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Molecular Species Composition of Phosphatidylglycerols from Chilling-Sensitive and Chilling-Resistant Plants

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The molecular species of phosphatidylglycerols from leaves of 9 species of chilling-sensitive plants and 12 species of chilling-resistant plants were analyzed by gas-liquid chromatography and gas chromatography-mass spectrometry. The sum of the contents of the dipalmitoyl plus the 1-palmitoyl-2-(*trans*-3-hexadecenoyl) species of phosphatidylglycerol ranged from 3 to 19% of the total of this lipid in the chilling-resistant plants, and from 26 to 65% in the chilling-sensitive plants. These findings suggest that these two molecular species of phosphatidylglycerols are closely associated with the chilling sensitivity of the plants. The biochemical difference between the chilling-sensitive and the chilling-resistant plants is discussed in terms of the activities of enzymes involved in phosphatidylglycerol biosynthesis in the chloroplasts.

Key words: Chilling-sensitive plant — Lipid molecular species — Phosphatidylglycerol.

In the previous paper (Murata et al. 1982), we reported that the sum of the 16:0 and 16:1^t contents relative to the total fatty acids in phosphatidylglycerol was higher in chilling-sensitive than in chilling-resistant plants, and suggested the possibility that the proportion of the 16:0/16:0 and 16:0/16:1^t species relative to the total molecular species of phosphatidylglycerol could be correlated with the chilling sensitivity of higher plants. The present investigation was designed to examine this hypothesis by GLC and GC-MS analysis of the molecular species of phosphatidylglycerol from leaves of chilling-sensitive and chilling-resistant plants.

Materials and Methods

Plant leaves were obtained and phosphatidylglycerols extracted and purified as previously described (Murata et al. 1982). Leaves of *Luffa cylindrica* (sponge cucumber) were obtained from plants growing in a farm field. The phosphatidylglycerols were treated with phospholipase C (Behringer-Mannheim), and the resultant diacylglycerols were acetylated with acetic anhydrate in pyridine (Kito et al. 1975). The monoacetyldiacylglycerols thus prepared were subjected to GLC (Shimadzu, GC-6AM) either directly or after hydrogenation of their unsaturation bonds. The molecular species of monoacetyldiacylglycerols were separated on a glass column (2 m×2 mm internal diameter) packed with 2% Silar 10C on Gaschrom Q run at 255°C and detected with a hydrogen flame ionization system. The flow rate of the nitrogen carrier gas was 40 ml min^{-1}. The monoacetyldiacylglycerols were also subjected to analysis by GC-MS (Hitachi, M-80) with the column conditions the same as in GLC, except that the carrier gas was

Abbreviations: GC-MS, gas chromatography-mass spectrometry; GLC, gas-liquid chromatography; 16:0, palmitic acid; 16:1^t, *trans*-3-hexadecenoic acid; 18:0, stearic acid; 18:1, oleic acid, 18:2, linoleic acid; 18:3, α-linolenic acid.

helium with a flow rate of 50 ml min^{-1}. The ionization voltage was 20 eV, and the ion source temperature was 200°C.

Results

Hydrogenated molecular species—The phosphatidylglycerols from plant leaves contain the fatty acids 16:0, $16:1^t$, 18:0, 18:1, 18:2 and 18:3 (Murata et al. 1982). In order to determine the possible combinations of these fatty acids, the monoacetyldiacylglycerols derived from the phosphatidylglycerols were analyzed by GLC and GC-MS after the unsaturation bonds in the fatty acids were hydrogenated. Fig. 1 shows a chromatogram of the hydrogenated monoacetyldiacylglycerols from the phosphatidylglycerols of taro leaves. Two peaks appeared at retention times of 11.9 and 16.0 min. Similar analyses were performed for the phosphatidylglycerols of leaves of sweet potato, maize, red clover, dandelion and spinach. In all these cases, two peaks appeared at the same retention times as in Fig. 1; however, the relative heights of the first and second peaks varied with plants.

