Rapid induction of microsomal $\Delta^{12}(\omega 6)$-desaturase activity in chilled *Acanthamoeba castellanii*

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The activity of microsomal Δ^{12}-desaturase in *Acanthamoeba castellanii* was increased after growing cultures were chilled from the optimal growth temperature (30 °C) to 15 °C. This increase was detectable in microsomes isolated from organisms subjected to only 10 min chilling. The mechanism of induction was investigated. The increase in activity on chilling was greatly reduced when protein synthesis was blocked before the temperature shift. Thus the major mechanism for the induction of Δ^{12}-desaturase is increased protein synthesis. Δ^{12}-Desaturase activity was higher when assayed at 20 °C than when assayed at 30 °C, but these changes were not due to the increased solubility of O_2 at 20 °C. The major substrate of Δ^{12}-desaturase was found to be 1-acyl-2-oleoyl phosphatidylcholine.

INTRODUCTION

The most rapid early change seen in the membrane lipid composition of *Acanthamoeba castellanii* in response to a shift to lower growth temperature is an increase in linoleate accompanied by a commensurate fall in oleate levels (Jones et al., 1991). Linoleate proportions in the microsomal membrane fractions of this amoeba double by comparison with other fatty acids within the first hour of a shift in growth temperature from 30 °C to 15 °C (Jones et al., 1993). This change is probably part of a complex concerted mechanism of homoeostatic adaptation which maintains the membrane fluidity of poikilothermic organisms during growth at lower temperatures.

In other protozoa and algae, responses to reduced temperature occur in specific sequences, and these have been studied in greatest detail in *Dunalliela salina* (Lynch and Thompson, 1984) and *Tetrahymena* species (Thompson and Nozawa, 1984). In these organisms, the initial emergency response appears to be a retailoring of the lipid molecular species, and this is followed by increased desaturation of lipid acyl chains. Over a longer time scale, changes in lipid class proportions eventually occur. In *D. salina*, the proportions of diunsaturated phospholipid molecular species (i.e. species with unsaturated acyl chains at both the *sn*-1 and *sn*-2 positions) increased at the expense of monounsaturated species (Lynch and Thompson, 1984). The increase in the total proportion of α-linolenate (at the expense of oleate and linoleate) observed in this alga took more than an hour to become significant, whereas changes in molecular species were apparent during the first hour after a shift in growth temperature from 30 °C to 12 °C. An increase in α-linolenate was also the response of *Neurospora crassa* to reduced growth temperature (Martin et al., 1981). This increase took longer to occur than the increase in linoleate in *A. castellanii*. Thus, in *N. crassa* cells shifted from higher to lower growth temperatures, the constant acyl chain composition of cells acclimated to the lower temperature was only attained 8 h after, whereas an analogous change occurred within the first 4 h after a temperature shift in *A. castellanii* (Jones et al., 1991). The observed increase in α-linolenate in *N. crassa* was abolished by the addition of cycloheximide to the cultures after a shifting to a lower growth temperature, presumably by prevention of increases in the levels of Δ^{15}-desaturase protein (Martin et al., 1981). In *Tetrahymena*, increases in the activities of both stearoyl-CoA desaturase and palmitoyl-CoA desaturase also followed a shift to lower growth temperatures and could be abolished by cycloheximide, suggesting that the rises in activity were the result of *de novo* synthesis of desaturase protein (Thompson and Nozawa, 1984).

Both of the above *Tetrahymena* desaturases use CoA derivatives as substrates (Thompson and Nozawa, 1984), whereas in many organisms Δ^{12}-desaturation, which is the principal adaptive response in *A. castellanii* (Jones et al., 1993), has been suggested to take place on a complex lipid substrate. This is the case in higher plants (Stymne and Appelqvist, 1978; Smith et al., 1990), fungi (Wilson et al., 1980) and in *Tetrahymena* (Kameyama et al., 1980). In contrast, the yeast *Candida lipolytica* has both oleoyl-phosphatidylcholine (PtdCho) and oleoyl-CoA Δ^{12}-desaturase activities (Pugh and Kates, 1973), and Cripps et al. (1990) showed that the house cricket, *Acheta domesticus*, one of a number of insects that can produce linoleate *de novo*, uses oleoyl-CoA as a substrate for Δ^{12}-desaturation.

