Phase Transitions in Thylakoid Polar Lipids of Chilling-Sensitive Plants

A COMPARISON OF DETECTION METHODS

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ABSTRACT

The phase behavior of thylakoid polar lipids from plants sensitive to chilling injury was investigated by calorimetry, electron spin resonance spectroscopy of spin labels, and fluorescence intensity after labeling with trans-parinaric acid. The plants used were oleander (Nerium oleander), mung bean (Vigna radiata L. var Mungo), and tomato (Lycopersicon esculentum cv Grosse Lisse). For all plants the initiation temperature for the calorimetric exotherm was coincident (±1°C) with the transition determined by the increase in the temperature coefficient of spin label motion and fluorescence intensity of trans-parinaric acid. For oleander plants, grown at 45°C, the transition was at 7°C while for plants from the same clone, grown at 20°C, it was at −2°C. For mung bean and tomato the transition was between 9 and 12°C. The similarity in the transition detected by spin labeling and fluorescence intensity suggest that spin labels, like the fluorescent label trans-parinaric acid, preferentially partition into domains of ordered lipid. The coincidence of the temperature for initiation of the transition, determined by the three techniques, shows that each is a valid method of assessing a phase transition in membrane polar lipids.

It has been proposed that chilling-injury is initiated by a thermally-induced transition in the structure or phase state of some of the lipids which constitute the bilayers of cell membranes (11, 12, 23). Evidence supporting this hypothesis has relied mainly on the correlation between the critical temperature below which the plant is injured and the temperature of the lipid phase transition detected indirectly using either spin (21, 22) or fluorescent (24) probes.

This explanation of the primary cause of chilling injury has not been universally accepted. The principal objection is that the phase transition is inferred from a change in the temperature coefficient of motion of a spin probe (28) intercalated with either mitochondria or chloroplast membranes or liposomes formed from the lipids of these membranes (6, 22). Furthermore, this inference relating the change in the temperature coefficient of probe motion to a phase transition has been criticized on the grounds that probes can form impurity pools within the host lipids and have the potential to perturb the system being analyzed (3, 28). In addition, motion of the spin probe is usually calculated using an equation which depends on the probe undergoing isotropic motion (9) but it has been noted that motion of the nitroxide-type probes, commonly used in these studies with membrane lipids, is rarely isotropic (28). Thus, the motion parameter calculated from the electron spin resonance spectra of probes in membrane lipids could represent the summation of segmental, diffusion, and vibrational motion as well as a component influenced by the ordering of the probe in the membrane lipids (28). Under these conditions the motion parameter could increase markedly as membrane lipids become more ordered at low temperature even though no transition occurred in the host lipids.

Another problem encountered in spin labeling is that the phase transition is assumed to occur at the temperature below which the temperature coefficient of spin label motion increases (22). This temperature is usually derived from the intersection of two straight lines fitted to Arrhenius-type plots of the data (i.e. the logarithm of the motion parameter against the reciprocal of absolute temperature). However, this assumption has also been criticized (2, 4, 32) by the proposal that a curve might be a better fit to the data. If this were the case it could be argued that there is no abrupt change in the temperature coefficient of probe motion and therefore no phase transition occurs in the host lipids (2, 17, 32).

Even though more recent fluorescent (24) and calorimetric studies (26) support the earlier views derived using spin probes (12, 22), the controversy of whether a phase transition occurs above 0°C in the membrane lipids of plants and whether the transition correlates with the sensitivity of the plant to chilling injury has, nevertheless, been perpetuated in two recent publications. In one, the authors failed to detect a transition above 0°C in membrane lipids of soybean (16), a chilling-sensitive plant. In the other, the authors found qualitatively similar, broad transitions in mitochondrial lipids from the fruit of two ecotypes of tomato having differing responses to chilling (6).

This paper compares the temperature of the phase transition detected by spin labeling, calorimetry, and fluorescence intensity of some plant species sensitive to chilling. It also examines the thermodynamic properties governing the temperature dependence of spin label motion and the partitioning of the label between gel and fluid phases of bilayer lipids. In addition, it investigates the validity of the interpretation of Arrhenius-type plots of label motion in terms of a phase transition in the host lipids.

