Molecular Cloning of the Chicken Oviduct Ecto-ATP-Diphosphohydrolase*

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The chicken oviduct ecto-ATP diphosphohydrolase (ATPDase), a member of the ecto-ATPase family, was purified to homogeneity previously (Strobel, R. S., Nagy, A. K., Knowles, A. F., Buegel, J., and Rosenberg, M. O. (1996) J. Biol. Chem. 271, 16323–16331). It is an 80-kDa glycoprotein with high specific activity (approximately 1,000 μmol/min/mg with MgATP as the substrate) and hydrolyzes both nucleoside triphosphates and diphosphates. Using amino acid sequence information obtained from the purified enzyme, two partial cDNA clones were obtained using reverse transcriptase-polymerase chain reaction and library screening. This is the second ecto-ATPase family member and the first ecto-ATPDase to be cloned from information derived from purified proteins. The deduced primary sequence of the chicken oviduct ecto-ATPDase indicates a protein of 493 amino acid residues with a molecular mass of 54 kDa. The predicted orientation shows it to be anchored to the membrane by two transmembranous segments near the NH2 and COOH termini with very short intracytoplasmic peptides at either end. The bulk of the protein is extracelluar and contains 12 potential N-glycosylation sites, several potential phosphorylation sites, and five sequences that are conserved in seven other related membrane proteins. Four of the conserved sequences, designated as apyrase conserved regions, are present in both ecto-ATPases and soluble E-type ATPases. The fifth conserved region, which occurs near the COOH terminus of the eight proteins, is observed only in the membrane-bound ecto-ATPases. Unexpectedly, sequence comparison revealed that the chicken oviduct ecto-ATPDase is equally distant from the two ecto-ATPases, which exhibit low activity toward ADP, and the four putative ecto-ATPDases, which are closely related to CD39.

The chicken oviduct ATP diphosphohydrolase (ATPDase)

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) AF 04155.

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1 The abbreviations used are: ATPase, ATP-diphosphohydrolase; PVD, polyvinylidene difluoride; PCR, polymerase chain reaction; bp, base pair(s); CD39, a cell surface glycoprotein of activated lymphoid cells; ACR, apyrase conserved region; NTPase, nucleoside triphosphatase.

2 A. F. Knowles and A. K. Nagy, submitted for publication.

The chicken oviduct ATP diphosphohydrolase (ATPDase) is a member of the ecto-ATPase family. Ecto-ATPases are ubiquitous integral membrane proteins that are characterized by their extracellular orientation and enzymatic properties, which are clearly different from the F-, P-, V-type ATPases (1, 2). Soluble ATPases, which are similar to the ecto-ATPases with respect to broad substrate and ion specificities, also exist. Together, they constitute a new ATPase family, the E-type ATPases (1). The physiological function of the ecto-ATPases has not been established firmly, although many investigators have proposed that they are involved in terminating the signals of purinoceptors by hydrolyzing their ligands, i.e. ATP and ADP. Strong experimental evidence has been presented which indicates that in the platelet system the endothelial cell ecto-ATPDase regulates platelet aggregation by affecting the concentration of the P2 receptor ligand, ADP (3–5). Resolution of the issue of functions of the ecto-ATPases can be aided greatly by a better understanding of the biochemical and molecular nature of these enzymes. However, most ecto-ATPases are present in small amounts in the plasma membrane and are easily inactivated by detergents commonly used to solubilize membrane proteins, both of which have made their isolation a difficult task. Successful purification of several ecto-ATPases has only been accomplished in recent years. Ecto-ATPases from rabbit transverse tubules (6), human umbilical vessel (7), chicken oviduct (8, 9), chicken liver (8), chicken gizzard (10), and human placenta (11) have been purified to homogeneity. Extensive purification of ecto-ATPases from pig pancreas (12), bovine lung (13), bovine aorta (14), and chicken stomach (15) have also been reported.

The purified chicken oviduct ecto-ATPDase is an 80-kDa glycoprotein with a high specific activity, i.e. approximately 1000 units/mg with MgATP as the substrate (8, 9). It hydrolyzes both ATP (and other nucleoside triphosphates) as well as ADP (and other nucleoside diphosphates) in the presence of Mg2+ or Ca2+ and is thus an ATPase. It is inhibited by millimolar concentration of azide (9), a characteristic exhibited by most ATPases (17, 18). Of the purified and partially purified ecto-ATPases mentioned above, all are ATPases except for the ecto-ATPases from rabbit transverse tubule and chicken gizzard, which do not hydrolyze ADP and are not inhibited by azide (19). The chicken gizzard ecto-ATPDase has been cloned recently (19). Its deduced amino acid sequence shows considerable homology with that of CD39 (20), a putative lymphoid cell activation antigen that is expressed on mitogen-activated or Epstein-Barr virus-transformed but not resting B-lymphocytes (21).

