The ecto-ATPase from chicken gizzard (smooth muscle) was solubilized, and the 66-kDa cell membrane ecto-ATPase protein was purified. The protein was then subjected to both enzymatic and chemical cleavage, and the resultant peptides were purified by reverse phase high pressure liquid chromatography and sequenced. Several of these internal peptide sequences were used to design oligonucleotides to screen a chicken muscle library to identify the cDNA encoding the ecto-ATPase. Two overlapping partial clones were sequenced, yielding the complete coding region and a long 3’-untranslated sequence. The deduced amino acid sequence is in agreement with the N-terminal and peptide sequences obtained from the purified protein. The chicken muscle ecto-ATPase is a slightly basic (predicted pI = 7.93) 494-amino acid protein (54.4 kDa), containing a single transmembrane domain at each end of the protein. The majority of the protein is predicted to be extracellular, making it a Type Ia plasma membrane protein. There are four putative N-glycosylation sites, a single potential cAMP/cGMP-dependent protein kinase phosphorylation site, as well as a single putative tyrosine kinase phosphorylation site. Analysis of the sequence using the BLAST programs demonstrated homology with other ecto-ATPases and ecto-apyrases, including those from the parasitic protozoan Toxoplasma gondii, potato tubers, and garden pea, as well as a guanosine diphosphohydrolase from yeast. However, the most striking homology observed was to the human and mouse lymphoid cell activation antigen 39 (CD39), a molecule now known to have apyrase activity. The chicken ecto-ATPase showed considerable amino acid sequence homology with CD39 over the entire length of the sequence, excluding about 30–40 amino acids at the extreme ends of the protein (which include the two membrane-spanning helices). The sequence homology between the gizzard ecto-ATPase and CD39 was confirmed by Western blots demonstrating immunoreactivity with monoclonal antibodies against the chicken ecto-ATPase and two commercially available monoclonal antibodies against the human CD39 protein. The results suggest that the muscle ecto-ATPase may be involved in cell adhesion, since the highly homologous CD39 protein is involved in homotypic adhesion of activated B lymphocytes.

Cell membrane ecto-ATPases are millimolar divalent cation-dependent, low specificity enzymes that hydrolyze all nucleoside triphosphates (NTPases). They are integral membrane glycoproteins that can be distinguished from ecto-apyrases (ecto-ATP Diposphohydrolases or ecto-ATPases) by their inability to hydrolyze ADP and other nucleoside diphosphates at rates that are more than ~1–2% that of their ATP hydrolysis rates. A recent review summarizes the properties and postulated functions of the ecto-ATPases and ecto-apyrases (1). From the results of work on the single-celled parasitic protozoan Toxoplasma gondii, it appears that the ecto-ATPase and ecto-apyrase enzymes from that source are closely related as judged by sequence analysis (2, 3). However, significant differences in enzymology and susceptibility to inactivation by detergents exist between ecto-ATPases and ecto-apyrases from a variety of species (for a review, see Ref. 1), suggesting that they may not be as closely related to each other as is suggested by the sequence homology of the T. gondii enzymes.

The physiological functions of the ecto-ATPases and ecto-apyrases are not known. However, many functions have been hypothesized, including roles in cellular adhesion, termination of purinergic signaling, and purine recycling (for a review, see Ref. 1), as well as secretion (4) and vesicle trafficking (5). The goal of this study was to clone and sequence the cDNA encoding the chicken muscle ecto-ATPase to gain information about the structure and physiological function of the whole class of ecto-ATPase enzymes by analysis of sequence homologies with proteins of known function. The sequence reported here represents the first vertebrate ecto-ATPase to be cloned and sequenced. (The rat liver cellular adhesion molecule of 105 kDa “ecto-ATPase” sequence (6) apparently does not encode the ATPase (7), and the rat liver enzyme is classified as an ecto-apyrase since it hydrolyzes ADP as well as ATP.) The results reported here suggest that at least one physiological function of the ecto-ATPase is involvement in the process of cell adhesion, since it is highly homologous with the lymphoid cell activation antigen (CD39), which is known to be involved in homotypic activated B-cell adhesion (8). This conclusion is consistent with several previous reports obtained from different species and tissues that suggested by indirect methods that the ecto-ATPase may be involved in cellular adhesion in rat liver (6),

1 The abbreviations used are: CD39, lymphoid cell activation antigen 39; BLAST, basic local alignment search tool; PAGE, polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography; ACR, apyrase conserved region.
chicken muscle (9, 10), and rat brain (11), as well as the recent finding that the CD39 protein, which is involved in homotypic lymphocyte adhesion (8), has ecto-apyrase activity (12).

