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PURIFICATION AND CHARACTERIZATION OF AN EXTRACELLULAR ATPase FROM OVIDUCTAL SECRETIONS

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Summary

Oviductal secretions include an ATPase (EC 3.6.1.3) that is tranferred from the outer surface of the secretory cells to the surface of the ovulated oocyte. The enzyme has been purified and is a highly labile, very high molecular weight lipoprotein complex ($>4 \cdot 10^6$). It consists of 47% protein and 53% lipid. Lipid composition is limited to phosphatidylcholine, phosphatidylethanolamine and sphingomyelin. The basic protein subunit has a molecular weight of 170 000. The enzyme exhibits many of the characteristics of ectoenzyme ATPase. The enzyme is Mg^{2+} or Ca^{2+} dependent; the Mg^{2+}-ATPase has pH optima at 6.0 and 7.8 and the Ca^{2+}-ATPase at 9.0. Substrate specificity is limited to ATP with lesser activity towards GTP, CTP, UTP and ADP. K_m for ATP is 0.88 mM and the enzyme is inhibited at substrate concentrations greater than 3 mM ATP.

Introduction

ATPases (ATP phosphohydrolase, EC 3.6.1.3) appear to fall into four general classes, namely, energy transducers found in mitochondria, chloroplasts and bacteria, transport enzymes, contractile enzymes and a poorly defined fourth class including ectoenzymes whose functions remain unclear (for reviews see refs. 1—5). In general, these ATPases are intracellular and associated with a membranous or filamentous subcellular structure. This report describes and extracellular ATPase that does not easily fit the above classification. Preliminary information on its purification and characterization have been presented [6,7]. This paper details the methods for its partial purification and several of its physical and chemical characteristics.

In earlier research work we reported that this ATPase is first localized to the

outer surface of luminal secretory cells in the proximal part of the oviduct in several vertebrates such as chickens, mice and humans [8—12]. The enzyme is bound by weak bonds, readily transfers and is bound by strong bonds [11] to the surface coat of the avian ovum [13]. Formation of the enzyme in the oviduct is affected by hormonal changes [14]. The ATPase has some of the characteristics of a transport ATPase and contractile ATPase [9]. This extracellular protein first appears at the outer surface of the cells responsible for its synthesis and can be viewed as a weakly bound ectoenzyme that serves as a transmitter molecule for cell-cell interaction.

Materials and Methods

Chemicals. Enzyme substrates were obtained from Sigma Chemicals. Random samples of nucleotides were tested for purity on an HPLC Zipax strong anion-exchange column. Acrylamide, *N,N'*-methylene-bis-acrylamide (BIS), *N,N,N',N'*-tetramethylethylenediamine (TEMED) and *N,N'*-diallyltartardiamide (DATD) were purchased from Eastman-Kodak, Triton X-100 was obtained from Rohm and Haas Co. and sodium dodecyl sulfate (SDS) was obtained from Gallard-Schlesinger. Agarose was purchased from Seakem Corp. Other materials were obtained from laboratory suppliers.

Preparation of oviductal secretions. For each collection approx. 300 oviducts were obtained from freshly killed chickens (*G. domesticus*) 8—16 months of age. For most preparations White Leghorns were used. The proximal 30 cm of each oviduct was dissected free of overlying tissues and everted such that the secretory cells faced outward. Ends of the tubes were tied with surgical silk and the oviducts were immersed in a buffer (25 ml per oviduct) consisting of 25 mM imidazole · HCl, pH 7.4, and 0.22 M sucrose. The preparations were placed on a shaker bath (approximately two oscillations per s) for 60 min. All procedures were carried out at 4°C. The bathing medium was centrifuged at 50 000 × *g* for 30 min and the supernatant separated into 20-ml aliquots and quickly frozen to −70°C. Aliquots were later rapidly thawed and used for enzyme purification as described in this report.

Enzyme isolation. The general scheme for purification of the oviductal ATPase is indicated in Scheme 1, p. 201.

Enzyme activity. ATPase assays were performed at pH 8.5. Final concentrations of components were 25 mM Tris · HCl, pH 8.5, 10 mM $MgCl_2$, 2 mM ATP. Final volume was 1 ml. The mixture was incubated in a shaker bath for 5 min at 37°C and the reaction stopped with 0.1 ml of 50% cold trichloroacetic acid. Following centrifugation of the precipitate, 0.5 ml of protein-free supernatants were analyzed by the method of Baginski et al. [15]. Adenylate kinase activity was measured by the method of Colowick [16] using excess hexokinase. Hexokinase was assayed by the method of Joshi and Jagarmathan [17].

Protein determination. Proteins were determined by one or more of three methods; the method of Lowry et al. [18], the biuret reaction [19] and the Fluram assay [20]. Bovine serum albumin was used as a standard.

Lipid analysis. Lipids were extracted by the Folch technique [21] using some modifications described below. For several extractions 0.01% butylated hydroxytoluene was added to the organic phase as an antioxidant. Following

separation from the aqueous phase the organic phase was evaporated under vacuum almost to dryness and then stored under nitrogen atmosphere at −20°C. All procedures except for evaporation at 23°C were carried out at 4°C. All glassware was pre-cleaned with chloroform.

Thin-layer chromatographic plates consisted of silica gel H and magnesium silicate in a ratio of 9/1 (w/w). Aliquots of the extracted lipids were run in a carrier of chloroform/methanol/water (65 : 25 : 4, v/v) for non-polar lipids, or hexane/ethyl ether/glacial acetic acid (8 : 1 : 1, v/v) for non-polar lipids. For detection of lipids, staining methods included iodine vapor, charring with sulfuric acid-dichromate (55% H_2SO_4 containing 0.6% $K_2Cr_2O_7$) at 200°C, Zinzadze reagent for phosphorus (Supelco), 0.2% ninhydrin, diphenylamine for glycolipid (Supelco), resorcinol plus orcinol for ganglioside (Supelco), Dragendorff reagent for choline, and phosphotungstic acid stain for cholesterol and cholesterol esters. Polar lipids were determined by the methods of Bartlett [22] using perchloric acid oxidation of phospholipids with the release of inorganic phosphate which is assayed by the reaction with ammonium molybdate. Trace amounts of suspected neutral lipids were analyzed by gas- liquid chromatography-mass spectroscopy.

