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Localization of ATP-Dependent Calcium Transport Activity in Mouse Pancreatic Microsomes

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Summary. Electron-dense deposits representing calcium oxalate crystals which result from ATP-dependent calcium uptake have been localized within vesicles of a heavy microsomal fraction prepared from mouse pancreatic acini. In the absence of either ATP or oxalate, no electron-dense deposits could be observed. By subfractionation of microsomes on discontinuous sucrose gradients, it could be shown that the highest energy-dependent calcium transport activity was associated with the rough endoplasmic reticulum. In rough microsomes, the $^{45}Ca^{2+}$-uptake measured was 7 times greater than that of smooth microsomes in the presence of ATP and oxalate and about 3 times greater in the presence of ATP alone. When ribosomes were released from the rough endoplasmic reticulum vesicles by treatment with KCl in the presence of puromycin, the stripped microsomes showed a 40% increase in the specific $^{45}Ca^{2+}$-uptake activity measured in the presence of ATP and oxalate and an increase of 80 to 90% in the presence of ATP alone. From these results it can be concluded that the calcium transport activity of microsomes prepared from mouse pancreatic acini is located predominately in the rough endoplasmic reticulum membrane.

Key Words: pancreas · calcium transport · rough endoplasmic reticulum

Introduction

Microsomes, isolated from mouse pancreatic acini, take up $^{45}Ca^{2+}$ by an ATP-dependent process (Lucas, Schmidt, Kromas & Loffler, 1978; Ponnappa, Dormer & Williams, 1981) probably by means of a calcium-activated ATPase located in the microsomal membrane (Ponnappa et al., 1981). This microsomal uptake system shows a K_m for Ca^{2+} of about 1 μM and is distinct from the mitochondrial Ca^{2+} uptake system in that it is not inhibited by azide or ruthenium red (Ponnappa et al., 1981). It is believed that the calcium is sequestered within the vesicular lumen, as $^{45}Ca^{2+}$-uptake is potentiated by oxalate and because calcium can be rapidly released from preloaded microsomes by addition of the calcium ionophore A23187 but not upon addition of the calcium chelator EGTA.

It is likely that this microsomal calcium transport system plays a role in the process of exocrine pancreatic stimulus-secretion coupling. Recent results showed that stimulation of intact pancreatic acini with secretagogues leads to a fall in the Ca^{2+} content of subsequently isolated microsomal vesicles (Dormer, Ponnappa & Williams, 1980) and to an increase in their ability to take up $^{45}Ca^{2+}$ (Ponnappa & Williams, 1981). The nature of the organelles in the microsomal fraction taking up Ca^{2+} is unknown, although the specific $^{45}Ca^{2+}$-uptake activity was much higher in a heavy as compared to a light microsomal fraction.

The present study was designed to further identify the microsomal component associated with calcium transport activity. The first approach made use of the fact that in vesicles of sarcoplasmic reticulum, intravesicular calcium oxalate precipitates could be visualized in the electron microscope (Agostini & Hasselbach, 1971*a, b*). In a second approach, a total microsomal fraction was subfractionated on discontinuous sucrose gradients to separate rough from smooth membrane vesicles, and $^{45}Ca^{2+}$-uptake activity was determined in both fractions. Both approaches indicated that the microsomal $^{45}Ca^{2+}$-uptake activity was predominantly associated with vesicles of rough endoplasmic reticulum.

Materials and Methods

Chemicals

The following chemicals were obtained from Sigma Chemical Corp., St. Louis, Mo.: soybean trypsin inhibitor type 1-S; puromycin, sodium azide, aprotinin, DL-dithiothreitol, benzamidine, phenylmethylsulfonylfluoride, orcinol, yeast RNA and ATP (disodium salt from equine muscle, vanadium free). $^{45}CaCl_2$ (33 mCi/mg) and butyl-PBD (scintillation grade) were purchased from New England Nuclear, Boston, Mass.; chromato-

graphically purified collagenase and purified chymotrypsin from Worthington Biochemical Corp., Freehold, N.J.; bovine serum albumin from Miles Laboratories, Elkhart, Ind.; minimal Eagle's medium essential amino acid supplement from GIBCO, Grand Island, NY.; and Bio-Rad protein assay dye reagent concentrate from Bio-Rad Laboratories Corp., Richmond, Calif.

