Characterization of Expression of a Plant Oleate Desaturase in Yeast

Adrian P. Brown, Rachel Dann, Steve Bowra, and Matthew J. Hills*

Department of Brassica and Oilseed Research, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, United Kingdom

ABSTRACT: We have expressed the oleate desaturase gene of *Arabidopsis thaliana* in *Saccharomyces cerevisiae* by means of a galactose-inducible promoter. Transfer of yeast cells to galactose-containing media and growth at 30°C resulted in the production of active oleate desaturase, as shown by the appearance of the diunsaturated octadecadienoic acid (18:2) and hexadecadienoic acid (16:2). The highest level of these two fatty acids was 46.2%. In induced cultures, 18:2 appeared before 16:2, and we show that both of these fatty acids were present first on phosphatidylcholine and then appeared in other phospholipids and a neutral lipid fraction. We raised antibodies against the C-terminal 100 amino acids of oleate desaturase and demonstrated that this protein was in the microsomal fraction of induced cells.
JAOCS 75, 77–82 (1998).

KEY WORDS: *Arabidopsis*, desaturase, fatty acids, linoleate, oleate, phosphatidylcholine, yeast.

In recent years, the biochemistry and molecular genetics of fatty-acyl desaturation in plants has been extensively investigated (1–4). Initial desaturation reactions are carried out by soluble enzymes that are present in plastids, and the monounsaturated fatty acids produced can either be incorporated into plastid membrane lipids or be exported from the plastid to form acyl-CoA molecules. Further desaturation can occur in the endoplasmic reticulum and is performed by membrane-bound enzymes, which act on fatty-acyl groups esterified to phospholipids (5). The first extra-plastidial desaturation in plants is catalyzed by oleate desaturase (1-acyl-2-oleoyl-*sn*-glycerol-3-phosphocholine Δ12-desaturase) enzyme. This enzyme converts oleic acid esterified on the *sn*-2 position of phosphatidylcholine (PC) to linoleic acid with the reduction of molecular oxygen to water, and requires the presence of NADH and an electron transport chain that consists of cytochrome b_5 and NADH:cytochrome b_5 reductase (6,7). Analysis of mutants allowed isolation of a gene that encodes an oleate desaturase from *Arabidopsis thaliana* (8). There has been great interest in studying the structure/function relationships of desaturases from a number of sources, to try to understand the reaction mechanisms and basis of specificity for both their substrates and point of insertion of double bonds (9,10). In the future, desaturases may be engineered and expressed in transgenic oilseed crops to produce oils that contain novel desaturated fatty acids. We investigated the possibility of using yeast as a system to investigate plant oleate desaturases. Yeast lipids contain 70% monounsaturated fatty acids and, when grown under standard laboratory conditions, no polyunsaturates (11). During the course of our work, it was reported that oleate desaturase can be expressed in yeast and produce up to 11% diunsaturated fatty acids at low temperatures (12). The results described here have substantially improved upon this, and growth of cells at 30°C, the optimum for growth, results in expression of a functional oleate desaturase gene product and accumulation of diunsaturates to typically 30–35% of total fatty acids. In addition, we show that 18:2 appears in the cells before 16:2, and both of these diunsaturates appear on PC first.

*To whom correspondence should be addressed at Department of Brassica and Oilseed Research, John Innes Centre, Norwich Research Park, Colney Lane, Norwich NR4 7UH, United Kingdom. E-mail: hillsm@bbsrc.ac.uk.

EXPERIMENTAL PROCEDURES

Materials. All chemicals, with the exception of those listed below, were from Sigma Chemical Company (Poole, United Kingdom). Yeast nitrogen base without amino acids and casamino acids were from Difco (Detroit, MI). Plasmid pBluescript KS^+ and a Prime it II® DNA labelling kit were from Stratagene (Cambridge, United Kingdom), and Hybond N^+ transfer membrane was from Amersham (Amersham, United Kingdom). Ultma® enzyme for polymerase chain reaction (PCR) was purchased from Perkin Elmer (Warrington, United Kingdom), and the vector pYES 2.0 from Invitrogen (Leek, The Netherlands). Plasmid pET15b and thrombin were from Novagen (Abington, United Kingdom), and Western blue® stabilized substrate for alkaline phosphatase from Promega (Madison, WI). Sodium methoxide in methanol was purchased from Aldrich (Poole, United Kingdom). The gas–liquid column (GLC) used was from Chrompack (Fisher Scientific Loughborough, United Kingdom), and silica gel G thin-layer chromatography (TLC) plates (0.25 mm thickness) were from Analtech (Anachem, Luton, United Kingdom).

