Chicken Oviductal Ecto-ATP-Diphosphohydrolase  
PURIFICATION AND CHARACTERIZATION*

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An ecto-ATP diphosphohydrolase (ATPDase) was purified to homogeneity from vesiculosomes shed from chicken oviduct. First, the ecto-ATPDase-enriched vesiculosomes were concentrated by filtration, differential centrifugation, and exclusion chromatography. Next, the nonionic detergent, Nonidet P-40, was used to extract the ecto-ATPDase from vesiculosomal membranes, and the solubilized enzyme was further purified by ion exchange (DEAE-Bio-Gel) and lentil-lectin-Sepharose 4B chromatography. In the final stage, immunoaffinity chromatography was utilized to obtain purified ecto-ATPDase. More than 25,000-fold purification was achieved. Specific activity of the purified enzyme was greater than 800 μmol/min/mg of protein with MgATP as the substrate, the highest ever reported for an ATPDase. The enzyme also hydrolyzed other nucleoside triphosphates in the presence of magnesium at similar rates and CaATP and MgADP at lower rates. The molecular mass of the purified glycoprotein was 80 kDa as determined by SDS-polyacrylamide gel electrophoresis and Western blot analysis. Based on its enzymatic properties, the relationship of the chicken oviduct ecto-ATPDase with other reported ATPDases and ecto-ATPases is discussed.

The chicken oviduct has been used as a model system for studying secretion (1). Enzymes which are postulated to be involved in the egg formation process, such as an ecto-protein kinase (2), various phosphatases (3), and an ATPase have been investigated by Rosenberg et al. (4). The ATPase, first described by Haaland and Rosenberg (5), showed extensive kinetic similarities to the Mg$^{2+}$-ATPase of the outer oocyte (vitelline) membrane (6, 7). While studying the origin of this Mg$^{2+}$-ATPase, Rhea et al. (8) showed that Mg$^{2+}$-ATPase activity was shed within microvesicles into the oviductal fluids from the apical membranes of the secretory cells of the oviduct infundibulum. It was also found that, during ovulation, part of this enzyme becomes immobilized on the vitelline membrane.

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indeterminable specificity, and there have been no reports of production of monoclonal antibodies to any of these ATPDases. In this study, we describe the preparation of an effective immunogen from partially purified oviduct ecto-ATPDase and the development of six specific monoclonal antibodies, as well as the complete purification of the chicken oviduct ecto-ATPDase utilizing one of the antibodies in immunoaffinity chromatography. Enzymatic characteristics of the purified ecto-ATPDase were determined and compared to other ecto-ATPDases.

EXPERIMENTAL PROCEDURES

Materials—Hybrimax fetal calf serum, autodavable RPMI 1640 medium, protein G-agarose, anti-mouse monoclonal antibody, goat anti-mouse IgG alkaline phosphatase, Sephadex G-25, CNBr-activated Sepharose 4B and polyethyleneimine cellulose were from Sigma, Bio-Gel A1.5, DEAE-Bio-Gel A, and Affi-Gel HZ hydrazide gel were obtained from Bio-Rad, and Nonidet P-40 was from Fluka (Ronkonkoma, NY). Polyvinyl chloride microtiter assay plates were from Costar. Outbred Swiss mice and nonimmune mouse serum were obtained from Biobag Inc., White Bear Lake, MN. N51 myeloma cells were from the American Type Culture Collection. 187.1 rat anti-mouse κ-chain hybridoma cells were the generous gift of Dr. Matthew Scharff, Albert Einstein College of Medicine, Bronx, NY.

Oviductal Washings—Chicken oviducts were collected at a slaughterhouse during the months of May, June, August, or September when the amount of enzyme in the secretions was highest (10). The collected oviducts were placed on ice and allowed to sit at 4°C overnight. This waiting period gave higher yields of enzyme than if the oviducts were processed immediately. After the infundibulum and magnum were isolated, the oviducts were inverted with a plastic rod and swirled on a platform shaker (60 rpm) at 4°C in 20 ml Tris-Cl, pH 7.4, 0.2 M sucrose for approximately 1 h. A standardized mixture contained 300 ml of loosely packed oviducts in sufficient buffer to give 800 ml total volume, in a 1-liter Erlenmeyer flask. At the end of the incubation, the oviducts were removed by filtration through cheesecloth, and the filtrate was centrifuged at 6000 rpm in a Beckman Spinco rotor and resuspended in TBS to form a slurry of approximately 50% insoluble material. The supernatant was again filtered through cheesecloth and stored in 175-ml aliquots at –70°C. The final filtrate was referred to as oviductal washings, and was the starting material for subsequent purification steps.

