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IS THERE A PLASMA MEMBRANE-LOCATED ANION-SENSITIVE ATPase?

IV. DISTRIBUTION OF THE ENZYME IN RAT PANCREAS *

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Summary

The intracellular localization of anion-sensitive Mg^{2+}-ATPase in rat pancreas was studied by differential centrifugation, density gradient centrifugation and by the use of inhibitors of mitochondrial Mg^{2+}-ATPase.

The anion-sensitive Mg^{2+}-ATPase appears to be localized almost exclusively in a mitochondrial (15 min, 15 000 × *g*) fraction which shows two peaks after density gradient centrifugation. Both peaks coincide with the highest levels of cytochrome *c* oxidase activity, but not with alkaline phosphatase, $(Na^+ + K^+)$-ATPase and leucine aminopeptidase activities or RNA. They appear to be equally sensitive to inhibition by oligomycin, aurovertin D and the rat liver mitochondrial inhibitor protein, at least when 1 mM EDTA is present in the isolation media.

We conclude that no significant plasma membrane-located anion-sensitive Mg^{2+}-ATPase activity is present in rat pancreas.

Introduction

In pancreas it is still a matter of controversy whether the transport of HCO_3^- is passive and linked to an active sodium transport or is active [1]. Wizemann and Schulz [2] have presented a model in which a Na^+-H^+-exchange pump, presumed to be identical to the HCO_3^--ATPase, is the driving force in the $NaHCO_3$ secretion of the pancreas. This model appears to be supported by the finding of a parallel distribution of HCO_3^--ATPase activity and secretory capacity of HCO_3^- in the ducts of the cat pancreas [3]. In addition, a role of this enzyme in fluid secretion is suggested, namely on account of the inhibitory

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effect of thiocyanate on the enzyme activity [4] as well as on the secretory flow rate [5], and of the stimulatory effects of valinomycin and nigericin on both parameters [2]. Obviously, localization of the enzyme in the plasma membrane is a prerequisite for such a model.

After fractionation of cat pancreas homogenate by density gradient centrifugation and gel electrophoresis Simon et al. [4,6] found a coincidence of anion-sensitive Mg^{2+}-ATPase with both alkaline phosphatase and leucine aminopeptidase, but not with ($Na^+ + K^+$)-ATPase activity. However, Milutinović et al. [7] find a parallel enrichment of ($Na^+ + K^+$)-ATPase and HCO_3^--ATPase activities in a plasma membrane fraction of the same tissue. Recently, Lambert and Christophe [8] have mentioned the presence of the enzyme in a microsomal plasma-membrane fraction of rat pancreas.

Several reports thus seem to support the presence of a plasma membrane-located anion-sensitive Mg^{2+}-ATPase in the pancreas. However, contamination with mitochondria, which have a high anion-sensitive Mg^{2+}-ATPase activity [9,10], may not always have been adequately excluded. Previously we have shown that the 'microsomal' anion-sensitive Mg^{2+}-ATPase activity in rainbow-trout gill [11], rabbit gastric mucosa [11] and rabbit kidney [12] is due to mitochondrial contamination and we have presented explanations for possibly wrong conclusions made in the past. In addition, we have recently shown the enzyme activity in rabbit erythrocyte to be part of the ($Ca^{2+} + Mg^{2+}$)-ATPase system [13], thus precluding a role of this activity in anion transport. In view of these findings we have now studied the localization and characteristics of anion-sensitive Mg^{2+}-ATPase activity in rat pancreas.

Materials and Methods

Tissue preparation

From 10 Wistar rats, killed by a blow on the neck, the pancreas is immediately and carefully dissected out, rinsed and cooled on ice. The tissue is then minced with scissors and homogenized in 6 vols. 0.25 M sucrose, 0.2 mg/ml soybean trypsin inhibitor, 25 mM Tris-maleate (pH 7.6) in a Teflon/glass homogenizer (Potter/Elvehjem) with 3—4 strokes at 850 rev./min. All operations are performed at 0—4° C.

Centrifugal fractionation

The homogenate is filtered through four layers of surgical gauze and diluted with another 3 vols. of homogenization buffer. It is then separated in a nuclear (10 min, 180 × *g*), mitochondrial (15 min, 15 000 × *g*) and microsomal (60 min, 100 000 × *g*) fraction and a remaining supernatant. Samples of these fractions, to be used for determination of anion-sensitive ATPase activity, are stored immediately at −18° C after isolation.

Density gradient centrifugation

The mitochondrial and microsomal pellets are further fractionated by resuspending them in homogenization buffer and layering them on top of a linear gradient of 30 to 55% (w/v) sucrose in 25 mM Tris-maleate (pH 7.6) enriched with 0.2 mg/ml soybean trypsin inhibitor. Gradients are routinely

centrifuged for 16 h at 24 000 rev./min (64 000 × g_{av}) in the SB 110 rotor of the IEC type B 60 ultracentrifuge. Approximately 25 fractions are collected and processed as previously described [11]. The effects of oligomycin and aurovertin D are determined in the fractions containing peak activities of anion-sensitive Mg^{2+}-ATPase. The effect of rat liver mitochondrial inhibitor protein is tested on appropriate fractions, selected on the basis of the anion-sensitive Mg^{2+}-ATPase distribution pattern (peak II, fraction numbers 14—16, and peak II, fraction numbers 11—12 in Fig. 2). These fractions are pooled, diluted with an equal volume of homogenization buffer, centrifuged for 60 min at 100 000 × *g* and resuspended in the appropriate solution.

