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THERMAL PHASE TRANSITIONS IN THE POLAR LIPIDS OF PLANT MEMBRANES

THEIR INDUCTION BY DISATURATED PHOSPHOLIPIDS AND THEIR POSSIBLE RELATION TO CHILLING INJURY

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The phase behaviour of leaf polar lipids from three plants, varying in their sensitivity to chilling, was investigated by differential scanning calorimetry. For the lipids from mung bean (*Vigna radiata* L. var. Berken), a chilling-sensitive plant, a transition exotherm was detected beginning at 10 ± 2°C. No exotherm was evident above 0°C with polar lipids from wheat (*Triticum aestivum* cv. Falcon) or pea (*Pisum sativum* cv. Massey Gem), plants which are insensitive to chilling. The enthalpy for the transition in the mung bean polar lipids indicated that only about 7% w/w of the lipid was in the gel phase at −8°C. The thermal transition of the mung bean lipids was mimicked by wheat and pea polar lipids after the addition of 1 to 2% w/w of a relatively high melting-point lipid such as dipalmitoylphosphatidylcholine, dipalmitoylphosphatidylglycerol or dimyristoylphosphatidylcholine. Analysis of the polar lipids from the three plants showed that a dipalmitoylphosphatidylglycerol was present in mung bean (1.7% w/w) and pea (0.3% w/w) but undetected in wheat, indicating that the transition exotherm temperature of 10°C in mung bean, 0°C in pea and about −3°C in wheat correlates with the proportion of the high melting-point disaturated component in the polar lipids. The results indicate that the transition exotherm, observed at temperatures above 0°C in the membranes of chilling-sensitive plants, could be induced by small amounts of high melting-point lipids and involves only a small proportion of the membrane polar lipids.

Introduction

Many tropical plants are injured and eventually die when exposed to non-freezing (0–12°C), chilling temperatures [1]. The susceptibility of these plants to chilling injury correlates with a change in the molecular ordering of their membrane lipids, detected by spin labelling, at a temperature coincident with that below which injury develops [2,3]. More recent studies using the fluorescence polarization of *trans*-parinaric acid [4], show that the change in ordering of membrane lipids corresponds with the onset of phase separation [5]. The membrane lipids of plants insensitive to chilling also exhibit changes in ordering [3] and phase separation [4] but in contrast to chilling-sensitive plants these events occur at temperatures at or

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Abbreviations: DGDG, digalactosyl diacylglycerol; DMPC, dimyristoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; HPLC, high performance liquid chromatography; MGDG, monogalactosyl diacylglycerol; PLPC, 1-palmitoyl-2-linoleoylphosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine.

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below 0°C.

Changes in molecular ordering and phase separation detected by adding probes to membrane lipids are not necessarily indicative of thermal phase transitions [6]. Furthermore, given the relatively high proportion of polyunsaturated fatty acids in plant membrane lipids, such transitions, in the temperature range of chilling, have been considered an unlikely occurrence [7]. Thermal transitions above 0°C have, however, been reported in the lipids of some plant membranes [8,9]. These observations raise the question of what lipid component(s) and what type of interaction between such component(s) and the unsaturated lipids of plant membranes could initiate phase transitions in plant membranes, especially at temperatures within the chilling range.

The phase transition temperatures of complex lipids depends on the chain length of the fatty acid, the position of fatty acid substitution, the position and extent of unsaturation as well as the type of headgroup [10]. Membranes of some plants contain a small proportion of disaturated lipids [11] with melting points up to 40°C. It is thus reasonable to suppose that these relatively high melting-point lipids could solidify and phase separate at temperatures above 0°C, providing the solidification temperature of the high melting-point component was not greatly depressed by co-crystallisation with low melting-point lipids of plant membranes.

This paper describes experiments designed to investigate the phase behaviour of membrane polar lipids and mixtures of these lipids and with high melting point lipids to gain some insight into how these systems might interact. The results show that a phase transition occurs in the polar lipids of a chilling-sensitive plant at temperatures above 0°C and in two chilling-insensitive plants at temperatures at or below 0°C. Furthermore, they show that phase transitions above 0°C can be induced in the polar lipids of the chilling-insensitive plants by the addition of about 1% to 2% of a high melting-point phospholipid. Analyses show high melting-point phospholipids are present in the membrane lipids of the chilling-sensitive plant but absent or in relatively low proportion in those of the chilling-insensitive plants.