EXPERIMENTAL PROCEDURES

Materials—Chicken membranes were isolated as described previously from gizzards obtained from a local slaughterhouse (13). Endoprotease-Lys-C and endoprotease-Glu-C were obtained from Pro-mega and Boehringer Mannheim, respectively. Cyanogen bromide was from Pierce, and the reverse phase HPLC columns were from Vydac. The chicken gizzard phage cDNA library was obtained from Clontech, and a chicken muscle lambda Zap® phage cDNA library and Epicurian Coli ultracompact bacteria were purchased from Stratagene. The GeneTrapper® cDNA positive selection kit was purchased from Life Technologies, Inc. Synthetic oligonucleotides were obtained from the DNA core facility at the University of Cincinnati. Sequenase version 2 kits were from U. S. Biochemical Corp. The 3’-oligo labeling system and the ECL detection reagents were from Amersham.

Internal Peptide Sequence Generation—The 66-kDa ecto-ATPase protein was purified either using a column chromatography scheme (13) or by immunoprecipitation with monoclonal antibody 6 (10). Using either methodology, a final step of preparative SDS-PAGE was employed to obtain a pure 66-kDa ecto-ATPase protein. The protein was then blotted onto a polyvinylidene difluoride membrane (14), the membrane was blocked, and the protein bound to the membrane was cleaved enzymatically with endoprotease-Glu-C, endoprotease-Lys-C, or a combination of the two enzymes as described (15, 16). Alternatively, the ecto-ATPase was subjected to CNBr cleavage subsequent to electrophoresis from the preparative SDS-PAGE gel and acetone precipitation. Approximately a 100-fold excess of CNBr over methionine residues was added, and cleavage was performed in 70% formic acid for 24 h at 22 °C and was light-protected and under N2.

Purification and Sequencing of Peptides—The soluble peptides resulting from the enzymatic and CNBr cleavage reactions were injected onto a reverse phase HPLC column and eluted using a gradient of acetonitrile with 0.1% trifluoroacetic acid as the ion pairing reagent, resulting from the enzymatic and CNBr cleavage reactions were injected from Pierce, and the reverse phase HPLC columns were from Vydac. The GeneTrapper® cDNA positive selection kit was purchased from Stratagene. The GeneTrapper® cDNA positive selection kit was purchased from Life Technologies, Inc. Synthetic oligonucleotides were obtained from the DNA core facility at the University of Cincinnati. Sequenase version 2 kits were from U. S. Biochemical Corp. The 3’-oligo labeling system and the ECL detection reagents were from Amersham.

RESULTS

Several attempts to isolate the chicken gizzard ecto-ATPase cDNA from the Clontech gizzard cDNA library using conventional phage plating and screening with various radioactively labeled degenerate oligonucleotide probes proved to be unsuccessful. However, the desired clones were obtained by using a chicken muscle lambda Zap® phage cDNA library. This phage library was mass-excised and converted into a plasmid cDNA library, which is the required starting material for the Gene-Trapper cDNA positive selection system kit. The GeneTrapper system then converts the double-stranded plasmid library into a single stranded library and enriches the cDNA encoding the ecto-ATPase by affinity purification using, in this case, a biotinylated degenerate probe designed from a cyanogen bromide peptide. (A chicken skeletal muscle library was used in the GeneTrapper system since no chicken gizzard (smooth muscle) cDNA library was commercially available that could be readily converted to the plasmid cDNA library required as the starting material for the GeneTrapper cDNA positive selection system.) A 2.1-kilobase pair clone (designated 19(2nd)), which hybridized to several degenerate probes designed from internal protein sequences of the 66-kDa ecto-ATPase, was sequenced. This clone was found to have approximately 750 bases of 3’-untranslated sequence and did not encode the known N-terminal sequence of the protein (13). However, the deduced amino acid sequence did agree with many internal peptide sequences obtained from the purified gizzard protein. A non-degenerate probe was designed from near the 5’-end of the first clone (19(2nd)), and after rescreening the same library in the same manner, a second overlapping partial clone was found (CS-Q, 0.83 kilobase pairs). Except for approximately 20 bases at the extreme 5’-end of clone 19(2nd), the sequences in the approximately 610-base pair region of overlap between the two clones were identical, and the deduced amino acid sequence of the overlapping cDNA sequence agreed with the sequences determined from the purified protein (see Fig. 1). There is one peptide sequence (the protein N terminus) encoded by the non-overlapping 5’-end of clone CS-Q, seven peptide sequences encoded in cDNA overlap sequence between the two partial clones, and six peptide sequences encoded by the non-overlapping coding region of clone 19(2nd) (see Fig. 1). Thus, there are a total of 14 peptide sequences (including the N terminus and the membrane-spanning region very close to the predicted C terminus of the protein) that agree with the protein sequence deduced from the cDNA sequence that was spliced together from the two partial clones.

