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BIOSYNTHESIS OF ACID PHOSPHATASE OF BAKER'S YEAST

CHARACTERIZATION OF A PROTOPLAST-BOUND FRACTION CONTAINING PRECURSORS OF THE EXO-ENZYME

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Summary

1. Yeast protoplasts, secreting acid phosphatase (orthophosphoric-monoester phosphohydrolase (acid optimum) EC 3.1.3.2) contain a small amount of firmly bound enzyme, even after lysis (Van Rijn, H.J.M., Boer, P. and Steyn-Parvé, E.P. (1972) Biochim. Biophys. Acta 268, 431—441). The major part (70%) of this protoplast-bound acid phosphatase can be solubilized by non-ionic detergents, such as Triton X-100.

2. The kinetics of radioactive amino acid incorporation in the solubilized and in the secreted enzyme has been estimated by pulse-chase labelling of secreting protoplasts, followed by fractionation and counting radioactivity in the enzyme band in polyacrylamide gels after electrophoresis at pH 5.0. A precursor-product relationship between the Triton X-100-extractable fraction of the protoplast-bound acid phosphatase and the secreted enzyme is apparent.

3. The solubilized acid phosphatase is essentially indistinguishable from the secreted enzyme with regard to a number of enzymatic properties and its stability towards pH and temperature. Both enzymes also behave alike on polyacrylamide-gel electrophoresis, producing a single acid phosphatase band with glycoprotein character and comparable mobility.

4. A striking difference is seen in isopycnic equilibrium sedimentation in CsCl: the secreted acid phosphatase is homogeneous, with a buoyant density of $\rho = 1.47$ g/cm^3, while the Triton X-100-extractable part of the protoplast-bound acid phosphatase is heterogeneous; besides heavier material a major component with buoyant density of $\rho = 1.37$ g/cm^3 is always visible.

Introduction

In previous work on the biosynthesis of acid phosphatase (ortho phos-

phoric monoester phosphohydrolase, EC 3.1.3.2) in baker's yeast we have studied the secretion of this enzyme by protoplasts. We determined optimal conditions for secretion of acid phosphatase, and were able to show the secreted enzyme to be identical with the acid phosphatase located in the cell wall of the intact yeast cell. Furthermore we observed that secreting protoplasts always retain a small amount of acid phosphatase, firmly bound, even after lysis [1].

It was of interest to know whether this protoplast-bound acid phosphatase is a precursor of the exocellular enzyme, and if so, whether this is the case for the whole fraction or only for part of it. For not all protoplast-bound, internal enzyme molecules are necessarily precursors of the secreted enzyme, as studies on a similar yeast exo-enzyme, β-fructofuranosidase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26; formerly known as invertase) have pointed out [2—5].

We report here (for the first time) the results of a more detailed study of the protoplast-bound acid phosphatase. We were able to solubilize most of the bound enzyme. We have studied the kinetics of the incorporation of radioactive amino acids into the solubilized and the secreted enzyme, and we have compared several specific properties of both enzymes.

Materials and Methods

Materials

All chemicals used were analytical grade.

The yeast used in the experiments was *Saccharomyces cerevisiae* (Koningsgist), which was inoculated monthly in a Wickerham medium and kept at 4°C. The cultivation of this yeast and the preparation of acid phosphatase-secreting protoplasts has been described previously [1].

The incubation medium for protoplasts was a medium according to Markham et al. [6], from which inorganic phosphate had been omitted and containing 12% (w/v) mannitol, indicated in this paper as Markham-mannitol medium.

A sample of Lubrol PX has kindly been supplied by I.C.I., Rotterdam. Radioactively labelled amino acids, [^{14}C]leucine (spec. act. 342 Ci/mol) and [^{14}C]tyrosine (spec. act. 507 Ci/mole), were purchased from The Radiochemical Centre, Amersham, U.K.