Although animals cannot usually synthesize linoleate *de novo*, *A. castellanii* and *Tetrahymena* are both exceptional in this respect. In *Tetrahymena*, Δ^{12}-desaturation does not appear to be as important in adaptation to lowered growth temperature as it is in *A. castellanii*; in the former organism the microsomal desaturases which produce palmitoleate and oleate are far more significant (Thompson and Nozawa, 1984).

As the increase in Δ^{12}-desaturation occurs so rapidly in *A. castellanii*, the mechanism by which the enzyme activity is increased appears very important in the adaptation of this organism to lowered growth temperatures. In this paper we report experiments aimed at increasing our understanding of this process. A preliminary report of some of the data has been published (Jones et al., 1992).

EXPERIMENTAL

Cell cultures

A. castellanii was grown at 30 °C in 200 ml of PGY medium (proteose peptone, 0.75 %; glucose, 1.5 %; yeast extract, 0.75 %).

Abbreviations used: PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; FAME, fatty acid methyl esters.

Cultures (200 ml) in 500 ml Ehrlenmeyer flasks were shaken at 200 rev./min (Edwards and Lloyd, 1977). Organisms were used experimentally at the mid-exponential phase of growth at a cell count of about 5×10^6 organisms/ml, when their generation time was 8 h. Cultures were shifted to 15 °C for 1 h to induce Δ^{12}-desaturase activity unless otherwise indicated in the Results section.

Isolation of microsomes

Cells were harvested after the appropriate treatment by centrifugation at 250 *g* for 5 min at 4 °C in the 6 × 250 ml rotor of a Sorvall RC5B centrifuge. All subsequent procedures were at 4 °C. Cells were washed twice in 20 mM Tes, pH 7.4, and the final pellet was resuspended in 1 vol. of 20 mM Tes, pH 7.4, and 1 vol. of glass beads (150–212 μm diameter, acid-washed), and disrupted in a Braun shaker (Bosch) at 4000 Hz for 10 s. After beads had settled, the homogenate was decanted and 2 M sucrose in 20 mM Tes, pH 7.4, was added to a final concentration of 0.30 M sucrose. The homogenate was centrifuged at 18000 *g* in the SS34 rotor of a Sorvall RC5B centrifuge for 20 min. The supernatant was decanted and centrifuged at 105000 *g* in a Beckman L8-M ultracentrifuge for 60 min (55.2T_i rotor). The microsomal pellets were resuspended in microsomal homogenization buffer (0.1 M potassium phosphate, pH 7.2, 2000 units of catalase/ml, 0.2% BSA, 0.30 M sucrose) and used immediately for desaturase assays.

Δ^{12}-desaturase assay

Equal volumes of resuspended microsomes and incubation buffer [0.1 M potassium phosphate (pH 7.2), 0.30 M sucrose, 2 mM NADH, 600 nmol of CoA] were used in each incubation (unless otherwise detailed in the Results section), to which oleoyl-CoA and [1-^{14}C]oleoyl-CoA (specific radioactivity 1.96 GBq/mmol; Amersham International, Amersham, Bucks, U.K.) were added as detailed in the individual experiments.

Assays were usually stopped by the addition of 0.1 ml of 60% KOH to 1 ml of incubation mixture. Hydrolysis was carried out at 70 °C for 30 min. Acidification with 20% (v/v) H_2SO_4 (0.4 ml/ml) was followed by the addition of 4 ml of chloroform and sonication for 10 min in a Kerry sonicating water bath. The clean separation of phases was ensured by centrifugation in a Baird and Tatlock Auto bench centrifuge mark IV. The lower phase was removed and filtered through glass wool. A further 4 ml of chloroform was added to the aqueous phase and the procedure was repeated. The total organic phase was combined and evaporated to dryness under N_2. Fatty acid methyl esters were prepared by transmethylation with 1 ml of 2.5% (v/v) H_2SO_4 in anhydrous methanol at 70 °C for 1 h.

