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Characterization of Ca^{2+}-binding proteins from Ehrlich ascites tumor cell cytoplasm and their binding to membranes

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A set of proteins in the 33–37 kDa range have been isolated from the cytoplasm of the Ehrlich ascites tumor cell. The proteins are characterized by their Ca^{2+}-dependent binding to cell membranes. This property has been used for isolation of the proteins by Ca^{2+}-dependent affinity binding to inside-out vesicles of the human red cell membrane. The proteins display Ca^{2+}-binding properties as shown by gel-filtration studies. The Ca^{2+}-dependent binding of the 33 and 34 kDa proteins to red cell membranes was studied after labelling of the proteins with tritium by reductive methylation. The average number of Ca^{2+} bound per protein molecule was 4.8 with a K_d of $3.4 \cdot 10^{-4}$ M Ca^{2+}. The proteins are distinct from most other Ca^{2+}-binding proteins of comparable molecular weights by not incorporating phosphate.

Introduction

Diverse cellular activities are regulated by calcium. The regulatory action is achieved via a Ca^{2+}-dependent interaction between a Ca^{2+}-receptor protein and the target protein. For example, the striated muscle responds to a rise in intracellular Ca^{2+} concentration by contraction, the Ca^{2+}-receptor protein being troponin-C. Calmodulin is believed to be one of the major intracellular Ca^{2+}-receptor proteins implicated in the regulation of a number of enzymatic activities. New classes of Ca^{2+}-binding proteins have been described lately, such as the calcimedins, which copurify with calmodulin by fluphenazine-affinity chromatography [1] or may be isolated by phenyl-Sepharose chromatography. In addition, proteins have been isolated by their Ca^{2+}-dependent association with membranes. A group of these proteins have been described as chromobindins [2], and others are the proteins I, II and III from liver and intestine [3] and the calelectrins from mammals and the electric organ of *Torpedo marmorata* [4,5]. Some of the proteins described appear to be related to one another, both immunologically and in amino acid sequences. Most of these new proteins fall into two distinct size classes, 67–71 and 30–40 kDa and may be involved in cellular processes regulated by Ca^{2+} and phospholipids [6].

In the present study we report three groups of proteins in the 33, 34 and 37 kDa range from the cytoplasm of Ehrlich ascites tumor cells. The proteins were isolated by Ca^{2+}-dependent binding to inside-out vesicles of human red blood cell membrane. Properties of the proteins were investigated for comparison with similar proteins from other sources.

Materials and Methods

Preparation of cell membranes and cytoplasm. Ehrlich ascites tumor cells were maintained by weekly intraperitoneal transfer into white female mice. The cells were harvested, suspended in an isotonic saline solution containing heparin and washed twice by centrifugation. The saline solution contained 150 mM NaCl/5 mM KCl/1 mM $MgCl_2$/1 mM $CaCl_2$/1 mM Na_2HPO_4/3.3 mM Mops/3.3 mM Tes/5 mM Hepes (pH 7.4). The cells were ruptured at 8% cytocrit in isotonic medium with or without 1 mM EDTA by nitrogen cavitation in a cell-disruption bomb (Parr Instrument) and sudden reduction of pressure. Proteolytic activity was inhibited by the addition of 0.1 mM PMSF.

Nuclei and other large structures were removed by centrifugation at $1000 \times g$ for 5 min, and mitochondria

Abbreviations: Mops, 4-morpholinepropanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IEF, isoelectric focussing; p*I*, isoelectric point; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonyl fluoride; TES, 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-amino}ethane sulfonic acid.

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were precipitated at $4000 \times g$ for 10 min. The supernatant was then centrifuged at $100000 \times g$ for 75 min to obtain the cytosolic fraction and sediment the membranes, which were further washed in 140 mM NaCl/10 mM Tris (pH 7.4) and finally in 5 mM Tris (pH 7.4), both containing 0.1 mM PMSF [7]. The cytosolic fraction constituting an approx. 13-times dilution of the original cytoplasm was deep-frozen for later use.

Inside-out vesicles were prepared from human red cells, two-thirds of the vesicles normally being inside-out, as determined by measuring acetylcholine esterase activity [8].

Membrane-affinity purification of proteins. Inside-out vesicles were used as affinity material for isolating cytoplasmic proteins which bind Ca^{2+}-dependently to membranes. Ca^{2+} was added to the Ehrlich ascites tumor cell cytoplasmic solution to a final concentration of 1 mM. Red cell inside-out vesicles were suspended in the cytoplasmic solution and incubated for 10 min (1–2 mg vesicle protein per ml cytoplasm). The vesicles were washed twice in a Ca^{2+}-containing medium (2 mM NaCl/5 mM KCl/0.5 mM EGTA/2 mM Tris (pH 7.4) and the appropriate amounts of $MgCl_2$ and $CaCl_2$ required to produce a 1 mM final concentration for each divalent ion as determined by calculation). Proteins were released from the membranes by washing with the medium, omitting $CaCl_2$. After centrifugation, the supernatant containing Ca^{2+}-dependent membrane-binding proteins was dialyzed against water and lyophilized. The pellet of vesicles was resuspended in new cytoplasmic solution and reused several times.