The protein sequence deduced from the cDNA is a 494-amino acid protein with a calculated protein molecular mass of 54,402 Da, a pI of 7.93, and four potential N-glycosylation sites (Fig. 2). The protein sequence deduced from the cDNA was compared to the non-overlapping 5’-end of clone CS-Q, seven peptide sequences encoded in cDNA overlap sequence between the two partial clones, and six peptide sequences encoded by the non-overlapping coding region of clone 19(2nd) (see Fig. 1). Thus, there are a total of 14 peptide sequences (including the N terminus and the membrane-spanning region very close to the predicted C terminus of the protein) that agree with the protein sequence deduced from the cDNA sequence that was spliced together from the two partial clones.

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The Kozak consensus sequence (22) is indicated by double underlining of the DNA sequence, and the initiation methionine is marked. There is a single potential cAMP/cGMP phosphorylation site and a single potential tyrosine kinase phosphorylation site (see Fig. 1). The hydrophilicity plot analysis of the protein sequence is shown in Fig. 2, indicating the likelihood of the following membrane topology: a putative membrane-spanning region that

Fig. 1. The cDNA sequence and the deduced amino acid sequence of the chicken muscle ecto-ATPase. The Kozak consensus sequence (22) is indicated by double underlining of the DNA sequence, and the initiation methionine is marked with a line through the letter M. Peptide sequences determined from cleavage of the purified protein are underlined. Potential N-glycosylation sites are indicated by asterisks (*). The potential cAMP/cGMP phosphorylation site (a) and the potential tyrosine kinase phosphorylation site (1) are also labeled. The position of the termination codon is also indicated (–*–). Clone CS-Q consists of bases 1–829, and clone 19(2nd) extends from base 189 to base 2352 (2164 bases).
appears to be an uncleaved signal sequence at the N terminus, a large extracellular loop containing approximately 85% of the total protein as well as all of the carbohydrates and including the active site(s), and a putative membrane-spanning region very close to the C terminus. Thus, the ecto-ATPase has the properties of a Type Ia membrane protein. Only approximately 10 amino acids are predicted to be intracellular. Also shown in Fig. 2 are the “antigenic index” and the “surface probability” as predicted by the Mac Vector computer program. These analyses reveal several potential antigenic “hot spots,” suggesting possible regions containing the epitopes recognized by existing antibodies (10, 19), as well as indicating sequences that may be useful for the design of anti-peptide antibodies.

The deduced protein sequence shows homology with several known ecto-ATPases and aryls, as shown in Fig. 3. The greatest amino acid homology is with mouse CD39, which is homologous to the entire ecto-ATPase amino acid sequence with the exception of the N- and C-terminal putative membrane-spanning regions (Figs. 3 and 4).

The close relationship of the chicken muscle ecto-ATPase with the human CD39 protein suggested by the sequence analysis was confirmed by demonstrating immunocross-reactivity of a gizzard ecto-ATPase affinity-purified polyclonal antibody (19), a gizzard ecto-ATPase monoclonal antibody (6 (10)), and two commercially available anti-human CD39 monoclonal antibodies using two mammalian species (pig coronary artery tissue and mouse 7gg7 (B-cell hybridoma) cells), as seen in Fig. 5.

**DISCUSSION**

The GeneTrapper “affinity enrichment” technique of screening a cDNA library proved successful in isolating chicken ecto-ATPase clones after several failed attempts using conventional phage plating and screening techniques. Two overlapping partial clones were isolated that encode the entire protein sequence, as judged by the agreement of both clones with many peptide sequences obtained from the gizzard ecto-ATPase purified protein (Fig. 1). The protein encoded by the cDNA sequenced is consistent with the known molecular mass of the isolated protein (54.4 kDa for the deduced sequence versus 53 kDa estimated by SDS-PAGE for the purified protein (13)). The initiation methionine occurs directly before the N-terminal sequence of the mature protein and is preceded by a Kozak consensus sequence for initiation in higher eukaryotes (GC-CGCC(A/G)CCATGG (22)). The slightly basic pI (7.93) predicted is also consistent with the behavior of the ecto-ATPases on ion exchange columns (13, 23) and with the assertion that much of the net negative charge on the ecto-ATPase at physiological pH is due to sialic residues on the glycan chains (24).