Lipid analysis

To extract complex lipids, reactions were terminated by the addition of 0.15 M acetic acid and the lipids extracted by a modification (Griffiths and Harwood, 1991) of the method of Bligh and Dyer (1959). The chloroform phase was removed, filtered through glass wool and evaporated to dryness under N_2. The residue was resuspended in a minimum volume of chloroform/methanol (2:1, v/v) and the lipids were separated by t.l.c. on precoated silica-gel plates (Merck; silica gel 60) in a solvent system of chloroform/methanol/water (65:25:4, by vol.). Lipids were detected by spraying with 0.2% (w/v) 8-anilino-1-naphthalenesulphonic acid in aq. 95% methanol and viewing under u.v. light. Lipid areas were scraped from the plate and methylated directly from the silica by transmethylation as above.

$AgNO_3$ t.l.c. was performed using precoated silica-gel G plates (Merck), soaked for 1 h in 4% (w/v) $AgNO_3$ in acetone/water (9:1, v/v). The fatty acid methyl esters (FAMEs) were separated in a solvent system of hexane/diethyl ether (9:1, v/v) and detected by spraying with 0.5% (w/v) 2,7-dichlorofluorescein in aq. 95% ethanol and viewing under u.v. light. Separated FAMEs were eluted from the silica, and silver ions and dichlorofluorescein were removed by the method of Christie (1982).

FAMEs were analysed by g.l.c. in a column of 15% (w/w) EGSS-X on Chromosorb WAW (100–120 mesh) (2.1 m × 0.4 cm, internal diameter) or SP2330 (3%) on Supelcoport (100–120 mesh) (2.1 m × 0.4 cm internal diameter) with a Pye–Unicam GCD gas chromatograph attached to a LabLogic RAGA radiogas proportional counter. Pentadecanoate was used as an internal standard. Results were analysed using LabLogic (Sheffield, Yorks., U.K.) RAMONA software. Radioactive samples were counted in Optifluor (Packard) in an LKB Rackabeta 1209 liquid-scintillation counter.

Protein estimations

Protein estimations were routinely performed by the method of Bradford (1976); when required, more accurate determinations were obtained using the method of Petersen (1977).

Inhibition of protein synthesis

Cells were harvested at mid-exponential growth phase and resuspended in fresh medium or 0.9% NaCl. Inhibitor was added and the cells were preincubated for various times before the addition of [^{3}H]leucine (1.78 TBq/mmol; Amersham International); incorporation was for 1 h. Incubations were stopped by the addition of an equal volume of 20% trichloroacetic acid, and incorporation of [^{3}H]leucine into protein was measured by collecting acid-precipitable counts by the method of Grollman (1968).

RESULTS AND DISCUSSION

Mechanism of induction

A number of mechanisms have been suggested for the induction of desaturase enzymes on chilling in various organisms. These include: (1) activation of pre-existing enzyme by conformational changes within the membrane; (2) the effect of increased O_2 concentration, as O_2, a substrate of the reaction, is more soluble at lower temperatures; (3) synthesis of new enzyme protein.

The mechanism proposed for the increase in Δ^{12}-desaturase activity seen on chilling in *C. lipolytica* (Kates et al., 1984) invoked increased temperature-dependent membrane viscosity; this would allow the proteins of the desaturase electron-transport chain to adopt a conformation more favourable for desaturase activity. Recent data obtained using *in situ* catalytic hydrogenation of microsomal components suggest that this type of mechanism may provide feasible autoregulation (Horvath et al., 1991). Thus, as more unsaturated acyl chains are introduced into the membrane, its fluidity is restored and the desaturase will become less active again. In *A. castellanii* microsomes, initial rates of Δ^{12}-desaturation are higher at incubation temperatures of 20 °C than at 30 °C, for both induced and non-induced cells (Table 1). However, desaturation was linear only for 20 min at 30 °C whereas it was linear for over an hour at 20 °C (results not shown: see the Experimental section). Moreover, it was of interest that the proportional increase in activity for the two preparations (i.e. induced and non-induced) when incubated at 20 °C was different. Presumably the microenvironment of the desaturase complex in shifted-cell microsomes was significantly