Extraction and analysis of lipids. After incubation at 30°C for appropriate lengths of time, yeast cells were washed once with water and resuspended in water to a final volume of 1

mL. Lipids were extracted from the cell suspension for 30 min after the addition of 3.75 mL MeOH/$CHCl_3$ (2:1, vol/vol), and cell debris was removed by centrifugation. After addition of a further 1 mL chloroform and 1 mL 0.7% (wt/vol) KCl, the extract was vortexed and centrifuged at $1000 \times g$ for 2 min. The organic phase was then removed to a fresh tube and evaporated to dryness under N_2, and the lipids were dissolved in 1 mL MeOH that contained 0.25 M sodium methoxide. The mixture was incubated for 2 min at 65°C, and the fatty acid methyl esters (FAME) produced were extracted into hexane. FAME were analyzed by GLC in a Perkin Elmer 8310 Gas Chromatograph with a 2 m × 2 mm packed column, which contained 10% CP-Sil 58 on 100/120 Chromosorb WHP. The column was run isothermally at 240°C, and the injector and flame-ionization detector were held at 310°C. The retention times of peaks were compared to authentic methyl ester standards. Methyl esters of the fatty acids extracted from yeast were separated according to the number of double bonds by argentation thin-layer chromatography. Silica gel H layers, containing 4% $AgNO_3$, were developed hexane/diethyl ether (85:15, vol/vol), and the regions containing methyl esters were revealed with fluorescein stain. Methyl esters of 18:0, 18:1, and 18:2 were used as standards. The appropriate regions of the plates were scraped, and methyl esters were eluted with water-saturated diethyl ether before analysis by GLC as described above. Separation of phospholipid classes and neutral lipids from yeast was performed by TLC on silica gel G layers, developed with chloroform/methanol/acetic acid/water (25:15:4:2, by vol). Phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and triolein were used as standards, and the positions of these were visualized with iodine. The separated yeast lipids were extracted into chloroform/methanol (1:1, vol/vol), and the fatty acids were methylated and analyzed by GLC as described above.

Expression of oleate desaturase in yeast. DNA that encoded the oleate desaturase from *Arabidopsis thaliana* was amplified by PCR by using the proofreading enzyme Ultma® from a full-length cDNA (EST number 48F12T7), identified by the EST program at Michigan State University, East Lansing and obtained from the *Arabidopsis* Biological Resource Center, Columbus, Ohio, USA. The primers used were 5′ CGGAATTCACATGGGTGCAGGTGG 3′ and 5′ GCTCTAGACGTCATAACTTATTGTTGTACC 3′, which included Eco RI and Xba I restriction sites at their 5′ ends, respectively. The PCR product was cloned into suitably prepared pBluescript KS^+. This plasmid was digested with Hind III and Not I, and the resulting 1200 bp fragment was cloned into suitably prepared pYES 2.0. The orientation of the insert was checked by using an internal Bam HI site, which was 700 bp from the 5′ end of the insert. The vector alone (pYES) or the oleate desaturase expression vector (p12DES) was transformed into yeast strain YPH499 by the lithium acetate/PEG method (13) and selected for growth on SD-glucose plates minus uracil. All subsequent growth was in media without uracil to maintain pYES or p12DES. Liquid cultures of the transformed yeast were grown in SD-glucose, and expression of oleate desaturase was induced by transfer of the cells to SD-galactose medium. Cultures were grown at 30°C in SD-glucose to an OD_{650} of about 1.0, and the cells were pelleted by centrifugation at $750 \times g$ for 3 min, washed once with distilled water, and resuspended in fresh distilled water (1 mL per 100 mL culture volume). The cells were added to fresh SD-galactose (0.5 mL of resuspended cells per 100 mL medium) and grown with vigorous shaking at 30°C.

