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Recovery of *Dunaliella salina* cells following hydrogenation of lipids in specific membranes by a homogeneous palladium catalyst

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Unsaturated fatty acyl chains of *Dunaliella salina* membrane lipids can be catalytically reduced by the homogeneous hydrogenation catalyst palladium di(sodium alizarine monosulphonate), $Pd(QS)_2$, under conditions permitting full recovery of the cells within 24 h. The hydrogenation is accomplished by incubation of cells with the hydride form of $Pd(QS)_2$ under 1 atmosphere of H_2 and for 2 min or less. Following this protocol, hydrogenation reduces only those fatty acids located in the plasma membrane and other membranes located near the cell surface. The limited reactivity in vivo is due to the fact the $Pd(QS)_2$ permeates into the living cells more slowly than it does into liposomes prepared from extracted *Dunaliella* membrane lipids. While *Dunaliella* is completely unaffected by exposure to the oxygenated, inactive catalyst, hydrogenated cells cease growth for approximately 12 h, during which time the hydrogenated acyl chains are being enzymatically retroconverted to their original unsaturated form. When the lipid composition approaches its prehydrogenation values, growth resumes, presumably due to the restoration of normal membrane functions. The system shows promise for studying the metabolic regulation of membrane microviscosity.

Introduction

The physical state of biological membranes is determined primarily by two factors: their lipid composition and their physical environment. Extensive studies [1] have shown that most cells can compensate for the effects of environmental changes, especially changes in temperature, by enzymatically altering their membrane lipids. In this way membrane physical properties may be maintained in a range compatible with normal function of membrane-bound proteins despite marked changes in a cell's surroundings.

The molecular mechanisms responsible for these compensatory responses are still poorly understood [2]. It is not known, for example, whether fluctuations in the activity of fatty acid desaturases known to occur in chilled cells are regulated by a direct effect of temperature per se or indirectly, as a result of the temperature-induced rise in the microviscosity of the membranes containing the desaturase enzymes.

Through the use of homogeneous hydrogenation catalysts, it has been possible to chemically reduce the double bonds of fatty acids in isolated organelles and in intact cells [3–7] and examine the consequences of the resulting reduced lipid

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Abbreviations: DLnPC, dilinolenoylphosphatidylcholine; FDA, fluorescein diacetate; $Pd(QS)_2$, palladium di(sodium alizarine monosulphonate); MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PG, phosphatidylglycerol, PE, phosphatidylethanolamine; PC, phosphatidylcholine; DGTS, diacylglyceryltrimethylhomoserine.

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flexibility on cell function over short periods of time. However, there have been no reports of cells being able to recover from this treatment. In the present study we describe the in vivo hydrogenation of the unicellular green alga *Dunaliella salina*, an organism whose responses to environmental stresses such as chilling temperature have been extensively studied (see, for example, Refs. 9, 19). Under the mild conditions used here, the cells recover from the hydrogenation and resume active growth. This system affords an excellent opportunity to examine the effects of altered membrane physical properties on metabolism in the absence of temperature change.

Materials and Methods

Chemicals

Dilinolenoylphosphatidylcholine (DLnPC) was purchased from Serdary Research Laboratories and used after confirming its purity by TLC. Fluorescein diacetate (FDA) was a Sigma product. The catalyst, palladium di(sodium alizarine monosulphonate), $Pd(QS)_2$, was prepared by F. Joó, as described in Ref. 8. $Pd(QS)_2$ is now available from Molecular Probes, Eugene, OR. All other chemicals were of commercial laboratory grade.

Cell growth

Axenic cultures of *Dunaliella salina* (UTEX 1644) were grown under continuous light (100 $\mu E \cdot m^{-2} \cdot s^{-1}$) in Erlenmeyer flasks containing 500 ml synthetic medium bubbled with 0.5% CO_2-enriched air at 30 °C [9]. Cells were harvested in the middle to late logarithmic phase.

