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Life Sciences 73 (2003) 2071–2082
Life Sciences
www.elsevier.com/locate/lifescie

ATP and ADP hydrolysis in brain membranes of zebrafish (*Danio rerio*)

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Received 6 January 2003; accepted 3 April 2003

Abstract

Nucleotides, e.g. ATP and ADP, are important signaling molecules, which elicit several biological responses. The degradation of nucleotides is catalyzed by a family of enzymes called NTPDases (nucleoside triphosphate diphosphohydrolases). The present study reports the enzymatic properties of a NTPDase (CD39, apyrase, ATP diphosphohydrolase) in brain membranes of zebrafish (*Danio rerio*). This enzyme was cation-dependent, with a maximal rate for ATP and ADP hydrolysis in a pH range of 7.5–8.0 in the presence of Ca^{2+} (5 mM). The enzyme displayed a maximal activity for ATP and ADP hydrolysis at 37 °C. It was able to hydrolyze purine and pyrimidine nucleosides 5′-di and triphosphates, being insensitive to classical ATPase inhibitors, such as ouabain (1 mM), N-ethylmaleimide (0.1 mM), orthovanadate (0.1 mM) and sodium azide (0.1 mM). A significant inhibition of ATP and ADP hydrolysis (68% and 34%, respectively) was observed in the presence of 20 mM sodium azide, used as a possible inhibitor of ATP diphosphohydrolase. Levamisole (1 mM) and tetramisole (1 mM), specific inhibitors of alkaline phosphatase and P1, P^5-di (adenosine 5′-) pentaphosphate, an inhibitor of adenylate kinase did not alter the enzyme activity. The presence of a NTPDase in brain membranes of zebrafish may be important for the modulation of nucleotide and nucleoside levels, controlling their actions on specific purinoceptors in central nervous system of this specie.

Keywords: Zebrafish; Adenosine; Extracellular ATP; NTPDase; Nucleotidases

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doi:10.1016/S0024-3205(03)00596-4

Introduction

Zebrafish is a small freshwater teleost belonging to Cyprinidae family. It has a number of features which make it attractive as a laboratory animal model such as, low cost and space requirement for maintenance, rapid generation cycle (egg to mature adult in 2–3 months), translucent embryos well suited for experimental manipulation and microinjection (Lele and Krone, 1996). For these characteristics, the zebrafish is a model system in studies involving developmental biology, toxicology, transgenic research, vertebrate genome evolution, teratology and neuroscience (Lele and Krone, 1996; Vascotto et al., 1997; Ivetac et al., 2000). The majority of its genes is already known, and its genome has a syntenic relationship with the human genome (Barbazuk et al., 2000; Woods et al., 2000). Furthermore, zebrafish may be an ideal vertebrate model system for numerous human diseases, where genetic and biological mechanisms of the diseases may be studied (Dodd et al., 2000; Gerlai et al., 2000; Ivetac et al., 2000).

Since the purinergic neurotrasmission was proposed (Burnstock, 1972), there has been growing interest in its role in synaptic transmission. ATP is stored in nerve terminals and co-released at synaptic cleft with several neurotransmitters, including acetylcholine, noradrenaline and serotonin (Di Iorio et al., 1998; Burnstock, 1999; Rathbone et al., 1999). Extracelular ATP evokes responses through two general classes of extracellular receptors, the ionotropic P2X receptors and the metabotropic P2Y (Ralevic and Burnstock, 1998). However, few studies about purinergic neurotransmission were conducted in zebrafish. Some studies characterized the presence of an ionotropic P2X3 purinoceptor in this specie (Boué-Grabot et al., 2000; Egan et al., 2000; Norton et al., 2000). Recently, the cloning and characterization of two P2X receptor subunits genes from the zebrafish were conducted. It was suggested that one cDNA encodes an orthologue of the mammalian P2X(4) subunit and the second cDNA encodes the orthologue of the mammalian P2X(5) subunit (Diaz-Hernandez et al., 2002).