Table 1 Desaturation of [1-^{14}C]oleoyl-CoA by *A. castellanii* microsomes

Cells were either grown at 30 °C continuously or at 30 °C and then shifted to 15 °C for 1 h before fractionation. Results are initial rates expressed as nmol of [^{14}C]linoleate produced/h per mg of protein. Data are expressed as means ± S.D. where $n = 3$. The analysis of Δ^{12}-desaturase activity using [^{14}C]oleoyl-CoA desaturation underestimates total activity of the enzyme in microsomal preparations because of the large pool of endogenous substrate (see the text). Analysis of the total mass of fatty acids before and after desaturation indicated actual desaturation rates of around 10–12 times the rates found for exogenously added substrate. For instance, in this experiment the total mass of oleate converted into linoleate on incubation at 20 °C for shifted cells was 91.9 ± 3.6 nmol/h per mg of protein.

	Δ^{12}-Desaturase activity	
Growth temperature	30 °C	20 °C
30 °C	1.2 ± 0.3	3.2 ± 0.6
30 °C to 15 °C	5.4 ± 0.3	8.1 ± 0.2

Table 2 Inhibition of the incorporation of [^{3}H]leucine into proteins by *A. castellanii in vivo*

Whole *A. castellanii* cells were preincubated with inhibitor for 2 h. They were then harvested, washed twice with 0.9% (w/v) NaCl/20 mM TES (pH 7.4) and incubated in the same buffer containing 1 μCi of [^{3}H]leucine. Data were measured as acid-precipitable counts (Grollman, 1968) with values showing means ± S.D.

Inhibitor	Protein labelling (% of control)
None	100.0 ± 3.2 ($n = 12$)
Cycloheximide (1 mg/ml)	56.8 ± 1.6 ($n = 9$)
Puromycin (1 mg/ml)	101.6 ± 3.6 ($n = 3$)
Emetine (1 mg/ml)	76.4 ± 3.8 ($n = 6$)
Anisomycin (1 mg/ml)	37.7 ± 5.1 ($n = 3$)
Anisomycin (0.1 mg/ml)	41.9 ± 2.6 ($n = 3$)

Table 3 Effect of anisomycin on Δ^{12}-desaturase activity of *A. castellanii* microsomes isolated from cells shifted from 30 °C to 15 °C growth temperature

Anisomycin (0.1 mg/ml) was added to cultures 2 h before the temperature shift. Microsomes were then isolated and incubations carried out at 20 °C in a final volume of 1 ml. Data are presented as means ± S.D. where $n = 3$. Results are expressed as nmol/h per mg of protein, but see the note in the legend to Table 1. Because of the large volume of the cell suspension, the temperature of the culture only reached 15 °C after about 1 h. The approximate temperatures after 10 and 25 min were 25 °C and 20 °C respectively (see the text).

	Time after temperature shift (min)...	Δ^{12}-Desaturase activity			
Treatment		0	10	25	60
Control		3.2 ± 0.2	4.8 ± 0.1	6.1 ± 0.2	6.9 ± 0.1
+ Anisomycin		—	4.4 ± 0.2	4.0 ± 0.2	4.4 ± 0.6

different in membranes isolated from cells grown at 30 °C and this accounted for the differences. Table 1 also demonstrates the induction of Δ^{12}-desaturase activity on shifting the growth temperature to 15 °C for 1 h, after continuous growth at 30 °C. Therefore it seems that there are two separate contributions: (1) existing enzyme (at least as measured in microsomal preparations) has higher activity at 20 °C than at 30 °C; (2) a large increase in total activity occurs after the growth temperature is lowered. It was also noticeable that little further metabolism of [^{14}C]linoleate (e.g. to γ-linolenate) was seen in the incubations. This was in keeping with the low rates of conversion of [^{14}C]oleate into intermediates in the pathway leading to C_{20} polyunsaturates. In whole cells, significant changes in the proportions of such acids were not seen until at least 4 h after a growth temperature shift (Jones et al., 1993).