Preparation of Immunogen—A partially purified chicken oviduct ecto-ATPDase preparation (G-25 fraction, see “Results”) which had a specific activity of approximately 40 units/mg of protein was used to prepare the immunogen. To 2 ml of the G-25 fraction (containing approximately 20 units of activity) was added 0.2 ml of sample buffer consisting of 50% sucrose, 0.05% bromophenol blue, and the sample was electrophoresed on a 5% nondenaturing, prepartive (10 cm) polyacrylamide gel according to Laemmli (32) with the modification that the gel was run at 25 V for 1.5 h at 4°C. After electrophoresis, the gel was washed in a tray of TBS-7.4, 0.1% Nonidet P-40, 2 mM CaCl2 for 1 h, and the ATPase band was cut out of the gel and used to prepare immunogen for injection into mice as described under “Experimental Procedures.”

wells of 10-96-well microtiter plates. This procedure led to predominately single clones growing in wells, which greatly simplified the recovery and subcloning of positive colonies. After wells of hybridomas were visually identified, the cells were transferred into 24-well dishes. Typically 96 colonies from a fusion were propagated and later subjected to the ATPase capture assay. Positive colonies were subcloned twice by limiting dilution. To obtain large quantities of antibody, hybridomas were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and 20 μM glutamine (10 times the standard concentration of glutamine). When the viability of the cultures as indicated by trypan blue exclusion fell to less than 50% (due to overgrowth), culture fluids were collected; they are referred to as “overgrown supernatants.” PurrigG was obtained by affinity chromatography on rabbit anti-mouse IgG-agarose or rat monoclonal anti-mouse (187.1) IgG-agarose according to standard procedures (35).

ATPase Capture Assay—In identifying hybridomas secreting anti-ATPDase antibodies, a sensitive ATPase capture assay was developed (Fig. 2) which allowed the screening of large number of hybridoma colonies with highly diluted, partially purified oviduct ecto-ATPDase preparations as the enzyme source. Three washes with TBS containing 0.1% Nonidet P-40 occurred between each step in the following procedure. 96-well polyvinyl chloride microtiter plates were first coated with 100 μl of affinity-purified rabbit anti-mouse IgG (10 μg/ml) at 4°C overnight, followed by blocking with 225 μl of 1% bovine serum albumin in TBS at 4°C overnight, which could be stored at 4°C until use. Next, 100 μl of any chosen hybridoma supernatant were added to the wells, and the plates were incubated at 37°C for 1 h to immobilize the anti-ATPDase monoclonal antibodies. Finally, 100 μl of a partially purified oviduct ATPDase preparation (DEAE fraction with an activity of approximately 0.9 unit/ml) were added to each well and incubated again for 1 h at 37°C. Wells containing anti-ATPDase monoclonal antibodies “captured” the ATPDase from the oviduct DEAE fraction, the activity of which was determined as follows. A reaction mixture consisting of TBS, 0.1% Nonidet P-40, 4 mM ATP, 4 mM MgCl2, was added to each well (200 μl/well) and incubated at room temperature overnight. An aliquot (150 μl) of the reaction mixture was transferred into a test tube, and 4 μl of ammonium molybdate reagent (36) were added. Samples from positive wells turned bright yellow. For screening purposes, visual identification of the positive tubes was adequate. For quantification, absorbance of the samples was measured at 355 nm. Alternatively, a reaction mixture containing 4 mM ATP and 20 mM CaCl2 was used in which case a white precipitate formed in those wells containing positive colonies. However, this assay could not be readily quantified.

