Partial purification of $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -dependent ATPase from pig smooth muscle and reconstitution of an ATP-dependent Ca^{2+} -transport system

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$(\text{CaMg})\text{ATPase}$ [$(\text{Ca}^{2+} + \text{Mg}^{2+})$ -dependent ATPase] was partially purified from a microsomal fraction of the smooth muscle of the pig stomach (antrum). Membranes were solubilized with deoxycholate, followed by removal of the detergent by dialysis. The purified $(\text{CaMg})\text{ATPase}$ has a specific activity (at 37°C) of 157 ± 12.1 (7) $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ of protein, and it is stimulated by calmodulin to 255 ± 20.9 (7) $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$. This purification of the $(\text{CaMg})\text{ATPase}$ results in an increase of the specific activity by approx. 18-fold and in a recovery of the total enzyme activity of 55% compared with the microsomal fraction. The partially purified $(\text{CaMg})\text{ATPase}$ still contains some Mg^{2+} - and $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase activities, but their specific activities are increased relatively less than that of the $(\text{CaMg})\text{ATPase}$. The ratios of the $(\text{CaMg})\text{ATPase}$ to Mg^{2+} - and $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase activities increase from respectively 0.14 and 0.81 in the crude microsomal fraction to 1.39 and 9.07 in the purified preparation. During removal of the deoxycholate by dialysis, vesicles were reconstituted which were capable of ATP-dependent Ca^{2+} transport.

Ca^{2+} plays an important role in the regulation of smooth-muscle contraction (Nonomura & Ebashi, 1980). The estimated amount of Ca^{2+} that has to be removed from the cytoplasmic compartment in order to achieve full relaxation, as well as the gradients against which this Ca^{2+} is transported, are similar in smooth muscle and in skeletal muscle (van Breemen, 1977). Therefore an active Ca^{2+} -transport system must be present in smooth muscle. Because of its slower relaxation, however, it can be assumed that the rate of removal of Ca^{2+} from the cytoplasm will be lower in smooth muscle than in fast skeletal muscle. We have previously shown that smooth-muscle microsomes (microsomal fraction) can accumulate Ca^{2+} in an ATP-dependent way (Raeymaekers *et al.*, 1977; Wuytack *et al.*, 1978) and that the $(\text{CaMg})\text{ATPase}$ activity present in this microsomal fraction might be related to the Ca^{2+} -transport system (Wuytack & Casteels, 1980). We now report a partial purification of this $(\text{CaMg})\text{ATPase}$ from a smooth-muscle microsomal fraction. This purification relies on the use of deoxycholate and is comparable with the method used by Meissner & Fleischer (1974) on fragmented sarcoplasmic reticulum of skeletal muscle. This technique has also

been applied to the partial purification and reconstitution of the Ca^{2+} -transport system in erythrocytes (Gietzen *et al.*, 1980). By removing carefully the deoxycholate from the solubilized $(\text{CaMg})\text{ATPase}$ of smooth muscle, vesicles were reconstituted which are capable of ATP-dependent Ca^{2+} transport.

Methods

Preparation of the microsomal fraction

Although pig antral smooth muscle is a less pure smooth-muscle tissue than the media-intima preparation of the pig coronary artery routinely used in our laboratory (Wuytack *et al.*, 1978), we have selected this tissue for the purification of $(\text{CaMg})\text{ATPase}$ because it can easily be obtained in large quantities. Antral parts of pig stomachs were obtained at the slaughterhouse and transported to the laboratory with the mucosal side resting on ice. In the laboratory the mucosa was removed by scissors, together with fat, blood vessels and mesenteric membranes. The remaining smooth muscle was cut into strips approx. 1 cm wide, which were quickly frozen in liquid nitrogen, and stored at -80°C . Freezing of the tissues allowed us to collect more material before starting the purification of the $(\text{CaMg})\text{ATPase}$.

For homogenization, approx. 100 g of frozen

Abbreviations used: $(\text{CaMg})\text{ATPase}$, $(\text{Ca}^{2+} + \text{Mg}^{2+})$ -dependent ATPase, $(\text{NaK})\text{ATPase}$, $(\text{Na}^+ + \text{K}^+)$ -dependent ATPase; MgATPase , Mg^{2+} -dependent ATPase.

tissue was thawed in 7 vol. of homogenization medium, containing 100 mM-sucrose, 0.5 mM-phenylmethanesulphonyl fluoride, 0.1 mM-7-amino-1-chloro-3-L-*p*-tosylamidoheptan-2-one ('TLCK'), 0.5 mM-benzamidine and 1 mM-dithiothreitol. The thawed tissues were taken out of the medium and passed through a tissue press, equipped first with a sieve plate with 1 mm holes and then with a sieve plate with 0.5 mm holes.

