Solubilization and partial purification of vacuolar ATPase of yeast *Saccharomyces carlsbergensis*

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Vacuolar ATPase *Purification* *Membrane marker* (Saccharomyces carlsbergensis)

1. INTRODUCTION

Yeast vacuoles are currently believed to possess transport systems for basic amino acids (lysine, arginine, etc) [1], nitrogenous bases [2], K^+, Mg^{2+} and orthophosphate [3]. The energization of the transport of these compounds across the vacuolar membrane needs further investigation. It is postulated that a H^+-ATPase is involved in the transport energization [1]. Though yeast vacuoles have long been known to possess ATPase activity [4], neither the pH-optimum nor the substrate specificity have been determined so far. In view of this, as well as of the existence of various phosphohydrolases in vacuoles [5], the presence of a specific ATPase in these organelles remains unproved. Still, if an ATPase is present in yeast vacuoles, it is associated with membranes [6]. We have recently shown [7] that the vacuolar ATPase activity differs from the plasmalemma ATPase activity since it is not inhibited by orthovanadate. But the two enzymes are similar in other characteristics: they both have a pH optimum of 6.5 and are insensitive to oligomycin and azide.

The present paper deals with the properties of the solubilized and partially purified vacuolar ATPase and demonstrates that this enzyme is different from ATPases of other yeast organelles.

2. MATERIALS AND METHODS

Yeast *Saccharomyces carlsbergensis* IBPhM-366 was grown as previously described [3]. Spheroplasts and vacuoles were isolated according to [8] and [7], respectively. To solubilize the vacuolar ATPase, the suspension of organelles in 0.1 M sorbitol and 10 mM Na–citrate buffer (pH 6.8), was mixed with the same volume of the solution containing: 20% glycerol, 10 mM Tris–HCl (pH 7.5), 1 mM EDTA–Na_2, 2 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride and zwitterionic detergent TM-314 (1 mg/mg protein). Solubilized proteins were centrifuged for 16 h in a continuous glycerol gradient (20–40%) at 37 000 rev./min (Beckman rotor SW-41). Protein was estimated according to [9]; all other analytical methods were described in [7].

3. RESULTS AND DISCUSSION

According to the results presented in table 1 (column A) vacuoles contain enzymes which hy-

Table 1

Specific phosphohydrolase activities of the isolated vacuoles and partially purified ATPase

Substrate	Specific activities	
	A Vacuoles (%)	B combined fractions 4–6, fig.1
ATP	100%[a]	100%[b]
GTP	210	53
UTP	250	41
CTP	180	25
ADP	18	0
AMP	21	0

[a] 100% corresponds to 400 mU/mg.
[b] 100% corresponds to 1700 mU/mg.

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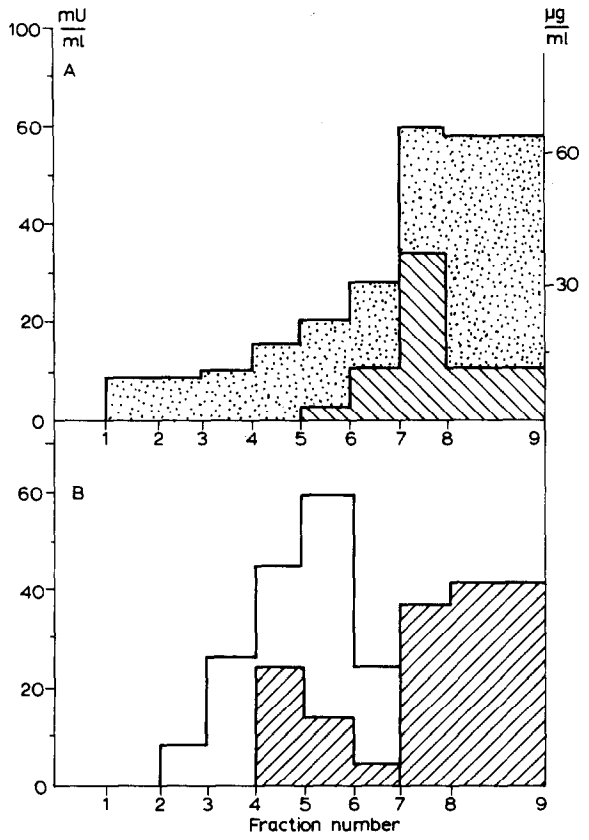

Fig.1. (A) Distribution of protein (dotted area) and alkaline phospatase (hatched area) after solubilization and glycerol gradient centrifugation. (B) ATPase activity (empty area) and GTPase activity (hatched area).

drolyze both ATP and other nucleoside triphosphates. Neither these findings nor the data of Wiemken et al. support 'the presence of a high specific activity of Mg^{2+} dependent ATPase' [5]. However, the experiments on the solubilization and partial purification of the vacuolar ATPase demonstrate that the vacuoles contain a specific ATPase. By glycerol gradient centrifugation, the solubilized ATPase is almost completely purified from alkaline phosphatase and, partially, from GTPase (fig.1).