After exert its actions, the neurotransmitter ATP can be inactivated through hydrolysis by enzymes known as ectonucleotidases. In the last years, several studies have demonstrated that members of several families of nucleotidases can contribute to the hydrolysis of nucleotides (for review, see Zimmermann, 2001). Nucleoside 5′-tri and diphosphates may be hydrolyzed by members of the NTPDase family (nucleoside triphosphate diphosphohydrolase family), whereas nucleosides 5′-monophosphates are mainly subjected to hydrolysis by ecto-5′-nucleotidase (Zimmermann and Braun, 1999; Zimmermann, 2001), producing the respective nucleoside. Therefore, the final product of ATP degradation promoted by the nucleotidase pathway is adenosine, an important neuromodulator that acts as agonist in a group of receptors named adenosine A_1, A_{2A}, A_{2B} and A_3 receptors (Cunha, 2001; Dunwiddie and Masino, 2001; Fredholm et al., 2001). These enzymes may play an important role in the modulation of the nucleotide and nucleoside levels, controlling the activation and the availability of the ligands for purinoceptors (Zimmermann et al., 1998; Bonan et al., 2001). Several studies have demonstrated the presence of ectonucleotidases in other species of fishes, such as *Torpedo marmorata*, *Carassius auratus* L. and *Rhandia quelen* (Sarkis and Saltó, 1991; Schwarzbaum et al., 1998; Schetinger et al., 2001; Alleva et al., 2002). Considering the presence of purinoceptors in zebrafish and the importance of nucleotidases in the control of ligands for these receptors, the objective of the present study was to perform the enzymatic characterization of a NTPDase activity in brain membranes of this vertebrate.

Material and methods

Experimental animals

Zebrafish (*Danio rerio*) were obtained from commercial suppliers and were kept in 50 L aquarium at 25 ± 5 °C for at least 7 days before the experiments.

Brain membranes

The brain membranes were prepared as described by Barnes et al. (1993). Briefly, whole zebrafish brains were homogenized in 60 volumes (v/w) of chilled Tris-citrate buffer (50 mM Tris-citrate, 2 mM EDTA, 2 mM EGTA, pH 7.4, adjusted with citric acid) in a motor driven Teflon-glass homogenizer. The homogenate was centrifuged at 1000×*g* for 10 min and the pellet was discarded. After removing the nuclear and cell debris, the supernatant was centrifuged for 25 min at 40,000×*g*. The resultant pellet was frozen in liquid nitrogen, thawed, resuspended in Tris-citrate buffer, and recentrifuged for 20 min at 40,000×*g*. This fresh-twaw-wash procedure was used to ensure lysis of the membranes. The final pellet, containing a mixture of intracellular and extracellular brain membranes, was resuspended and used in the enzyme assays. However, it is possible to exclude the presence of intracellular ATPases, since classical inhibitors of these enzymes did not alter ATP and ADP hydrolysis in this fraction. The material was maintained at 2–4 °C throughout preparation.

Enzyme assays

After membrane preparation, the optimum conditions for nucleotide hydrolysis were determined. Brain membranes of zebrafish (3–5 μg protein) were added to the reaction mixture containing 50 mM Tris-HCl (pH 8.0) and 5 mM $CaCl_2$ or $MgCl_2$ (or other cation as indicated) in a final volume of 200 μl. The samples were preincubated for 10 min at 37 °C. The reaction was initiated by the addition of substrate (ATP, ADP or other, as indicated) to a final concentration of 1 mM and stopped by adding 200 μl 10% trichloroacetic acid. The samples were chilled on ice for 10 min before assaying for the release of inorganic phosphate (Pi) (Chan et al., 1986). Incubation times and protein concentrations were chosen in order to ensure the linearity of the reactions. Controls with the addition of the enzyme preparation after mixing with trichloroacetic acid were used to correct non-enzymatic hydrolysis of substrates. Specific activity is expressed as nmol of Pi released.min^{-1}.mg of $protein^{-1}$. All enzyme assays were run in triplicate.

Protein determination

Protein was determined by Coomassie Blue method using bovine serum albumin as a standard (Bradford, 1976).

Statistical analysis

Data were analyzed by Student's t-test or one-way analysis of variance (ANOVA), followed by a Duncan multiple range test, considering a level of significance of 5%.