Protein determination

Protein concentrations are estimated by the method of Lowry et al. [14]. Bovine serum albumin, dissolved in homogenization buffer, serves as standard.

In gradient fractions protein concentrations are determined by measuring 280 nm absorbance (A_{280nm}) after dilution with 2 vols. of 2% sodium dodecyl sulphate to eliminate light scattering. Corrections are made for the absorbance of sucrose and trypsin inhibitor.

Enzyme assays

Cytochrome *c* oxidase, ($Na^+ + K^+$)-ATPase and alkaline phosphatase are estimated as previously described [11,12]. In the ($Na^+ + K^+$)-ATPase assay the ouabain concentration is raised to $2 \cdot 10^{-3}$ M in view of the low sensitivity of the enzyme in rat tissues to this inhibitor. Leucine aminopeptidase is measured according to Nagel et al. [15]. Anion-sensitive Mg^{2+}-ATPase is measured at pH 8.4 in the presence of ouabain in media of constant ionic strength, containing predominantly either HCO_3^-, Cl^- or SCN^- [11].

When inhibitors in ethanolic solution are applied, controls are run containing the same ethanol concentration (<1.5% v/v). Preincubation for 15 min at 0°C and 5 min at 37°C in the absence of ATP precedes the incubation in this case. The rat liver mitochondrial inhibitor protein is isolated and tested as described by Chan and Barbour [16].

Materials

Na_2-ATP is obtained from Boehringer (Mannheim, G.F.R.), sodium dodecyl sulphate (SDS) and trypsin inhibitor (soybean, type II-S) from Sigma (St. Louis, Mo., U.S.A.). Oligomycin (Sigma) consists of 15% oligomicyn A and 85% oligomycin B (average molecular weight 400). Aurovertin D (isolated by Dr. R. Bertina and donated by the BCP Jansen-Institute, University of Amsterdam) is dissolved in absolute ethanol and the concentration is determined spectrophotometrically at 367.5 nm using an absorbance coefficient of 42.7 $mM^{-1} \cdot cm^{-1}$ [17].

All other reagents are from E. Merck (Darmstadt, G.F.R.) and are of analytical grade.

Results

ATPase activities in main fractions

Fig. 1 shows the distribution of Mg^{2+}-ATPase activity in the main fractions

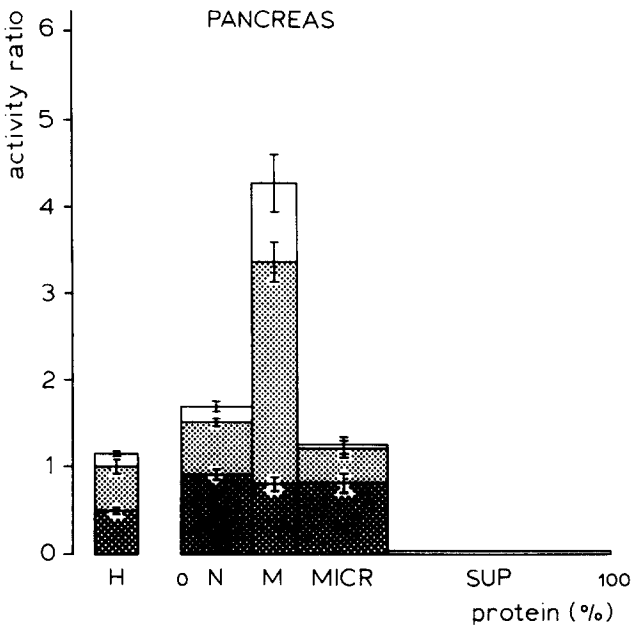

Fig. 1. Distribution of anion-sensitive ATPase activities in main fractions of pancreas. The relative specific activities in HCO_3^- medium (total column), Cl^- medium (lightly shaded part of column) and SCN^- medium (darkest part of column only) are plotted against the protein distribution in percent. The specific activity of the homogenate in Cl^- medium (6.5 μmol ATP · h^{-1} · mg^{-1} protein, S.E. 0.6, n = 4) is set at 1.0. Abbreviations: H is the homogenate; N, M and MICR are the fractions sedimenting at 10 min 180 × g, 15 min 15 000 × g and 60 min 100 000 × g, respectively. SUP is the remaining supernatant. Mean of 4 experiments with standard errors are given.

of rat pancreas. The largest stimulatory effect of HCO_3^- is observed in the mitochondrial (15 min, 15 000 × g) fraction, but little or no effect is seen in the nuclear (10 min, 180 × g) and microsomal (60 min, 100 000 × g) fractions. The Mg^{2+}-ATPase activity in the supernatant is negligible, which indicates that the enzyme activity is membrane bound.

In order to obtain a closer insight in the localization of the anion-sensitive Mg^{2+}-ATPase, we have further resolved the mitochondrial and microsomal fractions by means of density gradient centrifugation.