Solubilization of protoplast-bound acid phosphatase

Secreting protoplasts were washed twice with a solution of 12% (w/v) mannitol in 1 mM sodium acetate (pH 3.8) and then suspended in 1 mM sodium acetate (pH 3.8) containing 1% (w/v) Triton X-100. The suspension was kept at room temperature for 20 min, while shaking vigorously and repeatedly on a Vortex mixer. Subsequently the suspension was centrifuged (10 min, 1000 × *g*, 4°C). The supernatant forms the so-called TE (Triton-extractable) enzyme fraction. Before use the TE-fraction was dialysed (24 h) against 1 mM sodium acetate (pH 3.8) at 4°C, and, if necessary, concentrated against 20% (w/v) polyethylene glycol.

Polyacrylamide-gel electrophoresis

Polyacrylamide gels (7.5 cm × 0.6 cm) consisted of 5% (w/v) acrylamide, 0.25% (w/v) *N,N'*-methylene bisacrylamide, 0.1% (v/v) *N,N,N',N'*-tetramethylenediamine and 0.1% (w/v) ammonium persulphate in 10 mM sodium acetate (pH 5.0).

We always applied a pre-electrophoresis during 18 h at 1.5 mA/gel (4°C). Hereafter samples were electrophorized during 6 h at 2.5 mA per gel (4°C).

The gels were stained as follows:

Acid phosphatase activity was detected by incubating the gels for 15 min at room temperature in a 0.4% (w/v) solution of α-naphtyl phosphate in 10 mM sodium acetate (pH 3.8), followed by development of the red colour by incubating the gels during 10 min in a 0.2% (w/v) solution of Fast Red B in 1 mM sodium acetate (pH 3.8).

For protein staining gels were placed in a solution of 1% (w/v) Amido Black in 7% (v/v) acetic acid and incubated for 30 min at 50°C. Destaining was performed by gently shaking the gels in 7% (v/v) acetic acid.

Staining for carbohydrate was performed according to Zacharius et al. [7].

Isopycnic equilibrium sedimentation in CsCl

The density of enzyme preparations in 0.1 M sodium acetate (pH 4.0) was adjusted with solid CsCl to 1.4026 g/cm^3. Samples were centrifuged for 64 h at 35 000 rev./min in a Spinco SW-50 rotor (5°C). Fractions of 20 drops were collected for measurement of enzyme activity and the refractive index. The buoyant density was calculated from the refractive index with the aid of an expression published by Ifft et al. [8].

Sample treatment and counting of radioactivity

Samples obtained by precipitation of material with trichloroacetic acid were solubilized in 1 ml Soluene TM-100 (Packard) by incubation at 60°C for 1 h.

After gel electrophoresis of radioactive material gels were sliced (1 mm) and each slice was solubilized by shaking it in a counting vial in 0.5 ml 30% (w/v) H_2O_2 at 37°C for 24 h.

To the solubilized samples 14.5 ml of a scintillation fluid was added, consisting of a mixture of 7 vol. Triton X-100 and 23 vol. toluene (+ 0.5% (w/v) PPO and 0.005% (w/v) POPOP).

Radioactivity was measured in a Nuclear Chicago liquid scintillation counter (Mark I or Mark II).

Analytical methods

Activity of acid phosphatase towards the substrates *p*-nitrophenyl phosphate and β-glycerophosphate was determined as described by Van Rijn et al. [1].

Protoplasts were counted in a Bürker chamber as the difference between the number of cells before and after lysis. The protoplast preparations we used always contained less than 1% intact yeast cells.

Results

Solubilization of the protoplast-bound acid phosphatase

To facilitate examination of the protoplast-bound acid phosphatase we first tried to solubilize this enzyme fraction. 1 M NaCl or KCl had no effect, but with 1% (w/v) Triton X-100 we obtained 70% of the enzyme activity in solution. Other non-ionic detergents in the same concentration gave about the same results: Lubrol PX: 65%, Brij 58: 65% and Cetavlon: 60%. Higher detergent concentrations (up to 5% (w/v)), did not detach more acid phosphatase but led to loss of enzyme activity. So did detergents such as 0.5% (w/v) sodium dodecylsulphate or 1.25% (w/v) sodium taurocholate. Sonication of the protoplasts did not solubilize more acid phosphatase than treatment with 1% (w/v) Triton X-100 did.