MATERIALS AND METHODS

Plants. Oleander (Nerium oleander) plants were cloned from a single genotype. The plants were grown in growth cabinets at either 20/15°C or 45/30°C (day/night) temperatures at a light intensity of 400 µE m⁻² s⁻¹. Mung beans (Vigna radiata L. var Mungo) were germinated and grown in moist vermiculite at 28/25°C at a light intensity of 350 µE m⁻² s⁻¹ for 10 to 12 d. Tomato (Lycopersicon esculentum cv Grosse Lisse) plants were grown in...
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a glass house at 25/20°C.

Polar Lipids. Thylakoids were isolated from oleander leaves as previously described (25) and from mung bean and tomato by the method of Anderson et al. (1). Lipids were extracted from the thylakoids and fractionated by column chromatography using silica gel as described by Raison and Wright (26). The complete removal of phospholipids from the column was ensured by eluting with 10% acetic acid in methanol.

Electron Spin Resonance Spectroscopy. Polar lipids were dispersed in 0.02 M Tris-acetate buffer (pH 7.2) containing 2 mM EDTA by brief sonication to give a final concentration of 10 to 12 mg ml⁻¹. Spin label was added to give a molar ratio of label to lipid of 1:150. Spectra were recorded using a Varian E4 spectrometer (Varian Associates, Palo Alto, CA). The instrument was fitted with a Deltron temperature control unit (model TCM20, Deltron Pty Ltd, Sydney, Australia) which maintained the temperature of the N₂ gas flow at ±0.01°C of the set temperature. The aqueous dispersal of lipid with label (50–70 µl) was added to a 2 mm diameter glass tube and placed in the spectrometer cavity such that the tip of a thermocouple probe, when inserted into the sample, was 1 cm above the cavity. The temperature gradient in the sample tube was less than ±0.2°C deg over the 4 cm length of the cavity. This was achieved by inserting a Teflon baffle plate into the top of the heating dewar (Varian part No. 96542) to create turbulence in the flow of N₂. Without this modification, temperature gradients of up to 5°C deg were detected over the 4 cm length of sample at temperatures 15°C deg above and below ambient. Gas flow was maintained at 10 L min⁻¹. For most samples the temperature was increased automatically at a rate of 0.5 or 0.25°C deg min⁻¹. The detector current was maintained at 300 µamp by automatic adjustment of the cavity iris by a servo system linked to the detector current meter. The spectrometer was interfaced with a HP 1000 E computer (School of Mathematics and Physics, Macquarie University). Spectra, consisting of 2100 data points were recorded at a scan rate of 20 Gauss min⁻¹. Temperature was recorded 30 times during each scan and averaged. The time constant for the spectrometer was 0.3 s or less and the spectra were smoothed using the method described by Savitsky and Golay (27). The motion parameter τ₀ was calculated as

\[ \tau_0 = 6.25 \times 10^{-10} \times W_0 \left( (h_0/h_s)^{-1/3} + (h_0/h_s)^{-1/3} - 2 \right) s. \]

where \( h, h_0, \) and \( h_s \) are the heights of the low-, mid-, and high-field lines, respectively, and \( W_0 \) is the width of the mid-field line in Gauss. The software for smoothing, selecting maxima and minima, and the calculation of \( \tau_0 \) and temperature were written by Mr. G. Roberts, of the CSIRO Division of Mineral Chemistry, North Ryde.

Fluorescence. Fluorescence intensity and polarization of \( \text{trans} \)-paraninic acid was determined using a fluorimeter where the intensity of the emission (420 nm), parallel (Iₚ) and perpendicular (Iₜ) to the excitation beam (320 nm), were measured and recorded simultaneously. The intensity and polarization were calculated as described by Raison et al. (24).

Labels. The spin labels were oxazolidenyloxy derivatives of methyl stearate of the general formula:

\[
\text{CH}_3 - [\text{C}_2 \text{H}_5]_n - \text{C} - (\text{CH}_2)_m - \text{C} - \text{O} - \text{CH}_3 \quad (1)\]

They were synthesized by the general method of Kena et al. (8), and purified by preparative TLC. The \( \text{trans} \)-paraninic acid was obtained from Molecular Probes Inc., and was used without further purification.

Differential Scanning Calorimetry. This was carried out using a Perkin-Elmer, model DSC-2, calorimeter essentially as described by Raison and Wright (26). Samples of polar lipids (4–10 mg of lipid in chloroform) were transferred to stainless steel pans (170 µl), solvent was removed under vacuum, and 150% v/w of aqueous buffer (0.02 M Tris/aceta [pH 7.2] containing 2 mM EDTA), added. The pans were sealed and the lipid hydrated for at least 16 h at 36°C.

