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Role of Lipids in the *Neurospora crassa* Membrane: III. Lipid Composition and Phase Transition Properties of the Plasma Membrane, and its Components

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Summary. The *Neurospora crassa* mutant *slime*, which lacks a cell wall, was used to determine the lipid composition and phase transition properties of the plasma membrane and its components. The lipid composition and phase transition properties of the plasma membrane were then compared to the same parameters of whole cell extracts of *Neurospora slime*. Phospholipids extracted from whole cells and plasma membranes have similar fatty acid profiles, but differ somewhat in phospholipid base compositions. These differences in phospholipid base profiles were not sufficient to cause differences in the heat content (ΔH) or mid-point temperature of the phospholipids' gel-liquid crystal phase transitions as detected by scanning calorimetry. Phase transition temperatures and heat contents for plasma membrane and whole cell phospholipid extracts were -23.8 and -22.5 °C and 0.3 and 1.7 cal/g, respectively. Commercially available phospholipids when mixed in the ratios of phospholipids found in whole cell and plasma membrane extracts yielded similar results: The "model" system of whole cell extract had a transition temperature of -22 °C and $\Delta H = 1.1$ cal/g, compared to the "model" system for plasma membrane with a transition temperature of -21 °C and $\Delta H = 1.24$ cal/g. The neutral lipid fraction of plasma membranes also exhibits endothermic activity in the calorimeter at temperatures below 0 °C with a $\Delta H \sim 9$ cal/g. Total lipid preparations (neutral + phospholipids) and freeze-dried plasma membranes scanned in excess water do not exhibit any endothermic events in the calorimeter. Neutral lipids probably interact with phospholipids to abolish the calorimetrically detectable phase transition of phospholipids. Whole cell and plasma membrane extracts are sufficiently similar in phospholipid base composition to permit the use of whole cell extracts as a model system for studying the effect of altered lipid composition on the physical properties of the phospholipids in the *Neurospora crassa* plasma membrane.

The lipid composition of plasma membranes has become an area of intense biomedical research since the advancement of the fluid mosaic model of membrane structure and the hypotheses that: (i) the fluidity of the membrane influences its transport properties (Chapman, 1976), and (ii) the lipid environment influences enzyme activity (Gennis & Jonas, 1977). The majority of work in this area has utilized either non-nucleated or prokaryotic organisms. Our efforts have been directed at exploring these concepts with a nucleated organism in which it is possible to control the various components of membrane lipid composition: fatty acid composition, phospholipid base composition, and percent sterol content. For example, we have been utilizing the cel$^-$ (chain elongation) mutant of *Neurospora* to monitor the physical state of phospholipids with altered fatty acid composition (Friedman, 1977*a*) and have evaluated membrane electrophysiological properties of cells with altered fatty acid composition (Friedman, 1977*b*).

Implicit in our previously published work are the assumptions that: (i) phospholipid extracts of intact *Neurospora* have fatty acid profiles indistinguishable from the fatty acid profile of the plasma membrane, and (ii) the phase transition properties of extracted phospholipids serve as a model for the phase transition properties of the plasma membrane. To evaluate these assumptions, we have isolated the plasma membrane fraction from a different *Neurospora* mutant, *slime*, and: (i) compared the fatty acid composition of the isolated membrane fraction with the fatty acid composition of whole cell extracts, (ii) compared phospholipid base composition of the isolated mem-

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brane fraction with whole cell extracts, and (iii) performed differential scanning calorimetry on the isolated membrane and its lipid fractions. Our results, which are the subject of this report, indicate that the fatty acid profile of plasma membrane phospholipids is indistinguishable from that of whole cell phospholipid extracts. Plasma membrane extracts and whole cell extracts do differ somewhat in phospholipid base profile. Despite these differences, the two lipid extracts exhibit similar calorimetric behavior. We have also obtained differential scanning calorimetry data suggesting gel-liquid crystal phase transitions are present in the neutral lipid and phospholipid fractions of the plasma membrane, but not in the total lipid extract or the isolated plasma membrane.

Materials and Methods

Growth of Slime Cells

Slime cells (kindly supplied by Dr. E. Scarborough) were grown at 31 °C on Vogel's minimal medium supplemented with 2% (wt/vol) mannitol, 0.75% (wt/vol) yeast extract, and 0.75% (wt/vol) nutrient broth as previously described by Scarborough (1975). Cultures grown in 50 ml of media contained in 125-ml Erlenmeyer flasks, or 800 ml of media contained in 2-liter flasks, or 1.5 liters of media contained in 4-liter flasks were found to have similar fatty acid compositions. Cultures grown in 4-liter flasks were adopted for routine use. All cultures were grown on a rotary shaker at 160 rpm.