Preparation and Incubation of Acini

Pancreatic tissue was obtained from male White Swiss mice, weighing 20 to 25 g, which were fasted for 16 to 18 hr prior to decapitation. Isolated pancreatic acini were prepared by a modification of a previously described enzymatic digestion method (Williams, Korc & Dormer, 1978). Briefly, the digestive enzyme solution consisted of a modified Krebs-Henseleit bicarbonate buffer (KHB) as specified below, but with the $CaCl_2$ concentration reduced to 0.1 mM, to which was added 0.11 mg/ml purified collagenase, 0.015 mg/ml chymotrypsin and 2 mg/ml bovine serum albumin. The enzyme solution (5 ml/g pancreas) was injected into pancreatic parenchyma by means of a 27 gauge needle and replaced after 10 min with an equal volume of fresh medium. After incubation at 37 °C for a total of 50 min with shaking (120 cycles/min), acini were dissociated by repeated passage through polypropylene pipettes of decreasing orifice and purified by centrifugation through KHB containing 4% bovine serum albumin. The isolated acini were incubated for 60 min in KHB prior to homogenization. The composition of the KHB was (in mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, Na_2HPO_4 1, $MgCl_2$ 1.13, $CaCl_2$ 1.28, and glucose 5.5. The medium was supplemented with 0.1 mg/ml soybean trypsin inhibitor, 1% bovine serum albumin and minimal Eagle's medium essential amino acid supplement (Williams et al., 1978), and was equilibrated with a mixture of 95% O_2/5% CO_2.

Preparation of Microsomal Membranes

The acinar suspension was centrifuged (50 g for 3 min), and the pellet washed once in KHB without $CaCl_2$ before resuspension in 10 volumes of ice-cold homogenization medium of the following composition: sucrose, 0.3 M; Hepes-NaOH (pH 7.0 at 4 °C), 10 mM; dithiothreitol (DTT), 2 mM; and the protease inhibitors benzamidine, 1 mM, phenylmethylsulfonylfluoride (PMSF), 0.5 mM (in 0.5% ethanol), and 150 to 200 units/ml of the kallikrein inhibitor, aprotinin. Homogenization was carried out by 30 strokes of a motor-driven Potter-Elvehjem homogenizer. The homogenate was first centrifuged at $1000 \times g$ for 10 min and subsequently at $12{,}000 \times g$ for 10 min to remove all cell debris, zymogen granules, nuclei and mitochondria (Dormer et al., 1980). The resultant postmitochondrial supernatant was subjected to one of the following procedures:

1) Centrifugation at $27{,}000 \times g$ for 10 min to obtain a heavy microsomal fraction, which contains mainly rough microsomes (Ponnappa et al., 1981).

2) Centrifugation at $150{,}000 \times g$ for 30 min to obtain total microsomes.

3) Centrifugation at $100{,}000 \times g$ for 60 min of a gradient prepared by layering 4 ml of postmitochondrial supernatant on top of a 2-ml cushion of 1.3 M sucrose, buffered with 10 mM Hepes and supplemented with 1 mM $MgCl_2$, 2 mM DTT and protease inhibitors. Purified rough microsomes were obtained as a pellet at the bottom of the tube (Walter, Ibrahimi & Bloble, 1981).

Total microsomes, as prepared by the second procedure, were used to separate rough from smooth microsomes according to Tartakoff and Jamieson (1974) with slight modifications. The microsomal pellet was resuspended in 1.1 ml of 1.25 mM sucrose buffered with 10 mM Hepes and supplemented with DTT, PMSF and benzamidine by three strokes of a motor-driven homogenizer. The following discontinuous sucrose gradient was then prepared which contained from top to bottom: 1.25 ml 0.3 M sucrose, 1 ml 1.2 M sucrose, 0.5 ml 1.25 M sucrose plus microsomes, 1.25 ml 1.35 M sucrose and 1 ml 2 M sucrose. The gradient was centrifuged in a swinging bucket rotor (type SW 50.1) in a Beckman ultracentrifuge (type L8–70) at $233{,}000 \times g$ for 90 min. The smooth microsomes were collected at the 0.3 to 1.2 M sucrose interface, and the rough microsomes at the 1.35 to 2 M sucrose interface. The material at each interface was diluted to 0.3 M sucrose, and smooth microsomes pelleted by centrifugation at $100{,}000 \times g$ for 60 min. Rough microsomes were either pelleted the same way or by centrifugation at $48{,}000 \times g$ for 30 min; the latter procedure reduced the amount of protein, but did not affect the ratio between RNA content and protein, nor the calcium transport activity.

In some experiments purified rough microsomes were suspended for 15 min in 0.3 M sucrose medium containing in addition 500 mM KCl and 1 mM puromycin, a treatment known to remove ribosomes from rough endoplasmic reticulum in a nondestructive manner (Adelman, Sabatini & Bloble, 1973). The stripped membranes were collected by centrifugation at $48{,}000 \times g$ for 30 min and the pellet resuspended in homogenization medium.

