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LIPIDS OF CHILL-SENSITIVE AND -RESISTANT *PASSIFLORA* SPECIES: FATTY ACID COMPOSITION AND TEMPERATURE DEPENDENCE OF SPIN LABEL MOTION

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Abstract—Polar lipids were extracted from the leaves of *Passiflora* species which varied in their resistance to chilling injury. The fatty acid compositions of the 8 major polar lipid classes from *P. caerulea* (chill-resistant) were generally similar to those of the corresponding lipids from *P. flavicarpa* (chill-sensitive). Using ESR spectroscopy, the motion of spin-labelled molecules was measured in phospholipids isolated from a range of *Passiflora* species. The temperature dependence of the motion of the spin labels showed a change at 1° for lipids of the most chill-resistant species and at 9° for the lipids of the most chill-sensitive species. Lipids from other species showed changes at intermediate temperatures, and the greater the chilling sensitivity of the species, the higher was the temperature of the change. It is concluded that pronounced differences in chilling sensitivity of the *Passiflora* species are correlated with physical differences in their membrane lipids; however, the degree of unsaturation of the lipids is not a reliable guide to chilling sensitivity.

INTRODUCTION

Comprehensive evidence that the structure of cell membranes is adapted to their environmental temperature derives from experiments with microorganisms. For example, the membranes of *Bacillus stearothermophilus* adapted to lower growth temperatures have a high proportion of unsaturated and/or shorter chain-length saturated acyl groups than the membranes of organisms adapted to higher temperatures [1, 2]. Such adaptations influence both the fluidity of the membrane lipids and the temperature of the transition between a relatively ordered state of the membrane lipids and a more disordered state at higher temperatures. Individual species of higher plants are not adaptable to such wide ranges of temperature as are many bacteria, but they are genetically adapted to particular temperature ranges. For instance, species which originate from the tropics and sub tropics are usually injured by chilling temperatures, i.e. between 0° and ~10°, while species from cooler regions are resistant to chilling [3].

The lipid membranes of plants resemble those of bacteria in that they undergo order–disorder transitions, which however are characteristic of the species rather than their growth temperature [4]. These transition temperatures can be detected using electron spin resonance (ESR). Below the transition temperature the increased order of the hydrophobic fatty acyl chains causes an increase in the temperature coefficient of motion of the spin label molecule [5]; the transition is also coincident with changes in the temperature coefficient of viscosity of the lipids [6].

When the logarithm of spin label motion parameter τ_0 is plotted against the reciprocal of absolute temperature, straight lines are obtained, which intersect at the transition temperatures. In all chill-sensitive plants which have been examined the temperature of the order–disorder transition is several degrees above 0°. In contrast, the temperature of this transition in the membrane lipids of most chill-resistant plants is close to or below 0° [7, 8].

Membranes of chill-resistant plants are richer in unsaturated fatty acids than most chill-sensitive plants [7]. However, the difference is not consistent. For example, the lipids of the chill-sensitive sweet potato (*Ipomaea batatas*) are more unsaturated than the lipids of the chill-resistant potato (*Solanum andigena*) [9]. Low temperatures have also been reported to increase the proportion of unsaturated fatty acids in the membranes of some plants [10]; whether this is a general mechanism for changing membrane fluidity in response to changes in temperature, as in bacteria, is unknown.

To determine if indeed there is any correlation between the chill-sensitivity of plants, the fatty acid composition of their polar lipids and the temperature of the order–disorder transition, species of the genus *Passiflora* offer particular advantages. Some of these species are native to the tropical lowlands, while others originate from cool climates in tropical mountains [11, 12]. Comparisons of lipid behaviour as a function of temperature within this group would therefore be more likely to reflect adaptations to climatic regions than would comparisons between unrelated plants which are sensitive or resistant to chilling. Electrolyte leakage, an early symptom of chilling injury, has been measured using leaf tissues of a number of *Passiflora* species and the results used to rank species in order of their chill-resistance [13]. These results confirmed the wide range of tolerance to chilling

in this genus which had been suggested by horticultural data [14].

In the present work measurements were made of the fatty acid composition and the temperature dependence of spin label motion in the phospholipids from leaves of the same series of *Passiflora* varieties used previously to study electrolyte leakage [13]. In addition, the fatty acid composition of the 8 major polar lipid classes was determined.