Materials and Methods

Mung bean (*Vigna radiata* L. var. Berken), wheat (*Triticum aestivum* cv. Falcon) and pea (*Pisum sativum* var. Massey Gem) were grown in vermiculite at about 20°C in a glasshouse. The synthetic lipids DPPC, DPPG and DMPC were purchased from Serdary Research Laboratories Inc., London, Ontario, Canada and used without further purification.

Lipids were extracted from leaf tissue of 7-day-old plants by method A of Fishwick and Wright [12]. The lipids were fractionated by sequential elution from a silica gel column; neutral lipids with chloroform (8 ml/g of silica gel), and 10% v/v acetone in chloroform (4 ml/g), glycolipids with acetone (32 ml/g) and phospholipids with methanol (10 ml/g) followed by 10% v/v water in methanol (12 ml/g). The polar lipid fraction was obtained by eluting with the methanol and 10% v/v water in methanol immediately after removal of the neutral lipids with chloroform and 10% acetone in chloroform. The column was finally eluted with chloroform/methanol/acetic acid/water (170 : 30 : 20 : 6, v/v) to check that all polar lipids were removed by the water/methanol mixture. The various lipid fractions used for data shown in Fig. 5 were obtained from the polar lipid fraction by sequential elution from a silica gel column by acetone (32 ml/g) to give, in the initial volumes F_1, containing predominantly MGDG, and in latter volumes F2, containing DGDG, some acylated sterol glycoside, sterol glycosides and a trace of MGDG. F_3, which consisted mainly of sulphoquinovosyl diacylglycerol (sulpholipid) and phospholipids with a trace of DGDG and MGDG was eluted with methanol (10 mg/g). F_4, which contained only phospholipids, was obtained from F_3 by selectively removing the sulpholipid, DGDG and MGDG by elution from silica gel with acetone. The phospholipids were recovered by eluting with methanol and 10% v/v water in methanol. The lipid components of each fraction were determined by TLC.

Calorimetric scans were performed with a differential scanning calorimeter (Perkin-Elmer, Model DSC-2). Lipid samples were prepared as hydrated dispersions by two methods. Initially, polar lipids were dispersed in 20 mM Tris-acetate

buffer, pH 7.2, containing 2 mM EDTA by sonication for 2 min at 40°C. This mixture was frozen in liquid nitrogen and thawed three times and the hydrated lipid concentration by centrifuging at 170000 × *g* for 1 h. Portion of the pellet (2 to 3 mg of lipid) was transferred and sealed in an aluminium sample pan. The amount of lipid was determined by weighing after extracting and drying the lipid from the pan after calorimetry. In subsequent experiments it was desirable to obtain larger quantities of lipid in a sample pan and to achieve this, lipid (about 7 mg for aluminium and 13 mg for steel pans) was added directly to the pan as a solution in chloroform. Most of the solvent was evaporated in a stream of nitrogen and the remainder removed under vacuum for 3 h. Buffer was added to the lipid to give a 300% w/w excess. The lipid was hydrated by heating the sealed pan at either 25°C (membrane polar lipids) or 40°C (mixtures containing added DPPC or DPPG) for 16 h. Both methods gave the same qualitative result. The samples were scanned at 10 K/min. The reference chamber contained an empty aluminium pan or a steel pan containing 6 mg of moist Sephadex. The Sephadex provided similar convection conditions as the mixture in the steel pans [13].

Qualitative and quantitative analyses of disaturated lipids were carried out using 2 to 3 mg of either mixed phospholipids or individual phospholipids separated by TLC. The phospholipids were converted to 1,2-diacylglycerols by treatment with phospholipase C and then to the *p*-methoxybenzoate derivative before separation by HPLC [14]. Disaturated components were identified by their retention time compared to standard 1,2-diacylglycerols (Serdary Research Laboratories Inc. London, Ontario, Canada) and by the effects of bromination [14]. For quantitative analysis 1,2-distearylglycerol was added to the assay system and the amount of other diacylglycerols calculated relative to the recovery of the added diacylglycerol.