Results

Previous experiments demonstrated that Ca^{2+}-ATP and Ca^{2+}-ADP hydrolysis in brain membranes of zebrafish increased as a function of protein concentration. The product formation was linear in the range of 3–15 μg protein in the incubation medium. The time course for both substrates was linear up to 30 min in the presence of Ca^{2+} (data not shown).

Like other NTPDases, this enzyme was demonstrated to be divalent cation-dependent (Zimmermann, 2001) and its sensitivity to Ca^{2+}, Mg^{2+} and Zn^{2+} is shown in the Fig. 1. The patterns for ATPase and ADPase activities in brain membranes of zebrafish in the presence of the three divalent cations differed: (i) ATPase and ADPase activities in the presence of Ca^{2+} were dose-dependent, reaching a plateau at 5 mM, being the better activator for ADP hydrolysis (Fig. 1A and B); (ii) ATPase activity exhibited the greatest activation in the presence of 1 mM Zn^{2+} (Fig. 1A). Although Zn^{2+} was able to promote a stimulatory effect on ADPase activity, this cation was not the best activator of ADP hydrolysis (Fig. 1B).

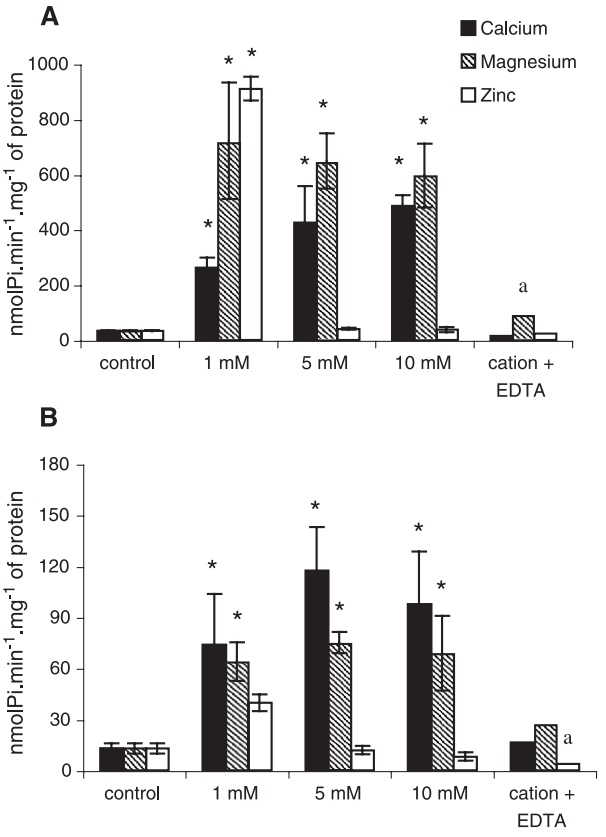

Fig. 1. Effect of different concentrations of $CaCl_2$, $MgCl_2$ and $ZnCl_2$ on ATP (A) and ADP (B) hydrolysis in brain membranes of zebrafish. Enzyme assays were carried out as described in Material and Methods. Control group was incubated without addition of cation. Cation + EDTA group was incubated in the presence of 1 mM $ZnCl_2$, 5 mM $CaCl_2$ or 5 mM $MgCl_2$ plus 5 mM EDTA. Data represent means ± S.D. of four different experiments, each in triplicate. * indicates difference when compared to the control and cation + EDTA groups ($P < 0.05$). [a] indicates difference when compared to the control group ($P < 0.05$).

(iii) ATPase and ADPase activities were significantly activated by Mg^{2+}, but this effect was not concentration-dependent (Fig. 1A and B). Cation dependency was confirmed by a dramatic decrease in ATP and ADP hydrolysis in the absence of cation added or in the presence of Ca^{2+}, Mg^{2+} or Zn^{2+} plus 5 mM EDTA (Fig. 1). Considering these results, a concentration of 5 mM Ca^{2+} was selected for subsequent enzyme assays.