Hydrogenation procedure

Hydrogenation of lipid dispersions made from DLnPC, whole cell total lipids or mixed phospholipids and glycolipids was performed at 20 °C as described previously [10]. Lipid multibilayer vesicles were prepared by brief sonication in 20 mM deoxygenated sodium phosphate buffer (pH 6.4); the lipid and catalyst concentrations were 0.25 mg/ml and 0.1 mg/ml, respectively.

Experiments with living cells. After the cells were harvested by centrifugation, they were resuspended at 20 °C in 900 mM mannitol/500 mM sorbitol/300 mM NaCl/20 mM sodium phosphate buffer (pH 6.4), designated as 'hydrogenation medium', and the concentration was adjusted to $1 \cdot 10^6$ or $3 \cdot 10^6$ cells/ml. A stock solution containing 10 mg catalyst per ml of degassed 20 mM sodium phosphate buffer was prepared simultaneously. Part of the catalyst solution was preactivated before use by converting it to its hydride form by incubation under a hydrogen atmosphere for 5 min. Aliquots (40 ml) of *Dunaliella* cells, preincubated in hydrogenation medium for 30 min, were placed in 100 ml custom designed reaction vessels, and transferred to a water bath at 20 °C. The reaction vessels were connected to a manifold and the gas phase evacuated and replaced by oxygen-free nitrogen (controls) or hydrogen, each maintained at a pressure of 1 atmosphere. Aliquots of the inactive or the preactivated form of $Pd(QS)_2$ were then injected into the cell suspension through a rubber septum to initiate the reaction. The final concentration of either form of the complex was 0.1 mg/ml. During the hydrogenation procedure (lasting a maximum of 20 min), reaction vessels were rotated at 45 rev/min. At the end of the desired incubation *Dunaliella* cells were removed from the vessel, and the reaction was stopped by bubbling the suspension with air for 20–30 s. The retroconversion of the activated complex to its oxidized form could be ascertained by the color changes (from brownish-green to brownish-red). Finally the cell suspension was filtered through a 30 μm filter and spun down at $370 \times g$ for 7 min. After washing once with the hydrogenation medium to remove remaining catalyst, cells were harvested for lipid extraction or cell fractionation. To assess the effect of the different conditions involved in the hydrogenation process on the rate of cell growth, cell density, initially adjusted to $5 \cdot 10^5$ cells/ml, was measured following the manipulations using a Coulter Counter model ZB. The integrity of the cells immediately after hydrogenation was tested by fluorescence microscopy with the stain fluorescein diacetate (FDA) [11]. The FDA solution (0.01%, w/v) was prepared by diluting a 5 mg/ml acetone solution with *Dunaliella* hydrogenation medium. After mixing 1 drop of the dye solution with 1 drop of a cell suspension on a glass microscope slide, fluorescence in live cells became detectable after about 2 min.

Cell fractionation, lipid isolation and analysis

Cells ($(1-3)\cdot 10^6$ cells/ml) were harvested by centrifugation at $370\times g$ for 7 min and resuspended in cold 400 mM mannitol containing 25 mM Tris-HCl (pH 8.2), 2 mM EDTA, and 1 mM $MgCl_2$. 40 ml of the concentrated cell suspension ($(20-50)\cdot 10^6$ cells/ml) were placed in a cold Parr pressure bomb and equilibrated for 15 min at 120 $lb\cdot inch^2$ in N_2 gas. The suspension was released from the bomb at a flow rate of 200 ml/min and then centrifuged at $2000\times g$ for 5 min. The resulting pellet was highly enriched in chloroplasts [9]. Centrifugation of the $2000\times g$ supernatant at $20\,000\times g$ for 15 min yielded a pellet (intermediate fraction). The $20\,000\times g$ supernatant was designated the microsomal fraction since most of the lipids there are recoverable in the pellet following the more conventional $100\,000\times g$ centrifugation for 60 min. Lipids were extracted using the procedure of Bligh and Dyer [12]. For the isolation of total phospho- and glycolipids, the lipid extract was chromatographed on silicic acid (100 mesh), eluting neutral lipids with chloroform, galactolipids with acetone and phospholipids with chloroform/methanol (1 : 1, v/v). The individual lipid classes: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulpholipid, phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), and diacylglyceryltrimethylhomoserine (DGTS), were isolated by two-dimensional TLC. Lipid extracts were spotted on pre-coated silica gel plates (Kieselgel 60 F_{254}, Merck) and developed in the first dimension in chloroform/acetone/methanol/acetic acid/water (50 : 20 : 10 : 10 : 5, by vol.). Plates then were dried under vacuum and developed in the second dimension in acetone/benzene/water (91 : 30 : 8, by vol.). Lipid components, visualized under ultraviolet light after spraying with primuline, were identified using specific test reagents and by comparing their mobilities with those of authentic standards. Fatty acid methyl esters were prepared from the complex lipids by scraping the spots into HCl/MeOH and reacting for 120 min under N_2 at 80 °C. The analysis of fatty acids was performed as previously described [10]. All the data presented here are the mean of three or more independent sets of experiments.

