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Purification and characterization of the Ca^{2+}-ATPase of plasma membranes from Ehrlich ascites mammary carcinoma cells

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Ca^{2+}-ATPase was isolated from plasma membranes of Ehrlich ascites mammary carcinoma cells by means of calmodulin affinity chromatography. The purification procedure included removal of endogenous calmodulin from a Triton X-100 solubilizate of the membranes by DEAE ion-exchange chromatography as an essential step. With respect to its molecular mass, activation by calmodulin, Ca^{2+}-dependent phosphorylation and highly sensitive inhibition by orthovanadate, the purified enzyme resembles the Ca^{2+}-ATPase of erythrocyte membranes. In contrast to the strong calmodulin dependence of the isolated enzyme the Ca^{2+}-ATPase in native Ehrlich ascites carcinoma cell membranes cannot be remarkably stimulated by added calmodulin. It is suggested that the membrane-bound Ca^{2+}-ATPase in the presence of Ca^{2+} is activated by interaction with endogenously bound calmodulin.

Introduction

Ca^{2+}-pumping ATPases have been described in plasma membranes of a wide variety of cell types (for a review, see Ref. 1). Many of these Ca^{2+}-ATPases have been shown to be regulated by calmodulin. The interaction between calmodulin and the Ca^{2+}-ATPase has been used to purify the respective enzymes from erythrocytes [2], heart sarcolemma [3], smooth and skeletal muscle sarcolemma [4,5] and brain synaptosomes [6].

Several reports deal with Ca^{2+} transport and Ca^{2+}-ATPase activity of transformed cells. A participation of these enzyme activities in some derangements of the intracellular Ca^{2+} metabolism was supposed [7,8]. The most intensively investigated tumor cells with respect to membrane-associated Ca^{2+}-ATPase activity are Ehrlich ascites mammary carcinoma (EAC) cells. Hinnen et al. [9] have analyzed ATP-driven Ca^{2+} fluxes in EAC cells and plasma membrane preparations. A Ca^{2+}-dependent phosphoprotein of molecular mass of 135 kDa was first identified by us in purified plasma membranes from EAC cells [10]. Recently Klaven et al. [11] have reinvestigated Ca^{2+}-ATPase activity and Ca^{2+} transport of EAC cell membranes. In contrast to the study by Hinnen et al. and to our results, these authors observed a slight enhancement of Ca^{2+}-ATPase activity and Ca^{2+} transport after addition of calmodulin.

In the present paper we present strong evidence

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Abbreviations: EAC, Ehrlich ascites mammary carcinoma; EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid; SDS, sodium dodecyl sulfate.

that the Ca^{2+}-ATPase in EAC cell membranes interacts in a Ca^{2+}-dependent fashion with an endogenous calmodulin fraction which does not dissociate from the membranes at very low Ca^{2+} concentration ($pCa \geqslant 9$). After removal of this calmodulin fraction from a Triton X-100 solubilizate of EAC cell membranes the Ca^{2+}-ATPase characterized by its phosphorylated intermediate can be isolated by means of calmodulin affinity chromatography. The purified Ca^{2+}-ATPase is markedly stimulated by added calmodulin and resembles in many features the calmodulin-dependent Ca^{2+}-ATPase in erythrocyte membranes.

Materials and Methods

Materials

All chemicals were of reagent grade. [γ-^{32}P]ATP and $Na^{125}I$ were products of the Zentralinstitut für Kernforschung, Dresden, G.D.R., and the Institute of Isotopes, Budapest, Hungary, respectively. CNBr-activated Sepharose, Sephadex A-25 and phenyl-Sepharose were obtained from Pharmacia, Uppsala, Sweden. Calmodulin was purified from ram testis according to Ref. 12. Iodination of calmodulin was performed by the Bolton and Hunter method [13]. The labeled calmodulin was freed from contaminations by hydrophobic affinity chromatography on phenyl-Sepharose CL-4B [14]. The product obtained had a specific activity of about 15 TBq/mmol and exhibited an unchanged potency to activate the Ca^{2+}-ATPase of erythrocyte membranes. Coupling of calmodulin to CNBr-activated Sepharose 4B was performed as described by Niggli et al. [2].