Carbohydrate and nucleic acid analyses. DNA was assayed by reaction with diphenylamine as modified by Giles and Myers [23]. RNA was assayed by reaction with orcinol and ferric ion [24]. The phenol-sulfuric acid method [25] was used to determine carbohydrate. Total hexoses were determined by the anthrone reaction [26,27]. The thiobarbituric acid assay described by Warren [28] was used to measure sialic acid.

Gel electrophoresis. Gel electrophoresis was carried out under non-denaturing and denaturing conditions. For the former a modification of the agarose-bis-acrylamide polymer described by Peacock and Dingman [29] was used. The bis-acrylamide solution consisted of an 8.1% solution of bis-acrylamide in a Tris/borate buffer, pH 8.3, with 1% dimethyl aminopropicnitrile and 0.3% Triton X-100. The agarose (Seakem) consisted of a 1.7% solution that was refluxed with vigorous stirring for 15 min. Agarose and bis-acrylamide were mixed at 40°C with swirling to prevent localized gelling or polymerization. $(NH_4)_2S_2O_8$ was added to 0.05%. The final concentration was 2.5% bis-acrylamide and 0.6% agarose. Gel tubes were maintained at 18°C while the 40°C solution was poured such that the agarose gelled prior to polymerization of the bis-acrylamide. Tris/borate, pH 8.3, was used for upper and lower baths. The sample solution contained 20% glycerol, tracking dye, and 0.3% Triton X-100. Initial current for 10 min was 1 Ma per tube with final current of 2 Ma per tube. Upon completion of electrophoresis, enzymatic activity was assayed in the presence of 15 mM ATP substrate and 15 mM $MgCl_2$. Color reaction was obtained with Ames reagent. The high substrate concentration was necessary here to allow for the restricted accessibility of the gel matrix.

To run samples under denaturing conditions the method of Laemmli [30] was used with the following modification. The sample was first delipidized with four volumes of diethyl ether, centrifuged at 1000 × *g* for 10 min [31], dialyzed against water and lyophilized. This sample was incubated at 37°C for 2 h in sample buffer consisting of 125 mM Tris · HCl, pH 6.8, 2.5% SDS, 8 M urea, 10% β-mercaptoethanol, and 20% glycerol. The gels consisted of 4% bis-acryl-

amide or 8% DATD-acrylamide, or a 3—16% slab gradient of bis-acrylamide. For the slab a current of 11 Ma was used.

Results

Electron microscopic and enzymatic controls have been used to show that the oviductal secretory cells are intact [11] and that the microtubules in the cilia have not been released or distorted. The bathing medium was chosen to reduce any possible release of dynein or cilia as described by Anderson [32]. As noted earlier, the enzyme is loosely attached to the surface of the secretory cell. Fig. 1 illustrates its release into the wash solution. The amount of protein released gradually increases with time in the shaker bath, the absolute enzyme activity reaches a plateau after 1 h, and a peak of specific activity is attained in approx. 1 h. In all experiments the washing procedure was standardized at 60 min.

The isolation procedure is shown in Scheme 1. The choice of a 50 000 $\times g$ for 30 min supernatant is based upon the observation that the bulk of the enzyme activity of interest remains in the supernatant. The sucrose step gradient was established following measurement of enzymatic activity in fractions from linear gradients. The fraction at the 1.07/1.18 step exhibits a considerable increase in specific activity (see Table I). Concentration of the fraction in preparation for exclusion chromatography is beset with problems.

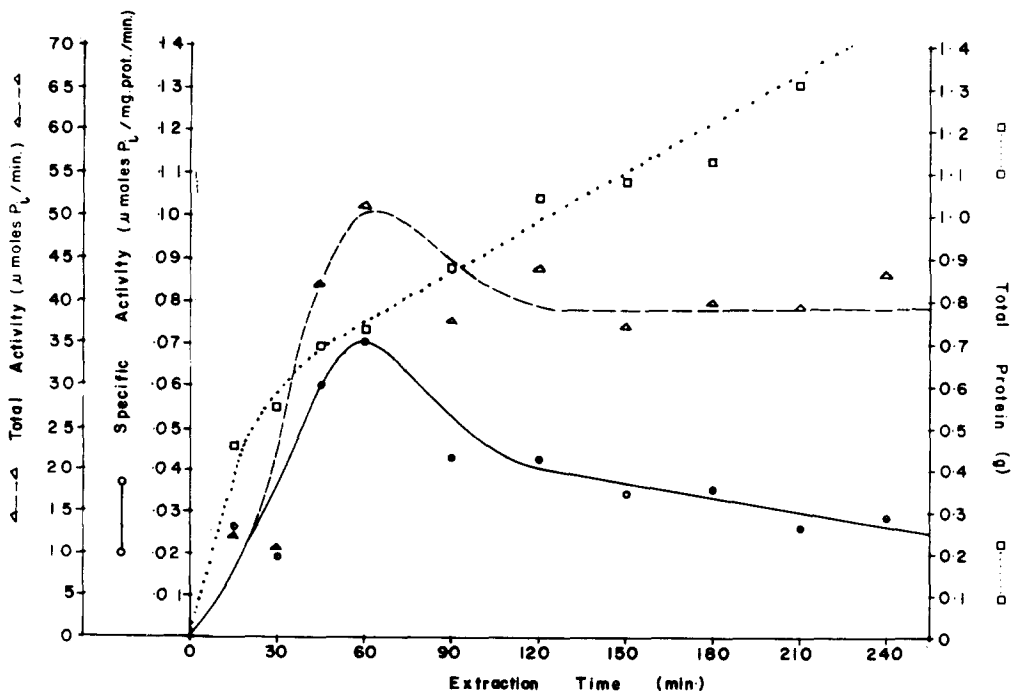
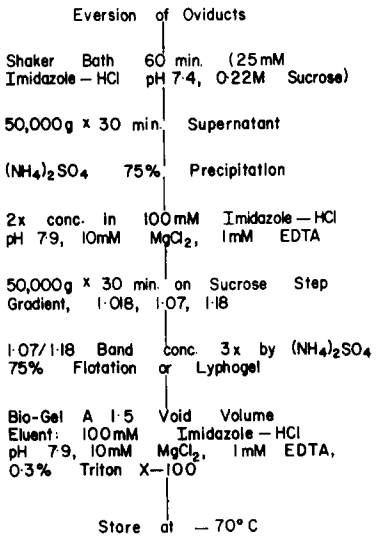


Fig. 1. Release of protein and enzyme into wash buffer as a function of time (amount of protein and absolute activity per oviduct).

