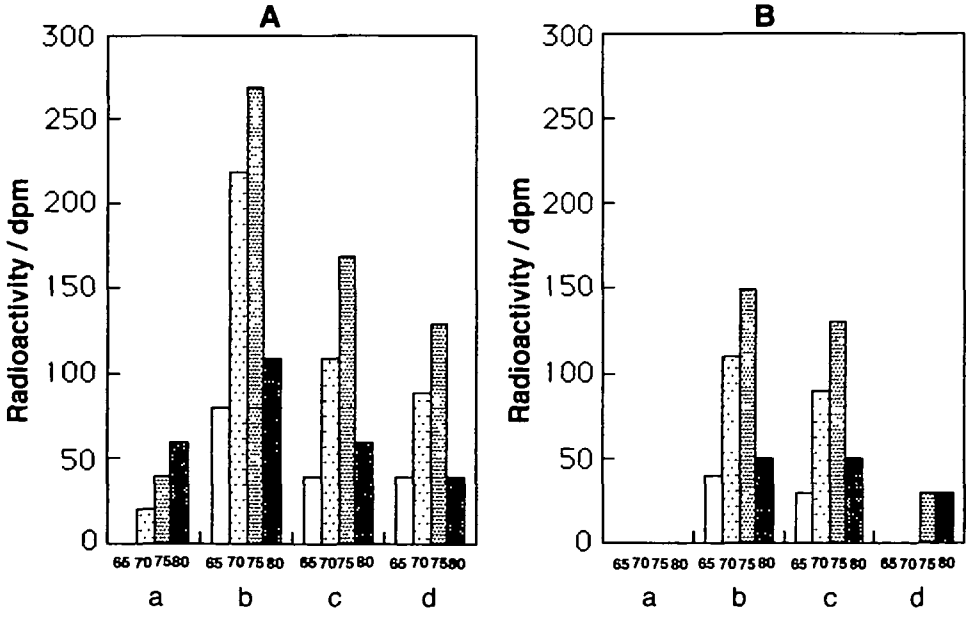

Fig. 5. **Allylic substrate specificity of microsomal polyprenyl diphosphate synthase.** The assay was carried out in the absence (A) or presence (B) of *N,N*-dimethyaminoethyl-PP with [1-^{14}C]IPP alone (a), [1-^{14}C]IPP plus *E,E*-FPP (b), [1-^{14}C]IPP plus *E,E,E*-GGPP, or [1-^{14}C]IPP plus *Z,E,E*-GGPP (d). The enzymatic products were treated as described under "EXPERIMENTAL PROCEDURES" and then subjected to two-plate TLC, and the spots of radioactivity corresponding to the polyisoprenoid alcohols were quantitatively analyzed. The numbers (65, 70, 75, and 80) refer to the carbon chain lengths of polyprenols.

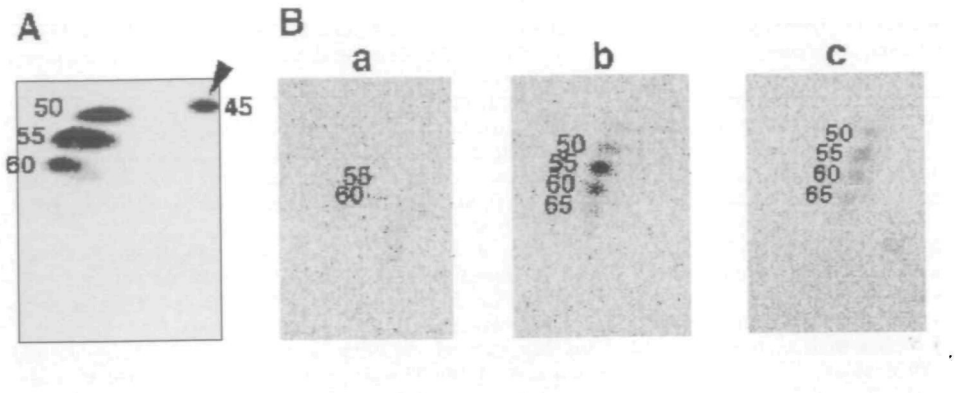

Fig. 6. **Naturally occurring polyisoprenoid alcohols in leek (A) and the enzymatic polyprenyl products with the leek homogenate (B).** In A, the numbers refer to polyisoprenoid alcohols, and the arrowheads indicate the ficaprenol (a) and solanesol (d) families. In B, the enzyme assay was carried out with [1-^{14}C]IPP alone (a), [1-^{14}C]IPP plus *E,E*-FPP (b), or [1-^{14}C]IPP plus *E,E,E*-GGPP (c). The numbers (50, 55, 60, and 65) refer to the carbon chain lengths of polyprenols. Spot number (45) is that of solanesol.

tain three internal *trans* isoprene residues (*E,E,E*-geranylgeranyl residue) and two internal *trans* isoprene residues (*E,E*-farnesyl residue) at the ω-end of the prenyl chain, respectively.

