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Purification and characterization of a Ca^{2+}- or Mg^{2+}-stimulated ATPase from plasma membrane enriched fractions of *Dictyostelium discoideum*

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Evidence is presented for the presence of both diethylstilbestrol (DES)-sensitive and DES-insensitive Mg^{2+}-ATPase activities in plasma membrane enriched fractions of *Dictyostelium discoideum*. When removed from the membrane, the DES-sensitive activity is markedly less stable than the DES-insensitive activity, and the two activities display a number of quite distinct properties. The DES-sensitive enzyme has a decided preference for Mg^{2+} over Ca^{2+}, displays saturation kinetics in response to ATP as substrate (K_m = 0.2 mM) and has a narrow pH optimum range. In contrast, the DES-insensitive activity is stimulated equally by Mg^{2+} or Ca^{2+}, is not saturable by ATP within the mM concentration range and has a much broader pH optimum. The DES-insensitive activity has been purified extensively. The purified enzyme is inhibited by vanadate and fluoride, but is insensitive to *N,N'*-dicyclohexylcarbodiimide (DCCD), *N*-ethylmaleimide and thimerosal. In the absence of divalent cations, the enzyme displays a sigmoidal activity curve in response to substrate concentration, which is abolished by addition of either Mg^{2+} or Ca^{2+}, suggesting a binding site for a divalent cation and a positive cooperative interaction. The enzyme is capable of hydrolyzing other nucleotide triphosphates and ADP, but is without activity on AMP, *p*-nitrophenyl phosphate and pyrophosphate. The enzyme has an apparent molecular weight of approximately 64000.

Introduction

There have been several reports describing the existence of plasma membrane ATPases in *Dictyostelium discoideum*. Initially, Parish and Weibel showed the presence of an ectopic ATPase activity that hydrolyzed extracellular ATP to ADP and P_i [1] and Blanco provided evidence for a Na^+/K^+-stimulated, azide-sensitive activity [2]. Somewhat later, we characterized Mg^{2+}-stimulated ATPase activity in plasma membrane enriched fractions, that was partially inhibited by vanadate, diethylstilbestrol (DES), *N,N'*-dicyclohexylcarbodiimide (DCCD) and thimerosal but was insensitive to the mitochondrial ATPase inhibitors azide and oligomycin [3]. Pogge-von Strandmann et al. [4] and Serrano et al. [5] subsequently presented similar evidence for a plasma membrane ATPase activity that was sensitive to vanadate and DES. Moreover, DES-sensitive proton translocation was observed when lysophosphatidylcholine-solubilized ATPase was reconstituted into phosphatidylcholine vesicles [4].

In this paper, we extend our studies on the plasma membrane ATPases of *D. discoideum*. Evi-

Abbreviations: DES, diethylstilbestrol; DCCD, *N,N'*-dicyclohexylcarbodiimide; pNPP, *p*-nitrophenyl phosphate; PMSF, phenylmethylsulfonyl fluoride.

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dence is provided for the existence of distinct DES-sensitive and DES-insensitive ATPases in plasma membrane enriched fractions, and the DES-insensitive activity has been purified extensively. This enzyme has the characteristics of a low-affinity Mg^{2+}- or Ca^{2+}-ATPase. While this work was in progress, reports were published characterizing an ATPase activity with the properties of a high-affinity calcium pump [6,7] properties quite distinct from those of the enzyme described here.

Materials and Methods

Materials

Bacteriological peptone and yeast extract were from Oxoid. Acrylamide, bis-acrylamide, ammonium persulfate, glycine, TEMED and the silver staining kit were from Bio-Rad. Sephacryl S-300 and DEAE-Sephacel were purchased as pre-swollen beads from Pharmacia and lysophosphatidylcholine and Z-14 were from Calbiochem. All other chemicals were of reagent grade from Fisher Scientific or Sigma Chemical Co.

Organism and culture conditions

D. discoideum, strain Ax-2, was grown in HL-5 media [8] at 22°C on a gyratory shaker, to a density of $5 \cdot 10^6$–$1 \cdot 10^7$ cells/ml. The cells were harvested by centrifugation at $700 \times g$ and washed twice with Bonners salts [9]. Cells at various stages of differentiation were obtained by plating cells on non nutrient agar as previously described [3].

Plasma membrane preparation and solubilization

Plasma membrane enriched fractions were prepared as previously described [3] with the exception all buffers contained 1 mM *p*-aminobenzamidine and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and were freshly prepared immediately before use. Unless indicated otherwise in the figure legends, solubilized extracts were prepared by resuspending the membrane preparation at a protein concentration of 1.5–2.0 mg/ml in 10 mM Tris-HCl (pH 7.5), 20% (v/v) glycerol, 1 mM *p*-aminobenzamidine and 0.1 mM PMSF. Detergent was added from a 10% (w/v) stock solution to give the desired final concentration and the suspension was stirred on ice for 15 min. The suspension was centrifuged at $100\,000 \times g$ for 30 min and the supernatant containing the solubilized ATPase fraction was removed. The remaining pellet was resuspended in 10 mM Tris-HCl (pH 7.5), 20% (v/v) glycerol, 1 mM *p*-aminobenzamidine and 0.1 mM PMSF to a volume equal to that of the original suspension.