Results

Hydrogenation of purified Dunaliella lipids

Before conducting experiments with living *Dunaliella* cells, preliminary studies were carried out on lipids extracted from the cells. The glycerolipids were very susceptible to hydrogenation when multilamellar liposomes of whole cell polar lipids (phospholipids and glycolipids) were incubated in buffer at 20 °C under 1 atm of H_2 pressure. The rates of hydrogenation (Fig. 1) were generally similar to those observed under like conditions using dilinolenoylphosphatidylcholine or other synthetic phospholipids [10]. The various *Dunaliella* glycolipids and phospholipids were all hydrogenated rapidly (Table I). Interestingly, in the initial stages of hydrogenation MGDG and PE, lipid classes that are both prone to form a non-bilayer conformation that seems somewhat resistant to reduction by the catalyst [10], were the slowest components to react.

Effect of hydrogenation on the survival of Dunaliella cells

Initial experiments with the living cells were designed to test their ability to survive the reaction. Growing cultures ($5\cdot 10^5$ cells/ml) of *Duna-*

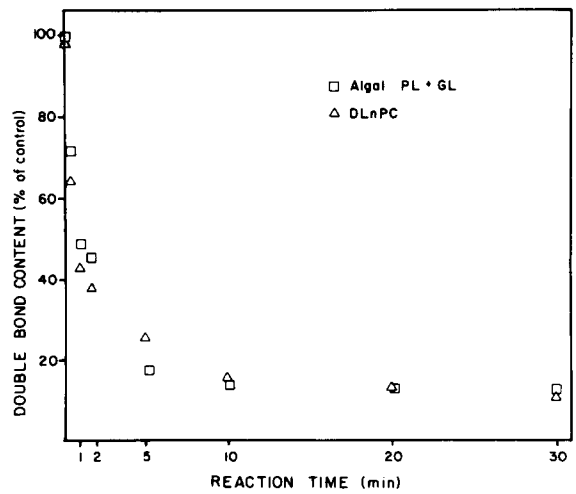

Fig. 1. Hydrogenation of multilamellar liposomes prepared from *Dunaliella* whole cell polar lipids (phospholipids and glycolipids) (□) and synthetic dilinolenoylphosphatidylcholine (△). The lipids were suspended at 20 °C in hydrogenation medium at a concentration of 0.25 mg/ml. Catalyst was present at a level of 0.1 mg/ml. For further details see the text.

TABLE I

SUSCEPTIBILITY TO HYDROGENATION OF DIFFERENT LIPID CLASSES OF *D. SALINA* PRESENT IN WHOLE CELL POLAR LIPIDS EXTRACTED, FORMED INTO MULTILAMELLAR LIPOSOMES, AND REACTED WITH CATALYST FOR TWO SELECTED TIME PERIODS

The mixed polar lipids at 0.25 mg/ml (equivalent to approximately $2.4 \cdot 10^6$ cells/ml) were hydrogenated as described in Materials and Methods. SL, sulpholipid.