General

Plasma membranes from EAC cells were prepared as previously described [10]. The Ca^{2+}-ATPase activity of membranes was assayed by P_i liberation [15]. Determination of ATPase activity of the solubilized enzyme was performed spectrophotometrically using a coupled enzyme system [16]. For assay of endogenous calmodulin in plasma membranes of EAC cells the samples were kept for 2 min in boiling water, cooled and centrifuged. The resulting supernatants were subjected to determination of calmodulin by measuring their ability to activate the Ca^{2+}-ATPase of human erythrocyte membranes [15].

The Ca^{2+}-ATPase from human erythrocyte membranes was purified as described by Niggli et al. [2]. For protein determination the method of Lowry et al. [17] was used. Protein concentration of Triton-containing solutions was assayed by the method of Bradford [18] using bovine serum albumin as a standard.

^{125}I-calmodulin gel overlay procedure

The method was performed essentially as described by LePeuch et al. [19]. Labeling of calmodulin-binding proteins separated on SDS-polyacrylamide gels according to the method of Laemmli [20] was carried out by incubating the whole gel with buffer containing 37 kBq/ml ^{125}I-calmodulin in the presence of 1 mM Ca^{2+} or 1 mM EGTA.

Isolation of Ca^{2+}-ATPase from plasma membranes of EAC cells

The method described by Niggli et al. [2] was used, optimized for the special properties of these membranes. Plasma membranes from EAC cells were centrifuged for 15 min at $105000 \times g$. The resulting pellet was suspended in a medium containing 1 mg Triton X-100/mg membrane protein, 300 mM KCl, 1 mM $MgCl_2$, 2 mM dithioerythritol, 0.1 mM $CaCl_2$ and 10 mM Tris at pH 7.4 and incubated at 4°C for 10 min. Solubilized membranes were then centrifuged for 15 min at $105000 \times g$. Asolectin (0.1 mg/ml, final concentration) and EGTA (1 mM, final concentration) were added to the clear supernatant.

To remove endogenous calmodulin, 1 ml DEAE-Sephadex A-25 gel equilibrated with buffer A (300 mM KCl, 1 mM $MgCl_2$, 2 mM dithioerythritol, 10 mM Tris (pH 7.4), 1 mM EGTA and 0.5% Triton X-100) was added to 5 ml of the supernatant. The mixture was stirred for 20 min at 4°C and then sucked through a nylon gauze. After addition of $CaCl_2$ (1.5 mM, final concentration) the clear filtrate was applied to a calmodulin affinity column (1 ml total volume) which had been equilibrated with buffer B (same composition as buffer A, but with 1 mg/ml asolectin and 0.1 mM Ca^{2+} instead of 1 mM EGTA). The column was washed with 100 ml buffer C (same composition as buffer B, but with 0.1% Triton instead of 0.5%). In

one experiment (Table II) after elution with 50 ml buffer C the column was washed with 50 ml buffer D (same composition as buffer C but with 1 mg/ml phosphatidylcholine instead of asolectin). Protein bound to the affinity column was eluted with buffer E (same composition as buffer C or D, 0.1 mM $CaCl_2$ was omitted and 1 mM EGTA added). To the fraction eluted by EGTA, $CaCl_2$ was added to a final concentration of 1 mM $CaCl_2$. All fractions were assayed for Ca^{2+}-ATPase activity and protein content immediately after column chromatography. The material obtained by elution of the affinity column with EGTA contained approx. 0.3% of the protein and 2% of the Ca^{2+}-ATPase activity of the membranes applied to the isolation procedure. The yield is considerably lower if the elution is performed in the presence of phosphatidylcholine instead of asolectin.

Polyacrylamide gel electrophoresis of purified enzyme was carried out according to Laemmli [20].