ISOLATION OF OVIDUCTAL ATPase



Scheme 1. Flow diagram for purification of $(Ca^{2+} + Mg^{2+})$-ATPase.

The enzyme is extremely labile and rapidly loses activity upon contact with surfaces. Activity is markedly decreased following concentration by means of dialysis or filtration. The most useful techniques for concentration are flotation in 75% $(NH_4)_2SO_4$ (the lipoprotein aggregates at the air-supernatant interface) or Lyphogel (Gelman) concentration as described in Scheme I. Resolubilization of the flotation fraction can be facilitated by the presence of Triton X-100. Concentrations less than or greater than the critical micelle concentration are used (0.01 and 0.3%, respectively). In general Triton X-100 has proved to be superior to Lubrol or deoxycholate. The relationship between enzyme activity and Triton X-100 concentration is shown in Fig. 2. Peaks in activity occur below or above the critical micelle concentration. Similar results for other enzymes have been described [33]. The concentrated fraction is then passed through an exclusion column containing Bio-Gel Al.5 and enzyme activity, as

TABLE I

BALANCE SHEET OF PROTEIN AND ENZYME ACTIVITY IN SUBFRACTIONS DURING PURIFICATION

Specific activity in μmol P_i/mg protein per min.

	Total absolute activity (U)	Total protein (mg)	Specific activity	% Yield (activity)	% Yield (protein)	Purification
Original sample	4.05	557.1	0.007	100	100	1
Sucrose step	2.50	10.4	0.24	62	1.9	32.8
$(NH_4)_2SO_4$ float	1.08	4.3	0.25	27	0.8	34.6
Al · 5	0.63	0.3	2.08	15.7	0.05	286.7

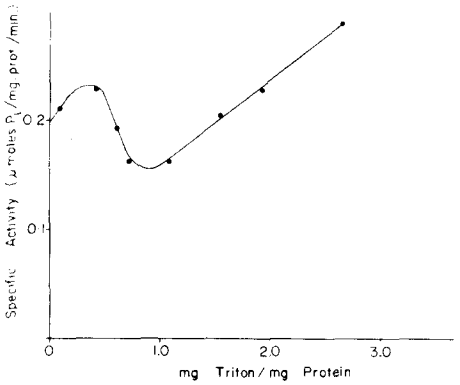


Fig. 2. Variation in enzyme specific activity with concentration of Triton X-100.

shown in Fig. 3, is confined to the exclusion volume. The choice of Al.5 stems from the lability of the enzyme. Whenever the enzyme has been chromatographed such that it remained in the retardation volume in exclusion or ion-exchange chromatography, activity has been lost. The usual protective agents such as 10—20% glycerol, 1 mM dithiothreitol, 0.1% bovine serum albumin, 0.05% butylated hydroxytoluene, 0.05% ascorbic acid, proteolytic inhibitors, ATP, 2 mM EDTA, non-ionic detergents and nitrogen atmosphere were not effective. The enzyme is stable at −70°C but labile at −20, 4, 20 and 37°C. The balance chart in Table I shows that the partially purified fraction has been enriched 287-fold. In a large number of repeat experiments enrichment has

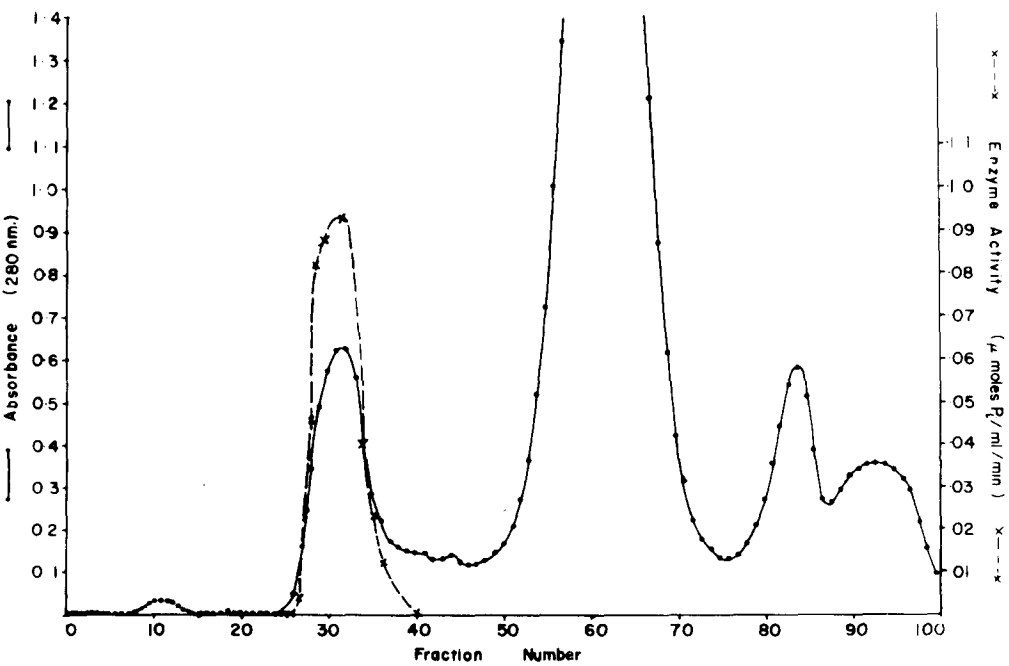


Fig. 3. Exclusion chromatography of semi-crude fraction on Bio-Rad Agarose Al.5. Column, 1.1 × 80 cm. Flow rate, 0.7 ml/min; 2-min fractions. Eluent buffer, 100 mM imidazole · HCl, pH 7.9, 10 mM $MgCl_2$, 1 mM EDTA, 0.3% Triton X-100.

varied between 200- and 300-fold. Yield in terms of total original activity is of the order of 16%. These data show that the enzyme is less than 0.35% of the total original protein.