The second possible general mechanism for increasing desaturase activity is as a consequence of elevated O_2 solubility at low temperatures. This route was first proposed by Harris and James (1969), who found that the extent of desaturation in sunflower and caster oil seeds could be kept constant regardless of the incubation temperature provided that O_2 concentrations were comparable. However, this is not always an important mechanism, as no effect of O_2 concentration on the stearate or oleate desaturases of safflower microsomes was found (Browse and Slack, 1983), and, moreover, the low K_m value (56 μM) for O_2 in purified safflower stearoyl-acyl carrier protein desaturase makes it unlikely that that enzyme would be subjected to regulation by O_2 availability *in vivo* (McKeon and Stumpf, 1982).

In order to test for O_2 effects in the *Acanthamoeba* system, we measured the Δ^{12}-desaturase activity in fully aerated systems first (O_2 concentrations in fully aerated systems were 230 μM at 30 °C and 305 μM at 15 °C). Even at much lower concentrations, O_2 had no significant effect on rates of desaturation to linoleate. Activity at 3.5 μM O_2 (8.1 ± 0.6 nmol of linoleate/h per mg of protein) was comparable with that at 107 μM O_2 (7.8 ± 0.5 nmol of linoleate/h per mg of protein). Therefore, although the solubility of O_2 is lower at 30 °C than at 15 °C, it is nevertheless high enough at both temperatures to sustain full desaturase activity. We conclude therefore that the availability of O_2 is not a factor influencing increased desaturase activity at low temperatures in *Acanthamoeba*.

The third route for increased desaturase activity is the formation of new protein. In order to test this possibility, it was first necessary to find a suitable inhibitor of protein synthesis. Table 2 demonstrates that some common protein-synthesis inhibitors were not very effective at inhibiting protein synthesis in intact *A. castellanii*. Puromycin was totally ineffective and cycloheximide and emetine [an ipecac alkaloid which also binds to 80S ribosomes (Chakrabarti et al., 1972)] produced less than a 50 % inhibition of protein synthesis during the time-course of the experiments. Of the range of protein-synthesis inhibitors tested, anisomycin (Lietman, 1970) proved to be the most active inhibitor for *A. castellanii* and was essentially as effective at 0.1 mg/ml as at 1.0 mg/ml concentrations, giving about a 60 % inhibition of protein synthesis.

To investigate the possibility that increased Δ^{12}-desaturase activity was parallelled by the synthesis of new protein, we preincubated cells for 2 h with anisomycin to inhibit protein synthesis, before carrying out a temperature-shift experiment. Table 3 shows the results of such an experiment. Clearly the desaturase activity is being induced rapidly by small temperature decreases; changes can be seen even at 10 min when the temperature had reached 25 °C (the final temperature of 15 °C was attained after 1 h). The major part of the increase in desaturase activity was blocked when protein synthesis was inhibited. In the experiment shown in Table 3 the rise in desaturase activity in the presence of anisomycin was reduced by about 68 % compared with the control values after 1 h. At the same concentration of anisomycin, protein synthesis was inhibited by about 60 % (Table 2).

Δ^{12}-Desaturase activity from other protozoa is known to require an electron-transport chain involving cytochrome b_5, NADH reductase and the desaturase protein, with O_2 as the final electron acceptor (Kates et al., 1984). We measured the amount

of cytochrome b_5 present in microsomes and found it comparable in cells grown continuously at 30 °C and in those shifted to 15 °C for 60 min. In both cases, around 30 % of the cytochrome b_5 could be reduced by the addition of NADH (A. L. Jones, D. Lloyd, J. L. Harwood and O. T. G. Jones, unpublished work). Therefore there was no evidence that increased cytochrome b_5 levels contributed to the induction of desaturase activity by providing increased electron flow. Our results are consistent with the induction of the desaturase protein itself, a conclusion also reached for the *Tetrahymena* stearoyl-CoA desaturase (see below).

The first report of low-temperature-induced synthesis of a desaturase enzyme was in *Bacillus megaterium* (Fulco, 1972). Induction of desaturases by protein synthesis as a response to decreased culture temperature has been observed previously in eukaryotes, including *Tetrahymena* (Thompson and Nozawa, 1984). Here inhibition of protein synthesis by cycloheximide before isolation of the microsomes largely prevented the usual rise in activity of the palmitoyl-CoA desaturase and stearoyl-CoA desaturase. The increase in activities of these *Tetrahymena* microsomal desaturases reached a maximum in the first 2 h after the lower temperature had been achieved and then declined. Increased activity in *A. castellanii* occurs much faster than this.

