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YEAST PLASMA MEMBRANE GHOSTS

AN ANALYSIS OF PROTEINS BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

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Summary

We have examined yeast cell ghost preparations to assess their value in obtaining plasma membrane proteins. Ghosts prepared by two methods involving stabilization of spheroplast envelopes had similar protein patterns by two-dimensional gel electrophoresis, and approximately 200 proteins were resolved. Spheroplasts were lactoperoxidase iodinated, and recovery of label in ghost preparations was greater than 60%. Spheroplasts appeared to be impermeable to the lactoperoxidase reagents as judged by an examination of two-dimensional gel electrophoretic patterns of ghost proteins that had been iodinated in spheroplasts or in unsealed ghosts. Spheroplasts were also impermeable to pronase proteases. Surface iodination and surface proteolysis allowed us to identify exposed ghost proteins; the major ghost glycoprotein was exposed in spheroplasts.

Two-dimensional patterns of ghost proteins were not heavily contaminated ($\leqslant$25% of all proteins) by proteins present in soluble or promitochondrial fractions, and estimates of surface label and total cell protein recovery suggested that the ghost fraction represents a cell envelope enrichment of 8—10 fold over whole cells.

Resolution of ghost proteins by two-dimensional gel electrophoresis appears to be a powerful aid toward identifying membrane proteins.

Abbreviation MTE, mannitol/Tris/EDTA (0.25 M mannitol/20 mM Tris-acetate, pH 7.5/0.1 mM EDTA).

Introduction

Yeast cell ghosts containing the plasma membrane can be isolated from spheroplasts which have been stabilised with concanavalin A [1—3]. Our aim has been to explore the value of ghost preparations as a source of plasma membrane proteins. The yeast system is potentially valuable for identifying plasma membrane proteins as mutants altered in plasma membrane function exist. To analyse the ghost proteins obtained by the concanavalin A stabilisation technique and by a related method we have used the two-dimensional polyacrylamide gel electrophoresis procedure developed by O'Farrell [4] and Ferro-Luzzi Ames and Nikaido [5]. We have also examined the topology of ghost proteins by surface labeling using lactoperoxidase-catalysed iodination and by surface proteolysis using pronase.

Materials and Methods

Media. The growth medium (YEPD) was the minimal medium of Halvorson [6] supplemented with yeast extract and peptone, each 0.5%, and glucose, 2%.

Yeast strains. Strains of *Saccharomyces cerevisiae* used were A8207BNK, α, his4C-864 from G.R. Fink; FL-100, *a*, (wt) from F. Lacroute; K19/10, *a*, trp5, leu 1 from J.M. Somers.

Materials. Yeast extract and peptone were from Difco. Concanavalin A, lactoperoxidase, sodium dodecyl sulphate and Tris were from Sigma Chemical Co. Glusulase was from Endo Laboratories and zymolyase 5000 was from Kirin Brewery Co., Takasaki, Japan. Nonidet P-40 was from BDH Chemicals, Ampholines were from LKB, electrophoresis reagents were from Bio-Rad laboratories. Pronase was grade B from Calbiochem. L-[U-^{14}C]protein-hydrolysate (CFB.25) was from Amersham Corp., Na^{125}I (carrier free) was from New England Nuclear, D-sorbitol (reagent grade) was from Fisher.

Assay for marker enzymes. Invertase was assayed as described previously [7]. Acid phosphatase was determined by the method of Schurr and Yagil [8]. Alcohol dehydrogenase was assayed as described by Racker [9]. Succinate dehydrogenase was measured by the method of Ells [10]. Glucose-6-phosphate dehydrogenase was assayed as described by Kornberg and Horecker [11]. Protease A was assayed by a modification of the procedure of Lenney et al. [12]; the reaction was stopped after 60 min by adding 200 μl of trichloroacetic acid, and the supernatant was assayed by the Folin procedure [13] using L-tyrosine as a standard.

Isolation of yeast cell ghosts. Concanavalin A-stabilised ghosts. Concanavalin A-stabilised ghosts were obtained by modifications of the methods of Scarborough [1] and Duran et al. [2]. To obtain spheroplasts, 200 ml of cells were grown to a density of approximately $2 \cdot 10^7$ cells/ml. Cells were harvested at $1500 \times g$ for 5 min, washed twice with 40 ml of distilled water and resuspended in 1.2 M sorbitol, to approximately $2 \cdot 10^8$ cells/ml (22 ml/g wet weight of cells). Glusulase was added to 1% (v/v) and the mixture incubated at 30°C for 2 h at 75—80 rev./min in a rotary shaker. Spheroplasts were harvested at 20—22°C in an HB-4 rotor at $650 \times g$ for 15 min. The spheroplasts were resuspended in 1.2 M sorbitol, 10 mM Tris-HCl (pH 7.5) plus 0.25 mg/ml con-

