Purification of pancreas type-I ATP diphosphohydrolase and identification by affinity labelling with the 5'-*p*-fluorosulphonylbenzoyl-adenosine ATP analogue

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The enzyme recently identified as type-I ATP diphosphohydrolase (ATPDase; EC 3.6.1.5) has been purified from the zymogen granule membrane of pig pancreas. After solubilization with Triton X-100 and chromatographies on ion-exchange and Affi-Gel Blue columns an approximate 3500-fold purification was obtained. The enzyme preparation with a specific activity of 45 units/mg of protein was much further purified by PAGE under non-denaturing conditions. The active band localized on the gel contained two proteins after SDS/PAGE and silver staining, corresponding to apparent molecular masses of 56 and 54 kDa. The identity of the ATPDase was confirmed by an affinity labelling technique with 5'-*p*-fluorosulphonylbenzoyl-

adenosine (FSBA) as an ATP analogue. The latter was detected by a Western blot technique. A strong reaction was observed with the band corresponding to 54 kDa. N-terminal sequence analysis revealed that the 56 kDa protein has significant similarities (50–72 %) with lipases, whereas the 54 kDa enzyme has no significant similarity with any known proteins. N-glycosidase F treatment confirmed the glycoprotein nature of the enzyme and suggested that the enzyme bears several N-glycosylation sites. Comparisons of molecular masses and biochemical properties show that this ATPDase is different from other reported mammalian ATPDases.

INTRODUCTION

ATP diphosphohydrolases, or apyrases (ATPDase; EC 3.6.1.5), constitute a family of enzymes that catalyse the hydrolysis of the γ - and β -phosphate residues from triphospho- and diphosphonucleosides. The first ATPDase was reported by Kalckar in potato tubers [1] in 1944 and, since then, ATPDases have been found in other plants, blood-feeding insects, ticks, bugs and mosquitoes, as well as leeches and other invertebrates (for reviews see Ribeiro [2] and Beaudoin et al. [3]). In 1980, LeBel et al. reported an ATPDase in the pig pancreas [4]. A couple of years later, Laliberté et al. purified the enzyme further and described some of its kinetic properties [5,6]. Since then, however, the catalytic unit of the enzyme has not been established with certainty. Other ATPDases have been found in vertebrate cells, including those of blood vessels [7–16]. Some evidence agrees with the view that the catalytic site of the ATPDase on endothelial and smooth muscle cells of blood vessels and trachea is a component of plasma membranes and would be responsible for the major part of the ectoADPase and ectoATPase activities at the cell surface [8,17]. Because of their low apparent K_m ($\sim 10 \mu\text{M}$), one is led to believe that ATPDases exert a control on ATP and ADP concentrations in blood and within the blood vessel wall [3,18,19]. Côté et al. have demonstrated that addition of a semi-purified ATPDase preparation to a platelet-rich concentrate could prevent or even reverse ADP-induced platelet aggregation [12].

Analysis of the biochemical properties of vertebrate ATPDases from different sources: pig pancreas [4–6], bovine aorta [11–13],

lung [20] and trachea [17], led us to propose the existence of at least three distinct types of ATPDases. The enzymes from these sources exhibit clear biochemical differences, but until recently little was known about their identification and their structure. In this work, type-I ATPDase has been purified and the identity of the catalytic unit has been confirmed by affinity labelling with 5'-*p*-fluorosulphonylbenzoyl-adenosine (FSBA), detected by an immunoblot technique.

EXPERIMENTAL

Materials

ATP, ATP-agarose, EDTA, glycerol (1,2,3-propanetriol), glycine, L-histidine, Nitro Blue Tetrazolium (NBT), Ponceau S, sodium deoxycholate, Tris, 1,4-dithiothreitol (DTT), 5-bromo-4-chloro-3-indolyl phosphate (BCIP), FSBA, mouse monoclonal antibodies to rabbit IgG (conjugated to alkaline phosphatase), and glutamate dehydrogenase (GDH) were all obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.; ADP, AMP, Triton X-100 and N-glycosidase F were purchased from Boehringer-Mannheim, Laval, Québec, Canada; CaCl_2 and Tween 20 were from Fisher Scientific Co., Montréal, Québec, Canada; microdialysis membranes (12000–14000 Da cut-off) were purchased from Bethesda Research Laboratories, Gaithersburg, MD, U.S.A.; dialysis membrane Spectra/Por (6000–8000 Da cut-off) was from Spectrum Medical Industries, Inc., Los Angeles, CA, U.S.A.; transfer membrane Immobilon-P

Abbreviations used: ATPDase, ATP diphosphohydrolase; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; DTT, dithiothreitol; FSBA, 5'-*p*-fluorosulphonylbenzoyl-adenosine; GDH, glutamate dehydrogenase; NBT, Nitro Blue Tetrazolium; ZGM, zymogen granule membrane.