Results

Differential scanning calorimetry provides a method of qualitatively determining the temperature of initiation of an exothermic phase transition as well as a quantitative estimation of the heat involved in the transition. For polar lipids from mung bean, a chilling-sensitive plant, Fig. 1, trace A, shows an exothermic phase transition initiated in the cooling mode at about 12°C. With a number of samples of polar lipids from mung bean the exotherm varied from 12 to 8°C probably due to either slight variation in growth conditions or to the effect of supercooling of the lipids. The area under the exotherm shown in Fig. 1 trace A, between 12°C and about −8°C, gives an estimate of the enthalpy of transition between these two temperatures. For mung bean this was 1.8 kJ/mol, assuming an average molecular weight of 750 for the 5 mg of lipid used in the assay. This represents about 7% of the enthalpy expected from the lipid present, assuming that the mean enthalpy for the mixed polar lipids is about 25 kJ/mol *. It is stressed that the figure of 7% is not considered a quantitative estimation of the lipid undergoing a transition and it is used only to demonstrate the relatively small proportion of lipid involved in the transition above 0°C. The transition for most of the mung bean polar lipids probably occurs well below 0°C since the leaf polar lipids contain a high proportion of MGDG and DGDG [16] and the transition for these lipids occurs at −50°C and −30°C, respectively [17].

The phase transition observed in the mung bean polar lipids is reversible. Completion of the endotherm, shown in the heating scan of Fig. 1, trace B at about 20°C, is however 8 K higher than the start of the exotherm (Fig. 1, trace A). This difference or hysteresis is similar to that found with binary mixtures of phospholipids [18] at comparable scan rates.

With polar lipids from either peas (Fig. 1, trace C) or wheat (Fig. 2a, trace A) the phase transition was detected close to or below 0°C, respectively. Neither of these plants is sensitive to chilling and

* The mean enthalpy of 25 kJ/mol is an estimate based on an enthalpy of about 38 kJ/mol for disaturated phospholipids and 32 kJ/mol for dioleoylphosphatidylcholine [15]. No data for the enthalpy of the major components of the polar lipids, MGDG and DGDG are available. However, since *cis* double bonds generally decrease the co-operativity and enthalpy of transitions [15] and linolenic acid is the major fatty acid of galactolipids [16], the enthalpy for the bulk polar lipids is estimated to be 25 kJ/mol.

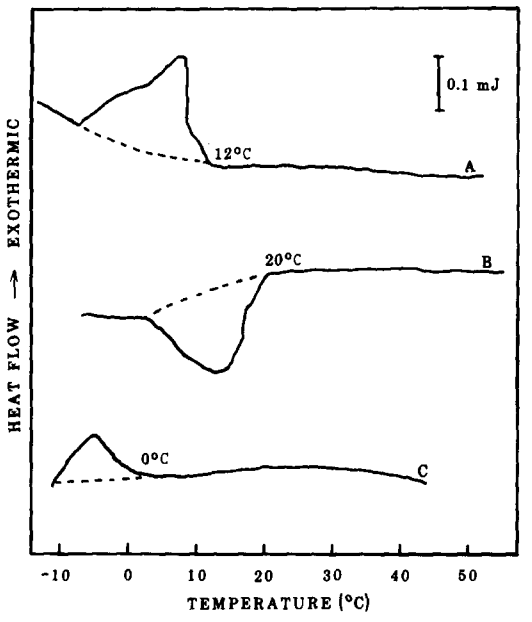

Fig. 1. Calorimeter traces showing the thermal behaviour of mung bean and pea polar lipids. Traces A and B are cooling and heating curves, respectively, for 5 mg of mung bean lipid and trace C is the cooling curve for pea lipids. Scanning rate was 10 K/min and sensitivity was 2.1 mJ/s.

the lower transition temperature is consistent with the absence of phase separation determined by fluorescence polarization [4] or a change in molecular order determined by spin labelling [3] above 0°C in polar lipids from chilling insensitive plants.

Transitions, in the temperature range observed with mung bean lipids, are produced with binary mixtures of a high melting-point lipid, such as DPPC (41°C), and a lower melting-point lipid which solidify to produce crystalline regions of the two components with little solid solution formation [10]. In mixtures of this type, where the lipids fail to co-crystallise, the transition temperature is raised as the proportion of the high melting-point component in the mixture is increased [10]. To determine if the transition observed in the plant polar lipids, could be initiated by a similar interaction between a small proportion of high melting-point lipid and the low melting-point lipids of plant membranes, DPPC was added to wheat polar lipids and the thermal properties of the mixtures analysed by differential scanning calorimetry (DSC).

