Subcellular Fractionation of Particles containing Acid Hydrolases from *Saccharomyces carlsbergensis*

By T. G. Cartledge and D. Lloyd

Department of Microbiology, University College, Newport Road, Cardiff CF2 1TA, U.K.

(Received 17 November 1971)

It has been shown that, under suitable conditions, the lysis of osmotically stabilized sphaeroplasts of yeast leads to the release of cell-vacuoles, which can then be purified on iso-osmotic gradients (Matile & Wiemken, 1967). On the basis of these fractionations these authors claim that the single large cell-vacuole is the only lysosome of the yeast cell. However, it has been shown that, by using rate-zonal separations on a mannitol–Ficoll gradient system, it is possible to demonstrate in addition the presence of a sedimentable population of acid-hydrolase-containing membranes that are distinct from these low-density vacuoles (Cartledge et al., 1971). The diversity of acid-hydrolase-associated membranes in yeast is here further confirmed by high-speed zonal fractionation on sucrose gradients.

Cultures of *Saccharomyces carlsbergensis* were grown to late exponential phase (5 × 10⁷ cells/ml) and harvested during glucose de-repression. Whole homogenates were prepared in 0.25M-sucrose–10mM-tris–HCl–2mM-MgCl₂ medium, pH7.4, and subjected to zonal centrifugation. All these procedures were identical with those described by Cartledge & Lloyd (1972), as were those for assays of protein, cytochrome c oxidase and acid p-nitrophenyl phosphatase. Alkaline p-nitrophenyl phosphatase was assayed by a method similar to that used for the corresponding acid hydrolase, except that tris–HCl buffer, pH10.0, replaced acetate buffer. β-Glycerophosphatase, acid deoxyribonuclease and acid ribonuclease were assayed as described by Müller et al. (1966), and acid protease was assayed as described by Vaes & Jacques (1965). Assays of the other hydrolases were carried out in media containing the appropriate p-nitrophenol derivatives (1.0mM), 40mM-sodium acetate buffer, pH5.0, and Triton X-100 (0.02%, w/v).

Enzymes assayed but not present at detectable levels of activity included α-l-fucosidase and α-D-mannosidase; very low N-acetyl-β-D-galactosaminidase and β-D-mannosidase activities were observed. Distributions of enzymes after zonal centrifugation are shown in Fig. 1. Cytochrome c oxidase is entirely sedimentable, and mitochondria have attained their equilibrium density (ρ > 1.20; Fig. 1b); profiles of E₅₂₀, E₆₆₀ and protein (Fig. 1a) indicate the presence of a variety of non-mitochondrial membranes (ρ < 1.20). More than 80% of the β-glycerophosphatase (Fig. 1c), α-glucosidase (Fig. 1e) and β-glucuronidase (Fig. 1e) are non-sedimentable, as are β-galactosidase and β-glucosidase (97% and 92.2% respectively; not shown). Acid p-nitrophenyl phosphatase (Fig. 1c) and acid protease (Fig. 1d) both show similar complex distributions. The sedimentable portion of alkaline p-nitrophenyl phosphatase (Fig. 1c) shows a distinctly bimodal distribution, and is different from the corresponding acid hydrolase. Three distinct populations of particles containing deoxyribonuclease are separated under these conditions (Fig. 1d); the distribution of ribonuclease (Fig. 1d) is also complex but differs from those of all the other acid hydrolases examined. α-Galactosidase (Fig. 1e) shows the most heterogeneous distribution of all the enzymes assayed. Thus it appears doubtful whether all the acid hydrolases detectable in homogenates of *Saccharomyces carlsbergensis* are associated in vivo with a single species of organelle, as suggested by Matile (1969) and Matile et al. (1969); these enzymes may also be associated with (or enclosed by) membrane systems other than those of the cell-vacuole. For instance, it has been shown that isolated yeast cell walls have acid p-nitrophenyl phosphatase activity (Nurminen et al., 1970); in our fractionations this enzyme is partially lost on washing of the sphaeroplasts, and that proportion remaining after centrifugation (to remove plasma membranes) is 50% latent (T. G. Cartledge & D. Lloyd, unpublished work). Further work is necessary to characterize organelles containing acid hydrolases in the intact yeast cell, in order to identify yeast lysosomes positively.

This work was carried out during the tenure of a Medical Research Council Research Studentship by T. G. C. The authors acknowledge the encouragement of Professor D. E. Hughes.


Matile, Ph. & Wiemken, A. (1967) *Arch. Mikrobiol.* 56, 148–155


Vol. 126
Fig. 1. Fractionation of a whole homogenate of aerobically grown glucose-de-repressed *Saccharomyces carlsbergensis*

The whole homogenate (30 ml), containing 168 mg of protein, was diluted 1:20, and volumes of diluted homogenate and of fractions taken for assays were as follows: cytochrome c oxidase, 0.1 ml; all others, 0.2 ml. Centrifugation was at 35000 rev./min for 165 min in the BXIV zonal rotor (6 × 10⁶ g-min at the sample zone; ω²·dt = 1.45 × 10⁻¹¹ rad²·s⁻¹). (a) Sucrose density gradient (○), E₅₂₀ (——) and E₂₆₀ (——). (b) Protein (●) and cytochrome c oxidase (○). (c) Acid p-nitrophenyl phosphatase (●), alkaline p-nitrophenyl phosphatase (pH 10.0) (○) and β-glycerophosphatase (▲). (d) Acid deoxyribonuclease (●), acid ribonuclease (○) and acid protease (▲). (e) α-Glucosidase (●), α-galactosidase (○) and β-glucuronidase (▲). The specific activities of enzymes (nmol/min per mg of protein) in the whole homogenate were as follows (recoveries in parentheses): protein (104%); cytochrome c oxidase, 10.3 (88%); acid p-nitrophenyl phosphatase, 4.5 (96.4%); alkaline p-nitrophenyl phosphatase, 7.4 (97.6%); β-glycerophosphatase, 35.9 (99%); acid deoxyribonuclease, 0.07 (432%); acid ribonuclease, 1.57 (38%); acid protease, 62 (ng of protein hydrolysed/min per mg of homogenate protein) (53%); α-glucosidase, 0.2 (124%); α-galactosidase, 0.3 (136%); β-glucuronidase, 1.67 (113%). Values on the diagram refer to % of total units sedimented to density >1.10 (‘% sedimentability’).