The lipid composition and membrane fluidity of *Dictyostelium discoideum* plasma membranes at various stages during differentiation

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Abstract There are only minor changes in the amounts of the major lipid constituents of *Dictyostelium discoideum* plasma membranes during the early stages in the differentiation of this organism. By the time cells reach the pseudoplasmodial stage of development there are small increases in the amounts of phosphatidylinositol, phosphatidylglycerol, and lysophosphatidylethanolamine, and small decreases in the amounts of phosphatidylethanolamine and its plasmalogen form. There is also a slight decrease in the total amount of sterol in the plasma membrane during the transition from aggregation to pseudoplasmodium formation. However, no significant change in membrane fluidity as determined by electron paramagnetic resonance (EPR) accompanies these minor changes in lipid composition. It can be concluded that the establishment of cell–cell interaction in *D. discoideum* does not involve gross changes in plasma membrane fluidity or lipid composition. It was found that the plasmalogen form of phosphatidylethanolamine is a major phospholipid constituent in *D. discoideum*, and that this species is somewhat enriched in the plasma membrane.—**Weeks, G., and F. G. Herring.** The lipid composition and membrane fluidity of *Dictyostelium discoideum* plasma membranes at various stages during differentiation. *J. Lipid Res.* 1980. **21:** 681–686.

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Two types of circumstantial evidence suggest that plasma membrane lipid may be intimately involved in the cell–cell interactions that accompany cellular differentiation. First, a number of reports indicate that manipulation of membrane lipid composition can profoundly influence cell–cell interaction (1–6). Second, differentiation of some cell types is accompanied by alterations in membrane fluidity (7–10). In addition, differences in membrane fluidity between normal and malignant cells have also been detected (11–14).

Since cell–cell contacts are essential for the differentiation of *Dictyostelium discoideum* but are not required for growth, this organism is a convenient one for studies on the molecular basis of cell–cell interaction. Although total cellular lipid composition changes during differentiation (15, 16), electron paramagnetic resonance (EPR) studies have indicated no concomitant changes in membrane fluidity (17, 18). However, since these latter studies were on intact cells, changes in plasma membrane fluidity may have been masked by the incorporation of the fatty acid spin probe into internal membrane structures (19).

In order to determine conclusively if plasma membrane fluidity remains constant during differentiation, we have determined the fluidity of purified plasma membrane preparations by EPR, and we have analyzed the plasma membrane lipid composition at various stages of differentiation.

MATERIALS AND METHODS

Materials

Bacteriological peptone and yeast extract were obtained from Oxoid Ltd. Lipid samples were obtained from Serdary and Supelco. Other reagents were the best available grade from Fisher Scientific and Sigma Chemical Co. The spin probe, 5-doxyl stearic acid, was obtained from Syva Associates, Palo Alto, Calif.

Organism and growth conditions

The axenic mutant, *D. discoideum*, Ax-2 was used throughout these studies. The strain was grown on HL5 medium as previously described (20) to a density of $5–7 \times 10^6$ cells/ml. At this stage amoebae were harvested as vegetative cells (0 hr) by centrifugation at 700 *g* for 3 min.

To obtain aggregation component (8 hr) or pseudoplasmodial (16 hr) cells, vegetative cells were washed

Abbreviation: EPR, electron paramagnetic resonance.

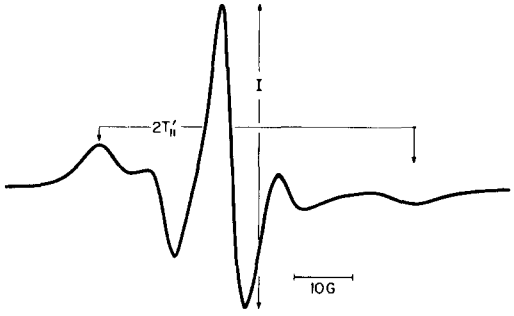

Fig. 1. EPR spectrum of 5-doxyl stearic acid in plasma membranes of vegetative cells of *D. discoideum*. The two measurements made ($2T'_{11}$ and I), are indicated.

with distilled water and spread on a 2% agar plate containing 10^{-3} M NaCl, 10^{-3} M KCl, and 10^{-4} M $CaCl_2$ at a density of 2×10^6 cells/sq cm. The plates were incubated at 22°C for the appropriate times. The aggregates or pseudoplasmodia were harvested from the plates and either analyzed directly for phospholipid composition or used for membrane preparation.