Purification of ATPase activity

All buffers contained 1 mM *p*-aminobenzamidine and 0.1 mM PMSF.

(i) Sephacryl S-300 gel filtration. Sephacryl S-300 was packed into a 3×80 cm column and equilibrated with 25 mM Tris-HCl (pH 7.5), 0.5% CHAPS, 0.3 M NaCl. No more than 4 ml of a 1% $C_{12}E_9$ solubilized membrane preparation (16–20 mg protein) was applied to the column and eluted with the equilibration buffer. Fractions expressing enzyme activity were pooled and dialyzed for 12 h against at least 100 volumes of 10 mM Tris-HCl (pH 7.5) containing 10% (v/v) glycerol and 0.1% CHAPS. The buffer was changed once during the dialysis procedure.

(ii) Ion-exchange chromatography on DEAE-Sephacel. Approximately 5 ml of DEAE-Sephacel was poured into a 1×10 cm column and equilibrated with 100 ml of 10 mM Tris-HCl (pH 7.5), 0.5% CHAPS. Enzyme preparations were loaded onto the column and the unbound protein was removed by washing with 75 ml of equilibration buffer. Bound protein was eluted by successively washing the column with four to six column volumes of 0.1, 0.3 and 0.5 M NaCl in equilibration buffer. Fractions containing ATPase were pooled and dialyzed against 100 volumes of Tris-HCl (pH 7.5) containing 10% (v/v) glycerol and 0.1% CHAPS.

(iii) Glycerol gradient centrifugation. Approximately 0.5 ml of the ATPase preparation was layered onto each of six 9 ml linear glycerol gradients (15 to 35% (v/v) glycerol, 0.25% (w/v) CHAPS in mM Tris-HCl (pH 7.5)) and centrifuged at $120\,000 \times g$ for 24 to 36 h in a Beckman SW41 rotor. The tubes were then punctured at the bottom and 20 drop fractions were collected. Following the removal of glycerol by overnight dialysis against 10 mM Tris-HCl (pH 7.5), fractions

were assayed for ATPase activity and protein as described below.

Enzyme assays and protein determination

The ATPase activity was assayed at 30°C by the release of inorganic phosphate. Unless indicated otherwise in the figure legends, the reaction mixtures contained 3 mM ATP, 10 mM $MgCl_2$, 10 mM Mes-Tris (pH 6.8) and enzyme protein in a final volume of 1.0 ml. After a 10 min pre-incubation reactions were initiated by the addition of ATP and were terminated after 15 min, unless otherwise indicated, by the addition of 0.1 ml 10% (w/v) trichloroacetic acid and 1% (w/v) SDS. Inorganic phosphate was measured by a slight modification of the method of Ames [10]. Aliquots of the terminated reactions (0.1 ml) were added to 0.2 ml 1% (w/v) SDS and 0.7 ml ammonium molybdate reagent (six parts of 0.42% (w/v) ammonium molybdate, 0.5% (w/v) SDS, in 0.5 M H_2SO_4 to one part 1% (w/v) SDS, 1% (w/v) ascorbic acid). Solutions were incubated at 45°C for 15 min and the absorbance was read at 660 nm. The rate of reaction was constant throughout the incubation period.

Protein was determined throughout by a modified Folin procedure [11], except that column and gradient fractions were monitored by absorbance at 280 nm.

Polyacrylamide gel electrophoresis

Sodium dodecylsulfate polyacrylamide gel electrophoresis was performed by the procedure of Laemmli [12]. The separation gel contained 7% acrylamide, 0.1% SDS in 0.15 M Tris-HCl (pH 8.8) and the stacking gel contained 1% acrylamide, 0.1% SDS in 0.083 M Tris-HCl (pH 6.8). The running buffer consisted of 0.2 M glycine, 0.083 M Tris in Tris-base and 0.1% SDS and the sample buffer contained 2% (v/v) glycerol, 0.083 M Tris-HCl (pH 6.8) and 2% (w/v) SDS. The gels were subjected to 5 mA for 1 h and then 30 mA until the Bromophenol blue tracking dye had reached the bottom of the gel. The gels were fixed overnight in methanol/glacial acetic acid/deionized water (0.8 : 0.2 : 1.0, v/v/v) and then silver stained as described by the Bio-Rad technical bulletin, except that the oxidizing step was replaced by treatment with 3 mg/ml dithiothreitol as described by Morrissey [13].

Results

Identification of separate DES-sensitive and DES-insensitive ATPases

In an earlier paper, we reported the existence of a Mg^{2+}-stimulated, DES-sensitive ATPase activity that was present in plasma membrane enriched fractions [3]. When these fractions were detergent solubilized, the extracted activity was less sensitive to DES and the results of a systematic analysis of the effects of detergent are shown in Fig. 1.

$C_{12}E_9$ extraction had essentially no effect on the DES-insensitive activity and only a slightly inhibitory effect on the DES-sensitive activity (Fig. 1C). The results for CHAPS, octyl glucoside, Triton X-100 and deoxycholate were similar except that the deleterious effects on the DES-sensitive activity were slightly more pronounced (data not shown). Lysophosphatidylcholine also had a slightly inhibitory effect on the DES-sensitive activity, but a marked stimulatory effect on the DES-insensitive activity (Fig. 1A). In contrast Z-14 had no effect on the DES-insensitive activity, but abolished the DES-sensitive activity (Fig. 1B). These results suggested that there were two distinct ATPase activities, one sensitive to DES and one insensitive to DES.