canavalin A at $2 \cdot 10^8$ cell equivalents/ml, and incubated for 10 min at room temperature. The aggregated spheroplasts were centrifuged at $650 \times g$ for 10 min and washed twice with 1.2 M sorbitol, 10 mM Tris-HCl, pH 7.5. The pellet was resuspended in 10 ml of cold lysis buffer containing 10 mM Tris-HCl, pH 7.5, 5 mM $MgSO_4$, 0.6 mM phenylmethylsulphonyl fluoride, 100 μg/ml DNAase (Sigma II bovine spleen), and 100 μg/ml RNAase (Sigma IIA bovine pancreas). The lysate was stirred on ice in a Sorvall omnimixer, setting 5 (half-maximum speed), for 1 min, then incubated at 30°C for 15 min at 75—80 rev./min in a rotary shaker. The lysate, 10 ml, was layered onto 30 ml of ice-cold buffer B (0.1 M Tris-HCl, pH 7.5, 0.5 M sorbitol) and centrifuged at $250 \times g$ for 30 min at 4°C in an HB-4 head. The ghost pellet was washed in 10 ml of 10 mM Tris-HCl, pH 7.5, then centrifuged at $2500 \times g$ for 5 min. The pellet was resuspended in 2 ml of the same buffer and kept on ice or at −20°C until needed.

Our ghosts preparations remained contaminated with whole cells, and the contamination varied with the strain: less than 0.1% for strain K19/10, 0.2% for A8207BNK and 3—4% for strain FL-100, as estimated by phase contrast microscopy of lysates.

Zymolyase ghost. Many yeast strains are resistant to glusulase action, and form only a low proportion of osmotically sensitive spheroplasts. We attempted to extend the usefulness of the ghost method by obtaining ghosts from spheroplasts that had been prepared from yeast cells digested with zymolyase [14] an endo-β-1,3-glucanase-containing preparation from *Arthrobacter luteus.*

Yeast strains were grown, harvested and washed as for the glusulase method. Cells were resuspended at 22 ml/g wet weight in zymolyase spheroplast medium containing 1.2 M sorbitol, 10 mM Tris-HCl, pH 7.5, 10 mM $CaCl_2$. 2 mM dithiothreitol and 100—200 μg/ml zymolyase 5000 were added and the mixture incubated as for the glusulase method. Spheroplasts were centrifuged and washed twice in zymolyase spheroplast medium at $169 \times g$ for 5 min. Subsequent steps were as for glusulase ghosts; the concanavalin A step was omitted as it was found to be unnecessary. Microscopic examination of lysed zymolyase spheroplasts showed a cell-sized ghost remnant, easily distinguished from spheroplasts in having no bright halo under phase contrast. The ghosts contained a visible cell wall remnant and this presumably stabilised the envelope. Consistent with this explanation zymolyase ghosts did contain more polysaccharide and were denser than concanavalin A-stabilised ghosts made from glusulase prepared spheroplasts. Recovery of ghosts polysaccharide as a percentage of total cellular carbohydrate was 1.2% for concanavalin A-stabilised ghosts and 6.1% for zymolyase ghosts, determined as in [15]. Zymolyase ghosts pelleted in the 5.8—50% Renografin gradient method of Duran et al. [2], and could be efficiently separated from whole cells. Strains used in this work gave low whole cell contamination after zymolyase treatment (<0.1% for A8207B, K19/10 and FL-100 by microscopic counts of lysates), and the Renografin step was not used routinely. Protein recovery in zymolyase ghosts of A8207BNK by the Folin procedure [13] after sodium hydroxide solubilization of ghost material was 6.6% (average of five determinations, range 5.5—8.5%), and was similar to the recovery of L-[U-^{14}C]protein-hydrolysate incorporated into protein, 8.1% (average of two determinations).

For concanavalin A-stabilised ghosts similar recoveries of L-[U-^{14}C]leucine were obtained.

Two-dimensional polyacrylamide gel electrophoresis of ghost proteins. We have adapted the Ferro-Luzzi Ames and Nikaido [5] modification of the O'Farrell procedure [4] to the yeast ghost system.

