Distribution of Plasma-membrane Fragments during Zonal Centrifugations of Homogenates from Aerobic *Saccharomyces cerevisiae*

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INTRODUCTION

During zonal centrifugations of homogenates from glucose-repressed *Saccharomyces cerevisiae*, the buoyant densities of various plasma-membrane fragments greatly depend on their content of heavy glycoprotein particles (Nurminen, Taskinen & Suomalainen, 1976). Experiments with homogenates from aerobic *S. cerevisiae* are described here. A complicating factor in the isolation of the yeast plasma membrane is that the only marker enzyme known for it, Mg²⁺-dependent adenosine triphosphatase (Mg-ATPase), is also present in mitochondria. The mitochondrial Mg-ATPase, however, differs from the Mg-ATPase of the plasma membrane in being oligomycin-sensitive. In aerobic yeast, fully developed mitochondria are readily detectable in electron micrographs and the activity of the typical mitochondrial enzymes is high. Therefore the possible presence of mitochondrial membranes in the preparations of plasma membranes is easier to detect than in the glucose-repressed yeast.

METHODS

Organism. This was commercial baker's yeast (*Saccharomyces cerevisiae*), from the Rajamäki Factories of the Finnish State Alcohol Monopoly (Alko).

Preparation of subcellular fractions. Fresh cells were disrupted in a Mini-mill disintegrator, high-speed zonal centrifugations were performed in a B XV zonal rotor, and several of the membrane fractions obtained by zonal centrifugation on buffered sucrose were further fractionated on gradients of iso-osmotic Urografin as described previously (Nurminen *et al.*, 1976).

Biochemical determinations. Enzyme assay procedures and analytical methods were as described by Nurminen *et al.* (1976).

Electron microscopy. Electron micrographs were obtained as described by Nurminen *et al.* (1976).

RESULTS AND DISCUSSION

Content of marker components in whole homogenates

The contents of the marker components in the whole homogenates [have been reported (Nurminen *et al.*, 1976, Table 1), apart from the total carbohydrate which was 103 mg (100 mg homogenate protein)⁻¹.

Fig. 1. Fractionation of a whole homogenate of aerobic baker's yeast (*S. cerevisiae*) by high-speed zonal centrifugation in a gradient of buffered sucrose. Centrifugation was at 20000 rev. min⁻¹ for 360 min in a B XV rotor (approx. 5×10^8 g-min at the sample zone). The density distributions of the following components are shown: (a) protein (○) and RNA (●); (b) acid phosphatase (△), NADPH-cytochrome *c* oxidoreductase (▲) and cytochrome *c* oxidase (○); (c) phospholipid (△) and sterol (▲); (d) α-mannosidase (△) and Mg-ATPase with (●) and without (○) oligomycin. Results are expressed as percentages of the total amounts recovered in the density range 1.00 to 1.29 g cm⁻³.

Fractionation by high-speed zonal centrifugation in a Ficoll gradient

In buffered iso-osmotic Ficoll, about 75 % of the protein was distributed at the densities 1.04 to 1.08 (g cm⁻³), as was most of the alkaline pyrophosphatase (98 %), AMPase (94 %), alkaline phosphatase (88 %) and β-fructofuranosidase (80 %). The RNA showed only one maximum, at $\rho = 1.08$, whereas phospholipid and sterol distributed in a broad band at $\rho = 1.04$ to 1.10, with two minor peaks at $\rho = 1.12$ to 1.13 and $\rho = 1.16$. NADPH-cytochrome *c* oxidoreductase was largely non-sedimentable, but did have a high activity coincident with the peak of the mitochondrial marker, cytochrome *c* oxidase, and with the main peak of oligomycin-sensitive Mg-ATPase at $\rho = 1.08$ to 1.10.

The proportion of Mg-ATPase that was oligomycin-insensitive was about 15 % around $\rho = 1.08$, 30 to 40 % at $\rho = 1.09$ to 1.11, and about 70 % at $\rho = 1.12$ to 1.13 and above. Electron microscopy showed that smooth-surfaced, triple-layered membranes were the dominant objects at around $\rho = 1.12$. These membranes were evidently fragments of the plasma membrane, for this material contained oligomycin-insensitive Mg-ATPase.