Enzyme distribution in density gradients

The enzyme distribution pattern of the mitochondrial (15 min, 15 000 × g) fraction is shown in Fig. 2. Three peaks of Mg^{2+}-ATPase activity can be observed. The first Mg^{2+}-ATPase activity peak (peak I) occurs at the top of the gradient below 28% (w/v) sucrose, is anion-insensitive (even slightly inhibited by HCO_3^-) and coincides with high activities of the plasma-membrane marker enzyme activities, alkaline phosphatase and leucine aminopeptidase and also 5′-nucleotidase (not shown). This region of the gradient is the only one having a rather low ($Na^+ + K^+$)-ATPase activity. Two HCO_3^--stimulated Mg^{2+}-ATPase activities (peak II and III), which are not well separated, are seen between 38 and 43% (w/v) sucrose and between 43 and 48% (w/v) sucrose, respectively. Both coincide with the highest levels of cytochrome *c* oxidase activity, but not

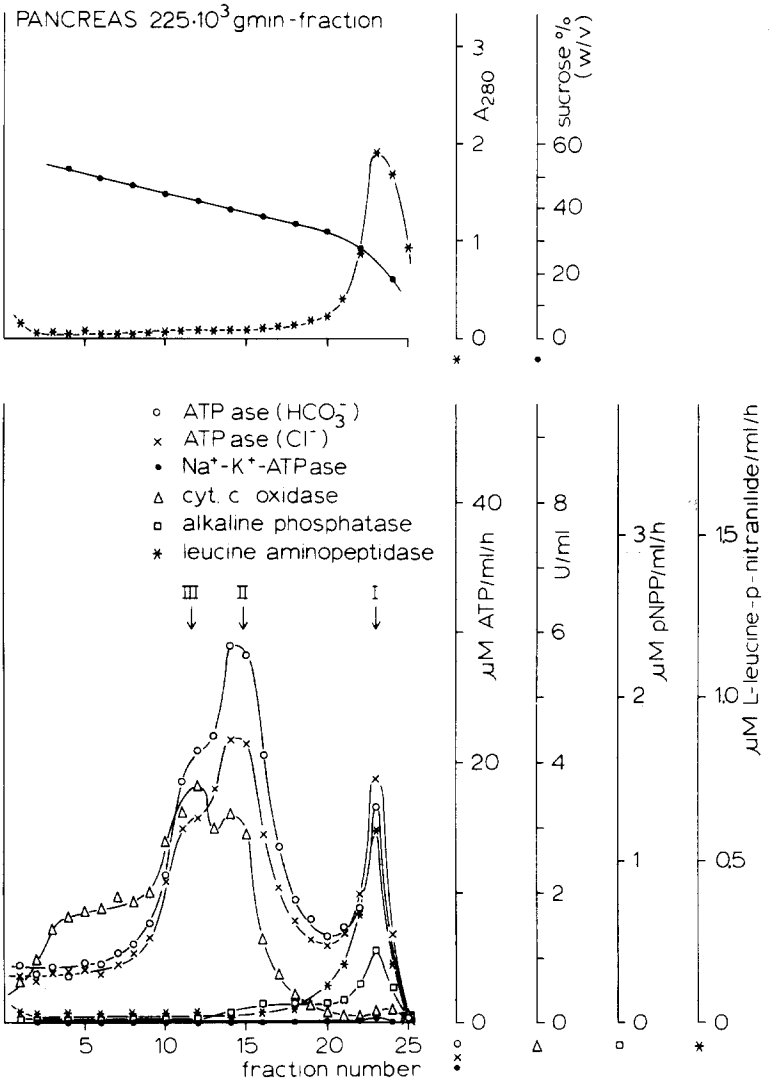

Fig. 2. Enzyme distribution patterns after density gradient centrifugation (16 h, 64 000 X *g*) of a mitochondrial fraction (15 min, 15 000 X *g*) of rat pancreas. Typical experiment representative for 4 experiments. ●———●, distribution of sucrose; *———*, (top figure), 280 nm absorbance after dilution and correction; ○———○, ATPase activity in HCO_3^- medium; X———X, ATPase activity in Cl^- medium; ●———●, ($Na^+ + K^+$)-ATPase activity; △———△, cytochrome *c* oxidase activity; □———□, alkaline phosphatase activity; *———*, leucine aminopeptidase activity.

with any of the plasma-membrane marker enzyme activities mentioned above. The ratio of HCO_3^--stimulated Mg^{2+}-ATPase to cytochrome *c* oxidase activity is higher in peak II than in peak III. Previously [11] we have suggested that this may be due to artefactual causes without indicating a non-mitochondrial origin for the material with the higher ratio.

A considerable but variable cytochrome *c* oxidase activity is observed at the higher sucrose densities (below fraction number 9), which does not appear to coincide with an equivalent level of HCO_3^--stimulated Mg^{2+}-ATPase

activity. There are two possible explanations for this finding. First, pancreatic zymogen granules, containing massive amounts of proteolytic and lipolytic proenzymes, are known to sediment at high sucrose densities [18]. Contamination with these enzyme activities of this part of the density gradient may lead to preferential loss of anion-sensitive Mg^{2+}-ATPase activity. Supporting this explanation is our observation that fractionation in the presence of 1 mM EDTA (see below), which is inhibitory to the zymogen granule enzymes, gives activity ratios close to those of peak II and III. Secondly, this high-density region of the gradient may contain virtually intact mitochondria, which will display a lower ATPase activity. Since cytochrome *c* oxidase is assayed in the presence of the detergent Tween-80 [11] this enzyme will show its full activity. In both cases, a low ATPase to cytochrome *c* oxidase ratio will be found.