Considering the fact that the shorter-chain *Z,E*-mixed polyprenols (C_{50-60}) are localized in chloroplasts and that the chloroplast fraction has the ability to synthesize the polyprenyl compounds, we next compared the occurrence of *Z,E*-mixed polyprenols (C_{50-60}) between leek and etiolated leek tissues. The lack of chloroplasts in the etiolated leek tissue was microscopically confirmed. Lipid analysis of the homogenates of the light yellow leek indicated no shorter-chain *Z,E*-mixed polyprenols (C_{50-60}) (data not shown). This strongly suggests that the formation of microscopically detectable chloroplasts is accompanied by the synthesis of the shorter-chain *Z,E*-mixed polyprenols (C_{50-60}).

DISCUSSION

The present study indicated that two polyprenyl diphosphate synthase activities (tentatively assigned to polyprenyl diphosphate synthases I and II) responsible for the carbon-chain formation of the *Z,E*-mixed polyprenols and dolichols are present in chloroplasts and microsomes, respectively. Considering the stereochemistry of naturally occurring spinach *Z,E*-mixed polyprenols (C_{50-60}) and dolichols (C_{70-80}), we speculate that *Z,E*-mixed polyprenols (C_{50-60}) and dolichols (C_{70-80}) are synthesized through the action of two enzyme systems, GGPP synthase plus polyprenyl diphosphate synthase I localized in chloroplasts, and FPP synthase plus polyprenyl diphosphate synthase II localized in microsomes, respectively. Szkopinska *et al.* have proposed a model involving pairing of FPP synthase with polyprenyl diphosphate synthase II responsible for dolichol biosynthesis from the results of experiments concerning the effects of FPP synthase overexpression on dolichol biosynthesis in yeast (*15*). FPP and GGPP are known to be intermediates in the biosynthetic pathway for divergent end products. Therefore, if each pairing is the case, the occurrence of several FPP synthases and several GGPP synthases will be expected in each plant. In *Arabidopsis thaliana*, at least two FPP synthase genes and five GGPP synthase genes have been found (*12–14*).

In a previous report (*3*), we described the occurrence of shorter chain *Z,E*-mixed polyprenols in the leaves of angiosperms such as rubber, and gymnosperms such as ginkgo and pine. But the content of gymnosperm shorter-chain *Z,E*-mixed polyprenols was extremely low compared with that of angiosperm shorter-chain *Z,E*-mixed polyprenols. The present leek results indicate that shorter-chain *Z,E*-mixed polyprenols (C_{50-60}) exist in parallel with chloroplasts. Does the low content of shorter-chain *Z,E*-mixed polyprenols in gymnosperms reflect the metabolic activity or number of chloroplasts? Further experiments are necessary to determine the role of shorter-chain *Z,E*-mixed polyprenols occurring in plant chloroplasts.

Sato *et al.* (*16*) recently reported two yeast genes (*RER2* and *SRT1*) which encode polyprenyl diphosphate synthase. Since the *RER2* enzyme is responsible for yeast dolichol synthesis, one enzyme found in the present study, *i.e.* polyprenyl diphosphate synthase II localized in microsomes, seems to correspond to the *RER2* enzyme. The other enzyme, polyprenyl diphosphate synthase I, does not seem to correspond to the *SRT1* enzyme, because yeast contains no chloroplasts. Since double disruption of the *RER2* and *SRT1* genes is lethal (*16*), the yeast *SRT1* enzyme seems to play an important role in dolichol biosynthesis, although characterization of the *SRT1* enzyme has not been performed yet.

Cunillera *et al.* (*17*) suggested the occurrence of the polyprenyl diphosphate synthase gene family and an additional polyprenyl diphosphate synthase isoform in *Arabidopsis thaliana*. It is possible to assume that this polyprenyl diphosphate synthase isoform is polyprenyl diphosphate synthase I. To better understand the entire mechanism of dolichol biosynthesis in plants, the occurrence of polyprenyl diphosphate synthase I should be considered.

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