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PLASMA MEMBRANE FROM *CANDIDA TROPICALIS* GROWN ON GLUCOSE OR HEXADECANE

II. BIOCHEMICAL PROPERTIES AND SUBSTRATE-INDUCED ALTERATIONS

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Summary

Isolated plasma membranes from the yeast *Candida tropicalis* grown on two different carbon sources (glucose or hexadecane), had similar contents of protein (60% of total dry weight), lipid (21—24%) and carbohydrates (16—21%). Sodium dodecyl sulphate gel electrophoresis of the membrane proteins revealed 17 and 19 protein bands, respectively, for glucose and hexadecane grown cells. There were marked differences in R_F values and relative peak heights between the two gels. Sterols and free fatty acids were the major components of the plasma membrane lipids. Phospholipid content was less than 2% of total plasma membrane lipids. Membrane microviscosity, as determined by fluorescence polarization, was very high (16.6 P). Fatty acid determination of membrane lipids by gas chromatography showed a significant increase of C_{16} fatty acids in plasma membranes of cells grown on hexadecane.

Reduced-oxidized difference spectra demonstrated the presence of a *b*-type cytochrome in both *Saccharomyces cerevisiae* and *C. tropicalis* plasma membranes. Its concentration in *C. tropicalis* plasma membranes was three-fold greater in cells grown on hexadecane than in glucose grown cells.

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Introduction

Studies on *Tetrahymena* cells have revealed that membranes alter their composition and function in relation to cell cycle and various environmental changes [1]. Several reports have appeared [2—7] regarding the properties and chemical composition of yeast plasma membranes, but only a few investigations have dealt with plasma membrane alterations caused by environmental changes. Nurminen et al. [8] examined the lipid composition of plasma membranes from *Saccharomyces cerevisiae*, aerobically and anaerobically grown. The plasma membrane of aerobic cells contained more unsaturated fatty acids and more than ten times as much ergosterol as the plasma membranes from anaerobic cells. Marriot [6] investigated the plasma membranes from *Candida albicans* in the yeast and mycelical form. Marked differences were observed between these two forms in content of the phospholipids, free and esterified sterols and total fatty acids.

The yeast *Candida tropicalis* grows on hydrocarbons as the sole carbon source. The uptake and oxidation of such water insoluble substrates remains poorly understood. It is probable that the yeast plasma membrane, known to be involved in many solute transport functions, is responsible for these processes. It was thus of interest to compare plasma membranes from *C. tropicalis* grown on hexadecane and glucose and, for comparison, from *S. cerevisiae*, since the latter yeast is most extensively characterized. The results of these studies are reported here.

Materials and Methods

Organisms and cell culture. The following yeast strains were used for the isolation of plasma membranes: *Candida tropicalis* ATCC 32113, grown on glucose or hexadecane and, for comparison, *Saccharomyces cerevisiae* LBG H 1022 and *Saccharomyces cerevisiae* DGI 251 (Hefefabriken Hindelbank AG, Bern), grown on glucose.

Batch cultivation was carried out in an aerated bioreactor at 30°C on a carbon-limited medium according to Divjak and Mor [9]. The concentrations of carbon sources were 1% for the hexadecane and 2.3% for the glucose. The cells were harvested in the early stationary phase and were washed twice in distilled water and once with osmotic stabilizer solution (0.4 M KCl/20 mM triethanolamine, pH 7.0).

Isolation of plasma membranes. The method of plasma membrane vesicle preparation from *S. cerevisiae* has been described by Fuhrmann et al. [10]. For the isolation of plasma membranes from *C. tropicalis*, a slightly modified procedure was used as described in our previous paper [11].

Analytical methods. Protein was determined according to Lowry et al. [12] with bovine serum albumin as standard.

For the extraction of lipids the method described by Kaneko et al. [13] was used. About 10—30 mg of lyophilized membranes were treated with 5 ml chloroform/methanol (2 : 1, v/v) for 1 h at room temperature on a rotary shaker. The extraction was repeated twice for 2 h with fresh solvent. After rotary evaporation of the solvent, the dry residue of the combined extracts was

reextracted with chloroform for 1 h. Extraction was repeated twice for 30 min. The extracts were combined and filtered. After rotary evaporation of the chloroform and subsequent drying under N_2 at room temperature for 10 min the dry weight of the extract was determined.

Lipid phosphorus was measured by the method of Fiske and SubbaRow [14].

