Biochimica et Biophysica Acta, 422 (1976) 120—126

BBA 67674

RELEASE AND ACTIVATION OF A PARTICULATE BOUND ACID PHOSPHATASE FROM *TETRAHYMENA PYRIFORMIS*

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(Received June 9th, 1975)

Summary

A sedimentable form of acid phosphatase (EC 3.1.3.2) from *Tetrahymena pyriformis* was found to be solubilized by Triton X-100. The total enzyme activity in the insoluble cell fraction increased almost 200% upon solubilization with Triton X-100 or Nonidet P-40.

Removal of membrane lipids and Triton X-100 from the particulate wash solution with a chloroform extraction resulted in non-specific enzyme-protein aggregation which was reversible upon addition of Triton X-100. The results indicate that this acid phosphatase is an integral membrane protein.

The pH optima for this particulate bound acid phosphatase was 3.5 with *o*-carboxyphenyl phosphate and 4.0 with *p*-nitrophenyl phosphate as substrates. The K_m values of each substrate were 3.1 and 0.031 mM, respectively.

Introduction

Acid phosphatase (EC 3.1.3.2) has been shown to be associated with particles such as lysosomes, microsomes, and mitochondria in *Tetrahymena pyriformis* [1—6]. The latency of acid phosphatase in homogenates of *T. pyriformis* is strong indication that this hydrolytic enzyme is contained within these organelles [1—6]. In studying lysosomal physiology of *T. pyriformis* Muller et al. [2] indicated that low osmotic pressure, increasing temperature, and freezing and thawing of cell homogenates decreased the latency of the lysosomal enzymes. Allen and Lee [7] found that latency of acid phosphatase was decreased by exposure of cell homogenates to high concentrations of KCl. No decrease in sedimentability of acid phosphatase was found in the homogenates of *T. pyriformis* after exposure to either cycles of freezing and thawing or high concentrations of KCl [2,7]. Muller et al. [2] proposed that the sedimentable acid phosphatase may be a membrane-bound enzyme or a possible

adsorption artifact. Allen and Lee [7] suggested that this acid phosphatase is lysosomal and that the procedures employed damaged the lysosomes but were not sufficiently damaged to release the enzymes. This explanation, however, seems unlikely because the amount of activity of four other lysosomal enzymes decreased with the decrease in latency [2]. It was the purpose of this study to isolate and study the properties of this particulate acid phosphatase from *T. pyriformis* W.

Materials and Methods

T. pyriformis W was obtained from Dr. Louise H. Greenburg of Byrn Mawr College, Byrn Mawr, Pennsylvania. Stock cultures were maintained at 20°C in test tubes containing 2% proteose peptone, 0.1% yeast extract, and 90 μM Fe(II)-EDTA and were transferred weekly. Cultures were grown at 28°C in 500-ml Erlenmeyer flasks containing 200 ml of the same medium. Each flask was inoculated with 2—3 ml of a 2-day-old culture and incubated for 3 days before harvesting.

Cells were collected after incubation using a Sorvall RC2-B centrifuge with KSB continuous flow system. Rotor speed and flow rate were adjusted such that a cell-free supernatant was obtained. Pelleted cells were washed once by suspension in 500 ml of water and recentrifuged at 7000 $\times$ *g* for 1 h. The pellets, free of medium proteins, were diluted 1 : 8 (w/v) with water prior to the French pressure cell treatment.

The washed cell suspension of *T. pyriformis* was subjected to French pressure cell treatment at 16 000 lb/inch^2. After 10 min of stabilization, the flow rate was adjusted to 10 ml/min and a pressure of 15 000 lb/inch^2 was maintained until the entire content contained in the pressure cell were collected. The homogenate resulting from French pressure cell treatment was centrifuged at 27 000 $\times$ *g* for 1 h. The supernatant containing the cytosol was decanted and the pellet retained for enzyme release studies.

The presence of acid phosphatase in the sediment was determined by assaying a portion of the pellet prior to elution experiments. The elution experiment was performed by resuspension of sediment with a glass homogenizer in wash solution containing varying concentrations of either Triton X-100 or Nonidet P-40 at a ratio of 1 : 8 (w/v). The homogenates were centrifuged at 27 000 $\times$ *g* for 1 h, the supernatants were retained for enzyme assay, and pellets were subjected to further washings. Wash procedures were continued until no significant amount of enzyme was detectable in either pellet or wash supernatants.