Lipid class	Initial unsaturation (double bonds/100 FA)	% of original double bonds present at	
		0.5 min	20 min
MGDG	329	74	14
DGDG	204	50	14
SL	121	59	14
PG	123	55	11
PE	103	80	8
PC	81	60	11
DGTS	131	34	7

liella were concentrated, resuspended in hydrogenation medium, and exposed to the different conditions of the hydrogenation process one by one. Cell viability, as judged by comparing the cell density with that of control cultures 24 and 48 h after treatment, was not affected by the presence of hydrogen or of catalyst in its oxidized (inactive) form, or by rotation of the reaction flask, when each of these parameters was tested individually. Likewise, when cells were subjected to the full conditions needed for hydrogenation with the exception that H_2 was replaced by N_2, cell growth, as estimated by measuring cell density 24 h following the reaction, was not adversely affected.

However, when the catalyst was activated by H_2 before its addition to a cell suspension maintained under H_2, cell viability was markedly affected (Fig. 2). The survival of *Dunaliella* was dependent upon the ratio of active catalyst to cells. Irreversible damage to cells was delayed in experiments where the catalyst was introduced in an oxidized state and reduced in situ by H_2 (Fig. 2, upper curve).

Using the lower reduced catalyst/cell ratio (Fig. 2, middle curve), a more systematic analysis of cell viability was conducted. Immediately after hydrogenation of the cells for increasing periods of time

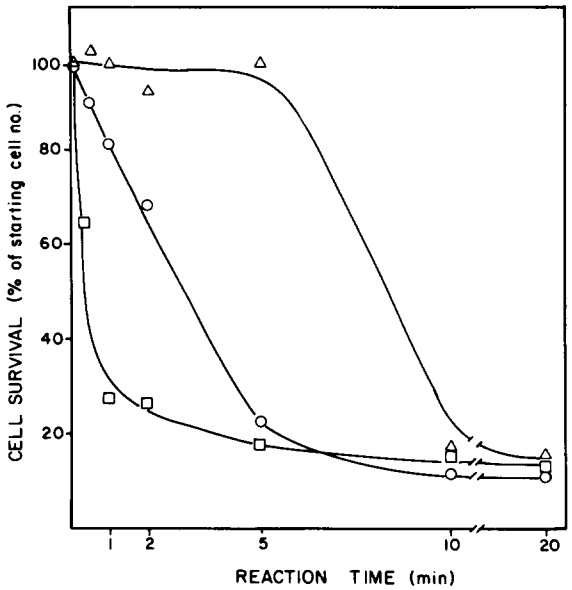

Fig. 2. Survival of *Dunaliella* cells as estimated by measuring the cell density 24 h following hydrogenation for different time intervals. All experiments utilized cultures grown to a density of $5 \cdot 10^5$ cells/ml, and then concentrated for use. Reactions involved either in situ activation of the catalyst by addition of hydrogen to vessels containing $Pd(QS)_2$ and $2.5 \cdot 10^6$ cells/ml (△) or the addition of the preactivated hydride form of $Pd(QS)_2$ to cells adjusted to a concentration of $1 \cdot 10^6$ cells/ml (□) or $2.5 \cdot 10^6$ cells/ml (○).

the integrity of the cells was tested with the vital dye fluorescein diacetate, which produces an intense fluorescence in living but not dead cells

TABLE II

THE INTEGRITY OF *DUNALIELLA* CELLS TESTED WITH FLUORESCEIN DIACETATE STAINING IMMEDIATELY AFTER HYDROGENATION

Catalyst was preactivated for 5 min and added to give a final concentration of 0.1 mg/ml in a suspension of $2.5 \cdot 10^6$ cells/ml.

Treatment	Viable cells (% of total)
Control [a]	94
Control [b]	94
Hydrogenation for 0.5 min	81
Hydrogenation for 1.0 min	78
Hydrogenation for 2.0 min	75
Hydrogenation for 5.0 min	14
Hydrogenation for 10.0 min	1

[a] No treatment.

[b] After degassing by vacuum three times for 20 s each, cells were rotated under nitrogen in the presence of inactive catalyst.

when examined microscopically [11]. By this criterion, the percentage of cells surviving a 2 min treatment was high, but exposure to the active catalyst for 5 min or more caused severe losses (Table II). Periodic cell counts made on hydrogenated cultures washed free of catalyst and resuspended in fresh growth medium confirmed that cells treated for 2 min or less were capable of recovering and resuming growth at normal rates (Fig. 3).