Properties of the membrane-bound DES-sensitive and DES-insensitive ATPase activities

In order to confirm that there were two distinct ATPase enzymes, the properties of the two activities in the original membrane preparations were examined.

The DES-sensitive ATPase was stimulated to a much larger extent by Mg^{2+} as compared to Ca^{2+}, whereas the DES-insensitive activity was stimulated equally well by both cations (Fig. 2), further suggesting the existence of two distinct enzymes. Both activities required mM concentrations of the divalent cations to produce maximum activity.

The response of enzyme activity to substrate concentration was also consistent with the hypothesis that there were two ATPases. In the presence of Mg^{2+} the DES-sensitive activity increased with ATP concentration, reaching saturation between 0.6 and 1.0 mM (Fig. 3A). When Mg^{2+} was replaced by Ca^{2+} the response to ATP concentration was similar, although the ac-

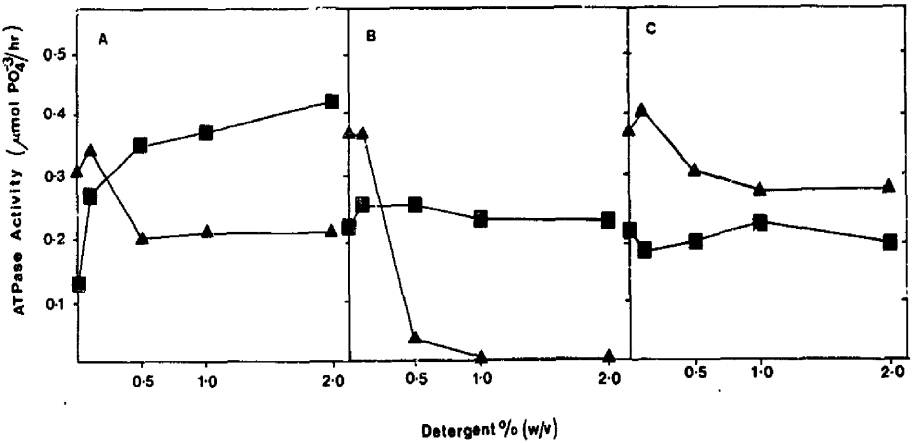

Fig. 1. Inactivation of the DES-sensitive ATPase upon treatment with various detergents: lysophosphatidylcholine (A); Z-14 (B); and $C_{12}E_9$ (C). Plasma membranes were treated with the indicated concentrations of detergent as described in Materials and Methods and the ATPase activities of 0.05 ml aliquots were determined in the presence and absence of 0.2 mM DES. The DES-insensitive activity (■) was provided by the former value, while the DES-sensitive activity (▲) was calculated as the difference between the activities obtained in the presence and absence of DES. The effects observed were obtained in three independent experiments. However, since the ATPase activity of plasma membrane fractions varied slightly from preparation to preparation, the data shown are from a single experiment.

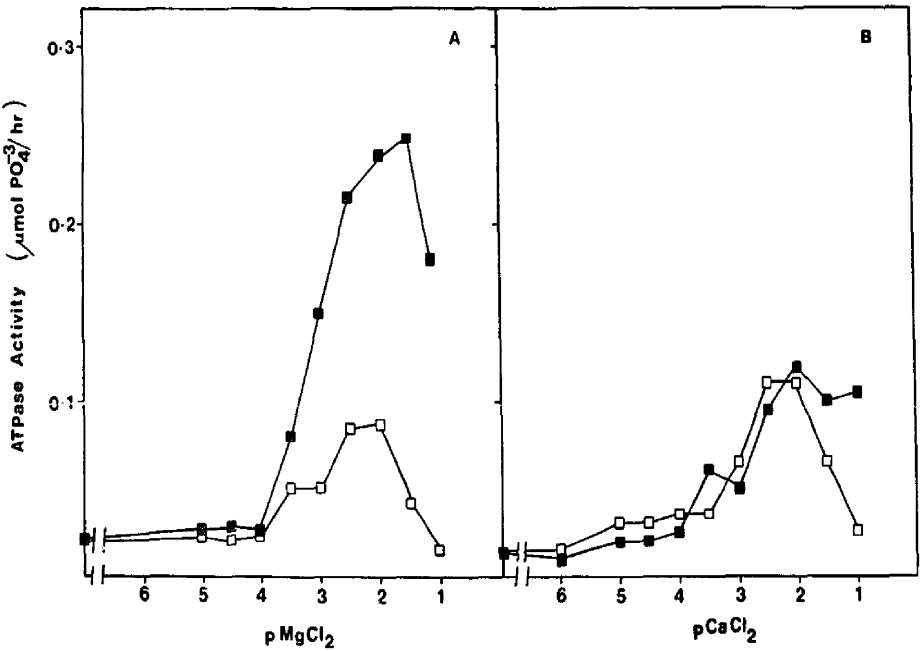

Fig. 2. Effect of Ca^{2+} and Mg^{2+} on the membrane bound DES-sensitive and DES-insensitive ATPase activity. Plasma membranes (0.1 mg protein) were incubated in the presence and absence of 0.2 mM DES in a mixture containing 3 mM Na_2ATP and the indicated concentrations of $MgCl_2$ (A) or $CaCl_2$ (B) in 10 mM Mes-Tris (pH 6.8). DES-sensitive (■) and DES-insensitive (□) ATPase activities were determined as described in Fig. 1. The data points are from a single experiment (see legend to Fig. 1).