Immunological Methods—Western immunoblots onto nitrocellulose were performed essentially according to Burnette (37). Bound antibody was detected by alkaline phosphatase-conjugated to goat anti-mouse IgG and NBT/BCIP (38). Determination of heavy chain isotypes of monoclonal antibodies was performed with the Sigma Immunotype kit. Determination of light chain isotype was by ATPase capture assay substituting the 187.1 rat anti-mouse κ-chain monoclonal antibody (39) as the capture antibody.

Immunoprecipitation was carried out as follows. To 1 ml of oviduct G-25 fraction, 100 μl of 1 mg/ml purified monoclonal antibody were added. After 1 h, the ATPase activity was determined. Protein-G-
agarose suspension was then added (50 μl), and the tubes were agitated by rotation at 4 °C overnight. The mixture was centrifuged at 1000 x g for 5 min, and ATPase activity remaining in the supernatant was determined.

Synthesis of Hydrazide-linked Immunoabsorbents—Purified monoclonal antibody (4 mg/ml) was dialyzed against 100 mM sodium acetate, pH 5.5, 150 mM NaCl. Solid sodium periodate was added to the antibody solution to a final concentration of 10 mM. The mixture was stirred in the dark for 30 min. Glycerol (0.05 volume) was added to stop the reaction. The oxidized antibody was desalted on a Sephadex G-15 column equilibrated in 100 mM sodium acetate, pH 5.5, and mixed with hydrazide activated gel (Affi-Gel HZ, Bio-Rad) at a ratio of 4 mg of antibody/ml of gel. The gel was swirled on a platform shaker at room temperature overnight. The amount of protein that did not couple to the gel was determined by absorbance at 280 nm; the amount coupled was determined by the difference. A matrix with 2.55 mg of immobilized MC22/ml of gel was produced by this method.

Preparation of Lentil-Lectin Affinity Columns—Lentil lectin was isolated from dried lentil beans by the method of Howard et al. (40). The purified lectin was coupled to CNBr activated Sepharose 4B at 4 mg/ml according to the directions of the manufacturer.

Gel Electrophoresis—Discontinuous SDS-polyacrylamide gels were prepared essentially according to Laemmli (32), except that 1% dicyclohexylcarbodiimide was used as a cross-linking agent, a modification which improved the silver staining of the gels (41). Protein samples for electrophoresis were dissolved in SDS sample buffer (5% glycerol, 3% mercaptoethanol, 2% SDS, 30 mM Tris-Cl, pH 8.6) and boiled for 5 min. Mercaptoethanol was usually left out if the samples were to be subjected to Western blot analysis. Mini gels of 7.5% acrylamide (1.5 mm thick) were routinely run at 150 V for 45 min. Some of the gels were stained in 0.1% Coomassie Blue R-250, 40% methanol, 10% acetic acid overnight and were destained in 40% methanol, 10% acetic acid. For highly sensitive and reproducible silver staining, a combination of several suggested modifications of the original procedure of Merrill et al. (42) was used. The gel was fixed overnight in 30% ethanol and 10% acetic acid. After washing six times for 10 min each in water, the gel was treated in 100 ml of 0.02% sodium thiosulfate for 1 min. It was washed again twice for 1 min each in water and incubated in 100 ml of solution containing 0.2 g of silver nitrate and 75 μl of 37% formaldehyde for 30 min. The gel was rinsed for 1 min in water, and the protein bands were developed in 100 ml of solution containing 0.6% sodium carbonate, 50 μl 37% formaldehyde, and 50 μl of freshly prepared 2% sodium thiosulfate for about 5 min or until the appearance of gel was satisfactory. Color development was stopped by direct addition of 3.5 ml of glacial acetic acid into the developing solution, and shaking was continued for 10 min. After washing in water four times for 30 min each, the gel could be stored in 20% ethanol.