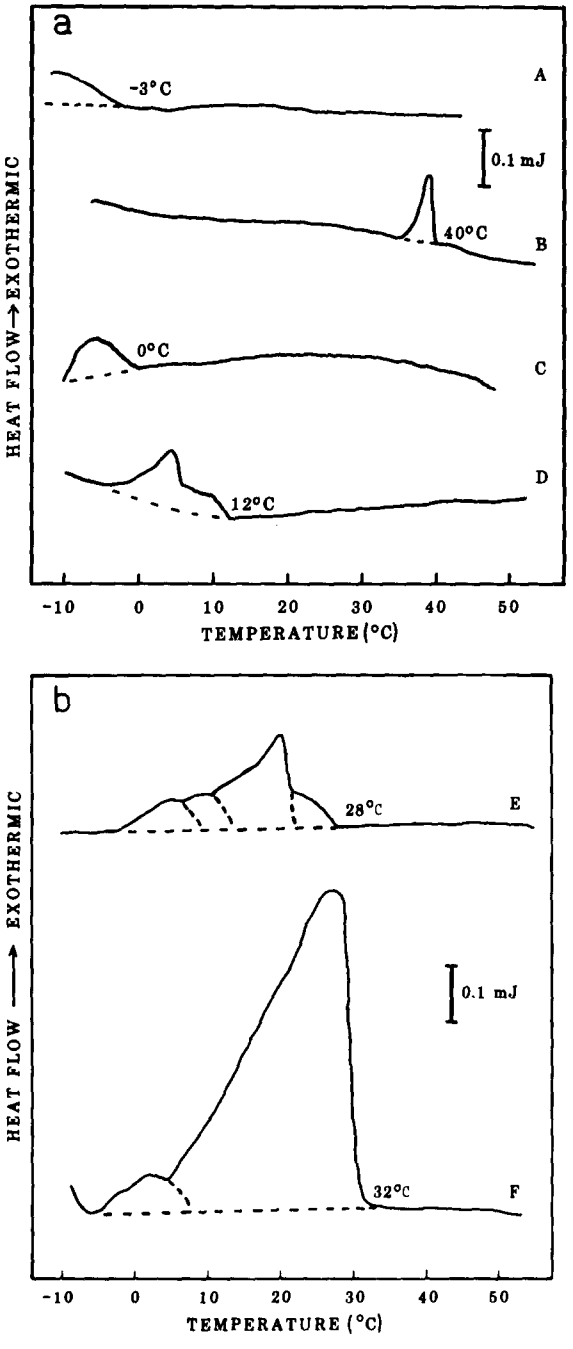

Fig. 2. Thermal response of wheat polar lipids, DPPC and various mixtures of the two. (a) Calorimeter traces of wheat polar lipids (Trace A), pure DPPC (Trace B), 0.5% DPPC in wheat polar lipids (Trace C), and 1% DPPC in wheat polar lipids (Trace D); (b) 17% DPPC in wheat polar lipids (Trace E) and 50% DPPC in wheat polar lipids (Trace F). Only the cooling curves are shown. Cooling rate was 10 K/min and sensitivity was 0.84 mJ/s.

As shown in Fig. 2a, trace B, DPPC undergoes a sharp transition at 40°C. In contrast the transition in the wheat polar lipids is at −3°C (Fig. 2a, trace A). However, when DPPC (0.5% w/w) is mixed with wheat polar lipids a transition is observed at 0°C, (Fig. 2a, trace C) similar to the transition observed with polar lipids from pea (Fig. 1, trace C). With 1% w/w of DPPC in wheat polar lipids the transition is increased to 12°C as shown in Fig. 2a, trace D and this is similar to the temperature for the transition observed with polar lipids from mung bean (Fig. 1, trace A). This shows that in the presence of wheat polar lipids an increase in the concentration of DPPC from 0.5% to 1.0% w/w raises the transition temperature of the mixture by 12 K to 12°C. Higher proportions of added DPPC resulted in transitions at higher temperatures as shown in Fig. 2b where 17% and 50% w/w induced transitions at 28 and 32°C, respectively. It should be noted that the exotherms produced by more than 1% w/w of added DPPC appear to consist of several, overlapping transitions indicating that DPPC might interact sequentially with several different components of the wheat polar lipids as the temperature is reduced.

Some indication of the type of interaction and thermal response which might occur when disaturated phospholipids are mixed with the predominantly unsaturated lipids of plant membranes was obtained by following the phase behaviour of different disaturated phospholipids with polar lipids from two different plants. The results are shown in Fig. 3. When DPPC (m.p. 41°C) or DPPG (m.p. 42°C) was added to wheat polar lipids at equivalent concentrations they produced transitions at about the same temperature indicating that both the dipalmitoyl lipids undergo similar interactions (Fig. 3, traces A and B). In comparison, DMPC (m.p. 23°C) at equivalent concentrations, elevated the transition temperature less (7 K with 1% w/w) indicating that the melting-point of the added disaturated phospholipid is a strong determinant of the transition temperature.