The molecular species of the hydrogenated monoacetyldiacylglycerols were determined by GC-MS. Upon electronic ionization, the first peak produced fragment ions having m/z values of 551, 355 and 239, which correspond to the structures $CH_2(OCOC_{15}H_{31})$-$CH(OCOC_{15}H_{31})$-CH_2, CH_2-$CH(OCOC_{15}H_{31})$-$CH_2(OCOCH_3)$ and $COC_{15}H_{31}$, respectively. The second peak produced fragment ions having m/z values of 579, corresponding to $CH_2(OCOC_{15}H_{31})$-$CH(OCOC_{17}H_{35})$-CH_2 and/or $CH_2(OCOC_{17}H_{35})$-$CH(OCOC_{15}H_{31})$-CH_2; 383, to CH_2-$CH(OCOC_{17}H_{35})$-$CH_2(OCOCH_3)$ and/or $CH_2(OCOC_{17}H_{35})$-CH-$CH_2(OCOCH_3)$; 355, to $CH_2(OCOC_{15}H_{31})$-CH-$CH_2(OCOCH_3)$ and/or CH_2-$CH(OCOC_{15}H_{31})$-$CH_2(OCOCH_3)$; 267, to $COC_{17}H_{35}$; and 239, to $COC_{15}H_{31}$. These data from GC-MS indicate that the first peak of GLC in Fig. 1 was due to $CH_2(OCOC_{15}H_{31})$-$CH(OCOC_{15}H_{31})$-$CH_2(OCOCH_3)$, and the second peak to $CH_2(OCOC_{17}H_{35})$-$CH(OCOC_{15}H_{31})$-$CH_2(OCOCH_3)$ and/or $CH_2(OCOC_{15}H_{31})$-$CH(OCOC_{17}H_{35})$-$CH_2(OCOCH_3)$. However, their molecular ions, which should have m/z values of 614 and 638, were not observed by GC-MS.

These findings suggest that the molecular species of phosphatidylglycerols should be in the form of either 1-C_{16}-2-C_{16}, 1-C_{18}-2-C_{16} or 1-C_{16}-2-C_{18}, but not in the form of 1-C_{18}-2-C_{18}. Since the C_{18} acids were localized at the C-1 position of *sn*-glycerol in phosphatidylglycerols from the chloroplasts and at both the C-1 and C-2 positions in the lipid from the whole leaves (Murata et al. 1982), the 1-C_{18}-2-C_{16} species should have originated from the chloroplasts and the 1-C_{16}-2-C_{18} species from the other membranes. Most or all of the 1-C_{16}-2-C_{16} species are considered to have also originated from the chloroplasts.

Unhydrogenated molecular species—The molecular species composition of unhydrogenated monoacetyldiacylglycerols was determined by GLC and GC-MS. Fig. 2 shows a gas chromatogram of the monoacetyldiacylglycerols derived from the phosphatidylglycerols from taro leaves. Five peaks were observed at retention times of 12.6, 16.7, 19.1, 22.2 and 27.7 min. The first and second peaks appeared at about the same retention times as the first and the second peaks of the hydrogenated monoacetyldiacylglycerols in Fig. 1.

The molecular species of monoacetyldiacylglycerols in the peaks in Fig. 2 were analyzed by GC-MS. Upon electronic ionization, the first peak in Fig. 2 produced fragment ions having m/z values of 551, 355 and 239, as in the first peak of the hydrogenated monoacetyldiacylglycerols (Fig. 1), and in addition, those with m/z values of 549, 353 and 237. These values indicate that the first peak in Fig. 2 contained molecular species of 16:0/16:0 and $16:0/16:1^t$ combinations. Since $16:1^t$ is localized at the C-2 position (Murata et al. 1982), the $16:0/16:1^t$ species should be in the form of 1-(16:0)-2-($16:1^t$).

The second peak in Fig. 2 produced fragment ions having m/z values of 579, 383, 355, 267