Chromatographic analysis of neutral lipids was performed on silica gel HR plates (Merck, Germany) in a one-dimensional thin-layer chromatography system developed by Kosaric and Carroll [15], with petroleum ether/ether/acetic acid (60 : 40 : 1, v/v/v) as solvent. The plates were sprayed with sulfuric acid (50%) and heated at 160° C for 10 min. Identification of the neutral lipids was achieved by comparison of their R_F values to lipid standards (Supelco, Inc., PA, U.S.A.). Polar lipids were separated in a one-dimensional system using chloroform/methanol/water (65 : 20 : 3, v/v/v) as developing solvent [15]. Phospholipids were stained by spraying the chromatograms wity a specific reagent for phosphatides developed by Vaskovski and Kostetsky [16].

Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis of the plasma membrane proteins was performed according to the method of Fairbanks et al. [17]. The acrylamide/bisacrylamide concentration was 7.06% and about 100 μg of protein was applied per gel. Molecular weight markers for SDS gel calibration were bovine serum albumin, ovalbumin, chymotrypsinogen A, myoglobulin from horse and cytochrome *c* (Serva Feinbiochemie, Heidelberg, F.R.G.). Methyl green was used as a tracking dye. Gels were stained with Coomassie Brilliant Blue or by the periodic acid-Schiff procedure [17] and scanned at 550 nm.

Gas chromatographic determination of fatty acids. The acids were transesterified from the extracted membrane lipids with 5 ml of 20% methanolic H_2SO_4 at 60° C overnight. The fatty acid methyl esters were extracted with pentane and samples were analysed with a Beckman GC 4 gas chromatograph fitted with DEGS-PS (10% on Supelcoport; 80/100 mesh) stainless steel columns (0.32 × 250 cm). The temperature was programmed from 120 to 200° C at a rate of 10° C/min. The nitrogen flow was 20 ml/min. Fatty acids were identified by comparing their retention time to fatty acid methyl ester standard test mixtures (Supelco, PA, U.S.A.).

Amino acids of the membrane proteins were analyzed according to the method of Moore and Stein [18]. Hydrolysis was carried out with 6 M HCl for 22 h in vacuo at 108° C with lyophilized membranes. Total carbohydrate, mannan, glucan and acid- and alkali-soluble glycogen were determined with the anthrone reagent, following the fractionation scheme developed by Trevelyan and Harrison [19]. Mannose was used as reference.

Microviscosity of the plasma membranes was estimated using the fluorescence polarization technique described by Shinitzky and Barenholz [10] with 1,6-diphenyl-1,3,5-hexatriene (DPH) as a fluorescent probe 50 μl of a 2 mM stock solution of DPH in tetrahydrofuran was sonicated at low energy in 25 ml of water. About 400 μg of membrane vesicles were incubated in this DPH suspension at 37° C for 30 min. Subsequently the fluorescence measurements were performed on a ELSINCT Microviscosimeter model MV-1a at 25° C.

Cytochromes were determined by difference spectroscopy of membrane

suspensions at room temperature. All vesicle preparations were suspended in 100 mM phosphate buffer at pH 7.4 to a concentration of 3—4 mg protein/ml. The contents of the sample cuvette were reduced by a few grains of sodium dithionite. Difference spectra were recorded on a Beckman ACTA C III spectrophotometer using a scattered transmission accessory 569599 for highly scattering samples. For quantitative determination of cytochrome *b* the wavelength pair 423, 409 nm and extinction coefficient 164 $mM^{-1} \cdot cm^{-1}$ [21] were used.

Results

Overall composition. In order to obtain reliable comparison of plasma membranes from various cell types, every effort was made to treat parallel samples identically. Table I gives the overall composition of theplasma membranes from *S. cerevisiae* and *C. tropicalis*, the latter grown on glucose or hexadecane. The largest membrane component was protein, which represented about 60% and 50%, respectively, of the dry weight of plasma membranes from *C. tropicalis* and *S. cerevisiae*. About 20% of dry weight was lipids and about 20% was carbohydrates in all three membrane types.

Differences in the overall composition of plasma membranes from *C. tropicalis* were not significant in relation to the different carbon sources, glucose and hexadecane.

Membrane proteins. The plasma membrane proteins were qualitatively analyzed by SDS polyacrylamide gel electrophoresis. The acrylamide/polyacrylamide concentration was 7.06% and permitted good resolution of proteins between 10 000 and 200 000 daltons.

The protein patterns obtained by densitometric scanning of gels stained with Coomassie Blue or by the periodic acid-Schiff procedure are shown in Fig. 1. The R_F values and the densitometer readings for the various protein bands are indicated by the solid peaks. In all, 20 protein bands were found for *S. cerevisiae*, 17 bands for glucose grown, and 18 bands for hexadecane grown *C. tropicalis*. The protein profiles of the three different plasme membranes are distinctly dissimilar. However, some protein bands show similar R_F values. Most

TABLE I

OVERALL COMPOSITION OF PLASMAMEMBRANES FROM *S. CEREVISIAE* AND *C. TROPICALIS*, GROWN ON GLUCOSE AND HEXADECANE

Results are given in percent of total dry weight of lyophilized membrane preparations and are expressed as means ± S.D. Number of experiments in parentheses.

