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Cloning, sequencing, and expression of a human brain ecto-ATPase related to both the ecto-ATPases and CD39 ecto-ATPases¹

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Abstract

An extracellular ATPase (E-type ATPase) clone was isolated from a human brain cDNA library and sequenced. The transcript shows similarity to the previously published chicken smooth muscle and rat brain ecto-ATPase cDNAs, human CD39L1 cDNA (putative human ecto-ATPase), and mammalian CD39 (lymphoid cell activation antigen, ecto-ATPase, ATPase, ATP-diphosphohydrolase) cDNAs. The full-length human brain cDNA encodes a 529 amino acid glycoprotein with a putative membrane spanning region near each terminus, with the majority of the protein found extracellularly. Expression of this clone in mammalian COS-1 cells yielded NaN₃-sensitive ATPase and ADPase activity detectable both on intact cells and cell membrane preparations. The nucleotide

hydrolysis ratio of the expressed protein is approx. 2.75:1 (ATPase:ADPase activity), classifying it as an ecto-apyrase. However, this hydrolysis ratio is intermediate between that observed for the ecto-ATPases and the CD39 ecto-apyrases (L. Plesner, *Int. Rev. Cytol.* 158 (1995) 141–214). Quantitative analyses of amino acid identities and similarities between this ecto-apyrase and other vertebrate E-type ATPases suggest that this human brain enzyme is nearly equally related to the ecto-ATPases and the CD39s, and phylogenetic analysis suggests that it could be an ancestral enzyme from which both ecto-ATPases and CD39 ecto-apyrases are derived.



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Keywords

Ecto-ATPase; Ecto-apyrase; CD39; Brain; Extracellular nucleotide; E-type ATPase

Abbreviations

ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; BLAST, basic local alignment search tool; cDNA, complementary DNA; CD39, lymphoid cell activation antigen (ecto-apyrase); ECL, enhanced chemiluminescence; ecto-ATPDase, ecto-ATP diphosphohydrolase (ecto-apyrase); EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycolbis(2-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; HB6, human brain E-type ATPase clone; MOPS, 3-(*N*-morpholino)propanesulfonic acid

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- 1 The nucleotide sequence reported in this paper has been submitted to the GenBank database and assigned Accession No. AF034840.

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