Solubilization. Ghosts were solubilised as described [5] except that the material was boiled for 3 min and centrifuged in an Eppendorf 3200 microcentrifuge at approximately 8000 × *g* for 2 min and the supernatant taken. Protein solubilization under these conditions for an SDS to protein ratio of 1.3 : 1 was 65—70%, as measured by recovery of incorporated L-[U-^{14}C]leucine or ^{125}I-iodinated material and was comparable to that described [5]. Variation in the SDS to protein ratios of 2.6—0.65 : 1 gave supernatants which when run in the two-dimensional system had similar gel patterns. The higher SDS to protein ratio gave better than 95% solubilization of incorporated L-[U-^{14}C]leucine, but gave a lower final protein concentration after NP-40 competition, so the 1.3 : 1.0 ratio was used as a compromise to maximise protein concentration. We have no evidence that this procedure results in selective solubilization of ghost proteins.

Sample preparation. This was as described [5]; we routinely added urea at 18 mg/20 μl of solubilised sample after addition of sample dilution buffer.

Isoelectric focusing. Procedures were as described by O'Farrell [4], except that we routinely loaded 200—300 μg of protein/gel. Estimation of the amount of solubilized L-(U-^{14}C)-labelled ghost proteins entering the isoelectric focusing gel was carried out as described by Ferro-Luzzi Ames and Nikaido [5]. Some 65% of the label applied to the gel was recovered. The pH range of the isoelectric gels was 3.8—6.6, as measured in [5]. The method for equilibrating isoelectric focusing gels with SDS prior to running on the second dimension was critical; 20 min at room temperature in 5 ml of SDS equilibration buffer on a roller drum at 180 rev./min. Longer equilibration led to increased horizontal streaking in the second dimension.

Sodium dodecyl sulphate gel electrophoresis. The Laemmli system [16] was used as described by O'Farrell with a 12% running gel. The concentration of Tris in both stacking and running gels was reduced to one-half. EDTA at 2 mM was added throughout and polymerization was catalysed by 0.05% TEMED and 0.1% ammonium persulphate. The electrode buffer contained twice the Laemmli concentration of Tris/glycine buffer. Slabs were cast in a Bio-Rad model 220 electrophoresis cell (with a bevel-edged plate to which the isoelectric focusing gel was attached with 0.5% agarose). Slab gels were 1.5 mm thick, or 0.75 mm when used for autoradiography. Samples: We used fresh ghost preparations for analysis; these were usually used the day after preparation after storage at −20° C and samples were never kept longer than 1 week. We did not get good separations after freezing isoelectric focusing gels, and would use them immediately after isoelectric focusing was complete. Proteins used as molecular standards were as in [5], beef heart lactate dehydrogenase and histidine-binding protein were omitted.

Staining and autoradiography. Gels were stained with Coomassie brilliant blue (Sigma R, 0.5%, w/v) in 25% isopropanol and 10% acetic acid for 1—2 h with gentle shaking. Gels were destained in 15% methanol and 7.5% acetic acid.

To prepare gels for autoradiography they were soaked in 3% glycerol with four 1 h washes, then dried for 2 h in a vacuum gel dryer (Bio-Rad 224). Autoradiograms of dried gels were made with Kodak XR 1 X-ray film.

Lactoperoxidase iodination. A modification of the method of Duran [2] was used. Spheroplasts or ghosts were suspended at $2 \cdot 10^9$ cell equivalents/ml in zymolyase spheroplast medium containing 100 μg/ml lactoperoxidase, 200 μCi/ml $Na^{125}I$, and 17.4 μM H_2O_2. After 10 min incubation at room temperature a further addition of H_2O_2 to a final concentration of 17.4 μM was made and the incubation continued for 10 min. After iodination, 9 ml of zymolyase spheroplast medium containing $1 \cdot 10^{-2}$ M sodium iodide was added/ml of reaction mix, and the mixture centrifuged at $164 \times g$ for 5 min. Iodinated spheroplasts or ghosts were washed and centrifuged a further three times with zymolyase spheroplast medium at 10 ml/$2 \cdot 10^9$ cell equivalents.

Pronase digestion of spheroplasts. Glusulase or zymolyase spheroplasts from $8 \cdot 10^9$ cells were incubated in 20 ml of 1.2 M sorbitol or zymolyase spheroplast medium plus 100 μg/ml pronase for 1 h at 37°C. The spheroplasts were centrifuged at $164 \times g$ for 5 min, resuspended in 20 ml of 1.2 M sorbitol or zymolyase spheroplast medium and washed three times in this way before being taken through the concanavalin A or zymolyase ghost procedure.