The material squeezed through the press was returned to the homogenization medium and homogenized in a blender (Braun type MX32GR; Braun A.G., Frankfurt, West Germany) for 20 s at maximal speed. Then the homogenate was given two up-and-down strokes of the pestle in a glass/Teflon Potter homogenizer (type S, 60 ml; B. Braun, Melsungen, West Germany) at 1500 rev./min. This homogenate was centrifuged in a Sorvall GS3 rotor at 6500 rev./min (7150 $g_{\max}$) for 15 min. The pellets and floating material were removed and the supernatant was again centrifuged in a Sorvall GSA rotor at 13000 rev./min for 20 min (27600 $g_{\max}$). The supernatant was then centrifuged in a Beckman type 19 rotor at 19000 rev./min (53700 $g_{\max}$) for 150 min and the pellet was resuspended in 40 ml of 0.1 M-sucrose with a Teflon/glass Potter homogenizer. A sample of this suspension was taken for further analysis (P_{crude}).

Extraction of the microsomal fraction with KCl

This crude microsomal fraction was mixed with an equal volume of 1.4 M-KCl in 10 mM-imidazole/HCl, pH 6.8, and gently stirred at 4°C for 1 h. The suspension was then centrifuged in a Kontron (Kontron, Zürich, Switzerland) TFT 50.38 rotor at 35000 rev./min (150000 $g_{\max}$) for 35 min, and the pellet resuspended with a Teflon/glass Potter homogenizer in 10 ml of 0.25 M-sucrose. This KCl-extracted fraction (P_{KCl}) was kept overnight at 4°C for extraction with deoxycholate the next day.

Solubilization of the microsomal fraction with deoxycholate

After measurement of the protein concentration in the P_{KCl} fraction, the microsomes were centrifuged for 30 min in a Beckman Ti 75 rotor at 50000 rev./min (223800 $g_{\max}$) and the pellet was resuspended in a volume of extraction buffer [10 mM-Tris/HCl (pH 7.9)/400 mM-sucrose/450 mM-KCl/1 mM-EDTA/1.5 mM-MgCl₂/0.1 mM-CaCl₂/1 mM-dithiothreitol] to obtain a calculated protein concentration of 10 mg/ml. The resuspended microsomes were distributed over a series of tubes and to each tube an equal volume of a deoxycholate solution in extraction buffer was added. The detergent/protein ratio obtained varied between 1:50 and 2:1 (w/w). The tubes were stirred slowly with a magnetic stirrer for 10 min at 4°C, and the unsolubilized material

was removed by centrifugation for 30 min in a Beckman Ti 75 rotor at 50000 rev./min (223800 $g_{\max}$).

Removal of the deoxycholate and membrane reconstitution

A sample of the supernatant (usually 1.5 ml) was dialysed against a large volume of dialysis buffer {10 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (pH 7.0)/250 mM-sucrose/400 mM-KCl/1 mM-EDTA/1.5 mM-MgCl₂/0.1 mM CaCl₂/1 mM-dithiothreitol}, first for 4 h at 22°C and thereafter overnight at 4°C. The dialysed fractions were diluted with 4 vol. of 0.25 M-sucrose and centrifuged for 30 min in the Beckman Ti 75 rotor at 50000 rev./min (223800 $g_{\max}$). The pellets were resuspended in 0.25 M-sucrose with a glass/Teflon Potter homogenizer.

Assays

ATPases were measured by using a coupled enzyme assay in which the ATP hydrolysis is coupled to NADH oxidation (Wuytack & Casteels, 1980). The reaction medium (1 ml) contained: 100 mM-KCl, 30 mM-imidazole/HCl (pH 6.8), 5.7 mM-MgCl₂, 5 mM-disodium ATP, 5 mM-Na₃, 1 mM-EGTA, 1.5 mM-phosphoenolpyruvate, 0.26 mM-disodium NADH, pyruvate kinase (40 units/ml), lactate dehydrogenase (36 units/ml) (both enzymes from rabbit muscle). After thermal equilibration, a small sample of ATPase was added to the cuvette to start the reaction. The decrease in A_{340} was recorded. Subsequently, each time after an interval of 2 min, 0.1 mM-ouabain, 0.87 mM-CaCl₂ and calmodulin (10 µg/ml) were added. The (NaK)-ATPase activity was calculated from the difference in slope before and after addition of ouabain, that of MgATPase from the slope in the presence of ouabain and that of (CaMg)ATPase from the difference in the slope before and after addition of CaCl₂. The free Ca²⁺ concentration in this condition amounted to 10 µM. A molar absorption coefficient, ϵ , of 6200 M⁻¹·cm⁻¹ was used.