We report here the molecular cloning of the chicken oviduct ecto-ATPDase. It is the second enzyme of the ecto-ATPase family and the first ecto-ATPDase to be cloned from information derived from purified enzyme.
EXPERIMENTAL PROCEDURES

Cloning of Chicken Oviduct Ecto-ATPase—Chicken oviduct ecto-ATPase was purified from vesiculosomes of chicken oviduct by ion exchange, lectin affinity, and immunoaffinity chromatography (9). The purified protein is soluble in 10 mM Tris-Cl, pH 7.4, and 0.1% Nonidet P-40. The purified enzyme protein (20 μg) was precipitated by 10% trichloroacetic acid in the presence of 0.02% sodium deoxycholate and left in dry ice for 3 min. The precipitate was collected by centrifugation in an Eppendorf microcentrifuge at 14,000 rpm for 30 min, washed twice with ice-cold acetone, and then dried thoroughly in a SpeedVac concentrator (Savant). The protein residue was dissolved in SDS-gel buffer containing 0.1% s-succrose, 3% SDS, 62.5 mM Tris-Cl, pH 6.9, 2 mM Na2EDTA, 5% m-mercaptoethanol, and 0.05% bromphenol blue and heated at 37 °C for 15 min. SDS-gel electrophoresis was carried out in 0.45% acrylamide gel according to a modified Laemmli protocol for protein sequencing described in the instruction manual of Bio-Rad PVDF protein sequencing membrane. All gel solutions were passed through 0.45-μm filters, and the gel was allowed to “age” for 24 h at room temperature before use. Thioglycolate (0.1 mM) was added to the running buffer in the upper reservoir to scavenge reactive compounds that might cause NH2-terminal blocking. After gel electrophoresis, the protein was transferred to the PVDF membrane and visualized by staining with Amido Black according to the manufacturer’s instruction. The protein residue was detected by anti-digoxigenin conjugated to alkaline phosphatase with chemiluminescent substrate. Positive substrates were used as templates to conduct nested PCR using two 5’-to-3’ primers synthesized from known sequences within the AT-480 cDNA and the M13 forward and reverse primers containing flanking vector DNA regions of λ Zap II. The first PCR was carried out using Thy DNA polymerase (Advantage Genomic Polymerase Mix; CLONTECH), 1 μg of sublibrary DNA, 0.2 μM M13 forward primer, and 0.2 μM gene-specific primer 5’-GAAGGATTGTCTTCCGCGC-3’ (sense, NH5, nucleotides 89–117 of AT-480). The following PCR protocol was used for the first reaction: 94 °C for 30 s, 67 °C for 10 s, and 70 °C for 3.5 min for 7 cycles; 94 °C for 30 s, 60 °C for 20 s, and 72 °C for 3.5 min for 32 cycles; then 94 °C for 30 s, 60 °C for 20 s, and 72 °C for 10 min for 1 cycle. The second round of PCR amplified 1 μl of a 1:50 dilution of the first reaction using Thy DNA polymerase, nested primers, i.e., the T7 primer and the second gene-specific primer 5’-AAGCTTAGAGCTTTCTGC-3’ (sense, NH6, nucleotides 407–426 of AT-480). The following thermal cycling protocol was used: for the nested PCR: 94 °C for 30 s, 67 °C for 10 s, and 70 °C for 3.5 min for 5 cycles; 94 °C for 30 s, 60 °C for 20 s, and 72 °C for 3.5 min for 20 cycles; then 94 °C for 30 s, 60 °C for 20 s, and 72 °C for 10 min for 1 cycle. The PCR products were separated by electrophoresis on a 1.2% agarose gel. An approximately 1,200-bp DNA fragment was obtained and subsequently cloned into PCR-Script Amp SK (+). The recombinant plasmid (AT-1200) was propagated in E. coli (XL-1 blue). Nucleotide sequence of the 1,200-bp partial cDNA was determined with Thermo Sequenase using standard sequencing primers and primers constructed from new sequence information.