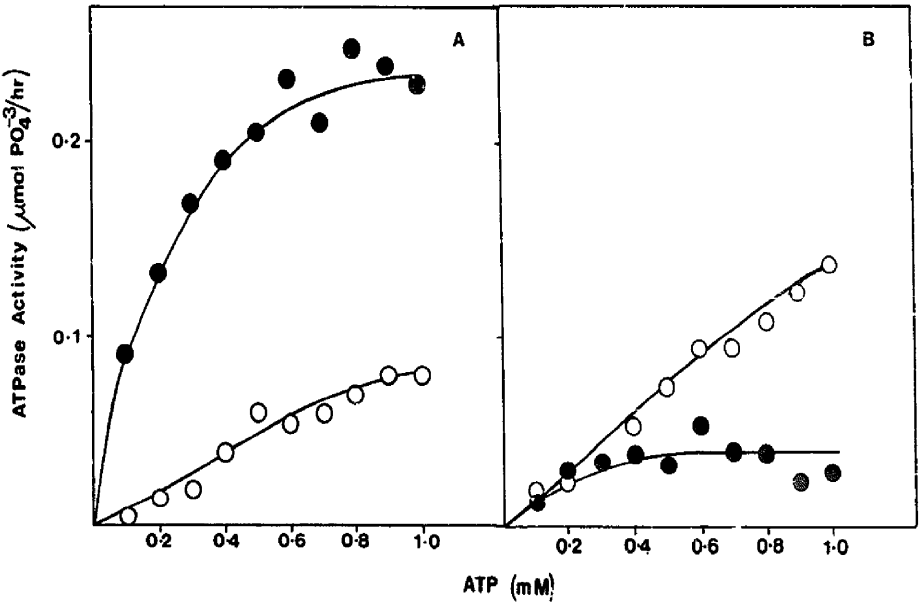

Fig. 3. Effect of ATP concentration on the membrane bound DES-sensitive and DES-insensitive ATPase activity in the presence of Mg^{2+} or Ca^{2+}. The reaction mixtures contained, in addition to the indicated concentrations of ATP, 10 mM $MgCl_2$ (A) or $CaCl_2$ (B) in 10 mM Mes-Tris (pH 6.8) and 0.1 mg of plasma membrane protein in the presence or absence of 0.2 mM DES. DES-sensitive (●) and DES-insensitive (○) activity was determined as described in Fig. 1. The data points are from a single experiment (see legend to Fig. 1).

tivity was considerably lower (Fig. 3B). Double-reciprocal plots of these data revealed that the K_m values were approximately the same (0.2 mM), regardless as to which cation was present. In contrast, the DES-insensitive ATPase was not saturated by substrate at concentrations up to 1 mM, and activities were similar with either Mg^{2+} or Ca^{2+} (Fig. 3). Double-reciprocal plots of these data were non-linear.

Finally, the DES-sensitive ATPase exhibited a bell-shaped pH curve with an optimum pH of 7.0–7.5, while the DES-insensitive enzyme displayed a much broader pH curve with an optimum of between pH 6.5 and 7.0 (Fig. 4).

Purification of the DES-insensitive ATPase

$C_{12}E_9$ was the detergent of choice for the initial purification experiments since it had the least deleterious effect on the DES-sensitive activity (Fig. 1C). Subsequently it was found that the use of CHAPS in all the column and gradient buffers improved the purification. When $C_{12}E_9$ extracts of plasma membrane enriched preparations were fractionated on a CHAPS-equilibrated Sephacryl S-300 column most of the ATPase activity was well separated from the major protein peak (Fig. 5). All the eluted ATPase, including the small amount of activity that co-chromatographed with the major main protein peak, was insensitive to DES, indicating a complete loss of the DES-sensitive ATPase during the fractionation. The substitution of $C_{12}E_9$ or other detergents during the purification procedure failed to prevent the complete loss of the DES-sensitive activity and resulted in a less purified product (data not shown).

The fractions from the main peak of ATPase activity were pooled, dialyzed and applied to a DEAE-Sephacel column. A major peak of ATPase activity was eluted with 0.3 M NaCl (Fig. 6). SDS-polyacrylamide gel electrophoresis of the purified ATPase revealed a single major protein band of approximately 64 000 M_r (Fig. 7). Table I

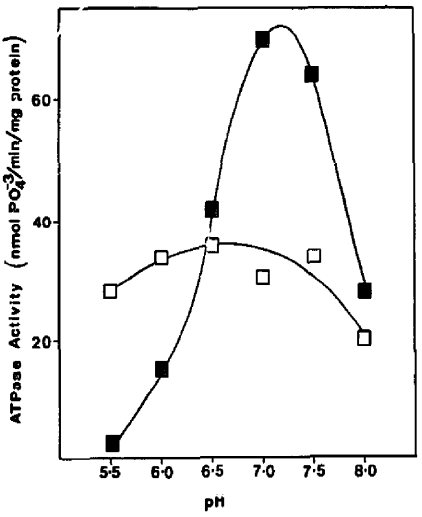

Fig. 4. Effect of pH on the membrane bound DES-sensitive and DES-insensitive ATPase activities. The reaction mixtures contained 2 mM ATP, 10 mM $MgCl_2$ and 10 mM Mes-Tris at the indicated pH values. DES-sensitive (■) and DES-insensitive (□) activities were determined as described in Fig. 1. The data points are from a single experiment (see legend to Fig. 1).