In order to obtain further insight in the intracellular localization of the (peak II) anion-sensitive Mg^{2+}-ATPase, this fraction is subjected to centrifugation under both rate and isopycnic conditions. This is performed by recentrifugation of peak II (fractions number 14—16 in Fig. 2), pooled after an initial separation in density gradients by the normal procedure in the presence of 1 mM EDTA. The peak II material is resuspended in homogenization buffer, and is then centrifuged for 2 h at 64 000 $\times g_{av}$ in a shallow gradient of 32 to 42% (w/v) or of 22 to 34% (w/v) sucrose. In four separate experiments no separation between anion-sensitive Mg^{2+}-ATPase and cytochrome *c* oxidase activity distribution has been obtained by this procedure, in contrast to the findings of Simon et al. [4] for dog and cat pancreas.

In Fig. 3 the enzyme distribution of the microsomal (60 min, 100 000 $\times g$) fraction is shown. At the top of the gradient an anion-insensitive Mg^{2+}-ATPase activity is found, which coincides with high levels of leucine aminopeptidase, alkaline phosphatase and $(Na^+ + K^+)$-ATPase activities. When a gradient of 15 to 40% (w/v) is employed in order to distribute this peak over the gradient, there is still only one Mg^{2+}-ATPase activity peak, which is again anion-insensitive. The specific activities of the plasma membrane marker enzymes in the microsomal fraction appear to be higher than in the mitochondrial fraction (Fig. 2), since their activities are from 4 to 29 times as high, whereas twice the amount of protein is applied to the density gradient. The relatively higher enrichment of $(Na^+ + K^+)$-ATPase activity in this microsomal plasma membrane fraction may indicate a preferential localization of $(Na^+ + K^+)$-ATPase in a distinct type of plasma membrane or a higher susceptibility of the enzyme for the contaminating pancreatic proteolytic or lipolytic enzymes.

The peak in 280 nm absorbance in the middle of the distribution pattern of the microsomal gradient (Fig. 3) coincides with a high level of RNA (not shown), and thus probably reflects the presence of rough endoplasmic reticulum. RNA is not detected in the distribution pattern of the mitochondrial density gradient (Fig. 2).

When the anion-sensitive Mg^{2+}-ATPase activity is measured at pH 7.6, which is reported to be optimal for the pancreas enzyme [6], rather than at the routinely used pH 8.4, no significant change in the distribution pattern of the enzyme is observed, either in the mitochondrial or in the microsomal fraction. In addition, measurement of the Mg^{2+}-ATPase activity before and