Ca²⁺ uptake was measured by using the Millipore filtration method at [Ca²⁺] = 10 µM, as described by Wuytack & Casteels (1980). (CaMg)ATPase and Ca²⁺ uptake were determined at 37°C.

Electrophoresis

Dodecyl sulphate/polyacrylamide-gel electrophoresis was done in a discontinuous buffer system as described by Laemmli (1970), with a stacking gel of 3% and a separating gel of 10% (w/v) acrylamide.

Protein

Protein was measured by the method of Lowry *et al.* (1951). To remove interference from buffers and

other additives with this technique, proteins were precipitated with trichloroacetic acid, followed by resolubilization in NaOH. Bovine serum albumin was used as a standard.

Materials

Deoxycholate (sodium salt), benzamidine hydrochloride, dithiothreitol and NADH were obtained from Sigma, 7-amino-1-chloro-3-L-*p*-tosylamidoheptan-2-one was from Serva, and phenylmethanesulphonyl fluoride, pyruvate kinase and lactate dehydrogenase were from Boehringer. Calmodulin was prepared from bovine brain as described by Sharma & Wang (1979).

Results

Microsomal fractions from smooth muscle of the antral part of the pig stomach have an ATP-dependent Ca^{2+} uptake which is stimulated by the presence of oxalate (Fig. 1), and also a (CaMg)-ATPase activity (Table 1).

Effect of KCl extraction on the microsomal fraction

Some 60% of the proteins present in the microsomal fraction appear to be not, or only loosely, associated with the membranes and can therefore easily be removed by a single wash in 0.7 M-KCl (see the Methods section). The specific activities of Ca^{2+} transport and of (NaK)ATPase, MgATPase and (CaMg)ATPase increase accordingly (Table 1).

Solubilization of the microsomal fraction by deoxycholate

KCl-extracted microsomes (P_{KCl}) were solubilized with deoxycholate at detergent/protein ratios between 1:50 and 2:1 (w/w), as described for fragmented sarcoplasmic reticulum by Meissner & Fleischer (1974). Fig. 2 gives values for the protein and for the total activity (in $\text{nmol} \cdot \text{min}^{-1}$) of the different ATPases solubilized from 10 mg of protein of P_{KCl} fraction as a function of the deoxycholate/protein ratio (w/w). The values given in Fig. 2 apply to the fractions after removal of detergent by dialysis, as described in the Methods section. The values for protein and for total activities of ATPases can be compared with the corresponding values for protein and total activity before extraction, which are also given in the legend of Fig. 2.

The following conclusions can be drawn.

(1) By increasing the deoxycholate/protein ratio, increasing amounts of protein are solubilized. At a ratio of 2:1, about 30% of the protein is in solution.

(2) The (CaMg)ATPase, measured in the absence or presence of saturating amounts ($10 \mu\text{g}/\text{ml}$) of calmodulin, shows the highest total activity after solubilization at a detergent/protein ratio of 0.2. At higher detergent/protein ratios the activity decreases again.

Fig. 1. ^{45}Ca transport measured in microsomal fractions from pig antrum smooth muscle

Ca^{2+} accumulation was measured at 37°C and $[\text{Ca}^{2+}] = 10 \mu\text{M}$ by the Millipore filtration technique (see the Methods section): □, in the absence of ATP; ●, in the presence of 5 mM-ATP and without oxalate; ○, in the presence of 5 mM-ATP and 5 mM-oxalate. No calmodulin was added in these experiments. (a) represents accumulation in P_{crude} (crude microsomal fraction), (b) in P_{KCl} (KCl-extracted microsomal fraction) and (c) in the $P_{\text{d0.2}}$ (reconstituted vesicles obtained after dialysis of material solubilized from P_{KCl} at a deoxycholate/protein ratio of 0.2). The symbols are mean values, with vertical bars representing $\pm$ S.E.M. The number of observations was eight for P_{crude} and P_{KCl} , and five for $P_{\text{d0.2}}$.

