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THE PLASMA MEMBRANE OF *SACCHAROMYCES CEREVISIAE*

MOLECULAR STRUCTURE AND ASYMMETRY

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The molecular structure of the plasma membrane of the haploid strain *Saccharomyces cerevisiae* X-2180 1A has been studied by means of sodium dodecyl sulfate polyacrylamide gel electrophoresis. Protein and glycoprotein components have been identified and their apparent M_r determined. A glycoprotein showing an apparent M_r of 27500 has been shown to be the main structural component. Treatment of the cells with cycloheximide prior to plasma membrane isolation resulted in a redistribution of the relative amounts of each protien band and a drastic reduction in the number of Schiff positive bands. It is postulated that treatment with this drug rids the plasma membrane of glycoprotein secretory components which are in the process of being secreted to the periplasmic space, thus allowing the study of the basic structural components of the organelle. The electrophoretic pattern of the internal membranes revealed close similarities with that of the plasma membrane and though two-dimensional electrophoresis might disclose greater differences, these similarities suggest a common origin for most of the components of both membranous systems. Finally, radioiodination techniques have been used in studying the asymmetric disposition of some of the components of the plasma membrane. At least five polypeptides were identified as located to the outer layer of the plasma membrane and two more glycopeptides were shown to span across the bilayer.

Introduction

Recent reports [1–4] support the contention that some of the most reliable procedures to isolate and purify the plasma membrane of *Saccharomyces cerevisiae* are those based on initial stabilization of spheroplasts with concanavalin A. Either open ghosts [1–3] or sealed, functional vesicles [4] can be easily obtained by this method thus opening wide possibilities for structural as well as functional studies of the isolated organelle. In this sense, yeast membrane vesicles have recently been shown to transport amino acid actively [4] and the ghosts have been used for one-dimensional [2] or two-dimensional [3] electrophoretic separations aimed at identifying the structural components of the plasma membrane.

Further insight into the molecular architecture of the yeast plasma membrane can be obtained by the use of different techniques that probe the asymmetric distribution of the molecular components of the bilayer [5,6]. Particularly, lactoperoxidase-catalyzed iodination of exposed proteins [7–13] or lipids [14–18] from biological membranes is a well-established method to study

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molecular asymmetry in those membranes. In the case of *S. cerevisiae* it has been demonstrated that the spheroplasts can be efficiently iodinated and most of the radioactivity of the surface-labeled spheroplasts is recovered in the ghost fraction [1,2]. In addition, the impermeability of the spheroplasts to the lactoperoxidase reagents has been shown [3].

In this work, we applied radiodination techniques and SDS-polyacrylamide gel electrophoresis analysis in order to identify the peptide and glycopeptide components as well as their asymmetric distribution in the bilayer of the plasma membrane of the haploid strain *S. cerevisiae* X-2180 1A. In addition, a comparison of the molecular components of the plasma membrane and the rest of internal membranous systems is presented.

Materials and Methods

Yeast strain and growth conditions. *Saccharomyces cerevisiae* X2180-1A received from the Department of Genetics, Seattle University (Washington, U.S.A.) was used throughout this investigation. The strain was maintained on slants of YED medium [19] solidified with 1.5% agar and subcultured periodically. Cells were grown with shaking (250 rev./min) in 1 liter Erlenmeyer flasks filled with 300 ml of YED liquid medum. The culture was incubated at 24°C and the cells were harvested in the early logarithmic phase.

Concanavalin A, DNAase (DN 25), mercaptoethanol and mannitol were from Sigma Chemical Co. Lactoperoxidase was purchased from CalBiochem and Sigma Chemical Co. Glusulase was from Endo Laboratories Inc.; sodium deoxycholate and sodium dodecyl sulfate were obtained from Merck (Darmstadt, F.R.G.). The components of the growth media were from Difco and all the electrophoresis reagents were obtained from Serva Fenbiochimica (Switzerland). $Na^{125}I$ (carrier free) was purchased from the Radiochemical Centre (Amersham, U.K.).

Protein determination was carried out by a modification [20] of the method of Lowry based on the reaction with the Folin's reagent as described previously [2].

Preparation of the plasma membrane. Cells harvested in the early logarithmic phase were treated with glusulase as described [2] and 100% spheroplasts were obtained after 50–60 min of the treatment. The plasma membrane of the spheroplasts were prepared then by the concanavalin A method as described by Santos et al. [2]. The term 'internal membranes' is applied in the text to all yeast membrane systems with the exception of the plasma membrane.

SDS-gel electrophoresis. Electrophoresis in sodium dodecyl sulfate was performed on 8% acrylamide gels as described by Zahler [21]. Proteins were detected by the method of Fairbanks et al. [22] and glycoproteins were stained according to Zacharius et al. [23]. The stained gels were scanned at 575 nm (for Coomassie blue stain) and at 560 nm (for carbohydrate stain) in a Pye-Unicam SP-1700 spectrophotometer equipped with a densitometer model Unicam SP-1809. The apparent molecular weight of the different bands in the gel were determined according to Santos et al. [2] from a plot relating molecular weights of known proteins versus their relative mobility in the electrophoretic system. Whenever needed, radioactivity was measured in the gels as described [24].