Under electron microscopic examination using negative staining the partially purified enzyme preparation consists of vesicles roughly 1500 Å in diameter (Fig. 4). The chemical composition of these vesicles (in the absence of Triton X-100 replacement) is approx. 53% lipid and 47% protein. Essentially no carbohydrate or nucleic acids are detectable. Table II lists the composition on the basis of dry weight. The lipids are limited to the three phospholipids, phosphatidylcholine 43%, phosphatidylethanolamine 40%, and sphingomyelin 17%.

Examination of the lipoprotein enzymic complex by gel electrophoresis proved very difficult. The large lipoprotein is not highly soluble and is restricted by the pore size of the gels. In addition, this lipoprotein is not easily solubilized. As described in Materials and Methods, non-denaturing gels using a carefully polymerized mixture of agarose and acrylamide can be used and a single band of enzymatic activity has been demonstrated (Fig. 5a). In a 4% acrylamide (bis cross-linked) non-denaturing gel the same sample yields one major and one minor band, both of high molecular weight (Fig. 5b). Up to 2.5% solutions of SDS in the presence of β-mercaptoethanol have not denatured and solubilized the protein subunits. Similarly, acetone extraction of the lipid followed by denaturation in 6 M guanidine · HCl and dialysis against a SDS solution was not successful. The procedure that proved most useful was diethyl ether extraction of lipid and detergent followed by solubilization and

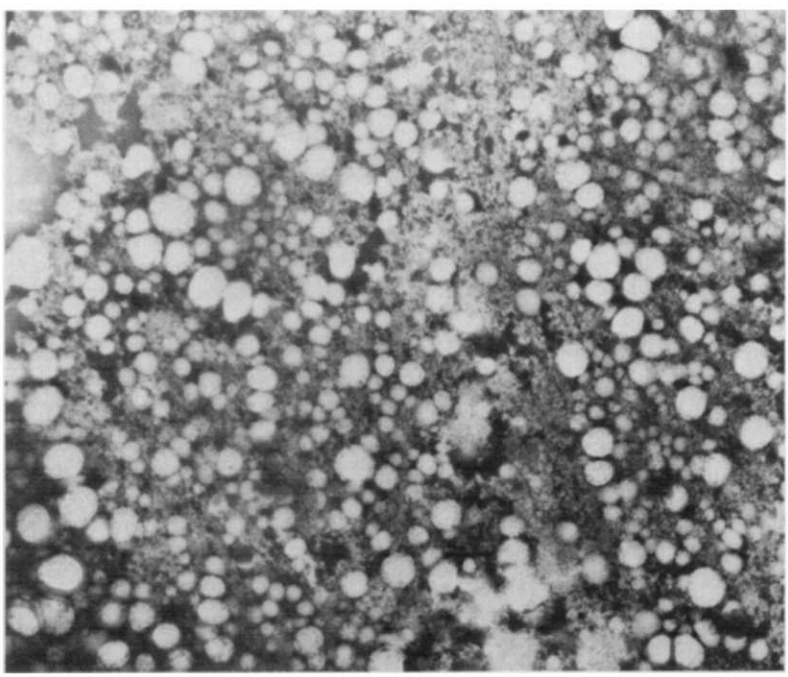


Fig. 4. Electron micrograph of negatively phosphotungstic acid-stained enzymatically active vesicles. Magnification 14 000×.

TABLE II

COMPOSITION OF PURIFIED ENZYMATIC PREPARATIONS (PERCENTAGES BY DRY WEIGHT)

Composition of isolated fraction (by weight)	
Protein	47%
Lipid	53%
Carbohydrate	<1%
Nucleic acids	0%

Lipids as percent of total lipid	
Phosphatidylcholine	43%
Phosphatidylethanolamine	40%
Sphingomyelin	17%
Cholesterol	Trace
Cerebroside	Trace

denaturation in SDS-urea as described under Materials and Methods. Initial runs were made in 4% acrylamide gels with DATD as the cross-linking agent. The use of DATD has the advantage of providing almost a 4-fold increase in pore size compared to bis-acrylamide on an equimolar basis [34]. For better resolution

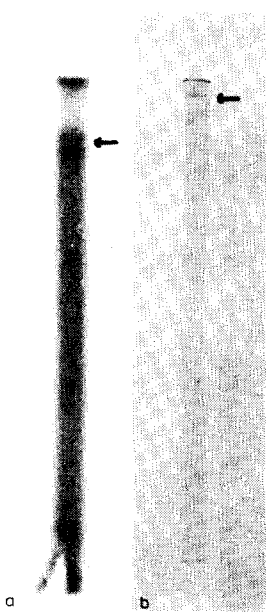


Fig. 5. Gel electrophoresis of enzymatically active lipoprotein complex under non-denaturing conditions in (a) agarose-bis-acrylamide tube gel (see Materials and Methods) and (b) 4% acrylamide (bis cross-linked).