Table 1. *Effect of KCl extraction of the microsomes on protein content and ATPase activities*
Protein content [$\text{mg of protein} \cdot (100\text{g wet wt. of tissue})^{-1}$] and specific activities ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ of protein) of the ATPases are given for crude microsomes (P_{crude}) and KCl-extracted microsomes (P_{KCl}). The last line gives also the mean increase in specific activity of (CaMg)ATPase in the presence of calmodulin. All values are means $\pm$ S.E.M. for the numbers of observations given in parentheses.

	P_{crude}	P_{KCl}	$\frac{P_{\text{KCl}}}{P_{\text{crude}}}$
Protein (mg/100 g of tissue)	298 ± 21.7 (9)	112 ± 2.6 (9)	0.37 ± 0.03 (9)
MgATPase	52 ± 4.7 (8)	118 ± 5.0 (8)	2.4 ± 0.16 (8)
(NaK)ATPase	10 ± 1.1 (8)	21 ± 2.0 (8)	2.2 ± 0.26 (8)
(CaMg)ATPase	11 ± 1.6 (8)	32 ± 3.5 (8)	3.2 ± 0.34 (8)
+ Calmodulin ($10\mu\text{g/ml}$)	19 ± 2.4 (8)	58 ± 5.0 (8)	3.4 ± 0.37 (8)
+ Calmodulin			
– Calmodulin	1.8 ± 0.7 (8)	1.9 ± 0.1 (8)

Fig. 2. *Solubilization of protein and ATPases from the microsomes as a function of the deoxycholate/protein ratio*
The total activity of ATPase ($\text{nmol} \cdot \text{min}^{-1}$) and protein (%) solubilized from 10 mg of P_{KCl} fraction as a function of the deoxycholate/protein ratio (w/w) is shown. The values are those obtained after dialysis (see the Methods section) and can be compared with the following total activities in 10 mg of protein of the P_{KCl} fraction ($\text{nmol} \cdot \text{min}^{-1}$): MgATPase, 1223; (NaK)ATPase, 227; (CaMg)ATPase, 417; (CaMg)ATPase in the presence of calmodulin ($10\mu\text{g/ml}$), 707. ■, Protein; □, MgATPase; △, (NaK)ATPase; ●, (CaMg)ATPase without calmodulin; ○, (CaMg)ATPase with calmodulin. The values are means of three observations.

(3) The (NaK)ATPase requires for maximal solubilization a higher detergent/protein ratio (0.5) than the (CaMg)ATPase. Like (CaMg)ATPase, the activity of the (NaK)ATPase is decreased again by a further increase in detergent.

(4) Most of the MgATPase activity is already solubilized at a detergent/protein ratio of 0.2. A further increase in this ratio does not diminish the total activity of MgATPase, as has been observed for the (NaK)ATPase and (CaMg)ATPase.

Solubilization of the KCl-extracted microsomes at a ratio of 0.2 mg of deoxycholate to 1 mg of protein

therefore yields the highest activity of (CaMg)ATPase in the dialysed supernatant ($P_{\text{do.2}}$). Table 2 gives the specific and total activities (per 100 g wet wt. of tissue) of the ATPase activities as well as the recovery during the purification procedure. These values of the (CaMg)ATPase activity both in the presence and in the absence of calmodulin clearly show the selective purification. Whereas we recover in the final $P_{\text{do.2}}$ preparation only slightly more than 7% of both the total MgATPase and (NaK)ATPase activities, we obtain more than 50% of the total (CaMg)ATPase activity. The recovery is greater for

Table 2. *Purification of the (CaMg)ATPase*

Specific activity ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ of protein), total activity [$\mu\text{mol} \cdot \text{min}^{-1} \cdot (100 \text{ g of tissue})^{-1}$] and recovery (%) of MgATPase, (NaK)ATPase and (CaMg)ATPase in the absence and presence of calmodulin during purification are shown. Values are means $\pm$ 1 S.E.M. for the numbers of observations shown in parentheses.

