Identification and Purification of a Soluble Adenosine Triphosphatase from Tetrahymena Pyriformis

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SUMMARY. An unusual ATPase isolated from the postribosomal supernatant fraction of Tetrahymena pyriformis has been purified to homogeneity. The purification procedure consisted of protamine sulfate and heat treatment; column chromatography successively on phosphocellulose, DEAE-cellulose and Sephadex G-150; and isoelectric focusing. The pure enzyme has a molecular weight of 89,000 and requires either Ca^{2+} or Ba^{2+} for maximum activation. Nucleoside triphosphates are hydrolized at decreasing rates in the order: ATP> GTP > ITP > CTP > UTP. The K_m for ATP is 2.5 mM. Because of its properties the enzyme is tentatively classified as a soluble Ca^{2+}-activated ATPase.

During the course of an investigation of protein synthesis in Tetrahymena pyriformis, an enzyme was detected in the postribosomal supernatant fraction of the cell which possessed high ATPase activity (1). Its predominant cytosolic location and somewhat unusual properties prompted further investigation. The following communications is a preliminary report of the purification and characterization of the homogeneous protein.

MATERIALS AND METHODS

Erlenmeyer flasks filled to 20% of capacity with an enriched proteose peptone medium (2) were inoculated with 1% of a stationary phase Tetrahymena Strain E culture and shaken at 150 rev/min at 28° on a New Brunswick gyrorotary shaker. The organisms were harvested after 72 hours of growth by low speed centrifugation. The cells were washed with distilled water, suspended in buffer containing 0.25 M sucrose and 50 mM Tris-HCl, pH 7.5, and disrupted with a motor-driven Potter-Elvehjem homogenizer. The homogenization and subsequent steps were carried out at 4°. The cell homogenate was freed of debris, nuclei and mitochondria by centrifugation at 20,000 x g for 15 min. The postribosomal supernatant fraction was obtained by further centrifugation at 105,000 x g for 90 min. Following

treatment with protamine sulfate, the resulting supernatant fraction was heated to 65° for 5 min and the precipitate removed by centrifugation at 20,000 x g for 5 min. The supernatant fraction was concentrated by lypholization, dissolved in 0.05 M dipotassium succinate, pH 5.8, and dialyzed against the same buffer. Subsequent purification steps consisted of chromatography on phosphocellulose, DEAE-cellulose, Sephadex G-150 and isoelectric focusing. Details of the enzyme purification will be published elsewhere.

ATPase activity was assayed in a 1.0 ml reaction mixture containing enzyme, 100 μmoles Tris-HCl, pH 7.5, 7.5 μmoles ATP and 10 μmoles $CaCl_2$. After 5 min incubation at 30° the reaction was terminated by addition of 0.1 ml ice cold 50% trichloracetic acid. The mixture was centrifuged and inorganic phosphate determined in the supernatant by the method of Ames (3). Linear rates with time and protein were obtained. Protein was determined according to a method of Lowry (4). All reagents were of the highest grade commercially available.

RESULTS AND DISCUSSION

A summary of the purification procedure including activity and recovery of the soluble ATPase which is representative of three separate experiments is presented in Table 1. By this procedure a 266-fold purification and a 16% overall yield were obtained. The specific activity of the pure enzyme was in the range of 20-25 μmoles P_i released/min/mg protein.

The enzyme obtained after isoelectric focusing was found to be homogeneous by polyacrylamide (5) and sodium dodecyl sulfate (6) gel electrophoresis (Figure 1). The molecular weight was determined to be 89,000 by the short column meniscus depletion sedimentation equilibrium method of Yphantis (7) assuming $\bar{v} = 0.72$. Treatment of the pure enzyme with 6 M guanidine HCl in the presence of dithiothreitol, prior to equilibrium sedimentation, indicated a molecular weight of about 30,000. Sodium dedocyl sulfate electrophoresis of the enzyme gave a molecular weight of approximately 26,000. The enzyme thus appears to be trimeric in structure.

ATP, in the presence of calcium, was the preferred substrate with a K_m of 2.5 mM (Table 2). The various nucleoside triphosphates were hydrolyzed at decreasing rates in the order:

Table 1

Purification of Soluble ATPase from Tetrahymena Pyriformis

Purification Step	Protein mg/ml	Specific Activity Units/mg	Total Units	Purification Factor	Yield %
S-100	36.48	0.077	450	1.0	100
Protamine Sulfate	33.60	0.085	428	1.1	95
Heat-treated Fraction	7.68	0.354	370	4.6	82
Phosphocellulose	2.00	4.00	330	52.0	66
DEAE-cellulose	3.60	5.11	179	66.0	39
Sephadex G-150	1.14	15.00	128	195.0	28
Isoelectric Focusing	1.00	20.50	72	266.0	16

ATP > GTP > ITP > CTP > UTP. There were no detectable alkaline or acid phosphatase, pyrophosphatase or transphosphorylase activities. However, there was a limited hydrolysis of ADP.