Centrifugation of the Triton X-100 extract at 100 000 × *g* for 1 h sedimented less than 5% of the enzyme activity, indicating a real solubilization of the enzyme [9].

Further experiments have been performed with Triton X-100 extracts, routinely obtained as described in Materials and Methods. These preparations are indicated as TE-fractions, while the secreted enzyme is called S-fraction.

Kinetics of radioactive amino acid incorporation in TE- and S-fractions

The TE-fraction is only an interesting object for further examination if it indeed contains precursors of the secreted enzyme. Therefore we have studied

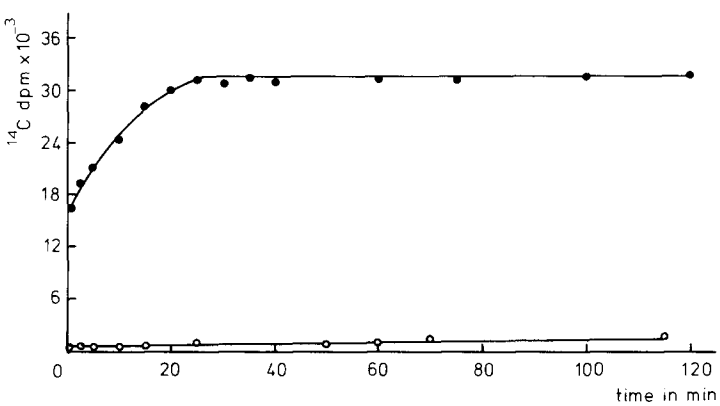

Fig. 1. Incorporation of [^{14}C]leucine in protoplasts (●———●) and in secreted material, precipitable with trichloroacetic acid (○———○). Secreting protoplasts were washed twice with 12% (w/v) mannitol in 1 mM sodium acetate (pH 3.8) and resuspended in fresh Markham-mannitol medium (10^8 protoplasts/ml, 30°C). After a pulse of 3 min with 0.5 μCi [^{14}C]leucine, unlabelled leucine was added (final concn: 1 mg/ml), followed by centrifugation (7 min, 1000 × *g*, 30°C) and resuspension in fresh Markham-mannitol medium (10^8 protoplasts, 30°C). Starting from then (t=0), periodically samples (2 ml) were taken, added to 0.06 ml cycloheximide (0.2 mg/ml) and chilled rapidly in ice. After centrifugation (7 min, 1000 × *g*, 4°C) 0.1 ml bovine serum albumin (20 mg/ml) was added to the supernatant. The sediment (protoplasts) was washed twice with 12% (w/v) mannitol in 1 mM sodium acetate (pH 3.8). To the combined washes and the supernatant 5 ml ice-cold 20% (w/v) trichloroacetic acid was added. The precipitate was washed twice with 1 ml ice-cold trichloroacetic acid. To the protoplast residue 1 ml ice-cold 10% (w/v) trichloroacetic acid was added. The sediment was washed twice with 1 ml 10% (w/v) trichloroacetic acid. Precipitates thus obtained from washed protoplasts and secreted material, were prepared for counting radioactivity as described in Materials and Methods. Abscissa: time of sampling, ordinate: dpm [^{14}C]leucine per 10^8 protoplasts.

the kinetics of incorporation of radioactively labelled amino acids in the TE- and the S-fractions.

Experiments as the one depicted in Fig. 1 shows that actively secreting protoplasts take up amino acid from the medium and incorporate it in higher molecular material. After a pulse with [^{14}C]leucine for 3 min, followed by addition of excess unlabelled leucine, centrifugation and resuspension of the protoplasts, incorporation continues for another 30 min and then reaches a plateau. This time-course might be explained by the fairly large amino acid pool in yeast [10]. A slight enhancement in the [^{14}C]leucine incorporation in secreted material, precipitable with trichloroacetic acid, can also be observed. The secretory status of the protoplasts was checked by resuspending them in fresh medium. This led to renewed and even more vigorous secretion of acid phosphatase (see also [1]).