Analytical Assays—For determinations of ATPase activity, samples were incubated at 37 °C in 50 mM Tris-Cl, pH 7.4, 4 mM MgCl2, and 4 mM substrate in a total volume of 500 μl (unless otherwise stated) for 5–30 min. When solubilized enzyme was assayed, the assay buffer also contained 0.1% Nonidet P-40. Inorganic phosphate released was determined by a modified method of Heinonen and Lahti (36). The enzyme reactions were terminated by the addition of 4 ml of AAM reagent (2 volumes acetone, 1 volume 5 N sulfuric acid, 1 volume 10 mM ammonium molybdate). After 5 min, 400 μl of 1 N citric acid was added to stabilize the phosphomolybdate complex, and the absorbance at 355 nm was determined. Alternatively, the released inorganic phosphate was determined by the method of Lanzetta et al. (43), using malachite green as a color reagent. All samples were run in duplicate or triplicate. 1 unit of activity is the amount of enzyme which releases 1 μmol of phosphate (P.) per min.

Products of ATP hydrolysis were also analyzed by thin layer chromatography. Samples were applied to polyethyleneimine cellulose-coated plates and developed with 1 N LiCl. The plates were photographed with indirect short wave UV illumination. The BCA method of Smith et al. (44) or the Lowry assay as modified by Peterson (45) was used to determine the protein content of the samples.

RESULTS

Generation of Monoclonal Antibodies to Chicken Oviduct ATPDase

Six anti-chicken oviduct ecto-ATPDase monoclonal antibodies were characterized in detail. They arose from two successful fusion experiments. The first fusion yielded three monoclonal antibodies designated MC1, MC2, and MC3. The second fusion yielded 51 antibodies. Three antibodies from this fusion were selected for further analysis and were designated MC18, MC22, and MC27. All six antibodies belonged to the IgG subtype (data not shown). MC27 had a single light chain which is rare in mouse monoclonal antibodies (37). The other antibodies had κ light chains.

Characterization of Monoclonal Antibodies

The ecto-ATPDase-binding capacity of the six antibodies was tested by the ATPase capture assay. Partially purified chicken oviduct ecto-ATPDase (DEAE fraction) was used as enzyme source. The antibody solutions were overgrown hybridoma supernatants. The use of rabbit anti-mouse IgG antiserum or rat IgG anti-mouse monoclonal antibodies as primary coating on the microplates increased the specificity and sensitivity of the assay. Under these conditions, all six antibodies “captured” ATPase activity from oviduct DEAE fraction at high dilution (Fig. 3).

Western blot analysis showed that all six anti-ATPDase monoclonal antibodies bound to an 80-kDa protein of the oviduct G-25 fraction (Fig. 4). The same antibodies also reacted with a 160-kDa band which was later shown to be a disulfide-linked homodimeric form of the enzyme. The two faintly stained, lower molecular mass bands on the blots could be proteolytic fragments of the 80-kDa polypeptide and were not detected in purified ATPDase preparations.

All six antibodies were able to deplete ATPase activity with various efficiency from the oviduct G-25 fraction in immunoprecipitation experiments (Fig. 5). MC2, MC18, and MC22 brought down >80% activity, whereas MC1 captured less activity (40%). The immunoprecipitated proteins were analyzed by SDS-PAGE. In a Coomassie Blue-stained gel (Fig. 6A), the heavy and light chains of the monoclonal antibodies were apparent. Close inspection of the gel showed a faint band at 80 kDa. In the Western blot probed with MC18, an intensely...
stained band was seen at 80 kDa in addition to the IgG heavy and light chains (Fig. 6B). Thus, all six antibodies immunoprecipitated the same 80-kDa polypeptide concomitant with depletion of ATPase activity from the oviduct G-25 fraction.

The functional properties of the antibodies are summarized in Table I. The results show that all six antibodies could generate positive signals in ATPase capture assays with partially purified oviduct ATPDase enzyme sample, they could immunoprecipitate ATPase activity from similar preparations, they bound to the same polypeptide in Western blots, and they were useful for immunoperoxidase localization of the ATPDase enzyme (data not shown). We subsequently utilized MC22 for immunoaffinity purification of the chicken oviduct ecto-ATPDase to homogeneity.