	S. cerevisiae H 1022	*C. tropicalis* grown on	
		Glucose	Hexadecane
Protein	47.2 ± 6.7 (9)	63.3 ± 4.6 (10)	59.3 ± 5.9 (8)
Lipid	24.3 ± 2.9 (6)	21.2 ± 2.4 (10)	24.5 ± 2.1 (8)
Carbohydrate	16.8 (2)	21.2 (2)	16.2 (2)
Total	88.3	105.7	100.0
Protein:lipid	2:1	3:1	2.5:1

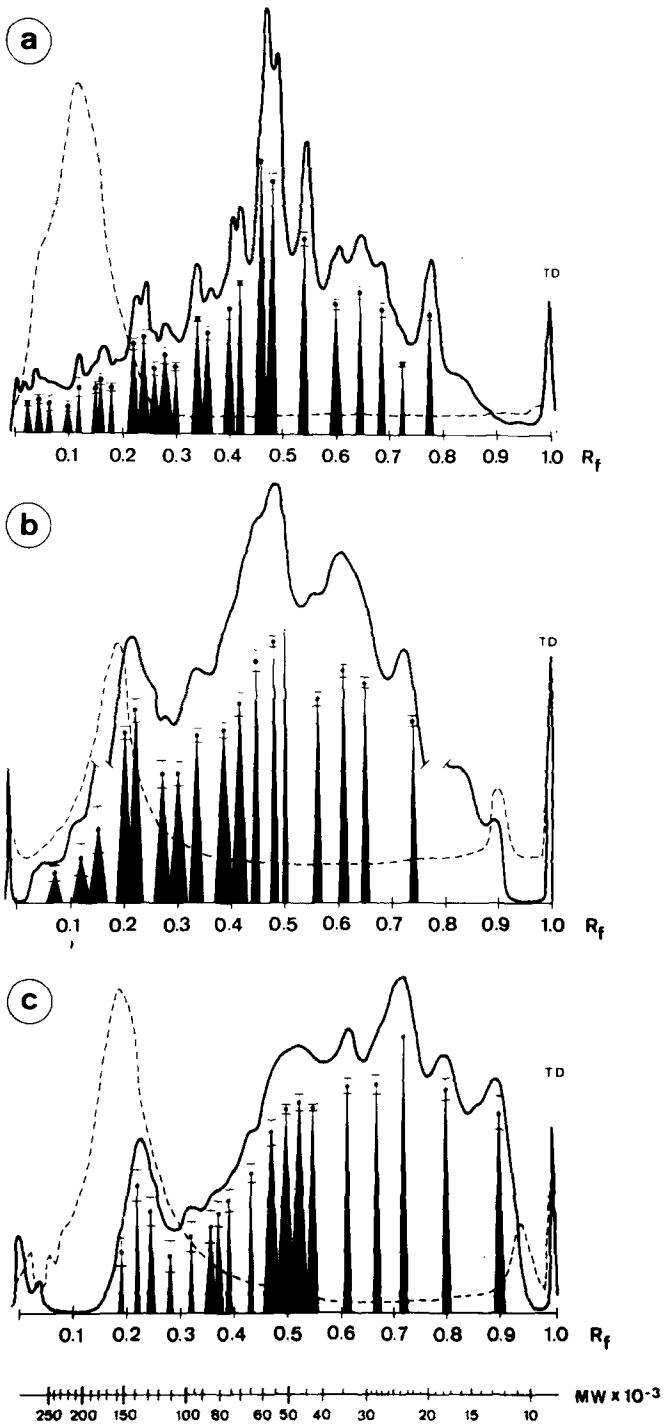

Fig. 1. SDS acrylamide gel electrophoresis of plasma membrane from *S. cerevisiae* DGI 251 (a) and *C. tropicalis*, grown on glucose (b) and hexadecane (c). The unbroken lines represent the scans after Coomassie Blue staining of proteins, whereas the broken lines show that periodic acid-Schiff areas. The triangles depict the single protein bands. The width of the base of the triangles represents the standard error of the average R_F value from 6—10 experiments and the height of the triangles represents relative protein concentration ± S.E. Td, tracking dye.

of the stained material from glucose grown cells of both yeast species exhibited molecular weight between 50 000 to 60 000 daltons, whereas the peak with highest density for *C. tropicalis* grown on hexadecane was at about 25 000. The plasma membranes of hexadecane grown cells showed at least one large low molecular weight protein band which was absent in the other two protein profiles.