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was obtained from Millipore Corp., Bedford, MA, U.S.A.; Bradford reagent, BSA fraction V, Affi-Gel Blue, DEAE Bio-Gel A agarose, SDS, molecular-mass standards and polyacrylamide were from Bio-Rad Laboratories, Mississauga, Ontario, Canada. All other reagents were of analytical grade.

ATPase purification

Pig pancreases, obtained from the 'Abattoir Bienvenue', St-Valérien, Québec, Canada, were removed within 2 min of death and kept on ice, in a Krebs-Ringer phosphate medium (pH 7.4) supplemented with 0.2% glucose. Purification of the zymogen granule membrane (ZGM) was carried out according to Pâquet et al. [21]. Membrane proteins were solubilized, at a protein concentration of 1 mg per ml, with 0.3% (v/v) Triton X-100/30 mM Tris, pH 8.0, and centrifuged for 1 h at 100 000 g in a Beckman rotor SW 41. Approximately 10–30 mg of proteins from the supernatant, containing most of the enzyme activity, were loaded on to an 8 ml DEAE-agarose column, pre-equilibrated with 7.5% (v/v) glycerin/0.1% (v/v) Triton X-100/20 mM Tris, pH 8.0. Proteins were eluted by a 0–0.13 M linear NaCl gradient in the same buffer, followed by a 2 M NaCl wash. Active fractions were pooled in 0.1 × buffer E [buffer E: 192 mM glycine/0.1% (v/v) Triton X-100/25 mM Tris, pH 7.5] and electrodialed in an ISCO electro-eluter according to the following technique. Buffer E was put in the apparatus and a 15 mA current was applied per cuvette. The buffer E was changed four times, at 50 min intervals. The dialysate was adjusted to pH 5.9 with a 200 mM L-histidine solution (pH 4.0), and loaded on to an Affi-Gel Blue column (6 ml of beads) pre-equilibrated with 7.5% (v/v) glycerin/0.07% (v/v) Triton X-100/25 mM Tris/L-histidine (pH 5.9). Proteins were eluted by a linear gradient of buffers A and B. [Buffer A (60 ml): 7.5% (v/v) glycerin/0.07% (v/v) Triton X-100/10 mM Tris, pH 6.8; buffer B (60 ml): 7.5% (v/v) glycerin/0.07% (v/v) Triton X-100/1 M NaCl/10 mM Tris, pH 7.5.] This was followed by a wash with 1 M NaCl/0.07% Triton X-100/100 mM Tris, pH 8.5. The ATPase fractions were pooled and dialysed overnight against 0.05% Triton X-100 in 1 mM Tris, pH 8.0, concentrated on a mini DEAE-agarose column of 1 ml as described above, eluted with 0.4 M NaCl and dialysed against distilled water. This purified fraction was used for FSBA labelling, digestion with N-glycosidase F and electrophoresis. All the purification steps were carried out at 4 °C and the column flow rate was 10 ml/h in all cases. Conditions of PAGE under non-denaturing conditions have been described previously [12].

ATPase assays

Enzyme assays were routinely carried out at 37 °C in 5 mM CaCl_2 /200 μM substrate (ATP or ADP)/50 mM Tris, pH 8.0, in a final volume of 1 ml. This concentration of substrate corresponds to about 25 times the apparent K_m of the enzyme and allowed low background readings for P_i determinations. Incubation was stopped with 0.25 ml of Malachite Green reagent, and P_i was estimated according to Baykov et al. [22]. One unit of ATPase activity corresponds to the release of 1 μmol of P_i /min at 37 °C. Proteins were estimated by the technique of Bradford using BSA as the standard of reference [23].

N-glycosidase F treatment

Deglycosylation was carried out according to the manufacturer's recommendations (Boehringer-Mannheim, Laval, Québec, Canada). Samples (6 μg) of protein from the Affi-Gel Blue

fraction were boiled for 10 min in 0.2% SDS/0.2% (v/v) 2-mercaptoethanol (25 μl total), the volume was then adjusted to 0.2 ml with a final concentration of 0.4% (v/v) Triton X-100/10 mM EDTA/50 mM sodium phosphate buffer, pH 7.4, and 1 unit of N-glycosidase F. After a 12 h incubation at 37 °C the medium was dialysed, lyophilized and fractionated by SDS/PAGE. A positive control was run with 15 μg of protein from the ZGM incubated under identical conditions (results not shown).