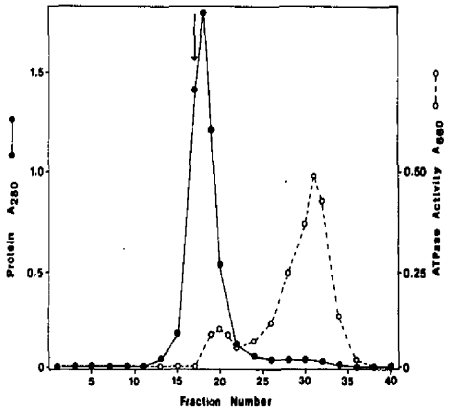

Fig. 5. Gel filtration of solubilized plasma membranes. Plasma membrane enriched fractions(4 mg/ml) were solubilized with 1% $C_{12}E_9$ and 4 ml (16 mg protein) of the 100000× *g* supernatant was applied to a Sephacryl S-300 column (3×80 cm) which had been equilibrated in 25 mM Tris-HCl (pH 7.5) containing 0.5% CHAPS, 0.3 M NaCl, 1 mM *p*-aminobenzamidine and 0.1 mM PMSF. Fractions of 2.5 ml were collected and protein (●) was monitored by absorbance at 280 nm. ATPase activity (○) was determined for a 0.1 ml sample of each fraction as described under Materials and Methods, except that a 1 h incubation time was used. Activity is expressed as absorbance at 660 nm. The arrow indicates the fraction at which a peak of Blue dextran eluted and is a measure of the void volume of the column.

shows the yield and enrichment attained during this purification procedure.

The ATPase was also purified by an alternative method involving an initial fractionation of solubilized plasma membranes by DEAE-Sephacel chromatography followed by centrifugation through a linear 15–35% glycerol gradient of the activity that eluted at 0.3 M NaCl. The peak ATPase fractions from the glycerol gradient contained the same 64000 M_r band (Fig. 7) although the yield of ATPase activity was lower than that obtained using the method summarized in Table I.

Properties of the purified DES-insensitive ATPase

Purified ATPase activity was stimulated by a wide variety of divalent cations (Table II), including Mg^{2+} and Ca^{2+}. Comparatively high concentrations (mM) of the divalent cations were required to elicit the stimulatory response. A detailed analysis of the effects of Mg^{2+} and Ca^{2+} on the purified enzyme revealed results that were very similar to those previously described (Fig. 2) for the membrane bound DES-insensitive enzyme

TABLE I

PURIFICATION OF THE DES-INSENSITIVE PLASMA MEMBRANE ATPase

Fraction	Protein (mg)	Total activity (nmol P_i/min)	Recovery (%)	Specific activity (nmol P_i/min per mg)
Plasma membranes	22.0	680.0	100	31.0
$C_{12}E_9$ extract	16.0	690.0	101	46.1
Sephacryl S-300 fraction	0.83	290.0	43	343.6
DEAE-Sephacel fraction	0.11	56.7	8.3	515.2

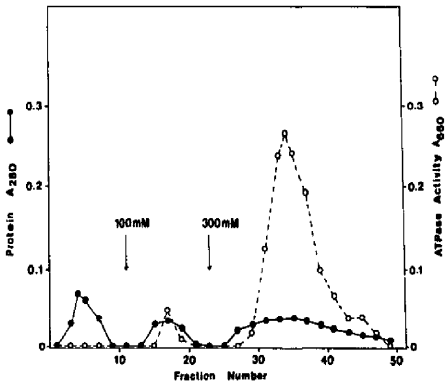

Fig. 6. DEAE-Sephacel chromatography. The dialyzed, pooled ATPase fractions from the Sephacryl S-300 column (0.8 mg protein) were applied to a 1×10 cm DEAE-Sephacel column which had been equilibrated as described under Materials and Methods. Unbound protein was removed by washing the column with 75 ml of equilibration buffer. Bound protein was eluted by successive applications of 5 column volumes of 0.1 M NaCl and 0.3 M NaCl in equilibration buffer. Protein was monitored by absorbance at 280 nm, (●) and ATPase activity (○) was determined as described under Materials and Methods, except that 0.1 ml aliquots of the fractions were assayed and incubations were for 2 h. Enzyme activity is expressed as absorbance at 660 nm.