Isolation of Plasma Membranes

Plasma membranes were isolated by slightly modifying the methodology detailed by Scarborough (1975). Our procedure differs from Scarborough's in that: (i) we wash our cells four times; (ii) we maintain the ratio of packed cells to washing volume of Buffer A at 1:10; (iii) the pelleted plasma membranes were freeze-dried overnight for biochemical analysis; and (iv) for scanning calorimetry, the pelleted membranes were subjected to an additional three washings with distilled water before freeze drying. The purity of our plasma membrane preparations was checked by transmission electron microscopy, and the micrographs obtained are analogous to those of Scarborough (1975).

Isolation of Phospholipid

Lipids were extracted from either freeze-dried washed cells, or freeze-dried isolated plasma membranes using the chloroform-methanol extraction and "Folch" wash procedures detailed by Christie (1973, p. 39). Lipid extracts were evaporated to dryness under nitrogen. Traces of remaining solvent were removed by freeze drying samples overnight. The dried, purified lipid samples were dissolved in chloroform and stored under nitrogen at −10 °C.

Neutral and phospholipid fractions were separated on a silicic acid column as previously described (Friedman, 1977*a*). Neutral lipids had to be eluted with a minimum of ten column volumes of chloroform, followed by the elution of total phospholipids in ten column volumes of absolute methanol.

Phospholipid Analysis

For qualitative analysis total lipids were extracted as described above and dissolved in chloroform (10 mg/ml). A 30 λ aliquot was spotted on thin-layer chromatography plates (silica gel H, "Redi-Coat", supplied by Supelco) under a stream of nitrogen. Thin-layer plates were activated before use by placement in a drying oven at 110 °C for 30 min. The plates were developed using a chloroform/methanol/ammonium hydroxide solvent system (65:35:5). Developed chromatograph plates were dried under a stream of nitrogen, and individual phospholipids were identified by spraying the plates with a 55% sulfuric acid, 0.6% potassium dichromate solution and heating the plates on a hot plate until color developed. Positive identification of unknowns was made by co-chromatography with known phospholipid standards.

Identified phospholipids were quantified by phosphorus determinations. We employed the methodology detailed by Christie (1973, p. 189), modified to obviate the possibility of silica gel interfering with an accurate, quantitative phosphorus determination (Doizaki & Zieve, 1963; Shen & Dyroff, 1962). Identified phospholipids were scraped from the chromatograph plates and an equal area of silica gel adjacent to each identified spot was scraped and used as a reference blank. Test tubes containing scraped phospholipids and reference blanks were incubated with 0.4 ml of 70% perchloric acid and refluxed for 20 min as described by Christie (1973, p. 222). A calibration curve was prepared by adding sodium dihydrogen phosphate to several tubes and treating them in a similar manner. All test tubes were then cooled, and 2.4 ml of ammonium molybdate and reducing reagent were added to each test tube. All tubes were thoroughly mixed and heated for 10 min in a boiling water bath (as described by Christie, 1973). The tubes were cooled and centrifuged at $3{,}000 \times g$ to remove the silica. Absorbance of the solutions was measured at 820 nm.

Fatty Acid Analysis

The fatty acid composition of the phospholipids was determined using the previously reported procedures (Friedman, 1977*a*). Phospholipid fatty acids were converted to their methyl esters via a transesterification procedure. Fatty acids were identified by comparison of the retention times of their methyl esters with standards chromatographed under identical conditions. The weight percentage of each fatty acid present was determined by peak integration.

Differential Scanning Calorimetry

The calorimetric behavior of (i) the isolated plasma membranes, (ii) the extractable lipid, (iii) the extractable neutral lipids, and (iv) the extractable phospholipids of *slime* plasma membranes was studied using the differential scanning calorimetry mode of a Dupont 990 Thermal Analyzer. For studies of extracted lipids, microgram quantities of lipid dissolved in chloroform were deposited in DSC pans, and evaporated to dryness under nitrogen. Open sample pans were freeze dried overnight to remove all traces of solvent. Water in excess (~ 1 λ water/mg lipid) was added to each pan before sealing. For studies of isolated plasma membrane, DSC pans were filled to capacity with isolated plasma membranes which had been washed in distilled water, freeze dried, and resuspended in excess water. The ratio of plasma membrane/water in these experiments was 1:10 on a weight basis. All samples were sealed in Dupont "volatile sample" pans and run against an empty sealed pan as standard.

Results

A. Comparison of the Fatty Acid Composition of Phospholipids Extracted from Slime Plasma Membrane and Whole Cells

The fatty acid profiles of phospholipids derived from the plasma membrane and whole cell extracts of *Neurospora slime* are shown and compared in Fig. 1. Values presented are per 100 moles ± SD. The values for fatty acids extracted from whole cells are the averaged values of four independent experiments, whereas the values presented for the plasma membrane phospholipids are the averaged values of five independent experiments.