Protein concentration was determined spectrophotometrically at 280 nm with lyophilized human serum as a standard. The absorbance at 280 nm by Triton X-100 and Nonidet P-40 was corrected by measuring the absorbance of the appropriate concentration.

Acid phosphatase activity was assayed in 0.15 M sodium acetate buffer of pH 3.5 using *o*-carboxyphenyl phosphate as substrate. The hydrolysis of *o*-carboxyphenyl phosphate to salicylic acid was followed by the increase in absorbance at 300 nm with a Beckman DU-2 spectrophotometer [8].

In order to remove the detergent and other lipids from the wash solution a

chloroform extraction was performed. Wash solution and cold chloroform were mixed (8 : 1, v/v), the resulting white cloudy precipitate removed by centrifugation at 13 000 × *g* for 20 min and the upper aqueous phase retained.

Polyacrylamide disc gel electrophoresis was performed as described by Davis [9]. Proteins were stained with amido black and acid phosphatase zymograms were obtained as described by Allen and Hynick [10] using α-naphthyl acid phosphate as substrate and fast blue RR as chromogen.

Results and Discussion

It was found that French pressure cell treatment completely disrupted the cells. The amount of acid phosphatase activity (40—60%) in the cytosol was considerably higher than that reported by other workers [2,4,11,12]. In previous studies the percent of total acid phosphatase activity associated with insoluble fragments of cell preparations were found to be about 80% [2,4,11,12]. The variations between present data and previous reports were probably due to differences in the homogenization procedure employed. The larger amount of acid phosphatase activity found in the cytosol indicated that the French pressure cell treatment ruptured the lysosomes. To insure that all lysosomes were ruptured prior to elution of membrane-bound acid phosphatase the pellet was resuspended with a glass homogenizer in hypotonic saline (0.25% NaCl) or water (1 : 8, w/v) since the lysosomes are known to be sensitive to low osmotic pressure [2]. No detectable amount of soluble enzyme activity was found with further water washes. It is obvious that a combination of French pressure cell and low osmotic pressure treatments insured rupture of all lysosomal membranes.

The non-ionic detergent Triton X-100 was found to be effective in elution of acid phosphatase from the insoluble cell fraction (Fig. 1). The concentration

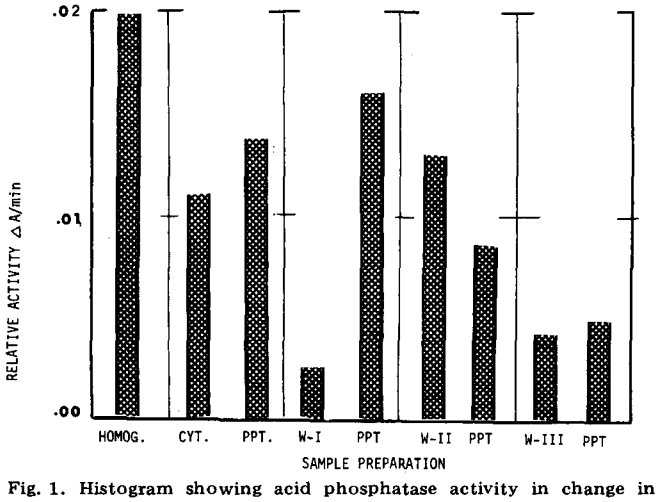

Fig. 1. Histogram showing acid phosphatase activity in change in absorbance units per minute as a function of sample preparation using *o*-carboxylphenyl phosphate - substrate. W-I (Wash 1) = saline; W-II (Wash 2) = 1% Triton X-100; W-III (Wash 3) = 1% Triton X-100; ΔA/min. = change in absorbance/min at 300 nm; HOMOG = homogenate; CYT = cytosol; PPT = the resuspended pellet after centrifugation.

TABLE I

EFFECTS OF TRITON X-100 AND NONIDET P-40 ON ACID PHOSPHATASE FROM THE PARTICULATE CELL FRACTION OF *T. PYRIFORMIS*

Enzyme activity, μmol of *o*-carboxylphenyl phosphate hydrolysed/min per ml solution. Specific activity, μmol of [S] hydrolysed/min per mg protein.