Deglycosylation of Chicken Oviduct ATPDase and Western Blot Analysis—Chicken oviduct ATPDase (1.4 μg in 100 μl) and carrier protein (20 μg of bovine serum albumin) in a total volume of 120 μl was mixed with 0.26 M sodium cacodylate and 0.26 M Na2CO3 (pH 9.1) to reduce to the protein prepared to test for deglycosylation. The mixture was heated at 37 °C for 30 min. The treated sample was separated on 0.1% SDS-gel and blotted. The membranes were incubated with goat anti-mouse IgG conjugated to alkaline phosphatase i.e., goat anti-mouse IgG conjugated to alkaline phosphatase (MC18) or goat anti-rabbit IgG conjugated to alkaline phosphatase (OVI-1). Western blot analysis was conducted with the BLASTP program (26) in the National Center for Biotechnology Information data base. The cDNA sequences and deduced amino acid sequences were analyzed for homology and relationship using the Genetics Computer Group (Wisconsin) software package.

RESULTS

The purified chicken oviduct ecto-ATPase is an 80-kDa glycoprotein. That the protein is glycosylated is deduced from the fact that it binds to lectin-sepharose, and this property was utilized in its purification (9). To determine the molecular mass of the ecto-ATPase peptide, the purified enzyme was biotinylated-N2-p-galactosamine-substituted and subjected to Western blot analysis. Fig. 1 shows that MC18, a monoclonal antibody developed against the active enzyme, detected 80-kDa protein as well as a 160-kDa protein in the nondeglycosylated sample that was not reduced before SDS-polyacrylamide gel electrophoresis (Fig. 1, lane 3). The 160-kDa protein is probably a disulfide-linked dimer of the 80-kDa protein because it was
not detected in samples that were reduced by β-mercaptoethanol (see Fig. 9 in Ref. 9). Deglycosylation decreased the molecular mass of the 160-kDa protein to 110 kDa and that of the 80-kDa protein to 54 kDa (Fig. 1, lane 4). These results indicate that MC18 cross-reacts with an epitope on the peptide rather than on the oligosaccharides of the native protein. The epitope is conformation-sensitive because no bands were detected when samples were reduced by β-mercaptoethanol before SDS-polyacrylamide gel electrophoresis (Fig. 1, lanes 1, 2, 5, and 6). Western blot analysis obtained with OVI-1, the polyclonal antibody developed against the NH2-terminal 21 amino acids of the protein, yielded results similar to those with MC18 with respect to the molecular mass of the protein before and after deglycosylation (Fig. 1, lanes 5–8). In general, the deglycosylated proteins produced a sharper band on the gel (compare lanes 4, 6, and 8 with lanes 3, 5, and 7). The reduced proteins appeared to be slightly retarded in their migration (compare lane 5 with lane 7, and lane 6 with lane 8). In contrast to MC18, OVI-1 bound most strongly to the reduced and deglycosylated protein (Fig. 1, lane 6) presumably because the NH2-terminal becomes more accessible after these treatments.

The NH2-terminal peptide sequence was utilized for the synthesis of degenerate oligonucleotides as probes for cDNA library screening. Initial screening of a chicken oviduct cDNA library yielded ambiguous results, so we decided to carry out reverse transcriptase-PCR utilizing the information of peptide sequences obtained from the purified enzyme. Oligonucleotide primers were generated corresponding to the seven amino acids at the NH2 terminus (MEYKGKV) and an internal tryptic peptide at an unknown position (EFPVDF). Reverse transcriptase-PCR was carried out with degenerate primers and template cDNA derived from chicken oviduct mRNA. The PCR product (approximately 480 bp) was subcloned into pCR-Script SK(+) vector. The nucleotide sequence of the insert, AT-480, was determined. The deduced amino acid sequence of the peptide of 156 residues contains both the 21 amino acids from the NH2 terminus as well as the internal tryptic peptide.
continued to obtain the remaining cDNA. This was accomplished by nested PCR using a chicken oviduct cDNA library as template. The partial cDNA obtained, AT-1200, overlaps with AT-480 by 61 nucleotides and contains a stop codon. Fig. 2 shows the nucleotide sequence assembled from AT-480 and AT-1200 and the deduced amino acids of the full-length cDNA. The 3′-untranslated region is probably incomplete. The coding sequence consists of 1492 nucleotides and is translated into a peptide of 493 amino acids. The calculated molecular mass is 54,034, which is in excellent agreement with the molecular mass of the deglycosylated enzyme. The protein contains 12 potential N-glycosylation sites with the consensus sequence Asn-X-Thr(Ser) at residues 65, 79, 133, 223, 234, 267, 324, 330, 361, 372, 382, and 445. A hydrophathy plot (27) of the deduced primary sequence indicated two transmembranous segments of 20 amino acids near the NH2 terminus (amino acids 7–28) and COOH terminus (amino acids 464–485) of the protein (Fig. 3). Analysis by the MEMSAT program (28) indicated that the short hydrophilic peptides connected to these segments are cytoplasmic. Thus, the peptides at either termini are situated inside the cell. The predicted orientation of the chicken oviduct ecto-ATPase is similar to that deduced for lymphoid cell CD39 (20) and chicken gizzard ecto-ATPase (19). Several potential casein kinase II phosphorylation sites (29) are detected in the extracellular portion of the peptide. Thus the chicken oviduct ecto-ATPase may be a candidate substrate protein for ecto-protein kinases that most resemble casein kinases in their enzymatic characteristics (30).