The phase behaviour of these mixed systems was also affected by the lipids of the host system. As shown in Fig. 3, trace C, the addition of DPPC to polar lipids from pea induced transitions at temperatures generally lower than the equivalent concentrations of DPPC with wheat polar lipids.

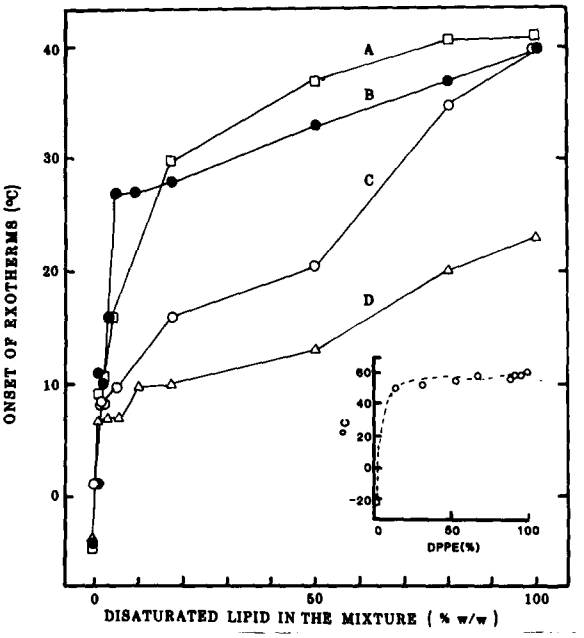

Fig. 3. Variations in the transition temperature for mixtures of disaturated phospholipids and plant polar lipids. The temperatures are for the onset of the exotherm in a cooling scan. The disaturated phospholipid was mixed with the plant polar lipids in the proportions shown and the mixture hydrated as described in Methods. (A) DPPG, (B) DPPC and (D) DMPC with polar lipids from wheat. (C) DPPC with polar lipids from pea. The inset shows the variation in transition temperature for binary mixtures of DPPE and DOPC (Data from Ref. 18).

In general the phase behaviour of mixtures of the plant polar lipids and added disaturated phospholipid resembled that of a mixture of DPPE (m.p. 63°C) with DOPC (m.p. −22°C) [18] as shown in the inset to Fig. 3. In this monotectic mixture, 10% w/w of the higher melting-point component elevated the transition more than 60 K above that of the lower melting-point component. The transition temperature of the lower melting-point component was altered little in this monotectic mixture where co-crystallisation formation does not occur (Inset Fig. 3 and Refs. 10 and 18). While the phase behaviour of the wheat polar lipids mixed with DPPC is generally similar to that of a monotectic mixture the possibility that some co-crystallisation occurs cannot be excluded. This would be indicated by an elevation of the transition of the lower melting-point component. However it was not possible to determine the transition

temperature of the wheat or pea polar lipids in mixtures with disaturated lipid (see Fig. 2a, traces D and Fig. 2b, traces E and F).

The quantitative aspects of the phase transition observed in the mixed systems, as well as in the plant polar lipids was obtained from the enthalpy of the transitions. As shown in Table I, the enthalpy of the transitions induced in the wheat polar lipids by either DPPC or DMPC in general exceeded that of the added disaturated lipid, up to a concentration of 3 to 4% w/w; after this the enthalpy was less than that predicted for the amount of disaturated lipid added. As pointed out earlier the enthalpy for the transition observed with mung bean polar lipids (Fig. 1, trace A) was 1.8 kJ/mol or about 7% of the polar lipids.

The induction of a transition at about 12°C in wheat polar lipids by the addition of about 1% w/w of disaturated lipid suggested that the transition in mung bean lipids might be induced by a small proportion of a high melting-point disaturated lipid. Lipids of this type occur in some plant membranes [11] and their presence in mung bean, wheat and pea phospholipids was therefore investigated. Fig. 4 shows chromatographs for the *p*-methoxybenzoate derivative of diacylglycerols from the polar lipids of mung bean (Fig. 4A) and pea (Fig. 4B). The lipids of both plants contain a 1,2-dipalmitoyl species which co-chromatographed with the *p*-methoxybenzoate derivative of 1,2-dipalmitoyl diacylglycerol and whose retention time was unaltered after bromination [14]. Quantitative

TABLE I

THE ENTHALPY FOR TRANSITIONS INDUCED IN WHEAT POLAR LIPIDS BY ADDED DISATURATED LIPID

Estimate of the amount of lipid involved in the transition induced in wheat polar lipids by adding various amounts of either DPPC or DPPG. The enthalpy ΔH of the transition was calculated assuming a mean molecular weight for the mixed polar lipids of 750 and a mean ΔH of 36 kJ/mol for DPPC and 32 kJ/mol for DPPG. The experimental values are the mean and standard deviation for the number of determinations shown in the brackets.