		Protein (mg/ml)			Enzyme activity			Specific activity			Total enzyme activity	Activity (%)
		Wash I	Wash II	Residue	Wash I	Wash II	Residue	Wash I	Wash II	Residue		
Water		—	—	55.46	0	0	0.197	—	—	0.00356	0.197	100
0.2%	Triton X-100	1	1.1	54.2	0.0926	0.0611	0.206	0.0926	0.0556	0.00380	0.360	183
0.5%	Triton X-100	1	0.8	50.4	0.113	0.0395	0.175	0.113	0.0494	0.00348	0.328	166
1.0%	Triton X-100	3	0.8	50.0	0.115	0.0020	0.194	0.0384	0.0250	0.00388	0.311	158
0.1%	Nonidet P-40	2	2.3	47.2	0.0800	0.0651	0.202	0.0400	0.0286	0.00427	0.347	176
0.2%	Nonidet P-40	3.1	2.3	47.8	0.113	0.0431	0.201	0.0366	0.0188	0.00420	0.357	181
1.0%	Nonidet P-40	3.4	1.4	43.2	0.126	0.0532	0.179	0.0368	0.0381	0.00415	0.358	182

of Triton X-100 which was found to be most efficient in releasing the acid phosphatase was 0.5% (Table I). An activation of enzyme activity (approx. 150—200%) was observed during the elution process (Table I). Similar results were obtained by employing another non-ionic detergent, Nonidet P-40 (Table I).

Considerable acid phosphatase activity was retained in the insoluble cell fraction after washing the sediment with solutions of high ionic strength, a chelating agent, or water. The acid phosphatase could only be released by treatment with non-ionic detergents (Triton X-100 and Nonidet P-40). The present results indicated that this acid phosphatase is an integral membrane protein.

Enzyme activation observed in the present study during solubilization by the non-ionic detergents was also reported for other integral membrane proteins. Integral membrane phosphatases and phosphotransferase from rat liver microsomes were activated up to 300% by NH_2OH, deoxycholate, and Triton X-100 [17]. Acetylcholinesterase from human brain was also activated by solubilization with Triton X-100 [21]. These workers suggested that activation may not be due to greater extraction of enzyme from membrane preparations but to changes in properties of the catalytic proteins. Solubilization of the protein by detergent may lead to unmasking of hidden catalytic sites by release of an allosteric inhibitory mechanism or by conformational changes in the molecule which render the enzyme more active [12,17]. Similarities between the present results and activation of other integral membrane enzymes indicate that this acid phosphatase from *T. pyriformis* is an integral membrane protein. However, the possibility that mechanical or steric factors present in the insoluble fraction resulting in blocking of substrate-enzyme binding and reduction of initial enzyme activity could not be ruled out.

Electrophoretic analysis of the 0.5% Triton X-100 wash revealed two discrete protein bands (Fig. 2). One band of acid phosphatase activity was

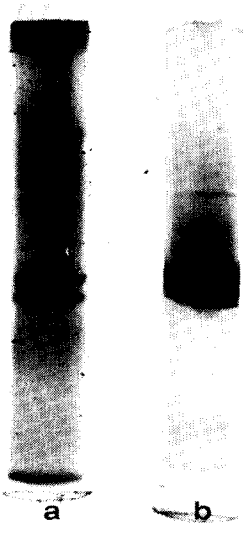

Fig. 2. Electrophoretic analysis of the 0.5% Triton X-100 wash solution; (a) protein stain, (b) zymogram for acid phosphatase.

observed (Fig. 2). Change in electrophoretic behavior of the enzyme was affected by the presence or absence of Triton X-100. Electrophoretic analysis of sample after removal of Triton X-100 by chloroform extraction (wash : chloroform, 8 : 1, v/v) indicated that none of the acid phosphatase entered the gel. However, if Triton X-100 was added to the sample the enzyme entered the gel. These results indicated that the acid phosphatase aggregated upon removal of Triton X-100 and other membrane lipids which were solubilized in the wash solution. It seems that acid phosphatase from *T. pyriformis* requires lipid or detergent to avoid aggregation. Integral membrane proteins by definition interact directly with the membrane lipid matrix [22]. Removal of lipid from association with integral enzyme proteins leads to non-specific aggregation with other proteins or a conformational change that results in enzyme deactivation [23].