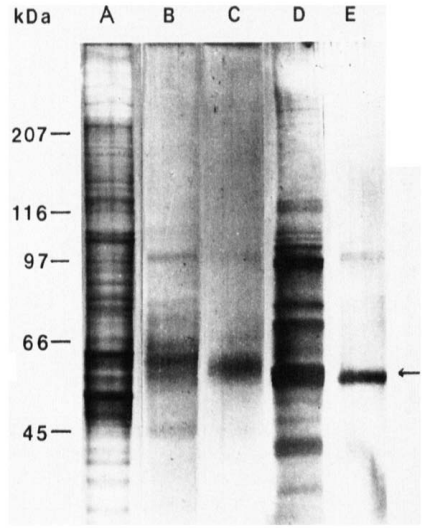

Fig. 7. SDS-polyacrylamide gel electrophoresis of ATPase containing fractions. Solubilized plasma membranes (Lane A, 20 μg protein), material eluted from Sephacryl S-300 (lane B, 10 μg protein), material from subsequent DEAE-Sephacel chromatography (Lane C, 2.5 μg protein), material obtained by direct DEAE-Sephacel chromatography of plasma membrane extracts (Lane D, 10 μg protein) and material from glycerol gradient (Lane E, 3 μg protein) were subjected to SDS-polyacrylamide gel electrophoresis as described under Materials and Methods. Proteins were visualized using the silver staining procedure described under Materials and Methods. Molecular mass markers were myosin (207 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa) and egg albumin (45 kDa).

(data not shown). Curiously, Mg^{2+} and Ca^{2+} inhibited enzyme activity when added together; concentrations of as low as 0.1 mM Ca^{2+} produced a slight inhibitory effect in the presence of 5 mM Mg^{2+} (Fig. 8). Neither the basal activity nor the divalent cation stimulated activity was inhibited by the inclusion of monovalent cations (data not shown).

The purified ATPase displayed a sigmoidal activity curve with increasing ATP concentration in the absence of added divalent cation (Fig. 9). Addition of 1.0 or 10.0 mM Mg^{2+} produced a positive allosteric effect abolishing the sigmoidal nature of the curve and resulting in an apparent increase in the affinity of the enzyme for ATP. However, a further increase in the cation concentration to 100 mM had no additional effect on ATP affinity and the reaction velocity was decreased (Fig. 9). Ca^{2+} had an almost identical effect to that of Mg^{2+} (data not shown) ATP was the most actively hydrolyzed substrate, although all the nucleoside triphosphates and ADP were

TABLE II

EFFECT OF VARIOUS CATIONS ON PURIFIED ATPase ACTIVITY

Addition [a]	Activity [b] (nmol P_i/min)	Relative activity [c] (%)
None	1.1±0.0	17.7
Mg^{2+}	6.2±0.1	100.0
Ca^{2+}	6.7±0.1	108.8
Ba^{2+}	3.1±0.1	50.0
Zn^{2+}	11.5±0.1	186.3
Mn^{2+}	9.2±0.1	148.6
Cu^{2+}	12.1±0.6	195.7

[a] In all cases the cation concentrations were 10 mM.

[b] The data represent the means ± S.D. of three separate experiments.

[c] The activity in the presence of Mg^{2+} was taken as 100% activity.

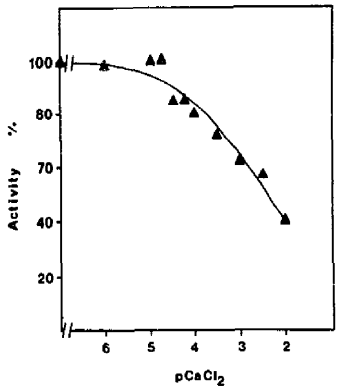

Fig. 8. Inhibition of purified ATPase activity by Ca^{2+}. Purified ATPase activity was assayed in the presence of 5 mM $MgCl_2$ and the indicated concentrations of $CaCl_2$, as described under Materials and Methods.

hydrolyzed to a significant extent (Table III). AMP, pyrophosphate and pNPP were not utilized (Table III).

In addition to its insensitivity to DES, the enzyme was also insensitive to DCCD (Fig. 10).

TABLE III

SUBSTRATE SPECIFICITY OF THE PURIFIED ATPase

Substrate [a]	% Activity
ATP	100
GTP	33.7±3.4
CTP	57.7±2.6
UTP	26.7±8.3
ADP	24.5±5.5
AMP	0.0
pNPP	0.0
Pyrophosphate	0.0

[a] The final substrate concentrations were 4 mM for the nucleotides and 1 mM for pNPP and pyrophosphate. The data are the means±S.D. of three separate experiments.

Only marginal inhibition was obtained with thimerosal but the ATPase exhibited pronounced sensitivity to vanadate and NaF (Fig. 10). The enzyme was insensitive to *N*-ethylmaleimide.

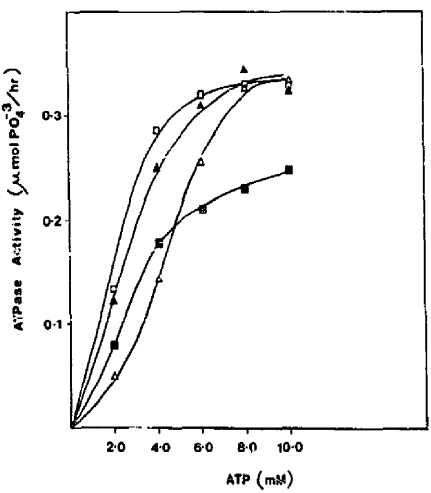

Fig. 9. Effect of ATP concentration on the activity of the purified ATPase. ATPase activity was determined at each of the indicated ATP concentrations in the absence of $MgCl_2$ (△) or in the presence of 1 mM $MgCl_2$ (▲), 10 mM $MgCl_2$ (□) or 100 mM $MgCl_2$ (■).