The hypothesis that the fatty acid composition of phospholipids extracted from plasma membranes is the same as that extracted from whole cells was tested by three statistical procedures. We tested the hypothesis that the mean value of each fatty acid

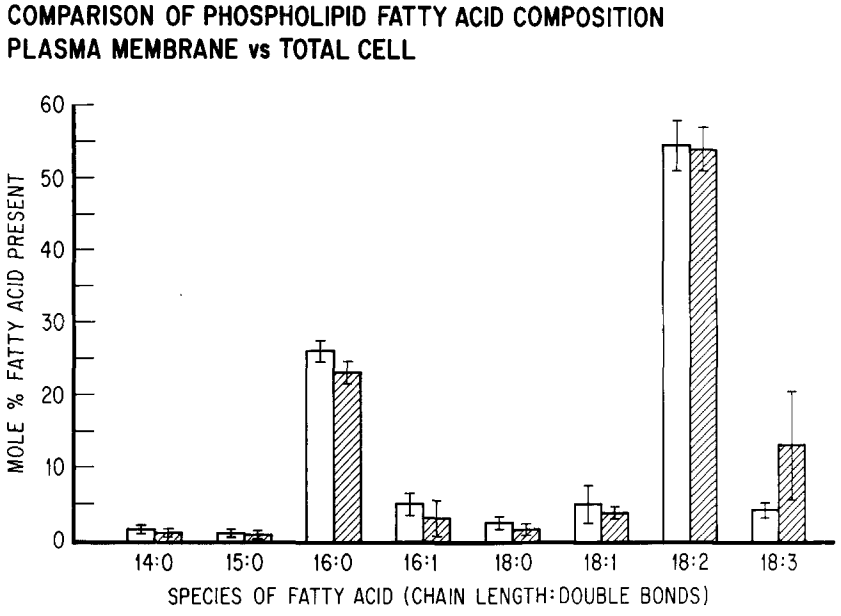

Fig. 1. Comparison of the fatty acid compositions of *Neurospora slime* phospholipids extracted from plasma membranes (clear bars), and whole cells (hatched bars). Values reported are the means of five experiments for plasma membranes and four experiments for whole cells ± SD

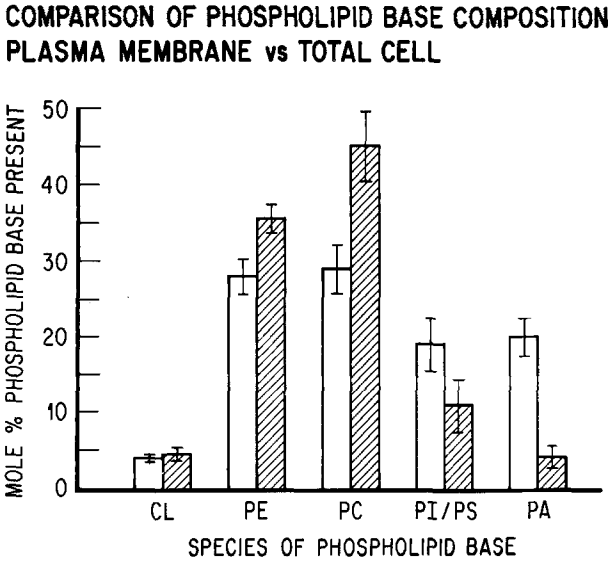

Fig. 2. Comparison of the phospholipid base profiles of *Neurospora slime* plasma membrane (clear bars) and whole cells (hatched bars). Values reported are the means of five experiments for plasma membranes and three experiments for whole cell extracts ± SD

obtained from plasma membranes is the same as the mean value obtained from whole cell extracts by evaluating the t statistic for each fatty acid using a two-sample test. No difference is found between the two groups at the 95% confidence level for all fatty acids except for palmitic acid (16:0) which was acceptable at the 99% confidence level.

The second statistical procedure we employed was a multivariate analog of a t test known as Hotelling's T^2. Due to the size of our sample we considered five (of the eight) fatty acids. The fatty acids considered were those found in the highest concentrations (i.e., 16:0, 16:1, 18:1, 18:2, and 18:3). The P value obtained for the five predominant fatty acids provided no basis for rejecting the null hypothesis, i.e., that the mean values for the five fatty acids tested from whole cell phospholipids are equal to the corresponding mean values of the five fatty acids tested in the plasma membrane phospholipid extracts. Finally, an arc sine transformation was performed on these data. Such a transformation is warranted when the data

