Temperature-induced membrane-lipid adaptation in *Acanthamoeba castellanii*

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A method has been developed for the separation of the major membrane fractions of *Acanthamoeba castellanii* after growth at different temperatures. The acyl-lipid compositions of individual membrane fractions, microsomal membranes, plasma membrane and mitochondria were analysed after a shift in culture temperature from 30 °C to 15 °C. The major change in lipid composition observed was an alteration in the relative proportions of oleate and linoleate. This reciprocal change was seen in all the membrane fractions, but occurred most rapidly in the phosphatidylcholine of the microsomal fraction. Thus, there appears to be a rapid induction of Δ12-desaturase activity in *A. castellanii* after a downward shift in growth temperature. Changes were also seen in the proportions of the $n-6$ C_{20} fatty acids, with a decrease in the proportions of icosadienoate and increases of icosatrienoate and arachidonate. However, unlike the alteration in oleate/linoleate ratios, this change was not seen in all the individual lipids of each membrane fraction.

INTRODUCTION

Poikilotherms, unlike birds and mammals, have no mechanisms for temperature regulation, and thus their membrane systems have to perform over the range of temperatures determined by ambient environmental conditions (Cossins and Raynard, 1987). In order to maintain the functional integrity of cell membranes and organelles they have developed a variety of adaptational responses (Kates and Manson, 1984; Harwood, 1991). Lower eukaryotes have served as excellent laboratory models in which to study the processes involved in temperature adaptation (Thompson and Nozawa, 1984; Lynch and Thompson, 1984a,b). One important aspect is the control of membrane fluidity because the lateral mobility of proteins and the diffusibility of hydrophobic components are essential for continued membrane function during temperature fluctuations.

Investigations of acclimatization to lowered temperatures (from 39 °C to 15 °C) in *Tetrahymena pyriformis* have indicated that within 1 h phospholipids of the microsomal fraction showed altered fluidity; in this case acyl-chain rearrangements were more evident than desaturation processes (Thompson & Nozawa, 1984). In *Dunaliella salina* low-temperature adaptation involved changes in the concentrations of molecular species of phospholipids (Lynch and Thompson, 1984a,b). These short-term modifications can occur rapidly in response to altered ambient conditions and, in these organisms, precede changes in unsaturation or alteration of overall acyl-chain-length composition which necessitates extensive *de novo* fatty acid synthesis.

Acanthamoeba castellanii is a small amoeba, ubiquitous in soils, where it is able to adapt to temperature fluctuations and is thus capable of vegetative growth over a wide range of temperatures (10 °C–32 °C) (Chagla and Griffiths, 1974). Encystation does not occur in rapidly growing axenic cultures, and so it is possible to characterize the biochemistry of temperature adaptation separately from processes of the life cycle. Furthermore, studies on membranes are facilitated by the ease with which subcellular fractionation of this organism can be achieved after gentle disruption (Morgan et al., 1973; Korn et al., 1974; Clarke et al., 1988). Earlier studies have established that *A. castellanii* contains high levels of $n-6$-unsaturated fatty acids (Korn, 1963a,b; Costas and Griffiths, 1984).

Like *T. pyriformis*, *A. castellanii* can synthesize linoleic acid *de novo*; this can then act as a precursor for other (mainly C_{20}) $n-6$-unsaturated fatty acids (Jones et al., 1991a). Increased unsaturation of phospholipids during growth at low temperatures has also been described for *A. castellanii*. Thus, by comparison with organisms grown at 30 °C, those proliferating at 15 °C showed decreased levels of oleate and palmitate, but increased levels of linoleate, icosatrienoate and arachidonate. In contrast, an upward temperature shift (from 15 °C to 30 °C) gave negligible changes in fatty acid composition over a 24 h period. Isotopic labelling studies directly showed that, at 15 °C, the organism converts stearate via oleate into further desaturation and chain-elongation products, and also that $n-6$ desaturation to produce linoleate is of key importance in the low-temperature adaptation of this soil amoeba (Jones et al., 1991a).

In this report we describe a procedure for the rapid separation of subcellular fractions which are highly enriched in plasma membrane, mitochondria and endoplasmic reticulum membranes after coating organisms with silica microbeads. Characterization of the phospholipids of each of the fractions, and the alterations consequent upon decreased growth temperature, emphasize the speed and extent of processes involved in this initial response. A preliminary account of this work has already appeared (Jones et al., 1991b).