Antibodies against oleate desaturase and Western analysis. Yields of full-length desaturase, expressed in *E. coli* as a His-tagged protein with the expression vector pET15b, were too low to be useful for raising antibodies. Therefore, DNA that encoded the C-terminal 100 amino acids was amplified by PCR and cloned into the expression vector pET15b. The peptide, including an N-terminal histidine tag, was expressed in *E. coli* strain BL21(DE3) by induction with isopropyl thio-β-D-galactoside in accordance with the manufacturer's instructions. The protein occurred as inclusion bodies and was solubilized with 6 M urea before being purified in a nickel affinity column as described by the manufacturer. The histidine tag was cleaved from the desaturase peptide by using thrombin with conditions for efficient cleavage, as described by the manufacturer, but including 2.2 M urea during a 24-h incubation at 16°C with 2.5 U of thrombin/mg protein. Antibodies were raised in a rabbit by standard protocols (14). Yeast cells from galactose-induced cultures were disrupted with glass beads as described (15) in buffer that contained 20 mM Tris Cl (pH 7.9), 1 mM EDTA, 5% glycerol, 1 mM DTT, and 100 mM KCl. After centrifugation at $750 \times g$ for 3 min to remove unlysed cells, the cell lysate was centrifuged at $20{,}000 \times g$ for 20 min, and the supernatant from this was centrifuged at $200{,}000 \times g$ for 30 min. The supernatant from the $200{,}000 \times g$ centrifugation and the 20,000 and $200{,}000 \times g$ pellets were used for Western blot analysis with the oleate desaturase antibodies. SDS-PAGE on a Laemmli gel system, transfer to nitrocellulose with wet blotting, and antibody binding studies were carried out by standard methods (14). The blots were incubated with 3% BSA as a blocking solution for the primary and secondary antibody. Binding of the alkaline phosphatase conjugated secondary antibody was visualized with Western Blue® stabilized substrate.

RESULTS AND DISCUSSION

Induction of an active oleate desaturase. The fatty acid profiles of yeast cells that expressed oleate desaturase in galactose medium were analyzed to see if induction of the oleate desaturase gene resulted in the production of an active oleate desaturase enzyme. The results (Fig. 1) clearly show that additional fatty acids are present in cells that contain oleate desaturase after induction for 21 h. The methyl esters present in control cells were identified on the basis of their retention times compared to 16:0, 16:1, 18:0, and 18:1 methyl ester

standards. To confirm the presence of two desaturated bonds in the extra FAME derived from the oleate desaturase-containing yeast lipids, a sample of FAME from these cells was separated by argentation TLC. When FAME, extracted from regions of the TLC plate corresponding to diunsaturated esters, were extracted and analyzed by GLC, the retention times of the resulting products were identical to the extra peaks seen in total FAME samples from oleate desaturase-containing cells. Therefore, growth of these cells in galactose resulted in the presence of the diunsaturated fatty acids 18:2 (identified by reference to standard methyl linoleate) and 16:2. These two peaks were previously shown by GC–MS to be 18:2 and 16:2 (13). For the analysis shown in Figure 1, the methyl esters of 16:2 and 18:2 fatty acids were 10.5 and 13.1%, respectively, of the total FAME produced. Transfer of cells into SD-galactose, as described in the Experimental Procedures section, produced *S. cerevisiae* that contained up to 33.2% diunsaturated fatty acids after 33 h of induction. The highest levels of diunsaturates we observed were obtained with SD-glucose and SD-galactose supplemented with 1% casamino acids, which still allows selection for the *URA3* marker in the vector. These cells contained 46.2% diunsaturates, 29.1% monounsaturates and 25.7% saturated fatty acids after 40 h of induction. There appears to be no absolute requirement for the high levels of monounsaturated fatty acids normally seen in *S. cerevisiae*. The ratio of saturated to unsaturated fatty acids does, however, appear to be regulated in yeast. In our experiments, the amount of saturated fatty acids present did not drop below 27%, and the maintenance of a balance between saturated and unsaturated fatty acids under differing conditions has been noted before (16,17). The ability of yeast to tolerate large amounts of linoleic acid in its lipids is reflected by the fact that induction of the oleate desaturase gene had no effect on the growth rate of YPH499 cells, in agreement with results described previously (12). However, in that report the induction of even modest amounts (11%) of linoleate required growth at reduced temperatures (15°C). The reason for the lack of such a temperature effect with our experiments is unclear, but it may be due to the host cell genotype. Our results suggest that the low levels of linoleic acid reported previously at 30°C (0.6%) are not due to an inherent decreased stability of oleate desaturase at this temperature. The system we have described here, in which we used pYES and yeast strain YPH499 for the overexpression of oleate desaturase in *S. cerevisiae*, is an improvement on that already described because growth at 30°C results in the presence of up to 75 times the total amount of linoleic acid. It is therefore an excellent model system for analysis of structure function relations of oleate desaturase, and maybe for membrane desaturases in general.