In order to evaluate the optimum pH for the enzyme activities, Ca^{2+}-ATP and Ca^{2+}-ADP were used as substrates in a medium containing 50 mM Tris-histidine (pH 6.0, 7.0, 7.2, 7.5, 8.0, 9.0) (Fig. 2). The maximal rate for ATP and ADP hydrolysis was observed at pH 8.0, similar to others NTPDases described in the literature (Plesner, 1995; Schetinger et al., 2001). Ca^{2+}-ATP and Ca^{2+}-ADP hydrolysis were also assayed at different temperatures (10, 20, 37 and 45 °C). In the temperatures tested, the results showed that the enzyme displayed the highest activity at 37 °C for both substrates.

Ca^{2+}-ATP and Ca^{2+}-ADP hydrolysis were determined at substrate concentrations in the range of 100–2000 μM. The enzyme activity increased with increasing concentrations of the nucleotide (fixed Ca^{2+} at 5 mM with variable concentrations of nucleotides). K(m) (Michaelis constant) and V_{max} (maximum velocity) values for Ca^{2+}-ATP and Ca^{2+}-ADP hydrolysis in brain membranes of zebrafish were estimated from the Lineweaver-Burk plots with at least five different enzyme preparations. The apparent K(m) values for Ca^{2+}-ATP and Ca^{2+}-ADP were 183 ± 16,8 μM and 96,5 ± 6,5 μM, respectively.

V_{max} values for Ca^{2+}-ATP and Ca^{2+}-ADP were 703 ± 186 and 127 ± 30,7 nmol Pi min^{-1} mg of $protein^{-1}$ for ATP and ADP, respectively (Fig. 3).

To investigate if the ATP and ADP hydrolysis occurs by the presence of a single enzyme, membrane preparations were incubated in the standard reaction medium containing 1 mM ATP, 1 mM ADP or 1 mM ATP + 1 mM ADP at the same time (Sarkis and Saltó, 1991). In the presence of 1 mM ATP + 1 mM ADP, the specific activity observed was 347,4 ± 89 nmol Pi. min^{-1}. mg of $protein^{-1}$, close to the arithmetic mean of the activities obtained with each individual nucleotide (563,6 ± 154,1 and 140,7 ± 21,4 nmol Pi. min^{-1}.mg of $protein^{-1}$ for ATP and ADP, respectively). This kinetic behavior indicates that a single active site is involved in the hydrolysis of both substrates. If two enzymes coexisted in the preparation, the rate of the mixed-substrate should to be sum of the values obtained for the individual substrates. To assure this result, we assay the combination of substrate concentrations in a Chevillard plot, a competition plot (Chevillard et al., 1993). Series of mixtures containing ATP and ADP were

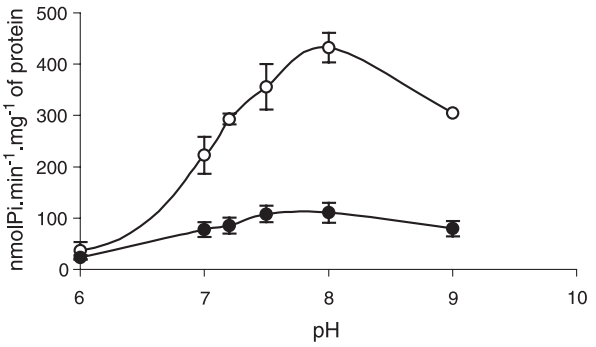

Fig. 2. pH dependence of Ca^{2+}-ATP (○) and Ca^{2+}-ADP (●) hydrolysis in brain membranes of zebrafish. Enzyme activity was determined as described in Material and Methods using the following buffers: 50 mM Tris-Histidine, pH 6.0, 7.0, 7.2, 7.5, 8.0 and 9.0. Data represent means ± S.D. of four different experiments, each in triplicate.