Lactoperoxidase iodination. A modification of the method of Santos et al. [2] was used. The lactoperoxidase used was a mixture of equal amounts of the enzyme obtained from both Sigma and CalBiochem. This precaution was taken due to the variability observed in different commercial batches of the enzyme. Spheroplasts obtained from 500 mg cells (dry weight) or membrane ghosts obtained from 250 mg cells (dry weight) were suspended in 6 ml of 0.1 M phosphate bufer (pH 7.5) containing 0.8 M sorbitol, 33 μg/ml lactoperoxidase and 1 μCi of carrier free $Na^{125}I$. The reaction was started by adding 10 μl of 0.2% H_2O_2 and the incubation was kept at 30°C. Successive additions of H_sO_2 were done every 2 min for a total period of 30 min. After that time the reaction was stopped by centrifugation and the resulting pellet was washed three times in the original buffer.

Results

Structure of the plasma membrane of S. cerevisiae X-2180 1A

As seen in Fig. 1A, the plasma membranes of

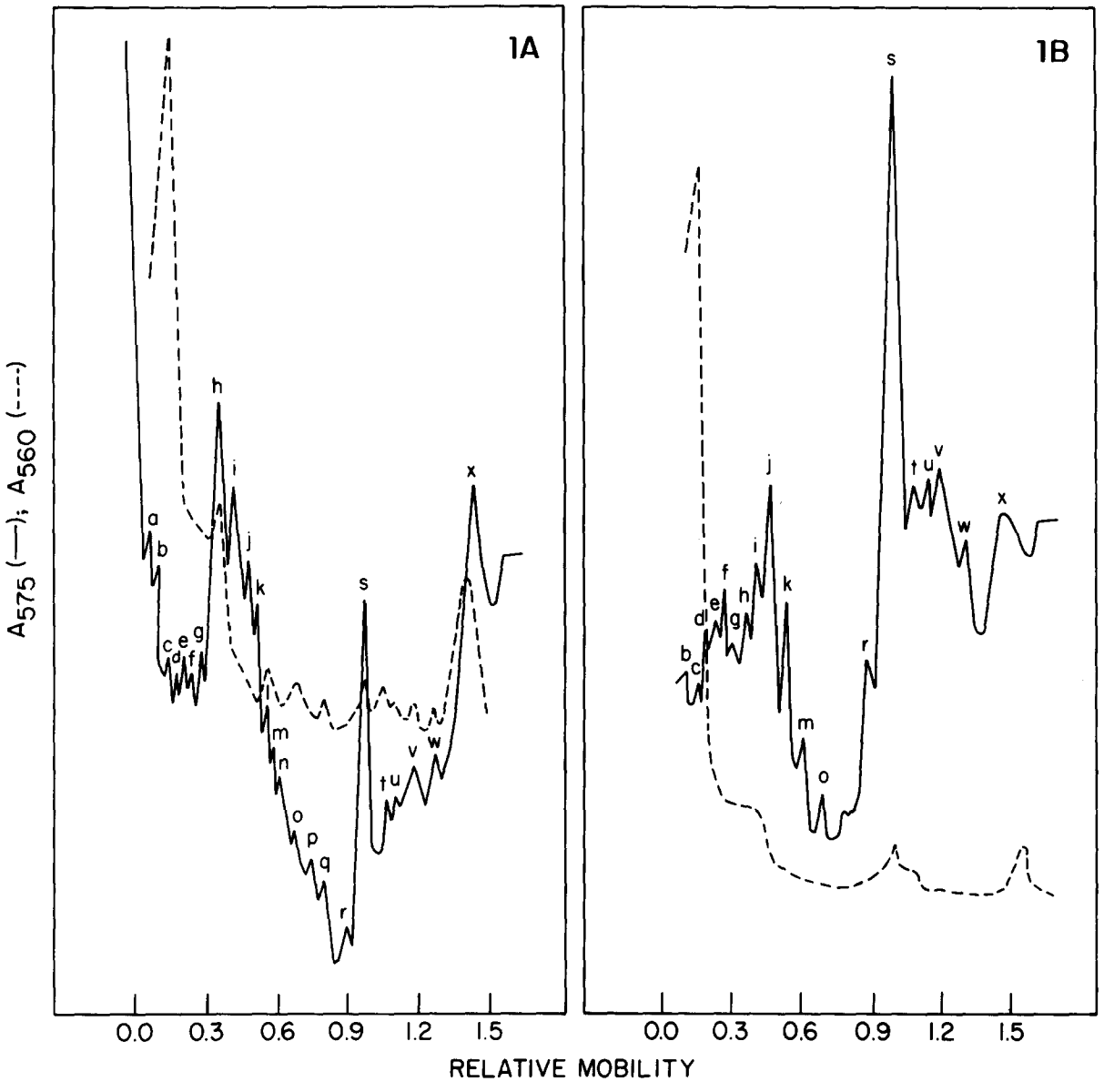

Fig. 1. Electrophoretic pattern of the plasma membrane of normally growing (A) and cycloheximide-treated cells (B). The figures correspond to densitometer tracings of sodium dodecyl sulfate polyacrylamide gels (8%, 12 cm long) showing the polypeptide (continuous line) and carbohydrate (broken line) electrophoretic pattern of the plasma membrane of normally growing (A) and cycloheximide-treated (B) yeast cells. The relative mobility of all bands is referred to that of band S, which is in the majority and shows an apparent M_r of 27500.