Hydrogenation of Dunaliella lipids in vivo

A detailed comparison was made of lipid hydrogenation rates in living cells and in liposomes prepared from whole cell total polar lipids. Initial findings showed that liposomes were much more susceptible to hydrogenation, as measured by the rates at which the most unsaturated fatty acids, 18 : 3 and 16 : 4, were reduced to more saturated acids (Fig. 4).

Following exposure to active catalyst for two selected time intervals, 1 min (ensuring cell survival) and 10 min (leading to gradual cell death), a more detailed lipid analysis revealed that certain lipid classes were virtually untouched by short term exposure of intact *Dunaliella* cells to active catalyst (Table III). These lipids included MGDG, DGDG and sulpholipid, all of which are located exclusively in chloroplasts. PG, a lipid found mainly in the chloroplast, was also slow to become hydrogenated. Although one of these lipids, MGDG, is also somewhat resistant to hydrogenation in liposome preparations (Table I), possibly due to its tendency to assume a hexagonal II conformation [10], our conclusion is that chloroplasts of the living cells are not readily accessible to the catalyst.

Intracellular sites of in vivo lipid hydrogenation

Following a 2 min hydrogenation of intact cells ($2.5 \cdot 10^6$ cells/ml and 0.1 mg catalyst/ml), chloroplasts, an intermediate fraction, and microsomes were isolated for lipid extraction. There was no statistically significant hydrogenation of the chloroplast or intermediate fraction by this short treatment (data not shown); however, microsomal lipids were appreciably reduced. Fig. 5 illustrates the

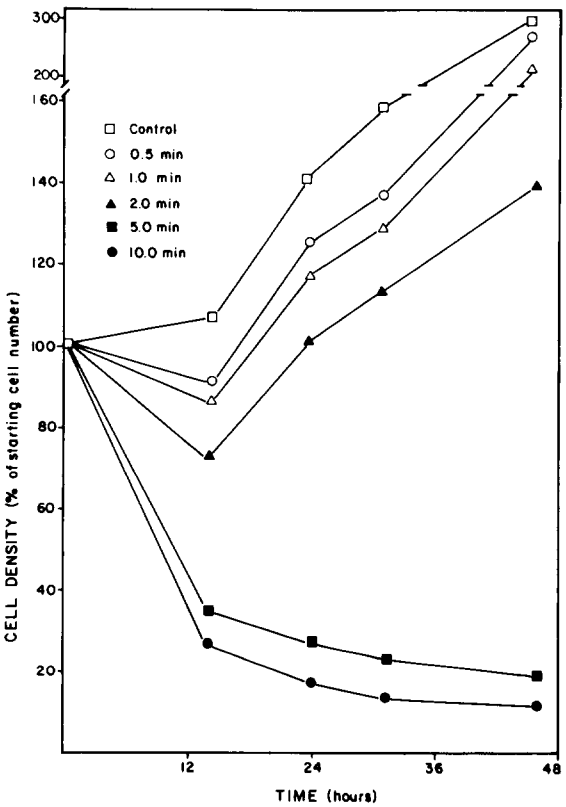

Fig. 3. Growth curves of *Dunaliella* cells following hydrogenation with preactivated catalyst for the indicated time periods. The cell density during hydrogenation was $2.5 \cdot 10^6$ cells/ml. Control cells (□) were rotated for 10 min under nitrogen in the presence of inactive catalyst prior to washing and resuspension in fresh medium for counting.