The periodic acid-Schiff-positive, carbohydrate containing area is represented by the dotted lines in Fig. 1. A glycoprotein peak is seen at around 150 000—200 000 daltons in all three gels. The small peaks at the front of the gels probably represent glycolipids overlapping with the lipid peak in the protein scan.

Amino acid composition. The amino acid compositions of all three plasma membrane types were, on the whole, similar (Table II). The amount of acidic amino acids was twice as much as that of basic amino acids. Tryptophan was absent due to its destruction during acid hydrolysis [18].

Lipid analysis. Table III shows the phospholipid portions in relation to total lipid content of the plasma membranes from *S. cerevisiae* and *C. tropicalis*. These results are based on phosphorus determinations according to Fiske and SubbaRow [14] after acid hydrolysis of lipids with perchloric acid.

The phospholipid content of *S. cerevisiae* plasma membranes was 8—9% of total lipid, whereas the phospholipid content of both *C. tropicalis* types was less than 2%. More accurate measurements at this low concentration were not possible due to lack of sufficient membrane starting material. This low phospholipid content was an unexpected finding. In order to verify further this observation, the residue remaining after chloroform extraction was reextracted with chloroform/methanol (2 : 1, v/v) for 3 h; however, no additional phospholipid was recovered as indicated by thin-layer chromatography.

TABLE II

OVERALL COMPOSITION OF PLASMA MEMBRANES FROM *S. CEREVISIAE* AND *C. TROPICALIS*

Hydrolysis were carried out as described in the text for 22 h. Results are expressed in percent of total amino acids as means ± S.D. of two analyses.

Amino acid	*S. cerevisiae* DGI 251	*C. tropicalis* grown on	
		Glucose	Hexadecane
Aspartic acid	9.7 ± 0.0	10.0 ± 0.0	10.4 ± 0.2
Threonine	5.9 ± 0.3	7.2 ± 0.0	7.5 ± 0.5
Serine	7.7 ± 0.3	8.4 ± 0.0	8.4 ± 0.4
Glutamic acid	10.3 ± 0.2	9.2 ± 0.1	9.4 ± 0.3
Proline	5.5 ± 0.1	4.4 ± 0.0	4.3 ± 0.1
Glycine	8.1 ± 0.8	8.4 ± 0.4	8.2 ± 0.0
Alanine	8.2 ± 0.1	9.2 ± 0.0	8.9 ± 0.3
Cysteic acid	0.4 ± 0.0	trace	trace
Valine	6.9 ± 0.2	6.9 ± 0.0	7.1 ± 0.1
Methionine	1.7 ± 0.2	1.8 ± 0.3	1.4 ± 0.0
Isoleucine	5.2 ± 0.1	5.3 ± 0.0	5.2 ± 0.1
Leucine	9.1 ± 0.5	9.2 ± 0.3	9.4 ± 0.1
Tyrosine	3.3 ± 0.0	3.3 ± 0.2	3.3 ± 0.5
Phenylalanine	5.2 ± 0.3	6.1 ± 0.1	5.8 ± 0.1
Histidin	1.7 ± 0.1	1.4 ± 0.0	1.5 ± 0.1
Lysine	7.1 ± 0.5	5.3 ± 0.1	5.7 ± 0.4
Arginine	4.5 ± 0.3	3.6 ± 0.1	3.7 ± 0.3

TABLE III

PHOSPHOLIPID CONTENT OR TOTAL LIPIDS FROM PLASMA MEMBRANES OF *S. CEREVISIAE* AND *C. TROPICALIS*

Experimental details are given in the text. Number of experiments in brackets. Results are expressed as means ± S.D.

	S. cerevisiae DGI 251	*C. tropicalis* grown on	
		Glucose	Hexadecane
Phospholipids (% of total lipid)	8.6 ± 0.7 (8)	<2 (5)	<2 (5)

Fig. 2 shows a one-dimensional thin-layer chromatogram of the neutral lipids of the three plasma membranes. The neutral lipid compositions were qualitatively very similar. Monoglyceride, triglycerides, ergosterol, sterol esters and free fatty acids were detected in extracts from all three membranes. Judged by the intensities of the spots on the chromatograms after treatment with sulphuric acid, sterol esters were present in greater amounts in the plasma membrane from *S. cerevisiae* compared to those from *C. tropicalis*. On the other hand the latter contained more triglycerides. Note the high content of free fatty acids which was found in all three samples.