MATERIALS AND METHODS

Growth and harvesting of organisms

A. castellanii were grown at 30 °C in medium containing 0.75% (w/v) proteose peptone, 1.5% (w/v) glucose, and 0.75% (w/v) yeast extract. Cultures (200 ml in 500 ml Erhlenmeyer flasks) were shaken at 200 rev·min^{-1}. Organisms were counted using a Fuchs–Rosenthal haemocytometer and were harvested in mid-

Abbreviations used: PtdCho, phosphatidylcholine; PtdEth, phosphatidylethanolamine.

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to late-logarithmic phase of growth at about 5×10^6–1×10^7 organisms/ml; they had a generation time at 30 °C of 8 h. Harvesting was by centrifugation at 250 *g* for 5 min at 4 °C in the 6 × 250 ml rotor of a Sorvall RC5B centrifuge.

Subcellular fractionation

All procedures were carried out at 4 °C. After washing the organisms obtained from a 200 ml culture twice in buffer A (20 mM Mes/150 mM NaCl, pH 6.5) and resedimenting, they were suspended in an equal volume of buffer B (20 mM Mes/140 mM sorbitol, pH 6.5). Silica beads (cationic colloidal silica, a gift from Dr. B. Jacobsen, University of Massachusetts, Amherst, MA), prepared at 6% (w/v) in buffer B, were added dropwise to an equal volume of the cell suspension with gentle vortexing. After dilution to 45 ml with buffer A organisms were centrifuged for 2 min at 650 *g*, and resuspended to 5 ml in buffer A. Then an equal volume of 2 mg/ml polyacrylic acid (90000-M_r) in buffer A (pH corrected to 6.5 with NaOH) was added. After dilution to 45 ml with buffer A, organisms were resedimented (2 min at 650 *g*), washed three times in buffer C (20 mM Tes, pH 6.9), resuspended in 1 vol. of buffer C and 1 vol. of glass beads (150–212 μm diam., acid-washed), and disrupted in a Braun shaker (Bosch, Germany) at 4 kHz for 10 s. After settling of the beads the homogenate was decanted, and buffer B containing 2 M sucrose was added to give a final concentration of 0.3 M sucrose. Low-speed centrifugation of the homogenate was performed in an SS34 (8 × 50 ml) rotor ($r_{av.}$ = 6.98 cm) on a Sorvall RC5B centrifuge; centrifuging at 100 *g* for 1 min removed large aggregates of silica beads. The supernatant was then centrifuged at 250 *g* for 10 min. The twice-washed sediment gave a fraction enriched in plasma membranes. After centrifugation of the supernatant at 1800 *g* for 20 min, and then a second centrifugation of that supernatant at 9000 *g* for 20 min, the resulting pellet provided the mitochondrial fraction, and this was washed three times in buffer C containing 0.30 M sucrose before assay. The 'post-mitochondrial' supernatant was centrifuged at 18000 *g* for 20 min before sedimentation of the endoplasmic reticulum-enriched fraction (Beckman L8-M centrifuge, Ti55.2 rotor) at 105000 *g* for 60 min ($r_{av.}$ = 7.35 cm).

Lipid analyses

Lipids were extracted using the method of Bligh and Dyer (1959) as modified by Griffiths and Harwood (1991). For fatty acid analysis, methyl esters were generated by acid-catalysed methanolysis [2.5% (v/v) H_2SO_4/methanol (v/v) at 70 °C for 2 h]. Phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEth) classes were separated by t.l.c. on silica-gel G plates (E. Merck, Darmstadt, Germany) using chloroform/methanol/water (65:25:8, by vol.) (Slomiany and Horowitz, 1970). After esterification (above) the methyl esters were extracted into light petroleum (60°–80 °C b.p. fraction), and aliquots analysed by radio-g.l.c. Heptadecanoate was used as an internal standard. Separations were routinely achieved with 15% (w/v) ethyleneglycol succinate silicone-X (EGSS-X) on Supelcoport WAW (Supelco, Elysian, MN, U.S.A.) packed into a glass column (2.1 m × 4 mm internal diameter). Fatty acids were usually identified by comparison with authentic standards, but were also analysed by m.s.–g.c. and by capillary g.c. to confirm their identities. Quantification of fatty acids by comparison with the internal standard was carried out by the method of Carroll (1961), or by using integrating software (LabLogic, Sheffield, U.K.).