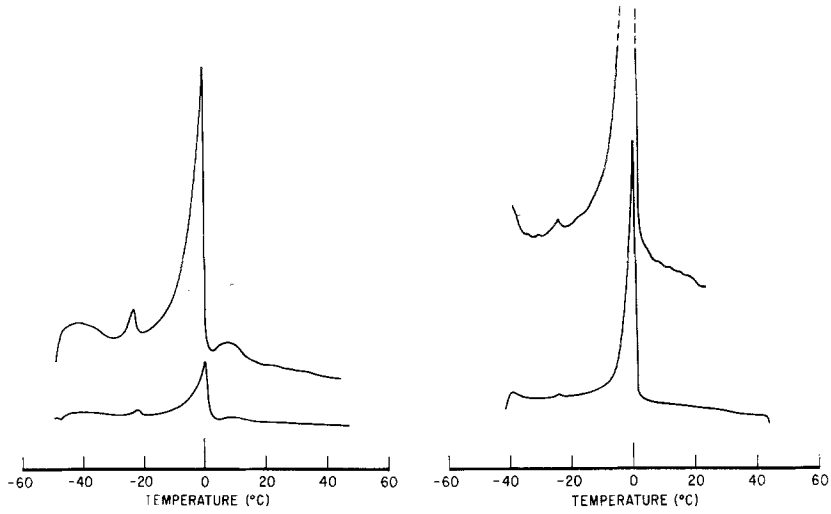

Fig. 3. Differential scanning calorimetry heating curves of *Neurospora slime* phospholipids. *Left panel:* 2 mg phospholipids extracted from whole cells in excess water. *Upper trace:* calorimeter sensitivity, 0.2 mcal/sec; *lower trace:* calorimeter sensitivity, 1.0 mcal/sec. Scan rate for both traces, 5 °C/min. *Right panel:* 2.5 mg phospholipids extracted from plasma membranes in excess water. *Upper trace:* calorimeter sensitivity, 0.1 mcal/sec; *lower trace:* calorimeter sensitivity, 0.5 mcal/sec. Scan rate for both traces, 5 °C/min

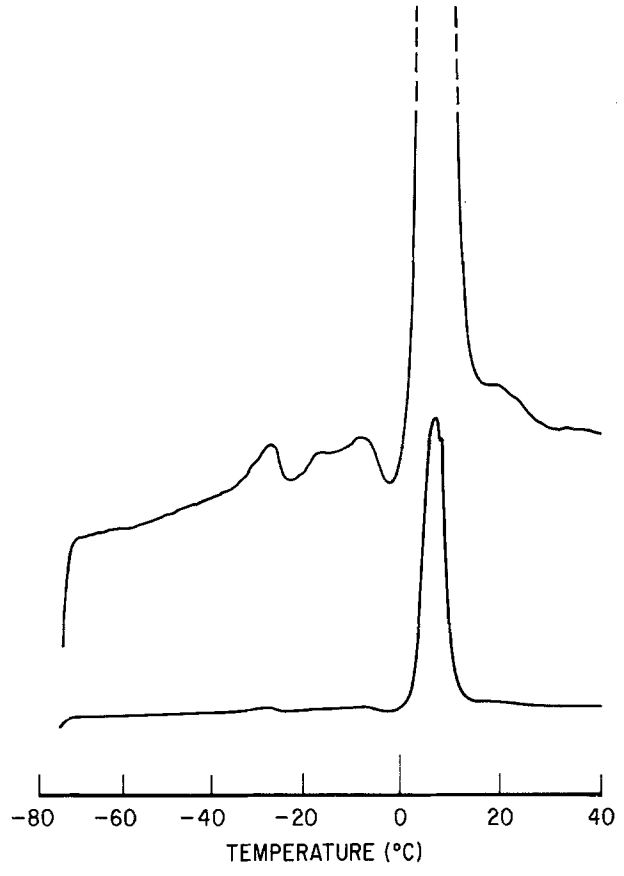

Fig. 4. Differential scanning calorimetry heating curve of neutral lipids extracted from *Neurospora slime* plasma membranes. 6.47 mg neutral lipids in excess water. *Upper scan:* calorimeter sensitivity, 0.5 mcal/sec; *lower scan:* calorimeter sensitivity, 5 mcal/sec. Scan rate for both traces, 5 °C/min

being analyzed is in proportional units. Repeating the multivariate analysis of variance on the transformed data yielded a nonsignificant P value. Thus the Hotelling's T^2 supports the hypothesis that the fatty acid profiles of plasma membranes and whole cell extracts are similar[1].

B. Comparison of the Phospholipid Base Composition of Slime Isolated Plasma Membranes and Whole Cells

The phospholipid base compositions of isolated plasma membranes and total cell extracts are shown and compared in Fig. 2. Values presented are per 100 moles $\pm$ SD as measured by phosphorus determinations. The values of the phospholipid profiles presented for plasma membranes are the averaged values derived from five independent experiments; the values for whole cell extracts are the averaged values derived from three independent experiments.