RESULTS

As shown in Figure 1 the exothermic transition, determined by differential calorimetry for thylakoid polar lipids of oleander grown at 45°C, started at 7°C and for the 20°C-grown plants, at -2°C. For the 45°C-grown plants the transition, initiated at 7°C, was complete at -20°C (Fig. 1). The enthalpy of the transition (\( \Delta H_{\text{cal}} \)) between 7 and -20°C was 13.3 mJ for the 9.4 mg of lipid in the pan. Assuming an average enthalpy of 25 kJ mol⁻¹ for the mixed, leaf polar lipids (26) it may be calculated that if all of the lipid is transformed via a first order phase transition from a fluid to a crystalline phase then the evolved enthalpy would be 312 mJ. This would indicate that less than 4% of the polar lipids are in the crystalline phase at -20°C. Such a model is probably an oversimplification of the behavior of the system. An alternative source of the thermal energy evolved on cooling below 7°C is the loss of entropy due to phase separation of the lipids rather than a change in their gross physical state. If some of the lipid (e.g. 10%) phase separates without undergoing a transition to the crystalline state the heat evolved may be approximated by:

\[ \text{Heat} = kT \ln(A_1/A_2) \text{ J·molecule}^{-1} \]

where \( k = \text{Boltzmann's constant, } A_1 \text{ the total area of lipid (100%) and } A_2 \text{ the area phase separated (e.g. 10%), and } T \text{ the absolute temperature (°K).} \)

For the example above:

\[ \text{Heat} = 1.38 \times 10^{23} \times 280 \times \ln 10 \text{ J·molecule}^{-1} = 0.889 \times 10^{-10} \text{ J·molecule}^{-1} \]

Fig. 1. Phase transitions in the polar lipids of oleander thylakoid membranes. The calorimetric traces were obtained at a cooling rate of 10°C deg min⁻¹, and a sensitivity of 0.2 mcal s⁻¹ for 9.4 mg and 6.8 mg of lipid from oleander grown at 45°C (trace A) and 20°C (trace B), respectively. The midpoint of the transition (Tₘ) for the 45°C-grown sample was taken as -6°C and width at half-height (Tₜ) 13°C deg. For the oleander grown at 45°C the transition between 7° and -19°C was 1.76 kJ mol⁻¹ assuming an average mol wt of 750 for the polar lipids.
For 10% of the 9.4 mg of lipid, i.e., \(1.25 \times 10^{-4}\) mol, the heat would be

\[0.889 \times 10^{-20} \times 1.25 \times 10^{-6} \times 6.022 \times 10^{23} \text{ J} = 6.69 \text{ mJ} \]

which represents about half the heat evolved (13.3 mJ). Thus, on the basis of heat evolved and assuming none of the lipid solidifies, 20% of the lipid could have phase separated.

Figure 1 also shows that the transition temperature of polar lipids from oleander grown at 20°C was at \(-2°C\) compared with that at 7°C for plants grown at 45°C, demonstrating that for this species the transition temperature varies with growth temperature. This shift in the transition temperature during acclimation is similar to that of the phase separation temperature determined by fluorescence intensity and fluorescence polarization of transparinic acid (24) and suggests that both methods are detecting the same physical event.

The effect of temperature on the motion of the spin label, \(I_{5,10}\), intercalated with polar lipids from oleander grown at the two temperatures is shown in Figure 2. For both lipid dispersions, the temperature coefficient of motion (slope of the line), increased significantly (P < 0.005), below 7°C for plants grown at 45°C and below \(-2°C\) for plants grown at 20°C, respectively. This discontinuity in the linear relationship between the logarthm of the empirical motion parameter, \(\tau_0\) and \(K^{-1}\) has been interpreted as the initiation temperature of a phase transition in the host lipids (22). ESR spectra of the probe \(I_{5,10}\) in the oleander lipids, show increased line broadening and anisotropic motion as the temperature declines (spectra not shown). Thus, the discontinuity in the Arrhenius-type plots, where \(\tau_0\) is greater than \(70 \times 10^{-10} \text{s (log } \tau_0 \text{ of 1.8 in Fig. 2)}, \) could be the consequence of a continuous decrease in the rate of probe motion, reflecting a decrease in fluidity, and/or an increase in the ordering of the host lipids and not necessarily indicative of a phase transition (28). If these conditions apply, the plots of probe motion, over the temperature range of about 0 to 35°C, would most likely be a curve as proposed by Patterson et al. (17) and Bishop et al. (4). Furthermore, if the data fit a curve it would be erroneous to interpret the intersection of two straight lines fitted to such data as representing an abrupt transition in lipid structure. These two assertions can be tested.