Added lipid	Amount (% of sample)	Enthalpy calculated (kJ/mol)	Enthalpy found (kJ/mol)
DPPC	0.5	0.2	0.2 ±0.004 (2)
	1	0.4	0.97±0.06 (4)
	2	0.7	0.81±0.01 (2)
	3	1.1	1.58±0.10 (11)
	5	1.8	1.39±0.08 (2)
	9	3.2	1.75±0.12 (5)
	17	6.0	3.5 ±2.6 (6)
	50	17.7	13.6 ±2.6 (6)
	80	28.4	21.2 ±0.2 (2)
	100	–	35.5 ±2.5 (2)
DPPG	1	0.3	0.46±0.6 (4)
	1.8	0.6	1.23±0.03 (3)
	4.5	1.4	2.14±0.23 (3)
	17	5.4	2.5 ±0.3 (4)
	50	16.0	10.5 ±1.9 (2)
	80	25.6	22.8 ±3.2 (2)
	100	–	32.0 ±1.1 (2)

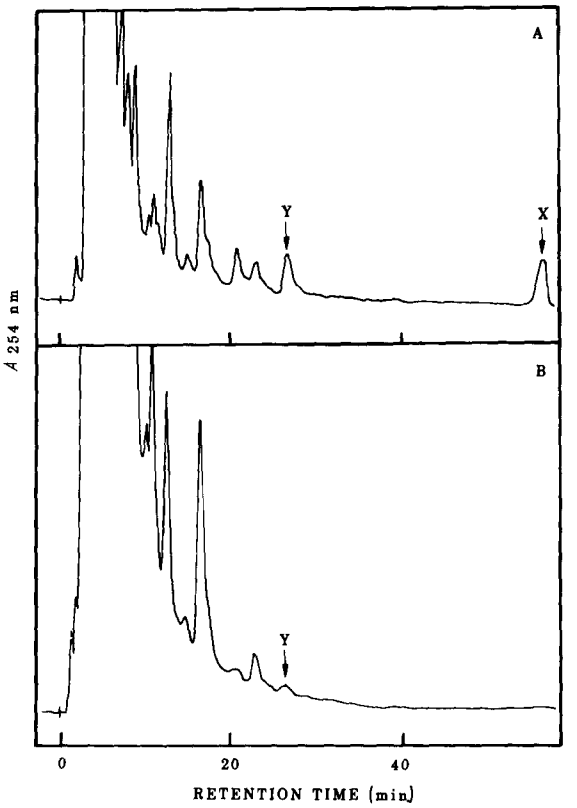

Fig. 4. HPLC separation of 1,2-diacylglycerol *p*-methoxybenzoates from (A) mung bean phospholipids and (B) pea phospholipids. Operating conditions: column, 25 cm RP-18 (10 μm); mobile phase 2-propanol/acetonitrile (25:75, v/v); column temperature, 25±1°C, flow rate 1 ml/min. The 1,2-di-18:0 diacylglycerol which was added as internal standard is the peak indicated 'X'. The retention time for 1,2-di-16:0 diacylglycerol is indicated by the peak 'Y'.

analysis showed that the mung bean lipids contained 1.7 ± 0.5% w/w and pea 0.3 ± 0.1% w/w of the dipalmitoyl species. No dipalmitoyl diacylglycerol species was detected in derivatives of wheat polar lipids.

The phospholipid species from which the dipalmitoyl diacylglycerol detected in the mung bean lipids was derived, were identified after separation of the phospholipids by TLC. The dipalmitoyl species was found mainly in the phosphatidylglycerols with a trace in the phosphatidylcholines. None was detected in phosphatidylinositols, phosphatidylethanolamines or phosphatidic acids.