We found that the primary sequence of the chicken oviduct ecto-ATPase is closely related to seven other sequences in GenBank release 104.0 (December 1997). These are human and mouse lymphoid CD39 (20), chicken gizzard ecto-ATPase (19), rat brain CD39 (31), bovine aorta endothelial CD39,3 a human CD39-like-1 gene (CD39L1) (33), and a rat brain ecto-ATPase (34). Alignment of the eight sequences was obtained using the PILEUP program and is shown in Fig. 4. Several conclusions can be drawn from the comparison of these sequences. 1) The four consensus sequences designated as apyrase consensus regions (ACRs) (35), i.e., DAGSSHT (amino acids 55–61, numbering corresponds to that in Fig. 4), ATAGMRL (amino acids 131–138), GXXEG (amino acids 172–176), and GGASTQ (amino acids 216–221), are present in all eight sequences. The ACRs were first revealed when sequence analysis was conducted with potato apyrase and several of the soluble E-type ATPases as well as human and mouse lymphoid CD39. The fact that they are conserved in all proteins that demonstrate E-type ATPase activity implied that they may be involved in the formation of the catalytic site. 2) An additional consensus sequence near the COOH terminus noted by Vasconcelos et al. (36) in all E-type ATPases, i.e., XXXWXXG, occurs in a stretch of contiguous sequence of marked identity in the eight sequences, i.e., GWXLGXMNLTNXIP (amino acids 457–471). It is noteworthy that this highly conserved region of 15 residues is only seen in the membrane-bound but not the soluble E-type ATPases. 3) An additional sequence with marked conservation is in the segment of amino acids 241–261, LYGXYYYTHSXL-CYG, which is also only found in the membrane-bound E-type ATPases. 4) The central hydrophobic region noted in the brain ecto-ATPase, brain ecto-ATPase (brain CD39), and chicken gizzard ecto-ATPase (34) contains several conserved hydrophobic amino acid residues in all eight peptide sequences (amino acids 351–376). 5) The 12 cysteine residues found in chicken oviduct ecto-ATPase include the 10 conserved cysteines as observed by Wang et al. in the CD39s of human and mouse lymphocytes and rat brain (31). These conserved cysteine residues are probably involved in disulfide bond formation. 6) The chicken oviduct ecto-ATPase, the rat brain and chicken gizzard ecto-ATPases, and human CD39L1 lack the first 7–9 amino acids that are present in the other four CD39 sequences. 7) Divergence among all of the sequences is most apparent near the NH2 and COOH termini, which coincide with the transmembranous segments, as well as amino acids 190–210, 262–300, and 376–396.