First, if the Arrhenius-type plots of spin label motion are curves the transition determined by the intersection of two straight lines would vary when data points at the extremities of either the high or low temperature segments of the data are excluded from calculations of the lines-of-best fit (2). To test this proposition, straight lines were fitted to data shown in Figures 2, 5C, and 6C and the point of intersection determined with up to 90% of the points, above the proposed transition, excluded from the calculation. As shown in Figure 3 the transition temperature, calculated using the points remaining after up to 90% were omitted, was reduced by only 2°C for the lipids from oleander (Fig. 2) and less than 1°C for those from mung bean (Fig. 5C) and tomato (Fig. 6C). For oleander grown at 45°C the deviation of 2°C was evident after 30% of the points were omitted but even after 60% of the points were omitted it did not exceed the error of the interval of the intersection of the two regression lines calculated at the 95% confidence level. Thus, these results do not support the view that data points of log \(\tau_0\) versus \(K^{-1}\) lie on a curve such that the transition temperature was dependent on the data points selected for analysis. Nor do they support the view that log \(\tau_0\) is a variable function of \(K^{-1}\). Therefore, the decrease in spin label motion at low temperature is unlikely to be due to a continuous, temperature-dependent increase in ordering of the host lipids.

Second, to gain some insight into the basis of the increase in the temperature coefficient of spin label motion at low temperature, spin label motion was studied in lipid systems containing various proportions of a high melting point lipid. Figure 4 shows plots for the effect of temperature on the motion of \(I_{5,10}\) in aqueous dispersions of pure DPPC, melting point 41°C, and in mixtures of DPPC and polar lipids from wheat roots which on their own show a transition at \(-6°C\) (19). Since the plots for the pure DPPC and the mixtures containing up to 50% DPPC appear to consist of two linear segments connected by a curve, the data were analyzed by two methods: (a) by fitting straight lines and determining the temperature at which there is a significant increase in the temperature coefficient of spin label motion (slope) as temperature decreases and (b) fitting the data to an equation developed by Klein (10) to define rate processes in membranes. This equation, shown in the caption to Figure 4, was proposed (10) as a model to describe Arrhenius plots consisting of linear segments with a discontinuity or curve related to a thermotropic transition of the membrane lipids. Application of the equation can provide values for the enthalpy and entropy of probe motion in the fluid (\(\Delta H_f\) and \(\Delta S_f\)) and solid \(\Delta H_s\) and \(\Delta S_s\) phases, the temperature of the midpoint of a transition (\(T_m\)), the width of the transition at half height (\(T_w\)), and a partition coefficient defining the partitioning of the probe between the fluid and solid phases (P). Since it has been shown (Fig. 1) that a thermotropic transition occurs in plant polar lipids the application of this model, in preference to other models described by Klein (10), seemed appropriate. Klein (10) has shown that the distribution of the Arrhenius plot in relation to the transition temperature is determined by \(P\). We have therefore determined a value of \(P\) for a nitroxide spin label in relatively defined systems.

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**Abbreviations:** ESR, electron spin resonance; DPPC, dipalmitoylphosphatidylcholine.

![Figure 2](https://example.com/figure2.png)

**Fig. 2.** Arrhenius-type plots of the effect of temperature on the motion parameter (\(\tau_0\)) of spin label with polar lipids from oleander. The label \(I_{5,10}\) was added to an aqueous dispersion of the polar lipids from (A) 45°C and (B) 20°C grown plants. The discontinuity determined from the minimum of the residual sum of squares for two straight lines fitted to the data as described by Pollard (18) is indicated.
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Fig. 3. The effect of deletion of data points on the temperature of the transition. The transition temperature was determined from the point of intersection of two straight lines fitted to the data points as described by Pollard (18) and shown in Figures 2, A and B; 5B; and 6B. The intersection was also determined after deletion of the indicated proportion of data points from the segment above the transition and the deviation from the transition temperature (ΔT) is shown as (O). The error bars represent the 95% confidence level for the intersection, calculated by the method of Pollard (18), for the proportion of points shown.