Membrane-bound acid phosphatases have been reported from a variety of organisms [13—20]. These studies indicated that acid phosphatase could be either peripheral membrane proteins or integral membrane proteins as defined by Singer [22]. The presence of a membrane-bound form of acid phosphatase in *T. pyriformis* has been suggested [2,3] but such an enzyme has never been isolated and characterized. The present study indicated that the acid phosphatase in the membrane fraction of cell homogenates of *T. pyriformis* was an integral membrane protein.

The pH dependence and some kinetic properties of this enzyme in the solubilized form were studied using *o*-carboxyphenyl phosphate and *p*-nitrophenyl phosphate as substrates. Buffers used for the pH dependence study were 0.1 M glycine · HCl (pH 2.0—4.5), 0.1 M sodium acetate (pH 3.0—6.0), 0.1 M succinate · HCl (pH 4.0—6.5), and 0.1 M Tris · HCl (pH 7.0—8.0). The pH optima for the hydrolysis of *o*-carboxyphenyl phosphate and *p*-nitrophenyl phosphate were 3.5 and 4.0, respectively (Figs 3 and 4). A difference in behavior of the enzyme reactions in glycine buffer as compared to other buffers was observed. Other reports of the pH optima of acid phosphatase from crude

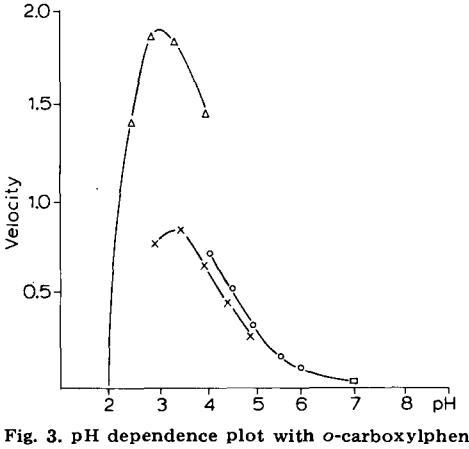

Fig. 3. pH dependence plot with *o*-carboxylphenyl phosphate as substrate, (using solubilized fraction of enzyme); Δ, Glycine/HCl buffer; X, Na/HAc buffer; □, Tris · HCl buffer; ○, Succinate/HCl buffer.

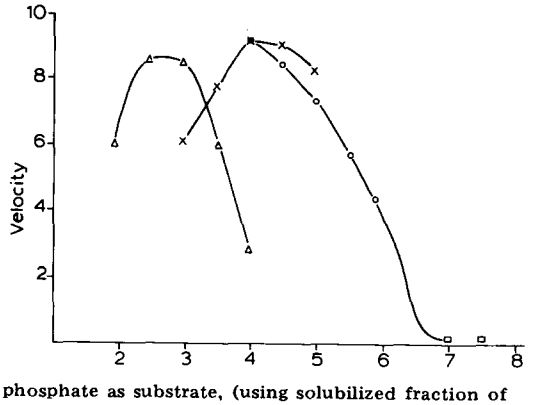

Fig. 4. pH dependence plot with *p*-nitrophenylphosphate as substrate (using solubilized fraction of enzyme); Δ, Glycine-HCl buffer; X, Na/HAc buffer; □, Tris · HCl buffer; ○, Succinate/HCl buffer.

homogenates from *T. pyriformis* indicated that a pH of 4.5 was the optimum using β-glycerophosphate, *p*-nitrophenyl phosphate, 5′-AMP and 3′-AMP as substrates [2,24,25]. Lineweaver-Burk plots of *o*-carboxyphenyl phosphate and *p*-nitrophenyl phosphate indicated that the enzyme obeyed Michaelis-Menten kinetics having correlation coefficients of 0.992 and 0.983, respectively. The K_m values calculated from these plots were 3.0 and 0.031 mM for *o*-carboxyphenyl phosphate and *p*-nitrophenyl phosphate, respectively. Lazarus and Scherbaum [24] found that the K_m of *p*-nitrophenyl phosphate was 7.0 mM with an acid phosphatase from a crude homogenate from *T. pyriformis* GL. It is apparent that the particulate bound enzyme found in the present study is different from those described previously.

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