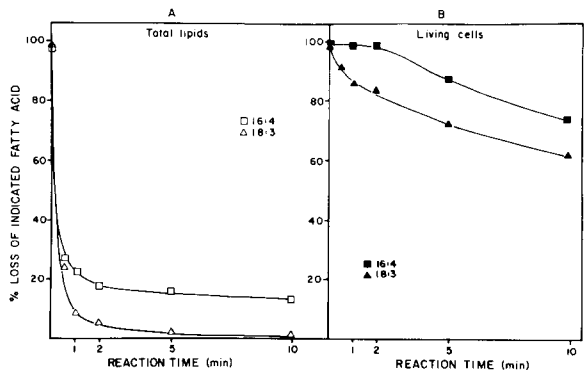

Fig. 4. Degree of lipid hydrogenation achieved in liposomes of whole cell polar lipids (A) and in living cells (B). The extent of hydrogenation is illustrated by the decrease in the level of two polyunsaturated fatty acids, measured as a percentage of their content in control cell lipids. Liposomes (part A) were present at a concentration of 0.25 mg/ml, and the cell density (part B) was $2.5 \cdot 10^6$ cells/ml.

TABLE III

CHANGE IN FATTY ACID UNSATURATION OF INDIVIDUAL POLAR LIPID CLASSES AFTER IN VIVO HYDROGENATION FOR 1 min AND 10 min

Reaction conditions were the same as in Table II.

Lipid class	% of initial double bond content	
	1 min	10 min
MGDG	96	75
DGDG	94	71
Sulpholipid	98	82
PG	92	55
PE	82	50
PC	86	77
DGTS	78	61

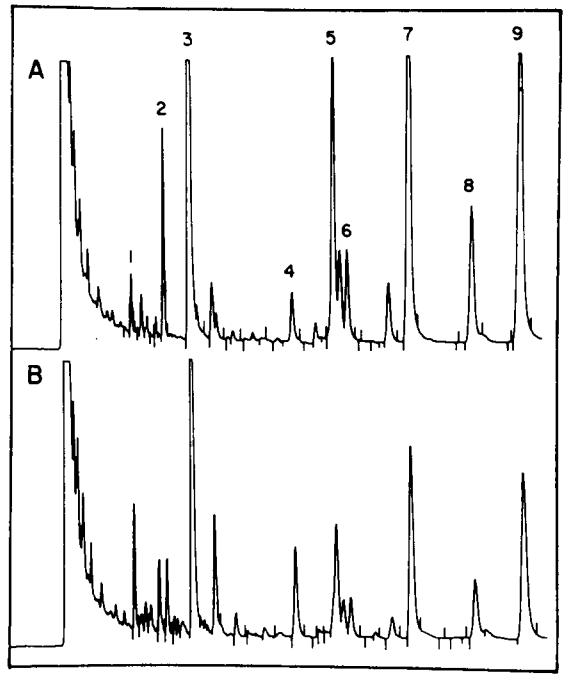

Fig. 5. Gas chromatographic tracings of fatty acid methyl esters prepared from total lipids of control cell (A) and 2 min hydrogenated cell (B) microsomes. Major peaks identified by number as follows: 1, 14:0; 2, 14:2; 3, 16:0; 4, 18:0; 5, 18:1; 6, 16:4; 7, 18:2; 8, γ-18:3; 9, 18:3.

fatty acid distribution in total microsomal lipids from control (upper tracing) and 2 min hydrogenated cells (lower tracing). Although cross contamination with chloroplast fragments (as detected by the presence of 16:4 (peak 6) in the microsomal lipids) was somewhat variable, it was clear from replicate experiments that the microsomal fraction was appreciably hydrogenated by a 2 min exposure to reduced catalyst.

The degree of hydrogenation following a 1 min exposure to active catalyst, although reproducible, was not as extensive as was found with isolated lipids (Fig. 4A). Because atomic absorption measurements of cells preincubated with inactive catalyst for various periods of time (data not shown), indicate that permeation of the catalyst into the *Dunaliella* cell is relatively slow, it appears that short term incubations may result mainly in hydrogenation of the surface membrane. In vitro hydrogenation of a plasma membrane-enriched fraction from *Dunaliella* under precisely

TABLE IV

EFFECT OF IN VITRO HYDROGENATION ON THE FATTY ACID COMPOSITION OF TOTAL LIPIDS FROM A *DUNALIELLA* PLASMA MEMBRANE-ENRICHED FRACTION

Plasma membrane enriched fraction was prepared by centrifuging the 20000 × *g* supernatant from the disrupted cells (see Materials and Methods) for 10 min at 660 × *g* in a two phase system composed of 6.7% w/v each of polyethylene glycol (average mol. wt. 3350, Sigma Chem. Co., St. Louis) and dextran (average mol. wt. 500000, Pharmacia, Uppsala, Sweden) according to Peeler et al. (Peeler, T.C., Stephenson, M.B., Einspahr, K.J., and Thompson, G.A., unpublished observations). Data are expressed as weight %.