Table I shows the distances of these eight sequences from each other, expressed as a fraction of identical and similar amino acids. As expected, the amino acid sequences of the mouse and brain CD39, obtained by cloning using human CD39 cDNA as a probe, as well as bovine CD39, exhibited greater than 70% identity and 80% similarity with human CD39. The most closely related pairs in this group are rat and mouse CD39. Table I also shows that the two ecto-ATPases from chicken gizzard and rat brain are more closely related to each other with 57% amino acid identity and 71% similarity. Interestingly, human CD39L1, which has a 23-amino acid deletion (404–426) but whose enzyme activity has not been assessed (33), is more closely related to the two ecto-ATPases than the four CD39s. The amino acid identity (82%) between human CD39L1 and the rat brain ecto-ATPase is quite remarkable considering the fact that they originated from two different species. The amino acid sequence of chicken oviduct ecto-ATPase has approximately 40% identity and approximately 60% similarity with all seven sequences. Because the substrate specificity and azide inhibition of the chicken oviduct ecto-ATPase and the expressed CD39s (4, 5, 37) are similar but distinctly different from that of the ecto-ATPases, it is surprising to find that the chicken oviduct ecto-ATPase appears to be equally distanced from the ecto-ATPases on the one hand and CD39s on the other. In fact, in analyses with four different programs designed to establish phylogenetic relationship of these proteins, the results indicate that the chicken oviduct ecto-ATPase is more closely related to the ecto-ATPases rather than the CD39-type ecto-ATPase proteins (data not shown).

**DISCUSSION**

The existence of a cell surface ATPase on avian erythrocytes was described by Engelhardt in 1957 (38), around the same time that the mitochondrial ATPase and Na+,K+-ATPase activities were first discovered. However, progress in ecto-ATPase research has lagged far behind the ion-motive ATPases. The major reason is that because of their low abundance and liability to detergents, it has been difficult to obtain pure enzymes for molecular characterization. Purification of a few of these membrane enzymes to homogeneity was only reported in the last few years, finally permitting the initiation of molecular biological approaches. At the same time, an unexpected link between a membrane protein, CD39, and several soluble ATPases was discovered by Handa and Guidotti when they com-

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3 A. S. Chang, R. L. Garcia, S. M. Chang, and W. P. Schilling, submitted for publication.
pared the cDNA sequence of potato apyrase with known sequences (35). Vasconcelos et al. (36) arrived at similar conclusions. The soluble ATPases that were shown to be related in their sequences to potato apyrase were a yeast Golgi luminal GDPase (39), a pea nuclear NTPase (40), and three sequences encoding the isoenzymes of nucleoside triphosphatase of *Toxoplasma gondii* (41, 42). The yeast Golgi GDPase, pea NTPase, and potato apyrase have a molecular mass of 47 kDa, whereas the tetrameric *Toxoplasma* NTPase consists of subunits of molecular mass of 66 kDa (42). Other than the yeast GDPase which is highly specific for GDP as the substrate, the enzymes from potato, pea, and *Toxoplasma* share similar enzymatic properties with the ecto-ATPases in their broad substrate specificity, ability to use both Mg$^{2+}$ and Ca$^{2+}$, and specific activities exceeding 1,000 units/mg of protein. These soluble enzymes and the membrane-bound ecto-ATPases are now collectively called the E-type ATPases (1).

In contrast to the soluble E-type ATPases from plants and microorganisms described above, CD39 is a 78-kDa cell membrane glycoprotein that has a protein core of 54 kDa (43). It was initially designated as lymphoid cell activation antigen because its expression was increased in activated lymphocytes and was first cloned from lymphoid cells (20). Although some sequence homology between CD39 and the yeast GDPase was noted (20), the possibility that CD39 possessed enzyme activity was not pursued. The recent revelation that this sequence homology extended to other soluble E-type ATPases prompted several studies in which CD39 cDNA was expressed in COS cells. Enzymatic assays and Western blot analysis showed a correlation of protein expression with increase of a cell surface ATPase activity, which also hydrolyzes ADP and is inhibited by high concentrations of azide (4, 5, 37). These results suggested that CD39 is an ATP diphosphohydrolase. However, attempts at isolating the CD39 protein as an active ATPDase from lymphocytes was not successful as 90% of the activity was lost when the cells were extracted by polyoxyethylene-9-lauryl ether (C12E9) and Triton X-100 to solubilize the enzyme (37). At present, although CD39 is considered to be synonymous with vascular ecto-ATPDase, the isolation of a CD39 protein possessing this biochemical activity has not been reported.

The first cloning of an ecto-ATPase with information obtained from purified protein was achieved with the chicken gizzard ecto-ATPase (19). This enzyme is a 63-kDa glycoprotein that could be deglycosylated to a 54-kDa peptide. It differs from the ecto-ATPDases in that it does not hydrolyze ADP. Immunohistochemical studies further showed that although both ecto-ATPase and ecto-ATPDase are present in the chicken stomach, they have distinctly different localization, implying possibly different functions (15). Nevertheless, the deduced amino acid sequence of the chicken gizzard ecto-ATPase revealed significant sequence homology to that of CD39. In addition, the protein cross-reacts with CD39 antibody in Western blot analysis (15).