One-dimensional separation of the polar lipids confirmed the almost complete absence of phospholipids in plasma membranes from *C. tropicalis* (Fig. 3). Phospholipids were determined by spraying the plates with molybdate reagent

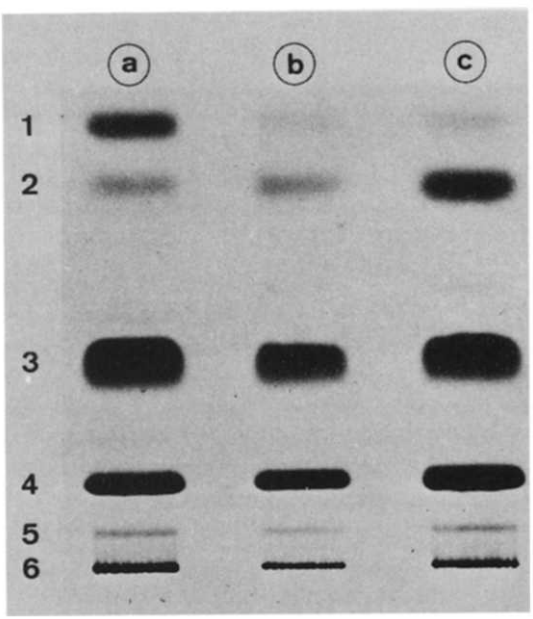

Fig. 2. Neutral lipids of plasma membranes from *S. cerevisiae* DGI 251 (a) and *C. tropicalis*, grown on glucose (b) and on hexadecane (c), separated by thin-layer chromatography. The solvent system was petroleum ether/ether/acetic acid (60 : 40 : 1, v/v/v). Lipids were detected by charring with sulphuric acid (50%). Abbreviations: 1, sterol ester; 2, triglyceride, 3, free fatty acids; 4, sterol; 5, monoglyceride; 6, base line.

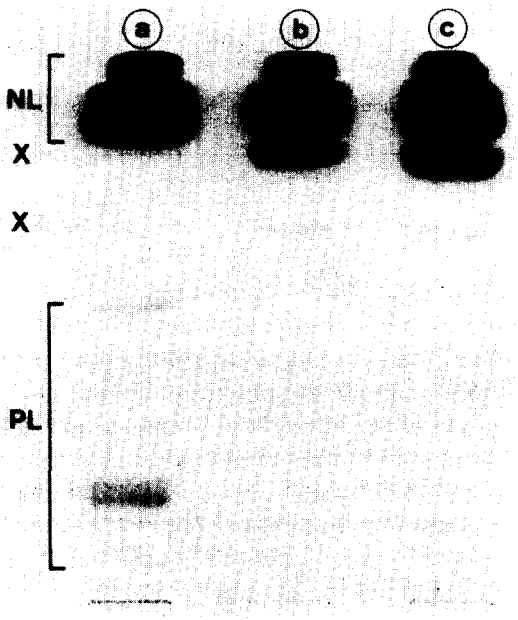

Fig. 3. Separation of polar lipids from plasma membranes from *S. cerevisiae* DGI 251 (a) and *C. tropicalis* on glucose (b) and hexadecane (c) ina a one-dimensional system with chloroform/methanol water (65 : 20 : 3, v/v/v). as developing solvent. Phospholipid were detected by spraying the chromatogram with acid molybdate reagent. PL, phospholipids; NL, neutral lipids; X, unidentified fractions.

[16]. Neutral lipids were identified by means of lipid standard. Unknown spots (X) were also detected and could represent glycolipids which remained at the origin during neutral lipid separation (Fig. 2). The fatty acid composition of the three plasma membranes is listed in Table IV. Only four major acids ($C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$) were found in the plasma membranes of *S. cerevisiae*, whereas seven fatty acids ($C_{16:0}$, $C_{16:1}$, $C_{17:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$) were present in both kinds of membrane from *C. tropicalis* was that the membranes of hexadecane-grown cells contained considerably more C_{16} fatty acids that those from glucose cells, which in turn have a higher amount of C_{18} acids.

Carbohydrates. In Table V the plasma membrane composition of *S. cerevisiae* and *C. tropicalis* is given with respect to total carbohydrate, mannan glycogen and glucan. Mannan represented the main fraction (45%) of the carbohydrates from *S. cerevisiae* plasma membranes, in contrast to those from *C. tropicalis*, which showed a high glycogen content (30—40%). In all three membrane types, glucan was found only in small amounts. No significant differences in the carbohydrate composition of both kinds of plasma membranes from *C. tropicalis* was observed.

Determination of microviscosity. In Table VI the microviscosity of plasma membranes was estimated by fluorescence polarization according to Shinitzky and Inbar [20]. For all three kinds of plasma membrane a very high microviscosity was found. The values for *C. tropicalis* membranes were even twice as high compared to those from *S. cervisiae*.