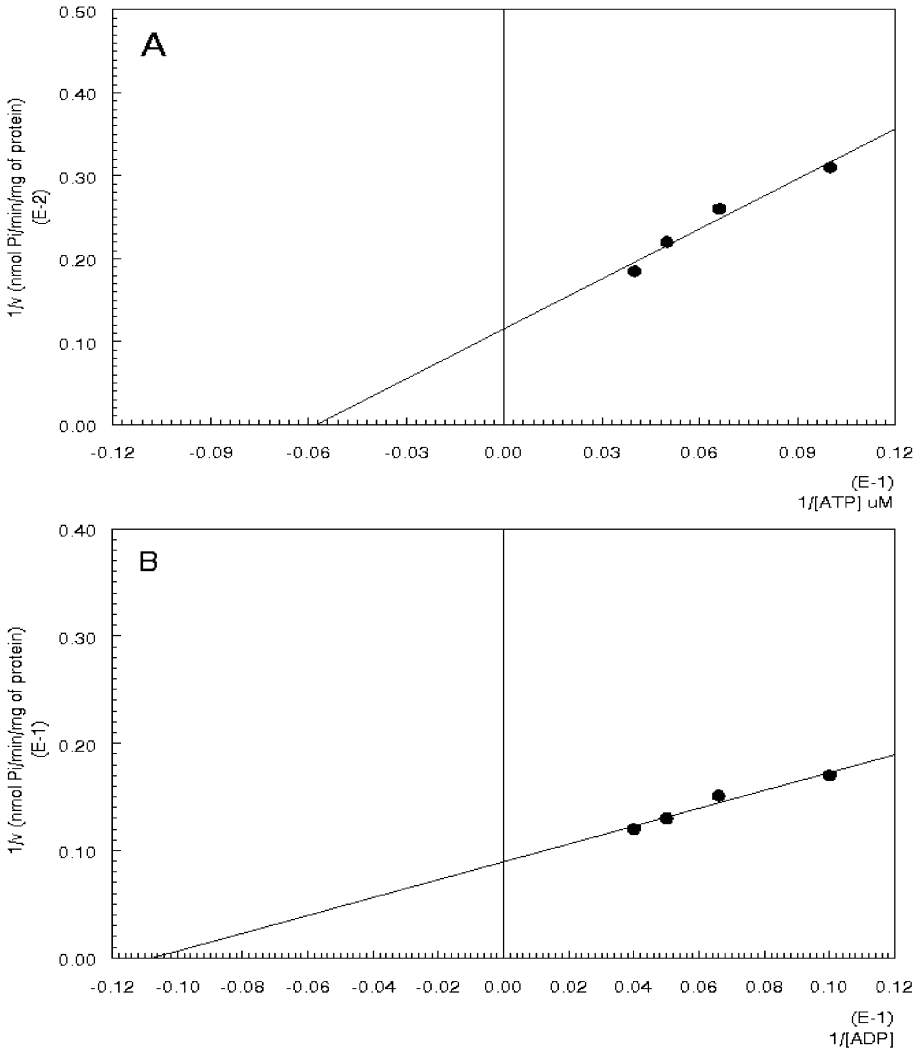

Fig. 3. Lineweaver-Burk plots for NTPDase activity. Brain membranes of zebrafish (3–5 μg protein) were incubated at 37 °C. The reaction medium and enzyme assays are described in Material and Methods. Results for plots were obtained with concentrations of ATP (A) and ADP (B) in the range of 0.1–0.25 mM. All experiments were repeated at least five times and similar results were obtained. Data presented were obtained from a representative experiment.

prepared. To assay the combination of substrate concentrations in a Chevillard plot we chose a concentration at which the rate of hydrolysis was the same when either ATP or ADP was used as substrate (Fig. 4). The *P* values ranged from 1 to 0. The horizontal straight line obtained in the competition plot indicates a constant hydrolysis rate at all substrate combinations tested. The interpretation is that ATP and ADP hydrolysis corresponds to the same active site of a single enzyme. For each point (two substrates) we chose incubation times to ensure the linearity of the reaction (Fig. 4).