Radioactive amino acids in the medium are also incorporated into newly-synthesized acid phosphatase and secreted as labelled enzyme by the protoplasts as can be seen in Fig. 2. This figure gives a typical example of the radioactivity pattern in an electropherogram of the S-fraction. A significant peak of radioactivity coincides with the acid phosphatase band. When extracts containing acid phosphatase are subjected to polyacrylamide-gel electrophoresis at pH 5.0, only the acid phosphatase moves to the positive pole, while other proteins do not penetrate into the gel (see comparison of properties of TE- and S-fractions). This favourable circumstance enables us to estimate incorporated radioactivity in the acid phosphatase, even in crude preparations.

A direct correlation between [^{14}C]leucine incorporation in the secreted acid phosphatase, sampled at a certain time, and its matching TE-fraction, could be obtained by processing samples of labelled, secreting protoplasts as outlined in Fig. 3. By integrating radioactivity in the gel slices containing the acid phosphatase band, and by using known volumes of samples and their sub-fractions throughout their processing, it was possible to express incorporated radioactivity as dpm in the acid phosphatase band per 10^8 protoplasts.

A typical pulse-chase experiment is depicted in Fig. 4, showing the time-course of [^{14}C]leucine incorporation of the TE- and the S-fractions. About 20 min after resuspension of the protoplasts, when the overall [^{14}C]leucine incor-

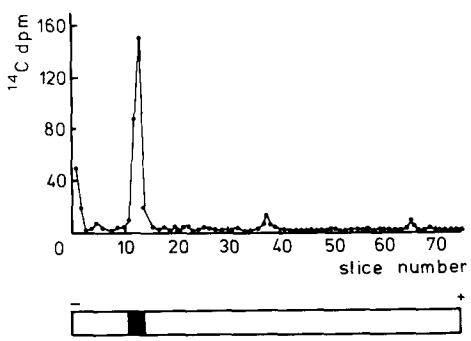

Fig. 2. Radioactivity pattern after polyacrylamide-gel electrophoresis of labelled S-fraction at pH 5.0. The lower schematic drawing represents a gel stained for acid phosphatase activity. Experimental conditions as described in Materials and Methods.

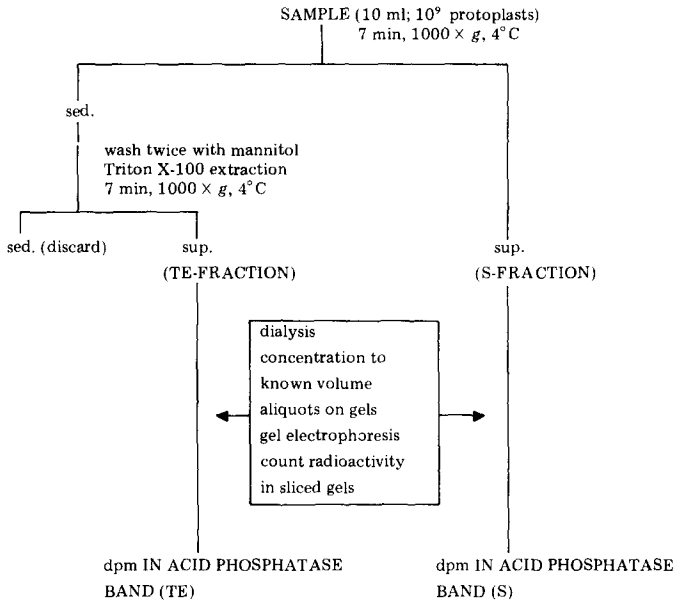

Fig. 3. Flow-sheet for processing labelled, secreting protoplasts for measurement of incorporated radioactivity in acid phosphatase of TE- and S-fractions. Procedures, briefly indicated in the outline, are described in detail in Materials and Methods.

poration in protoplasts reaches a plateau (Fig. 1), the [^{14}C]leucine incorporated in the TE-fraction begins to diminish. At the same time there is a comparable enhancement of the radioactive label in the S-fraction. We have repeated the experiment, this time using [^{14}C]tyrosine, with similar results.