Preparation of purified plasma membranes

Washed cell suspensions were broken by agitation with glass beads as described previously (21) and the broken cell suspension was centrifuged at 700 *g* for 2 min to remove unbroken cells. The cell-free extract was centrifuged at 30,000 rpm in a Spinco Type 30 rotor and the pelleted material was used as the crude membrane preparation. Pure plasma membranes and a crude internal membrane fraction were separated by isopycnic sucrose gradients as described previously (21, 22). The plasma membrane preparations are markedly enriched in the plasma membrane marker enzymes, alkaline phosphate and 5′-nucleotidase, and contain relatively little internal membrane marker enzymes (21, 22). The protein content of the membrane was determined by the method described by Lowry et al. (23).

Lipid extraction and phospholipid analysis

Lipid was routinely extracted from intact cells and purified plasma membranes by the method of Bligh and Dyer (24). In some experiments lipid was extracted by a procedure described by Ellingson (15), and by the acid-butanol procedure described by Bjerve, Daae, and Bremer (25).

Total phospholipid was determined by the method of Bartlett (26). The amounts of the individual phospholipid species were determined following fractionation of the total lipid extract by two-dimensional thin-layer chromatography on small Silica gel G plates as described by Yavin and Zutra (27), a method that conveniently separated phosphatidylethanolamine from its plasmalogen form. The positions of the separated phospholipids were determined by iodine vapor treatment, and the areas containing lipid were scraped off and assayed for phospholipid content by the method of Bartlett (17), except that samples were digested in 70% perchloric acid for 45 min at 180°C.

The fatty acid and sterol composition was determined, after saponification, by previously published procedures (21).

Electron spin resonance procedures

The incorporation of 5-doxyl stearic acid into the plasma membranes and the determination of the spin resonance spectrum have been described previously (19). Membrane fluidity was monitored by determining $2T'_{11}$, the separation between the outer extrema, (**Fig. 1**), as discussed previously. The rate of destruction of the spin probe was determined by measuring the height of the central line, I (Fig. 1) at various time intervals after the introduction of the probe.

RESULTS AND DISCUSSION

Phospholipid composition of vegetative intact cells

In previous studies on *D. discoideum* phospholipids, phosphatidylethanolamine and its plasmalogen form were not quantitatively separated (15, 28). We have separated the two species and have found that the plasmalogen form of phosphatidylethanolamine is a major phospholipid species in *D. discoideum* (**Table 1**). In addition, the phospholipid composition is somewhat different from that published by Ellingson (15) for the same strain. In particular we detected considerably more phosphatidylcholine and considerably less lysophosphatidylethanolamine. The reason for this discrepancy is not clear and cannot be explained by differences in the experimental procedures used. Repeating the extraction procedure used by Ellingson (15), we obtained results identical to those in Table 1, (data not shown). In addition, use of Ellingson's thin-layer chromatographic procedure (15) gave results similar to those in Table 1, with the exception that phosphatidylethanolamine and its plasmalogen form were not separated (data not shown).

We also tried an acid-butanol extraction procedure that has been shown in previous studies to be more efficient for the extraction of lysophospholipids (25). This procedure did extract slightly more phospholipid, but resulted in an almost total degradation of

TABLE 1. Phospholipid composition of plasma membranes and intact cells at different stages of development

Phospholipid	Intact Vegetative Cells	Plasma Membrane Preparations		
		Vegetative	Aggregation Competent	Pseudo-plasmodial
		percent total phospholipid[a]		
Lysophosphatidylcholine	2.3 ± 1.0	1.5 ± 0.3	0.7 ± 0.5	2.2 ± 0.4
Lysophosphatidylethanolamine	2.0 ± 0.9	1.4 ± 0.6	2.5 ± 0.3	2.4 ± 0.3
Phosphatidylinositol	6.3 ± 2.1	7.8 ± 2.0	10.6 ± 3.2	10.9 ± 1.2
Phosphatidylserine	2.0 ± 0.9	1.5 ± 0.8	0.6 ± 0.4	1.8 ± 0.2
Phosphatidylglycerol	1.3 ± 0.8	1.2 ± 0.1	2.0 ± 0.7	2.8 ± 0.4
Phosphatidic acid	0.9 ± 0.2	0.3 ± 0.3	0.8 ± 0.1	1.1 ± 0.2
Cardiolipin	0.4 ± 0.3	2.0 ± 0.8	1.1 ± 0.2	1.8 ± 0.5
Phosphatidylcholine	32.9 ± 3.1	28.8 ± 2.2	28.3 ± 4.2	28.5 ± 2.8
Phosphatidylethanolamine	31.8 ± 1.3	27.8 ± 4.2	24.0 ± 0.7	23.7 ± 1.2
Phosphatidylethanolamine (plasmalogen form)	14.3 ± 4.4	27.3 ± 3.8	26.1 ± 4.1	21.2 ± 3.2
Others[b]	4.0 ± 0.9	1.2 ± 0.8	3.1 ± 2.1	4.3 ± 2.1

[a] Each value is the mean ± standard deviation of three determinations.
[b] Several minor unidentified constituents.

the plasmalogen form of phosphatidylethanolamine to lysophosphatidylethanolamine (data not shown), and its possible applicability was not investigated further. In all subsequent experiments, lipid was extracted by the procedure of Bligh and Dyer (24).