Specific activity				
	MgATPase	(NaK)ATPase	(CaMg)ATPase	
			– calmodulin	+ calmodulin
P_{crude}	61.2 $\pm$ 7.5 (8)	10.6 $\pm$ 0.9 (8)	8.6 $\pm$ 1.7 (7)	15.1 $\pm$ 2.7 (7)
P_{KCl}	127 $\pm$ 6.2 (8)	24.2 $\pm$ 1.8 (7)	29.1 $\pm$ 3.1 (7)	56.9 $\pm$ 4.3 (7)
$P_{\text{d0.2}}$	113 $\pm$ 8.8 (7)	17.3 $\pm$ 1.9 (6)	157 $\pm$ 12.1 (7)	255 $\pm$ 20.9 (7)
Total activity				
	MgATPase	(NaK)ATPase	(CaMg)ATPase	
			– calmodulin	+ calmodulin
P_{crude}	15.6 $\pm$ 0.5 (7)	3.18 $\pm$ 0.24 (7)	2.94 $\pm$ 0.59 (7)	4.44 $\pm$ 0.81 (7)
P_{KCl}	13.7 $\pm$ 0.8 (7)	2.66 $\pm$ 0.18 (7)	3.05 $\pm$ 0.42 (7)	6.24 $\pm$ 0.67 (7)
$P_{\text{d0.2}}$	1.15 $\pm$ 0.11 (7)	0.21 $\pm$ 0.04 (7)	1.63 $\pm$ 0.22 (6)	2.67 $\pm$ 0.39 (7)
Recovery				
	MgATPase	(NaK)ATPase	(CaMg)ATPase	
			– calmodulin	+ calmodulin
P_{crude}	100	100	100	100
P_{KCl}	88.0	83.4	103	141
$P_{\text{d0.2}}$	7.4	7.3	55.3	60.1

the (CaMg)ATPase activity measured in the presence of calmodulin probably because calmodulin has been removed during the KCl-wash procedure. This also explains a recovery of 140% in the P_{KCl} fraction compared with the P_{crude} (Table 2).

This partial purification of the (CaMg)ATPase has resulted in an approx. 18-fold increase in the specific activity of the enzyme as compared with that in the crude microsomes. By contrast, the specific activities of the MgATPase and (NaK)ATPase were increased by a factor of less than 2.

Membrane reconstitution

Removal of the detergent by dialysis at 22°C resulted in a reconstitution of membranes. This is indicated by the increased turbidity during the dialysis and by the fact that after dialysis a fraction could be sedimented by centrifugation at 223 800g for 30 min. An electron-microscopic analysis of this fraction shows monolamellar vesicles. These vesicles are capable of an ATP-dependent Ca^{2+} uptake, which is further stimulated by oxalate (Fig. 1). A Ca^{2+} -transport system still functions after removal of the detergent.

Further purification of (CaMg)ATPase by density-gradient centrifugation

In some experiments we have attempted a further

purification of the reconstituted vesicles by density-gradient centrifugation. The $P_{\text{d0.2}}$ fraction (0.5 ml) was therefore put on a 5.2 ml linear sucrose density gradient prepared from solutions of 15% and 50% (w/w) sucrose in water, in Beckman SW 50.1 tubes. The tubes were centrifuged for 16 h at 37 000 rev./min (160 000 g_{max}). At the end of the run two bands were visible, a large band at a concentration of 20% (w/w) sucrose, which contained the (CaMg)ATPase, and a sharp band at 40% sucrose.

This procedure results in a further purification of the (CaMg)ATPase. Mean values for (CaMg)-ATPase activity of 216 and 314 $\text{nmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ were obtained respectively in the absence and presence of calmodulin. Most important, however, is the observation that microsomes equilibrating at a density of 20% sucrose were devoid of a polypeptide band of 220 000 daltons, which probably corresponds to contaminating myosin heavy chains. In the fraction equilibrating at 40% sucrose this 220 000-dalton band is clearly visible on the dodecyl sulphate/polyacrylamide gel.

Discussion

After demonstrating the presence of a (CaMg)-ATPase in smooth-muscle microsomal fractions,

which is probably related to the ATP-dependent Ca^{2+} -transport system (Wuytack & Casteels, 1980; Wuytack *et al.*, 1980), we decided to purify this enzyme further. The partial purification of the (CaMg)ATPase of the microsomal fraction of smooth muscle is described in the present paper.

Because the Ca^{2+} -transport ATPase is a membrane enzyme, detergents were used for its purification. The deoxycholate procedure described by Meissner & Fleischer (1974) was selected, because this detergent can be removed more easily than most other detergents. In view of the relatively low activity of the (CaMg)ATPase in smooth-muscle microsomes, a large amount of smooth muscle was required to start the isolation procedure, and therefore the antrum of the pig stomach was chosen. Several inhibitors of proteolysis were included in the homogenization system, because during homogenization and detergent treatment proteinases may be activated. The ensuing proteolysis could not only damage the Ca^{2+} -transport system, but could also interfere with the stimulation of the Ca^{2+} -transport ATPase by calmodulin. It has been reported that mild proteolysis activates calmodulin-dependent phosphodiesterase (Cheung, 1971) and Ca^{2+} transport in erythrocytes (Sarkadi, 1980), making those activities thereby less sensitive to calmodulin.