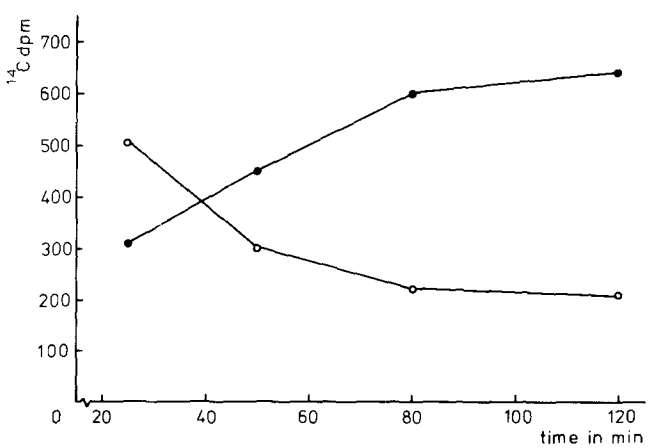

Fig. 4. Kinetics of [^{14}C]leucine-incorporation in the TE-fraction (○———○) and the S-fraction (●———●). The experiment was performed as described in the legend of Fig. 1, up to sampling. Now, starting 20 min after resuspension of the protoplasts, samples (10 ml) were processed according to the plan in Fig. 3. From the TE- and S-fractions thus obtained aliquots were subjected to polyacrylamide-gel electrophoresis at pH 5.0, and radioactivity was counted in the sliced gels. Experimental conditions as described in Materials and Methods. Abscissa: time of sampling, ordinate: dpm [^{14}C]leucine in the acid phosphatase band/10^8 protoplasts.

As has been described previously [1], the amount of protoplast-bound acid phosphatase, expressed as units/10^8 protoplasts, remains constant during the secretion process. Since the TE-fraction accounts for 70% of the protoplast-bound fraction, this implies that the total amount of enzyme activity in the TE-fraction remains constant, while the incorporated radioactivity in the TE-acid phosphatase decreases. Obviously the labelled acid phosphatase molecules are chased from the TE-fraction by unlabelled enzyme molecules. Since the increase in [^{14}C]leucine incorporation in the secreted enzyme is in good agreement with the decrease of the incorporation in the TE-fraction, we interpret these kinetics as a precursor · product relationship between the two fractions.

Comparison of properties of TE- and S-fractions

After having shown that the TE-fraction does indeed contain precursor molecules of the secreted acid phosphatase, we compared several specific enzymatic and physico-chemical properties of this fraction with those of the S-fraction. The results are summarized in Table I. There is a striking resemblance between both fractions with regard to enzymatic and stability properties. Therefore we concluded that in both fractions we are dealing with exactly the same enzyme.

Polyacrylamide-gel electrophoresis at pH 5.0 also suggests identity of the TE- and the S-fractions. On the electropherogram of the S-fraction (Fig. 5) only one sharp band can be observed after staining for acid phosphatase activity, carbohydrate and protein. Electrophoresis of the TE-fraction under identi-

TABLE I

COMPARISON OF PROPERTIES OF ACID PHOSPHATASE SECRETED BY PROTOPLASTS (S-FRACTION) WITH TRITON X-100-EXTRACTABLE PART OF PROTOPLAST-BOUND ACID PHOSPHATASE (TE-FRACTION)

Property		S-fraction	TE-fraction
pH optimum (*p*-nitrophenyl phosphate)		4.0—4.5	4.0—4.3
pH optimum (β-glycerophosphate)		4.0—4.3	4.0—4.3
K_m (*p*-nitrophenyl phosphate) (mM)		1.0	1.5
K_m (β-glycerophosphate) (mM)		3.0	3.5
K_i (inhibitor molybdate ions, substrate *p*-nitrophenyl phosphate) (mM)		0.01	0.06
pH · stability range*		3.0—5.5	3.0—5.5
Heat inactivation (%)**	23°C	100	100
	30°C	100	100
	40°C	83	90
	50°C	23	25
	60°C	2	2
Freezing-and-thawing (%)***		100	100

* Equal amounts of the same preparation were kept in 0.1 M sodium acetate solutions of different pH (1 h, 25°C). Thereafter enzyme activity was determined at pH 3.8 as described in Materials and Methods.