Measurement of $^{45}Ca^{2+}$-Uptake

$^{45}Ca^{2+}$-uptake into microsomes was measured as described previously (Ponnappa et al., 1981). Briefly, microsomes (10 to 90 μg protein) were incubated at 37 °C in 1 ml of the following medium (in mM): KCl 100, imidazole 20 (pH 6.8 at 37 °C), $MgCl_2$ 5, ATP 5 (adjusted to pH 6.8 with imidazole), with or without ammonium oxalate, 5 mM; $CaCl_2$, 20 to 100 μM; and $^{45}Ca^{2+}$, 0.2 μCi/ml. At specified incubation times $^{45}Ca^{2+}$-uptake was terminated by filtering the samples under vacuum through 0.45 μM membrane filters (HAWP, Millipore, Boston, Mass.), which were presoaked with medium containing 100 mM KCl, 20 mM imidazole (pH 6.8) and 5 mM $MgCl_2$. The filters were washed once with 5 ml of the same buffer and then dried at 60 °C for 45 min in scintillation vials. The radioactivity was determined by liquid scintillation spectrophotometry using a scintillation cocktail of 4 g/liter butyl-PBD in toluene. Background radioactivity due to $^{45}Ca^{2+}$ in the medium in the absence of microsomes was determined similarly and subtracted from the experimental samples.

For the ultrastructural localization of calcium oxalate crystals, the heavy microsomes prepared by method 1 were allowed to take up calcium for 60 min from the above medium containing 100 μM Ca^{2+}. Uptake was terminated by centrifugation at $12{,}000 \times g$ for 10 min. The supernatant was discarded and the pellet prepared for electron microscopy.

Electron Microscopic Procedures

Smooth, rough or heavy microsomal pellets were fixed for 30 min with 2 ml of 1.5% glutaraldehyde, 1% paraformaldehyde in 0.08 M sodium cacodylate buffer (pH 7.4). They were then rinsed twice for 1 min with cacodylate buffer and postfixed with 2% osmium tetroxide in 0.08 M sodium cacodylate buffer for 30 min all at 4 °C. The fixed pellets were rinsed again and then dehydrated through graded ethanol water mixtures at room temperature (70% ethanol, 5 min; 95% ethanol, 5 min; 100% ethanol, 3×10 min) followed by propylene oxide for 15 min and a mixture of propylene oxide-araldite (1:1) for 1 hr after which they were embedded in British araldite.

For fixation of microsomes loaded with calcium oxalate, all solutions through to 70% ethanol contained 10 mM ammonium oxalate to prevent the loss of calcium oxalate crystals from inside the vesicles. Ammonium oxalate was also added to the water bath on the surface of which gold thin sections were collected for electron microscopy.

The thin sections were either double stained with 4% uranyl acetate in 50% methanol and 4% lead citrate or left unstained. They were examined in a JEOL JEM 100 B electron microscope at 60 kV.

Gel Electrophoresis and Assays

SDS polyacrylamide gel electrophoresis utilized the system of Laemmli (1970) as modified to include a 10 to 18% acrylamide exponential gradient as the separating gel (Burnham & Williams, 1982). Proteins were stained with 0.2% coomassie brilliant blue R in glacial acetic acid/methanol/water (7:50:43). RNA was extracted from cell fractions according to the procedure of Munro and Fleck (1966) and determined as described by Kerr and Seraidarian (1945). Protein was estimated by the method of Bradford (1976).

Results

Calcium Localization

For the cytochemical localization of calcium oxalate crystals associated with vesicles of the endoplasmic reticulum, heavy microsomes were incubated in the presence of 100 μM calcium. This was the highest extracellular calcium concentration that could be used without the formation of calcium oxalate precipitates in the medium. Under these conditions calcium uptake was linear over a time period of about 60 min with a mean rate of 25 nmol·mg protein^{-1}·min^{-1} (Fig. 1) after which calcium uptake tended to level off. For an optimal loading of microsomes with calcium oxalate, therefore, a 60-min uptake period was used at which time the microsomes contained about 1.5 μmol Ca^{2+}/mg protein. Figure 2 shows an electron micrograph of heavy microsomes incubated in the presence of ATP alone (Fig. 2*A* and 2*C*) and in the presence of both ATP and oxalate (Fig. 2*B* and 2*D*). Microsomes incubated with oxalate but without ATP were essentially similar to those incubated with ATP but without oxalate. Only the incubation of microsomes in the combined presence of calcium, ATP and oxalate led to the appearance of dark needlelike or hexagonal structures, sometimes containing holes which gave them a Swiss-cheese appearance. When thin sections were floated on water without oxalate, the electron-dense material disappeared and was replaced by similarly shaped holes. In other microsomal preparations from muscle, similar deposits have been identified as calcium oxalate precipitates (Greaser, Cassens, Hoekstra & Briskey, 1969; Agostini & Hasselbach, 1971*b*; Carsten & Reedy, 1971).