Enzyme assays and electron microscopy

Enzyme assay methods were as follows: succinate dehydrogenase, catalase and acid phosphatase as described previously by Morgan et al. (1973), choline phosphotransferase (Lord et al., 1973), thiamine pyrophosphatase (Novikoff et al., 1962), lactate dehydrogenase (Pesce et al., 1964) and 5′-nucleotidase (Avruch and Wallach, 1971). Electron microscopy was performed on ultrathin sections after glutaraldehyde–osmium tetroxide fixation using a Phillips 1200 electron microscope.

RESULTS

For the quantification of membrane-lipid composition in *A. castellanii* it is important to be able to separate fractions which are enriched in mitochondria, endoplasmic reticulum, and plasma membrane in a single procedure. Table 1 shows the distributions of enzymes characteristic of these organelles and membranes in subcellular fractions obtained by differential centrifugation of homogenates prepared by disruption of silica-bead-coated organisms (Figures 1a and 1b). After centrifugation for 10 min at 250 *g* in the SS34 (8 × 50 ml) rotor of a Sorvall RC5B centrifuge, 25.6% of the 5′-nucleotidase-containing membranes of the whole homogenate was sedimented; this enzyme has been shown to be located in the plasma membrane of *A. castellanii* (Schultz and Thompson, 1969). This fraction (Figure 1) accounted for 15.3% of the homogenate protein but only contained 1.9% of the choline phosphotransferase activity and 5.8% of the succinate dehydrogenase activity [marker enzymes for endoplasmic reticulum (Lord et al., 1973) and mitochondria (Morgan et al., 1973) respectively].

When the supernatant was centrifuged further, first at 1800 *g* for 20 min and then at 9000 *g* for 20 min (in the above-mentioned rotor), the sedimentable fraction was highly enriched in mitochondria (56.1% of the total succinate dehydrogenase activity) with little contamination by plasma membrane or endoplasmic reticulum marker enzymes. This mitochondria-enriched fraction did, however, contain substantial proportions (49.7% and 23.9%) of the total catalase and acid phosphatase activities [markers for peroxisomes (Morgan et al., 1973) and contractile vacuole plus lysosomes (Bowers and Korn, 1973) respectively] (results not shown).

After a further intermediate centrifugation step, a final separation by Beckman L8-M centrifuge in a Ti55.2 rotor at 105000 *g* for 60 min ($r_{av.}$ = 7.35 cm) yielded an endoplasmic reticulum-enriched microsomal fraction. Although this sediment contained only 3.4% of the total protein and 12% of the 5′-nucleotidase total units, it showed 83.1% of the choline phosphotransferase activity and 67% of the thiamine pyrophosphatase activity [a Golgi apparatus marker in *A. castellanii* (Novikoff et al., 1962)] (results not shown). Succinate dehydrogenase activity was not detectable in this fraction. The final supernatant contained 97.6% of the cytosolic enzyme lactate dehydrogenase (Pesce et al., 1964) (results not shown).

In order to evaluate the extent of temperature-induced fatty acid adaptation in the different membranes of *A. castellanii*, subcellular fractionation was carried out using organisms grown at 30 °C and then at intervals after decreasing the temperature of growth to 15 °C. Table 2 shows the changes in the fatty acid composition of membranes in whole homogenate and in plasma membrane-, mitochondria- and endoplasmic reticulum-enriched fractions from organisms grown at 30 °C, and from organisms harvested 1 h, 4 h and 24 h after shift-down to 15 °C. Whereas oleate, the major fatty acid of the homogenate from organisms grown at 30 °C, was still predominant after growth for 1 h at 15 °C, after 4 h linoleate content had increased to equal the

Table 1 Purity of membrane fractions from *A. castellanii* obtained using cationic silica microbeads and differential centrifugation

Results are expressed as means ± S.D., where $n = 3$ (proteins, succinate dehydrogenase and 5′-nucleotidase) or $n = 2$ (choline phosphotransferase). This is a representative result from several experiments, using 30 °C cells. Underlined values indicate fractions with highest activities. Abbreviations: n.d., not determined; tr., trace.