Phospholipid composition of purified plasma membranes

The phospholipid composition of the *D. discoideum* plasma membrane is substantially different from that of the intact cell (Table 1). The plasma membrane preparation is enriched considerably in the plasmalogen form of phosphatidylethanolamine and this molecular species is in fact one of its major phospholipid constituents. This enrichment in plasmalogen phosphatidylethanolamine is offset by slight decreases in the amount of phosphatidylcholine, lysphosphatidylcholine, phosphatidylethanolamine, and lysophosphatidylethanolamine. The low levels of lysophospholipid and phosphatidic acid in the plasma membrane suggest that appreciable phospholipid degradation does not occur during the lengthy (≈20 hr) preparative procedure, despite the presence in *D. discoideum* cell-free extracts of high levels of phospholipase A and lysophospholipase (29). In addition, prolonged incubation of plasma membranes at 4°C does not alter either the total phospholipid content or the species composition (data not shown), further confirming the stability of the plasma membrane phospholipid. In contrast, an identical treatment of a crude membrane preparation resulted in a considerable increase in lysophospholipid and phosphatidic acid levels (data not shown) suggesting that the degradative enzymes are membrane-bound, but not associated with the plasma membrane.

Lipid composition of the plasma membrane during differentiation

The data in Table 1 and **Table 2** reveal that neither the total phospholipid content of the plasma membrane nor the proportions of the individual phospholipid species change appreciably during aggregation (8 hr development) and pseudoplasmodium formation (16 hr development), although there are slight increases in the amounts of lysophosphatidylethanolamine, phosphatidylinositol and phosphatidylglycerol, and slight decreases in the amounts of phosphatidylethanolamine and its plasmalogen form. There are also small increases in the amounts of the minor unidentified constituents. Since none of these changes is as pronounced as the developmentally regulated modification of the total cellular phospholipid composition that was previously reported (15), we also analyzed the intact cell phospholipid composition at various developmental stages. No major alterations in phospholipid composition were detected (data not shown).

TABLE 2. Total phospholipid and sterol content of purified plasma membranes from cells at various stages of development

Component	Plasma membranes		
	Vegetative	Aggregation Competent	Pseudo-plasmodial
	μg/mg membrane protein[a]		
Phospholipid	434.8 ± 46.4 (5)	395.0 ± 45.0 (2)	427.5 ± 40.0 (2)
Sterol	99.4 ± 11.4 (5)	101.0 ± 11.0 (2)	81.5 ± 21.2 (3)

[a] Each value is the mean of the number of determinations shown in parentheses ± the standard deviation.

TABLE 3. The fatty acid composition of the plasma membranes from various stages of differentiation

Fatty Acid	Vegetative	Aggregation Component	Pseudo-plasmodial
	percent composition		
	(5)[a]	(2)	(3)
14.0[b]	1.2 ± 0.2[a]	2.2 ± 0.1	1.9 ± 0.4
Palmitaldehyde	3.5 ± 0.9	4.6 ± 0.0	4.4 ± 1.1
16:0	7.5 ± 0.5	8.3 ± 1.9	7.4 ± 1.0
16:1 (Δ^9)	2.4 ± 0.2	1.7 ± 0.0	2.2 ± 0.5
16:2 ($\Delta^{5,9}$ and 17:0)[c]	1.3 ± 0.4	2.5 ± 0.1	2.5 ± 0.2
18:0	2.7 ± 1.0	5.0 ± 0.6	4.0 ± 1.5
18:1 (Δ^9 and Δ^{11})[c]	34.6 ± 1.7	28.5 ± 1.2	29.6 ± 3.0
18:2 ($\Delta^{5,9}$ and $\Delta^{5,11}$)[c]	41.4 ± 0.3	45.2 ± 2.0	45.3 ± 4.1
Others[d]	4.7 ± 1.8	1.0 ± 0.6	1.1 ± 0.4

[a] The standard errors are from the number of determinations indicated in parentheses at the head of each column.

[b] In all fatty acid abbreviations, the number preceding the colon is the chain length, the number following the colon is the number of double bonds, and the numbers following Δ denote the positions of the double bonds. Thus, 5,9-octadecadienoic acid is abbreviated to 18:2 $\Delta^{5,9}$ etc.

[c] The methyl esters of these two fatty acids are not resolved by the chromatographic conditions used in the present study.

[d] Several unidentified minor components.