Formation of the ^{32}P-phosphoprotein

The method described by Wuytack et al. [21] was used. 0.4 ml incubation medium contained 125 mM KCl, 12.5 μM $MgCl_2$, 10 mM Tris(pH 7.4), 8 μM [γ-^{32}P]ATP (spec. act. about 1 TBq/mmol), 100 μM $LaCl_3$, 0.1% bovine serum albumin, about 5 μg isolated Ca^{2+}-ATPase and 200 μM $CaCl_2$ or 1 mM EGTA. Phosphorylation was performed for 15 s at 4°C. The reaction was stopped by addition of 1 ml 1 mM ATP, 50 mM H_3PO_4 and 6% trichloroacetic acid and the pellet was washed with the same solution. SDS-polyacrylamide gel electrophoresis of the phosphorylated enzyme was performed according to Avruch and Fairbanks [22]. Analysis of dried gels was performed by autoradiography on HS11 film (ORWO Wolfen, G.D.R.).

Results

Ca^{2+}-ATPase activity and calmodulin content of EAC cell membranes

First, some basic characteristics of the membrane bound Ca^{2+}-ATPase were determined. Table I summarizes the kinetic parameters of the enzyme. Furthermore, the content of endogenous calmodulin in the membranes was determined. Both Ca^{2+} activation of the Ca^{2+}-ATPase and the calmodulin content are unaffected after washing the membranes in the presence of 1 mM EGTA (not shown). From this result it can be concluded that the endogenous calmodulin fraction is tightly bound to the membranes in a Ca^{2+}-independent way. Attempts to remove endogenous calmodulin by treating the membranes with saponin or non-solubilizing concentrations of Triton X-100 are likewise ineffective.

Addition of calmodulin to the membrane preparations had a negligible effect, if any, on the Ca^{2+}-ATPase activity. With respect to its high Ca^{2+}-affinity and sigmoidal dependence of ATPase activity on Ca^{2+} concentration the enzyme resembles the calmodulin-activated form of the Ca^{2+}-ATPase in erythrocyte membranes [23]. These results provide a possibility for the Ca^{2+}-dependent activation of the Ca^{2+}-ATPase of EAC cell membranes to be realized by interaction of the enzyme with endogenous calmodulin.

Detection of calmodulin binding proteins in EAC cell membranes

Interaction of membrane protein with calmodulin was examined by the ^{125}I-calmodulin gel overlay technique. The autoradiogram in Fig. 1A (lane 1) indicates Ca^{2+}-dependent calmodulin binding to various proteins of EAC cell membranes. The most prominent band has an M_r of about 150000, only slightly differing from the Ca^{2+}-pump related phosphoprotein [10]. If the gel overlay incubation with ^{125}I-calmodulin was per-

TABLE I

KINETIC PARAMETERS OF Ca^{2+}-ATPase AND ENDOGENOUS CALMODULIN CONTENT OF EAC CELL MEMBRANES

	− CaM	+ CaM [a]
Ca^{2+}-ATPase		
$k_{0.5\,Ca^{2+}}$ (μM)	0.4	0.4
V_{max} (μmol P_i/mg per h)	1.7	1.7
n_H	2.7	2.8
Calmodulin content (ng per mg membrane protein)	880 [b]	
	670 [c]	

[a] CaM, calmodulin; final concentration: 10 μg/ml.
[b] Determined by Ca^{2+}-ATPase activation.
[c] Determined by ELISA (Wenz, I., et al., unpublished results).