NTPDases have been described as enzymes with a broad substrate specificity (Sarkis and Saltó, 1991; Pilla et al., 1996; Anderson and Parkinson, 1997; Caldwell et al., 1999; Zimmermann, 2001). Therefore,

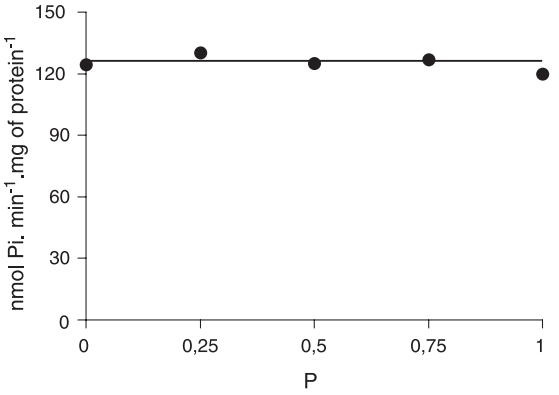

Fig. 4. Competition plot. Substrate A (ATP) at $P = 0$ was 0.04 mM. Substrate B (ADP) at $P = 1$ was 0.5 mM. Assay conditions are described in Material and Methods. Data represent a typical experiment run in triplicate.

the ability of the enzyme to hydrolyze others di-and triphosphate nucleosides was also investigated (Table 1). ATP was the best substrate and ADP was the second most hydrolyzed nucleotide (33,7% when compared to ATP). All others di-and triphosphate nucleosides tested were hydrolyzed at a similar rate by brain membranes of zebrafish (Table 1). The presence of 5′-nucleotidase and pyrophosphatase activities can be excluded because there are no significant hydrolysis of AMP and PPi in these assay conditions (Table 1). However, preliminary results obtained from our laboratory have demonstrated that Mg^{2+}-AMP hydrolysis in brain membranes of zebrafish increased as a function of protein concentration. The product formation was linear in the range of 5-15 μg protein in the incubation medium containing of 5 mM $MgCl_2$, pH 7.5, and 1 mM AMP at 37 °C. The time course for AMP hydrolysis was linear up to 60 min in the presence of Mg^{2+}. In the presence of 5 mM of $CaCl_2$, AMP hydrolysis was 8,3 ± 1,3 nmol Pi. min^{-1}. mg of $protein^{-1}$ of protein. However, $MgCl_2$ was a better activator, since AMP hydrolysis in the presence of 5 mM $MgCl_2$ corresponds to 14,5 nmol Pi. min^{-1}.mg of $protein^{-1}$ of protein. Further studies will be required to conclude that the enzyme that promotes AMP hydrolysis in brain membranes of zebrafish is an ecto-5′-nucleotidase.

Table 1
Substrate specificity of NTPDase activity in brain membranes of zebrafish

Substrate	% Control activity
ATP	100 ± 8,85
GTP	30,4 ± 4,70
CTP	29,7 ± 6,26
UTP	33,3 ± 3,45
ADP	33,7 ± 1,08
GDP	16,4 ± 3,55
UDP	21,9 ± 4,79
AMP	1,65 ± 0,33
PPi	6,0 ± 0,69

Data represent means ± S.D. of at least three experiments. Control ATPase and ADPase activities were 507,73 ± 63,36 nmol Pi.min^{-1}.mg $protein^{-1}$ and 158,06 ± 10,28 nmol Pi.min^{-1}.mg $protein^{-1}$, respectively. Results are expressed as percentage of control activity (ATP hydrolysis - 100%).

Table 2
Effects of inhibitors on ATP and ADP hydrolysis in brain membranes of zebrafish

Inhibitor	Concentration (mM)	ATPase (% of control activity)	ADPase (% of control activity)
Ouabain	1	104 ± 20	101 ± 24
Orthovanadate	0.1	104 ± 1	79 ± 23
Levamisole	1	78 ± 12	88 ± 19
Tetramisole	1	93 ± 13	89 ± 16
NEM	0.1	90 ± 19	100 ± 25
Ap5A	0.01	99 ± 17	85 ± 8
Sodium azide	0.1	84 ± 2	108 ± 39
	20	32 ± 6*	66 ± 9*

Control Ca^{2+}-ATPase and Ca^{2+}-ADPase activities were 510,7 ± 53,4 nmol $Pi.min^{-1}mg^{-1}$ protein and 160,1 ± 11 nmol $Pi.min^{-1}mg$ $protein^{-1}$, respectively. Results are expressed as percentage of control activities (100%). Data represent the means ± S.D. for at least four different experiments and were analyzed statistically by one-way ANOVA or by paired sample *t*-test.