The presence of four putative glycosylation sites is consistent with our unpublished observation of at least four N-linked glycan chains as demonstrated by quantum decreases in molecular mass on SDS-PAGE during time course studies of deglycosylation of the purified enzyme by peptide N-glycosidase-F. The topological prediction of only approximately 10 amino acids of the ecto-ATPase being located intracellularly suggests...
that it is unlikely that the ecto-ATPase is modulated by intracellular proteins or involved in signal transduction pathways mediated by direct interactions of ecto-ATPase with intracellular proteins.

As is strongly suggested by the hydrophilicity analysis and the fact that the chicken ecto-ATPase is indeed an ecto enzyme (9, 24, 25), the majority of the protein (including the active site) is located on the exterior of the cell membrane. This is confirmed by the location of the putative N-glycosylation sites, at least some of which must be glycosylated to account for purification (13) and lectin modulation of activity data (9, 13, 26). It seems likely that most of the cyst(e)ine residues in the large extracellular loop (11 or 12 cyst(e)ine residues depending on exactly where the N-terminal membrane-spanning sequence exits the membrane) are disulfide-bonded, since 1) they are exposed to the oxidizing extracellular media, 2) cysteine-selective chemical modification reagents are not inhibitors of chicken gizzard ecto-ATPase activity, and 3) dithiothreitol has a significant inhibitory effect on activity even though there are no intermolecular disulfide bonds in the chicken ecto-ATPase (19). The presence of the intramolecular disulfide bonds, as well as the glycosylation, would likely increase the stability of the protein and probably contribute significantly to the resistance to proteases that is characteristic of the ecto-ATPases. The N-terminal sequence has the properties of an uncleaved signal sequence, and the assignment of membrane-spanning regions (and therefore membrane topology), appears to be straightforward. A linear model of the ecto-ATPase consistent with the sequence-derived information described above is presented in Fig. 6.

The chicken muscle ecto-ATPase is homologous to ecto-ATPases and ecto-apyrases from a variety of plants, lower organisms, and mammals (Fig. 3). The deduced amino acid sequence contains the proposed apyrase-conserved regions (ACR1–ACR4 (27)). ACR1 and ACR4 sequences are similar to the actin-hap70-hexokinase β- and γ-phosphate binding motifs (27), but
no Walker consensus ATP binding motif sequences (28) are present. The only ecto-ATPase/apyrase cloned and sequenced that the deduced chicken muscle ecto-ATPase amino acid sequence does not share homology with is the mosquito saliva apyrase (29). The mosquito apyrase also does not have homology with other enzymes in the ecto-ATPase/apyrase family derived from many diverse species (27), suggesting that either the mosquito enzyme is unrelated to the others or that the mosquito clone sequenced does not represent the mosquito apyrase protein.

The chicken muscle ecto-ATPase-deduced amino acid sequence is most homologous with the lymphoid cell activation antigen CD39 (Figs. 3 and 4). The homology encompasses the entire extracellular domain of the chicken ecto-ATPase, accounting for approximately 85% of the protein. CD39 is involved in homotypic activated lymphocyte cell adhesion (8). Thus, this suggests that the physiological function of the ecto-ATPase is related to that of CD39-cell adhesion. This is consistent with our finding that a monoclonal antibody that recognizes integrin on Western blots is capable of immunoprecipitating gizzard ecto-ATPase (10), since integrin is a ligand for other known cell adhesion molecules. Also, there are several reports in the literature that the ecto-ATPases and ecto-apyrases from various sources are either associated with or identical to adhesion molecules (6, 9, 11). The hypothesis supported by the body of work generated by this laboratory over the last few years (10, 13, 19, 23, 30, 31) is that the ecto-ATPase is not an adhesion molecule itself but is in some (yet to be understood) way critical to cell adhesion or to the regulation of the adhesive process. This regulation may be by dephosphorylation of proteins phosphorylated by ecto-protein kinases or, more likely, by control of the concentrations of extracellular nucleotides serving as triggers for cell adhesion. Human ecto-apyrase (CD39) present in vascular endothelial cells has been speculated to serve such a function in the bloodstream, i.e. to keep the levels of ADP low during normal conditions so that platelet aggregation is not induced, which would lead to pathologies associated with vascular clot formation.