Preparation of crude promitochondrial fraction. The method of Needleman and Tzagoloff [17] was used, but modified as follows. Cells were grown as for spheroplast preparations, and washed twice with distilled water, and twice with MTE buffer. Washed cells were resuspended in a mixture of 2.4 ml of MTE buffer and 2.5 g of glass beads (0.25—0.3 mm)/g wet weight of cells and homogenized in a Braun homogeniser (2 times 90 s) with cooling. The homogenate was centrifuged twice at $600 \times g$ for 10 min in an HB-4 head. The supernatant was centrifuged at 13 000 $\times g$ for 20 min and the pellet taken as the crude promitochondrial fraction.

Soluble protein extract. Zymolyase spheroplasts were prepared, lysed in lysis buffer, and the lysate centrifuged at 27 000 $\times g$ for 10 min at 4°C. The supernatant was treated with 10% trichloroacetic acid, and the precipitate washed with acetone, and solubilised with SDS as described by Sato et al. [18].

Results and Discussion

Duran et al. [2], and Santos et al. [3] showed that a large fraction of the radioactivity associated with iodinated yeast spheroplasts was isolated in the ghost fraction. This provided evidence that the concanavalin A-stabilised ghosts did contain the plasma membrane. We have repeated the surface-labeling experiments by iodination of spheroplasts obtained by both the glusulase and zymolyase methods (Table I). The results are similar to those published [2,3].

Two-dimensional gel electrophoresis patterns of yeast ghost proteins

Using the modified Ferro-Luzzi Ames procedure, we have made two-dimensional gel separations of proteins in yeast ghosts from several strains. Both the concanavalin A-stabilised ghosts [2] and zymolyase ghosts have been used; some strains make spheroplasts poorly with glusulase and for these the zymolyase procedure offers a useful alternative.

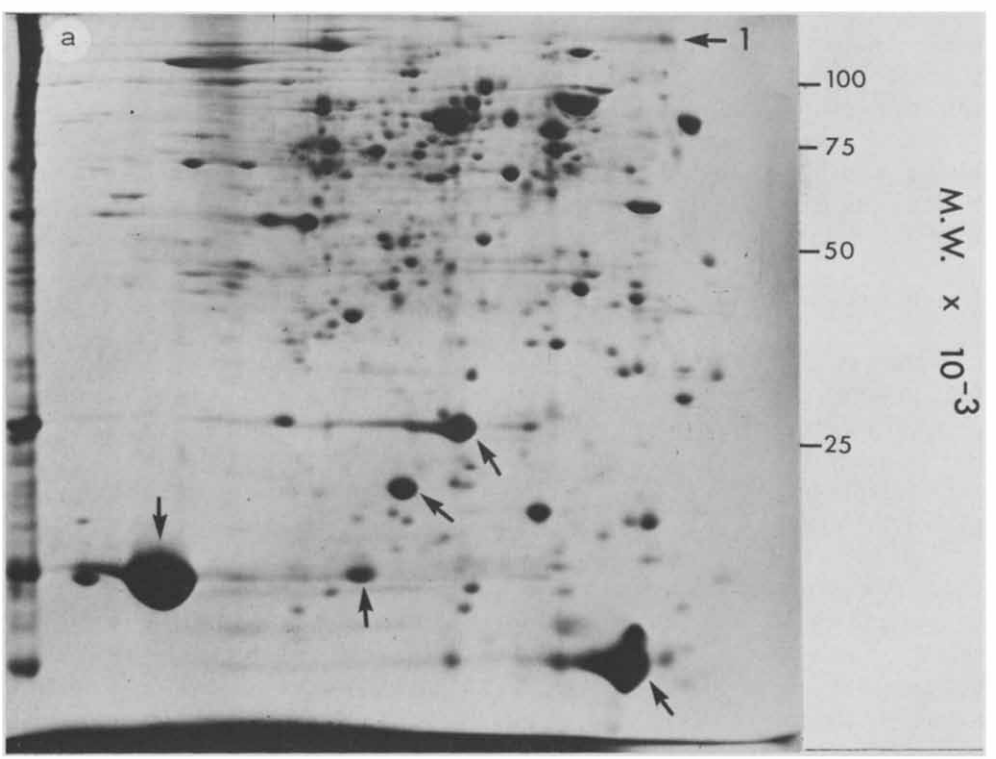
TABLE I

RECOVERY OF SPHEROPLAST ^{125}I-IODINATED MATERIAL IN GHOSTS

Conditions for the preparation of spheroplasts, lactoperoxidase iodination and ghost isolation are described in Materials and Methods. Av., average; *n*, number of experiments.