	Marker enzyme							
	Total protein		5′-Nucleotidase		Choline phosphotransferase		Succinate dehydrogenase	
Fraction	Concn. (mg/ml)	Yield (%)	Specific activity (μmol/mg of protein per min)	Yield (%)	Specific activity (pmol/mg of protein per min)	Yield (%)	Specific activity (μmol/mg of protein per min)	Yield (%)
250 *g* pellet	4.5 ± 0.3	15.3	323 ± 53	25.6	1.6 ± 0.2	1.9	0.9 ± 0.2	5.8
1800 *g* pellet	4.6 ± 0.1	15.2	383 ± 49	30.5	1.7 ± 0.4	1.9	2.5 ± 0.4	20.6
9000 *g* pellet	2.2 ± 0.2	2.2	616 ± 136	7.1	0.4 ± 0.1	0.3	6.9 ± 0.5	56.1
18000 *g* pellet	2.8 ± 0.1	4.7	252 ± 93	6.2	1.2 ± 0.6	4.3	n.d.	n.d.
105000 *g* pellet	2.0 ± 0	3.4	685 ± 219	12.2	49.6 ± 3.5	83.1	n.d.	n.d.
Supernatant	1.9 ± 0.2	35.7	16 ± 3	3.0	1.1 ± 0.1	1.3	n.d.	n.d.
Washes (250 *g* × 3, 9000 *g* × 1)	0.3 ± 0.1	19.8	59 ± 4	3.6	tr.	0.6	0.4 ± 0.3	22.4
Total yield		96.3		88.1		93.4		104.9

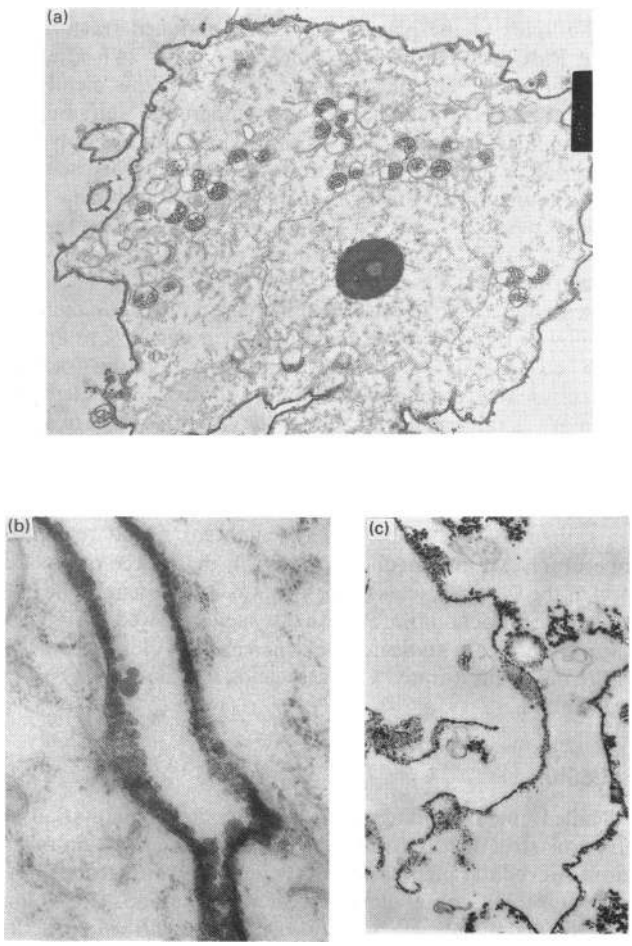

Figure 1 Electron micrographs of *A. castellanii* cells and homogenates

(**a**) Intact *A. castellanii* cell are shown after one coat of cationic silica microbeads. Magnification: × 3600. The mitochondria have shrunk because of the time taken to fix the cells once coated with microbeads. Normally they were sheared and resuspended in 0.30 M sucrose immediately. (**b**) Close-up of part of the plasma membrane of the intact cell with cationic silica microbeads attached. Magnification: × 38000. (**c**) Washed 250 *g* fraction of *A. castellanii* homogenate demonstrating the presence of large sections of plasma membrane with silica microbeads attached. Magnification: × 10000.

Table 2 Changes in fatty acid composition of *A. castellanii* membrane fractions after a shift in growth temperature from 30 °C to 15 °C

Others include $C_{14:0}$, $C_{16:1}$ ($n-7$), $C_{16:2}$, $C_{20:1}$ ($n-9$), $C_{20:4}$ ($n-3$). Results are expressed as means ± S.D. with $n = 2$, and are from a representative experiment. *$P < 0.05$, statistical analysis by Student's *t*-test.