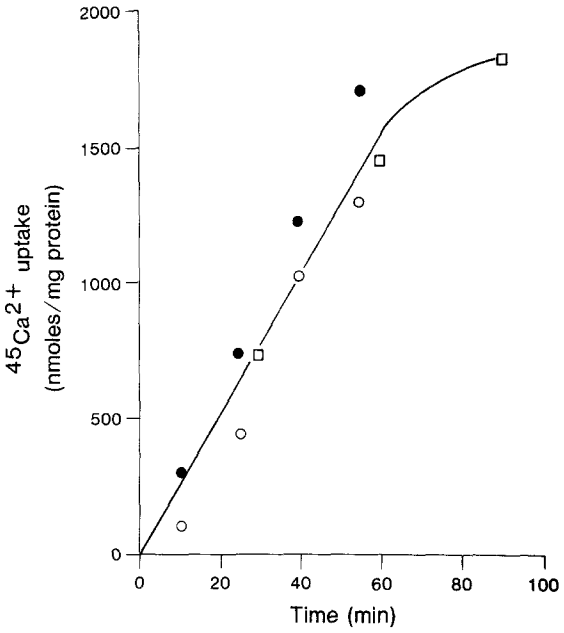

Fig. 1. Time course of ATP-dependent $^{45}Ca^{2+}$-uptake by heavy microsomes prepared from mouse pancreatic acini. The total calcium concentration was 100 μM and oxalate was present at a concentration of 5 mM. Different symbols (○ □ ●) are from three different experiments, in each of which, each point represents duplicate determinations

These calcium oxalate crystals are much better visualized in unstained sections, as can be seen from Fig. 2*D*, although the identification of the surrounding vesicular membranes is somewhat difficult. Staining of the sections led to a loss of calcium oxalate crystals and seemed to have some destructive effects on the membrane themselves (Fig. 2*B*). As the heavy microsomal vesicles consist mainly of rough endoplasmic reticulum, as can be most clearly seen from stained sections, we conclude that it is likely that rough microsomal vesicles rather than a small subpopulation of some other contaminating membrane vesicles have the ability to sequester $^{45}Ca^{2+}$.

We attempted to use the increased density of vesicles loaded with calcium oxalate to purify the Ca^{2+} transporting vesicles. When postmitochondrial supernatant was incubated with calcium, ATP and oxalate and then centrifuged at 12,000 × *g* for 10 min, the pellet was enriched for Ca^{2+}-uptake. However, the specific activity of $^{45}Ca^{2+}$-uptake was not greater than if heavy microsomes were first prepared by sedimentation at 27,000 × *g* for 10 min and then allowed to take up $^{45}Ca^{2+}$ under comparable conditions. This lack of further enrichment was due to the tendency of microsomes