Visual inspection of the data as well as Hotelling's T^2 test indicate statistically significant differences in phospholipid base composition between plasma membranes and total cell extracts. Plasma membranes have decreased amounts of phosphatidylcholine and phosphatidylethanolamine and increased amounts of phosphatidylinositol/serine and phosphatidic acid when compared to total cell extracts. Nevertheless, phosphatidylcholine and phosphatidylethanolamine appear to be the major phospholipids present in both plasma membranes and total cell extracts.

[1] We are grateful for and wish to acknowledge consultations with and statistical analyses performed by Messrs. R. Belling, M. Feuerman and Mrs. J. Chou of our Department of Information Processing.

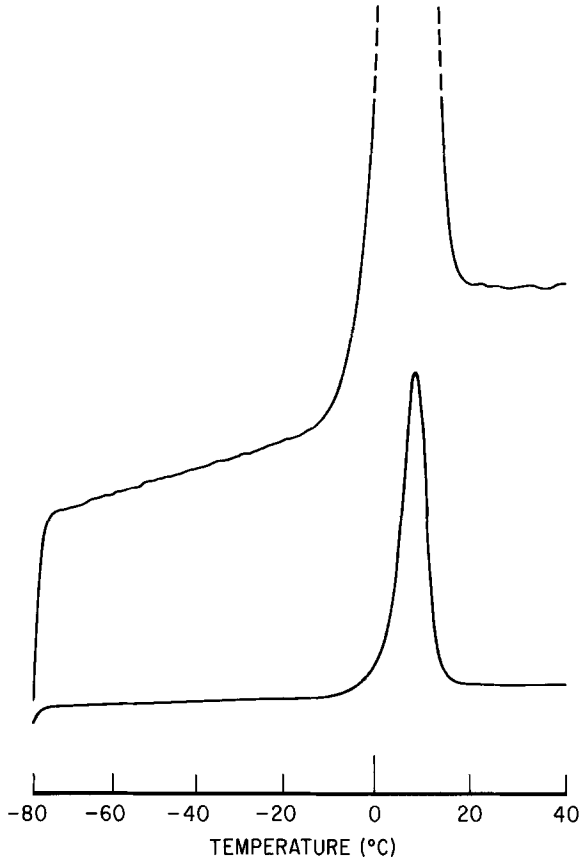

Fig. 5. Differential scanning calorimetry heating curve of total lipid extract of *Neurospora slime* plasma membrane. Lipid scanned was 3.31 mg in excess water. *Upper scan:* calorimeter sensitivity, 0.5 mcal/sec; *lower scan:* calorimeter sensitivity, 5 mcal/sec. Scan rate for both traces, 5 °C/min

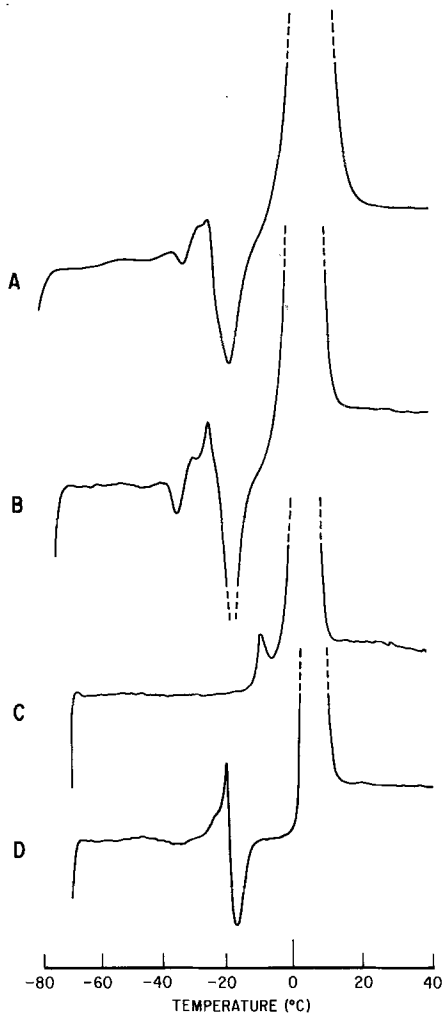

Fig. 6. Differential scanning calorimetry heating curves of isolated plasma membrane in Buffer B, Buffer B, and Buffer B components. (*A*): Isolated plasma membrane scanned in Buffer B. Calorimeter sensitivity, 1 mcal/sec. (*B*): Scan of Buffer B at calorimeter sensitivity 0.5 mcal/sec. (*C*): Scan of 3.27 mg of a 0.1 M Tris HCl solution, pH 7.5. Calorimeter sensitivity, 0.5 mcal/sec. (*D*): Scan of 3.34 mg of a 0.5 M mannitol solution at calorimeter sensitivity 0.5 mcal/sec. Scanning rate for all traces, 5 °C/min