** Equal amounts of the same preparation were kept at the temperature indicated in 0.1 M sodium acetate (pH 3.8) for 10 min. After rapid chilling in ice, acid phosphatase activity was determined and expressed as percentage of the activity measured at 23°C.

*** Test tubes containing aliquots of the TE- and S-fraction with equal acid phosphatase activity, were alternately immersed in a mixture of dry ice and acetone in the water of 30°C (5 times). Thereafter acid phosphatase activity was determined and expressed as percentage of the activity measured before this treatment.

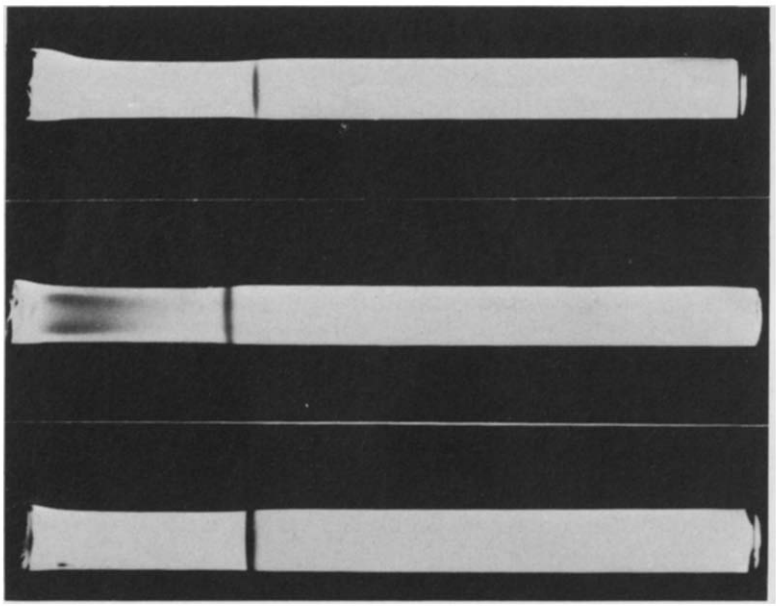

Fig. 5. Electropherogram of secreted acid phosphatase on 5% polyacrylamide-gel electrophoresis at pH 5.0. Electrophoresis and staining performed as described in Materials and Methods. The origin is on the left. Gels stained for acid phosphatase activity (upper gel), carbohydrate (middle gel) and protein (lower gel).

cal conditions shows a completely comparable pattern; i.e. one sharp band after stainings as mentioned above. We have checked the remarkable phenomenon that in the relatively crude preparations only one protein band is visible on the electropherogram by carrying out electrophoresis of the same preparations at pH 3.0 and 7.0. Both at the higher and the lower pH several additional protein bands are visible besides the acid phosphatase · protein band (which was identified by running electropherograms of purified acid phosphatase from intact cells [11]). Moreover, we were able to confirm the homogeneity of the acid phosphatase band from both preparations by cutting out that part of the gel, placing it on top of a 10% sodium dodecylsulphate-polyacrylamide gel and performing sodium dodecylsulphate-gel electrophoresis according to Summers et al. [12]. After staining for protein only one band could be detected in the sodium dodecylsulphate gel.

After isopycnic equilibrium sedimentation in CsCl (Fig. 6), the S-fraction appeared to contain only a single acid phosphatase component with a buoyant density ρ = 1.47 g/cm^3 (Fig. 6A). The very same value was found for acid phosphatase purified from intact yeast cells (Fig. 6B). The TE-fraction, however, has a quite different pattern (Fig. 6C); we have examined several, separately obtained preparations, and in every case we found an acid phosphatase activity peak with a buoyant density ρ = 1.37 g/cm^3, together with heterogeneous material of higher buoyant density. The relative amounts of material with higher density and the component with ρ = 1.37 g/cm^3 varied for several preparations: sometimes even a distinct peak was visible at ρ = 1.47 g/cm^3.