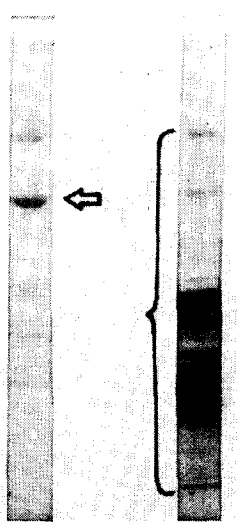


Fig. 6. SDS gel electrophoresis (see Materials and Methods) of delipidized protein in 3—16% gradient acrylamide (bis cross-linked) slab gel. The gels were calibrated with bovine serum albumin, heavy chain γ-globulin and light chain γ-globulin as markers. Heavy arrows point to the major subunit band of the enzymic protein. The bracket points to the subunits found in the initial 50 000 × *g* supernatant fraction containing the enzyme.

following the initial scan on DATD-acrylamide, the sample was run in 3—16% gradient acrylamide (bis cross-linked) slab gels as shown in Fig. 6. The subunits appear to have molecular weights of the order of 170 000, 340 000 and 760 000, with the major subunits being 170 000. A minor band of 76 000 is highly variable and may be a contaminant.

The isolated enzyme exhibits a divalent cation dependency for Mg^{2+} or Ca^{2+}. Fig. 7 illustrates this dependency with slightly higher activity found in the presence of calcium. Neither cation appears to inhibit the other. The ratio of Mg^{2+} to ATP must be greater than 1 :1 on a molar basis for optimum activity (Fig. 8). The specific activity of the enzyme exhibits different dependencies on pH, depending on the divalent cation present. Fig. 9 shows the double optima of activity (pH 6.0 and 7.8) when Mg^{2+}-ATP is the substrate compared to the single peak (pH 9.0) when Ca^{2+}-ATP is the substrate. This variation of activity with pH is similar to earlier reported data [9,11].

The enzyme is active towards ATP with lesser activity towards UTP, GTP, CTP and ADP as substrates but has little to no activity toward other substrates such as AMP, *p*-nitrophenyl phosphate, *o*-carboxyphenyl phosphate, pyrophosphate, β-glycerophosphate and phosphoenol-pyruvate. It appears that its activity (Table III) is limited to anhydride phosphate linkages. The Lineweaver-Burk plot shown in Fig. 10 yields a K_m and V of 0.88 mM and 0.69 μmol P_i/min for ATP, and 1.21 mM and 0.16 μmol P_i/min for ADP, respectively. Little inhibition of the ATPase activity is provided by ADP, AMP or β,γ-methylene-

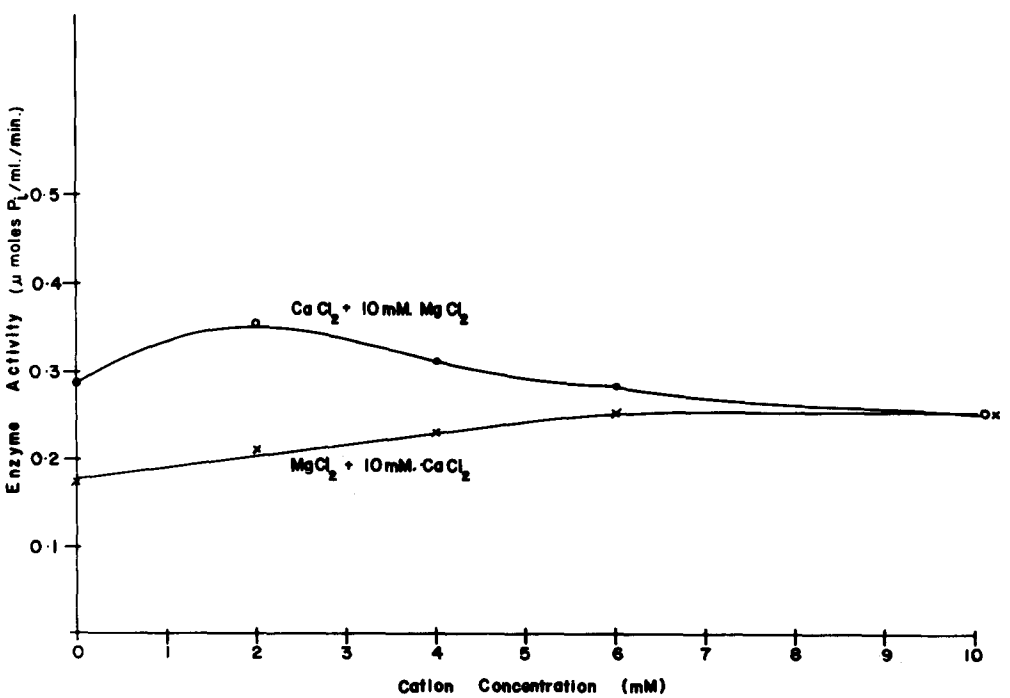


Fig. 7. ATPase activity as a function of Mg^{2+} and Ca^{2+} concentration. Protein concentration was 0.17 mg/ml

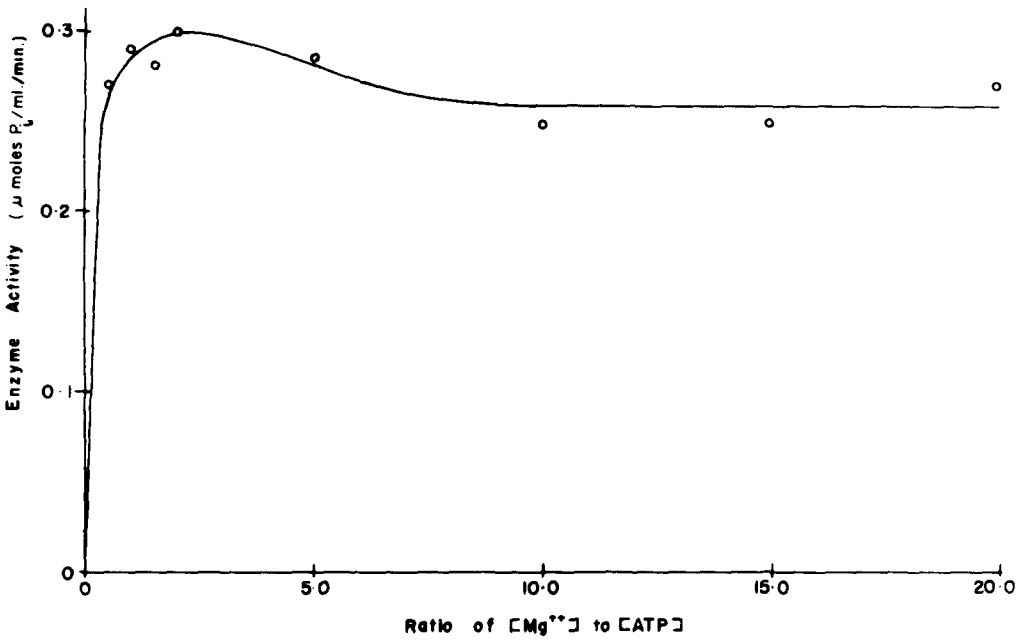


Fig. 8. ATPase activity as a function of Mg^{2+}/ATP ratio. Protein concentration was 0.15 mg/ml.