C. Phase Transitions of Intact Plasma Membrane and Lipid Fractions

Our observation of similar fatty acid but different base profiles of the phospholipids in plasma membrane and total cell extracts leaves open the possibility for differences in the physical chemistry of these two distinct phospholipid systems. Panels *A* and *B* of Fig. 3 provide calorimetric evidence that this is not the case. Both panels illustrate differential scanning calorimetry heating curves of extracted phospholipids in excess water. A scan of total cell phospholipids is shown in Panel *A*, whereas a scan of plasma membrane phospholipids is shown in Panel *B*. The larger peaks in these thermograms represent the melting of ice, whereas the smaller peaks to the left represent the bulk phospholipid gel-liquid crystal phase transition. The peak temperatures of these phase transitions are −22.5 and −23.8 °C for whole cell and plasma membrane extracts, respectively. The estimated heats of transition are 1.7 cal/g for total cell phospholipid extract and 0.3 cal/g for plasma membrane phospholipid extract.

The calorimetric behavior of the neutral lipid fraction of the plasma membrane was also explored. Neutral lipids were scanned from −75 to +40 °C in the presence of excess water. As shown in Fig. 4, we observed comparatively large endothermic events (ΔH=9 mcal/mg) in the approximate temperature range of −40 to 0 °C. Since we have not as yet characterized the neutral lipid fraction of *Neurospora* plasma membranes, we cannot state which compound(s) present in this fraction is (are) responsible for these events. However, it does not seem likely that ergoste-

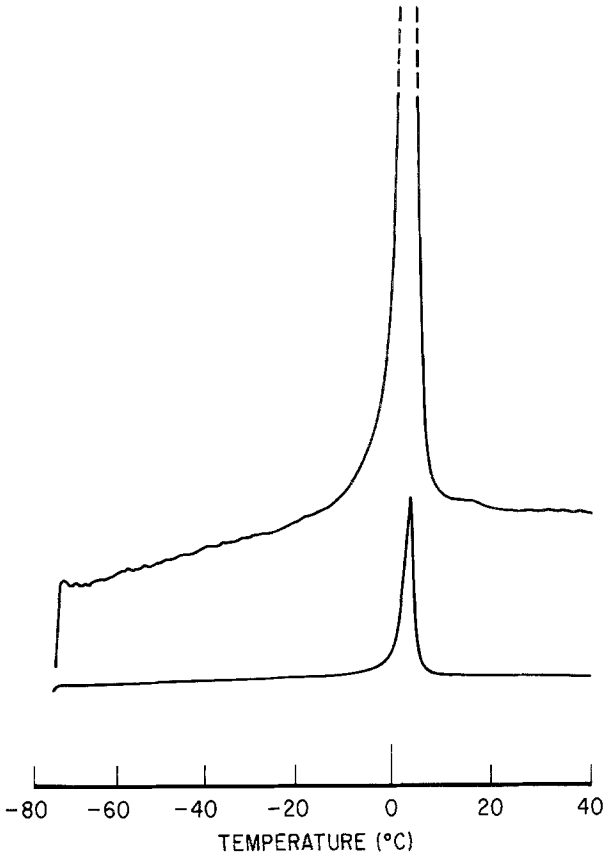

Fig. 7. Differential scanning calorimetry heating curve for isolated, freeze dried plasma membrane of *Neurospora slime* in excess water. Weight of dry plasma membrane was 5.64 mg. *Upper trace:* calorimeter sensitivity, 0.5 mcal/sec; *lower trace:* 5 mcal/sec. Scan rate, 5 °C/min

rol (the major sterol present in this fraction) is responsible for the observed endotherms. Calorimetric scans of ergosterol show no endothermic events in this temperature range (data unpublished).

The observance of endothermic events in both the neutral and phospholipid fractions of the plasma membranes prompted us to scan the total lipid extract (neutral + phospholipid). As shown in Fig. 5, we did not observe any endothermic events in scans of total lipid.

The ability to isolate *Neurospora* plasma membranes and pellet them tightly by centrifugation permitted us to look for bulk phase gel-liquid crystal transitions in these membranes with scanning calorimetry. We performed that experiment in the appropriate buffered saline between the temperatures of −75 to +40 °C. A representative scan of such an experiment is shown in Fig. 6*a*. The complex endo/exothermic events observed are not attributable to the membrane lipid, but rather to Tris and mannitol present in the buffered solution (Fig. 6*c* and *d*). To exclude the thermal events of the buffer solution from our scans of plasma membranes, the membrane pellets were washed free of buffer solution with quartz-distilled water. Washed membranes were freeze dried, then scanned in excess water. Figure 7 is a scan of 5.64 mg of plasma membranes, the phospholipid content of which should yield a calorimetrically detectable heat of transition of ~1 mcal. No such heat of transition was seen. We interpret this finding as indicating an interaction between the neutral and phospholipid components of the cell membrane which prevents an observable heat of transition.