consisting of either pure DPPC or mixtures of DPPC and polar lipids from wheat roots. As shown in Table I, the value obtained for P using DPPC is 0.2 and the distribution of the Arrhenius plot, shown in Figure 4A is consistent with a melting transition at 41°C and a transition width of 2°C deg. For the mixtures of DPPC and wheat lipids, Tm and Tc were determined by fitting the equation to the data in Figure 4, B and C with P varying between 0.1 and 0.5. As shown in Table I, P remains relatively constant as Tm is lowered and Tc increases with an increasing proportion of wheat lipid. When the proportion of wheat lipid exceeds 90% the error in determining Tc and P, by this method, increases as there are insufficient points defining the lower limit of the region containing both fluid and ordered (gel) phases. For the plant polar lipids shown in Table I values of Tm and Tc were also obtained using values for P of 0.5 to 0.1.

The data in Figure 4 have also been analyzed to determine the temperature at the intersect of two straight lines fitted to the points in the fluid phase and in the region containing both fluid and ordered phases (Fig. 4; Table I). An example is shown in the inset to Figure 4B. The temperature of the intersect is shown in Table I as T* with the enthalpy (ΔH*) for the motion in the fluid phase determined from the slope of the line of best fit. Also shown in Table I are the same parameters obtained from the data shown in Figures 2, A and B; 5, C and D; 6, C and D. Of particular interest is the consistency between Tm plus Tc and T*, indicating that when the phase limits are fairly well defined the temperature of initiation for the phase transition can be determined either from the thermodynamic parameters obtained from a curvilinear fit to the data or from the intersect of two straight lines. Table I also shows that the enthalpy of motion for the spin labels in the fluid phase (ΔH) is similar to the value of (ΔH*) obtained from the slope of the straight line fitted to data points in the fluid phase.

The consistency in the correlation between the temperature of the transition exotherm, determined by calorimetry (Fig. 1), the temperature for initiation of the phase change, detected by spin labeling (Fig. 2), and the temperature of phase separation, detected by fluorescence polarization (24) for oleander polar lipids, is also observed with lipids from tomato and mung bean, two chilling-sensitive plants. As shown in Figure 5 the exotherm for polar lipids from the thylakoids of mung bean begins at 10°C (Fig. 5A). The transition determined from spin label motion for this species is at 9°C, determined as the intersect of two straight lines (Fig. 5C) and at 12°C as determined by Tm plus Tc (Fig. 5D; Table I). From fluorescence measurements the transition is at 11°C (Fig. 5B). Similarly, as shown in Figure 6, the transition for the lipids from tomato is at 11°C by calorimetry (Fig. 6A) and at 11°C by spin labeling (Fig. 6C). The sum of Tm and Tc is 13°C (Fig. 6D; Table I) and phase separation, detected by fluorescence, begins at 11°C (Fig. 6B).

DISCUSSION

The calorimetric exotherm, detected in the polar lipids from 45°C-grown oleander at 7°C (Fig. 1), in mung bean at 10°C (Fig. 4), and in tomato at 12°C (Fig. 5), provides direct evidence of a thermally induced transition in leaf polar lipids of these plants at chilling temperatures. The initiation of the transition is similar to the temperature below which injury develops in tomato plants (5) and for mung beans it is similar to the temperature below which the growth rate of seedlings is markedly reduced (20) and the plants die (2). The relation between the temperature of the transition in the membrane polar lipids and the lower temperature limit for normal physiological function of plant cells, indicates that a transition in the physical and functional properties of cell membranes most likely occurs at the same temperature. Indeed, the osmotic response (14), the photochemical activity (29), and the ultrastructure (31) of chloroplasts of chilling-sensitive plants show abrupt changes at about 12°C, consistent with the view that the transition observed in the isolated polar lipids occurs in the chloroplast membrane at a similar temperature. For oleander the temperature of the exotherm depends on the growth temperature (Fig. 1). This confirms the earlier results, obtained using a fluorescent probe and spin labels (24), showing that the transition temperature of polar lipids from thylakoids of oleander shifted about 10°C deg during acclimation to a 25°C deg shift in growth temperature.