RESULTS AND DISCUSSION

Table 1 shows the fatty acid composition of 8 polar lipid fractions from *P. flavicarpa* (chill-sensitive) compared with those of *P. caerulea* (chill-resistant). Although these species resist chilling to quite different extents, and are adapted to different climates, comparisons of their lipid composition reveal only small differences in fatty acid composition. The slight trend towards greater unsaturation in some lipids of the more chill-resistant species, *P. caerulea*, is probably not significant, considering the limits of error for the analysis. A similar slight trend in unsaturation is apparent for the fraction containing sulphoquinovosyl diacylglycerol (SQDG) but the trend is reversed for the phosphatidyl inositol (PI) fraction. In the phosphatidyl glycerol (PG) fraction there are differences in the relative proportions of the unsaturated fatty acids 16:1, 18:2 and 18:3. However, the bulk phospholipid fraction before separating PG does not show these differences. Given the complex fractionation procedure required for fatty acid analysis and the close similarity in the composition of the unfractionated lipid classes, there seems little difference in the overall fatty acid compositions of *P. caerulea* and *P. flavicarpa* lipids.

Figure 1 shows the temperature dependence of spin label motion in phospholipids from two *Passiflora* species. The change in the logarithm of the motion parameter τ_0 is plotted as a function of the reciprocal of the absolute temperature. For *P. flavicarpa*, the most chill-sensitive species studied there is a change in a slope or 'break' in the plot at about 9°. For the lipids from *P. caerulea*, the 'break' occurs at 1°.

The temperature of the 'break', which represents a transition between a disordered and a more ordered state [4, 5] was determined using phospholipids from 6 other *Passifloras* with intermediate degrees of chilling sensitivity. The results are presented in Fig. 2 as a function of chilling resistance, which was measured by electrolyte leakage [13]. The plot shows a negative correlation ($P < 0.01$) between the temperature of the order–disorder transition and the resistance of the plant to chilling.

Comparing the patterns of fatty acid content in *Passiflora* lipids (Table 1) with those of the lipids from a wide variety of plants [15–22], the most obvious conclusion is that the patterns of fatty acid content in the two species of *Passiflora* resemble each other more than they resemble the patterns in lipids of unrelated plants. It therefore appears that during the evolution of temperature adaptation in *Passiflora* there has been little change in the proportions of the different fatty acids incorporated into the polar lipids.

Chill-sensitive plants, as a group, have less unsaturated fatty acid in their phospholipids than most chill-resistant plants [7, 23] and at first light our results would appear to conflict with these findings. However, Lyons *et al.* [23] showed that the correlation is not exact and there is considerable overlap in fatty acid composition between the two groups of plants. The unsaturation of the *Passiflora* species (Table 1) fits within the range of the overlap. Other published data for individual phospholipids from a variety of plants are also consistent with an overlap in the degree of unsaturation between the lipids of chill-sensitive and -resistant plants. For instance, using published data for the composition of phosphatidyl choline (PC) from a number of species [15–22], the mean content

Table 1. Fatty acid composition of lipids from chill-sensitive and -resistant *Passiflora* varieties. The content of individual fatty acids and the content of unsaturated fatty acid is expressed to the nearest mole per cent. Roman type: *P. caerulea*; italic type: *P. flavicarpa*

Fatty acid	Fatty acid content (mole per cent) 16:0	16:1	18:0	18:1	18:2	18:3	Total unsaturated fatty acid
Phospholipid + SQDG	32	6	2	3	34	23	66
(total)	*33*	*5*	*2*	*5*	*34*	*21*	*65*
PC	23	0	3	1	47	26	74
	25	*0*	*3*	*1*	*46*	*25*	*72*
PE	28	0	2	2	52	16	70
	30	*0*	*1*	*1*	*54*	*14*	*69*
DPG	25	4	2	2	44	23	73
	27	*0*	*2*	*2*	*47*	*22*	*71*
PI	48	0	2	1	38	11	50
	43	*0*	*2*	*0*	*43*	*12*	*55*
PG	30	28	2	8	20	14	70
	35	*14*	*2*	*4*	*14*	*31*	*63*
SQDG	43	1	2	1	28	25	55
	45	*1*	*4*	*0*	*32*	*16*	*49*
Galactolipid	5	0	1	0	5	89	94
(total)	*6*	*0*	*1*	*1*	*7*	*85*	*93*
MGG	1	0	0	0	4	94	98
	2	*0*	*0*	*0*	*7*	*91*	*98*
DGG	8	0	1	0	3	87	90
	8	*0*	*2*	*1*	*6*	*83*	*90*