Hydrogenation, time (min)	Fatty acid [a] composition											
	14:0	14:1	14:2	16:0	16:1	18:0	*t*18:1	18:1	il8:2	18:2	γ-18:3	18:3
0	1.5	1.9	15.1	45.0	1.8	1.4	–	6.6	4.5	11.1	3.8	7.1
2	6.4	4.0	4.8	47.0	3.5	14.1	5.4	6.5	1.0	4.0	1.2	2.0
10	7.7	2.7	1.5	49.8	0.5	22.3	5.3	4.0	1.6	2.4	0.7	1.4

[a] The number preceding the colon indicates the number of carbon atoms present while that following the colon depicts the number of double bonds.

the same conditions utilized to obtain whole cell data yielded very rapid reduction of all unsaturated fatty acids (Table IV).

Retroconversion of hydrogenated lipids to their original unsaturated form

The fact that briefly hydrogenated *Dunaliella* cells can survive and resume active growth (Fig. 3) implies that they are capable of reversing or overcoming the inhibitory effects of the lipid alterations. To investigate this point, microsomal lipids were prepared from cells at various times following their hydrogenation for 1 min – a time selected to achieve a measurable reduction of double bonds while assuring survival of nearly all the cells. The composition of microsomal total fatty acids isolated from cells incubated for 21 h following the 1 min hydrogenation (during which time the cell density declined to 93% and then increased to 97% of the starting value) had recovered to virtually the same degree of unsaturation found in the microsomal lipids prior to hydrogenation (Table V). Preliminary experiments to establish the time course of microsomal retroconversion have shown that replacement of the double bonds takes place mainly between 7 h (no retroconversion apparent) and 12 h (almost complete return to the natural fatty acid pattern). Further experimentation is underway to confirm the kinetics of the process.

TABLE V

FATTY ACID COMPOSITION OF TOTAL MICROSOMAL LIPIDS ISOLATED IMMEDIATELY AFTER 1 min HYDROGENATION OF CELLS OR FOLLOWING FURTHER INCUBATION FOR 21 h

	Fatty acid [a] composition				
	18:0	18:1	18:2	γ-18:3	18:3
Control [b]	3.0	11.3	28.2	10.4	47.1
Hydrogenated for 1 min	9.5	13.7	25.7	8.9	42.2
Hydrogenated 1 min + incubated for 21 h	3.3	11.2	26.9	10.0	48.6

[a] To highlight the effect of hydrogenation, only the fatty acid pattern of C_{18}-fatty acids is presented. Data are presented as weight %.

[b] After degassing by vacuum three times for 20 s each, cells were rotated under nitrogen in the presence of inactive catalyst.

Discussion

Many cells, when exposed to environmental stress, appear capable of regulating the composition of their membrane lipids so as to maintain the membranes in a physical state optimal for physiological function [1]. Regulation is imposed upon a variety of lipid metabolic pathways, but the most prominent and carefully examined is that controlling the degree of glycerolipid-bound fatty acid unsaturation [2,13,14].

The preferred experimental approach to the study of fatty acid desaturase control has involved perturbing cells by an environmental stress, often low temperature, and then analyzing changes in desaturase activity as the cell restores the physical state of its membrane from a relatively rigid condition to a physiologically adequate condition. Unfortunately, the stress employed to trigger a change in fatty acid desaturase activity usually affects cellular metabolism in a number of other ways as well, thereby complicating efforts to understand the molecular mechanisms involved in desaturase regulation. Increased acyl chain saturation and physiologically damaging increases in membrane microviscosity have also been achieved by massive dietary supplementation with saturated fatty acids [1], but this too can result in a variety of confusing metabolic imbalances.