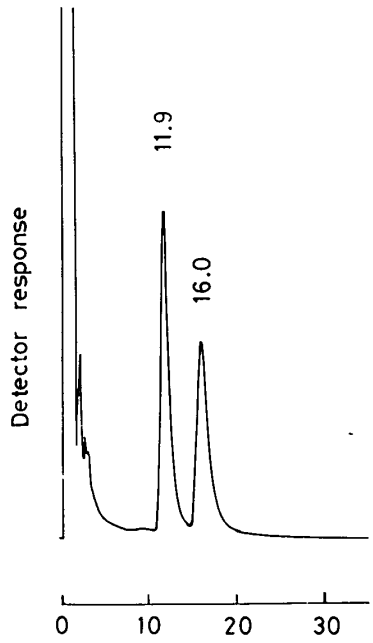

Fig. 1

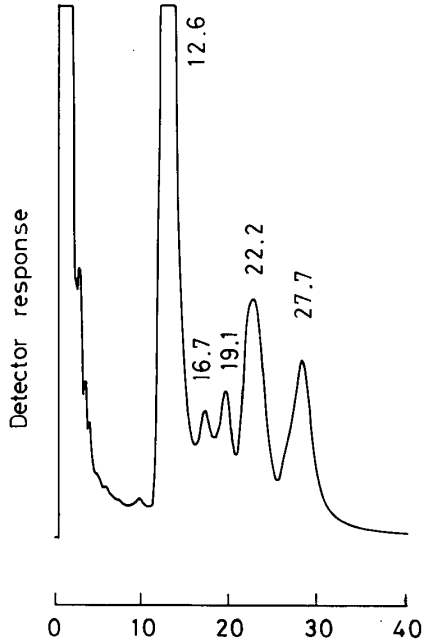

Fig. 2

Fig. 1 Gas chromatogram of the monoacetyldiacylglycerols derived from phosphatidylglycerols of taro leaves after hydrogenation of the unsaturation bonds.

Fig. 2 Gas chromatogram of the unhydrogenated monoacetyldiacylglycerols derived from phosphatidylglycerols of taro leaves.

and 239, as in the second peak of the hydrogenated monoacetyldiacylglycerols (Fig. 1), and in addition, those of 577, 353 and 237. Thus the second peak likely contained molecular species of 18:0/16:0 and 18:0/16:1^t combinations, i.e., the 1-(18:0)-2-(16:0), 1-(16:0)-2-(18:0) and 1-(18:0)-2-(16:1^t) species.

In a similar way, the molecular species in the other peaks in Fig. 2 were determined: 1-(18:1)-2-(16:0), 1-(16:0)-2-(18:1) and 1-(18:1)-2-(16:1^t) species in the third peak; 1-(18:2)-2-(16:0), 1-(16:0)-2-(18:2) and 1-(18:2)-2-(16:1^t) species in the fourth peak; and 1-(18:3)-2-(16:0), 1-(16:0)-2-(18:3) and 1-(18:3)-2-(16:1^t) species in the fifth peak. The molecular ions were observed in the polyunsaturated species such as the latter seven. It was clear that the retention time of the molecular species depended on the chain length of the fatty acids and the number of *cis* unsaturation bonds in the C_{18} acids. The effect of a *trans* unsaturation bond in the C_{16} acids, if present, was very small.

The mass chromatograms for the molecular and fragment ions provided the exact retention time for every molecular species of monoacetyldiacylglycerol, and this analysis revealed that the isomers having the molecular structures 1-C_{18}-2-(16:0) and 1-(16:0)-2-C_{18} appeared at the same retention time. The molecular species having 16:1^t appeared slightly later than the corresponding species having 16:0.

Molecular species composition—In the determination of the molecular species composition, the relative areas under the peaks in the gas chromatograms as in Fig. 2 were first measured. As mentioned above, each peak contained three molecular species having the same C_{18} fatty acid and either 16:0 or 16:1^t. The ratios between the 16:0/16:0 and 16:0/16:1^t species and between the C_{18}/16:0 and C_{18}/16:1^t species within the peaks were determined by comparing the areas on the mass chromatograms for the fragment ions $CH_2(OCOR_1)$-$CH(OCOR_2)$-CH_2^+. In this

Table 1 Molecular species compositions of phosphatidylglycerols from leaves of the chilling-sensitive and chilling-resistant plants

Plants	Molecular species composition (%)										
	[16:0 16:0]	[16:0 16:1^t]	[18:0 16:0]	[18:0 16:1^t]	[18:1 16:0]	[18:1 16:1^t]	[18:2 16:0]	[18:2 16:1^t]	[18:3 16:0]	[18:3 16:1^t]	[16:0 16:0]+[16:0 16:1^t]
Chilling-sensitive plants											
Sweet potato	26	39	3	3	7	8	4	4	2	4	65
Taro	20	42	1	3	3	3	11	4	7	6	62
Castor bean	20	22	7	1	15	29	3	1	1	1	42
Tobacco	19	20	0	5	1	15	11	7	7	16	39
Sponge cucumber	16	23	2	13	4	19	5	3	4	11	39
Maize	20	17	0	0	0	4	5	11	17	26	37
Kalanchoe	32	2	3	0	19	0	26	1	11	6	34
Squash	17	24	0	2	0	5	13	3	23	23	31
Cyclamen	5	21	0	3	11	34	8	8	4	5	26
Chilling-resistant plants											
Pea	12	7	1	0	27	6	15	10	15	7	19
Lettuce	13	4	0	1	1	1	26	4	26	24	17
Cluster amaryllis	6	5	0	2	6	3	28	7	23	20	11
Red clover	4	6	0	0	5	23	11	14	12	25	10
Japanese radish	5	3	1	0	3	1	5	4	51	26	8
Welsh onion	5	2	1	0	4	1	27	11	26	24	7
Chinese cabbage	4	3	1	0	2	0	9	3	47	32	7
Spinach	1	5	0	0	1	0	5	0	16	72	6
Oat	3	2	0	0	2	0	11	3	33	45	5
Cabbage	3	1	0	0	4	0	45	2	43	2	4
Dandelion	2	2	0	0	3	12	16	29	15	20	4
Wheat	1	2	0	0	2	0	11	5	32	47	3