Fractionation by high-speed zonal centrifugation in a sucrose gradient

After zonal centrifugation in sucrose gradients, about 65 % of the protein, including most of the alkaline pyrophosphatase (96 %), β-fructofuranosidase (94 %), alkaline phosphat-

Table 1. Chemical composition of the membrane fractions, and their oligomycin-insensitive Mg-ATPase and cytochrome *c* oxidase activities

Fractions S1 to S6 were isolated at the specified densities by high-speed zonal centrifugation in sucrose gradients. They were further purified by swing-out centrifugation on Urografin gradients. The chemical compositions [$\mu\text{g (mg protein)}^{-1}$], the oligomycin-insensitivity of Mg-ATPase [(units with oligomycin/units without oligomycin) $\times 100$] and the specific activity of cytochrome *c* oxidase [$\mu\text{mol min}^{-1} (\text{mg protein})^{-1}$] of each starting fraction (S1 etc.) and the main visible bands obtained from it on Urografin (U1a etc.) are shown.

Fraction	Density range (g cm^{-3})	RNA	DNA	Phospholipid	Sterol	Total lipid	Carbohydrate	Oligomycin-insensitivity of Mg-ATPase	Cytochrome <i>c</i> oxidase
S1	1.09–1.13	350	3	130	30	380	99	18	0.098
U1a	1.11–1.16	60	1	340	30	1460	74	38	ND
U1b	1.17–1.23	20	1	280	100	1080	56	75	ND
S2	1.16–1.17	60	8	270	50	ND	68	14	2.009
U2	1.15–1.19	30	4	250	60	750	70	48	ND
S3	1.18–1.19	60	12	280	40	ND	81	20	1.765
U3	1.15–1.19	10	6	210	30	780	43	ND	ND
S4	1.21–1.22	50	10	110	20	ND	278	38	0.613
U4	1.15–1.19	60	4	230	30	ND	823	89	ND
S5	1.23–1.24	90	12	100	30	ND	253	87	0.345
U5	1.15–1.19	30	8	50	40	ND	510	ND	ND
S6	1.25–1.27	140	3	150	50	ND	484	87	0.168
U6	1.19–1.23	60	1	190	40	1080	680	81	ND

ND, Not determined

ase (85 %), acid phosphatase (67 %) and AMPase (61 %), was at $\rho = 1.03$ to 1.06 (Fig. 1). About 65 % of the RNA was coincident with the second protein peak at $\rho = 1.10$. The fractions around $\rho = 1.10$ were rich in mixed light membranes, including membranes of the rough endoplasmic reticulum (the main peaks of RNA and NADPH-cytochrome *c* oxidoreductase), vacuolar membranes (the maximum of α -mannosidase, the marker for vacuolar membranes; van der Wilden *et al.*, 1973), and membranes containing Mg-ATPase. The main peaks of cytochrome *c* oxidase (about 70 %) and oligomycin-sensitive Mg-ATPase indicate the presence of mitochondria at $\rho = 1.16$ to 1.18 . Electron microscopy also showed mitochondria to be the dominant organelles in this density range.

About 20 % of the phospholipid and 40 % of the sterol were non-sedimentable ($\rho < 1.09$). Their peaks above $\rho = 1.09$ indicate the positions of the major part of the membrane material. Less than 10 % of Mg-ATPase was non-sedimentable, but otherwise its distribution resembled that of phospholipid and sterol. The proportion of oligomycin-insensitive Mg-ATPase increased towards the higher densities (Table 1), and was 80 to 100 % at $\rho = 1.23$ to 1.26 .

Electron micrographs of the material present in the last peak of phospholipid and sterol at $\rho = 1.26$ revealed smooth-surfaced, triple-layered single-unit membranes as the dominant objects. Because its Mg-ATPase was nearly completely oligomycin-insensitive and practically no NADPH-cytochrome *c* oxidoreductase or cytochrome *c* oxidase was present, it is reasonable to conclude that these membranes were heavy fragments of plasma membrane.