In the plant endoplasmic reticulum, there are few 16:1 acyl moieties because 18:1 and small amounts of 16:0 and 18:0 are the main acyl groups exported from the plastid, whereas phosphatidylcholine in the yeast endoplasmic reticulum will contain large amounts of palmitoleic acid (18). It is not unexpected that oleate desaturase is active on both 16:1 and 18:1 because other desaturases, such as *FAD* 6, *FAD* 7 and *FAD* 8, present in the plastid can utilize acyl chains of both 16 and 18 carbons also (2).

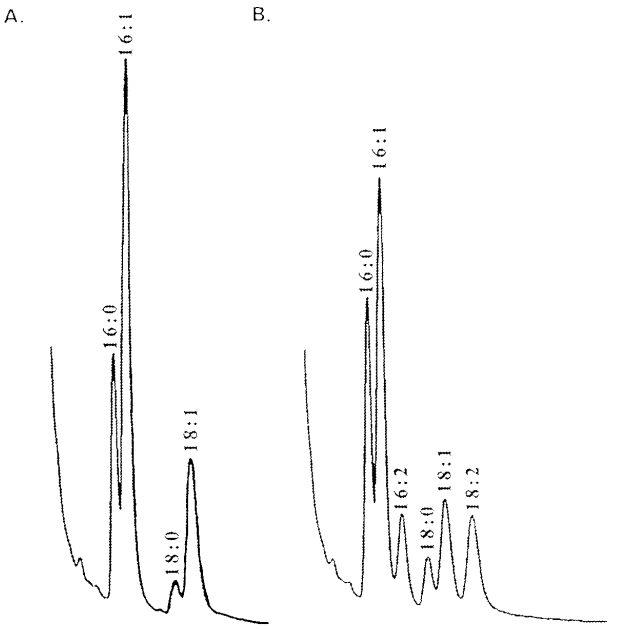

FIG. 1. Fatty acid profiles of yeast (strain YPH499) that contains pYES (vector control, panel A) and p12DES (*Arabidopsis* oleate desaturase cDNA in pYES, panel B). Lipids were extracted from 100 mL cultures grown in galactose medium, and methyl esters were made and analyzed by gas–liquid chromatography, as described in the Experimental Procedures section. The fatty acids from which the methyl esters are derived are marked above the peaks.

Analysis of fatty acids in cells that express oleate desaturase. Changes in the proportions of the six fatty acids present in induced oleate desaturase-containing cells with time are shown in Figure 2. The appearance of 18:2 fatty acids in the cells precedes that of 16:2 fatty acids, and the levels of 16:1 fall correspondingly after 18:1. It is unlikely that this is due to position effects on the membrane phospholipids because oleate desaturase has clearly been shown to act on oleate in both the *sn*-1 and *sn*-2 positions of phosphatidylcholine (19). The ratio between total saturated and total unsaturated fatty acids remains almost constant during the whole period of induction of the oleate desaturase.