determination, however, the molecular species of 1-C_{18}-2-(16:0) and 1-(16:0)-2-C_{18} could not be discriminated, so the sum of their contents is given instead (Table 1).

A comparison of the molecular species compositions of leaf phosphatidylglycerols in chilling-sensitive and chilling-resistant plants is presented in Table 1. The 16:0/16:0 species accounted for 5% (cyclamen) to 32% (kalanchoe) of the total phosphatidylglycerols in the chilling-sensitive plants, and from 1% (spinach and wheat) to 13% (lettuce) in the chilling-resistant plants. The proportion of 16:0/16:1^t species ranged from 2% (kalanchoe) to 42% (taro) in the chilling-sensitive plants, and from 1% (cabagge) to 7% (pea) in the chilling-resistant plants. Thus, the relative content of either of these species alone was not correlated with the chilling sensitivity of the plants. The sum of the relative contents of the 16:0/16:0 and 16:0/16:1t species, however, did correlate well with the chilling sensitivity. It varied from 3% (wheat) to 19% (pea) in the chilling-resistant plants, and from 26% (cyclamen) to 65% (sweet potato) in the chilling-sensitive plants. Among the chilling-sensitive plants, the sensitivity is greatest in sweet potato, taro and castor bean and least in cyclamen and kalanchoe. Among the chilling-resistant plants, wheat, oat and spinach are the most hardy, and pea and lettuce the least. It may be seen in Table 1 that the order of the 16:0/16:0 plus 16:0/16:1^t contents seems to correspond to the degree of chilling sensitivity of the plants.

Nishihara et al. (1980) determined the molecular species composition of phosphatidyl-

glycerols from the chloroplasts of higher plants. The 16:0/16:0 plus 16:0/16:1^t content was 14% of the total phosphatidylglycerols in spinach, a chilling-resistant plant, and 43% (soy bean), 37% (rice), 39% (mesophyll cells of maize) and 56% (bundle sheath cells of maize) in chilling-sensitive plants. These values fall within the ranges of the 16:0/16:0 plus 16:0/16:1^t contents of the chilling-sensitive and the chilling-resistant plants in the present work, showing consistency with the results in Table 1.

Discussion

The molecular species compositions of phosphatidylglycerols from chilling-sensitive and chilling-resistant plants in the present study indicate that the sum of the 16:0/16:0 plus 16:0/16:1^t species contents relative to the total phosphatidylglycerols correlates well with the chilling sensitivity of the plants. As discussed previously (Murata et al. 1982), the thermotrophic behaviour of the 16:0/16:0 and 16:0/16:1^t species of phosphatidylglycerol should be similar, with both species undergoing a gel to liquid crystalline phase transition at about room temperature. It is reasonable, therefore, that these two molecular species would form gel phase domains in the membranes at the chilling temperature and cause chilling injury in the plants. The molecular species 18:0/16:0 and possibly 18:0/16:1^t should also undergo the thermotrophic phase transition at about room temperature. The contents of these molecular species were very low in the chilling-resistant plants, and amounted to a considerable level in some chilling-sensitive plants. The other molecular species containing either 18:1, 18:2 or 18:3 would be expected to undergo the thermotrophic phase transition below room temperature, and therefore would not take part in chilling injury (Murata et al. 1982).

Since most of the phosphatidylglycerols from leaves originate in the chloroplasts, the molecular species compositions of the chloroplastic phosphatidylglycerols should be similar to those in Table 1. The chloroplastic phosphatidylglycerols contain either 16:0 or a C_{18} acid at the C-1 position and either 16:0 or 16:1^t at the C-2 position (Murata et al. 1982). This observation suggests that the relative content of 16:0 at the C-1 position should be equal to the sum of the 16:0/16:0 plus 16:0/16:1^t species contents, and should therefore be related to the chilling sensitivity of plants. Sparace and Mudd (1982) proposed that phosphatidylglycerols are synthesized from glycerol-3-phosphate and acetate in spinach chloroplasts, and that the primary products of phosphatidylglycerols contained 18:1 and 18:2 at the C-1 position, and 16:0 at the C-2 position of *sn*-glycerol.