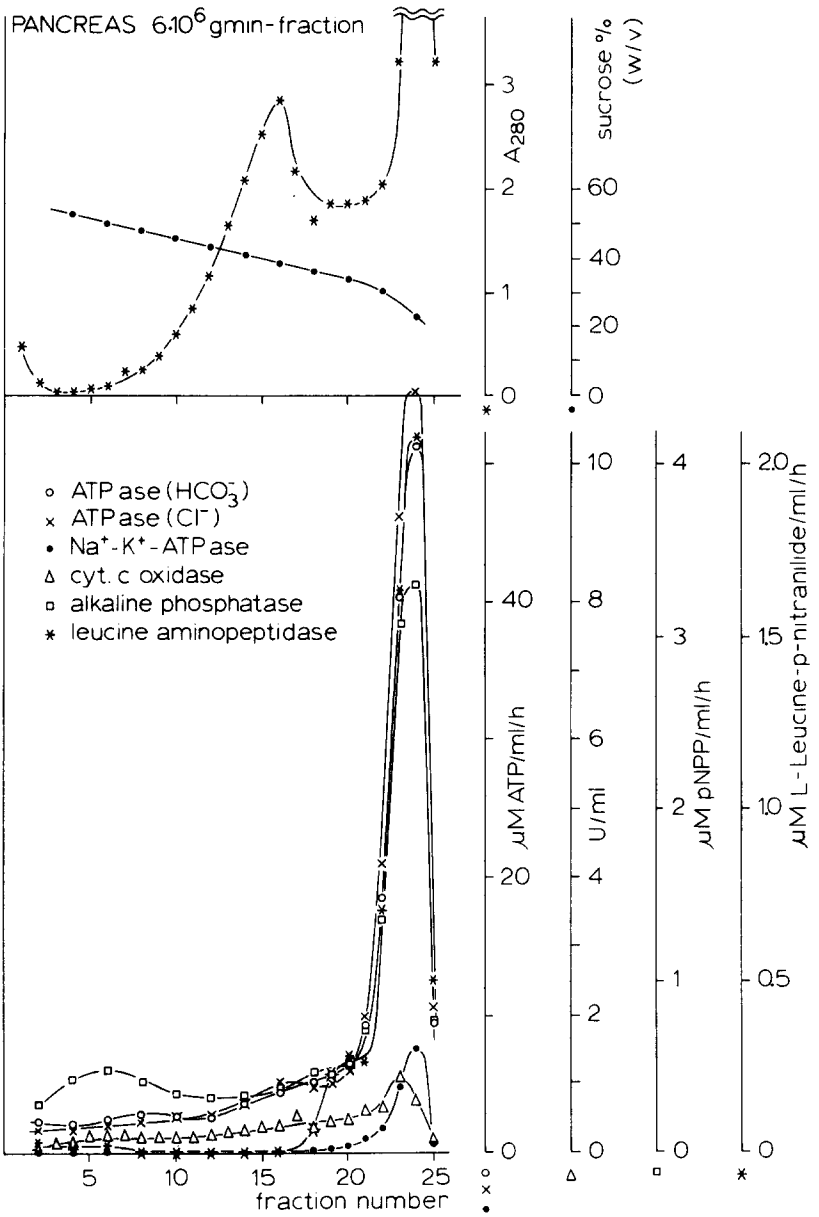

Fig. 3. Enzyme distribution pattern after density gradient centrifugation (16 h, 64 000 × *g*) of a microsomal fraction (60 min, 100 000 × *g*) of rat pancreas. Typical experiment representative for 4 experiments. Symbols as in Fig. 2.

after freeze-thawing does not result in changes in the characteristics of either the anion-sensitive or the anion-insensitive Mg^{2+}-ATPase activity peaks.

Inhibitor studies

The results, mentioned above, clearly suggest that no plasma membrane-located HCO_3^--stimulated Mg^{2+}-ATPase appears to be present in rat pancreas.

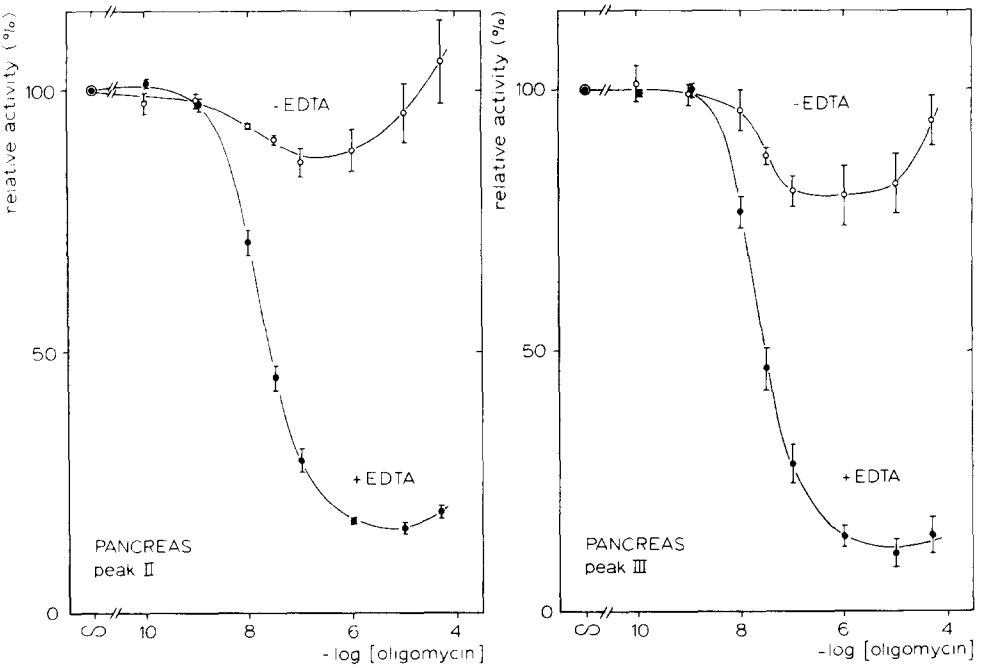

Fig. 4. Relative ATPase activity in HCO_3^- medium, as a function of the negative logarithm of oligomycin concentration in two subfractions from pancreas mitochondrial fraction prepared in the absence (○) or in the presence (●) of 1 mM EDTA. Mean with standard errors of percentage activity relative to the activity without added oligomycin (≡100%) are shown for 3 experiments in each case.

In order to test this point further, we have studied the effects of mitochondrial ATPase inhibitors on subfractions of the mitochondrial fraction. The microsomal fraction was not investigated, since it does not contain any HCO_3^--stimulated Mg^{2+}-ATPase activity.

The effects of oligomycin on the anion-sensitive Mg^{2+}-ATPase activities of rat pancreas (peak fractions II and III in Fig. 2), are shown in Fig. 4

TABLE I

EFFECTS OF OLIGOMYCIN AND AUROVERTIN D ON ANION-SENSITIVE Mg^{2+}-ATPase ACTIVITIES IN TWO SUBFRACTIONS FROM PANCREAS MITOCHONDRIAL FRACTION, PREPARED IN THE PRESENCE OF 1 mM EDTA

pI_{50} is the negative logarithm of the molar inhibitor concentration at half-maximal inhibition, pI_{99} that giving 99% of the maximal inhibition. Rest activity is percent ATPase activity remaining at maximal inhibition. The HCO_3^- medium was used in all experiments. Means are presented with the standard error.