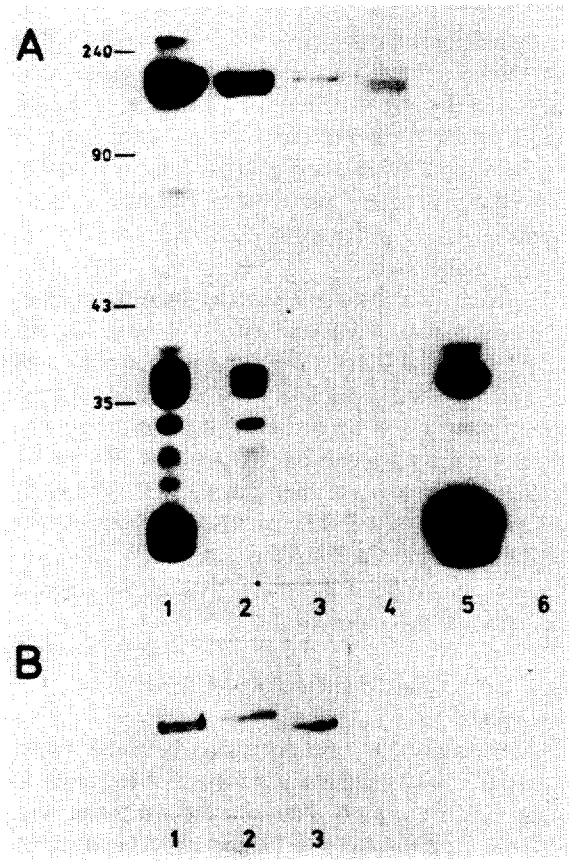

Fig. 1. Identification of calmodulin-binding proteins in Ehrlich ascites carcinoma cell membranes by the ^{125}I-calmodulin gel overlay technique. A. The following samples were subjected to SDS-polyacrylamide gel electrophoresis: 1, EAC cell membranes (100 μg membrane protein); 2, 25 μl of Triton X-100 solubilizate of the membranes; 3, 0.3 μg protein obtained after calmodulin-Sepharose affinity chromatography of the solubilizate; 4, 0.3 μg Ca^{2+}-ATPase isolated from erythrocyte membranes; 5, same as in lane 1; 6, same as in lane 3. After fixation and renaturation of the proteins, the gel was soaked in ^{125}I-calmodulin in the presence of 1 mM Ca^{2+} (lanes 1–4) or 1 mM EGTA (lanes 5 and 6). After unbound calmodulin had been removed by intensive washings, the calmodulin-binding proteins were visualized by autoradiography. Bars indicate the positions of standard proteins ($M_r \times 10^{-3}$ is given).
B. Proteins were subjected to prolonged electrophoresis in a 5–15% gradient gel. The ^{125}I-calmodulin gel overlay procedure was performed as in A. Lanes: 1, a mixture containing 0.3 μg Ca^{2+}-ATPase isolated from erythrocyte membranes and 0.3 μg Ca^{2+}-ATPase isolated from EAC cell membranes; 2, 0.3 μg Ca^{2+}-ATPase isolated from EAC cell membranes; 3, 0.3 μg Ca^{2+}-ATPase isolated from erythrocyte membranes. Only the relevant portion of the autoradiogram is shown.

formed in the absence of Ca^{2+} (with 1 mM EGTA), then the 150 kDa band on the autoradiogram disappeared completely (Fig. 1, lane 5). Under these conditions only some proteins of low molecular weight were able to bind ^{125}I-calmodulin.

Purification of the Ca^{2+}-ATPase from solubilized EAC plasma membranes by calmodulin affinity chromatography

As shown in Fig. 1A (lane 2), the ^{125}I-calmodulin-binding protein of about 150 kDa can be solubilized by treating EAC cell membranes with Triton X-100. The detergent/protein ratio of 1 : 1 was optimal for maximal recovery of Ca^{2+}-ATPase activity. First attempts to isolate the approx. 150 kDa protein from the solubilizate by affinity chromatography on a calmodulin-Sepharose column have failed. Neither calmodulin-binding protein nor Ca^{2+}-ATPase activity could be detected in the fractions eluted from the affinity column by EGTA (not shown).