normally grown cells could be separated into 25–30 discrete polypeptide bands, and at least 12 of them showed a positive reaction to Schiff staining. A band (S) showing an apparent molecular weight of around 28 000 and positive staining for carbohydrate was calculated to be the major individual component of the plasma membrane and the R_m of all other bands are referred to this one in Table I. The apparent molecular weights of all bands ranged from 10000 to 125000 with a predominance of those with higher molecular weights. Perhaps some of the high molecular weight bands

TABLE I

CHARACTERIZATION OF PEPTIDES AND GLYCOPEPTIDES OF THE PLASMA MEMBRANE OF NORMALLY GROWING AND CYCLOHEXIMIDE-TREATED CELLS

The individual bands separated in Fig. 1 were characterized in terms of staining properties (C, Coomassie blue stain; G, glycoprotein stain) and apparent M_r. The values of M_r were calculated according to Ref. 2 from a plot relating M_r of known proteins versus their relative mobilities in the electrophoretic system.

Band	R_m	Apparent M_r ($\times 10^{-3}$)	Cells			
			Normally growing		CH-treated	
			C	G	C	G
A	0.09	117.5	+	−	−	−
B	0.12	114.0	+	−	+	−
C	0.17	105.0	+	+	+	+
D	0.20	97.5	+	−	+	−
E	0.23	93.0	+	−	+	−
F	0.27	88.0	+	−	+	−
G	0.32	82.0	+	−	+	−
H	0.39	74.0	+	+	+	+
I	0.44	67.6	+	−	+	−
J	0.51	61.3	+	−	+	−
K	0.56	56.0	+	−	+	−
L	0.59	53.6	+	+	−	−
M	0.62	51.0	+	−	+	−
N	0.64	48.0	+	−	−	−
O	0.70	44.0	+	+	+	−
P	0.78	39.0	+	−	−	−
Q	0.85	36.0	+	+	−	−
R	0.92	30.0	+	−	+	−
S	1	27.3	+	+	+	+
T	1.08	23.7	+	+	+	−
U	1.15	22.0	+	+	+	−
V	1.22	19.6	+	+	+	−
W	1.31	16.25	+	+	+	−
X	1.45	12.80	+	+	+	+

represent mannan subunits, as they showed a heavily positive reaction to Schiff staining while the 28000 glycoprotein band may well be the main structural component of the yeast plasma membrane.

It is known that halting protein synthesis by means of cycloheximide produces a rapid inhibition of the synthesis of wall mannan glycoproteins at the level of peptide formation, whereas glycosylation and secretion of those peptides already formed does take place normally [25,26]. Furthermore, treatment with this antibiotic produces a dramatic decrease in the carbohydrate/protein ratio at the level of the plasma membrane [2]. When exponentially growing cells were treated with 100 μg/ml cycloheximide for 120 min in the growth medium before plasma membrane isolation, a dramatic change in the relative amount of the polypeptide bands was observed (Fig. 1B). The main bands were found to be of lower molecular weight than in normally growing cells and a better separation between them was evident. Many Schiff-positive bands disappeared, leaving only two major, high molecular weight bands along with a third reduced one corresponding to the major polypeptide of 28 kDa. The peptide and glycopeptide bands found after cycloheximide treatment might well represent the real structural components of the yeast plasma membrane, while the additional bands found in normally grown cells might then represent the nonstructural material which is actively secreted across the plasma membrane.

Structure of the internal membranes

The electrophoretic patterns obtained from the internal membranes of normally growing and cycloheximide-treated cells were very similar (we present here data of normally growing cells in Fig. 2 and Table II) and revealed to be close to that of the plasma membrane of cycloheximide-treated cells though two-dimensional electrophoresis might disclose greater differences. This similarity in composition suggests a common origin for both membranous systems. When the electrophoretic patterns of plasma and internal membranes were compared, all main bands with the excpetion of three were observed to be common in both types of membranes and only the relative amount of each band was at variance. As in the plasma membrane, the major component observed was a Coomassie blue-positive, Shiff-positive band with an apparent molecular weight of 28000. Two of the three missing bands corresponded to high molecular weight components bands A and B, apparent M_r 117500 and 114000, respectively, which are important in plasma membranes of normally growing cells and very reduced in