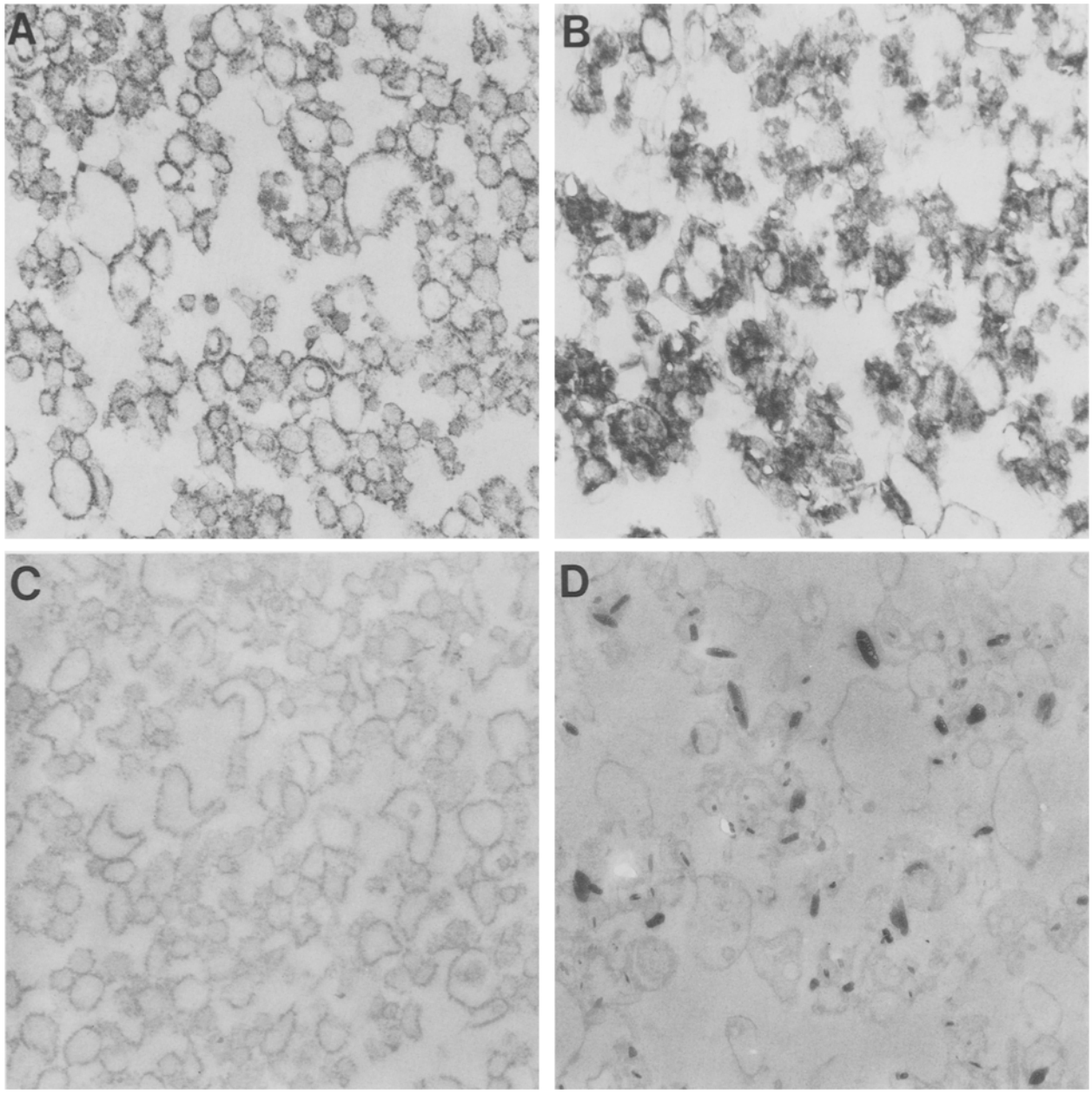

Fig. 2. Electron micrograph of heavy microsomes incubated with calcium in the presence or absence of oxalate. Heavy microsomes were incubated for 1 hr in the presence of 5 mM ATP and 100 μM $CaCl_2$ (*A* and *C*) or in similar medium containing in addition 5 mM oxalate (*B* and *D*). *A* and *B* were stained with lead citrate and uranyl acetate while *C* and *D* were unstained sections. Magnification 32,500 ×

to aggregate in the presence of Ca^{2+}, ATP and oxalate which could be visualized by negative staining of vesicles with uranyl acetate (*not shown*).

$^{45}Ca^{2+}$-Uptake into Fractions of Rough and Smooth Endoplasmic Reticulum

To better isolate those membranes associated with calcium transport activity, a total microsomal preparation was further subfractionated by the use of a discontinuous sucrose sandwich gradient to obtain fractions enriched in rough and smooth microsomal vesicles (Fig. 3). The rough microsomal fraction consisted of a homogenous population of vesicles studded with ribosomes. The smooth microsomal fraction was more heterogeneous including completely smooth membrane vesicles, flattened golgi cisternae and vesicles with a few at-