Assuming that Ca^{2+}-dependent interaction of Ca^{2+}-ATPase molecules with endogenous calmodulin prevented binding of the enzyme to the affinity column, we tried to remove the anionic calmodulin from the solubilizate by batchwise treatment with DEAE-Sephadex. As measured by adding trace amounts of ^{125}-calmodulin, more than 80% of calmodulin was bound to the anion-exchange resin in the presence of EGTA. After removal of endogenous calmodulin by this procedure, Ca^{2+} was added to the solubilizate and the mixture was applied to the calmodulin affinity column. Under these conditions the fractions eluted from the column by EGTA contained measurable amounts of protein and Ca^{2+}-ATPase activity. As shown by the ^{125}I-calmodulin gel overlay procedure (Fig. 1, lane 3) a single band of a calmodulin binding protein ($M_r \approx 150\,000$) (likely the Ca^{2+}-ATPase of EAC cell membranes) was obtained in the EGTA eluate. Significant amounts of other calmodulin-binding proteins originally present in the solubilizate did not appear. The affinity of these proteins for the calmodulin column seems to be relatively low. As shown in Fig. 1A, lane 6, interaction of ^{125}I-calmodulin with the isolated calmodulin-binding protein from EAC cell membranes was prevented by EGTA.

The electrophoretic mobility of the Ca^{2+}-

ATPase isolated from erythrocytes (Fig. 1, lane 4) seems to be slightly higher than that of the Ca^{2+}-ATPase of EAC cell membranes. In order to verify this finding both enzyme species were either separately or as mixture applied to a 5–15% gradient gel and electrophoresed for a prolonged time. Under these conditions the electrophoretic mobilities of the enzyme were found to be clearly different (Fig. 1B). The apparent difference of molecular masses of the Ca^{2+}-ATPase from erythrocytes and EAC cell membranes was estimated to be about 6 kDa.

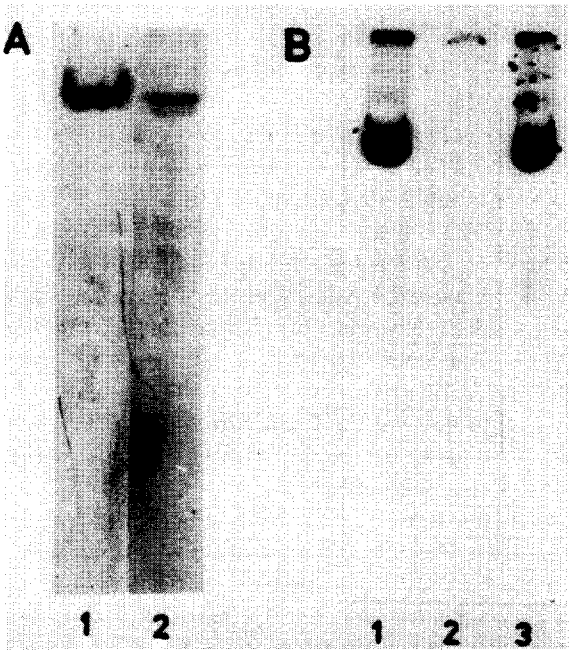

Fig. 2. Characterization of the fraction from calmodulin affinity chromatography by SDS-gel electrophoresis and demonstration of Ca^{2+}-dependent phosphorylation. A. Electrophoresis was performed with 7.5% gels according to Laemmli [20]. Proteins were stained with Coomassie brilliant blue. 1–2 μg protein is obtained after calmodulin-Sepharose affinity chromatography of the Triton X-100 solubilizate of EAC cell membranes. 2–2 μg Ca^{2+}-ATPase isolated from erythrocyte membranes. B. 5 μg protein eluted from the calmodulin affinity column by EGTA were phosphorylated in the presence of [γ-^{32}P]ATP and: 1–0.1 mM Ca^{2+} or 2–1 mM EGTA. For a comparison 5 μg of purified Ca^{2+}-ATPase from erythrocyte membranes were phosphorylated in the presence of 0.1 mM Ca^{2+} (lane 3). SDS-polyacrylamide gel electrophoresis was performed at pH 2.4 on 5.6% slab gels according to Avruch and Fairbanks [21].