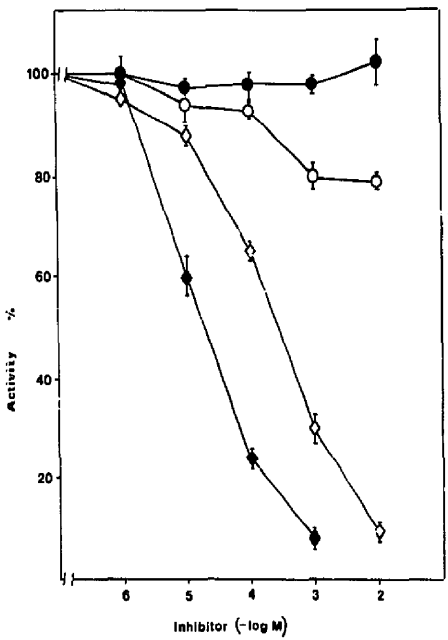

Fig. 10. Effect of various inhibitors on purified ATPase activity. Purified ATPase was assayed as described in Materials and Methods, with the indicated concentrations of vanadate (◆), thimerosal (○), DCCD (●) and NaF (◇). The data represent the means±S.D. for three separate experiments.

Discussion

In an earlier publication we described the existence of a Mg^{2+}-stimulated, DES- and vanadate-sensitive, ATPase activity in plasma membrane enriched fractions from *D. discoideum* [3]. The results of the present study indicate that these fractions contain both DES-sensitive and DES-insensitive activities, that differ with regard to pH optimum, divalent cation stimulation and substrate kinetics. The DES-sensitive ATPase was partially inactivated when removed from its native membrane-bound state by detergent treatment and the solubilized activity could not be fractionated without complete loss of enzyme activity.

Our results indicate that the DES-sensitive ATPase is always the activity that is preferentially lost and the possibility that the insensitive activity results from proteolytic cleavage of the sensitive activity must be considered. However, the fact that DES sensitivity, substrate kinetics, pH optima and divalent cation requirements are all so different makes it unlikely that the one activity is merely a proteolytic cleavage product of the other. Furthermore, in many experiments where the DES-sensitive activity decreased there was no concomitant increase in the DES-insensitive activity (e.g. Fig. 1B). Finally, the fact that all buffers contained proteinase inhibitors and were freshly prepared also makes proteolysis an unlikely possibility. The total loss of the DES-sensitive activity upon column chromatography is difficult to understand, in view of the fact that the activity was not markedly unstable following $C_{12}E_9$ or CHAPS detergent extraction (Fig. 1).

The DES-sensitive ATPase described in this report is probably identical to the DES-sensitive activity described by other workers [4,5] and is probably analogous to the well characterized proton pump Mg^{2+}-ATPase of yeast and other fungi [14–18], although these latter enzymes are considerably more stable. There has been no previous study of the DES-insensitive, Mg^{2+}- or Ca^{2+}-ATPase that we have identified, although the low-affinity Ca^{2+}-ATPase mentioned by Bohme et al. [6] may be the same enzyme. The DES-insensitive activity proved to be stable to detergent and was purified extensively. Preparations obtained by two different procedures were shown to contain a major constituent of M_r 64000, when fractionated by SDS-PAGE. In addition, active enzyme sedimented in a glycerol gradient with an estimated molecular mass of between 65 and 77 kDa (data not shown). The purified activity was markedly inhibited by vanadate and NaF, slightly inhibited by thimerosal and was insensitive to DCCD and *N*-ethylmaleimide.

The kinetic properties of the purified protein are noteworthy. When ATPase activity was measured as a function of the ATP concentration in the absence of a divalent cation a sigmoidal curve was obtained (Fig. 8). Addition of 1 or 10 mM Mg^{2+} or Ca^{2+} served to increase the affinity of the enzyme for substrate indicating a positive allosteric effect. From these data it is possible to postulate the presence of at least one distinct divalent cation binding site at which binding of Mg^{2+} or Ca^{2+} serves to activate the enzyme by increasing its affinity for substrate. The reduction in velocity observed with 100 mM divalent cation may indicate an additional lower-affinity binding site, occupancy of which inhibits rather than activates the enzyme, although the inhibitory concentration is well beyond the physiological range. The inhibition of enzyme activity is not simply a non-specific high ionic strength effect because enzyme activity was unaffected by high concentrations of NaCl or KCl. Interestingly, however, considerable inhibition occurred when both Mg^{2+} and Ca^{2+} were present together at concentrations that were stimulatory when each was present alone (Fig. 8). The underlying reasons for this inhibitory phenomenon are not known.

The purified ATPase does not display any of the properties of a high-affinity Ca^{2+} pump. Enzyme activity was not stimulated by low concentrations of Ca^{2+} (0.01–10 μM). In addition the molecular weight of the enzyme is considerably lower than those previously obtained for plasma membrane Ca^{2+} pumps [19–22]. It is therefore highly unlikely that the ATPase purified in this study is the same as the recently reported high-affinity Ca^{2+} pump [6,7]. However, in addition to the high-affinity Ca^{2+}-ATPase, Bohme and coworkers [6] did indicate the existence of an additional low-affinity Ca^{2+}-ATPase, which may be identical to the purified DES-insensitive enzyme described here.