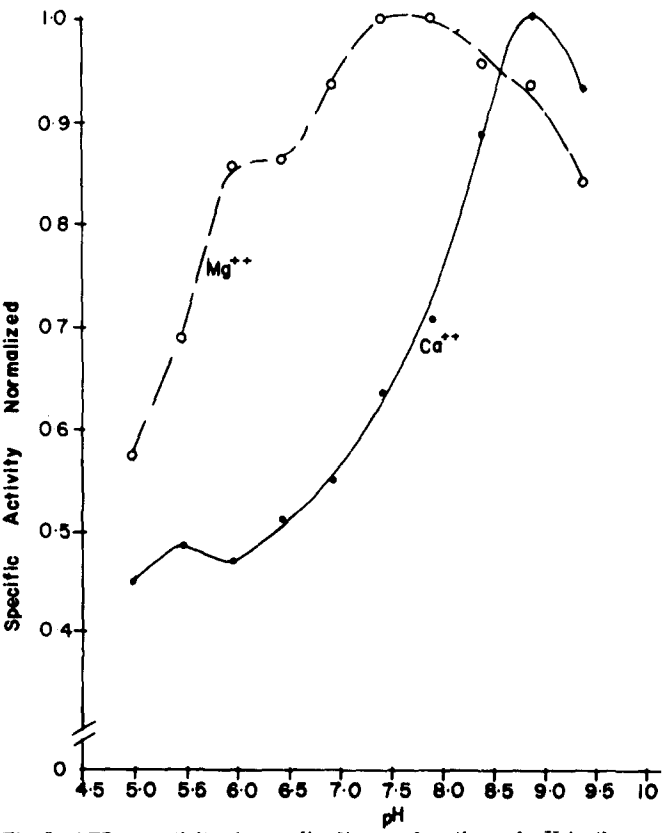


Fig. 9. ATPase activity (normalized) as a function of pH in the presence of Mg^{2+} or Ca^{2+}. The buffers consisted of a Tris/histidine · HCl system plus 50 mM KCl, 40 mM NaCl and 10 mM $CaCl_2$ or $MgCl_2$.

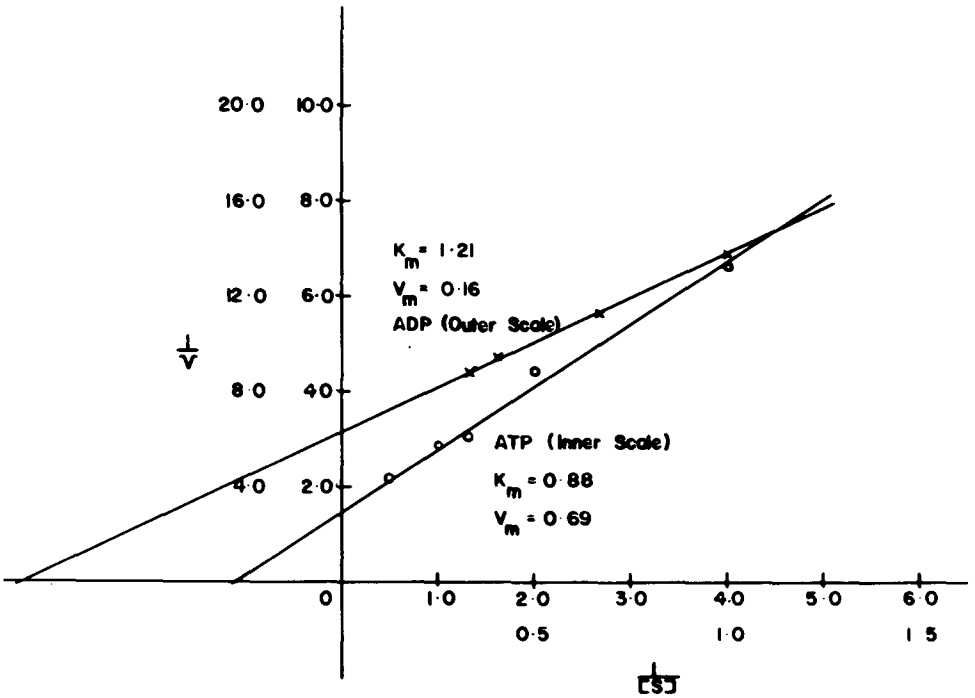


Fig. 10. Lineweaver-Burk plot of ATPase and ADPase activities. K_m and V values determined by extrapolation.