Thus we conclude that induction of Δ^{12}-desaturase activity in *A. castellanii* is mainly the result of the synthesis of new enzyme protein. This is probably a consequence of activation of the desaturase gene although increased translation of pre-existing mRNA cannot be discounted.

Desaturase substrate

In order to investigate desaturase substrate specificity in *A. castellanii* we carried out several experiments. This work was of interest in view of the phylogenetically diverse Δ^{12}-desaturases which have been reported. In higher plants (Stymne and Appelqvist, 1978; Smith et al., 1990), yeast (Pugh and Kates, 1975) and fungi (Wilson et al., 1980), the Δ^{12}-desaturase uses a phospholipid substrate. Most animals cannot carry out Δ^{12}-desaturation (which results in the formation of the essential fatty acid, linoleate) but recently four insect species have been found to contain the enzyme. Of these, the house cricket, *Acheta domesticus* was studied in most detail and oleoyl-CoA found to be the substrate (Cripps et al., 1990). By contrast, *Tetrahymena* has been proposed to use complex lipids (Kameyama et al., 1980).

Table 4 Distribution of counts from [^{14}C]oleoyl-CoA in lipid classes after incubation with *A. castellanii* microsomes in the absence or presence of Δ^{12}- desaturation

Data are expressed as means ± S.D. (where $n = 2$). Experimental details were as for Figure 1. Others includes phosphatidylserine, phosphatidylinositol, phosphatidic acid and traces of lysolipids. PtdEtn, phosphatidylethanolamine. Specific activity of total desaturation was 8.4 ± 1.3 nmol/h per mg protein in the presence of NADH but no desaturation could be detected in the absence of NADH.

	Distribution of radioactivity between lipid class		
Lipid class	−NADH (no desaturation)	+NADH (desaturation)	Desaturation on addition of NADH (%)
PtdCho	62.2 ± 3.0	59.4 ± 1.6	71.2
PtdEtn	17.3 ± 5.1	14.4 ± 5.8	48.7
Neutrals	12.0 ± 1.7	15.1 ± 2.4	51.3
Others	8.5 ± 0.9	11.1 ± 2.3	32.6

When microsomes were incubated with [^{14}C]oleoyl-CoA, radiolabel rapidly accumulated in the complex lipids. In fact, after 20 min, over 80 % of added radiolabel was recovered in such lipids (results not shown). It is possible that acyltransferase enzymes which transfer oleate into (and linoleate from) complex lipids are located close to the desaturase systems, as proposed for higher plants (Murphy et al., 1984; Jaworski, 1987). The acyl transfer took place in the absence of NADH at rates indistinguishable from those in its presence (Table 4). In both cases, PtdCho was most heavily labelled and accounted for about 60 % of the radioactivity of all lipids including unesterified fatty acids. NADH was omitted to prevent desaturation taking place, and Figure 1(a) shows that this was almost completely effective as far as PtdCho was concerned. Similar radio-g.l.c. traces were also obtained for other lipid classes (results not shown). The addition of fresh incubation medium (containing NADH, but no CoA or oleoyl-CoA) after acyl transfer had taken place then allowed desaturation to occur, as shown for PtdCho in Figure 1(b).

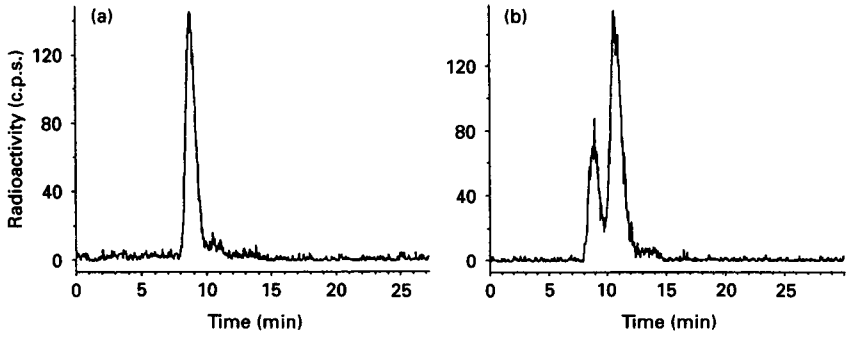

Figure 1 Conversion of [^{14}C]oleoyl-PtdCho into [^{14}C]linoleoyl-PtdCho by *A. castellanii* microsomes after the addition of NADH