Strain	Spheroplast method	% of total surface-labeled material recovered in ghost fraction		
		Av.	*n*	Range
A8207BNK	glusulase	67	1	—
A8207BNK	zymolyase	61.6	5	53—72
FL-100	glusulase	64.6	5	52—75

Comparison of the two-dimensional gel patterns of ghosts prepared by the two methods from strain A8207BNK (Fig. 1a and b) showed that some 86% of the proteins were common to both concanavalin A-stabilised ghosts and zymolyase ghosts. Similar comparisons for strains FL-100 and K19/10 gave figures of 90% and 85%, respectively. While the two ghost preparations are not identical their two-dimensional gel patterns reveal extensive homology, suggesting that the ghost fractions prepared by both methods are enriched for a similar set of proteins.



Complexity of ghost proteins

Most of our analyses have been made by simply staining gels of ghost proteins with Coomassie brilliant blue, which reveals approximately 200 spots. Staining for glycoproteins by the periodic acid-Schiff procedure gave four spots, in gels from concanavalin A-stabilised ghosts of strain A8207BNK. The major glycoprotein was a diffuse spot in the upper right hand corner of the gel which we have called the 'North Star' (see Fig. 1a).

This protein was far more prominent in concanavalin A-stabilised ghosts than in zymolyase ghosts, and was the most intensely labelled protein after surface iodination of spheroplasts. As the ghosts were prepared from spheroplasts obtained by enzymatic degradation of the cell wall, the possibility exists that polysaccharide was removed from ghost glycoproteins so our estimate is a minimal one.

Gross contamination of the ghost preparations with other cell components was checked by marker enzyme determinations (Table II). Zymolyase and

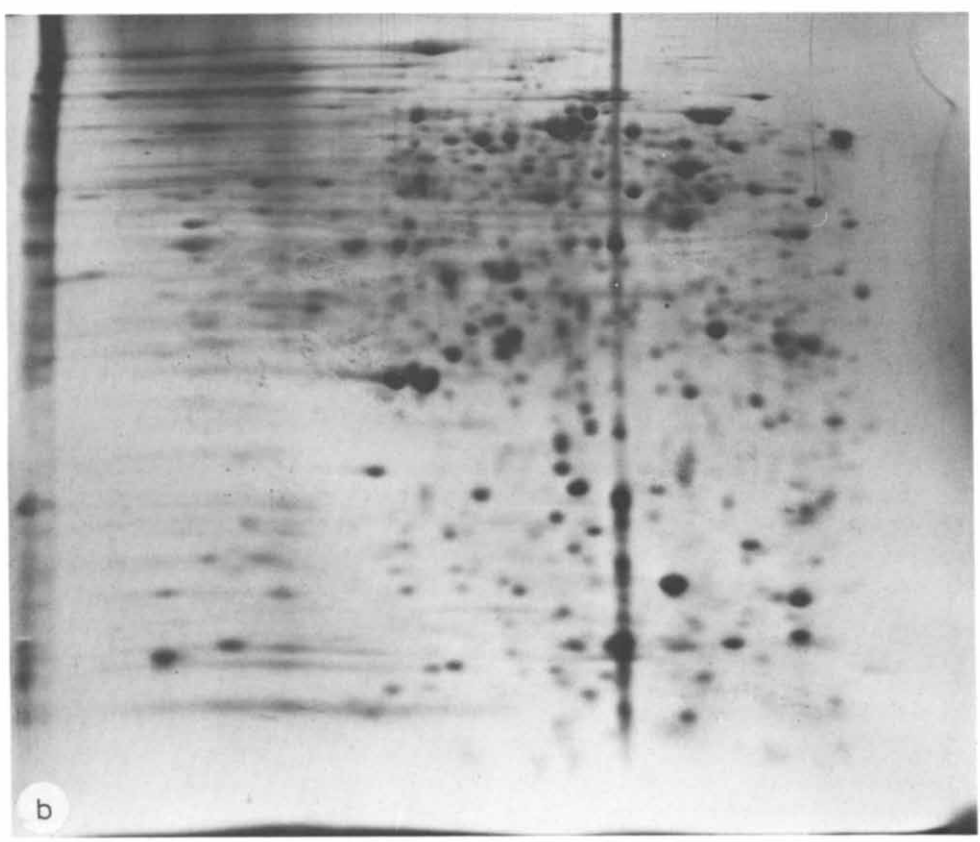


Fig. 1. Two-dimensional gel patterns of ghost proteins. In each case the isoelectric focusing first dimension was from the left to the anode on the right. The molecular weight separation in the second dimension was from the top to the anode at the bottom. Strain A8207BNK was used. (a) Concanavalin A-stabilised ghosts, 300 μg protein. The arrow No. 1 indicates the North Star glycoprotein, other arrows indicate the 27 000 mol. wt. concanavalin A subunits and major fragments present at approximately 100 μg protein. (b) Zymolyase ghosts, 200 μg protein.

concanavalin A-stabilised ghosts showed similar levels of contaminants; acid phosphatase, a cell wall component, was highest but was less than 4% of the total spheroplast activity. Levels of protease A (a vacuolar marker), soluble enzymes, and succinate dehydrogenase (a mitochondrial marker), were never greater than 3% of total spheroplast activity.