That the ecto-ATPases and ecto-ATPDases are related is illustrated further by recent studies where two different cDNAs were obtained from rat brain by using mouse CD39 cDNA as a probe in library screening (31, 34). The two cDNAs

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**FIG. 4.** Sequence comparison of the chicken oviduct ecto-ATPDase with related membrane proteins. Sequence alignment of the eight proteins was generated using the PILEUP program of Genetics Computer Group (Wisconsin) software package. Amino acids identical to those in the chicken oviduct ecto-ATPDase (cow ATPase) are indicated by *. Sequences originally designated as ACRs (35) are in bold letters. Conserved cysteine residues (31) are underlined. The seven peptides for comparison and their accession numbers are: chicken gizzard (UY4467) (19), rat brain ecto-ATPase (eATPase) (Y11835) (34), rat brain CD39 (U81295) (31), bovine aorta endothelial CD39 (AF005940), mouse CD39 (P57572) (20), human CD39 (P49961) (20), and human CD39L1 (U91510) (33).
Cloning of Chicken Oviduct Ecto-ATPDase

Table I

Comparison of the extent of identity and similarity of amino acids in primary sequences of chicken oviduct ecto-ATPDase and related proteins

|           | Gizzard ATPase | Rat e-ATPase | Rat CD39 | Bovine CD39 | Mouse CD39 | Human CD39 | Human CD39L1 |
|-----------|----------------|--------------|----------|-------------|------------|------------|---------------|
| cov ATPDase | 0.416 (0.500) | 0.430 (0.574) | 0.421 (0.576) | 0.420 (0.572) | 0.416 (0.562) | 0.412 (0.600) | 0.420 (0.701) |
| Gizzard ATPase | 0.568 (0.707) | 0.400 (0.590) | 0.390 (0.586) | 0.412 (0.572) | 0.390 (0.572) | 0.572 (0.701) | 
| Rat brain e-ATPase | 1.000 (0.685) | 0.398 (0.568) | 0.410 (0.570) | 0.416 (0.570) | 0.406 (0.556) | 0.824 (0.890) | 
| Rat CD39 | 1.000 (0.765) | 0.632 (0.943) | 0.902 (0.831) | 0.743 (0.547) | 0.390 (0.547) | 
| Bovine CD39 | 1.000 (0.763) | 0.649 (0.826) | 0.712 (0.845) | 0.403 (0.551) | 
| Mouse CD39 | 1.000 (0.763) | 0.763 (0.826) | 0.403 (0.551) | 
| Human CD39 | 1.000 (0.763) | 0.384 (0.536) | 
| Human CD39L1 | 1.000 | 

were designated, respectively, as brain CD39 and brain e-ATPase (for ecto-ATPase). The expression of the rat brain CD39 cDNA in COS-7 cells was correlated to increased calcium-dependent ecto-ATPase activity, but ADP hydrolysis was not assessed (31). Expression of the rat brain e-ATPase cDNA in Chinese hamster ovary cells was correlated to the increase of an ecto-ATPase activity with little ADP hydrolysis activity (34). Primary sequence comparison showed that the latter is also more closely related to the chicken gizzard ecto-ATPase than the CD39 proteins. The heterogeneity of ATPase composition in brain was noted previously in biochemical studies (44). The results obtained from expression studies described above suggest that at least two different types of E-type ATPases are present in rat brain. Cloning of human brain ecto-ATPases has not been reported. Thus we note with interest that the rat brain ecto-ATPase shows the highest protein homology with human CD39L1 that was isolated from a cDNA library enriched for transcripts of human chromosome 9q but was not expressed for biochemical characterization (33). It is likely that CD39L1 codes for one of the ecto-ATPases of human brain because strong signals were obtained with brain (poly)DNA in Northern blot analysis using a partial CD39L1 cDNA as a probe (33).

Thus far, published data on sequences of the ecto-ATPases suggest a subdivision of ecto-ATPases and ecto-ATPDases, with the latter more closely related to CD39. However, except for chicken gizzard ecto-ATPase, which was both purified and cloned, the conclusion that CD39-related DNAs encode E-type ATPases was obtained from expression studies only, and the expressed activities were generally low. For this reason, the cloning of another E-type ATPase using information obtained from purified protein is highly desirable. The work described in this paper met this goal. In addition, because the chicken oviduct enzyme is an ecto-ATPDase, comparison of its sequence with that of an ecto-ATPase from the same species, i.e., the chicken gizzard ecto-ATPase, may offer some insight as to their different selectivity with respect to substrates.