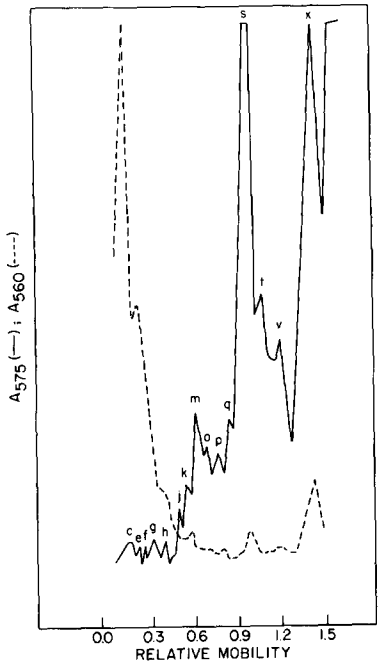

Fig. 2. Electrophoretic pattern of the internal membranes from yeast. Densitometer tracings of sodium dodecyl sulfate polyacrylamide gels (8%, 12 cm long) showing polypeptide (continuous line) and carbohydrate (broken line) staining are presented here. Denomination of the bands and values of R_m are as for Fig. 1. It can be observed that a group of high molecular weight components (apparent M_r 117000–75000) is significantly reduced as compared to the corresponding components of the plasma membrane (Fig. 1).

cycloheximide treated cells; these two bands probably represent mannan subunits on their way to the cell wall. On the other hand, it is to be noted that there is almost complete absence of a third component (band I), which is abundant in plasma membranes of both normally grown and cycloheximide treated cells. This band has an apparent molecular weight of 67000 and is a major structural polypeptide of the plasma membrane which is almost completely absent in internal membranes.

Asymmetry of the plasma membrane

Lactoperoxidase-catalyzed iodination was used as a means to ascertain the sideness of the polypeptides and glycopeptides previously identified by SDS gel electrophoresis. Viable protoplasts were labeled exhaustively with ^{125}I in the presence of lactoperoxidase and then the plasma membrane was purified using concanavalin A as described

TABLE II

CHARACTERIZATION OF PROTEIN AND GLYCOPROTEIN COMPONENTS OF THE INTERNAL MEMBRANES

The individual bands separated in Fig. 2 were characterized in terms of staining properties and apparent M_r following the procedure used in Table I.

Band	R_m	Apparent M_r ($\times 10^{-3}$)	Coomassie blue stain	Glycoprotein stain
C	0.17	105.0	+	+
E	0.23	93.0	+	−
F	0.27	88.0	+	−
G	0.32	82.0	+	−
H	0.39	74.0	+	+
I	0.44	67.5	+	−
J	0.51	61.3	+	−
K	0.56	56.0	+	+
M	0.62	51.0	+	−
O	0.70	44.0	+	−
P	0.78	39.0	+	−
Q	0.85	36.0	+	+
S	1	27.0	+	−
T	1.08	23.7	+	−
V	1.22	19.6	+	+
X	1.45	12.8	+	+

[2]. An aliquot of this preparation was washed with α-methylmannoside so as to elute the concanavalin A and then analyzed by SDS gel electrophoresis in order to characterize the externally exposed peptides and glycopeptides. Another aliquot of the externally labeled membranes, still bearing a coat of concanavalin A attached to its external surface (and therefore in the form of open sheets) was exhaustively iodinated again with ^{125}I and lactoperoxidase; this treatment allowed the labeling of those peptides exposed to the cytoplasmic side of the plasma membrane. A posterior treatment of the membranes with α-methylmannoside eliminated the concanavalin A fixed to the outside and yielded a preparation of plasma membranes labeled with ^{125}I in both the external and internal sides of the bilayer.

The fact that the specific activity of the membranes labeled both externally and internally ($3 \cdot 10^6$ cpm/mg protein) was 3-fold higher than that of membranes labeled only on the outside, strongly suggests an uneven distribution in the amount of proteins in both leaflets of the bilayer and implies a preferential enrichment of the inner leaflet in

terms of protein. These results are in accordance with previous reports [5] on membranes of different cell type.

The electrophoretic pattern of radioactive peptides and glycopeptides from both externally labeled memranes and membranes labeled on both sides were compared in order to ascertain their orientation in the bilayer. Only those protein or glycoprotein bands accounting for 1% or more of the total radioactivity applied to the gels were considered significant so as to obtain unequivocal results when ascribing them a particular orientation in the bilayer. Several criteria were applied in order to ascribe an orientation to each significant peptide and glycopeptide separated by SDS gel electrophoresis:

(a) A protein or glycoprotein band will be considered as located in the outer leaflet of the bilayer when its percentage of the total radioactivity found in membranes labeled from both sides is significantly lower than its percentage of total radioactivity present in membranes only externally labeled. The ratio (% radioactivity external side)/(% radioactivity both sides) should be equal or higher than 1.5.

(b) A peptide or glycopeptide band will be assigned to the inner leaflet of the bilayer when its radioactivity is not significant in membranes externally labeled (less than 1% of total radioactivity), but is significant (> 1.5% of total radioactivity) in membranes labeled from both sides. The ratio (% radioactivity both sides)/(% radioactivity external side) should be greater than 2.