Cytochrome spectra. As described in our previous report [11] the mito-

TABLE IV

FATTY ACID COMPOSITION OF TOTAL LIPIDS FROM PLASMA MEMBRANES OF *S. CEREVISIAE* AND *C. TROPICALIS*

The fatty acids are designated *x:y*, where *x* is the number of carbon atoms and *y* the number of double bonds/molecule. Number of experiments in brackets. Results are expressed as means ± S.D.

Fatty acid (% of total fatty acids)	*S. cerevisiae* DGI 251 (7)	*C. tropicalis* on glucose (6)	*C. tropicalis* on hexadecane (7)
16:0	12.3 ± 2.5	26.8 ± 1.5	40.9 ± 3.2
16:1	40.9 ± 3.8	7.1 ± 0.6	18.9 ± 2.2
17:1	trace	1.9 ± 0.4	1.6 ± 0.4
18:1	5.5 ± 1.2	1.0 ± 0.3	1.3 ± 0.5
18:1	37.9 ± 1.7	24.3 ± 1.0	19.0 ± 3.3
18:2	trace	31.0 ± 2.6	15.7 ± 2.4
18:3	—	7.2 ± 0.8	trace
Total	96.6	99.3	97.4

TABLE V

THE COMPOSITION OF PLASMA MEMBRANES FROM *S. CEREVISIAE* AND *C. TROPICALIS* WITH RESPECT TO TOTAL CARBOHYDRATE, MANNAN, GLYCOGEN AND GLUCAN

Results are expressed in percent of total carbohydrate (*) and in mg hexose/g dry weight of membranes (**) as means of two experiments.

Plasma membranes	Total carbohydrate *	Mannan **	Glycogen **	Glucan **
S. cerevisiae DGI 251	168	45	10	2
C. tropicalis (glucose)	212	7	30	9
C. tropicalis (hexadecane)	162	8	40	6

TABLE VI

MICROVISCOSITY OF PLASMA MEMBRANES FROM *S. CEREVISIAE* AND *C. TROPICALIS*

Measurements were carried out at 25°C. Results are expressed as mean of two experiments. η, Microviscosity in P; *P*, fluorescence polarization.

	P	η
S. cerevisiae DGI 251	0.350	7.6
C. tropicalis (glucose)	0.400	16.6
C. tropicalis (hexadecane)	0.400	16.6

TABLE VII

CONTENT OF CYTOCHROME *b* IN PLASMA MEMBRANES FROM *S. CEREVISIAE* AND *C. TROPICALIS*

Number of experiments are given in brackets. Results are expressed as means ± S.D.

	S. cerevisiae H 1022 (5)	*C. tropicalis* (glucose) (4)	*C. tropicalis* (hexadecane) (6)
Cytochrome *b* (nmol/mg protein)	0.175 ± 0.01	0.105 ± 0.02	0.325 ± 0.02

chondrial contamination of the purified plasma membrane fraction was no more than 6%, as estimated enzymatically. However, to exclude definitively that the cytochromes detected in the plasma membranes were not the result of mitochondrial contamination we recorded cytochrome spectra for three fractions: the fraction consisting of mitochondrial and plasma membrane vesicles; the aggregated mitochondrial fraction and the purified plasma membrane fraction as shown in Fig. 4. Reduced-oxidized difference spectra were assayed at room temperature, scanning from 650 nm to 400 nm. Curves A illustrate the spectra of the mixed fractions of plasma membrane vesicles and mitochondria from *S. cerevisiae* (a) and *C. tropicalis*, grown on glucose (b) and on hexadecane (c), respectively. Curves B show the same spectra for the aggregated mitochondria fractions with major peaks for cytochrome oxidase (peak centred at 598 nm; shoulder at 440 nm) and cytochrome *b* (peak centred at 557 nm).

There were slightly different absorption maxima for *S. cerevisiae* and *C. tropicalis* in the Soret band (423 nm for *S. cerevisiae*; 427 for *C. tropicalis*). Curves C represent the difference spectra of the plasma membrane fractions. Only one cytochrome was found, which could be identified as a *b*-type cytochrome as judged by the absorption maxima of 557, 523 and 423 nm for *S. cerevisiae* and 557, 523 and 427 nm for *C. tropicalis*. In Table VII the cytochrome *b* content was calculated according to Yoshida et al. [21]. The plasma membranes from *C. tropicalis* grown on hexadecane contained three times more cytochrome *b* than those from glucose grown cells. No reduction of this cytochrome *b* was obtained with NADH or NADPH under anaerobic conditions.