Discussion

Our recent studies (Friedman, 1977*a*, *b*) investigating the relationship between membrane physiology and membrane lipid composition have assumed that: (i) the fatty acid profiles obtained by extracting phospholipids from whole cells is reflective of the fatty acid profile of the plasma membrane, (ii) the phospholipid profiles obtained by extracting phospholipids from whole cells is reflective of the phospholipid profile of the plasma membrane, and (iii) phospholipids extracted from *Neurospora* can serve as models for the calorimetric behavior of *in situ* phospholipids in the plasma membrane. The present work addresses these assumptions by using *slime*, a cell wall mutant which permits the isolation of the plasma membrane.

The fatty acid profiles of plasma membrane and total cell phospholipid extracts are quite similar. This is evidenced by our data (Fig. 1), our statistical analyses, and the fatty acid profiles we and others have previously published for *Neurospora*, wild-type (*see* Table 1). The data argue for the similarity of fatty acid composition between the two strains.

Phospholipid base analyses of *Neurospora* and other fungi have been reported by other investigators. According to Weete (1974), the general pattern found in fungi is: phosphatidylcholine, 35 to 50%; phosphatidylethanolamine, 14 to 35%; phosphatidylserine and phosphatidylinositol <25%. Our data for total cell extracts fall within these limits. Kushwaha and Kates (1976), and Hubbard and Brody (1975) have reported the phospholipid base composition of wild-type *Neurospora*. We have recalculated the data of Kushwaha and Kates (1976) in terms of mole percent, and present them along with the data of Hubbard and Brody (1975) in Table 2. Although our data is in good agreement with these other published values, the phospholipid base profile of plasma membranes appears to differ from that of the total cell extract. This is evidenced by Fig. 2 and our statistical analyses. The major differences appear to be: (i) increased

Table 1. Comparison of fatty acid profiles of phospholipids extracted from *Neurospora crassa*[a]

	w/t 22 °C[b]	*sl* 31 °C	*w/t* 34 °C[c]
14:0	–	0.85	–
15:0	–	0.6	–
16:0	14.0	23.3	24.4
16:1	2.6	3.1	–
16:2	1.6	–	–
18:0	1.5	1.6	4.5
18:1	6.1	3.8	11.9
18:2	58.7	53.7	45.4
18:3	15.5	13.1	13.5
% Saturated	15.5	26.35	28.9
% Unsaturated	84.5	73.7	70.8
% 16 C	18.2	26.4	24.4
% 18 C	81.8	72.2	75.3

[a] Values given are per 100 mole.
[b] Friedman, 1977*a*
[c] Brody & Nyc, 1970

heterogeneity of bases present in plasma membranes, and (ii) a fourfold increase in the phosphatidic acid concentration present in plasma membranes.

The finding of 20 moles/100 moles phosphatidic acid in the plasma membrane seems unusually high. The data do not appear to be spurious, however, since our values for total cell extract (4.2 ± 1.8 moles per 100 moles) are in reasonably good agreement with other published values for other fungi. Jakovcic et al. (1971) report values as high a 2.2 moles per 100 moles for "Petite" yeast phosphatidic acid content, and Hubbard and Brody (1975) report approximately 3 moles per 100 moles for *Neurospora*.

In addition, we ruled out the possibility of the high phosphatidic acid content being caused by phospholipase D activity with the following experiments: (i) We increased the incubation time of our lysate to two hours at the temperature of homogenization (~ 5 °C) without any noticeable increase in plasma membrane phosphatidic acid content, and (ii) we raised the pH of our homogenization buffer from pH 7.5 to 8.5 (which should inactivate phospholipase D) without any noticeable decrease in plasma membrane phosphatidic acid content.

Despite some difference in phospholipid base composition, our calorimeter scans of total cell and plasma membrane phospholipid extracts indicate that the two extracts have similar calorimetric behavior. To confirm that the observed differences in phospholipid base composition do not significantly alter the bulk phase transition properties of these heterogeneous phospholipid systems, we "modeled" both total cell and plasma membrane phospholipid extracts with commercially available phospholipids[2]. The commercially prepared phospholipids were mixed in chloroform in proportion to their mole percentages in total cell and plasma membrane extracts. These "model" systems were then processed in a manner identical to that used for our *Neurospora* phospholipid extracts. Calorimetric scans of the "model" systems extracts are shown in Fig. 8. The model system for the total cell extract when scanned from -75 to $+40$ °C has its major endothermic event at -22 °C and a heat content (ΔH) of 1.1 cal/g. The model system for the plasma membrane extract, when similarly scanned, has its major endothermic event at -21 °C and a heat content (ΔH) of 1.24 cal/g. On this basis we conclude that total cell and plasma membrane phospholipid extracts exhibit sufficiently similar calorimetric behavior to permit us to use in future total cell phospholipid extracts as a method of screening the gel-liquid crystal phase transition temperatures of *Neurospora* mutants with altered membrane phospholipid base compositions. The data further suggest the importance of polar head groups, rather than the fatty acid composition, in determining the bulk phase transition properties of the phospholipids found in biological membranes.