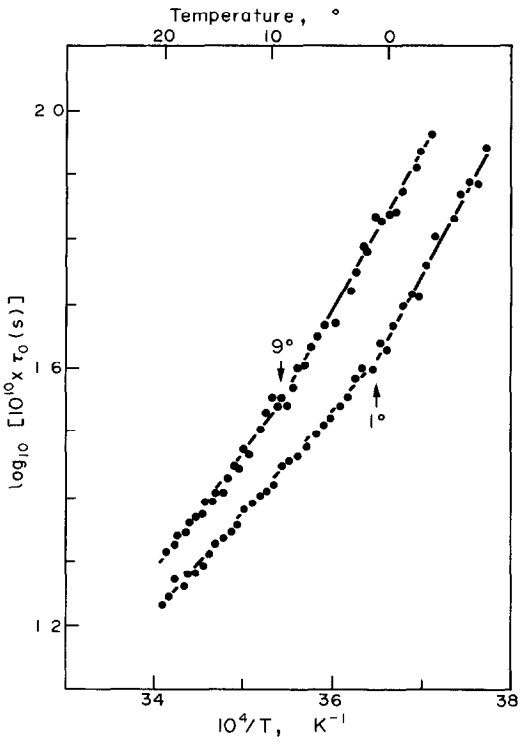

Fig. 1. The effect of temperature on the motion of the spin label (12 NS) in vesicles prepared from the lipids of *Passiflora* species. Changes in the temperature coefficients of motion are shown at 9° for *P. flavicarpa*, and at 1° for *P. caerulea*.

of unsaturated fatty acid in a group of chill-resistant plants was 75 mole % (s.d. 3 mole %) and in a group of chill-sensitive plants was 67 (s.d. 6 mole %). *P. flavicarpa* and *P. caerulea* gave values of 72 and 74 mole % respectively (Table 1). Some other chill-sensitive plants, such as *Episcia reptans* have relatively high levels of unsaturation (74 mole %) in PC [19].

The question arises whether the similarities of fatty acid composition between the two species of *Passiflora* are inconsistent with the differences in the temperature of the order–disorder transition of their membrane lipids detected by spin labelling. Our measurements of the overall fatty acid composition of, for instance, PC do not necessarily indicate differences in the molecular composition, however, because each PC molecule is esterified with fatty acid at two positions and many different molecular combinations can result in the same overall fatty acid composition. Phillips *et al.* [24], using synthetic phospholipids, have shown how inter- and intramolecular mixing of fatty acids in PC can give physical differences between mixtures which have the same overall fatty acid composition. A further possibility is that the differences in the physical behaviour of the membrane lipids are caused not by variation of the fatty acid composition but by variation of the relative proportions of the different head groups.

Genetic adaptation to environmental temperature during the evolution of *Passiflora* species appears to have resulted in an adjustment of the temperature of the lower order–disorder transition. This is suggested by the negative correlation between the temperature of the order–disorder transition in the lipid vesicles and the sensitivity to chilling of the *Passiflora* variety from which the lipids were extracted (Fig. 2). This correlation supports the data which show that chill-sensitive plants, as a group, have changes in the temperature coefficient of various metabolic events involving diverse membrane structures near 10° while the same changes appear at lower temperatures in chill-resistant plants [8]. While in *Passiflora* the evolution of resistance to chilling has been accompanied by proportionate changes in the temperature response of their polar lipids, the proportion of unsaturated fatty acids in the lipids has not changed markedly. The question remains open whether the physical differences as measured by ESR result from a relocation of the fatty acids into different molecular combinations.

EXPERIMENTAL

Lipid extraction. Leaves from *Passiflora* species which had been grown in a glass house (25° day, 18° night) were cut into small pieces after removing the main veins. Portions of *ca* 20 g were homogenized with 150 ml $CHCl_3$–MeOH (3:7), containing 0.2 mg butylated hydroxytoluene as antioxidant plus 45 mg of 2-butoxy-*N*-(2-diethylaminoethyl)-cinchonamide HCl to inhibit phospholipase activity [25]. The homogenate was heated to 50°, filtered and the residue washed with 200 ml $CHCl_3$. The filtrate was partitioned with 0.2 vol of 0.73% NaCl, the lower layer dried over Na_2SO_4 and the solvent evaporated under red. pres. Lipids were stored in $CHCl_3$ under liquid N_2.