While it is evident (Figs. 1 to 3) that a disaturated phospholipid can induce a transition above 0°C in wheat polar lipids, it was pertinent to determine if the transition detected in the mung bean polar lipids, at about 12°C, was a property of the phospholipids alone or a product of the interaction of a number of lipid components. To investigate these possibilities, mung bean polar lipids were separated into fractions consisting predominantly of MGDG (F1), DGDG with sterol glycosides (F2), sulpholipids and phospholipids (F3) and one containing only phospholipids (F4). An exothermic transition was detected above 0°C in fractions F3 and F4 and in a number of combinations of F3 with other fractions as shown in Fig. 5. For the phospholipids alone (F4) the transition was at 20°C (Fig. 5, F4), a higher temperature than the transition observed for the original polar lipids (Fig. 5). Sulpholipids with phospholipids (F3) produced a broad transition beginning at 30°C (Fig. 5, F3). This transition could be lowered to about 14°C when either the DGDG and sterol glycosides (F2) or the MGDG (F1) were mixed with the sulpholipids and phospholipids (F3) in the same proportion as in the original polar lipids (Fig. 5, F3 + F2 and F3 + F1). When fractions F1, F2 and F3 were recombined in the proportion present in the original polar lipids the transition was detected at the same temperature and had the same enthalpy and general shape as that of the original polar lipids (Fig. 5, F1 + F2 + F3). Thus while a disaturated phospholipid might be the component which induces a transition above 0°C it is evident that other lipid components interact with the disaturated phospholipids to produce transitions in the chilling temperature range.

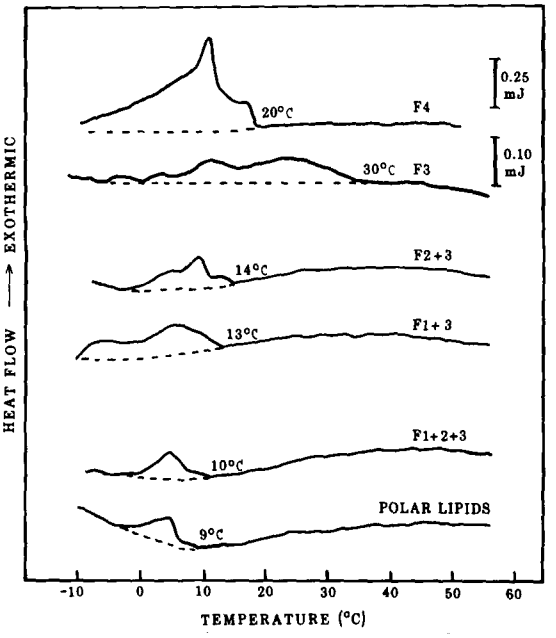

Fig. 5. Calorimeter traces showing the thermal behaviour of mung bean polar lipids and derived fractions. Only the cooling curves are shown. Scan rates were 10 K/min and sensitivities were 2.1 mJ/s for (F4) and 0.84 mJ/s for all other scans. The fractions contained: (F1) MGDG; (F2) DGDG, ASG, SG, and a trace of MGDG; (F3) sulpholipid, phospholipids, and a trace of DGDG and MGDG; (F4) phospholipids.

Discussion

The transition exotherm and endotherm observed in the polar lipids of mung bean provides unequivocal evidence for a reversible phase change in the membrane lipids of a plant, above 0°C and at a temperature consistent with that below which chilling injury becomes evident [19]. The onset of the exotherm (12°C, Fig. 1) coincides with the temperature at which a change in molecular ordering is detected by spin labelling [3] and is similar to the temperature at which lipid phase separation is detected by fluorescence polarization in chilling-sensitive plants [4]. The changes in lipid ordering detected by spin labelling at 23–28°C in mung bean membrane lipids [3] and at about 30°C in wheat [20] and a number of other plants [3] was not detected as an exothermic transition by calorimetry in the plant polar lipids (Figs. 1 and 2).

The enthalpy calculated for the exotherm in

mung bean polar lipids indicates that only about 7% of the polar lipid of mung bean is in the gel phase at −8°C. Considerably less lipid would be in the gel phase immediately below the temperature at which the phase change is initiated. Nevertheless, many tropical plants exposed to temperatures immediately below the phase change show increased ion leakage from cells, metabolic imbalance and physiological dysfunction characteristic of chilling injury [1]. Furthermore, the phase transition at about 12°C marks the lower limit for growth for mung bean [19]. This suggests that even small amounts of gel phase lipid can disrupt molecular ordering in a bilayer to the extent that functional properties of the membrane are seriously impaired.