To discover in which type of phospholipid(s) the di-unsaturated fatty acids first appear, total lipids from a number of different time points after induction were separated by TLC as described. Chloroform/methanol/acetic acid/water (25:15:4:2, by vol) separated the extracted lipids in to four classes: PC (R_f 0.36), PS/PI (R_f 0.6), PE (R_f 0.75), and neutral lipid (R_f 1.0). Component fatty acids in the four resolved classes were analyzed by GLC after extraction and methylester formation. The results demonstrate that 18:2 appears first in phosphatidylcholine, and is present 4 h later on all four types of lipids (Fig.

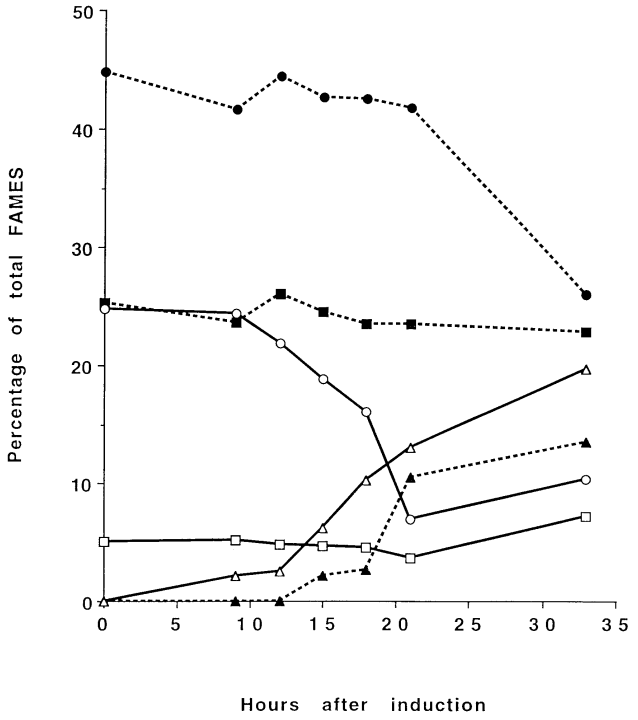

FIG. 2. Changes in the fatty acid composition in oleate desaturase-containing cells. The graph shows the amount of each fatty acid as a percentage of the total fatty acids as a function of time after induction of expression. Lipids were extracted from 100-mL cultures at various times after transfer to galactose medium. Methyl esters were made and analyzed by gas–liquid chromatography, as described in the Materials and Methods section. The fatty acids from which the methyl esters were derived are as follows: ■ — ■, 16:0; ● — ●, 16:1; ▲ — ▲, 16:2; □ — □, 18:0; ○ — ○, 18:1; △ — △, 18:2.