In purification stage II, four aliquots (volume, approximately 1200 ml) of the oviduct 1st peak fraction were thawed at room temperature, and Nonidet P-40 was added to a final concentration of 0.1% to solubilize the ecto-ATPDase. The sample was stirred at 4 °C overnight and then centrifuged at 12,000 rpm in the Sorvall SS-34 rotor for 20 min at 4 °C. The supernatant was filtered through Whatman No. 1 filter paper and applied to a 2.5 cm x 43-cm (210 ml) column of DEAE-Bio-Gel A equilibrated in 50 mM Tris-Cl, pH 7.4, containing 0.1% Nonidet P-40. All ATPase activity was retained on the column as no activity was detected in the flow-through. The column was washed with equilibrating buffer (Fig. 7, fractions 1–9), and the enzyme was eluted as a broad peak by a 0 to 100 mM NaCl gradient in the same buffer (Fig. 7, fractions 10–40). Fig. 7 also shows separation of irrelevant proteins from the ATPase as the majority of the protein was only eluted by 0.1 M NaCl (Fig. 7, fractions 41–80). Attempts to sharpen the activity peak using different elution strategies were not successful. Although only a 3-fold purification was achieved in this step, it was very useful, because the Nonidet P-40 concentration was reduced to 0.1% and contaminating material that tended to precipitate from the solubilized 1st peak fraction was removed.

The DEAE fraction was applied to a 1.5 cm x 14-cm (25 ml) column of lentil-lectin-Sepharose 4B equilibrated with 50 mM Tris-Cl, pH 7.4, containing 0.1% Nonidet P-40. All of the
The active fraction obtained after desalting was referred to as "oviduct G-25 fraction." The ATPase and ADPase activities had co-purified through this stage in the purification, but AMPase and nonspecific phosphatase activities were markedly reduced. The oviduct G-25 fraction was used as antigen to develop anti-ecto-ATPDase mouse monoclonal antibodies.

In the last purification step, the oviduct G-25 fraction was mixed with 3 ml of MC22-hydrazide column matrix and rocked end over end overnight at 4°C. The slurry was then poured into a small column and washed sequentially with 50 ml each of chromatography buffer (50 mM Tris-Cl, pH 7.4, 0.1% Nonidet P-40), chromatography buffer with 500 mM NaCl, and chromatography buffer again. The enzyme was eluted with 50 mM glycine, pH 2.5, 0.1% Nonidet P-40. Fractions of 1 ml were collected into tubes containing 100 μl of 1 M Tris-Cl, pH 8.0, 0.1% Nonidet P-40 and mixed immediately to minimize exposure to low pH. ATPase activity was eluted from the MC22-hydrazide column in a broad peak of approximately 2 column volumes of elution buffer (Fig. 8A). A strong 80-kDa and a weaker 160-kDa band were visualized in fractions coinciding with high ATPase activity. Both protein bands ran as broad bands which are characteristic of glycoproteins. Western blot analysis of the same fractions probed by another anti-ATPDase monoclonal antibody (MC18) revealed the same 80- and 160-kDa bands (Fig. 8C). The 160-kDa band could be easily detected if the purified enzyme was treated with nonreducing SDS sample buffer prior to gel electrophoresis (Fig. 9, lanes A and B), but disappeared if the samples were treated with β-mercaptoethanol (Fig. 9, lanes C and D). In some purified ecto-ATPDase preparations, the 80-kDa peptide was the only band detected on SDS gel, even without prior reduction.

Enzymic Characteristics of the Purified Ecto-ATPDase

Time Course—Hydrolysis of ATP by the purified chicken oviduct ecto-ATPDase was linear with time with either MgATP or CaATP as the substrate (data not shown here). Analysis of the hydrolysis products of ATP revealed that ADP was an intermediate and that AMP was the end product in the complete hydrolysis of ATP (Fig. 10).

Substrate Specificity—The purified oviductal ecto-ATPDase catalyzed the hydrolysis of ATP in the presence of either Mg2+ or Ca2+. Activity obtained with MgATP was usually twice as high as with CaATP (Fig. 11A). The low activity detected in the absence of divalent cations (Fig. 11A) was abolished by the addition of 1 mM EDTA, thus divalent cations are essential for activity. Table III shows that, in the presence of Mg2+ and at pH 7.4, the ecto-ATPDase hydrolyzed TTP, UTP, CTP, and GTP at similar rates as ATP. Nucleoside diphosphates were also hydrolyzed, although at much lower rates. However, the enzyme exhibited no activity toward AMP, β-glycerophosphate, or pyrophosphate.