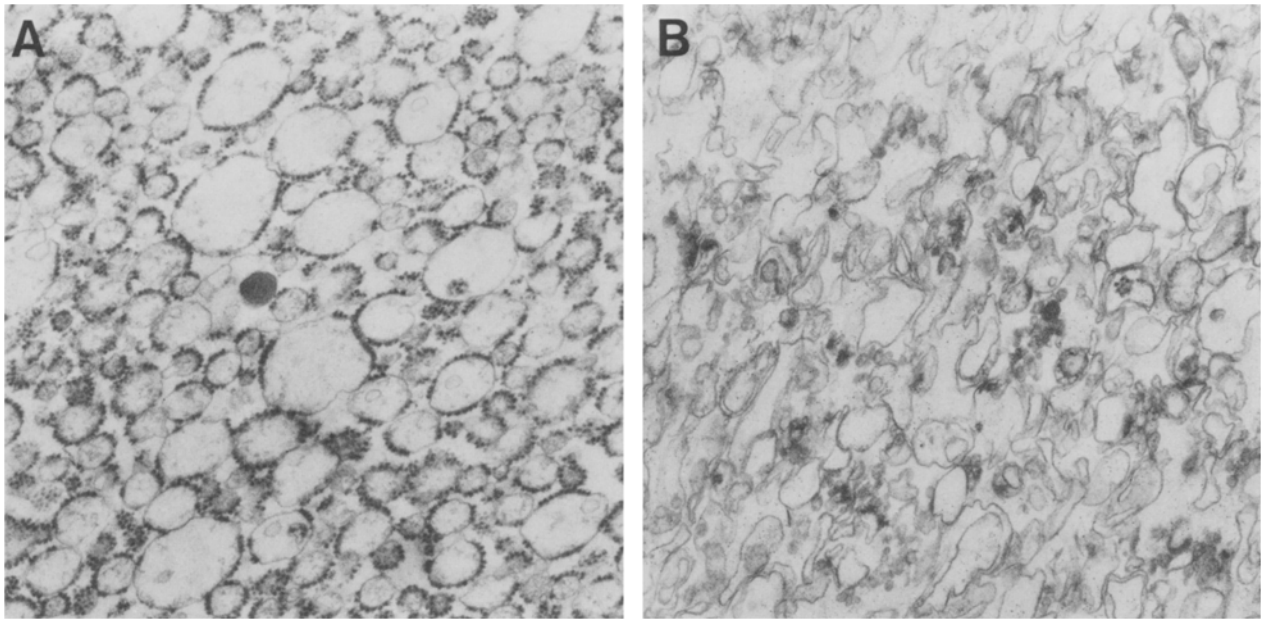

Fig. 3. Electron micrograph of rough (*A*) and smooth (*B*) microsomes prepared from mouse pancreatic acini. Magnification 32,500 ×

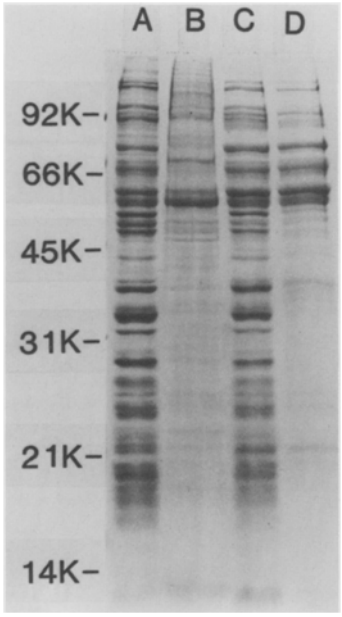

Fig. 4. SDS gel electrophoresis protein binding pattern of pancreatic acinar microsomal fractions. *A*. Total microsomes. *B*. Smooth microsomes. *C*. Rough microsomes. *D*. Rough microsomes treated with 0.5 M KCl – 1 mM puromycin. The position of molecular weight markers (Bio-Rad) are indicated

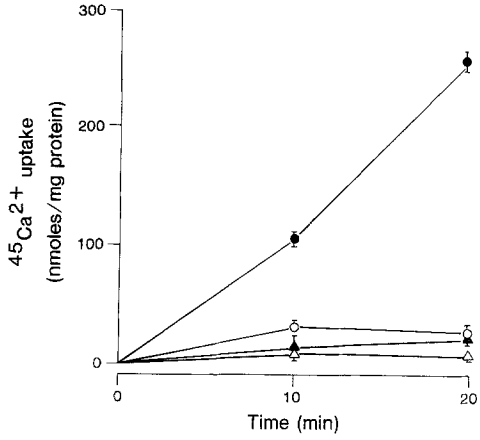

Fig. 5. Time course of $^{45}Ca^{2+}$-uptake by rough and smooth pancreatic microsomes. Circles, rough; triangles, smooth. Closed symbols, 5 mM oxalate present; open symbols, no oxalate present. All values are mean ± SE of three experiments, in each of which, each point was measured in triplicate

tached ribosomes. The ratio of RNA to protein was consistent with the morphological distinction being 0.64 ± 0.04 mg RNA/mg protein in the rough microsomal fraction compared to only 0.19 ± 0.04 mg RNA/mg protein in the fraction enriched in smooth membrane vesicles (mean ± SE, $n = 6$). Rough and smooth microsomal fractions also showed distinct protein banding patterns following SDS polyacrylamide gel electrophoresis (Fig. 4).