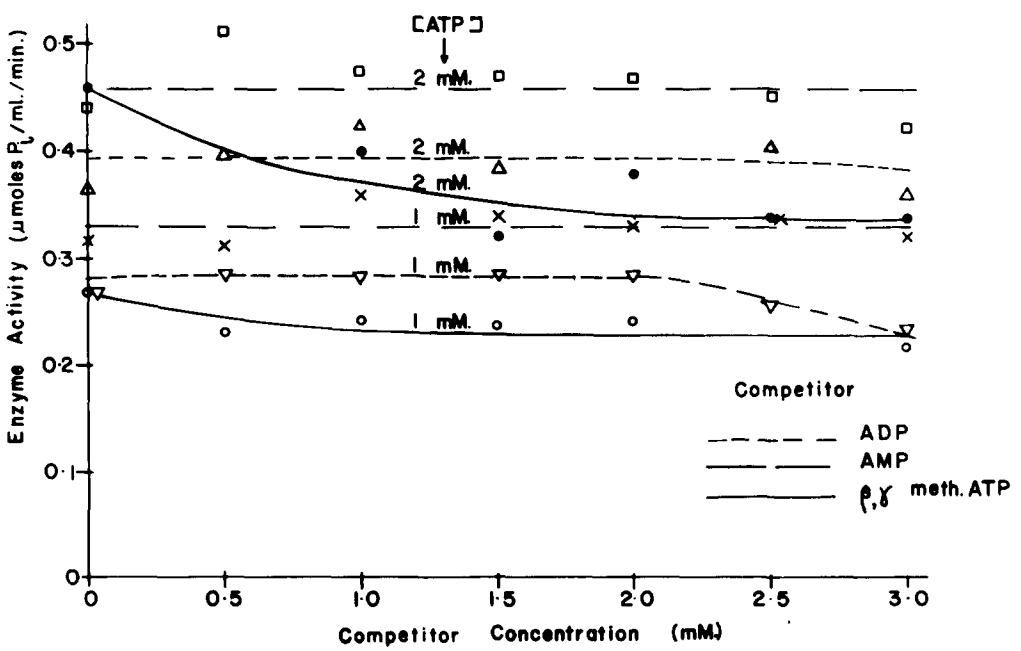


Fig. 11. Effects of ADP, AMP and β,γ-methylene-ATP on ATPase activity. Protein concentration was 0.15 mg/ml.

TABLE III

RELATIVE SPECIFICITY OF ENZYMATIC ACTIVITY TOWARD SEVERAL SUBSTRATES
Protein concentration was 0.17 mg/ml.

Substrate	Enzyme activity (μ mol P_i/min per ml)
ATP	0.27
UTP	0.14
GTP	0.11
CTP	0.11
ADP	0.12
Phosvitin	0.00
AMP	0.03
p-Nitrophenylphosphate	0.01
o-Carboxyphenylphosphate	0.00
Pyrophosphate, disodium	0.02
β-glycerophosphate	0.00
Phospho(enol)pyruvate	0.00

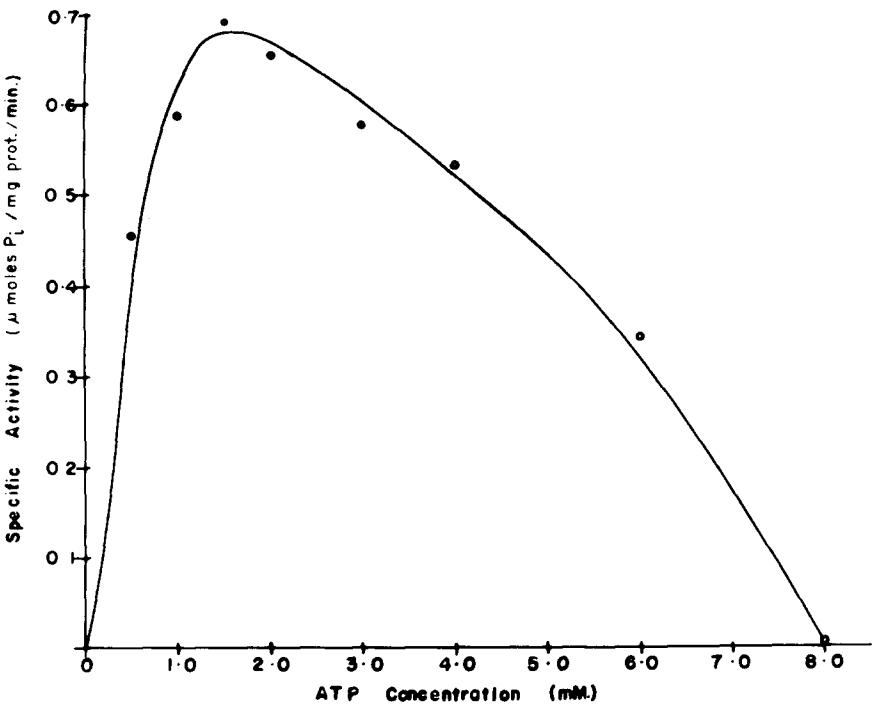


Fig. 12. ATPase activity as a function of ATP substrate concentration.

ATP as shown in Fig. 11. On the other hand, excess amount of the substrate ATP is inhibitory as shown in Fig. 12, with maximal activity occurring at a substrate concentration of approx. 2 mM ATP. The enzyme exhibits no adenylate kinase activity.

Discussion

The ATPase that has been partially purified from oviductal secretions appears to represent a unique class of nucleoside triphosphate hydrolases. The

enzyme is extracellular, unlike previously isolated ATPases. It is transferred from the surface of the secretory cells to that of the oocyte, a form of intercellular communication not previously described for such enzymes. It is a lipoprotein whose phospholipid composition is very similar to that described for Ca^{2+} transport ATPases yet does not exhibit a similar Ca^{2+} dependence [2]. It has a pH dependence similar to that described for contractile ATPases [4] yet is not related to a filamentous subunit. The enzyme exhibits substrate inhibition, a process that is often associated with multiple binding sites for substrate [35]. The enzyme is far more labile than other isolated or partially purified ATPases with respect to temperature, time, lipid replacement, adsorption and so-called stabilizing agents. Its production appears to be subject to hormonal influence [14].