Agent	Peak II ATPase			Peak III ATPase			*n*
	pI_{50}	pI_{99}	Rest activity (%)	pI_{50}	pI_{99}	Rest activity (%)	
Oligomycin	7.75 ± 0.05	5.83 ± 0.19	16 ± 1	7.67 ± 0.06	5.60 ± 0.20	11 ± 3	3
Aurovertin D ($1.6 \cdot 10^{-5}$ M)	—	—	44 ± 1	—	—	42 ± 2	4

(—EDTA). Both ATPase activities seem to be only slightly and variably inhibited by oligomycin, and the inhibition is lost again at higher concentrations of oligomycin. This rather strange phenomenon may be due to the action of proteolytic enzymes and (phospho)lipases, which are known to be present in high activities in the pancreas. These enzyme activities are able to influence the oligomycin sensitivity of sub-mitochondrial particles [19,20].

While the presence of soybean trypsin inhibitor in our media will curtail both trypsin and chymotrypsin effects, (phospho)lipase activity may still be active [21]. Since these enzymes seem to require Ca^{2+} [22], we have added 1 mM EDTA to the homogenization buffer and gradient solutions. This introduces only a slight change in the enzyme distribution pattern upon density gradient centrifugation, namely a shift of the two anion-sensitive Mg^{2+}-ATPase peaks to lower sucrose concentrations: peak II at 35—38% (w/v) sucrose and peak II at 38—41% (w/v) sucrose. The two anion-sensitive Mg^{2+}-ATPase activities have now become very sensitive towards inhibition by oligomycin (Fig. 4, +EDTA).

Aurovertin D, an inhibitor of the HCO_3^- stimulation of mitochondrial Mg^{2+}-ATPase activity [23], is equally effective on both activities. The effects

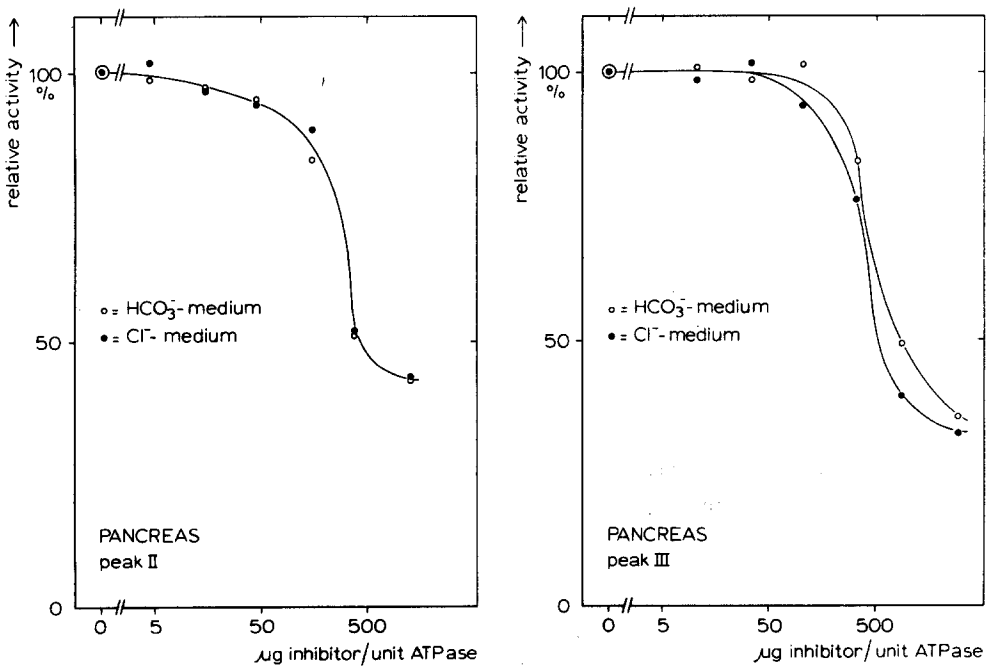

Fig. 5. Relative ATPase activity in HCO_3^- medium (○) and Cl^- medium (●), as a function of the ratio of rat liver mitochondrial inhibitor protein to ATPase activity in two subfractions from pancreas mitochondrial fraction prepared in the presence of 1 mM EDTA. A unit of ATPase is defined as the amount of enzyme which converts 1 µmol ATP per min at 37°C in HCO_3^- medium [16]. Ratios over the activity without inhibitor (≡100%) are shown in each case. The inhibitor is tested as described in Methods and Materials. A 50% inhibition of ATPase activity is obtained at 430 µg inhibitor/unit ATPase (peak II) or at 850 and 520 µg inhibitor/unit ATPase (peak III) in HCO_3^- and Cl^- medium, respectively. Typical experiment representative of 2 experiments.

of oligomycin and aurovertin D, summarized in Table I, closely agree with those observed for the anion-sensitive Mg^{2+}-ATPase activities in gastric mucosa [11] and kidney [12], which have previously been shown to be of mitochondrial origin [11,12,24,25]. The pI_{50} values for oligomycin indicate that the rat pancreas anion-sensitive Mg^{2+}-ATPase is about 1000 times more sensitive to this drug than the plasma membrane-located ($Na^+ + K^+$)-ATPase [26].

In addition, the effects of the mitochondrial inhibitor protein, isolated from rat liver [16], have been tested on the anion-sensitive Mg^{2+}-ATPase activities (Fig. 5). Both the peak II and the peak III activities are inhibited by this protein. The peak II activity appears to be somwhat more sensitive to this inhibitory agent, which may indicate that this fraction is more depleted of the inhibitor. Since the ATPase of intact mitochondria does not appear to be affected by the inhibitor protein [27], this may imply that after the density gradient centrifugation the mitochondrial anion-sensitive Mg^{2+}-ATPase activities from rat pancreas are not located in intact mitochondria.