A more sensitive measure of contamination was made by comparing the two-dimensional gel pattern of zymolyase ghosts of A8207BNK with those of soluble cellular proteins and of a promitochondrial fraction.

Analysis of soluble cellular material showed that of the approximately 160 major spots in the total soluble material no more than 18 were present in ghosts. While some major spots were common to both preparations, the overall patterns of the ghost and soluble fractions were distinct.

Another potential source of ghost contamination would be other membranes, for example those of mitochondria, and this was tested. In our experiments ghosts were obtained from cells grown in shake flasks in 2% glucose, conditions which repress mitochondrial systems [19]. An enriched promitochondrial fraction was prepared from such a culture and the two-dimensional gel pattern of the proteins obtained. The pattern was less complex than that of the ghosts; about 90 spots were visible, most were minor. Promitochondria and ghost patterns had spots in common: of the 90 spots in gels of promitochondria some 30—35 were visible in those of zymolyase ghosts. Some of the common spots were removed by surface proteolysis, and so were either common to both mitochondrial and plasma membranes or plasma membranes contaminated the promitochondrial fraction. In summary, the zymolyase ghost fraction contained proteins associated with both soluble and promitochondrial fractions and as many as 25% of all ghost proteins were of this type. The ghost fraction

TABLE II

MARKER ENZYME RECOVERIES IN GHOST FRACTIONS

Preparation of spheroplasts and ghosts, lysis of spheroplasts, and enzyme assays are described in Materials and Methods. Activity units are given as μmol/min per mg protein. Strain A2807BNK was used.

Enzyme	Spheroplasts		Ghosts		
	Total units	Specific activity	Total units	Specific activity	% spheroplast activity in ghosts
A. Glusulase ghost fraction					
Invertase	16.8	0.12	0.037	0.004	0.22
Acid phosphatase	11.4	0.08	0.43	0.046	3.8
Protease A	1179	8.30	22.5	2.42	1.9
Glucose-6-phosphate dehydrogenase	1.78	0.012	0.008	0.001	2.2
Alcohol dehydrogenase	167.0	1.18	0.73	0.078	0.44
Succinate dehydrogenase	not detectable				
B. Zymolyase ghost fraction					
Invertase	23.0	0.15	0.06	0.007	0.26
Acid phosphatase	13.5	0.09	0.53	0.061	3.9
Protease A	1106	7.18	32.67	3.79	3.0
Glucose-6-phosphate dehydrogenase	7.3	0.05	0.004	0.0005	0.057
Alcohol dehydrogenase	174.8	1.14	0 05	0.006	0.29
Succinate dehydrogenase	67.0	0.44	1 84	0.21	2 7

can then only be considered as one enriched for envelope proteins. Estimates of surface label recovery (Table I) and total cell protein recovery (6—8%, see Materials and Methods) suggest that the enrichment was 8—10-fold over whole cells.

Controls for surface labelling

Recovery of labelled material from iodinated spheroplasts in the ghost fraction was the main criterion for assuming that ghosts contained plasma membrane (Table I, [2,3]). One concern with iodination of spheroplasts is that all or a certain proportion of them may be permeable to the labelling reagents. We have tested the integrity of spheroplasts by examining their sensitivity to pronase digestion (see below). In addition, to directly examine spheroplast impermeability to the surface-labelling reagents we have compared the pattern of labelling of the two-dimensional gels of ghosts obtained from iodinated spheroplasts with that found when isolated ghosts were labelled by the same procedure. Some iodinatable proteins may not be exposed in spheroplasts but would be labelled in ghosts where both sides of the envelope are accessible to the labelling reagents [20].

Pronase digestion of spheroplasts

All of the proteins associated with zymolyase ghosts were removed by pronase digestion (100 μg/ml, 1 h at 37° C), based on one-dimensional SDS gels of the digests (data not shown).