Subfractionation of zonal fractions on a Urografin gradient

Fraction S1 (Table 1) was rich in light membranes. Sedimentation through Urografin increased its total lipid, phospholipid and sterol contents and decreased its RNA, DNA and protein contents. The removal of rough endoplasmic reticulum was indicated by the marked decrease in RNA. The proportion of oligomycin-insensitive Mg-ATPase increased to 75 % in fraction U1b. This suggests that an enrichment of the light vesicles of the plasma membrane was obtained. The presence of vacuolar membranes is unlikely, for these move slowly even through a flat Urografin gradient (van der Wilden *et al.*, 1973).

S2 and S3 were mitochondrial fractions as indicated by their high specific activity of cytochrome *c* oxidase. The proportion of carbohydrate in fractions S4, S5 and S6 was markedly higher than in the less dense fractions, S1, S2 and S3, and was further increased after purification on Urografin. The decrease in the specific activity of cytochrome *c* oxidase indicated that the amount of mitochondria in these fractions decreased towards the higher densities. Fraction U6 is representative of the purity of the plasma membrane preparations achieved in this work.

Fractions S1, S4, S5 and S6 contained mannose as the dominant sugar; in each fraction it formed about 60 % of the total amount of hexoses and inositol. A high content of mannose is a characteristic of yeast plasma membrane preparations isolated by mechanical means (Matile, Moor & Mühlethaler, 1967; Matile, 1970; Fuhrmann, Wehrli & Boehm, 1974; Nurminen *et al.*, 1976). Matile *et al.* (1967) found mannose as the only hexose in their preparation and showed that the globular particles of the plasma membrane are composed of mannanprotein. In addition to mannose, fractions S1, S4, S5 and S6 contained glucose, galactose and inositol. A certain amount of the three hexoses and inositol in our preparations can be attributed to the plasma membrane glycolipids (cf. Nurminen & Suomalainen, 1971; Työrinoja, Nurminen and Suomalainen, 1974). Most of the inositol in the plasma membrane is present as phosphatidylinositol. The proportion of mannose in fractions S4, S5 and S6 was similar to that in the heavy membranes isolated from glucose-repressed cells (Nurminen *et al.*, 1976). However, the total carbohydrate was considerably lower in the three aerobic fractions than in the preparation isolated at $\rho = 1.24$ to 1.26 from glucose-repressed yeast. These observations suggest that the heavy mannanprotein particles are largely responsible for the high density of the plasma-membrane fragments, but they are detached more easily from the plasma membrane of aerobic yeast. Alternatively, there may be fewer of these particles in the resting aerobic cells than in the budding glucose-repressed cells in which the particles may carry material for the new cell wall.

REFERENCES

- FUHRMANN, C. F., WEHRLI, E. & BOEHM, C. (1974). Preparation and identification of yeast plasma membrane vesicles. *Biochimica et biophysica acta* **363**, 295–310.
- MATILE, Ph. (1970). Properties of the purified cytoplasmic membrane of yeast. In *Membrane Structure and Function*. Federation of European Biochemical Societies, 6th Meeting, Madrid 1969, pp. 39–49. Edited by J. R. Villanueva and F. Ponz. London and New York: Academic Press.
- MATILE, Ph., MOOR, H. & MÜHLETHALER, K. (1967). Isolation and properties of the plasmalemma in yeast. *Archiv für Mikrobiologie* **58**, 201–211.
- NURMINEN, T. & SUOMALAINEN, H. (1971). Occurrence of long-chain fatty acids and glycolipids in the cell envelope fractions of baker's yeast. *Biochemical Journal* **125**, 963–969.
- NURMINEN, T., TASKINEN, L. & SUOMALAINEN, H. (1976). Distribution of membranes, especially of plasma-membrane fragments, during zonal centrifugations of homogenates from glucose-repressed *Saccharomyces cerevisiae*. *Biochemical Journal* **154**, 751–763.
- TYÖRINOJA, K., NURMINEN, T. & SUOMALAINEN, H. (1974). The cell-envelope glycolipids of baker's yeast. *Biochemical Journal* **141**, 133–139.
- WILDEN, W., VAN DER, MATILE, Ph., SCHELLENBERG, M., MEYER, J. & WIEMKEN, A. (1973). Vacuolar membranes: isolation from yeast cells. *Zeitschrift für Naturforschung, Teil C* **28**, 416–421.