(c) Finally, a peptide or glycopeptide band will be considered transmmebranal when (i) its percentage of the total radioactivity is significant (> 2%) in both externally labeled membranes and membranes labeled from both sides, and (ii), its percentage of total radioactivity in externally labeled membranes is similar or lower than in membranes labeled from both sides.

The application of the aforementioned criteria to the data displayed in Table III, which was obtained by integration of the percentages of radioactivity present in the bands separated by SDS gel electrophoresis (Fig. 3), led to the allocation of six glycopeptides and five peptides. A topographic model is depicted in Fig. 4a, where the peptide or glycopeptide nature of a given band as well as the molecular size and the relative amount of each individual band in the membrane are tentatively included. Four glycopeptides (bands H, O, L and X) and four peptides (bands E, G, P and I) were identified located in the outer layer of the membrane, while only one peptide (band A) was identified in the inner layer. In addition, three glyco-

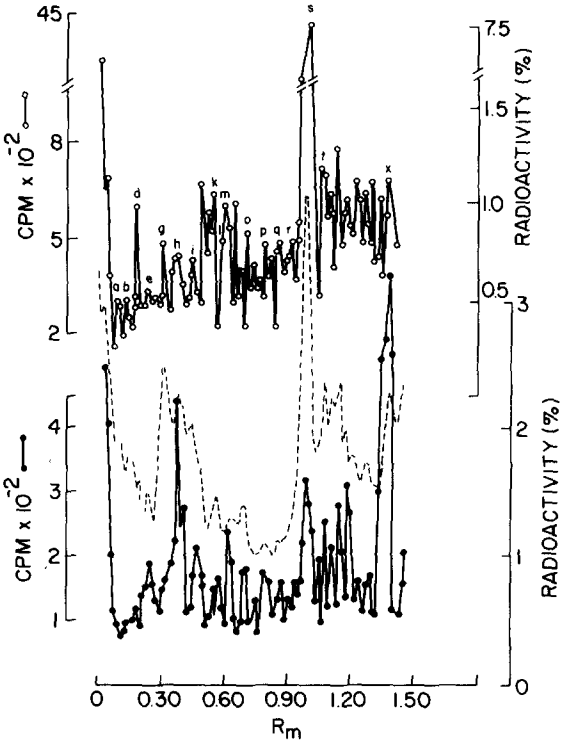

Fig. 3. Normally growing cells. Electrophoretic separation of components of the plasma membrane iodinated from the external side (●) or from both sides (○). Protoplasts (500 mg, dry weight) were iodinated as described in Methods and used to isolate the plasma membrane by the concanavalin A method [2]. An aliquot of these plasma membranes was treated with α-methylmannoside to eliminate the concanavalin A and applied to the gels (●) to characterize components labeled from the external side. Another aliquot of the plasma membranes still bearing concanavalin A attached to the external surface was iodinated again and applied to the gels to identify the components labeled from both sides (○). 100 μg protein were applied to the gels in each case; the gels and the characteristics of the run were exactly as for Fig. 1. Broken line represents Coomassie blue stain and the solid lanes correspond to amount of radioactivity present in 1 mm fractions of the gels. Total radioactivity per fraction and percentage of total radioactivity applied to the gel are represented in ordinates. Relative mobility is presented in abscissa.

TABLE III

CHARACTERIZATION OF ^{125}I-LABELED COMPONENTS OF THE PLASMA MEMBRANE OF NORMALLY GROWING CELLS

The individual peaks of radioactivity in Fig. 3 were identified according to the nomenclature used in previous figures and tables and characterized in terms of staining properties. Integration of the radioactivity present in each individual peak was done and a calculation of the percentage of total radioactivity present in each component is presented. The tentative sidedness assigned to each component by applying the criteria mentioned in the text is also included.

Band	R_m	Apparent M_r ($\times 10^{-3}$)	Glycoprotein staining	% Radioactivity both sides	% Radioactivity external side	Sidedness ascribed
A	0.09	117.5	−	1.5	0.5	inner
B	0.12	114.0	−	1.6	1.4	–
D	0.20	97.5	−	2.8	2.0	–
E	0.23	93.0	−	3.2	4.9	outer
G	0.32	82.0	−	2.2	3.2	outer
H	0.39	74.0	+	3.6	5.3	outer
I	0.44	67.6	−	3.3	5.3	outer
K	0.55	56.0	−	2.2	2.4	–
L	0.59	53.6	+	1.9	2.5	–
M	0.62	51.0	−	3.2	3.6	–
O	0.70	44.0	+	1.7	3.2	outer
P	0.78	39.0	−	1.8	3.5	outer
Q	0.85	36.0	+	2.4	3.2	–
R	0.92	30.0	−	3.5	2.2	transmembranal
S	1.00	27.3	+	26.6	7.6	transmembranal
T	1.08	23.7	+	3.8	2.3	transmembranal
W	1.31	16.2	+	2.6	4.2	–
X	1.45	12.8	+	3.4	14.3	outer