Zymolyase-prepared spheroplasts were incubated with pronase as above, ghosts were prepared and the two-dimensional gel patterns of proteins compared with those from untreated ghosts. For ghosts the overall protein pattern was largely unchanged by pronase digestion: examination of the gels showed selective loss of some ten spots and a further seven spots showed some disproportionate reduction in intensity. There was a general low-level reduction in spot intensity on two-dimensional gels of ghosts from pronase-treated spheroplasts. The selective loss of ghost proteins in the present of pronase is best

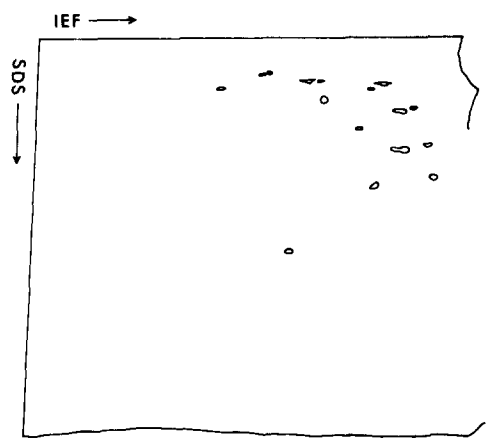


Fig. 2. Diagram showing a two-dimensional gel separation of proteins selectively removed by pronase digestion of zymolyase spheroplasts of strain A8207BNK. The control gel for comparison is Fig. 1b.

TABLE III

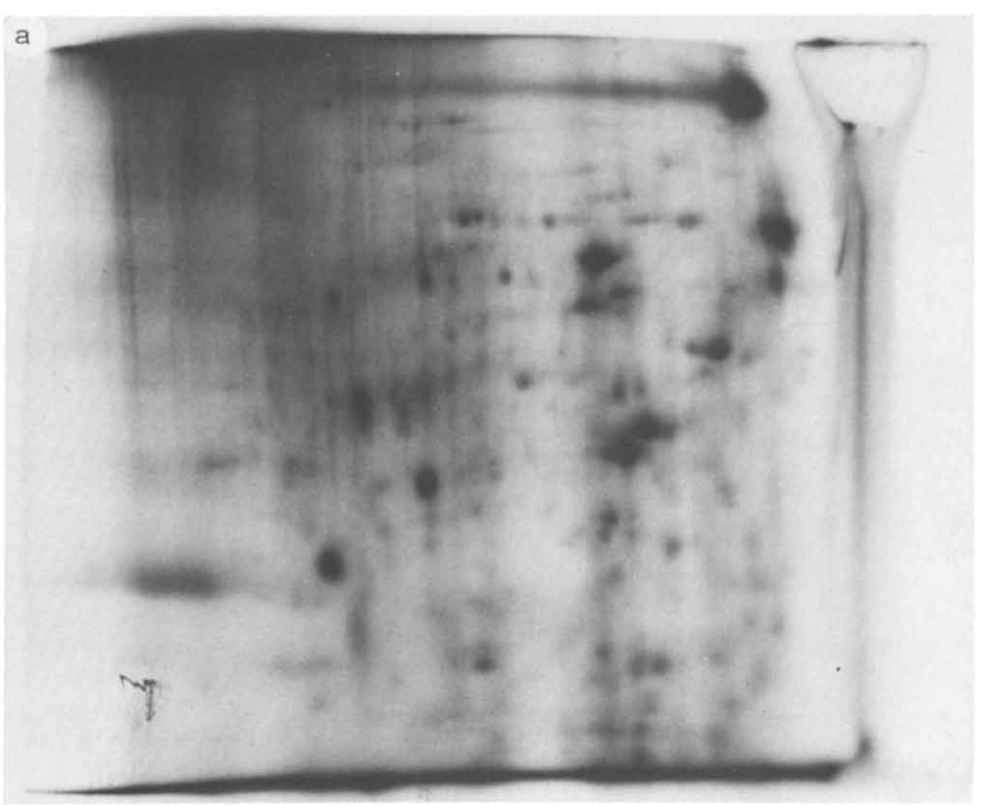
LACTOPEROXIDASE-CATALYSED ^{125}I IODINATION OF ZYMOLASE SPHEROPLASTS AND GHOSTS FOR STRAIN A8207BNK

	Iodination cpm $^{125}I/2 \cdot 10^9$ cell equivalents)		
	Ghosts from iodinated spheroplasts	Iodinated ghosts	Ratio ghosts/spheroplasts
Expt. 1	$1.5 \cdot 10^6$	$5.5 \cdot 10^6$	3.7
Expt. 2	$1.1 \cdot 10^6$	$4.9 \cdot 10^6$	4.4

explained by supposing that the digested proteins were exposed in spheroplasts. Proteins selectively removed by pronase treatment of spheroplasts are indicated in schematic form in Fig. 2.