(**a**) −NADH (before desaturation); (**b**) +NADH (after desaturation). Incubations were in a total volume of 3 ml with 0.5 μCi of [^{14}C]oleoyl-CoA/incubation. Microsomes were isolated and resuspended as detailed in the Experimental section to give protein concentrations in the range 1.2–1.5 mg/ml in the final incubation mixture, added to incubation medium without NADH and incubated at 20 °C for 20 min to allow the incorporation of label into complex lipids. The incubation mixture was then diluted to 30 ml with buffer [0.1 M potassium phosphate (pH 7.2), 0.3 M sucrose] to wash out unincorporated substrate, and the microsomes were repelleted at 105000 *g* for 1 h. The resulting microsomal pellets were rehomogenized in homogenization medium to a volume of 1.5 ml and added to 1.5 ml of incubation medium including NADH but no oleoyl-CoA or CoA, for 1 h at 20 °C for desaturation to take place. Complex lipids were extracted, separated and analysed as detailed in the Experimental section.

Table 5 Comparison of the specific radioactivity of [^{14}C]oleate and [^{14}C]linoleate in different lipid fractions during desaturation

Data are means ± S.E.M. of two experiments each performed in duplicate. Experimental details were as for Figure 1.

	Specific radioactivity (d.p.m./nmol)		
	Before desaturation	After desaturation	
Lipid class	18:1	18:1	18:2
Total lipids	31.3 ± 4.6	28.2 ± 3.2	15.6 ± 4.1
PtdCho	69.3 ± 6.4	78.1 ± 11.2	38.3 ± 3.6
PtdEtn	36.1 ± 2.1	17.3 ± 2.5	10.4 ± 5.6
Triacylglycerol	3.0 ± 0.3	5.3 ± 0.6	0.7 ± 0.8

Desaturation rates as well as the final proportion of desaturation were highest for PtdCho (Table 4). Labelled linoleate appeared in all fractions, although to a lesser extent than in PtdCho; neither oleoyl- nor linoleoyl-CoA were detected at the end of the final incubation. The omission of CoA from the final incubation medium should limit transesterification reactions and the amount of desaturation was identical in its presence or absence (results not shown).

The preferential transfer of [^{14}C]oleoyl-CoA into PtdCho is also reflected by the specific radioactivity of this moiety in PtdCho (Table 5), where it was over double that for the total lipids. PtdEtn, the next most abundantly labelled lipid class (Table 4), showed a decline in the specific radioactivity of its oleate moiety during desaturation (Table 5) possibly indicating two pools of oleoyl-PtdEtn, only one of which is accessible to acyltransferases. In contrast, triacylglycerol showed the opposite effect; radiolabelled oleate was gradually transferred into this neutral lipid during the incubations.

These results are most easily interpreted by postulating that PtdCho is the preferred substrate for Δ^{12}-desaturation in *A. castellanii*. It seems unlikely that oleoyl-CoA can be used by the Δ^{12}-desaturase, as desaturation is linear for 60 min under the standard incubation conditions at 20 °C, whereas transfer of the oleoyl moiety into PtdCho is much more rapid. Moreover, oleoyl-CoA could not be detected at the end of the incubation, presumably because of both enzymic and non-enzymic hydrolysis (results not shown). It is also possible that PtdEtn is also used as an (inferior) substrate. In their work with *C. lipolytica*, Pugh and Kates (1973) found that endogenous PtdCho (but not endogenous PtdEtn) was used as a desaturase substrate. In contrast, both PtdCho and PtdEtn were used when added exogenously. They also propose that it is the rise in the oleoyl-CoA desaturase activity (rather than PtdCho desaturation) which accounts for the increased desaturation seen upon chilling in this organism (Pugh and Kates, 1975). In higher plants, PtdEtn has also been suggested as a possible oleate desaturase substrate, at least in some species (Sanchez and Stumpf, 1984).