Polyclonal antibodies to FSBA

Polyclonal antibodies to FSBA conjugated to GDH were raised according to the method of Anostario et al. [24]. Aliquots (20 mg) of GDH were incubated in a potassium phosphate buffer, pH 7.1, with 10% (v/v) dimethylformamide and 2.6 mM FSBA, in a final volume of 10 ml, for 2 h at 37 °C. The FSBA-GDH conjugate was dialysed against a 10 mM phosphate buffer, pH 7.4, and kept at –20 °C. Five albino rabbits of approximately 2 kg each were injected in the leg muscle with a mixture of 0.5 ml (1 mg) of the GDH-FSBA conjugate and 0.5 ml of Freund's complete adjuvant. Two boosters were given at 4-week intervals with 0.75 mg of GDH-FSBA in PBS. Blood samples were taken daily, starting at day 4 after the second booster, and titred by dot blot assays with the GDH-FSBA conjugate (30 μg /dot). When titre reached 1:25 000, rabbits were anaesthetized with Somnotol (0.5–1 ml/2 kg), bled by canulation of the femoral artery, and their serum kept at –70 °C.

FSBA labelling and SDS/PAGE

A sample (6 μg) of the purified enzyme from the Affi-Gel Blue column fraction was mixed with 150 mM KCl/5% (v/v) DMSO/0.2 mM FSBA as estimated by absorbance coefficient ($1.35 \times 10^4 \text{ cm}^{-1} \cdot \text{M}^{-1}$ in ethanol) and 36 mM Tris/acetate, pH 7.5, in a final volume of 1 ml. The labelling reaction was carried out at 37 °C, starting with the addition of FSBA and stopped 35 min later with the addition of 100 mM DTT in 0.1% SDS. Control experiments were run with 10 mM ADP or ATP (substrate) added 5 min before FSBA. After incubation, the medium was dialysed for 8 h against water in a microdialysis apparatus (Bethesda Research Laboratories, Gaithersburg, MD, U.S.A.) at room temperature. The dialysate was lyophilized, resuspended in 150 μl of 1 × sample buffer [2% (w/v) SDS/1% (v/v) 2-mercaptoethanol/10% (v/v) glycerin/0.001% Bromophenol Blue/65 mM Tris, pH 6.8] and incubated for 20 min at 37 °C. FSBA-labelled proteins were separated by SDS/PAGE on a 7–12% polyacrylamide gradient according to the method of Laemmli [25] and detected by a Western blot technique with anti-FSBA antibodies (see below). Proteins fractionated on SDS/PAGE were detected by the Coomassie Blue and/or silver nitrate diamine staining methods [26].

Immunoblotting procedure

Proteins were transferred to Immobilon-P sheets as described by Towbin et al. [27]. Blots were stained with either Ponceau S (Millipore Corp., Bedford, MA, U.S.A.) or 0.1% Amido Black according to the method of Schaffner and Weissman [28]. Blots were incubated in 0.9% NaCl/0.5% skimmed milk powder/10 mM sodium phosphate, pH 7.4, for 1 h, washed (3 × 5 min) with solution B (0.1% BSA/0.9% NaCl/20 mM Tris, pH 7.4) and incubated for 2 h with the primary antibody diluted 1:10 000 with solution C [1% BSA/0.9% NaCl/0.03% (v/v) Tween 20/20 mM Tris, pH 7.4]. Excess antibody was removed by repeated washings (10 × 3 min) in solution B. Blots were then

incubated for 1.5 h with a mouse anti-(rabbit IgG) antibody conjugated to alkaline phosphatase at a dilution of 1:6000 in solution C. After washings (10×3 min) in 0.9% NaCl/0.03% (v/v) Tween 20/20 mM Tris, pH 7.4, antibodies were detected with NBT/BCIP according to the method of Harlow and Lane [29].