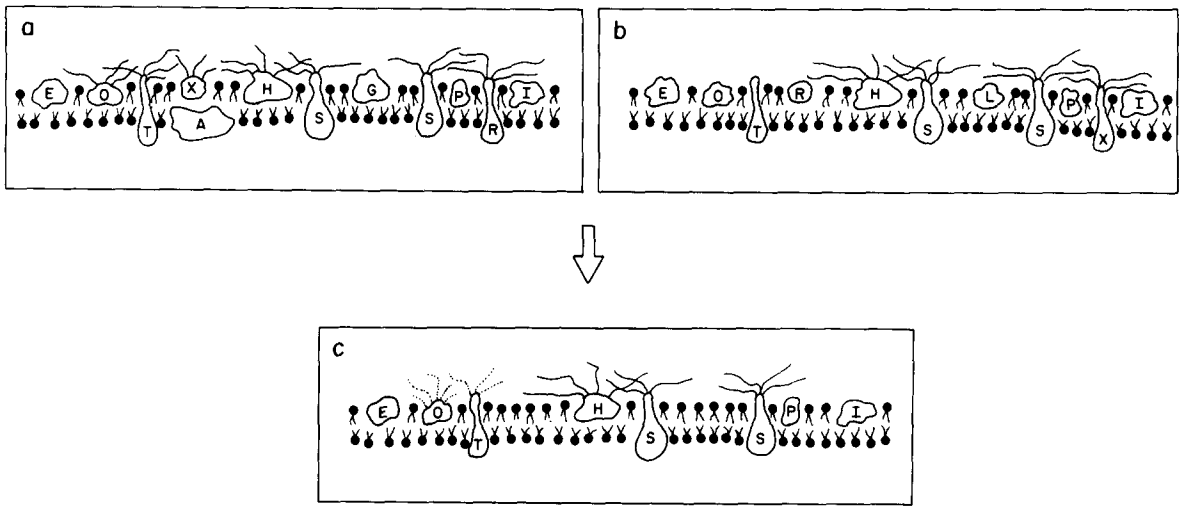

Fig. 4. Tentative topological model of the plasma membrane of *S. cerevisiae*. Application of the criteria mentioned in the text to data displayed in Tables III and IV led to the tentative models depicted here. (a) depicts results from normally growing cells and (b) represents results from cycloheximide-treated cells. Most of the components depicted in (a) and (b) are coincident and are presented in (c). It should be noted that almost all bands in (c) show identical staining properties for carbohydrate in normally growing and in cycloheximide treated cells with the exception of bands O and T that are Schiff negative in cycloheximide-treated cells and Schiff positive in normally growing cells. Denomination of the components is as for all other figures and tables. Glycoproteins are depicted with solid lines protruding from the body of the component; (broken lines in O and T). Molecular size of the component has been roughly represented but it is not intended to be at scale.

peptides (bands S, T and R) were considered as spaning across the bilayer of normally growing cells.

Asymmetry of the plasma membrane of cycloheximide-treated cells

The same experimental approach described above for membranes of normally growing cells was applied also to the plasma membranes of cycloheximide-treated cells. A value of $1.4 \cdot 10^6$ cpm/mg protein was obtained for the specific activity of membranes labeled only on their external surface and at least a 10-fold higher specific activity was observed in membranes labeled from both sides. the pattern of radioactive bands obtained from externally labeled membranes and membranes labeled from both sides is represented in Fig. 5. Integration of the percentages of total radioactivity found in each individual peak is summarized in Table IV. When these figures were evaluated according to the criteria mentioned in the previous section, a model consistent with that found for membranes of normally grown cells was found (Fig. 4b). Six polypeptides (bands E, I, O, L, P and R) were confidently assigned to the outer layer of the plasma membrane and only one glycopeptide (band H) remained to be assigned to this external leaflet of the plasma membrane. Five of these polypeptides (E, P, I, H and O) were ascribed the same orientation in normally growing cells but it is to note that polypeptide O is Schiff-negative in cycloheximide-treated cells as apposed to normally growing cells while polypeptides E, P, I and H retain the same staining properties as in normally growing cells. In addition, the figures obtained in these experiments allowed two more polypeptides (L and R) to be assigned to the outer leaflet of the plasma membrane. Polypeptide L was not ascribed an orientation in normally growing cells while band R was considered transmembranal in that case. A transmembranal character could be assigned to the glycopeptides S and T a result in agreement with data from normally growing cells; in addition, glycopeptide X (considered located to the outer surface in normally growing cells) was also considered as transmembranal in cycloheximide-treated cells. The figures obtained from cycloheximide-treated cells did not allow, however, to assign confidently any polypeptides to the internal side of the plasma membrane.