Figure 5 shows a typical time course of $^{45}Ca^{2+}$-uptake into rough and smooth microsomal vesicles. In the presence of ATP and oxalate $^{45}Ca^{2+}$-uptake into both fractions was almost linear over the 20-min time period tested. The calcium uptake activity in the fraction enriched in

smooth endoplasmic reticulum, however, was only 15.2 ± 2.0 nmol/mg protein after 10 min (mean $\pm$ SE, $n = 6$), about 7 times less than that of 106.8 ± 3.5 nmol/mg protein (mean $\pm$ SE, $n = 6$) measured in the rough endoplasmic reticulum. In the absence of oxalate, the ATP-dependent $^{45}Ca^{2+}$-uptake was already maximal at the 10-min time point. The mean values for $^{45}Ca^{2+}$-uptake measured were 30.1 ± 0.8 nmol/mg protein (mean $\pm$ SE, $n = 6$) in the rough and 9.8 ± 1.1 nmol/mg protein (mean $\pm$ SE, $n = 6$) in the smooth endoplasmic reticulum, which represents a threefold increase in calcium transport activity in rough microsomes.

$^{45}Ca^{2+}$-Uptake into Rough Microsomes Stripped of Ribosomes

From experiments investigating the transfer of proteins across microsomal membranes, it is known that ribosomes can be released from rough endoplasmic reticulum by treatment with high salt and puromycin (Adelman et al., 1973). It was of interest to see whether such ribosome-free membranes would still take up $^{45}Ca^{2+}$ and to determine if the specific activity of $^{45}Ca^{2+}$-uptake would be increased as a result of reducing the amount of protein not associated with calcium transport activity. Since the high salt treatment prolongs the isolation of rough and smooth membranes, and as it is rather difficult to preserve the $^{45}Ca^{2+}$-uptake activity for a long time, a shorter procedure for the isolation of rough microsomes was used (Walter et al., 1981).

Table. $^{45}Ca^{2+}$-uptake and RNA content of control and KCl-puromycin treated rough microsomes

	Prepared from total microsomes by sucrose sandwich gradient	Prepared directly from post-mitochondrial supernatant
$^{45}Ca^{2+}$-uptake (nmol/mg protein 10 min)		
Control:		
(ATP + oxalate)	108.3 ± 5.2 (4)	113.2 ± 3.2 (6)
(ATP alone)	30.3 ± 1.2 (4)	28.6 ± 1.3 (6)
KCl-puromycin:		
(ATP + oxalate)	151.9 ± 36.7 (4)	158.5 ± 3.9 (4)
(ATP alone)	53.3 ± 14.4 (4)	56.6 ± 1.4 (4)
µg RNA/µg protein		
Control	0.636 ± 0.04 (6)	0.571 ± 0.02 (4)
KCl-puromycin	0.264 ± 0.03 (4)	0.208 ± 0.03 (4)

All values are the mean $\pm$ SE of the number of preparations shown in parentheses, in each of which $^{45}Ca^{2+}$-uptake, RNA and protein were determined in duplicate.

The postmitochondrial supernatant was directly layered on top of a cushion of 1.3 M sucrose medium, and centrifuged at $100{,}000 \times g$ for 60 min. The rough microsomes could then be collected as a pellet at the bottom of the centrifuge tube. The ATP and oxalate-dependent specific $^{45}Ca^{2+}$-uptake activity into rough microsomes prepared by the sucrose sandwich gradient or the simpler sucrose gradient was the same (Table). If the rough microsomes were exposed for 15 min to sucrose medium containing 500 mM KCl and 1 mM puromycin, the subsequent $^{45}Ca^{2+}$-uptake into ribosome-depleted microsomes was increased by 40% in the presence of ATP and oxalate and between 80 and 90% in the presence of ATP alone (Table). The RNA-to-protein ratio of the stripped rough microsomes was decreased by a factor of 2.5, suggesting that at least part of the ribosomes had been released. In support of this, the SDS gel electrophoresis pattern (Fig. 4) showed a greatly simplified pattern in the stripped rough microsomes.