Some residual ATPase activity could be detected in the absence of added cation. The basal activity was stimulated over 5-fold by the addition of either $CaCl_2$ or $BaCl_2$ to the reaction mixture (Table 3). Other divalent cations gave lesser degrees of stimulation and monovalent cations tested produced no activation. A combination of Mg^{++} and Ca^{++} was not additive or mutually inhibitory.

The ATP hydrolytic activity which is soluble appears to be very different from the plasma membrane Na^{+}-K^{+} stimulated ATPase (8) since the addition of Na^{+} and K^{+} to the Mg^{2+} stimulated reaction mixture elicited no additional effect. Also the properties of the soluble enzyme do not correspond to those of the known mitochondrial ATPase (9, 10). Isolation of the ATPase in the 105,000 x g supernatant fraction after the cells were broken gently in isotonic buffer strongly supports the conclusion that it is a soluble protein. Distri-

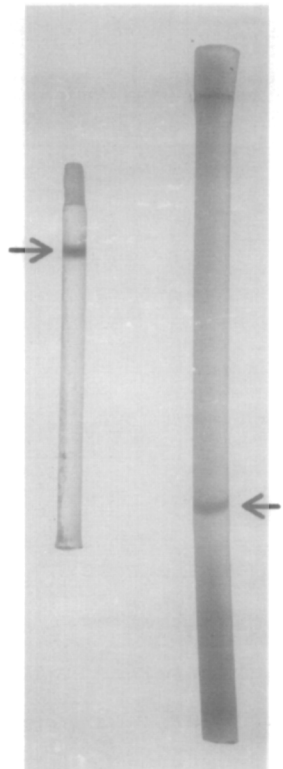

Figure 1

Electrophoretic Profile of the Soluble ATPase

Following purification through the isoelectric focusing 5 μg of protein was used for electrophoresis. Left, electrophoresis on 7.0% polyacrylamide gels according to Davis (6) (→). Right, electrophoresis on sodium dodecyl sulfate gel as described by Laemmli (5) (←).

bution studies indicated, in fact, that about one-third of the total ATPase activity of the cell was present in the postribosomal supernatant fraction and was not inhibited by oligomycin or aurovertin. The soluble enzyme has no resemblance to the Tetrahymena ciliary ATPase (11, 12), the ATPase activity associated with a specific 5S-RNA-protein complex (13), the DNA-dependent ATPase (14) or the RNA-dependent ATPase associated with Rho termination factor (15). It has been reported (16)

Table 2

Substrate Specificity of Soluble ATPase

ATPase was assayed as described under Materials and Methods. Different substrates at a final concentration of 5 mM were used for the assay procedure.

Substrate Tested	μmoles P_i Released	% ATP
ATP	0.848	100
GTP	0.662	78
ITP	0.574	67
CTP	0.473	55
UTP	0.261	30
dATP	0.688	81
dGTP	0.315	37
ADP	0.154	18
UDP	0.149	17
IDP	0.072	8
5'-AMP	0	0
Cyclic AMP	0	0
PP_i	0	0
Adenosine 5'-tetraphosphate	0.385	45
p-Nitrophenyl phosphate	0	0
o-Phospho-L-serine	0	0
DL-o-Phosphothreonine	0	0

that Chlamydomonas flagella contain, in addition to dynein, a distinct low molecular weight, Ca^{2+}-specific ATPase; however, in contrast to the enzyme under study, the K_m of this enzyme for ATP was 4×10^{-4} M and Mg^{2+} was inhibitory when added in the presence of Ca^{2+}.

Although the ATPase was detected in the cytosol throughout the growth cycle of Tetrahymena, the hydrolytic activity increased several fold at the termination of log phase growth

Table 3

Cation Activation of Soluble ATPase

ATPase was assayed as described under Materials and Methods. Different cations at final concentration of 5 mM were added to the reaction medium.

Cation	μmole P_i Released	Activation
None	0.090	1.0
$CaCl_2$	0.456	5.1
$MgCl_2$	0.308	3.4
$BaCl_2$	0.477	5.3
$MnCl_2$	0.361	4.0
$CoCl_2$	0.253	2.8
$ZnCl_2$	0.150	1.7
$CdSO_4$	0.093	1.0
$MgSO_4$ + $CaCl_2$	0.443	4.9
NaCl + KCl	0.125	1.4
NaCl + KCl + $MgSO_4$	0.322	3.6
NaCl + KCl + $CaCl_2$	0.445	5.1

and remained elevated into stationary phase growth. It was previously reported that as Tetrahymena enters stationary phase growth, either naturally or artificially induced, there is a decline in protein synthesis and an acceleration of gluconeogenesis (17). The ability of this soluble enzyme to hydrolyze ATP suggests that it may have as yet an unknown natural function and/or a more physiological substrate. The role of the soluble ATPase in the metabolism of Tetrahymena as well as other organisms remains to be elucidated and is presently under investigation.

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