	Time after shift-down			
Fraction	0 h	1 h	4 h	24 h
Homogenate				
$C_{18:1}$ ($n-9$)	36.8 ± 0.9	33.4 ± 0.6	22.5 ± 3.2	13.2 ± 1.4*
$C_{18:2}$ ($n-6$)	8.6 ± 0.1	13.2 ± 1.2	22.3 ± 0.9*	26.5 ± 1.2*
$C_{20:2}$ ($n-6$)	12.6 ± 1.2	12.0 ± 0	8.5 ± 0.2	7.0 ± 0.3
$C_{20:3}$ ($n-6$)	3.7 ± 0.2	4.1 ± 0.2	6.2 ± 0.4	7.7 ± 0.4
$C_{20:4}$ ($n-6$)	13.0 ± 0.1	16.0 ± 1.1	13.1 ± 0.6	18.3 ± 1.3
Others	25.3 ± 0.3	21.3 ± 0.4	27.4 ± 1.6	27.3 ± 0.4
Mitochondria				
$C_{18:1}$ ($n-9$)	36.8 ± 0.9	35.2 ± 2.6	28.7 ± 1.6	22.6 ± 1.9
$C_{18:2}$ ($n-6$)	8.7 ± 0.2	16.0 ± 0.8	23.0 ± 0.4*	28.6 ± 0.6*
$C_{20:2}$ ($n-6$)	10.2 ± 1.2	10.4 ± 0.7	6.1 ± 0.3	6.2 ± 0.1
$C_{20:3}$ ($n-6$)	3.4 ± 0.1	4.3 ± 0.1	5.7 ± 1.6	5.4 ± 0.3
$C_{20:4}$ ($n-6$)	16.1 ± 0.7	21.5 ± 0.9	17.9 ± 0.8	17.7 ± 1.3
Others	24.8 ± 0.6	12.6 ± 0.6	18.6 ± 0.2	19.5 ± 0.3
Endoplasmic reticulum				
$C_{18:1}$ ($n-9$)	48.7 ± 2.6	36.8 ± 1.9	25.5 ± 0.9*	15.0 ± 2.6*
$C_{18:2}$ ($n-6$)	12.3 ± 0.7	22.8 ± 3.6	26.3 ± 2.4*	30.1 ± 3.9*
$C_{20:2}$ ($n-6$)	13.6 ± 0.8	11.5 ± 0.6	8.8 ± 1.6	6.9 ± 0.1*
$C_{20:3}$ ($n-6$)	3.8 ± 0.5	4.8 ± 1.2	5.6 ± 0.9	8.2 ± 0.3
$C_{20:4}$ ($n-6$)	6.6 ± 1.6	8.7 ± 0.9	10.2 ± 1.2	17.6 ± 0.9*
Others	15.0 ± 0.3	15.4 ± 0.4	23.6 ± 0.8	22.2 ± 0.6
Plasma membrane				
$C_{18:1}$ ($n-9$)	34.7 ± 2.9	26.6 ± 3.2	23.7 ± 1.3	15.0 ± 0.9*
$C_{18:2}$ ($n-6$)	8.1 ± 2.1	15.2 ± 1.9	25.2 ± 2.6*	25.4 ± 2.6*
$C_{20:2}$ ($n-6$)	15.6 ± 1.1	11.2 ± 0.8	10.4 ± 0.6	8.8 ± 0.7*
$C_{20:3}$ ($n-6$)	3.4 ± 0.7	5.6 ± 1.1	5.0 ± 0.3	7.5 ± 1.1
$C_{20:4}$ ($n-6$)	10.7 ± 0.4	11.8 ± 0.4	8.0 ± 1.6	14.5 ± 0.9
Others	27.5 ± 2.1	29.6 ± 0.9	27.7 ± 0.5	28.8 ± 0.8

oleate content, and by 24 h the dienoic acid accounted for 26.5 % of the total fatty acids. Icosadienoate concentration decreased over the 24 h period. Concomitant increases in the icosatrienoate, arachidonate and icosapentaenoate levels confirmed the changes