Discussion

In analogy to the hypothetical model for the role of anion-sensitive Mg^{2+}-ATPase in the acid secretion of the stomach [28], Simon et al. [4] have presented a model describing the involvement of the enzyme in the bicarbonate secretion by the pancreas. This model presupposes that the enzyme is localized in the plasma membrane. Simon and Thomas [6] have reported that after polyacrylamide gel electrophoresis a Mg^{2+}-ATPase activity appears to be localized in a membrane fraction together with alkaline phosphatase and leucine aminopeptidase activities, but they do not present data on the Mg^{2+}-ATPase activity without a stimulating anion.

A potential source of wrong conclusions is the possibility of contamination of a so-called plasma membrane fraction with mitochondria or mitochondrial material. We have previously encountered this possibility in a study of the distribution of anion-sensitive Mg^{2+}-ATPase activity in rainbow-trout gill [11], rabbit gastric mucosa [11] and rabbit kidney [12]. In these papers we have presented evidence that the activity observed in microsomal and related fractions, and also in the brush border membrane fraction of rabbit kidney cortex, each of which has previously been reported by other investigators to contain non-mitochondrial anion-sensitive Mg^{2+}-ATPase activity, is due to mitochondrial contamination. In addition, the anion-sensitive Mg^{2+}-ATPase activity in rabbit erythrocyte has been shown not to be a separate enzyme but rather to form part of the ($Ca^{2+} + Mg^{2+}$)-ATPase system [13]. Since recent papers [7,8,29,30] indicate that in the pancreas the enzyme is thought to be involved in the process of bicarbonate secretion, we have made a similar study of the distribution and characteristics of the enzyme in rat pancreas.

The enzyme activity is found to be localized almost exclusively in the mitochondrial (15 min, 15 000 × *g*) fraction (Fig. 1). The fractionation method chosen is based on a technique developed by Svoboda et al. [31] for the isolation of plasma membranes from rat pancreas. With our media we find the microsomal (60 min, 100 000 × *g*) fraction to be a better source for plasma membranes than the mitochondrial fraction, considering the enrichment of

the plasma membrane marker enzymes alkaline phosphatase and $(Na^+ + K^+)$-ATPase in peak I of the microsomal fraction (cf. Figs. 2 and 3). However, no HCO_3^--stimulated Mg^{2+}-ATPase activity appears to be present in the microsomal (60 min, 100 000 × *g*) fraction, whether it is measured at pH 8.4 or pH 7.6. Hence, the enzyme does not appear to be located in the plasma membrane of rat pancreas.

This is in contrast to the findings of Simon et al. [4] who report a coincidence of alkaline phosphatase and anion-sensitive Mg^{2+}-ATPase activities in the distribution pattern of homogenates from cat and dog pancreas, resolved by centrifugation in a step-wise density gradient. In addition, these authors report that this anion-sensitive Mg^{2+}-ATPase activity does not show inhibition by oligomycin, in contrast to the activity coinciding with the highest level of succinate dehydrogenase activity. This conclusion must be considered with caution in view of our findings about the effect of EDTA on the inhibition of this enzyme activity by oligomycin (Fig. 4). Fractionation in the absence of EDTA gives weak and erratic inhibition of the anion-sensitive Mg^{2+}-ATPase activities of peak II and III of the mitochondrial fraction, but the presence of 1 mM EDTA during fractionation leads to strong and equal inhibition by oligomycin, aurovertin D and also by the rat liver mitochondrial inhibitor protein (Table I and Fig. 5). It is known that the oligomycin sensitivity of submitochondrial particles is decreased by treatment with trypsin [19] and increased by the addition of certain phospholipids [20]. Thus it seems likely that the protecting effect of EDTA on the oligomycin sensitivity of the enzyme is due to chelation of a divalent cation required by proteolytic or lipolytic enzymes present in pancreatic tissue fractions. Rutten et al. [21] have presented evidence that at the low temperatures used for cell fractionations, (phospho)lipases are still rather active in the pancreas. Milutinović et al. [7] have recently reported that in the absence of a Ca^{2+}-chelating agent the results of the fractionation of cat pancreas are rather unsatisfactory because of inactivation of hormone-stimulated adenylate cyclase during the course of the fractionation procedure. The inhibition values for oligomycin and aurovertin D in the presence of EDTA are comparable to those reported for the anion-sensitive Mg^{2+}-ATPase activities in rabbit gastric mucosa [11] and rabbit kidney [12]. The effects of the mitochondrial inhibitor protein are also comparable to the effects of a similar protein, isolated from rabbit liver mitochondria, on the microsomal anion-sensitive Mg^{2+}-ATPase activity of rabbit gastric mucosa [32].