Hydrogenation of membrane-associated fatty acid chains by the $Pd(QS)_2$ catalyst [3–7] and particularly the development of very mild conditions offers an alternative tactic making possible a significant rigidification of the membrane lipids under conditions which do not require increased fatty acid uptake and appear not to perturb other metabolic pathways. $Pd(QS)_2$ has been shown to be selective in hydrogenating the double bonds of long chain fatty acids but not those of chlorophyll, carotenoids and other isoprenoids [15]. For this reason it offers a new and potentially sensitive tool for determining how fatty acid desaturases are regulated.

Although hydrogenation by this catalyst is specific for fatty acyl chains, it does lead to complications of a different sort, namely, the generation of partly saturated fatty acid isomers not normally found in unreacted cells. These isomers can now be easily identified as a result of our

parallel studies of catalyst action on synthetic phospholipids [10]. Preliminary investigation of the short term *D. salina* response following in vivo hydrogenation (Vigh, L., Horváth, I. and Thompson, G., unpublished observations) showed that the unnatural isomers are present in only small amounts and are largely converted to more typical fatty acids within a few hours.

When the reaction time is limited to 2 min or less under the conditions employed here, the $Pd(QS)_2$ catalyst reduced the double bonds of only those lipids present in membranes near the cell surface. Thus the microsomal fraction, which includes elements of the endoplasmic reticulum and the Golgi apparatus as well as the plasma membrane, showed decreased unsaturation in its fatty acids (Fig. 5, Table V) while the chloroplast did not. In view of the ease with which an isolated plasma membrane-enriched fraction was hydrogenated in vitro (Table IV), it is likely that this membrane, accounting for approx. 30% of the microsomal lipids [16], was also the most extensively reduced structure in the intact cell. We have not yet made a satisfactory comparison of plasma membrane isolated from control and hydrogenated cells because in vivo hydrogenation renders the plasma membrane more fragile and difficult to purify. Improvements in the capacity of the dextran-polyethyleneglycol aqueous two phase system [17] to resolve hydrogenated as well as non-hydrogenated plasma membrane (Peeler, T., unpublished observations) will permit a better opportunity to determine how much hydrogenation of the cell surface lipids can be tolerated before irreversible damage occurs. Evidence for selective catalytic hydrogenation of a peripherally located membrane has been reported using the blue green alga *Anacystis nidulans* [4]. Here the cytoplasmic membrane lipids became hydrogenated immediately, while the inner thylakoid membranes were affected only at a much later stage.

The limited permeability of the plasma membrane for the catalyst may offer certain experimental advantages. *Dunaliella* can be loaded with inactive catalyst by a lengthy (up to 24 h) preincubation in oxygenated $Pd(QS)_2$ without any toxic effects (Horváth, I., Vigh, L. and Thompson, G., unpublished observations). Our recent trials have confirmed a greatly enhanced hydrogenation of cellular lipids following treatment of these preincubated cells with hydrogen. By washing such cells free of extracellular catalyst and exposing them to hydrogen after judiciously chosen times, different groups of organelles can be hydrogenated selectively, depending upon the extent of permeation into or, conversely, back out of the cells.

This system is well suited for studying the metabolic regulation of fatty acid desaturase action. The initial studies described here confirm that briefly hydrogenated cells can support a retroconversion of the reduced fatty acyl chains to their original unsaturated form in less than 21 h. During this period of time the recovering cells have not experienced enough new growth to significantly affect the lipid composition by dilution. Experiments are now underway to establish whether fatty acid desaturases of the endoplasmic reticulum are more active during this recovery period than they are in control cells and whether any increase in activity is due to the synthesis of new desaturases or to further activation of preexisting enzymes. These as well as other means of regulating the membrane's physical properties, such as glycerolipid molecular species retailoring [18] and agonist-stimulated phospholipid hydrolysis [19,20] can be fruitfully examined using the *Dunaliella* system perturbed by selective lipid hydrogenation.

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