Some properties of the Ca^{2+}-ATPase isolated from EAC cell membranes

First, purified Ca^{2+}-ATPases from EAC cell membranes and from erythrocyte membranes were compared by electrophoresis in SDS-polyacrylamide gels and stained with Coomassie blue (Fig. 2A). The electrophoretic mobilities of both enzyme species under the given conditions of gel electrophoresis seemed to be very close. To determine whether the 150 kDa protein is identical with the Ca^{2+}-dependent phosphoprotein of the Ca^{2+}-ATPase, the EGTA column eluate was incubated with [γ-^{32}P]ATP in the presence or absence of Ca^{2+}. The phosphorylated polypeptides were investigated by SDS-gel electrophoresis and autoradiography as shown in Fig. 2B. In lane 1 of this figure the phosphorylated intermediate of the enzyme isolated from EAC cell membranes is demonstrated. If incubation with [γ-^{32}P]ATP was performed in the presence of 1 mM EGTA the phosphoprotein disappeared (Fig. 2B, lane 2). For comparison, lane 3 demonstrates the phosphorylated Ca^{2+}-ATPase from erythrocyte membranes.

Strong dependence of ATPase activity in fractions eluted from the calmodulin affinity column on Ca^{2+} was detected; Mg^{2+}-ATPase was negligible. As shown in Table II, an approx. 5-fold activation of Ca^{2+}-ATPase by calmodulin was found, if the elution of the calmodulin-Sepharose column was performed in the presence of phos-

TABLE II

Ca^{2+}-ATPase ACTIVITY OF THE PROTEIN ELUTED FROM THE CALMODULIN COLUMN BY EGTA

Ca^{2+}-ATPase activity was measured spectrophotometrically at 2.4 μM Ca^{2+}. At p$Ca \geq 9$ ATPase activity was not measurable.

Additions	Ca^{2+}-ATPase activity (nmol per mg/min)
Enzyme reconstituted in phosphatidylcholine	
no additions	72
+10 μg/ml calmodulin	350
Enzyme reconstituted in asolectin	
no additions	200
10 μg/ml calmodulin	200
vanadate 0.5 μM	166
1 μM	112
2 μM	22

phatidylcholine. Addition of calmodulin to fractions of the affinity column eluted in the presence of asolectin, however, did not significantly affect Ca^{2+}-ATPase activity (Table II). This is probably due to the presence of acidic phospholipids in asolectin. As shown by previous work, acidic lipids are able to activate the calmodulin-dependent Ca^{2+}-ATPase of erythrocyte membranes [24], presumably by interacting with the calmodulin-binding site of the enzyme [25]. As further shown in Table II, the Ca^{2+}-ATPase in EAC cell membranes is strongly inhibited by micromolar concentrations of orthovanadate.

Discussion

The results described in this report clearly demonstrate that plasma membranes from EAC cells contain a calmodulin-dependent Ca^{2+}-ATPase. With respect to its molecular weight, the sensitivity to vanadate and the direct stimulation by calmodulin, the Ca^{2+}-ATPase resembles the analogous enzyme in erythrocytes and clearly differs from the Ca^{2+}-ATPase of sarcoplasmic reticulum and liver plasma membranes. The Ca^{2+} pumps of membranes form sarcoplasmic reticulum and from rat liver are insensitive to stimulation by calmodulin and exhibit molecular weights of 105 000 and 200 000, respectively [26–28]. Furthermore, Ca^{2+}-ATPase from EAC cell membranes is inhibited by vanadate at much lower concentrations than those described for calmodulin-independent ATPases [28,29]. Thus, the enzyme clearly belongs to a family of Ca^{2+}-pumps found hitherto in plasma membranes of non-transformed cells.

The reason for the slightly different electrophoretic mobilities of Ca^{2+}-ATPases from EAC cells on the one hand and erythrocyte membranes on the other hand is presently unknown. Additionally to (possibly species-dependent?) differences in the primary structure of the both enzyme species, distinct posttranslational modifications may give rise to the electrophoretic pattern observed.