These findings strongly indicate that these activities all derive from mitochondrial origin. On the other hand, the low density material of the microsomal fraction (subfraction 21—25 in Fig. 3), which clearly derives from plasma membranes, does not contain any bicarbonate-stimulated Mg^{2+}-ATPase activity. Why then did Milutinović et al. [7] find bicarbonate-stimulated ATPase activity in a light subfraction (d = 1.07) of cat pancreas without accompanying succinate dehydrogenase activity? A different in vivo localisation of the enzyme in rat and cat pancreas is theoretically possible, but seems unlikely to us. Due to the higher connective tissue content of the cat pancreas, they may have had to apply a more drastic homogenization to the cat pancreas than we used for the rat pancreas. This would give a more extensive destruction of organelles. Since

they pellet their fractions before the enzyme assays, this may have led to a release of succinate dehydrogenase and perhaps also of the mitochondrial ATPase inhibitor protein [16] from broken mitochondria.

Hence, we find no plasma membrane-located anion-sensitive Mg^{2+}-ATPase in rat pancreas, in contrast to previous reports for dog [4] and cat [4,7] pancreas. This makes a direct role of this enzyme in the bicarbonate secretion of the rat pancreas very improbable. The parallel distribution of whole homogenate HCO_3^--ATPase activity and secretory capacity in the ducts of the cat pancreas [3] may well be due to a parallelism between mitochondrial abundance and secretory capacity. Since the ductular cells represent only a small part of the total cell mass, a further study on the fractionation of an isolated duct cell preparation would be desirable.

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References

1 Schulz, I., Yamagata, A. and Weske, M. (1969) Pflügers Arch. 308, 277—290
2 Wizemann, V. and Schulz, I. (1973) Pflügers Arch. 339, 317—338
3 Wizemann, V., Christian, A.L., Wiechmann, J. and Schulz, I. (1974) Pflügers Arch. 347, 39—47
4 Simon, B., Kinne, R. and Sachs, G. (1972) Biochim. Biophys. Acta 282, 293—300
5 Schulz, I. (1972) in Gastric Secretion (Sachs, G., Heinz, E. and Ullrich, K.J., eds.), pp. 363—379, Academic Press, New York
6 Simon, B. and Thomas, L. (1972) Biochim. Biophys. Acta 288, 434—442
7 Milutinović, S., Sachs, G., Haase, W. and Schulz, I. (1977) J. Membrane Biol. 36, 253—279
8 Lambert, M. and Christophe, J. (1976) Arch. Int. Physiol. Bioch. 84, 1084—1085
9 Lambeth, D.O. and Lardy, H.A. (1971) Eur. J. Biochem. 22, 355—363
10 Ebel, R.E. and Lardy, H.A. (1975) J. Biol. Chem. 250, 191—196
11 van Amelsvoort, J.M.M., de Pont, J.J.H.H.M. and Bonting, S.L. (1977) Biochim. Biophys. Acta 466, 283—301
12 van Amelsvoort, J.M.M., de Pont, J.J.H.H.M, Stols, A.L.H. and Bonting, S.L. (1977) Biochim. Biophys. Acta 471, 78—91
13 van Amelsvoort, J.M.M., van Hoof, P.M.K.B., de Pont, J.J.H.H.M. and Bonting, S.L. (1978) Biochim. Biophys. Acta 507, 83—93
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
15 Nagel, W., Willig, F. and Schmidt, F.H. (1964) Klin. Wschr. 42, 447—449
16 Chan, S.H.P. and Barbour, R.L. (1976) Biochim. Biophys. Acta 430, 426—433
17 Baldwin, C.L., Weaver, L.C., Brooker, R.M., Jacobsen, T.N., Osborne, Jr., C.E., and Nash, H.A. (1964) Lloydia 27, 88—95
18 White, D.A. and Hawthorne, J.N. (1970) Biochem. J. 120, 533—538
19 Bulos, B. and Racker, E. (1968) J. Biol. Chem. 243, 3891—3900
20 Bulos, B. and Racker, E. (1968) J. Biol. Chem. 243, 3901—3905
21 Rutten, W.J., de Pont, J.J.H.H.M., Bonting, S.L. and Daemen, F.J.M. (1975) Eur. J. Biochem. 54, 259—265
22 de Haas, G.H., Postema, N.M., Nieuwenhuizen, W. and van Deenen, L.L.M. (1968) Biochim. Biophys. Acta 159, 103—117
23 Ebel, R.E. and Lardy, H.A. (1975) J. Biol. Chem. 250, 4992—4995
24 Soumarmon, A., Lewin, M., Cheret, A.M. and Bonfils, S. (1974) Biochim. Biophys. Acta 339, 403—414
25 Katz, A.J. and Epstein, F.H. (1971) Enzyme 12, 499—507
26 Inturrisi, C.E. and Titus, E. (1968) Mol. Pharmacol. 4, 591—599
27 Chan, S.H.P. and Barbour, R.L. (1976) Biochem. Biophys. Res. Commun. 72, 499—506
28 Durbin, R.P. and Kasbekar, D.E. (1965) Fed. Proc. 24, 1377—1381

29 Simon, B. and Knauf, H. (1976) Klin. Wschr. 54, 97—104
30 Koenig, C.S., Santelices, L.C. and Vial, J.D. (1976) J. Histochem. Cytochem. 24, 1065—1075
31 Svoboda, M., Robberecht, P., Camus, J., Deschodt-Lanckman, M. and Christophe, J. (1976) Eur. J. Biochem. 69, 185—193
32 Bonting, S.L., van Amelsvoort, J.M.M. and de Pont, J.J.H.H.M. (1978) Acta Physiol. Scand. Special Suppl., pp. 329—340