The calorimetric data for lipids from oleander grown at 45°C show that only about 4% of the lipids could be in the gel phase at −20°C, where this transition is almost complete. The transition for the bulk of the polar lipids probably occurs below −20°C, consistent with the large proportion of unsaturated lipids in the thylakoid membranes of oleander plants (25). At the midpoint of the transition (−6°C, Fig. 1) the width at half-height is 14°C deg and it can be calculated (13) that the van' Hoff enthalpy (ΔHvH) is 145 kJ mol⁻¹. Since the heat evolved in the transition (ΔHev) was 1.76 kJ mol⁻¹ it can be calculated, from ΔHvH/ΔHev that the cooperative unit for a solidification process would be about 82 molecules. From the above data it can be predicted that, at about −6°C, gel phase lipids might coexist with fluid phase lipids in domains consisting of about 80 molecules. The size of these domains is significant in terms of interpreting the effect of temperature on spin label motion.

When a spin label of the type used here is associated with fluid phase lipid the logarithm of the motion parameter, (log r0), is a linear function of the reciprocal of absolute temperature (K⁻¹), at least to within a few degrees of the transition exotherm determined by calorimetry. This conclusion is supported by the lack of a significant shift in the transition temperature determined by the intersect of two lines fitted to a decreasing number of data points (Fig. 3) and by the thermodynamic properties obtained when data are analyzed as a curvilinear function based on the equation described by Klein (10). When this equation is used it is possible to determine the transition midpoint (Tm), the transition width (Tw), the partition ratio (P) for label in the fluid and solid phases, as well as the enthalpy and entropy for motion.
in the fluid and solid phases. As shown by Klein (10) the distribution of the Arrhenius plot depends on the values of \( P \). For \( P \) less than 1 the first or high temperature "break of slope" will occur some degrees above \( T_m \) depending on the value of \( T_m \). In applying the equation to data obtained using plant lipids, \( P \) was varied between 0.5 and 0.1 in steps of 0.05. The assignment of these limits to \( P \) was based on the values for \( P \) obtained using the data for pure DPPC and mixtures of DPPC and wheat lipids (Table I) where \( T_m \) and \( T_w \) could be estimated. From the Klein equation (see caption to Fig. 4) it is apparent that in the temperature range where the lipids are all in fluid phase the rate of spin label motion is influenced only by the enthalpy and entropy of motion, and thus log \( \tau_0 \) is linearly related to "K". Deviation from this linear relationship occurs when the rate of spin label motion is influenced by the phase transition and partitioning of the label between the two phases. This explains the general agreement between the temperature for initiation of the exotherm, determined as the intersection of two straight lines (\( T^* \) in Fig. 4) and the temperature defined by the sum of \( T_w \) and \( T_m \) determined by the thermodynamic parameters of the Klein equation. It is also consistent with the view that graphs of log \( \tau_0 \) against "K" can be analyzed as two linear segments with the point of intersection representing the initiation of a transition. This view is, however, contrary to that of Patterson et al. (17) and Bishop et al. (4). These workers considered that log \( \tau_0 \) is a continuously variable function of "K" and concluded that the increase in the temperature coefficient of motion was not evidence of a phase transition.

An important observation arising from the values of the thermodynamic parameters obtained from the use of the Klein (10) equation is that \( P \) is less than one. This suggests that the spin label preferentially partition into gel phase lipids and is contrary to what was expected. Oldfield et al. (15) found that when liposomes, consisting of gel phase lipid and labeled with \( I_{10}^{31} \),
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Table 1. Values for the Thermodynamic Parameters Derived from the Effect of Temperature on Spin Label Motion

The data in Figure 4 were analyzed either as (a) a curvilinear function defined by the equation shown in Figure 4 or (b) as two straight lines (18). The parameter $T^*$ is the temperature of the intersection of the two straight lines and $\Delta H^*$ the Arrhenius activation enthalpy calculated from the slope of a straight line fitted to data points in the fluid phase.