The peak at ρ = 1.37 g/cm^3 is not due to a shift in the buoyant density caused by the presence of Triton X-100, or to components from polyethylene

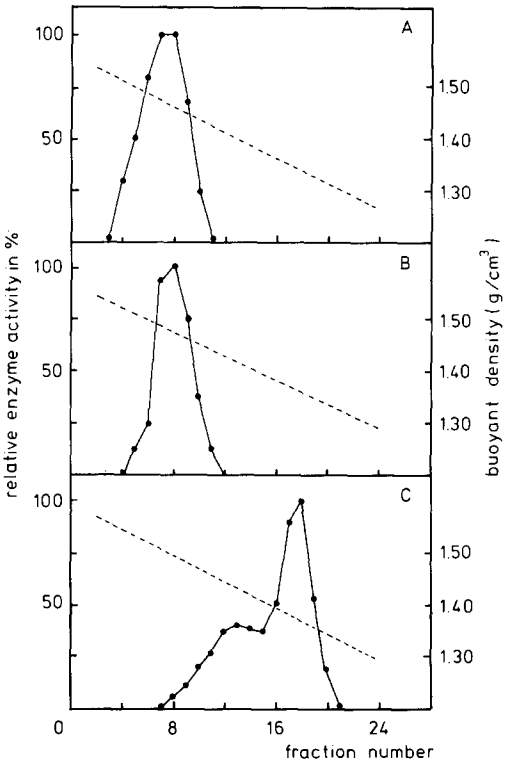

Fig. 6. Isopycnic equilibrium sedimentation in CsCl of S-fraction (A), purified acid phosphatase from intact cells (B) and TE-fraction (C). Experimental conditions and procedures for determination of acid phosphatase as described in Materials and Methods.

glycol used for concentration of the TE-fraction, because addition of Triton X-100 or polyethylene glycol (1% (w/v)) to purified acid phosphatase from intact yeast cells does not shift the acid phosphatase activity peak. Moreover, TE-preparations concentrated against solid sucrose and against polyethylene glycol give identical patterns. It therefore seems very unlikely that the acid phosphatase activity peak at ρ = 1.37 g/cm^3 would be an artifact. Further supporting evidence will be presented in the Discussion. Centrifugation for 64 h is sufficient for obtaining equilibrium, since centrifugation for 92 h does not change the buoyant density values for the components in the S- and TE-fraction, or the pattern of the TE-fraction.

From these experiments we conclude that in spite of the great similarity between the S- and the TE-fractions, they are not completely identical, as the latter fraction definitely contains acid phosphatase molecules with a lower buoyant density than that of the secreted enzyme.

Discussion

In order to examine whether the protoplast-bound acid phosphatase contains precurs ›rs of the secreted enzyme this fraction had to be solubilized. Since it was not possible to release acid phosphatase from protoplasts by treat-

ment with high salt concentrations, we can rule out the possibility that the protoplast-bound enzyme is merely acid phosphatase adsorbed during the secretion process. After lysis of protoplasts no more than about 5% of the enzyme activity is set free [1]; the remainder is firmly bound, but non-ionic detergents solubilize 60—70% of this bound activity. However, up to now we have not been able to solubilize all enzyme activity. The residual fraction is inaccessible for intensive examination, but we were able to study a number of its enzymatic and stability properties, as was done with the TE- and S-fractions (Table I). We could not detect any essential differences in these respects between the residual fraction and the soluble TE- and S-fractions (Van Rijn, H.J.M., unpublished). The Triton X-100-extractable fraction of the protoplast-bound acid phosphatase must consist of enzyme molecules, released by this detergent from either membranes or certain cell compartments. We cannot explain why some acid phosphatase molecules are resistant to solubilization.

As has already been mentioned, we have interpreted experiments such as that depicted in Fig. 4, as evidence for a precursor · product relationship between the major part of the protoplast-bound acid phosphatase and the secreted enzyme. Much research has already been done on the internal fraction of the acid phosphatase [13—16], and naturally this internal fraction will contain precursors of the exo-enzyme. However, such a relationship has never been established up to now. Although our data provide good supporting evidence, they are not yet conclusive. To further solve this (essentially morphological) problem, we are (in collaboration with the Biological Ultra-Structural Research Unit of this University) performing cytochemical studies of acid phosphatase-secreting protoplasts, the results of which will be published elsewhere.