The specific activity of the ATPase (fractions 4–6); (table 1, column B) increases 4-fold, whereas this is not the case with GTPase, UTPase and CTPase. The ATPase does not hydrolyze AMP, ADP, pyro- or polyphosphates. On the other hand, fractions 8 and 9 exhibit pyro- and polyphospha-

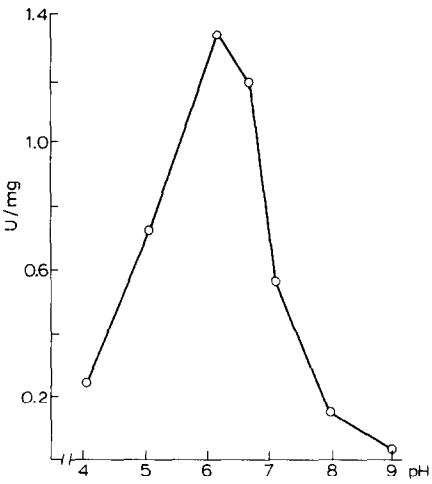

Fig.2. pH-dependence of the solubilized ATPase (fractions 4–6, fig.1).

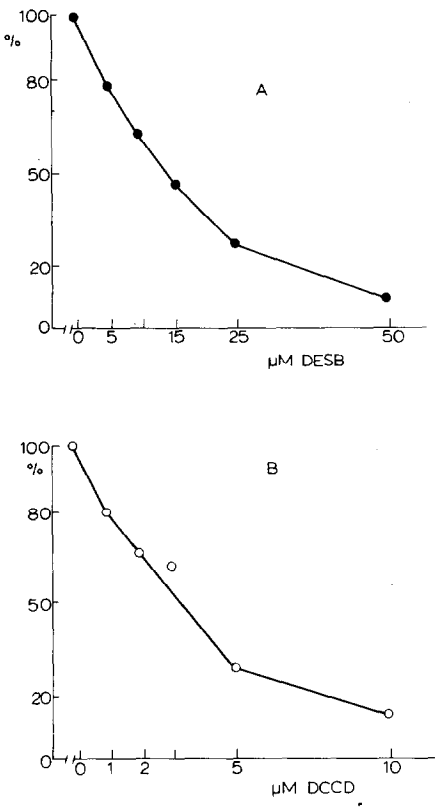

Fig.3. The effect of diethylstilbestrol (A) and dicylclohexylcarbodiimide (B) on the solubilized ATPase activity (fractions 4–6, fig.1). DESB = diethylstilbestrol; DCCD = dicyclohexylcarbodiimide.

Table 2

Specific activities of the ATPase (pH 6.5) and α-mannosidase (mU/mg protein) at different steps of the ATPase purification

Enzyme	Spheroplast lysate[a]	Isolated vacuoles	Fractions 4–6
ATPase	12	400	1700
α-mannosidase	0.63	48	not done

[a] The ATPase was assayed in the presence of 5 mM NaN_3 and 50 μM Na_3VO_4

tase activities. The specific activities of pyrophosphatase in these two fractions are 1140 and 1380 mU/mg protein, and those of polyphosphatase are 1400 and 1100 mU/mg protein, respectively.

The second glycerol gradient centrifugation leads to a further purification of the ATPase so that the specific activity of the GTPase drops to 30% of that of the ATPase (data not shown).

The solubilized ATPase (fractions 4–6, fig.1) has a pH-optimum of 6.0 (fig.2). It is effectively inhibited by diethylstilbestrol and dicyclohexyl carbodiimide (fig.3) and is not sensitive to orthovanadate (data not shown).

The insensitivity of the vacuolar ATPase to orthovanadate and azide may be used to distinguish vacuolar membranes from plasmalemma or mitochondria. Noteworthy is that the vacuolar ATPase may serve as a marker enzyme of vacuolar membranes due to its insensitivity to orthovanadate, oligomycin or azide at pH 6.0–6.5. Both the ATPase and α-mannosidase specific activities [5] increase as a result of the isolation of vacuoles (table 2). The ATPase sensitive to orthovanadate and insensitive to azide or oligomycin may be used as a marker enzyme of plasma membranes.

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NOTE ADDED

While this work was in preparation, the excellent paper of Y. Anraku's group was published [Y. Kakinuma et al. (1981), J. Biol. Chem. 256, 10859–10863]. The authors demonstrated the ATP-dependent formation of an electrochemical potential difference of protons in right-side-out vacuolar membrane vesicles. However, they failed to determine whether the specific ATPase or nucleoside triphosphatase create this gradient.

REFERENCES

[1] Matile, Ph. (1978) Ann. Rev. Plant Physiol. 29, 193–213.
[2] Svihla, G., Dainko J.L. and Schlenk F. (1963) J. Bacteriol. 85, 399–409.
[3] Okorokov, L.A., Lichko, L.P. and Kulaev I.S. (1980) J. Bacteriol. 144, 661–671.
[4] Wiemken, A. (1969) Eigenschaften der Hefevakuole. Thesis ETH Nr 4340, Swiss Federal Institute Technol. Zurich.
[5] Wiemken, A., Schellenberg, M. and Urech K. (1979) 123, 23–35.
[6] Shabalin Yu.A. (1979) Biosynthesis of high molecular polyphosphates conjugated with mannan biosynthesis in *Saccharomyces carlsbergensis* yeast. PhD thesis, Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Pushchino, USSR (in Russian).
[7] Okorokov, L.A., Kulakovskaya, T.V., Lichko, L.P., Petrov, V.V. (1982) submitted (in Russian).
[8] Tsiomenko, A.B., Vagabov, V.M., Kulaev, I.S. (1975) Doklady Acad. Nauk SSSR, 220, 250–253 (in Russian).
[9] Bradford M.M. (1976) Anal. Biochem. 72, 248–254.