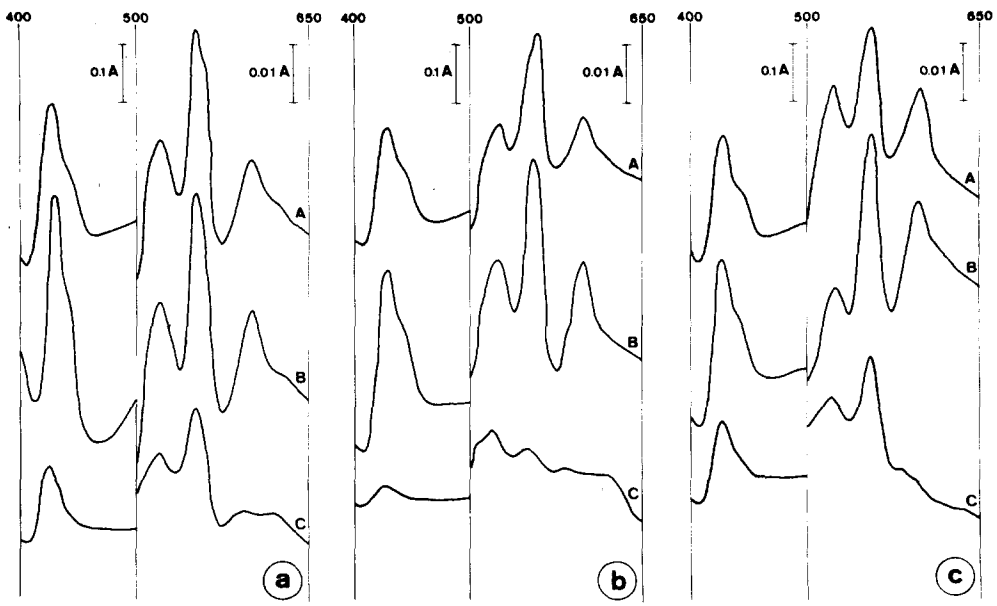

Fig. 4. Reduced-oxidized difference spectra of the mixed fraction of plasma membrane vesicles and mitochondria (A), the aggregated mitochondria (B) and the plasma membrane preparation (C) from *S. cerevisiae* H 1022 (a) and *C. tropicalis* on glucose (b) and on hexadecane (c). Reduction was carried out with dithionite. Experimental details are described in the text.

Discussion

Several reports have appeared regarding the chemical composition of plasma membranes from various types of yeast [2—7]. Generally, our observations of *C. tropicalis* plasma membranes are consistent with previously published data in terms of protein, lipid and carbohydrate content. We estimated a somewhat higher protein:lipid ratio and a substantially lower phospholipid content. However, most of the previous studies had omitted to document the purity of their plasma membrane fractions by performing a quantitative estimation for contaminating organelles. Undetected contamination, especially by mitochondrial membranes, could explain the disparity of our observations with others. For our plasma membrane preparations we measured a maximal contamination by mitochondria of 6% as shown by our previous report [11].

SDS gel electrophoresis of the three plasme membrane (*S. cerevisiae*, grown on glucose and *C. tropicalis*, grown on either glucose or hexadecane) showed 17—20 different polypeptide bands. The patterns were quite distinctly different, but some protein bands appeared to be identical in all three gels. It is noteworthy that *C. tropicalis* plasma membranes grown on either glucose or hexadecane are as different from one another as they are from *S. cerevisiae*, a different species. However, protein profiles from different strains of the same yeast species, grown similarly, are comparable. The pattern of membrane proteins from *S. cerevisiae* LBH H 1022, shown by Kramer et al. [7] corresponds exactly to our protein profile from *S. cerevisiae* DGI 251.

The difference in the protein pattern of *C. tropicalis* plasma membranes from cells, grown on different carbon sources, are probably caused by induction of specific plasma membrane proteins for uptake and degradation of *n*-alkanes [22,23]. The results of SDS gel electrophoresis for each membrane type were highly reproducible, provided phenylmethyl sulfonyl fluoride (PMSF) was included in the storage buffer to inhibit proteolytic activity. Yeast contains several proteolytic enzymes but PMSF does not inhibit all of them [24,25]. Therefore, it is possible that some modifications of membrane proteins has occured.

Periodic acid-Schiff staining was similar in gels from all three membranes, indicating similar distribution of glycoproteins, probably mannan glycoproteins [26]. The membrane proteins in this high molecular weight region above 10 000 were only slightly stained by Coomassie Blue. Similar results were previously reported for the sialo glycoproteins in human erythrocytes [27].

The overall similarity between the amino acid composition of all three plasma membrane types suggests that the specialized functions of membrane proteins do not confer any peculiar amino acid composition on these proteins as was previously mentioned by Longley et al. [4].