In further experiments, we examined the positional distribution of [^{14}C]oleate in PtdCho and PtdEtn before desaturation and [^{14}C]oleate and [^{14}C]linoleate in the phospholipids after desaturation. The data were consistent with a preferential esterification of oleate at the *sn*-2 position, but some radiolabelled substrate was also found at the *sn*-1 position (Figure 2). In both PtdCho and PtdEtn, the contribution of the desaturation at the *sn*-2 position to the total observed desaturation is far greater than that of desaturation at the *sn*-1 position, but no clear specificity for the *sn*-1 or *sn*-2 position can be inferred from the data. Desaturation in these experiments was conducted in microsomes that had been preincubated with the [^{14}C]oleate and subsequently washed, so the available desaturase substrate is in the form of complex lipids. It is possible that during the 1 h incubation used in this experiment substantial acyl transfer of [^{14}C]linoleate has occurred. In *C. lipolytica*, Pugh and Kates (1975) found the activity of the oleoyl-PtdCho Δ^{12}-desaturase to be higher at the *sn*-2 position than the *sn*-1 position. In higher plants, desaturation has also been shown to occur at the *sn*-2 position of PtdCho (e.g. Griffiths et al., 1985).

Conclusion

We have shown that the temperature-induced increase in linoleate in the membranes of *Acanthamoeba* is associated with a rapid

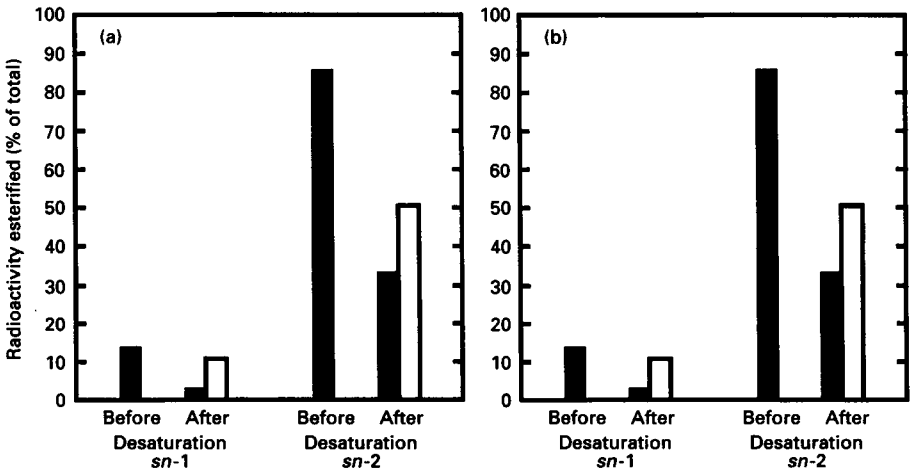

Figure 2 Comparison of desaturation occurring at the *sn*-1 and *sn*-2 positions of PtdCho and PdtEtn in *A. castellanii* microsomes

Experimental details were as for Figure 1. Data are means when $n = 2$. For the experiment shown, the amount of desaturation on PtdCho (**a**) and PtdEtn (**b**) was 62% and 33% of the total measured respectively. Desaturation at the *sn*-2 position of PtdCho contributed 51% and of PtdEtn 29% of the total desaturation measured in all lipid classes. ■, 18:1; □, 18:2.

increase in Δ^{12}-desaturase activity. Although existing enzyme can probably be activated by temperature-induced changes in membrane fluidity, the major source of desaturase activity is due to new protein synthesis. The increased activity can be detected within 10 min of a temperature shift; this is a very rapid response. Furthermore, the desaturase appears to act on the main membrane lipid of the endoplasmic reticulum, PtdCho. In view of its rapid induction and high activity, the *Acanthamoeba* Δ^{12}-desaturase would seem an excellent system in which to probe further the features of linoleate synthesis.

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