RESULTS

ATPDase purification

Isolation of the ZGM was the first step of the purification procedure of pancreas type-I ATPDase. A relatively high specific activity was found in this membrane preparation as compared with the homogenate, approximately a 160-fold purification as previously reported [5]. The membrane was solubilized in Triton X-100, which caused a loss of about one-third of the enzyme activity, with a recovery of about 85% of the protein in the soluble fraction. A further 4-fold purification was obtained by DEAE-agarose column chromatography, with a 75% recovery of the enzyme activity (Figure 1, Table 1). The active fraction was fractionated on an Affi-Gel Blue column, which further purified the ATPDase by a factor of six, with a 50% recovery of

Figure 1 DEAE-agarose chromatography of the particulate fraction solubilized with Triton X-100

The left-hand arrow indicates the initiation of the linear NaCl gradient (0–0.13 M), and the right-hand arrow, the 2 M NaCl wash. Conditions of chromatography are described in the Experimental section. Fractions of 3 ml were collected. (....), Protein concentration; (—), ATPase activity.

Table 1 ATPDase purification

The data shown are representative of three independent experiments.

Steps	Total protein (mg)	Total activity (units)	Specific activity (ATP) (units/mg of protein)	Yield (%)	Purification factor (fold)	Hydrolysis rates (ATP/ADP)
ZGM	20.0	60.8	3.0	—	(160)*	1.3
ZGM + Triton X-100	20.0	40.6	2.0	100	1	1.3
100 000 g supernatant of solubilized ZGM	17.6	37.0	2.1	91	1.1	1.3
DEAE-agarose column	3.5	28.8	8.3	71	4.2	1.3
Affi-Gel Blue column	0.31	13.8	45	34	23	1.3

* Laliberté et al. showed a 160-fold purification for the ZGM compared with the homogenate using ADP as the substrate [5].

Figure 2 Affi-Gel Blue chromatography of the active fraction from the DEAE-agarose column

The left-hand arrow indicates the initiation of a linear gradient of NaCl and change of pH. The right-hand arrow indicates the beginning of the wash. Conditions of chromatography are described in the Experimental section. Fractions of 3 ml were collected. (....), Protein concentration; (—), ATPase activity.

activity (Figure 2, Table 1). Throughout these purification steps the relative hydrolysis rates of ATP to ADP remained constant, indicating that no other nucleotidase was eliminated during purification (Table 1).

The final step of our analytical purification procedure was PAGE under non-denaturing conditions. Analysis of the enzyme activity in the gel confirmed the presence of the ATPDase, which specifically catalysed the hydrolysis of ATP and ADP, but not AMP (Figure 3). The activity band was excised from the gel and fractionated by SDS/PAGE. This activity band contained two polypeptides as shown after SDS/PAGE and silver nitrate overstaining: a sharp 56 kDa band and a diffuse band of about 54 kDa slightly below and overlapping with the 56 kDa band (Figure 4, lane H). We also analysed by SDS/PAGE an additional band excised just above the active band on the non-denaturing gel and only the 56 kDa band could be seen (Figure 4, lane I). This observation indicated that the 56 kDa band was not the ATPDase, leaving the 54 kDa diffuse band as the only presumptive candidate for the enzyme.

N-glycosidase F treatment

We then investigated the possibility that the enzyme is a glycoprotein, since previous reports had shown that the enzyme

Figure 3 Localization of the ATPase of the Affi-Gel Blue fraction by PAGE under non-denaturing conditions

Electrophoresis was carried out on the active fraction from the Affi-Gel Blue column as described in the Experimental section. A sample (6 μ g) of protein was loaded in each well of lanes A–D and separated on a 4–7% polyacrylamide gel gradient. ATPase was localized by incubating the gel for 10 min at 37 °C in: 10 mM CaCl_2 /100 mM Tris/imidazole (pH 7.5)/4 mM of the nucleotide ATP, ADP or AMP. Released P_i forms a white Ca^{2+} precipitate. Lane A, ATP; lane B, ADP; lane C, AMP; lane D, Coomassie Blue staining; lane E, Coomassie Blue staining, 80 μ g (new gel).

Figure 4 SDS/PAGE protein patterns at the different steps of the purification procedure and after N-glycosidase F digestion of the Affi-Gel Blue fraction

Protein samples were fractionated on a 8–13.5% polyacrylamide gradient and deglycosylation was carried out as described in the Experimental section. Lanes A, B and C show the results of N-glycosidase F treatment of the Affi-Gel Blue fraction (silver nitrate staining). Lane A, N-glycosidase F alone; lane B, control without N-glycosidase F; lane C, with N-glycosidase F. Lanes A', B' and C' were as above but stained with Coomassie Blue. Lanes D, E, F and G show the results of the purification procedure (Coomassie Blue staining). Lane D, molecular-mass standards: 97.4, 66.2, 45.0, 31.0, 21.5, 14.4 kDa; lane E, ZGM (100 μ g); lane F, DEAE-agarose column (25 μ g); lane G, Affi-Gel Blue column (6 μ g). Lanes G', H and I show the last steps of the purification procedure (silver nitrate overstaining). Lane G', same as lane G; lane H, activity band from the non-denaturing gel (refers to Figure 3); lane I, control band of gel taken just above the activity band of the non-denaturing gel. The excised bands from the non-denaturing gel were incubated for 1 min in 0.4% SDS/0.5 M Tris, pH 6.8, boiled for 5 min with 50 μ l of 3 $\times$ sample buffer and the mixture was loaded in the well for SDS/PAGE.