Effect of pH—Fig. 11B shows that the pH optima for the MgATP activity of the purified oviduct ecto-ATPDase were in the range of 7.5 to 8.5. More than 50% of the maximal activity was obtained at pH as low as 5. Interestingly, ADP hydrolysis by the purified enzyme exhibited a lower pH optimum around 6 and ADPase activity was 70% of the ATPase activity. At pH 7.4 which is used in most assays, the ADPase activity is only 30% of the ATPase activity.

Kinetic Constants—When the rate of ATP hydrolysis was measured as a function of ATP concentration at fixed divalent concentrations, we found that the true substrate of the enzyme...
the activity of the chicken oviduct ecto-ATPase. The activity was also not affected by mercaptoethanol, AMP, adenosine, P$_{1-3}$-di(adenosine-5')pentaphosphate, an inhibitor of adenylyl kinase (46), and DIDS, which inhibits ecto-ATPases of human tumor cells (47). Azide and fluoride (10 mM), which were shown to inhibit ATP diphosphohydrolase activity (13, 14, 17, 20, 25), as well as vanadate at high concentration (1 mM), and pyrophosphate (5 mM) decreased the chicken oviduct ATPDase activity. Inhibition of ADP hydrolysis by these latter four compounds was significantly greater than inhibition of ATP hydrolysis by the purified oviduct ATPDase.

**DISCUSSION**

Purification of membrane enzymes usually involves the following steps: 1) isolation of membrane fractions, 2) solubilization of enzyme using detergents which retain enzyme activity, 3) ion-exchange chromatography, and 4) affinity chromatography on columns with lectins or biologically relevant ligands covalently bound to the matrix. In the purification of the oviduct ecto-ATPase, membrane fractionation from the oviduct tissue homogenate was not necessary since simple washing of inside-out oviduct strips with a buffer solution resulted in shedding of vesiculosomes which were abundant in ecto-ATPase. Previous work established that the asymmetric orientation of the ecto-ATPase (48) was preserved in these 150-nm microvesicles (4). Nonidet P-40 was found to be a suitable detergent for subsequent purification, because it effectively solubilized the enzyme without loss of activity, was compatible with ion-exchange chromatography, and was also not harmful to the activity of lentil lectin (49), which was immobilized to Sepharose and used as a chromatographic matrix for purification in this study. Using a combination of chromatographic separations, a 1,400-fold increase in the specific activity of ecto-ATPase was achieved. However, the preparation (oviduct G-25 fraction) still contained other peptides. Further purification of the enzyme by conventional methodology proved to be difficult. In the absence of specific affinity ligands, anti-ecto-ATPase monoclonal antibodies were produced intended for subsequent use as ligands in immunoaffinity chromatography.

The G-25 fraction was not used directly for injection in mice but was first subjected to native gel electrophoresis to yield the
immunogen. The ATPDase remained active after electrophoresis and was easily detected in a zymogram. Because of its ability to hydrolyze CaATP as well as MgATP, the location of the active ATPDase in the gel was indicated by the formation of a white band resulting from the precipitation of calcium phosphate when the reaction mixture contained high concentration of calcium (200 mM). The procedure described in this report for the preparation of immunogen had three beneficial effects: 1) native gel electrophoresis resulted in further separation of the ATPDase from some of the contaminating proteins, 2) subsequent treatment of the gel reduced the amount of Nonidet P-40 injected into the mice, and 3) polyacrylamide has been shown to be an effective adjuvant (50).

We further developed an ATPDase capture assay for screening hybridomas. Immobilization of antibodies in the hybridoma culture supernatant was facilitated by prior coating of the microtiter plates with a rat anti-mouse Ig monoclonal antibody. While inhibitory antibodies may be overlooked in this screening procedure, it is highly suitable for selection of binding antibodies which can be used to prepare immunoaffinity matrix, the major goal of this endeavor. Using the ATPDase capture assay, a large number of hybridomas were selected from two different fusions. The characteristics of six monoclonal antibodies are described in this report and their performance in several immunochemical assays compared. All six antibodies recognized an 80-kDa protein in partially purified oviduct ATPDase fraction shown by both Western blot analysis and immunoprecipitation. One monoclonal antibody, MC22, was selected for the preparation of an immunoaffinity column. Chromatography of the G-25 fraction on this column yielded an 80-kDa protein with an ATPase activity of about 800 units/mg of protein, the highest specific activity ever reported for an ATPDase.