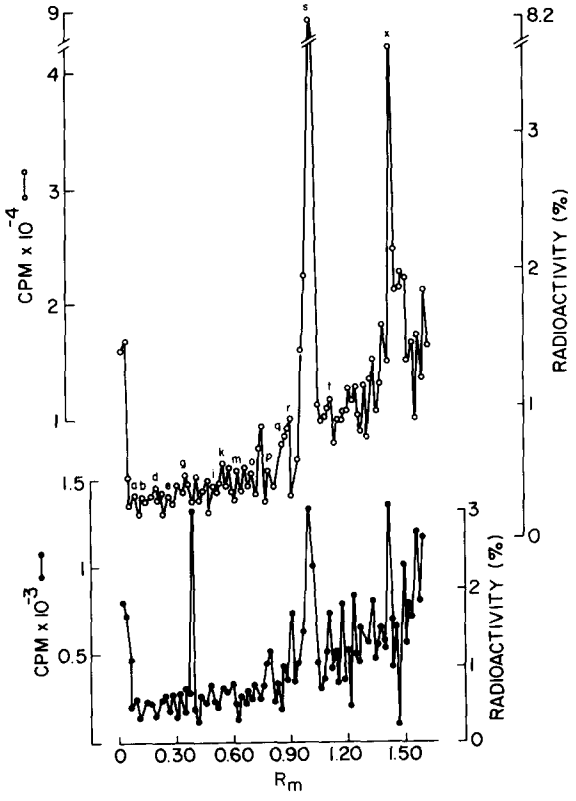

Fig. 5. Cycloheximide-treated cells. Electrophoretic separation of components of the plasma membrane iodinated from the external side (●) or from both sides (○). Protoplasts prepared from cycloheximide-treated cells were used to prepare plasma membranes iodinated from the external side (●) or from both sides (○) following the procedure described in Fig. 3: Approx. 200 μg protein were applied to the gels in each case and the radioactivity of 1 mm long gel fractions was counted. Total radioactivity per fraction and percentage of total radioactivity applied to the gel is presented in ordinates. Relative mobility is presented in abscissa.

It should be mentioned that the topological conclusions derived from studies with normally growing cells and cycloheximide-treated cells were coincident for most of the polypeptides assigned an orientation (seven) and only contradictory results were found for bands R and X. The fact that these low molecular weight bands show a very fast turnover rate (unpublished results) suggest that they may be a mixture of degradation and/or secretory components. This composition makes it easy to understand that these bands present a very

TABLE IV

CHARACTERIZATION OF ^{125}I-LABELED COMPONENTS OF THE PLASMA MEMBRANE OF CYCLOHEXIMIDE-TREATED CELLS

Individual peaks of radioactivity in Fig. 5 were identified according to nomenclature used in previous figures and tables and characterized for staining properties. The radioactivity present in each peak was integrated and the percentage of total radioactivity present in each individual component calculated. The tentative sidedness atributed to some of the bands is also presented.

Band	R_m	Apparent M_r ($\times 10^{-3}$)	Glycoprotein stain	% Radioactivity both sides	% Radioactivity external face	Sidedness ascribed
A	0.09	117.5	−	0.8	1.7	−
B	0.12	114.0	−	0.8	1.1	−
D	0.20	97.5	−	1.3	1.6	−
E	0.23	93.0	−	1.1	2.3	outer
G	0.32	82.0	−	1.5	1.7	−
H	0.39	74.0	+	1.0	4.6	outer
I	0.44	67.6	−	0.98	2.8	outer
K	0.56	56.0	−	1.8	2.2	−
L	0.59	53.6	−	1.6	2.6	outer
M	0.62	51.0	−	1.3	1.4	−
O	0.70	44.0	−	1.8	2.6	outer
P	0.78	39.0	−	1.6	4.6	outer
Q	0.85	36.0	−	2.3	2.2	−
R	0.92	30.0	−	2.9	4.4	outer
S	1.00	27.3	+	25.3	10.8	transmembranal
T	1.08	23.7	−	4.7	5.0	transmembranal
W	1.31	16.3	−	2.9	3.2	−
X	1.45	12.8	+	9.5	6.7	transmembranal

different degree of exposure to iodination when they are present in normally growing cells than when they are studied in glycoprotein-depleted, cycloheximide-treated cells. The peptide and glycopeptide bands assigned the same orientation in normally growing cells and in cycloheximide-treated cells are drawn in Fig. 4c. Two of these bands (O and T) show glycoprotein nature in normally growing cells but not in cycloheximide-treated cells.

Discussion

Molecular analysis by means of SDS-polyacrylamide gel electrophoresis has allowed us to identify and characterize a number of peptides and glycopeptides present in the yeast plasma membrane.