was bound by a concanavalin A-agarose column [4]. When the Affi-Gel Blue preparation was submitted to digestion by N-glycosidase F, both bands (56 and 54 kDa) were displaced. One showed only a small shift in its molecular mass of about 4000 Da, which would correspond to one or two N-linked oligosaccharide chains (assuming a molecular mass contribution of 2000–4000 per oligosaccharide [30]), whereas a very pronounced decrease to about 35 kDa was observed for the other band (Figure 4, lanes C and C'). Controls run in the absence of N-glycosidase F failed

Figure 5 Characterization of rabbit anti-FSBA antibodies

FSBA labelling, blot and immunodetection were carried out as described in the Experimental section. Aliquots (50 μ g) of unlabelled GDH or labelled GDH-FSBA were loaded in each well as indicated. The anti-FSBA was purified on an ATP-agarose column as described by Anostario et al. [24]. (a) Preimmune serum, 1:10 000; (b) immune serum, 1:10 000; (c) purified anti-FSBA, 1:250.

Figure 6 Western blot of FSBA-labelled samples from the Affi-Gel Blue column fraction

Labelled samples were loaded on an SDS/7–12% polyacrylamide gel, transferred to Immobilon-P membrane, incubated with the rabbit anti-FSBA antibody and detected by a secondary antibody conjugated to alkaline phosphatase as described in the Experimental section. Aliquots (6 μ g) of the Affi-Gel Blue-purified fraction were used in lanes A, B and C. Molecular-mass standards are indicated on the left: 97.4, 66.2, 45.0, 31.0, 21.5, and 14.4 kDa; lane A, FSBA; lane B, FSBA + ADP; lane C, no labelling.

to show any shift in those bands, ruling out the possibility of a potential proteolytic cleavage (Figure 4, lanes B and B').

FSBA labelling

The identity of the 54 kDa protein as the ATPase was confirmed by affinity labelling with FSBA, an ATP analogue. We have previously reported that FSBA is a strong and irreversible inhibitor of ATPase type-II from the bovine aorta [13] which forms a covalent bond with ATP-binding sites of proteins [31–33]. To label the ATP-binding protein we used the conditions described by Côté for the bovine aorta ATPase [34]. Labelled proteins were localized by a Western blot technique with FSBA-specific rabbit polyclonal antibodies. Figure 5(b) shows a specific reaction with GDH-FSBA at a dilution of 1:10 000. Polyclonal antibodies specific to the FSBA residue of the GDH-FSBA complex were purified on an ATP-agarose column and, as

shown in Figure 5(c), no detectable reaction was found with GDH. Preimmune serum was unreactive under the same conditions (Figure 5a). These antibodies could efficiently recognize the 54 kDa FSBA-complex diffuse band (Figure 6, lane A). Controls, where labelling was carried out in the presence of ATP (results not shown) or ADP, showed no signal, confirming the specificity of FSBA for the active site of the enzyme (Figure 6, lane B). Specificity of the antibody was also demonstrated by the absence of reactivity with the control where the Affi-Gel Blue-purified ATPDase was unlabelled (Figure 6, lane C).