It is difficult to estimate the molecular weight of the enzyme since it is a lipoprotein complex. The electron micrographs indicate that the purified fractions are micellar in shape with molecular weights of the order of four million. These magnitudes are consistent with the results obtained by exclusion chromatography using A5.0 and A1.5 exclusion columns. Other workers [36] have recently shown that ovovitelline nucleoside triphosphate hydrolase solubilized with various non-ionic and ionic detergents remains in the void volume of Sepharose 4B exclusion chromatographs and has a similar molecular weight. Only with concentrations of 3% deoxycholate and high centrifugation ($9 \cdot 10^6$ $g \cdot$ min) could an additional smaller molecular weight unit of 260 000 be obtained. Our data indicate that the major subunit of protein has a molecular weight of 170 000. The lipoprotein which we have described appears to be the same as the enzyme obtained from the ovovitelline membrane [37,9]. SDS gel electrophoresis shows that the ATPase is a high molecular weight minor component of the less pure 50 000 $\times g$ supernatant from which it is isolated. The purified ATPase is free of the major lower molecular weight subunits found in this less pure fraction. The lipids can be replaced with detergents such as Triton X-100 at a concentration of 0.3%. This concentration is well above the critical micelle concentration of 0.015% for Triton X-100 even allowing for a binding of 0.5 mg of Triton per mg protein [38]. Delipidation and replacement with Triton X-100 or incorporation of the enzyme into detergent micelles results in an increase in activity.

We suspect that high lability of the enzyme is due to configurational change in the protein with alteration in lipid content and considerable hydrophobicity of the protein. Thus, ion-exchange chromatography, adsorption, and filtration result in configurational changes and a marked decrease to total loss in activity. The reasons for cold (−20°C, 4°C) and heat (23°C) lability are not clear. Hopefully, further experiments using lipid exchange and phospholipid vesicles will provide some of these answers.

At the moment little is known about the functions of this enzyme. Several possibilities must be considered. The ATPase may be an ectoenzyme partially freed during the exocytosis of oviductal secretions. There is good evidence to support this hypothesis. Ectoenzyme ATPases described by Trams and Lauter [39] require concentrations of Mg^{2+} of the order of 10 mM, display pH maxima at 6.5 and 8.0, have a K_m of approx. 1.7 mM, and are not wholly specific, displaying lesser activity toward other nucleoside triphosphates. The

enzyme which we describe in this report is very similar. Garnett and Kemp [40] described a (Ca^{2+} + Mg^{2+})-activated ATPase in the plasma membrane of mouse liver cells that was not studied for ectoenzymatic activity, but exhibited a dependency on Mg^{2+} or Ca^{2+} and ATP substrate concentration that is comparable to our ATPase. They report a K_m of 0.88 mM which is comparable to ours. They feel that the (Ca^{2+} + Mg^{2+})-ATPase is associated with contractile proteins in the surface region. Thus, the evidence at the moment indicates the enzyme reported in this paper has the characteristics of an ectoenzyme yet does not fully satisfy the criteria established by DePierre and Karnovsky [5] in that the enzyme is not tightly bound to the cell surface, but is part of an extracellular matrix and transfers to the surface of another cell. The enzyme is not within the plasma membrane and there is no 5′-nucleotidase activity; another ectoenzyme noted by Trams and Lauter [39].

Trams [41] has proposed that ATP is translocated from the inner region of cells across the plasma membrane following a pertinent stimulus. He suggests that the extracellular ATP impinges on the membranes of adjacent cells and effects a biphasic change in permeability with extrusion of K^+ and possibly of cytoplasmic ATP, with the ectoenzyme needed to limit this biphasic change. He also proposes that that entire process would require an additional phosphorylation reaction on the outer aspect of the membrane. Extracellular ATP in concentrations of the order of 3 mM have been implicated in numerous pharmacological actions including traumatic shock [42]. The control of extracellular ATP may be of considerable significance in cell interactions and pathology. Regarding extracellular phosphorylation mechanisms we have demonstrated that the oviductal secretions not only include the ATPase described in this report, but also a protein kinase that is cyclic AMP activated (Berliner, A.F. and Rosenberg, M.D., unpublished), a phosphoprotein phosphatase (Lee, K. and Rosenberg, M.D., unpublished) and a cyclic AMP-binding protein [43]. We propose that these proteins, enzymatic and non-enzymatic (including the ATPase), are part of an extracellular phosphorylating-dephosphorylating system in oviductal secretions and assuming that the ATPase is an ectoenzyme, feel that the function of this system is more complex than that proposed by Trams and Lauter [39]. In addition to affecting the levels of extracellular ATP, the complex may control the activation and synthesis of other enzymes and provide a mechanism for stabilizing the highly labile ATPase. For example, egg white [44] contains a nucleoside triphosphate phosphohydrolase which is a high molecular weight glycoprotein ($>4 \cdot 10^6$) that cannot be transformed to active subunits. The lipoprotein ATPase might be delipidated and glycosylated upon binding to the ovovitelline since the glycosylated enzyme appears to be more stable. Another possibility is that this phosphorylating-dephosphorylating complex functions in the production of the viscous coat of mucopolysaccharides surrounding the oocyte. Increased viscosity of secretions of the reproductive system generally occurs at the time of ovulation when the secreted ATPase is maximal. Some researchers [45] have proposed that a phosphorylation-dephosphorylating mechanism is of importance in fertilization and early embryonic development, but the mechanism to which they refer is intracellular and whether it is related to the mechanisms proposed here is not known.

Whether similar extracellular ATPases are present in other organ systems is not clear. An ATPase of very low specific activity can be found in peritoneal fluid, presumably due to reflux from the oviduct [11]. None has been detected in sera. Much of the difficulty is due to the marked lability of the extracellular ATPase. Were it to be present in other extracellular fluids and act in intercellular processes, the enzyme would not be detected by generally used methods of isolation.

Oviductal fluids provide a relatively abundant source of unusual extracellular ATPases and other enzymes and proteins of interest from both a basic research point of view and potential applicability in reproductive biology for the regulation of early zygote cleavage. The ATPase activity described in this report can be inhibited by increases in the concentration of its natural substrate ATP, presumably by saturating the multiple binding sites. The enzyme can be purified and antisera and antibodies have recently been prepared [46] that reduce the enzymatic activity by more than 85%. The similarity of this enzyme to ectoenzyme ATPase suggests that oviductal fluids may provide an extracellular source of ectoenzymes enabling the purification, characterization and determination of function for these highly important macromolecules.

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