The fatty acid composition of the plasma membranes does not change appreciably during the early stages of differentiation (**Table 3**), although there is a slight increase in the amounts of the diunsaturated fatty acids and a corresponding decrease in the amount of the monounsaturated fatty acids. There was an approximately 20% decline in the total sterol content during the transition from aggregation to pseudoplasmodium formation (Table 2).

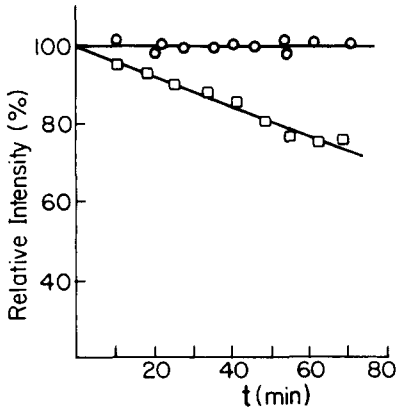

Fig. 2. The degradation of 5-doxyl stearic acid by a crude internal membrane fraction (□) and by plasma membranes (○). The height of the central line, I (Fig. 1) was measured at the indicated times after the introduction of the probe.

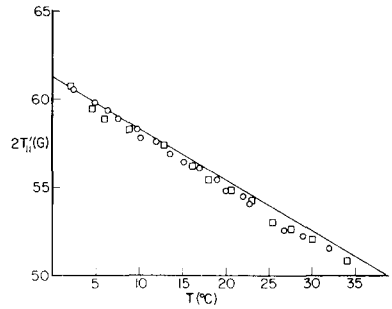

Fig. 3. The temperature profiles of $2T'_{II}$ for plasma membranes from vegetative cells (——), aggregation phase cells (○), and pseudoplasmodial phase cells (□). The values plotted are the means of 3 determinations for the aggregation phase membranes and 2 determinations for the pseudoplasmodial phase membranes. Error bars have been eliminated for clarity, but in no case were errors greater than 0.3 G.

EPR studies using 5-doxyl stearic acid as spin probe

In order to determine whether the small developmental changes in lipid composition reported above were sufficient to modify plasma membrane fluidity, we undertook a detailed EPR study of the purified membrane preparations. EPR of a fatty acid spin probe has been used previously to monitor developmental changes in plasma membrane fluidity in *D. discoideum* (17, 18) but, because the measurements were on intact cells, their significance is questionable. Our previous studies (19) showed that intact cells of *D. discoideum* internalize the fatty acid spin probe. The resonance signal associated with the intact cells rapidly decayed (19) and the addition of $K_3Fe(CN)_6$ (30) failed to revive the EPR signal. In addition, the 5-doxyl-stearic acid spin probe decayed when introduced into a crude membrane fraction, but remained stable in a purified plasma membrane preparation (**Fig. 2**). These results suggest that intact cells of *D. discoideum* rapidly incorporate the spin probe into internal membranes where the probe is degraded. In all subsequent experiments the motion of the 5-doxyl stearic acid was therefore determined in purified plasma membrane fractions. The temperature profiles of $2T'_{II}$ for plasma membranes of vegetative, aggregation phase, and pseudoplasmodial phase cells are shown in **Fig. 3.** The data indicate that there is possibly a slight increase in the fluidity of the plasma membrane during differentiation when measured at or above physiological temperatures (≃22°C). However, since the error on each determination is ±0.3 G, we do not feel that it is a significant change. Thus the absence of major changes in the phospholipid membrane composition agrees with the unaltered

membrane fluidity during development. We have recently shown (31) that $2T'_{11}$ determinations for 5-doxyl stearate and fluorescence depolarization measurements of diphenyl hexatriene yield qualitatively similar assessments of plasma membrane fluidity in *D. discoideum,* and we are confident that the present measurements of $2T'_{11}$ are valid indicators of membrane fluidity and demonstrate that there is no major change in the fluidity of *D. discoideum* plasma membranes during differentiation.

There were no discontinuities in the plots of $2T'_{11}$ against temperature (Fig. 3) indicating the absence of detectable phase transitions in *D. discoideum* plasma membrane from cells of any of the developmental stages studied, results in contrast to those of Kawai and Tanaka (18), but in agreement with those of Von Dreele and Williams (17). This absence of a phase transition in the range of 0°C to 35°C is probably a reflection of the highly unsaturated nature of the majority of the acyl chains (Table 3) and the high sterol content (Table 2) (see also ref. 19).

In summary, although the possibility remains that as yet undetected changes in minor lipid components are important for the differentiation of *D. discoideum,* there are no gross changes in either plasma membrane fluidity or lipid composition during the establishment of cell–cell contacts. It is possible that the small changes in lipid composition might conceivably be of physiological significance for the establishment of cell–cell contacts. Furthermore, there might be important localized changes in membrane fluidity during development that are not detected by the spin label approach used in this study. Considerably more work will be needed however to substantiate or eliminate these possibilities.

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