The calorimetry data reported here indicate that plasma membrane and total cell phospholipid extracts of *Neurospora slime* undergo their gel-liquid crystal phase transitions at approximately -22 °C. This agrees with our previously reported data from phos-

[2] Cardiolipin, phosphatidylethanolamine and phosphatidic acid were obtained from Supelco, phosphatidylcholine and phosphatidylinositol were supplied by Sigma. Phosphatidylserine was obtained from Applied Science. The fatty acid composition of these phospholipids was not determined.

Table 2. Comparison of phospholipid base composition (per 100 mole)

	Cardiolipin	Phosphatidyl ethanolamine	Phosphatidyl choline	P-inositol/ P-serine	Phosphatidic acid
Plasma membrane	4.03 ± 0.6	28.08 ± 2.25	28.98 ± 3.3	18.88 ± 3.6	20.01 ± 2.39
Total cell	4.48 ± 0.67	35.59 ± 1.77	44.97 ± 4.5	11.08 ± 3.6	4.15 ± 1.78
Kushwaha and Kates	2.75	25.04	46.58	4.7[a]	20.87
Hubbard and Brody	5–8	20–25	40–45	11–16	3

[a] Serine only, no value for inositol given.

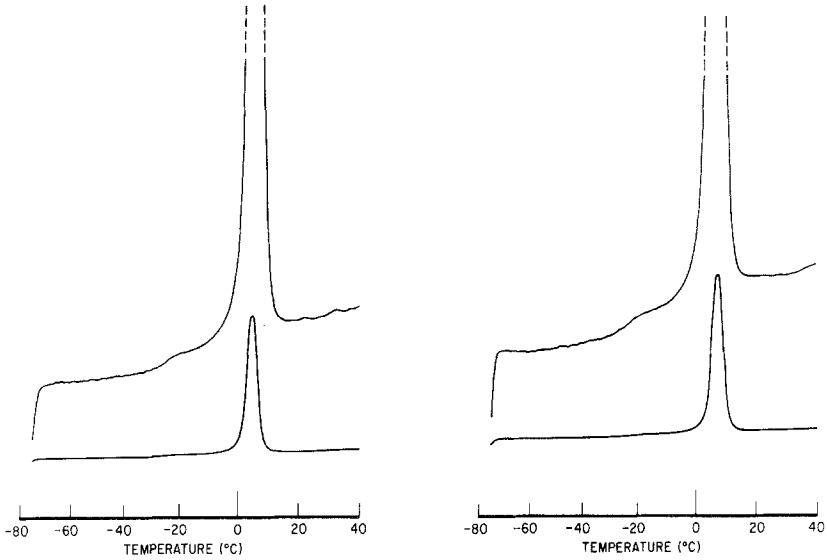

Fig. 8. Differential scanning calorimetry heating curves of *Neurospora* phospholipid "models". *Left panel:* "Model" of *Neurospora slime* whole cell extract. 3.36 mg phospholipid in excess water scanned at 0.5 mcal/sec sensitivity (upper trace); 5 mcal/sec sensitivity (lower trace). *Right panel:* "Model" of *Neurospora slime* plasma membrane. 3.89 mg phospholipid in excess water scanned at 0.5 mcal/sec (upper trace); 5 mcal/sec (lower trace). All traces scanned at a rate of 5 °C/min

pholipid extracts of other strains of *Neurospora*. Mendelsohn and Van Holten (1979) have recently used Raman Spectroscopy to observe an onset temperature of −19 °C and a completion temperature of −6 °C for *Neurospora* phospholipid phase transitions. This compares favorably with our calorimetrically detected phase transition at approximately −20 °C for the same phospholipids (data unpublished).

Our data indicate that calorimetry is useful in monitoring interactions between membrane neutral and phospholipids. We have monitored an interaction between plasma membrane neutral lipids and phospholipids, the effect of which is to maintain the physical state of the membrane lipids constant over a wide temperature range. A similar finding of neutral lipids influencing phospholipid phase transitions has been recently reported by McKersie and Thompson (1979). They used wide angle X-ray diffraction to demonstrate that the neutral lipids of bean cotyledon microsomes influence the phase transition temperature of phospholipids.

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