The primary sequence of chicken oviduct ecto-ATPDase reveals the presence of the four ACRs present in all E-type ATPases, whether soluble or membrane-bound. ACR1 (DAGST) and ACR4 (DLGGASTQ) contain the phosphate 1 and phosphate 2 motifs of the sugar kinases-actin-hsp70 family (45). The connect 1 motif, which occurs between phosphate 1 and phosphate 2 motifs, was located in the pea NTPase (40), but it was difficult to determine in the chicken oviduct ATP-Dase sequence. It would appear that the presence of the four ACRs is essential for enzyme activity. However, it has been reported that only ACR 4 is present in the pig pancreas ecto-ATPDase, which is a 35-kDa protein after deglycosylation (12). Thus the pancreas ATPDase represents a truncated protein compared with the other known membrane-bound E-type ATPases. Nevertheless, the pancreas protein is active as an ATPDase and can be labeled by fluorosulfonylbenzoyl adenosine (12); thus one must conclude that the ATP and ADP binding site is present in this protein, which lacks the first three ACRs. It will be of interest to determine if an ATPDase activity can be obtained when a partial cDNA containing only the fourth ACR is expressed.

We had expected the chicken oviduct ATPDase sequence to be more closely related to those of the four CD39s than to the two ecto-ATPases. Thus it was a surprise to find that this was not the case. This cannot be explained as resulting solely from species differences because the ecto-ATPases of chicken and rat share 57% identical amino acids, which is significantly greater than the amino acid identity (41%) between chicken ecto-ATPase and rat ecto-ATPDase (CD39). At present, it is not clear if there may be further division within the ecto-ATPDase subfamily. This issue can be resolved only when sequence data of other purified ecto-ATPases become available. Recently, the chicken stomach ecto-ATPDase has been partially purified and its NH2-terminal sequence determined (15). Interestingly, the first 17 amino acids MEYKKKVAGLLTATWV are nearly identical to those of the chicken oviduct ATPDase (Fig. 1), suggesting that the ATPDase proteins in these two tissues are likely to be the same.

In comparing the chicken oviduct ecto-ATPDase and the gizzard ecto-ATPase, we noted several differences despite their similar molecular mass and sequence homology (42% amino acid identity and 58% amino acid similarity). 1) The chicken oviduct ecto-ATPDase has a significantly lower isoelectric point (pI), 6.37, than that of the chicken gizzard ecto-ATPase, 7.94. 2) There are 12 N-glycosylation sites in the oviduct ecto-ATPDase but only 3 N-glycosylation sites in the gizzard ecto-ATPase. This agrees well with the finding that the oviduct ecto-ATPDase is more highly glycosylated because the oviduct ecto-ATPDase is an 80-kDa protein, whereas the gizzard ecto-ATPase is a 66-kDa protein. 3) The potential tyrosine kinase phosphorylation site seen in the gizzard ATPase is also present in the oviduct sequence (amino acids 116–121 TPTYLG); how-
however, the potential protein kinase A phosphorylation site is missing in the oviduct ATPDase. Some of the differences may be related to the ability of the gizzard and other ecto-ATPases to oligomerize (16), whereas the ecto-ATPases appear not to have this propensity (32). However, none of these obvious differences offers an explanation of the inability of the chicken gizzard ecto-ATPase to hydrolyze ADP. This is analogous to the example of the two NTPases of T. gondii, which showed markedly different abilities in hydrolyzing NDP, but differ in only 16 amino acid residues throughout the sequence of a protein of more than 600 amino acid residues (42). An alternative explanation of the extensive sequence homology of proteins that have different substrate specificities is that the ADP hydrolysis activity of ecto-ATPases is not manifested under the standard assay conditions.

In summary, the sequence information of the chicken oviduct ecto-ATPase, which is both purified and cloned, provided better insight in the relationship of the several members of the ecto-ATPase family which have been cloned. There is an obvious need of a more detailed characterization of the enzymatic properties of these proteins, most of which have only been studied in terms of expression. This information together with mutagenesis and structural studies should ultimately be useful for defining the functions of the ecto-ATPases and ecto-ATPases in different systems.

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