ATPDases, often referred to as ATP diphosphohydrolases or apyrases, hydrolyze both ATP and ADP. The rate of ADP hydrolysis often approaches that of ATP hydrolysis, i.e., they exhibit low ATPase/ADPase ratios. However, isoforms with high ATPase/ADPase ratios were found to exist for potato apyrases, the prototype of ATPDase (51, 52). In contrast to plant enzymes, ATPDases of mammalian cells are usually membrane-bound and are inhibited by 5–10 mM azide (13, 14, 17, 20, 24, 25, 53). The ATPDase activities from different sources exhibit a variety of ATP/ADPase ratios. Purification of ATPDases from pig pancreas (14), bovine aorta (30), and bovine spleen (31)
were reported previously. Specific activities of these purified enzymes were approximately 60–100 units/mg of protein, an order of magnitude lower than the activity of the purified chicken oviduct ATPDase. Compared to the other ATPDases, the chicken oviduct ATPDase was more active in the presence of Mg\(^{2+}\) than Ca\(^{2+}\) and had a higher ATPase/ADPase ratio at pH > 7. The \(K_m\) values for ATP and ADP were significantly higher than those of the other ATPDases. It was also interesting to note that the ADPase activity of the oviduct enzyme had a lower pH optima (around 6) than that for the ATPase activity (7.5–8.5; Fig. 11B) and was more sensitive to inhibition by azide, fluoride, and pyrophosphate (Table IV). The latter may be related to a lower affinity of the enzyme for ADP than ATP or may reflect a lower sensitivity of ATP hydrolysis than ADP hydrolysis to these inhibitors.

The molecular masses of the four purified ATPDases were also different. The molecular mass of the native pig pancreas ATPDase was estimated to be 134 kDa based on inactivation by \(^{60}\)Co irradiation (23), but SDS-PAGE analysis of the purified protein showed the presence of two peptides of 58 and 28 kDa (14). The bovine aorta ATPDase preparation appeared to contain a single protein of 110 kDa (30), and the bovine spleen enzyme preparation contained a major band of 100 kDa (31). Both SDS-PAGE and Western blot analysis indicated that the molecular mass of chicken oviduct ATPDase was 80 kDa. In some preparations, a 160-kDa band could be detected if the sample was not reduced fully by \(\beta\)-mercaptoethanol prior to gel electrophoretic separation. When this preparation was deglycosylated by N-glycosidase F, two protein bands of 90 and 45 kDa were detected by Western blot analysis (data not shown). It is concluded that the 160-kDa protein is most likely a dimer of the 80-kDa ATPDase. However, dimerization is not necessary for activity, since preparations containing only the 80-kDa protein were just as active.

The only other purified ecto-ATPase which exhibits a higher specific activity (6,700 units/mg of protein) was obtained from rabbit skeletal muscle transverse tubules (54). The enzyme is also an E-type ATPase, and like other T-tubule ATPases (reviewed in Ref. 55), is labile to detergent inactivation, exhibits little activity toward ADP, and is not inhibited by azide. The purified rabbit T-tubule ATPase (54) is a 67-kDa glycoprotein which can be deglycosylated to a 52-kDa protein. The same laboratory also reported the isolation of a protein of similar molecular mass from chicken gizzard with an ATPase activity of 90 units/mg of protein in the absence, and 1700 units/mg of protein in the presence of concanavalin A (56). N-terminal amino acid sequences and lack of immunoreactivity indicated that the chicken oviduct ATPDase differs from both the rabbit T-tubule ecto-ATPase and the chicken gizzard ecto-ATPase (data not shown). In spite of the differences, all three enzymes have broad substrate specificity and display markedly higher turnover numbers compared to the intracellular ion-motive ATPases. Future structural analysis of the active sites of these proteins might reveal the basis for their unique characteristics.

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