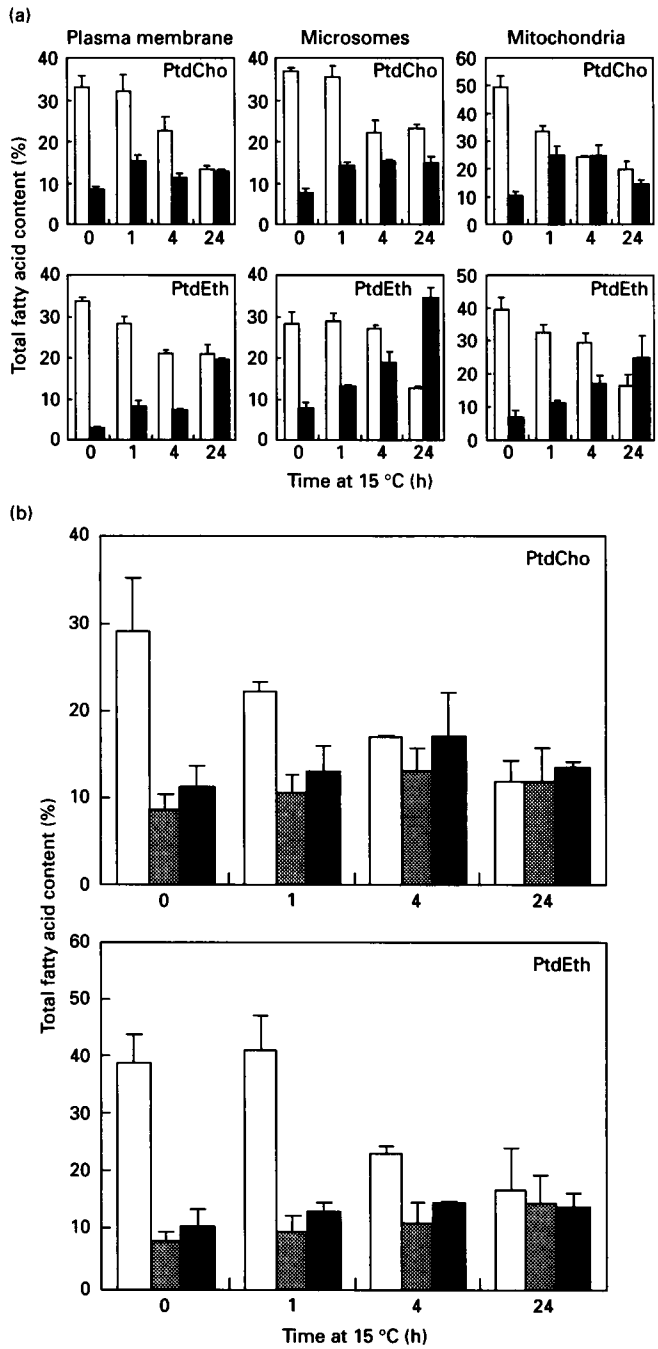

Figure 2 Changes in the relative proportions in PtdCho and PtdEth lipids of oleate and linoleate in different membrane fractions (a), and icosadienoate, icosatrienoate and arachidonate in the microsomal fraction (b) purified from *A. castellanii* after a shift in culture temperature from 30 °C to 15 °C

(**a**) Key to shading: □, oleate; ■, linoleate. (**b**) Key to shading: □, icosadienoate ($C_{20:2}$); ⊡, icosatrienoate ($C_{20:3}$); ■, arachidonate ($C_{20:4}$).

previously reported for whole cells (Jones et al., 1990, 1991a).

In the plasma membrane-enriched fractions similar changes in fatty acids were evident. Again, those organisms grown at 30 °C contained predominantly oleate and during the 24 h of the temperature shift the level of this fatty acid decreased as the amount of linoleate increased. In addition, icosadienoate decreased as the other polyunsaturated C_{20} fatty acids increased. A significant increase in palmitate levels accompanied these changes towards increased unsaturation.

In the mitochondria-enriched fraction the most pronounced trend was again in the replacement of oleate by linoleate. The endoplasmic reticulum-enriched fraction showed this precursor–product relationship most clearly, and also the increasing unsaturation of the C_{20} fatty acids.