The first purification step consists of a KCl wash. The data in Table 1 indicate that such a wash decreases the protein content of the microsomal fraction and that it increases the specific activities of the ATPases. The mean increase in the (CaMg)-ATPase activity is slightly larger than that of the other ATPase activities. We find after a KCl wash of the microsomal fraction an increase in plateau value of Ca^{2+} accumulated in the absence of oxalate as well as an increased rate of Ca^{2+} transport in the presence of oxalate. These changes in transport characteristics appear smaller than one would expect from the diminution in protein content. Washing the microsomes with KCl might increase the Ca^{2+} -permeability of some vesicles, resulting in an increase of the Ca^{2+} -transport activity which is relatively smaller than the concomitant increase in ATPase activity.

The deoxycholate/protein ratio was chosen in such a way as to increase selectively the (CaMg)-ATPase activity. Whereas in the control preparation the (CaMg)ATPase activity (measured in the presence of calmodulin) is only one-quarter of the MgATPase activity, in the final preparation it is about twice the corresponding value for the MgATPase (Table 2). The origin of this MgATPase in our microsomes is unclear. It is unlikely that mitochondrial fragments contribute considerably to this activity, because we measure the ATPase activity in the presence of azide. Even at a detergent/protein ratio of 2, only 30% of the protein

is solubilized. We have not used higher detergent/protein ratios because of its inactivating action on the (CaMg)ATPase activity. The nature of the unsolubilized protein material has not been investigated, but one could assume that a considerable part of this protein fraction may consist of the cytoskeletal matrix and of membrane-associated matrix (Mescher *et al.*, 1981).

Dodecyl sulphate/polyacrylamide-gel electrophoresis of the smooth-muscle microsomes shows peptide bands of 220 000 and 45 000 daltons, which probably indicate the presence of myosin heavy chains and of actin respectively. KCl extraction does not completely remove these bands. Even in our final $\text{P}_{\text{d}0.2}$ fraction corresponding bands can still be observed. The question therefore arises whether a Ca^{2+} -regulated actin-stimulated myosin ATPase could be responsible for the observed (CaMg)-ATPase activity. In a previous paper (Wuytack & Casteels, 1980) we concluded that this is not the case in coronary-artery smooth muscle and that most of the (CaMg)ATPase activity is probably related to the Ca^{2+} -transport system. We have now more arguments in favour of this hypothesis. Under two experimental conditions we obtained (CaMg)ATPase preparations that on dodecyl sulphate/polyacrylamide-gel electrophoresis no longer showed the 220 000-dalton myosin bands, but nevertheless still had a high (CaMg)ATPase activity which could be stimulated by calmodulin. (1) The first preparation is obtained by isopycnic equilibration of $\text{P}_{\text{d}0.2}$ fractions on sucrose gradients. The (CaMg)ATPase activity equilibrating at 20% sucrose has a higher specific activity of (CaMg)-ATPase than does the fraction before isopycnic equilibration, but it does not contain the 220 000-dalton band. (2) When microsomes (fraction P_{KCl}) are extracted with Triton X-100 the 220 000-dalton myosin band is not solubilized. The solubilized protein does, however, show (CaMg)ATPase activity, which can be activated by calmodulin (results not shown).

The presence of an ATP-dependent Ca^{2+} -transport activity in the $\text{P}_{\text{d}0.2}$ fraction further indicates that the (CaMg)ATPase activity in this fraction may be due to a Ca^{2+} -transport ATPase. That the specific activity of the Ca^{2+} transport in this fraction is not increased to the same extent as the (CaMg)ATPase activity could be due to the presence in the detergent-treated fractions of a larger proportion of vesicles permeable to Ca^{2+} .

The present preparation contains MgATPase and (NaK)ATPase activities, but the (CaMg)ATPase activity has been selectively enriched over the other two ATPases. If the pure (CaMg)ATPase from smooth muscle has a specific activity comparable with that of skeletal-muscle or erythrocyte (CaMg)-ATPase (Sarkadi, 1980), it would require another

10-fold purification step in order to approach 100% purity.

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