Related to this is the recent finding that inhibition of the vascular ecto-ATPase/apyrase by free radical-induced oxidative damage is thought to be involved in triggering the clotting responsible for the acute cessation of blood supply to xerografts, resulting in death of the transplanted tissue (32). However, it seems unlikely that the chicken muscle ecto-ATPase described in this work could fulfill such a function in the circulatory system since the true ecto-ATPases, unlike the ecto-apyrases, do not hydrolyze ADP, and ADP is the nucleotide most important for the maintenance of circulatory homeostasis.

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REFERENCES

1. Plemer, L. (1995) Rev. Cytol. 158, 141–214
2. Asai, T., Miura, S., Sibley, L. D., Okabayashi, H., and Takeuchi, T. (1995) J. Biol. Chem. 270, 11391–11397
3. Born, D., Perk, R. H., Afflit, M. A., Becker, C. J. M., and Joiner, K. A. (1994) J. Biol. Chem. 269, 29252–29260
4. Strobel, R., and Rosenberg, M. (1993) Annu. Rev. Physiol. 57, 487–489
5. Kittel, A., and Bacey, E. (1994) Cell. Biol. Int. Rep. 18, 875–880
6. Lin, S.-H., and Guidotti, G. (1989) J. Biol. Chem. 264, 14408–14414
7. Knowles, A. F. (1995) Biochim. Biophys. Res. Commun. 207, 529–535
8. Kania, G. S., Wood, G. S., and Tedder, T. F. (1991) J. Immunol. 146, 2235–2244
9. Cunningham, H. B., Yarazi, P. J., Domingo, R. C., Oades, K. V., Bohlen, H., Sabbadini, R. A., and Dahms, A. S. (1993) Arch. Biochem. Biophys. 303, 32–43
10. Stout, J. G., Strobel, R. S., and Kirley, T. L. (1995) J. Biol. Chem. 270, 11845–11850
11. Dahanazhugazyan, K., and Bock, E. (1993) FEBS Lett. 336, 279–283
12. Wang, T. F., and Guidotti, G. (1996) J. Biol. Chem. 271, 9898–9901
13. Stout, J. G., and Kirley, T. L. (1994) J. Biochem. Biophys. Methods 29, 61–75
14. Matsuura, F. (1987) J. Biol. Chem. 262, 10035–10038
15. Fernandez, J., DeMott, M., Atherton, D., and Mische, S. M. (1992) Anal. Biochem. 201, 255–264
16. Fernandez, J., Andrews, L., and Mische, S. M. (1994) Anal. Biochem. 218, 112–117
17. Kirley, T. L., Wallick, E. T., and Lane, L. K. (1984) Biochim. Biophys. Res. Commun. 125, 767–773
18. Lennmli, U. K. (1970) Nature 227, 680–685
19. Stout, J. G., and Kirley, T. L. (1996) Biochemistry 35, 8289–8298
20. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
21. Wolery, K. C., Wiese, B. A., and Smith, R. F. (1995) Genome Res. 5, 173–184
22. Kazak, M. (1987) Nucleic Acids Res. 15, 8125–8132
23. Stout, J. G., Brittsan, A., and Kirley, T. L. (1994) Biochim. Biophys. Int. 33, 1091–1098
24. Treuehit, M. J., Vaghy, P. L., and Kirley, T. L. (1992) J. Biol. Chem. 267, 17777–17782
25. Saberido, A., Moro, G., and Megias, A. (1991) J. Biol. Chem. 266, 23490–23498
26. Moulton, M. P., Sabbadini, R. A., Norton, K. C., and Dahms, A. S. (1986) J. Biol. Chem. 261, 12244–12251
27. Handa, M., and Guidotti, G. (1996) Biochem. Biophys. Res. Commun. 218, 916–923
28. Walker, J. E., Saraste, M., Runswick, M. J., and Gay, N. J. (1982) EMBO J. 1, 945–951
29. Champagne, D. E., Smartt, C. T., Ribeiro, J. M., and James, A. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 694–698
30. Stout, J. G., and Kirley, T. L. (1994) Biochem. Mol. Biol. Int. 32, 745–753
31. Stout, J. G., and Kirley, T. L. (1995) Biochem. Mol. Biol. Int. 36, 927–934
32. Robson, S. C., Candinas, D., Siegel, J. B., Kopf, C., Millan, M., Hancock, W. W., and Bach, F. H. (1996) Transpl. Proc. 28, 536