Accepting that the TE-fraction contains precursors, the question arises whether these precursors are complete enzyme molecules, ready for secretion, or whether the TE-fraction also comprises molecules in an earlier phase of synthesis, with less carbohydrate. Judging from the identical behaviour of the enzyme in the TE- and S-fractions as regards enzymatic properties, stability (Table I) and mobility in polyacrylamide-gel electrophoresis, we were at first inclined to conclude that in both fractions we have to do with the same, essentially complete, glycoprotein enzyme molecules. But this conclusion is no longer tenable since examination of the fractions with the aid of isopycnic equilibrium sedimentation in CsCl has revealed distinct differences between the acid phosphatase molecules in the TE- en S-fractions.

The possibility of applying this well-known biochemical technique [17] to the study of differences in the carbohydrate/protein ratio of glycoproteins was recently brought to our notice by Drs J.E. Varner and S. Huang, who have used it, inter alia, for studying forms of acid phosphatase occurring in different yeast strains (personal communication). The rationale of this approach is the fact that carbohydrates have a higher buoyant density than proteins. Therefore the buoyant density of glycoproteins consisting of the same kind of protein and carbohydrate will depend primarily on their carbohydrate/protein ratio [18].

Application of isopycnic equilibrium sedimentation in CsCl to our problem has established beyond doubt the already highly probable identity of the acid phosphatase secreted by yeast protoplasts (S-fraction) with the purified

enzyme obtained from the cell wall of intact cells. In both cases we have the same homogeneous glycoprotein. Its buoyant density (ρ = 1.47 g/cm^3) is in good agreement with the value (ρ = 1.46 g/m^3) reported for acid phosphatase in another strain of *S. Cerevisiae* (Varner, J.E. and Huang, S., personal communication).

The TE-fraction, on the contrary, turns out to be heterogeneous: besides heavier material it contains a component with lower buoyant density (ρ = 1.37 g/cm^3). Most likely this component represents a form of acid phosphatase containing less carbohydrate than the secreted enzyme. This supposition is supported by experiments in which acid phosphatase, purified from yeast mutant X2180-1A-5 [19] was treated with α-mannanase from Arthrobacter GJM-1 [20] to remove attached mannan chains. This treatment caused a shift of the buoyant density of the acid phosphatase from ρ = 1.45 g/cm^3 to ρ = 1.38 g/cm^3 (Boer, P., unpublished).

Neither Lampen and co-workers [5,21] nor we [16] have ever been able to detect a smaller form of the acid phosphatase in baker's yeast besides the exo-enzyme. We now have good evidence for the existence of another, probably smaller, form of this enzyme in a detectable concentration.

Knowing now that the acid phosphatase in the TE-fraction is heterogeneous, we may wonder why it behaves like a homogeneous glycoprotein in polyacrylamide-gel electrophoresis. We can think of two possible explanations: either all the enzyme molecules have the same mobility, regardless of their carbohydrate content, and so are all concentrated in a single band in the gel, or this band only contains the essentially complete molecules, the lighter ones either having run through the gel or not yet run into it under our circumstances.

The biological role of the newly detected acid phosphatase in the TE-fraction is another intriguing problem. It is tempting to regard the component with lower buoyant density as an acid phosphatase intermediate requiring (further) mannosylation to the complete glycoprotein before it can be secreted. But the experience with yeast β-fructofuranosidase, an internal, carbohydrate-free form of which was first considered as a precursor of the exo-enzyme [2—4], but later proved to be a metabolic side-product [5], dictates caution.

The discovery of this "lighter" acid phosphatase in the TE-fraction would also appear to complicate the precursor · product relationship between TE-fraction and secreted enzyme. Further work is in progress to establish a possible function of the "lighter" enzyme as a precursor of the exocellular enzyme, its relationship to the other molecular species in the TE-fraction and their place in the chain of events leading to the synthesis of yeast acid phosphatase.

Acknowledgements

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