Lipids were fractionated by chromatography on acid-washed Florisil [26]. Pigments and neutral lipids were eluted with $CHCl_3$, galactolipids with Me_2CO and phospholipids with MeOH (8 ml/g of adsorbent in each case). Galactolipids were further separated on acid-washed Florisil by eluting monogalactosyl diacylglycerol (MGDG) with 35% Me_2CO in $CHCl_3$, unidentified lipids with 55% Me_2CO in $CHCl_3$ and digalactosyl diacylglycerol (DGDG) with Me_2CO (10 ml/g

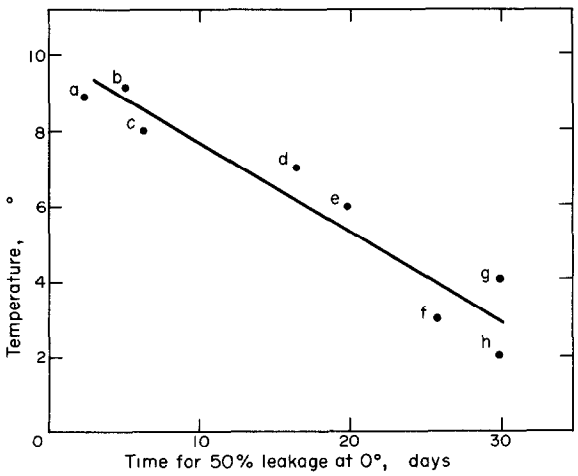

Fig. 2. The relationship between the temperature of the lower change in the coefficient of spin label motion and the time for half of the electrolyte to leak from leaf tissues (13) for different *Passiflora* species. The temperature of the lower change in the temperature coefficient of spin label motion was determined as in Fig. 1. The letters refer to the varieties as follows: a, *P. flavicarpa*; b, *P. flavicarpa* hybrid (unknown parentage); c, *P. maliformis*; d, *P. flavicarpa* × *cincinnata* F_1; e, *P. cincinnata*; f, *P. edulis*; g, *P. flavicarpa* × *caerulea* F_2; h, *P. caerulea*.

adsorbent). Phospholipids were further separated on acid-washed Florisil by eluting sulpholipid (SQDG), phosphatidyl glycerol (PG) and diphosphatidyl glycerol (DPG) with 15% MeOH in $CHCl_3$ and phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) and phosphatidyl inositol (PI) with 50% MeOH in $CHCl_3$ and some remaining PC with MeOH (5 ml/g of adsorbent). All fractions containing more than one phospholipid were fractionated by preparative-TLC on 0.5 mm Si gel HR plates with $CHCl_3$–MeOH–HOAc–H_2O (85:15:10:3) containing 4 μg/ml butylated hydroxytoluene. Phospholipids were detected with 0.2% 2,7-dichlorofluorescein in EtOH and eluted from the Si gel with $CHCl_3$–MeOH (2:1).

Analysis of fatty acids. Fatty acid Me esters were prepared by transesterification of lipid (2–3 mg) with 14% BF_3 in MeOH (2 ml) at 60–65° for 30 min. After adding H_2O (3 ml), Me esters were extracted ×3 with 5 ml petrol (bp 40–60°). The combined extracts were dried over Na_2SO_4 and the solvent removed under N_2. The Me esters were adsorbed on a Florisil column containing 7% H_2O and eluted with 5% Et_2O in petrol (bp 40–60°) [27]. Fatty acid Me esters were quantitated by GLC at 170° using a 2 m × 4 mm glass column containing 25% diethylene glycol succinate—2% phosphoric acid. Components were identified by comparison with standards

Measurement of chilling resistance. Ten leaf strips 3 cm × 1 mm were cut from between the main veins, washed and suspended in 3 ml H_2O at 0° [13]. The conductivity of the bathing soln was measured daily at 0° and the mean time taken for the conductivity of 10 replicates to reach 50% of their max values was used as a measure of relative chilling resistance. Max values of conductivity were obtained after killing the tissue at 100° and allowing the tissue electrolyte to equilibrate with the bathing soln for 24 hr [13].

ESR measurements. The lipid fraction containing total phospholipid and SQDG, after removal of neutral lipid and galactolipid, was suspended at a concn of 10 mg/ml in 0.1 M Tris–HOAc buffer, pH 7.4, containing 5 mM EDTA and 10^{-4} M nitroxide spin label. The spin label used was 2-(10-carbmethoxydecyl)-2-hexyl-4,4-dimethyl-3-oxazolidinyloxy (12 NS) [4]. The empirical motion parameter τ_0 was calculated from the first derivative absorption spectra as described in ref. [28]. The temp at which the coefficient of spin label motion changes, i.e. the change in slope of a plot of the logarithm of τ_0 against the reciprocal of the absolute temp, was determined by the statistical method described in ref. [4].

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