The finding that the addition of only 1% w/w of DPPC induced a phase transition in the polar lipids of pea and wheat, at temperatures near that of the transition in mung bean lipids, is pertinent to understanding the thermal behaviour of plant polar lipids. When DPPC was added to wheat lipid at concentrations less than 5%, there is a large increase in the transition temperature with increasing concentration of the disaturated lipid. However at concentrations exceeding 20% the transition was only a few degrees below that of the added disaturated lipid (Fig. 3). This thermal response is similar to that of a mixture of phospholipids such as DPPE and DOPC (Ref. 18 and Fig. 3). These lipids are structurally dissimilar and form a monotectic mixture rather than a cogel structure on cooling. Thus it is reasonable to suppose that the transition in the mung bean lipids (Fig. 1) is induced by a small amount of high melting-point lipid forming a monotectic mixture with some lower melting-point and structurally dissimilar lipid. The finding of about 2% w/w of a dipalmitoyl lipid species in the polar lipids of mung bean is consistent with this view. The lesser amount (0.3% w/w) of the dipalmitoyl species in pea polar lipid and its absence in wheat polar lipids correlates with lower transition temperatures of these plants and provides further support for the view that the transition detected above 0°C in the mung bean polar lipids is induced by the high melting-point component forming a monotectic mixture with some lower melting-point lipids in the plant polar lipids.

It should be stressed, however, that as the temperature is reduced some plant polar lipids could co-crystallise with the gel phase lipids forming domains of cogel. This would explain why the enthalpy for the transition in the mung bean lipids (equivalent to 7% of the lipid) exceeds that expected considering that mung bean polar lipids contain only about 2% w/w of the high melting-point, dipalmitoyl phospholipid. This view is further supported by the observation that the enthalpy for the transition induced in the wheat polar lipids (Table I) with less than 5% w/w of added DPPC or DPPG exceeds that of the added disaturated lipid. Co-crystallisation occurs with lipid varying only slightly in chain length [10] and hence melting points and possible candidates for forming co-crystals with a disaturated lipid like DPPC would be 1-oleoyl-2-stearoylphosphatidylcholine (m.p. 8.6°C) [21] and dipalmitoyl sulpholipid (melting-point probably greater than about 35°C) [17]; both of which are present in thylakoid membranes [22].

The heterogeneity of plant membrane lipids with regard to type, headgroup and acyl chain composition precludes a precise description of phase behaviour. However some insight into how the transition temperature is influenced by interaction between the major types of membrane lipids can be gained from studies on the thermal response of the separate and recombined fractions. For example the transition for a fraction containing only phospholipids occurs about 10 K higher than the mixed polar lipids. However, the transition of the phospholipids was increased by about 10 K by the inclusion of the sulpholipids. This is not unexpected considering the transition of algal sulpholipids with more than 50% palmitic acid, is as high as 35°C [17] and dipalmitoyl sulpholipid is a component of the chloroplast envelope [11]. As with the phospholipids, the relatively high transition temperature for the fraction containing the phospholipids and sulpholipids was reduced when this fraction was mixed with either of the galactolipid fractions. It should be pointed out that the fraction containing the DGDG also contained sterol glycosides and these have been shown to broaden phase transitions [23]. Thus the results show that low melting point lipids and lipids which can reduce the cooperativity of a

phase transition, interact in the mixed plant polar lipid systems in the manner predicted. In addition the observation that the transition temperature of the recombined fractions is similar to that of the original polar lipids and to that for the change in molecular ordering detected in membranes by spin labelling [2] shows that the membrane polar lipids are randomly distributed. This also indicates the thermal response of the membrane polar lipids is not influenced significantly by membrane proteins.

Studies on the molecular mechanism of chilling injury in plants have established that for plants sensitive to chilling, metabolic dysfunction and cellular damage become evident when tissue is exposed to temperatures below that at which changes in molecular ordering [3] or phase separation [4] of membrane lipids occurs. The coincidence in temperature of the physiological changes and the disruption to membrane lipid ordering led to the proposal that the latter was the primary molecular event in the initiation of chilling injury [2]. The present results are consistent with this view and further show that the phase transition above 0°C involves a relatively small proportion of the membrane lipids. This finding explains the lack of correlation between the temperature of the phase transition and the proportion of unsaturated fatty acids in membrane lipids [24]. Whilst results of earlier studies [25,26] had indicated that the thermal response of mitochondrial membranes of chilling-sensitive plants might be explained by a relative high proportion of saturated fatty acids, this was not supported by subsequent analysis [24]. From the results presented it is clear that attempts to explain the occurrence of phase transitions, of the type described here, in terms of membrane lipid composition, necessitate determining the positional distribution of acyl chains for individual lipid types. Even with this information, relating thermal behaviour and composition will depend on knowing the type of lipid with which the disaturated lipid interacts.

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