In order to characterize these changes during temperature adaptation more fully, analyses of the proportions of unsaturated C_{18} and C_{20} fatty acids in the major membrane lipids, PtdCho and PtdEth, were performed on subcellular fractions. Figure 2 shows that shifting the temperature of growing cultures from 30 °C to 15 °C yields microsomal fractions (mainly endoplasmic reticulum) with a progressively decreasing degree of saturation. Thus, PtdCho fatty acids showed rapid and reciprocal changes in oleate and linoleate (within the first hour). After 4 h, concentrations of linoleate decreased. The C_{20} fatty acids showed a progressive decline in icosadienoate levels between 1 h and 24 h of shift-down. A similar change occurred in the PtdEth fatty acids; in this case the C_{20} fatty acids accounted for a larger proportion of the total, and the trend towards an increased degree of unsaturation (both in C_{18} and C_{20}) continued for the entire 24 h period.

In the lipids of the plasma membrane-enriched fraction the ratio of PtdCho to PtdEth remained at 1:1 for 24 h after the temperature shift. The oleate in the PtdCho of this membrane fraction decreased for 24 h after the temperature shift but the level of linoleate in this lipid class showed an increase only over the first 4 h. A decrease in the proportion of C_{18} fatty acids by 24 h was compensated for by an increase in that of the $n-6$ C_{20} fatty acids, especially by a rise in icosadienoate. The level of linoleate in PtdEth from plasma membrane increased slowly at first, then between 4 h and 24 h of chilling its proportion was increased almost 3-fold. An initial increase in the icosadienoate was followed by a decrease, paralleled by a decrease in arachidonate. Icosatrienoate levels increased over 24 h in this fraction.

The mitochondria-enriched fraction showed a higher proportion of C_{20} fatty acids than either the endoplasmic reticulum- or plasma membrane-containing fractions. The PtdCho fatty acids of mitochondria did not show such rapid changes as those observed in lipids of the endoplasmic reticulum and the $C_{18:1}/C_{18:2}$ ratio remained stable after growth for 4 h at the lower temperature. An intermediate increase in the degree of unsaturation of the C_{20} fatty acids (after 4 h) was not evident in the 24 h sample. The mitochondrial PtdEth fatty acids showed progressive desaturation; again linoleate levels increased as oleate decreased and the more unsaturated C_{20} fatty acids rose at the expense of icosadienoate.

DISCUSSION

A detailed understanding of low-temperature adaptation of membrane structure and function necessitates separation of the various subcellular membrane components. Several groups have previously used subcellular-fractionation methods to obtain organelles and membrane preparations from *A. castellanii*. Thus, Schultz and Thompson (1969) showed that 5′-nucleotidase is a plasma membrane marker enzyme in this organism, and Korn and colleagues (Korn and Wright, 1973; Korn et al., 1974) pioneered studies of the macromolecular composition of the plasma membrane with special reference to lipophosphonoglycan. Morgan et al. (1973) showed how zonal centrifugation provides a large-scale separation method, and Clarke et al. (1988) used self-generating Percoll gradients to isolate plasma membranes. We have found that the most convenient procedure

for separation of plasma membranes from mitochondria and endoplasmic reticulum from *A. castellanii* relies on coating the organism with silica beads before disruption. This method, developed for use with *Dictyostelium discoideum* (Chaney and Jacobson, 1983), and more recently applied successfully to yeasts (Schmidt et al., 1983) presents several advantages. Thus, in addition to providing a rapid single procedure for the separation of large open sheets of plasma membrane free from contamination by endoplasmic reticulum, it does not require density-gradient centrifugation. Preliminary experiments showed that the buoyant densities of membranes and organelles from *A. castellanii* are markedly affected by growth temperatures (A. L. Jones, J. L. Harwood and D. Lloyd, unpublished work) and this characteristic hinders experimental design of a consistent separation strategy using gradients.