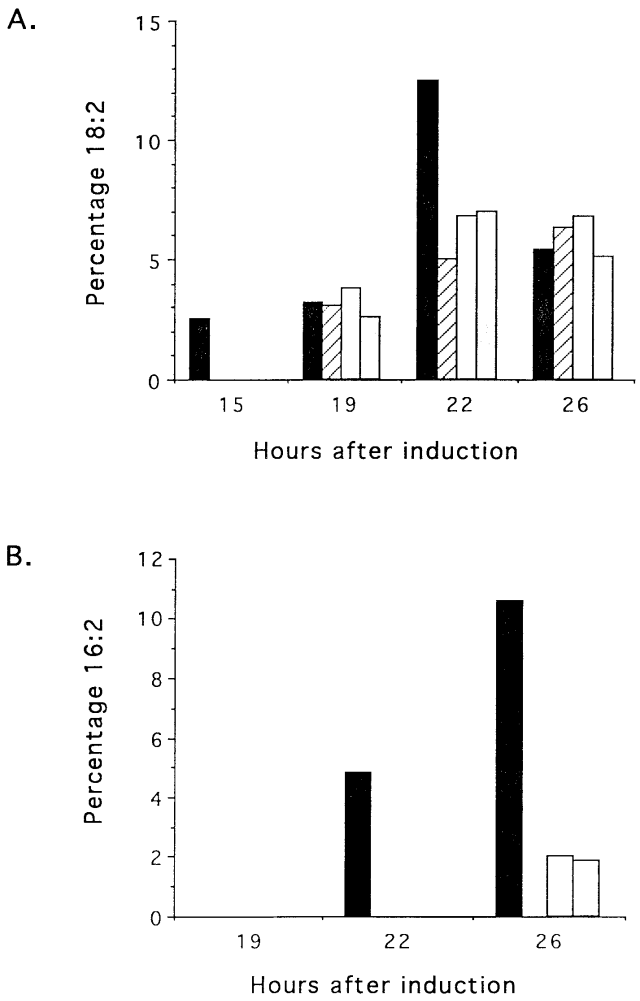

FIG. 3. Occurrence of diunsaturated fatty acids in different phospholipid classes of *Arabidopsis* oleate desaturase containing yeast cells. The graphs show the percentages of 18:2 (panel A) and 16:2 (panel B) in the total FAME from different lipid classes. Filled bars correspond to PC, hatched bars to PS/PI, clear bars to PE, and grey bars to neutral lipids.

3A). Similarly, 16:2, which appears after 18:2 in agreement with Figure 2, is first apparent in PC, and during the next 4 h it becomes dispersed among other types of lipids (Fig. 3B). The timing of induction of an active oleate desaturase gene product by transfer to media that contain galactose was too variable between different experiments to allow the results to be presented on one graph, but both 16:2 and 18:2 first appeared in phosphatidylcholine in the three separate oleate desaturase induction experiments performed. It is thought that the desaturases present in plant endoplasmic reticulum use phosphatidylcholine as a substrate, although some other phospholipids, such as phosphatidylethanolamine, may also be substrates (20). The results we obtained demonstrate that oleate desaturase acts at least preferentially on phosphatidylcholine, and the reaction products then appear on other phospholipids. It is not clear whether this is the result primarily of glycerophospholipid headgroup exchange or fatty-acyl exchange that involves a lysophosphatidylcholine acyltransferase as occurs in plants. There is no report of such an acyltransferase in yeast, although phospholipase A_2-catalyzed deacylation and reacylation of glycerophospholipids at the *sn*-2 position have been reported (18).

Localization of the oleate desaturase. The cellular localization of induced oleate desaturase protein was investigated by analysis of cell fractions obtained by centrifugation after cell lysis. Protein profiles shown by Coomassie Blue staining of the 20,000 × *g*, 200,000 × *g* and 200,000 × *g* supernatant fractions of induced oleate desaturase-containing and control cells were not qualitatively different, even in cultures grown in media supplemented with casamino acids and $FeCl_3$ (data not shown). $FeCl_3$ was included because expression of some iron-binding proteins is compromised if there is insufficient iron in the medium, causing incorrect folding and rapid degradation of the protein. Western analysis of samples from cells grown in SD-galactose without casamino acids or $FeCl_3$ demonstrated that oleate desaturase was present in induced cells that contained p12DES (Fig. 4a). The protein detected by antibodies raised against the C-terminal 100 residues of oleate desaturase was apparently full-length, with a molecular mass of ap-