Surface-labelled spheroplasts

Zymolyase method. Zymolyase ghosts were more heavily labelled by lactoperoxidase iodination than were zymolyase spheroplasts (Table III), a finding suggestive of reduced availability of sites for iodination in spheroplasts. The two-dimensional gel pattern of ghosts obtained from surface-labelled spheroplasts is shown in Fig. 3.



Iodinated zymolyase ghosts showed a more complex pattern than ghosts isolated from iodinated spheroplasts; about 110 spots for labelled ghosts and about 75 spots, many weakly labelled, for ghosts from labelled spheroplasts. Some 20 spots were preferentially labelled in spheroplasts and were barely detectable in ghosts. These spots were in the acidic region of the gel and represented minor protein species by Coomassie staining. The most prominent iodinated species was the North Star, the major glycoprotein associated with the ghosts. The difference in labelling pattern between spheroplasts and ghosts was not consistent with zymolyase spheroplast permeability to the lactoperoxidase iodination reagents, and strongly suggests that iodination of spheroplasts represents labelling of exposed proteins.

The experiments reported here indicate that both the concanavalin A-stabilised ghosts and zymolyase ghosts are enriched 8—10-fold for surface-labelled proteins, and are recovered in high yield. These ghosts seem a useful starting point for examining yeast plasma membrane proteins, though further purification of the ghosts should be explored. Our two-dimensional gel electrophoresis studies of ghost proteins have indicated classes of proteins exposed to protease digestion or lactoperoxidase-catalysed iodination in spheroplasts; these are good candidates for membrane proteins. The two-dimensional gel

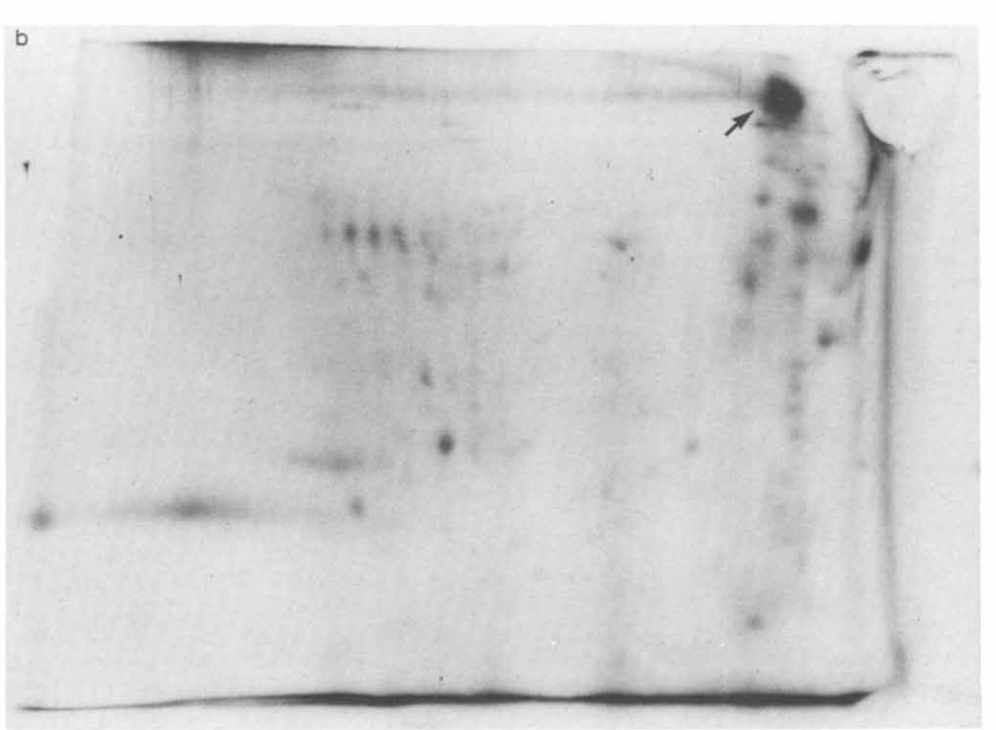


Fig. 3. Two-dimensional gels of iodinated proteins of zymolyase ghosts obtained from an iodinated ghost fraction, and from iodinated spheroplasts of strain A8207BNK. (a) Iodinated ghost fraction, 200 μg protein, $9.9 \cdot 10^5$ cpm. (b) Ghosts from iodinated spheroplasts, 200 μg protein, $2.2 \cdot 10^5$ cpm. Autoradiography was eleven weeks for (a) and twelve weeks for (b). Arrow indicates the position of the North Star glycoprotein.

electrophoretic analysis of ghost proteins should aid identification of membrane proteins if they can be altered by mutation or by differential expression during changes in environmental conditions or in the yeast developmental sequence.

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