Methodological effects may be responsible for the different absolute values of the molecular masses previously determined by us for the phosphorylated EAC Ca^{2+}-ATPase [10] (135 kDa) and now estimated for the purified protein as shown in Fig. 1 (about 150 kDa). As reported by De Jonge et al. [30], the molecular weights of calmodulin-dependent Ca^{2+}-ATPases ascertained by SDS-gel electrophoresis differ in dependence on the electrophoretic buffer system used.

In contrast with the high calmodulin sensitivity of the purified Ca^{2+}-ATPase embedded in phosphatidylcholine liposomes, the membrane-bound enzyme of EAC cells is not significantly [9,10] or only slightly [11] stimulated by added calmodulin. The calmodulin-binding site of the membrane-bound Ca^{2+}-ATPase seems to be occupied by the endogenous calmodulin fraction of the EAC cell membranes. An activation of the enzyme by endogenous acid lipids is unlikely, since these activators have relatively low affinities for calmodulin-dependent Ca^{2+}-ATPase [25], insufficient to prevent the Ca^{2+}-dependent binding of the enzyme to the calmodulin affinity column. Furthermore, the sigmoidal activation of the Ca^{2+}-ATPase in EAC cell membranes by Ca^{2+} points to a(n) (endogenous) calmodulin-dependent reaction step of the enzyme. As shown for the Ca^{2+}-ATPase of erythrocyte membranes, the apparent Ca^{2+}-cooperativity is a characteristic feature of the calmodulin-dependent enzyme [23]. These data strongly suggest that the high Ca^{2+} affinity and the sigmoidal activation of the Ca^{2+}-ATPase in EAC cell membranes by Ca^{2+} are consequences of the Ca^{2+}-dependent interaction of the enzyme with endogenous calmodulin.

As demonstrated by the ^{125}I-calmodulin overlay experiment (Fig. 1), the interaction of calmodulin with the Ca^{2+}-ATPase depends on Ca^{2+}. Thus, it can be concluded that endogenous calmodulin is not an integral subunit of the Ca^{2+}-ATPase as found for the phosphorylase kinase [31]. Ca^{2+}-independent binding of endogenous calmodulin to EAC cell membranes should be realized by some low molecular weight membrane proteins detectable by ^{125}I-calmodulin overlay in the absence of Ca^{2+}, as shown in Fig. 1A, lane 5. Other proteins whose calmodulin affinity in the absence of Ca^{2+} is even higher than in its presence were recently found in secretory granule membranes [32] and cerebral cortex membranes [33].

In contrast to the Ca^{2+}-ATPase in EAC cell membranes, the enzyme in erythrocytes and other cells can be strongly activated by added calmodulin [1]. Regulation of the Ca^{2+}-ATPase activity by

endogenous calmodulin in the plasma membranes of these cells seems to play a minor role, if at all. One reason for the obvious different ability of endogenous calmodulin to interact with membrane-bound Ca^{2+}-ATPase could be variations in the membrane content of the Ca^{2+}-independently bound calmodulin. Erythrocyte membranes contain about 5 pmol endogenous calmodulin per mg membrane protein [12], whereas the content of Ca^{2+}-ATPase is about 25 pmol per mg membrane protein [34]. Thus, for stoichiometric reasons endogenous calmodulin is unable to contribute substantially to Ca^{2+}-ATPase activation in the red cell. The consequences of obvious alternative regulation of membrane-bound Ca^{2+}-ATPase by endogenous or cytosolic calmodulin for enzyme function remain to be elucidated.