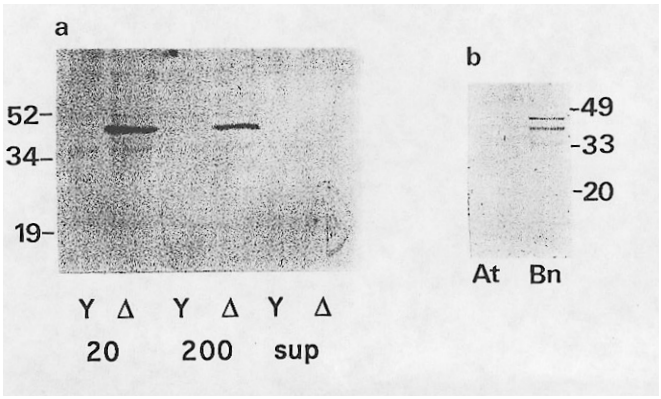

FIG. 4. (A) Western blot analysis of fractions from yeast cells. Vector control and oleate desaturase-containing cells were transferred to SD-galactose and induced for 64 h before cell lysis and centrifugation. Electrophoresis through a 12% gel was carried out with 50 μg of each protein sample before Western analysis, as described in the Experimental Procedures section. Y corresponds to samples from YPH499 (pYES) cells and Δ to those from YPH499 (oleate desaturase) cells. The number 20 denotes proteins in the 20,000 × *g* pellet, 200 those in the 200,000 × *g* pellet, and sup the supernatant from the 200,000 × *g* centrifugation. (B) Western blot of microsomal membrane proteins (200,000 × *g* pellet), isolated from leaves of *Arabidopsis thaliana* (At) and developing embryos of *Brassica napus* (Bn). The locations of molecular mass markers (as kDa) are indicated.

proximately 42 kDa, and was present in the 20,000 and 200,000 × *g* pellets of oleate desaturase-expressing cells. No oleate desaturase was apparent in the cytoplasmic fraction, and secretion of large amounts of incorrectly targeted oleate desaturase had apparently not occurred because no protein was detectable by Bradford assay in the culture medium. The binding of antibodies raised against part of oleate desaturase to a microsomal fraction from induced cells was to be expected, because oleate desaturase is present in the endoplasmic reticulum of *A. thaliana*, and the electron transport chain required for its activity is also located there. We interpret the presence of oleate desaturase in the 20,000 × *g* pellet to be due to the pelleting of endoplasmic reticulum with the nuclear membrane, with which it is contiguous. To assess the molecular mass of the desaturase in plant extracts, a Western blot of proteins in a microsomal fraction, isolated from young *Arabidopsis* leaves and developing *Brassica napus* embryos, was probed with the desaturase antibodies (Fig. 4b). The oleate desaturase from *Brassica* has 96% amino acid sequence similarity to the *Arabidopsis* oleate desaturase in the final 100 bp used to raise the antibody and is also predicted to have a molecular mass of 42 kDa (21). The leaf microsomes from *Arabidopsis* showed little binding of antibodies, although the *Brassica* embryo microsomes showed two bands at 43 kDa and 39 kDa. The band at 43 kDa is within the error observed for that expressed in yeast (Fig 4a). At this stage, it is not clear whether the band at 39 kDa is another oleate desaturase or a processed product of the 43 kDa protein. Because developing seeds synthesize lipids at much greater rates than leaves, it would be expected that enzymes involved are present at much higher levels in the membranes. Indeed, such a large difference in expression between leaves and embryos has been described previously for the stearoyl-ACP desaturase in oilseed rape (22).

ACKNOWLEDGMENTS

We thank Dr. M. Bevan for providing yeast strain YPH499 and for his advice, and Dr. D.J. Murphy for critical reading of the manuscript. This work was supported by the Biotechnology and Biological Sciences Research Council UK through its competitive strategic grant to the John Innes Centre.