The distribution of proteins and glycoproteins in the plasma membrane of the haploid strain X-2180A showed an almost complete similarity to that of a diploid strain previously studied in our laboratory [2]. These results strongly suggest that no major changes in the composition of the plasma membranes of yeast are determined by the haploid or diploid condition of a given strain. In normally growing cells a predominance of high molecular weight components (90000–120000) has been observed in the plasma membrane and it is particularly noteworthy that the most abundant components are of a glycoprotein nature. These results confirm previous quantitative observations [2] showing a high carbohydrate content of these plasma membranes. In that report the suggestion was made that part of that carbohydrate did not account for structural components but for secretory material caught in the process of being secreted across the plasma membrane. That possibility has been proven valid in the present paper when the plasma membrane of cycloheximide-treated cells was analyzed by means of SDS electrophoresis. It was observed that the elimination

of secretory products from the plasma membrane by means of cycloheximide [25,26] produced a dramatic rearrangement of the peptides and glycopeptides observed in he electrophoretic pattern. An important number of Schiff-positive bands disappeared (with this treatment) and they may well represent those secretory glycoproteins mentioned before. Among the bands remaining, a 28 kDa glycopeptide showed to be majoritory and this is probably the main structural component of the plasma membrane of *S. cerevisiae*.

The electrophoretic analysis of the internal membranes' fraction showed a very similar pattern to that found in plasma membranes of cycloheximide-treated cells. Thus, the main difference between the internal membrane and the plasma membrane of normally growing cells lies in the secretory glycoproteins found in the latter fraction. Despite this difference, the presence of the 28 kDa majoritory glycopeptide and the close similarities in most of the other detected components suggests that most of the components of the plasma membrane are originate from the internal membranes. This concept of 'membrane flow' has been proposed as the most important process involved in the building up of the plasma membrane in a number of eukaryotic systems [27,28]. Nevertheless, a complete similarity in the biogenesis of the plasma membrane and the other membranous systems of the cells is to be discarded as one of the most abundant polypeptides of the plasma membrane (band I, apparent M_r 67000) is practically absent from the internal membranous fraction.

Radioiodination has been used in the present study to determine the asymmetric location of several peptides and glycopeptides in the membrane. The validity of this approach to study membrane asymmetry in *S. cerevisiae* has been proven by previous reports in which the impermeability of the reagents for lactoperoxidase-catalyzed iodination has been demonstrated [1–3] and a partial, preliminary identification of externally exposed glycoproteins has been achieved [3]. In order to obtain statistically unequivocal results only a few peptides and glycopeptides accounting for a significantly high percentage ($> 1\%$) of the total ^{125}I fixed in the membrane were considered here for allocation on either or both sides of the bilayer. The criteria mentioned in Results could only be confidently applied to the quantitative data obtained from those most prominent bands and unfortunately, more sensitive techniques of separation are to be applied in order to be able to apply those criteria to the rest of the identified bands within a statistic range of confidence. Coincident results show the occurence of two transmembranal glycopeptides on the plasma membrane of both normally growing cells and cells treated with cycloheximide. One of those glycopeptides (M_r 28000) has also been shown to be the majoritory structural component of the plasma membrane. On the other hand, at least five other components of the electrophoretic pattern have also been assigned to the outer side of the bilayer; it is interesting to note that two of the externally exposed components (bands O and T) show glycoprotein nature in normally growing cells but not in cycloheximide-treated cells. Finally, our data only permitted the identification of one peptide located to the cytoplasmic side of the plasma membrane of normally growing cells. The models depicted in Fig. 4 represent the well characterized membrane proteins only and are therefore preliminary and incomplete. It is important to underline that the topographic disposition discussed above agrees with current ideas on the molecular arrangement of the components of eukaryotic plasma membranes [5,6]. Thus, regarding the known asymmetry of carbohydrates in biological membranes all the glycopeptides assigned an orientation in our study were found to be accessible to iodiation from the outer side of the membrane and the one peptide allocated to the cytoplasmic side of the bilayer was clearly Schiff negative. Furthermore, the considerably higher specific activities (3–10-fold) obtained in plasma membranes iodinated in both sides than in membranes labeled only in the external surface strongly suggest that most of the protein components of the plasma membrane are located in the inner leaflet of the bilayer. This has also been shown to be the case with the red cell membrane proteins and some other membranes [5]. An interesting aspect to be noticed concerns the glycoprotein nature of a high number of the components of the plasma membrane. In this sense, it is noteworthy to mention previous electron microscopic studies [2] using the electron-dense complex concanavalin A-ferritin that suggested that at

least part of the glycoproteins found in plasma membranes of yeast cells might be arranged forming a 'glycoprotein hair' all around the spheroplast. The occurrence of mannosyltransferase activity located on the outer side of the plasma membrane (results to be described elsewhere) along with the non-availability of phospholipids of the surface of protoplasts from cycloheximide-treated cells to the action of phospholipase C or derivation by trinitrobenzene sulfonate (unpublished results), provide increasing evidence that the outer surface of the plasma is rich in glycoproteins that expand across the periplasmic space towards the cell wall. This structure might be somehow equivalent to the glycocalyx of mammalian cells.

The application now in course in our laboratory of some complementary techniques such as specific proteolysis, crossed immunoelectrophoresis, and circular dicroism will provide additional information in the future on the molecular structure of the plasma membrane of yeast.

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