*Significant difference from control activity by one-way ANOVA ($P < 0.05$).

To evaluate the correlation between the enzyme activity described in this study with ATPases and alkaline phosphatase, inhibitors of these enzymes were tested. The following inhibitors had no effect upon ATP and ADP hydrolysis in brain membranes of zebrafish (Table 2): (a) the Na^+, K^+-ATPase inhibitor, ouabain; (b) orthovanadate, an inhibitor of transport ATPases, acid phosphatases and phosphotyrosine phosphatases; (c) N-ethylmaleimide (NEM), a compound that modifies sulphydril groups; (d) levamisole and tetramisole, inhibitors of alkaline phosphatase and unespecific phosphatases; (e) P^1, P^5-di(adenosine 5′-)pentaphosphate (Ap_5A), an inhibitor of adenylate kinase; (f) sodium azide, an inhibitor of the mithocondrial ATPase (I_{50} = 0.04 mM, a drug concentration which produce 50% inhibition) (Pullman et al., 1960). However, sodium azide, at 5–20 mM, is considered a possible inhibitor of ATP diphosphohydrolases from several sources (Plesner, 1995; Knowles and Nagy, 1999). Table 2 shows a significant inhibition of ATP and ADP hydrolysis in the presence of 20 mM sodium azide (68% and 34% inhibition, respectively) in brain membranes of zebrafish.

Discussion

The results of the present study demonstrate a NTPDase activity in brain membranes of zebrafish. This enzyme was divalent cation-dependent and presents parallel behavior when ATP and ADP are used as substrates. $CaCl_2$, at 5 mM, was used in the experiments of this study in order to exclude the significant interference of the Mg^{2+}-dependent enzymes, such as Na^+, K^+-ATPase and adenylate kinase. Despite the stimulatory effect produced by 1 mM Zn^{2+}, we did not use this cation due the contrasting effects produced on ATPases activities as described in the literature. Zinc presents opposite effects on ATPases, activating or inhibiting these enzyme activities (Gandhi and Ross, 1988; Sarkis et al., 1995). Caldwell et al. (1999) observed an inhibitory effect on ATP hydrolysis from chicken gizzard smooth muscle and liver in the presence of 5–10 mM Zn^{2+}. Other investigators have been concerned with this effect and showed zinc as a non-specific inhibitor of Na^+, K^+-ATPase from rat brain (Shafiq-ur-Rehman, 1986). These interesting effects of zinc could be

explored in future studies, since a better understanding of the metal actions can be a useful tool for toxicological studies.