Discussion

It has recently become apparent that nonmuscle cells possess at least two distinct Ca^{2+} sequestering mechanisms. Besides mitochondria whose Ca^{2+}-uptake can be blocked with ruthenium red or azide (for review, *see* Bygrave, 1978), a nonmitochondrial system has been described in synaptosomes (Blaustein, Ratzlaff, Kendrick & Schweitzer, 1978; Rahamimoff & Abramovitz, 1978) and squid axons (Henkart, Reese & Brinley, 1978). ATP-dependent uptake with properties similar to the nonmitochondrial system has also been observed in crude microsomal fractions prepared from salivary gland (Alonso, Bazerque, Arrigo & Tumilasci, 1971), kidney (Moore, Fitzpatrick, Chen & Landon, 1974), liver (Moore, Chen, Knapp & Landon, 1975), adipocytes (Bruns, McDonald & Jarett, 1976), platelets (Käser-Glanzmann, Jakábová, George & Luscher, 1977), and pancreas (Ponnappa et al., 1981). In most of the above cases, the component of the microsomal fraction involved in Ca^{2+}-uptake has not been identified although many of the authors have assumed it to be smooth endoplasmic reticulum. By contrast, a coated vesicle preparation prepared from posterior pituitary was also shown to take up $^{45}Ca^{2+}$ (Torp-Pedersen, Saermark, Bundgaard & Thorn, 1980).

In the present study, we were able to visualize the site of Ca^{2+}-uptake by mouse pancreatic microsomes by making use of the fact that calcium taken up in the presence of oxalate forms electron-dense deposits as first shown in sarcoplasmic re-

ticulum vesicles by Hasselbach (1964). After incubating pancreatic heavy microsomes for 1 hr in 100 μM Ca^{2+} they had accumulated about 1.5 μmol Ca^{2+}/mg protein, and calcium oxalate crystals could be visualized in about 50% of the microsomal vesicles. The general appearance of these deposits was similar to those in the studies with skeletal muscle (Hasselbach, 1964) and cardiac muscle (Carsten & Reedy, 1971) microsomes. The lack of calcium oxalate crystals in the absence of ATP strongly supports the hypothesis that calcium is only precipitated by oxalate inside the lumen of the microsome after its energy-dependent transport across the vesicle membrane.

Since the heavy microsome fraction is composed predominantly of rough endoplasmic reticulum vesicles, we expected to observe either widespread small electron-dense deposits in a large number of vesicles, or much more highly concentrated deposits in a small subpopulation of smooth vesicles. The former appeared to be the case. Although not all vesicles contained oxalate deposits, the absence of visible deposits in some vesicles could be due to the plane of sectioning or to the fact that those vesicles were leaky.

That the majority of the pancreatic microsomal Ca^{2+}-uptake is present in rough microsomes was also indicated by the experiments in which a total microsomal pellet was prepared and then fractionated into rough and smooth vesicles on the basis of density. A clear distinction could be made both on morphological grounds and the difference in RNA-to-protein ratios which was 3 times higher in rough than in smooth microsomes. This is less than the sevenfold difference reported for guinea pig pancreas (Meldolesi, Jamieson & Palade, 1971; Tartakoff & Jamieson, 1974). While the relatively high RNA content of our smooth microsomes could be due to the species, it more likely is due to the fact that the separation procedure was shorter than the one described by Tartakoff and Jamieson (1974).

In any case, our results clearly indicate that for pancreatic acinar cell microsomes, both the specific activity and total Ca^{2+} sequestering capacity is much greater for rough microsomes. The Ca^{2+}-uptake activity that is present in the smooth microsomes could be due to contaminating rough microsomes or be intrinsic to smooth vesicles such as those from the Golgi or plasma membrane. A low ATP-dependent $^{45}Ca^{2+}$-uptake activity into plasma membrane vesicles isolated from mouse pancreatic acini has been reported (Ponnappa et al., 1981).

The high specific $^{45}Ca^{2+}$-uptake activity in the purified rough microsome fraction together with the increase in calcium transport activity upon removal of ribosomes, strongly supports the contention that it is the rough endoplasmic reticulum that sequesters calcium in an energy-dependent manner. Similarly, in submaxillary gland microsomes, Ca^{2+}-uptake was by microsomes which appeared morphologically to be almost entirely rough (Alonso et al., 1971). Since in muscle, and probably liver (Moore et al., 1975), Ca^{2+}-uptake is by smooth microsomes, this suggests that in different cells, different components may act as a nonmitochondrial Ca^{2+} buffer.

The importance of the endoplasmic reticulum, whether smooth or rough, as the organelle that is involved in the regulation of calcium-dependent processes such as excitation-contraction coupling in muscle (Hasselbach, 1964; Greaser et al., 1969) and stimulus-secretion coupling in glands (Alonso et al., 1971; Sehlin, 1976; Dormer et al., 1980; Ponnappa et al., 1981) is well documented. However, as the calcium transport activity in the pancreas is localized in the membrane of the rough endoplasmic reticulum, further work is necessary to determine whether there may be a link between calcium sequestration and other functions of the endoplasmic reticulum, such as protein synthesis.

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