DCCD inhibition has been suggested as a general probe for proton translocating ATPases [23], and the absence of inhibition of the Mg^{2+}- or Ca^{2+}-ATPase by DCCD would suggest that the enzyme is not involved in this function. Furthermore, the lack of sensitivity to *N*-ethylmaleimide would indicate that the enzyme is not a vacuolar type ATPase [24].

The purified ATPase displays many of the characteristics of the non-specific, low-affinity Ca^{2+}- or Mg^{2+}-ATPases that have been identified in the plasma membranes of a variety of mammalian cells [25–33]. These properties include equal stimulation by Ca^{2+} and Mg^{2+}, tolerance to other divalent cations and relatively low substrate specificity. The physiological function of these enzymes are not known although several of them have been shown to be ecto-ATPases [31–33] and as such may be involved in nucleotide triphosphate degradation as an initial step in nucleoside formation. Whether the *Dictyostelium* enzyme is an ecto-enzyme will require further study, but it is noteworthy that the existence of an ecto-ATPase has been reported previously [1] and it is possible that the two enzymes are identical.

There have now been described several membrane bound ATPases in *D. discoideum* and more detailed comparisons of the various activities will be necessary for a clear picture to emerge. The delineation of distinct DES-sensitive and DES-insensitive activities in plasma membrane enriched fractions is an initial step along the path to a comprehensive understanding.

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References

1 Parish, R.W. and Weibel, M. (1980) FEBS Letts. 118, 263–266.
2 Blanco, M. (1982) Biochim. Biophys. Acta 687, 94–96.
3 MacDonald, J.I.S. and Weeks, G. (1984) Arch. Biochem. Biophys. 235, 1–7.
4 Pogge-von Strandmann, R., Kay, R.R. and Dufour, J.-P. (1984) FEBS Lett. 175, 422–428.
5 Serrano, R., Cano, A. and Pestana, A. (1985) Biochim. Biophys. Acta 812, 553–560.
6 Böhme, R., Bumann, J., Aeckerle, S. and Malchow, D. (1987) Biochim. Biophys. Acta 904, 125–130.
7 Milne, J.L. and Coukell, M.B. (1988) Biochem. J. 249, 223–230.
8 Weeks, C. and Weeks, G. (1975) Exp. Cell Res. 92, 372–382.
9 Bonner, J.T. (1947) J. Exp. Zool. 106, 1–26.
10 Ames, B.N. (1966) Methods Enzymol. 8, 115–118.
11 Sanderman, H. and Strominger, J. (1972) J. Biol. Chem. 247, 5123–5131.
12 Laemmli, U.K. (1970) Nature 227, 680–685.
13 Morrissey, J.H. (1981) Anal. Biochem. 117, 307–310.
14 Addison, R. and Scarborough, G. (1981) J. Biol. Chem. 256, 13165–13171.
15 Bowman, E.J., Bowman, B.L. and Slayman, C.W. (1981) J. Biol. Chem. 256, 12336–12342.
16 Dufour, J.P. and Goffeau, A. (1978) J. Biol. Chem. 253, 7026–7032.
17 Perlin, D.S., Kasamo, K., Brooker, R.T. and Slayman, C.W. (1984) J. Biol. Chem. 259, 7884–7892.
18 Villalobo, A., Boutry, M. and Goffeau, A. (1981) J. Biol. Chem. 256, 12081–12087.
19 Penniston, J.T. (1983) Plasma membrane Ca^{2+}-ATPases as active Ca^{2+} pumps in calcium and cell function, Vol. 4 (Cheung, W.Y., ed.), Academic Press, New York.
20 De Smedt, H., Parys, J.B., Borghgraef, R. and Wuytak, F. (1983) Biochim. Biophys. Acta 728, 409–418.
21 Debetto, P. and Cantley, L.C. (1984) J. Biol. Chem. 259, 13824–13831.
22 Ansah, T.-A., Molla, A. and Katz, Z. (1984) J. Biol. Chem. 259, 13442–13450.
23 Soliz, M. (1984) Trend Biochem. Sci. 9, 309–312.
24 Pedersen, P.L. and Carafoli, E. (1987) Trends Biochem. Sci. 12, 146–150.
25 Shami, Y. and Radde, I.C. (1971) Biochim. Biophys. Acta 249, 345–352.
26 Parkinson, D.K. and Radde, I.C. (1971) Biochim. Biophys. Acta 242, 238–246.
27 Garnett, H.M. and Kemp, R.B. (1975) Biochim. Biophys. Acta 382, 526–533.
28 Lotersztajn, S., Hanoune, J. and Pecker, F. (1981) J. Biol. Chem. 256, 112-09-11215.
29 Verma, A.K. and Penniston, J.T. (1981) J. Biol. Chem. 256, 1269–1275.
30 Ochs, D.L. and Reed, P.W. (1984) J. Biol. Chem. 259, 102–106.
31 Coetzee, G.A. and Gevers, W. (1977) Biochem. J. 164, 645–652.
32 Carraway, C.A.C., Corrado, F.J., IV, Fogle, D.D. and Carraway, K.L. (1980) Biochem. J. 191 45–51.
33 Hamlyn, J.M. and Senior, A.E. (1983) Biochem. J. 214, 59–68.