REFERENCES

1. Murphy, D.J., Biogenesis, Function and Biotechnology of Plant Storage Lipids, *Prog. Lipid Res. 33*:71–85 (1994).
2. Ohlrogge, J., and J. Browse, Lipid Biosynthesis, *Plant Cell 7*:957–970 (1995).
3. Somerville, C., and J. Browse, Dissecting Desaturation—Plants Prove Advantageous, *Trends in Cell Biol. 6*:148–153 (1995).
4. Lindqvist, Y., W. Huang, G. Scneider, and J. Shanklin, Crystal Structure of Delta-9 Stearoyl-Acyl Carrier Protein Desaturase from Castor Seed and Its Relationship to Other Di-Iron Proteins, *EMBO J. 16*:4081–4092 (1995).
5. Stobart, A.K., and S. Stymne, The Regulation of the Fatty Acid Composition of the Triacylglycerols in Microsomal Preparations from Avocado Mesocarp and the Developing Cotyledons of Safflower, *Planta 163*:119–125 (1985).
6. Smith, M.A., A.R. Cross, O.T.G. Jones, W.T. Griffiths, S. Stymne, and K. Stobart, Electron-Transport Components of the 1-Acyl-2-Oleoyl *sn*-Glycero-3-Phosphocholine Delta-12 Desaturase in Microsomal Preparations from Developing Safflower (*Carthamus tinctorius*) Cotyledons, *Biochem. J. 272*:23–29 (1990).
7. Kearns, E.V., S. Hugly, and C.R. Somerville, The Role of Cytochrome b_5 in Delta-12 Desaturation of Oleic Acid by Microsomes of Safflower (*Carthamus tinctorius*), *Arch. Biochem. Biophys. 284*:431–436 (1991).
8. Okuley, J., J. Lightner, K. Feldmann, N. Yadav, E. Lark, and J. Browse, *Arabidopsis* FAD2 Gene Encodes the Enzyme That is Essential for Polyunsaturated Lipid Synthesis, *Plant Cell 6*:147–158 (1994).
9. Shanklin, J., E. Whittle, and B.G. Fox, Eight Histidine Residues are Catalytically Essential in a Membrane-Associated Iron Enzyme, Stearoyl-CoA Desaturase and are Conserved in Alkane Hydroxylase and Xylene Monooxygenase, *Biochemistry 33*:12787–12794 (1994).
10. Avelange-Macherel, M.-H., D. Macherel, H. Wada, and N. Murata, Site Directed Mutagenesis of Histidine Residues in the Delta-12 Acyl Lipid Desaturase of *Synechocystis, FEBS Lett. 361*:111–114 (1995).
11. Bossie, M.A., and C.E. Martin, Nutritional Regulation of Yeast Delta-9 Fatty Acid Desaturase Activity, *J. Bacteriol. 171*:6409–6413 (1989).
12. Covello, P.S., and D.W. Reed, Functional Expression of the Extraplastidial *Arabidopsis thaliana* Oleate Desaturase Gene (Fad2) in *Saccharomyces cerevisiae, Plant Physiol. 111*:223–226 (1996).
13. Ito, H., Y. Fukuda, K. Murata, and A. Kimura, Transformation of Intact Yeast Cells Treated with Alkali Cations, *J. Bacteriol. 153*:163–168 (1983).
14. Harlow, E., and D. Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988.

15. Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, K. Struhl, L.M. Albright, D.M. Coen, and A. Varki, *Current Protocols in Molecular Biology Unit 13.13*, John Wiley & Sons, New York, 1993.
16. Stukey, J.E., V.M. McDonough, and C.E. Martin, Isolation and Characterization of Ole1, a Gene Affecting Fatty Acid Desaturation from *Saccharomyces cerevisiae, J. Biol. Chem. 264*:16537–16544 (1989).
17. McDonough, V.M., J.E. Stukey, and C.E. Martin, Specificity of Unsaturated Fatty Acid Regulated Expression of the *Saccharomyces cerevisiae* Ole1 Gene, *J. Biol. Chem. 267*:5931–5936 (1992).
18. Wagner, S., and F. Paltauf, Generation of Glycerophospholipid Molecular Species in the Yeast *Saccharomyces cerevisiae* Fatty Acid Pattern of Phospholipid Classes and Selective Acyl Turnover at *sn*-1 and *sn*-2 Positions, *Yeast 10*:1429–1437 (1994).
19. Sperling, P., M. Linscheid, S. Stöcker, H.-P. Mühlbach, and E. Heinz, *In Vivo* Desaturation of *cis* Delta-9 Monounsaturated to *cis* Delta-9,12 Diunsaturated Alkenylether Glycerolipids, *J. Biol. Chem. 268*:26935–26940 (1993).
20. Gennity, J.M., and P.K. Stumpf, Studies of the Delta-12 Desaturase of *Carthamus-tinctorius*, *Arch. Biochem. Biophys. 239*:444–454 (1985).
21. Lightner, J.E, and J.J. Okuley, Genes for Microsomal Delta-12 Fatty Acid Desaturases and Related Enzymes from Plants, Patent WO 94/11516 (1994).
22. Piffanelli, P., Molecular and Biochemical Studies of Stearoyl-ACP Desaturase in Gametophytic and Sporophytic Generations of Plants, Ph.D. Thesis, University of East Anglia, United Kingdom, 1997.

[Received April 22, 1997; accepted August 27, 1997]