DISCUSSION

In 1980, LeBel et al. demonstrated the existence of a mammalian ATPDase in pig pancreas [4]. Subsequent cytochemical observations suggested that the enzyme was localized in both ZGM and plasma membranes of the pancreas exocrine cell [35,36]. Laliberté et al. [5] further purified the enzyme by 5'-AMP-Sepharose chromatography. This procedure yielded a highly purified fraction consisting of three bands after SDS/PAGE and detection by silver staining or radio-iodination techniques. Detailed kinetic studies were carried out on this purified preparation [5,6]. Vachereau [37] later attempted to identify the polypeptide carrying the catalytic activity of the enzyme by a different procedure on the same ZGM preparation. After dialysis in the presence of ADP and mercaptoethanol, saponin treatment and electrophoresis under non-denaturing conditions, the enzyme was localized on the acrylamide gel. Elution of the active band from the non-denaturing gel revealed the same bands obtained by Laliberté et al. after SDS/PAGE and silver staining: 58, 46 and 28 kDa [37]. These proteins were poorly antigenic in rabbits and mice as indicated by the weak affinities and low titres of the antibodies obtained [37,38]. Anterior studies of Pâquet et al. described the ZGM-protein composition from the rat pancreas by combined isoelectric focusing and SDS/PAGE [21]. Their pattern was very similar to the one reported by Ronzio et al. several years before [39]. Among these proteins, four glycoproteins were found corresponding to 130 (GP1), 80 (GP2), 54 and 52 kDa (GP3 and GP4?) respectively. The two latter bands could not be clearly dissociated from each other by unidimensional electrophoresis.

The present purification procedure led to a fraction containing a doublet of proteins as assessed by SDS/PAGE and silver staining. One was a sharp band with a molecular mass evaluated at 56 kDa whereas the other (54 kDa) was a more diffuse band overlapping and slightly below the 56 kDa band. Assuming that one of the two bands was the ATPDase type I, we proceeded to an analysis of the amino acid sequence. N-terminal sequences of 20 amino acids were obtained from both proteins, i.e. 56 and 54 kDa. Screening of the 56 kDa protein sequence with EMBL and GenBank revealed more than 50% identity with human lipases and the ZGM 'lipase-like' protein from rat pancreas identified as GP3 [40]. There was no significant similarity of the N-terminal sequence of the 54 kDa protein with any known protein. This observation ruled out the 56 kDa band, leaving the 54 kDa protein as the only presumptive candidate for ATPDase type I. N-glycosidase F digestion of the Affi-Gel Blue-purified fraction (54–56 kDa) resulted in a marked shift in the molecular mass down to 35 kDa for one band, and a smaller shift to about 52 kDa for the other. The observations on the previously described rat GP3 'lipase', showing only one site of N-glycosylation, lead us to believe that the 35 kDa band corresponds to the deglycosylated form of ATPDase I. This marked shift would indicate 4 to 10 N-glycosylation sites. The identity of the ATPDase catalytic unit was corroborated by affinity labelling

with the ATP analogue FSBA. Indeed, the signal corresponded to the diffuse 54 kDa instead of the sharp well-defined band of the 56 kDa protein. FSBA is recognized as an efficient reagent for the affinity labelling of ATP-binding sites of proteins [31–33]. Labelling could be specifically prevented by competing with an excess of ATP or ADP. The immunochemical approach provides an easy and inexpensive alternative to the use of radiolabelled ligand, i.e. ^3H - or ^{14}C -labelled FSBA [24]. It is noteworthy that our results on labelling with FSBA are in agreement with labelling experiments with 8-azido- $[\alpha\text{-}^{32}\text{P}]\text{ATP}$ on the ZGM proteins of pig pancreas [37,41]. The biochemical properties and the molecular mass of the ATPDase type I differ from those reported for other mammalian ATPDases described in the literature [7,9,42].

The purification of ATPDase I brings about the questions of localization and functions of this protein. This ATPDase is associated with the ZGM in the pancreas but this localization may be transitory, since during the exocytotic process the ZGM becomes an integral part of the plasma membrane. The enzyme is most probably inactive in the mature zymogen granules, because of the acidic conditions and the small amount of free water within these organelles. In contrast, the biochemical conditions are much more favourable for the enzyme after exocytosis, when the catalytic site is exposed to the alkaline extracellular lumen which matches the optimum pH for catalysis by the ATPDase. Hence, it is logical that the function of the enzyme would be exerted on the external face of the luminal plasma membrane. Cytochemical studies have confirmed that an ATPDase activity is localized on both luminal and basolateral portions of the plasma membrane of the acinar cell of rat pancreas [35,36]. In a more general way, the function of ATPDases is to dephosphorylate extracellular ATP and ADP which interact with P_2 purinoceptors at the cell surface [3,19]. The reaction product AMP may be converted by the ecto-5'-nucleotidase into adenosine, which is responsible for the so-called P_1 effect. The understanding of the function of the enzyme is closely linked to its localization, which can only be investigated with specific, high-affinity antibodies, and so far this has not been possible. The sequencing of the protein will provide valuable information for this purpose. Knowledge of the distribution and the characterization of both nucleotide receptors and ATPDases will facilitate the elucidation of their functional relationships and will clarify the role of the ATPDases.

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