\[
\begin{array}{cccccccccc}
\text{Lipid mixtures} & \Delta H_f & \Delta H_s & \Delta S_f & \Delta S_s & T_m & T_w & P & k & T_m + T_w & T^* & \Delta H^* \\
\text{kJ mol}^{-1} & \text{J} \text{K}^{-1} \text{mol}^{-1} & ^\circ\text{C} & ^\circ\text{C} & ^\circ\text{C} & & & & & & \text{kJ mol}^{-1} \\
\text{DPNPC/wheat} \\
100/0 & 32 & 23 & 12 & 39 & 2 & 0.2 & 0.5 & 41 & 41 & 33 \\
80/20 & 33 & 24 & 18 & 31 & 8 & 0.15 & 0.55 & 39 & 39 & 35 \\
50/50 & 35 & 26 & 20 & 22 & 11 & 0.15 & 0.35 & 33 & 31 & 36 \\
0/100 & 22 & 21 & 20 & -14 & 14 & 0.3 & 0.35 & 0 & -5 & 20 \\
\text{Plant lipids} \\
\text{Oleander} \\
45^\circ\text{C} & 34 & 18 & 11 & -7 & 13 & 0.15 & 0.35 & 6 & 7 & 32 \\
\text{Oleander} \\
20^\circ\text{C} & 34 & 19 & 15 & -15 & 11 & 0.15 & 0.35 & -4 & -2 & 31 \\
\text{Mung bean} & 38 & 26 & 22 & -2 & 14 & 0.15 & 0.35 & 12 & 11 & 36 \\
\text{Tomato} & 38 & 26 & 22 & -1 & 14 & 0.25 & 0.35 & 13 & 11 & 34 \\
\end{array}
\]

*It is conventional to represent the effect of temperature on the phase transition as a graph of log $\tau_0$ against \( ^\circ\text{K}^{-1} \). However, $\tau_0$ is the time for the label to rotate once a radian and the rate of motion is the reciprocal of $\tau_0$. The thermodynamic parameters have therefore been determined using plots of $\log 1/\tau_0$ against \( ^\circ\text{K}^{-1} \) hence the values for enthalpy are positive.

![Graphs](https://www.plantphysiol.org)

FIG. 5. A comparison of the temperature of the phase transition of leaf polar lipids from mung bean determined by (A) calorimetry, (C) and (D) spin label motion, and (B) fluorescence intensity. For (A) the sample weight was 6.9 mg, scan rate 5 \( ^\circ\text{K} \text{ min}^{-1} \), sensitivity 0.2 mcal s \(^{-1} \). For (B) and (C) the change in a slope was determined by linear regression (18). For (D) the transition was determined from spin label motion by fitting the equation (10); the parameters were: $\Delta H_f$ and $\Delta H_s = 34 \text{ kJ mol}^{-1}$; $\Delta S_f = 26$ and $\Delta S_s = 22$ J mol\(^{-1} \)

were mixed with liposomes consisting of fluid phase lipid, the motion of the label increased. This was interpreted as being due to label preferentially moving to the liposomes consisting of fluid lipids. However, the increase in label motion observed in these studies could also be due to the fluid phase liposomes fusing with the gel phase liposomes. That the labels $I_{1,0}$ and $I_{1,4}$ preferentially partition into gel phase domains in plant polar lipids is consistent with two important observations. The first is that the slope of the plot of log $\tau_0$ against \( ^\circ\text{K}^{-1} \) increases in the region where gel and fluid phase lipids coexist (Figs. 2, A and B, 4B; 5C; 6C). In the case of pure DPPC where the proportion of gel phase lipids increases from 0 to 100% over 4 \( ^\circ\text{C} \) deg, the slope is steep (Fig. 4). For the other mixtures of DPPC and wheat lipids the slope in the mixed fluid plus gel phase is reduced as the width
of the transition increases. However, in all of the examples shown (Table I), a significant increase in slope occurs at a temperature corresponding to the sum of $T_m$ and $T_r$, which is consistent with the label preferentially locating in domains which restrict motion. The second observation is that the transition temperature detected by the spin label is the same as that detected by trans-parinaric acid (Figs. 5, B and C; 6, B and C). Sklar et al. (30) have shown that trans-parinaric acid preferentially partitions into gel phase with a $P$ of approximately 0.25. Since the labels $I_{10(10)}$ and $I_{10(14)}$ show similar $P$ ratios of 0.1 to 0.35 (compare Figs. 5 and 6 and Table I) it is concluded that they also preferential partition into gel phase.

The coincidence of the initiation of a phase transition, detected by calorimetry and spin labeling, as well as the detection of phase separation by the fluorescent probes, demonstrates the suitability of using any one of these techniques to detect a phase transition in aqueous dispersions of leaf polar lipids. The temperature of the phase transition of membrane lipids correlates well with the temperature below which injury becomes evident in plant tissue (21). Thus, each of these techniques can provide a valid method for assessing the sensitivity of plants to chilling, where chilling sensitivity is defined in terms of the temperature below which injury develops rather than the time-course for development of injury at a fixed temperature.

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