The insensitivity to classical inhibitors of ATPases and the ability to hydrolyze nucleoside 5′-triphosphates and diphosphates are characteristics of E-NTPDases family (Zimmermann, 2001). There were no significant changes on ATP and ADP hydrolysis in the presence of classical inhibitors of intracellular ATPases, such as P-type, F-type, V-type ATPases and alkaline phosphatase. The influence of a Na^+, K^+-ATPase activity was excluded because neither ouabain nor orthovanadate inhibited ATP and ADP hydrolysis in brain membranes of zebrafish. The possibility that ADP hydrolysis occurs by a prior conversion of ATP, catalyzed by adenylate kinase and later hydrolysis by an ATP-specific enzyme, should be ruled out since this enzyme combination could mimic NTPDase activity (Sarkis et al., 1995). The influence of a contaminating adenylate kinase on ADP hydrolysis in our assay condition was also excluded since 10 μM P^1, P^5-di(adenosine-5′-)pentaphosphate (Ap_5A), an adenylate kinase inhibitor, did not alter either ATP and ADP hydrolysis in brain membranes of zebrafish (Table 2). Tetramisole and levamisole, inhibitors of alkaline phosphatase, did not inhibit the hydrolysis of both substrates tested. The association of an ATP pyrophosphohydrolase (EC 3.6.1.8) and an inorganic pyrophosphatase (EC 3.6.1.1) was excluded because no significant amount of Pi was released when 1 mM pyrophosphate (PPi) was used as substrate instead of ATP (Table 1). Sodium azide is a well known inhibitor of the mithocondrial ATPase, acting on both F_oF_1 and soluble F_1, indicating that the inhibitory site of azide is on F_1, with an I_{50} of 0.04 mM (Pullman et al., 1960). Our results showed that sodium azide, in the μM range, did not promote significant changes on ATP and ADP hydrolysis, excluding the participation of a mithocondrial ATPase in our experiments (Table 2). However, the hydrolysis of both substrates were significantly altered in the presence of 20 mM azide, known as a possible inhibitor of NTPDases from several sources (Plesner, 1995; Knowles and Nagy, 1999). Studies have demonstrated that the variable inhibition of NTPDases promoted by azide has been related when different sources or combinations of assay pH, ATP or ADP and Mg^{2+} or Ca^{2+} were employed (Knowles and Nagy, 1999; Caldwell et al., 1999). Furthermore, several studies have used the Chevillard plot, which is a classical method used to determine if two substrates are hydrolyzed at the same active site of an enzyme (Chevillard et al., 1993; Kettlun et al., 1994; Pilla et al., 1996; Matos et al., 2001). The constant velocity at all substrate combinations tested suggests the participation of a single active site involved in ATP and ADP hydrolysis in brain membranes of zebrafish (Fig. 4).

The release of cellular nucleotides and nucleosides results in myriad physiological responses through approximately 20 different G protein-coupled and ligand-gated ion channel receptors (Ralevic and Burnstock, 1998; Khakh et al., 2001). Studies have described and characterized P2X purinoceptors from zebrafish (*Danio rerio*) (Egan et al., 2000; Norton et al., 2000; Boué-Grabot et al., 2000; Diaz-Hernandez et al., 2002). Neurotransmitter signaling pathways require effective mechanisms for removing or metabolizing extracellular signaling molecules, and in the case of extracellular nucleotide signaling, a broad range of nucleotide-degrading and interconverting ecto-or extracellular enzymes have been identified (Zimmermann, 2001). Studies have characterized and identified enzymes involved in the degradation of the extracellular ATP in several species of fishes. Sarkis and Saltó (1991) characterized a synaptossomal ATP diphosphohydrolase from the electric organ of *Torpedo marmorata*. Schetinger et al. (2001) demonstrate that the synaptosomal NTPDase response to several factors is similar in fish, chickens and rats and that the enzyme presents functional homology. Furthermore, it has been described that addition of extracellular ATP to intact goldfish hepatocytes resulted in total dephosphorylation of ATP to adenosine (Schwarzbaum et al., 1998). Recently, two different members of the E-NTPDases

family have been reported in goldfish liver (Alleva et al., 2002). Considering the presence of neuronal purinoceptors in zebrafish, it becomes essential the control of this signaling pathway by NTPDase family, which is widely viewed as the major nucleotidase family responsible for the hydrolysis of nucleotides. Considering that the NTPDase activity described here has a 3–4 fold preference for ATP over ADP as a substrate, it is possible that this enzyme presents characteristics similar to a NTPDase 3, which is a functional intermediate and hydrolyzes ATP approximately three times better than ADP (Zimmermann, 2001). Further experiments will be required in order to determine the member of NTPDase family involved in ATP and ADP hydrolysis in this fraction. In conclusion, here we describe an enzyme in brain membranes of zebrafish that shares several kinetic properties, which characterize a NTPDase. The presence of a NTPDase in central nervous system of zebrafish may be a key component for nucleotide cell signaling that could lead to better understanding of the role of the purinergic system in this specie.

Acknowledgements

This work was supported by Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Third World Academy of Sciences (TWAS). E.P.R. was a recipient of fellowship from FAPERGS. M.R.S. was a recipient of fellowship from PIBIC/CNPq. The authors would like to thank the Instituto de Pesquisas Biomédicas (PUCRS) for the technical support and Dr. João J.F. Sarkis for critical reading of the manuscript.

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