Although there was no difference in the lipid composition of the two types of plasma membrane from *C. tropicalis*, it was remarkable that these membranes exhibited an almost complete lack of phospholipids. When we simultaneously extracted and analyzed the plasma membrane lipids of *S. cerevisiae*, we observed similar amounts of phospholipids as described by Kramer et al. [7] and Rank et al. [28]. The action of lipolytic enzymes in the plasma membrane fractions as demonstrated in *S. cerevisiae* by Nurminen and Suomalainen [29]

could be one explanation of the near absence of phospholipids. However, we found little evidende for lyso compounds by two dimensional separation of the phospholipids. In addition, no difference in the phospholipids was observed when the plasma membrane preparation and the lipid extraction was performed in the presence of EDTA [7] which is known to inhibit the membrane-bound phospholipases of the A_2 type.

Since the free fatty acids we detected occur also in whole cells [30], and since only minor amounts of mono- and diglycerides were found by thin-layer chromatography (Fig. 2), there appears to be no triglyceride lipase activity, either.

For the extraction of membrane lipids we used a method shown to be highly suitable for the analysis of yeast lipids [31]. Furthermore, controls of our extraction method by reextracting the remaining residue after chloroform extraction did not show any evidence for additional phospholipids as checked by thin-layer chromatography. Thus, we rule out the possibility that the unexpected low phospholipid content was caused by an inadequate extraction procedure. In addition, the differences in the phospholipid content of *C. tropicalis* and *S. cerevisiae* appear to correlate with changes in the membrane viscosity as discussed below.

The fatty acid compositions of the total membrane lipids was found to be specific for the particular plasma membrane types, and were highly reproducible. Palmitoleic acid and oleic acid represented the major components of *S. cerevisiae* membranes in accordance with the results of Suomalainen and Nurminen [5], whereas in plasma membrane of *C. tropicalis* palmitic and linoleic acid were present in considerable amounts. Differences between the two types of *C. tropicalis* membranes were apparent. Plasma membranes from hexadecane grown cells contained significantly more C_{16} acids. This finding agrees with the results of Hug and Fiechter [30], who observed a direct incorporation of fatty acids with corresponding chain length of the alkane substrate into the total lipids of cells cultivated on hydrocarbons. The concomitant increase of unsaturated C_{16} acids might be explained by direct dehydration of the oxidized substrate [32]. C_{18} acids probably were produced by chain elongation. It was established by Meyer and Schweizer [33] that the endogenous fatty acid biosynthesis in the two yeast species *S. cerevisiae* and *C. lipolytica* was completely repressed by addition of long-chain fatty acids to the growth medium.

The content of carbohydrates in the yeast plasma membrane is much greater than in any intracellular membrane. This might be explained by the suggestion that cell wall synthesis is located in the plasma membrane [2,34,35]. Moreover, it is well established that yeast protoplasts have the capacity for regenerating the cell wall [36] and Cortat et al. [37] described the presence of mannan synthetase in plasma membrane fragments from *S. cerevisiae*.

In *S. cerevisiae* plasma membranes we determined in agreement with Matile et al. [2] and Fuhrmann et al. [38] that mannan is the major carbohydrate, whereas in both membrane types from *C. tropicalis* mostly glycogen was found. This might be due to marked differences in the chemical composition of the cell walls in the two yeast species as described by Käppeli [39].

The plasma membranes from cells grown on hexadecane contained three

times more cytochrome *b* than did plasma membranes from glucose grown cells. This increase of cytochromes might be related to the assimilation of hydrocarbons by *C. tropicalis*. Gallo et al. [44] observed an induction of cytochrome *b* and cytochrome *P*-450 in the microsomal fraction of alkane assimilating cells from *C. tropicalis*.

In the degradation of alkanes the paraffinic hydrocarbon is first transformed into a primary alcohol by monoterminal oxidation [45]. According to several authors, this reaction is catalyzed by enzyme systems involving cytochromes *P*-450 and b_5 [44—48]. Al already mentioned above, it was reported by Liu and Johnson [22] and Rhode et al. [23] that the plasma membrane may represent the membrane system for alkane oxidation.

Our observation of an increase in cytochrome *b* induced by hydrocarbon assimilation supports this assumption. On the other hand, it has to be noted that plasma membrane from *S. cervisiae*, which is not able to grow on alkanes, also contained cytochrome *b*. Therefore, it is possible that these cytochromes are involved in other processes, such as fatty acid desaturation [49].

With regard to differences observed in the plasma membranes of *C. tropicalis* grown on glucose or hexadecane, significant alterations were found in the qualitative determination of the membrane proteins by SDS gel electrophoresis, in the fatty acid composition and in the cytochrome content. However, it is not known whether these changes reflect a modified membrane synthesis during alkane assimilation or a functional role of the plasma membrane in uptake and oxidation of hydrocarbons.

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