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References

1 Penniston, J.T. (1983) in Calcium and Cell Function (Cheung, W.Y., ed.), Vol. 4, pp. 100–144, Academic Press, New York
2 Niggli, V., Penniston, J.T. and Carafoli, E. (1979) J. Biol. Chem. 254, 9955–9958
3 Caroni, P. and Carafoli, E. (1979) J. Biol. Chem. 256, 3263–3270
4 Wuytack, F., De Schutter, G. and Casteels, R. (1981) FEBS Lett. 129, 297–300
5 Michalak, M., Famulski, K. and Carafoli, E. (1984) J. Biol. Chem. 259, 15540–15547
6 Hakim, G., Itano, T., Verma, A.K. and Penniston, J.T. (1982) Biochem. J. 207, 225–230
7 Cittadini, A., Dani, A.M., Wolf, F., Bossi, D. and Caviello, G. (1982) Biochim. Biophys. Acta 686, 27–35
8 Veigl. M.L., Vanaman, T.C. and Sedwick, W.D. (1984) Biochim. Biophys. Acta 738, 21–48
9 Hinnen, R., Miyamoto, H. and Racker, E. (1979) J. Membrane Biol. 49, 309–324
10 Spitzer, E., Böhmer, F.-D. and Grosse, R. (1983) Biochim. Biophys. Acta 728, 50–58
11 Klaven, N.B., Pershadsingh, H.A., Henius, G.V., Lavis, P.C., Long, J.W. and McDonald, J.M. (1983) Arch. Biochem. Biophys. 226, 618–628
12 Klinger, R., Wetzker, R., Wenz, I., Dinjus, U., Reissmann, R. and Frunder, H. (1984) Cell Calcium 5, 167–175.
13 Bolton, A.E. and Hunter, W.M. (1973) Biochem. J. 133, 529–538
14 Gopalakrishna, R. and Anderson, W.B. (1982) Biochem. Biophys. Res. Commun. 104, 830–836
15 Klinger, R., Wetzker, R., Fleischer, I. and Frunder, H. (1980) Cell Calcium 1, 229–240
16 Klinger, R., Wetzker, R., Fleischer, I. and Frunder, H. (1981) Cell Calcium 2, 553–560
17 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
18 Bradford, M. (1976) Anal. Biochem. 72, 248–254
19 LePeuch, C.J., LePeuch, D.A.M., Katz, S., Demaille, J.G., Hincke, M.T., Bredoux, R., Enouf, J., Levy-Toledano, S. and Caen, J. (1983) Biochim. Biophys. Acta 731, 456–464
20 Laemmli, U.K. (1970) Nature 227, 680–685
21 Wuytack, F., Raeymaekers, L., DeSchutter, G. and Casteels, R. (1982) Biochim. Biophys. Acta 693, 45–52
22 Avruch, J. and Fairbanks, G. (1972) Proc. Natl. Acad. Sci. USA 69, 1216–1220
23 Wetzker, R., Klinger, R., Cumme, G., Hoppe, H. and Frunder, H. (1982) Biochem. Int. 4, 385–390
24 Niggli, V., Adunyah, E.S. and Carafoli, E. (1981) J. Biol. Chem. 256, 8588–8592
25 Wetzker, R., Klinger, R. and Frunder, H. (1983) Biochim. Biophys. Acta 730, 196–200
26 Tada, M., Yamamoto, T. and Tonomura, Y. (1978) Physiol. Rev. 58, 1–79
27 Lotersztaijn, S., Hanoune, J. and Pecker, F. (1981) J. Biol. Chem. 256, 11209–11215
28 Iwasa, Y., Iwasa, T., Higashi, K., Matsui, K. and Miyamoto, E. (1982) Biochem. Biophys. Res. Commun. 105, 488–494
29 Wang, T., Tsai, L.-T., Solaro, R.J., Grassi de Gende, A.O. and Schwarz, A. (1979) Biochem. Biophys. Res. Commun. 91, 356–361
30 De Jonge, H.R., Ghijsen, W.E.J.M. and Van Os, C.H. (1981) Biochim. Biophys. Acta 647, 140–149
31 Cohen, P., Burchell, A., Foulkes, J.G., Cohen, P.T.W., Vanaman, T.C. and Nairn, A.C. (1978) FEBS Lett. 92, 287–293
32 Geisow, M.J. and Burgoyne, R.D. (1983) Nature 301, 432–435
33 Andreasen, T.J., Luetje, C.W., Heidemann, W. and Storm, D.R. (1983) Biochemistry 22, 4615–4618
34 Stieger, J. and Luterbacher, S. (1981) Biochim. Biophys. Acta 641, 270–275