The changes in the proportions of oleate and linoleate over the time-course of the experiments show similar trends in all the membrane fractions, and reflect the changes seen in whole cells after a downward shift in temperature (Jones et al., 1991a). In the homogenate (total cellular lipid) fraction there is a steady rise in the proportion of linoleate, with a commensurate reduction in that of oleate. These changes in the oleate/linoleate ratio are even more marked for individual membrane fractions. Thus in the plasma membrane- and endoplasmic reticulum-enriched fractions there is a doubling of linoleate proportions only 1 h after temperature shifting (Table 2). For the endoplasmic reticulum this probably reflects the location of the Δ12-desaturase responsible for the conversion of oleate into linoleate, since animal desaturases are thought to be located generally in the endoplasmic reticulum (Bishop and Bell, 1988). Since there is evidence for rapid movement of various lipid species from the endoplasmic reticulum to the plasma membrane by vesicular transport in *Acanthamoeba* (Mills et al., 1984; Bishop and Bell, 1988), the increase in linoleate levels in the plasma membrane fraction may be the result of such a process. If the changes in oleate/linoleate ratios in the complex lipids are examined (Figure 2a), the most rapid initial shift occurs in PtdCho from the endoplasmic reticulum fraction. This rapid change may indicate the involvement of PtdCho in the desaturation of oleate. Oleoyl PtdCho is known to be the substrate for Δ12-desaturation in a number of systems which carry out this desaturation, including higher plants (Stymne and Appelqvist, 1978), fungi (Wilson et al., 1980) and other eukaryotic micro-organisms (Koudelka et al., 1983). Experiments with *Candida lipolytica* suggested that both an oleoyl-PtdCho desaturase and an oleoyl-CoA desaturase were present (Pugh and Kates, 1973), with the major pathway being via oleoyl PtdCho. *A. castellanii* is unusual because most animals cannot synthesize linoleate, although *Tetrahymena* spp. do so, possibly via oleoyl PtdCho (Thompson and Nozawa, 1984). Subsequent to the rapid initial shift, the proportions of linoleate remained steady for up to 4 h after chilling in PtdCho from endoplasmic reticulum and then decrease by 24 h, although oleate proportions continued to fall throughout the time-course. There are several possible explanations for this: (1) The linoleate produced is the precursor for the $n-6$ family of fatty acids, and is being used as a substrate for further elongation/desaturation after its initial production on PtdCho in the endoplasmic reticulum. Although the amount of $n-6$ C_{20} fatty acids in PtdCho in endoplasmic reticulum does not increase during the time-course studied, there are overall increases in these fatty acids in other fractions in keeping with this possible explanation. (2) The linoleate produced in the endoplasmic reticulum is transferred to other membrane systems. This explanation would certainly agree with the results shown in Table 2 where the proportion of linoleate in mitochondrial or plasma membranes rises after the initial rise in its percentage in the endoplasmic reticulum. (3) Alternatively, the transfer of linoleate could be from PtdCho into other lipids. Figure 2(a) shows that the proportion of linoleate in PtdEth continues to rise throughout the experimental period, presumably as the fatty acid is synthesized on PtdCho. Thus, there is evidence for all three of the above processes, which may well all be significant in the adaptive process in *Acanthamoeba*.

At growth times longer than 24 h after the temperature shift, linoleate levels are much higher as a proportion of the total cellular acyl chains than they would be in *A. castellanii* grown continuously at 15 °C (15% of total fatty acids): this has been observed previously in whole cells (Jones et al., 1991a). This overproduction of linoleate may be an initial response to decreased temperature, when an increase in unsaturation is necessary to restore the fluidity of the membranes so that other, longer-term adaptations can take place. When other responses (e.g. changes in the proportions of complex lipids or changes in other acyl chains) have taken place then linoleate levels may fall back to reflect a steady-state level of desaturase activity at the new lower growth temperature.

While changes in the proportions of oleate/linoleate are rapid, changes in the proportions of C_{20} fatty acids are neither so rapid nor so clear cut. In whole cells there is a decrease in icosadienoate as seen previously (Jones et al., 1991a). This decrease is, again, most marked in the microsomal fraction (Figure 2).

It appears from this study that a major and rapid response of *A. castellanii* to a shift to a lower temperature is an induction of Δ12-desaturase ($n-6$) activity. The increase in the proportion of linoleate, at the expense of oleate, is most rapid in the endoplasmic reticulum- and plasma membrane-enriched fractions, particularly in the PtdCho from the endoplasmic reticulum-enriched fraction. This may indicate the location in these membranes of a Δ12-desaturase which uses a phospholipid substrate. In view of the obvious importance of Δ12-desaturation in the temperature adaptation of *A. castellanii*, further work is being directed at investigating the mechanism(s) by which Δ12-desaturase activity is increased.

We are grateful to the N.E.R.C. for financial assistance and to Dr. Bruce Jacobsen (Department of Biochemistry, University of Massachusetts, MA, U.S.A.) for the gift of the cationic silica microbeads.

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Received 27 July 1992/11 September 1992; accepted 16 September 1992