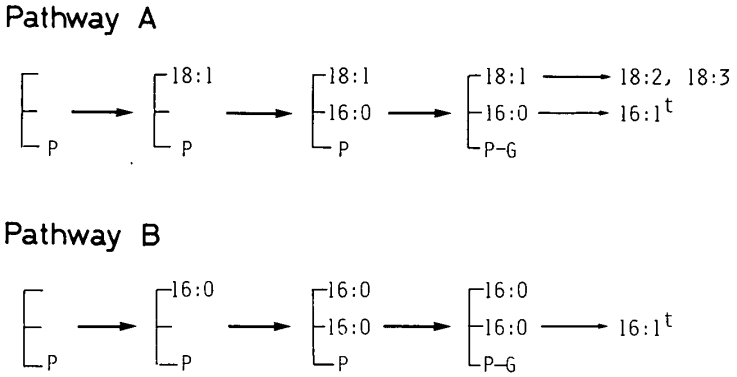

Fig. 3 A proposal for two pathways of biosynthesis for the phosphatidylglycerol molecular species in the chloroplasts of chilling-sensitive and chilling-resistant plants. Pathway A is dominant in the chilling-resistant plants, whereas both pathways A and B are of comparable activity in the chilling-sensitive plants. P, phosphate; P-G, glycerophosphate.

On the basis of the results of the present and previous (Murata et al. 1982) studies and the proposal of Sparace and Mudd (1982), it may be speculated that two pathways can occur in the chloroplasts for the biosynthesis of the phosphatidylglycerol molecular species. As shown in Fig. 3, in pathway A, 18:1 is esterified to the C-1 position of glycerol-3-phosphate, and 16:0 to the C-2 position. After the phosphatidic acid thus produced is converted to phosphatidylglycerol, most of the 18:1 at the C-1 position is desaturated to 18:2 and 18:3, and some of the 16:0 at the C-2 position to $16:1^t$. The combinations of the fatty acids thus produced form a variety of molecular species. In pathway B, 16:0 is esterified to both the C-1 and C-2 positions of glycerol-3-phosphate. After the transformation of the phosphatidic acid into phosphatidylglycerol, some of the 16:0 at the C-2 position is desaturated to $16:1^t$, but the 16:0 at the C-1 position is not desaturated at all, resulting in the formation of only two molecular species, 1-(16:0)-2-(16:0) and 1-(16:0)-2-($16:1^t$), in this pathway.

The observed differences in the molecular species compositions of phosphatidylglycerols in chilling-sensitive and chilling-resistant plants can be accounted for by the preferential activities of the synthetic pathways of their phosphatidylglycerols. In chilling-resistant plants, in which the sum of the 16:0/16:0 plus $16:0/16:1^t$ contents is low, the activity of transferring 16:0 to the C-1 position of glycerol-3-phosphate (pathway B) should be much lower than that which transfers 18:1 to the C-1 position (pathway A), resulting in a small proportion of the C_{16}/C_{16} species. In chilling-sensitive plants, on the other hand, the activity of transferring 16:0 to the C-1 position (pathway B) should occur at a rate comparable to that transferring 18:1 to the C-1 position (pathway A), resulting in a large proportion of C_{16}/C_{16} species.

If such a scheme is valid, the difference in the sum of the 16:0/16:0 plus $16:0/16:1^t$ species contents, which apparently accounts for the variability in chilling sensitivity, should result from the preferential transfer of 16:0 and 18:1 to the C-1 position of glycerol-3-phosphate. Such a preference is possible if an enzyme (acyltransferase), which transfers the acyl group to the C-1 position of glycerol-3-phosphate, has a different selectivity for 18:1 and 16:0 between the chilling-sensitive and the chilling-resistant plants; the enzyme in the chilling-resistant plants has a rather strict specificity for 18:1 than for 16:0, whereas that in the chilling-sensitive plants is unspecific for either of the fatty acids and transfers both 18:1 and 16:0 in comparable rates. It is more likely if there are two acyltransferases, one specific for 18:1 and the other for 16:0; in the chilling-resistant plants the former enzyme has a much greater activity than the latter, whereas in the chilling-sensitive plants both enzymes are almost equally active.

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