Polyacrylamide gel electrophoresis and isoelectric focussing. One-dimensional SDS-gel electrophoresis was performed in 12% 1.5×110 mm slab gels or 0.75×70 mm mini slab gels following the procedure of Laemmli [9]. Isoelectric focussing (IEF) and two-dimensional polyacrylamide gels were prepared by the method of O'Farrell [10] using 2.5 mm diameter tubes or capillary tubes and 9 M urea and 2% Nonidet P-40 detergent. LKB Ampholines were used in the pH range 3–10. pH gradients were determined by slicing focussing gels into 0.5 cm segments, incubating each slice with 1 ml of boiled deionized water, and measuring the pH of the water. Gels were stained with Coomassie Blue. LKB electrophoresis system and Bio-Rad Mini Protean™ II Cells were used. Gels were scanned in an LKB ultroscan XL gel scanner.

Calcium-binding determination. Ca^{2+}-binding to protein was determined by the procedure described by Hummel and Dreyer [11]. The isolated cytoplasmic proteins of Ehrlich ascites tumor cells were extensively dialyzed against distilled water and lyophilized. Samples (0.5 mg) were dissolved in 200 µl of a 40 mM Tris-HCl buffer (pH 7.5) containing 100 mM NaCl/1 mM $MgCl_2$/2 µM $^{45}CaCl_2$ and then added to a Sephadex G-25 Pharmacia PD-10 prepacked column equilibrated and eluted with the same buffer. 650-µl fractions were collected and aliquots were counted in a liquid scintillation spectrometer (Packard Model 3330). Calmodulin and bovine serum albumin were used as positive and negative controls, respectively [12].

^3H labelling of Ca^{2+}-dependent membrane-binding proteins. The soluble 33 and 34 kDa Ca^{2+}-binding proteins were labelled with ^3H by reductive methylation to a high specific activity [13,14] and an assay for calcium-dependent binding of the proteins to inside-out vesicles of the human red cell was performed, according to similar experiments with calmodulin [15,16]. Samples containing 100 µg of the inside-out vesicle protein were incubated with 750 ng ^3H-labelled protein for 10 min at room temperature in 0.5 ml assay medium consisting of 2 mM NaCl/5 mM KCl/0.5 mM EGTA/2 mM Tris (pH 7.4) and the appropriate amounts of $MgCl_2$ and $CaCl_2$ required to produce the final concentration 1 mM for free Mg^{2+} and in the range of 10^{-8}–10^{-3} M for free Ca^{2+}, as determined by calculation [17]. The final Ca^{2+} activity of each solution was checked using a calcium-sensitive electrode. After incubation the samples were centrifuged and the pellets resuspended and washed in ice-cold incubation medium with Ca^{2+}-binding protein omitted. The pellets were transferred to count the radioactivity.

Results

The pattern of Ehrlich ascites tumor cell membrane proteins differed, as analyzed by SDS-polyacrylamide gel electrophoresis, depending on the absence or presence of Ca^{2+} in the buffer medium during preparation of the plasma membranes. Additional bands appeared in the 33–37 kDa range when the membrane preparation took place in a Ca^{2+}-containing medium, as compared to membranes prepared in an EDTA-containing medium. Membranes prepared in a medium containing Mg^{2+} instead of Ca^{2+} showed no appearance of new proteins, indicating that Mg^{2+} cannot substitute for Ca^{2+} (see Fig. 1).

Fig. 2 shows a gel scan of lanes 1 and 2 in Fig. 1. The arrows indicate the position of peaks representing additional proteins presumably picked up from the cytoplasm by Ca^{2+}-dependent binding to the membranes (B). Fig. 3 shows SDS-polyacrylamide gel electrophoresis of human red cell inside-out vesicle proteins (lane 1) and the protein profile of vesicles with Ca^{2+}-binding protein attached to the membrane after incubation in Ca^{2+}-containing cytoplasm of the Ehrlich ascites tumor cell (lane 2). Two additional bands appear comparable to those appearing in membranes of the Ehrlich cells treated in the same way. The Ca^{2+}-binding proteins were released from the membranes by washing the vesicles in an EGTA-containing medium (lane 3). Minor

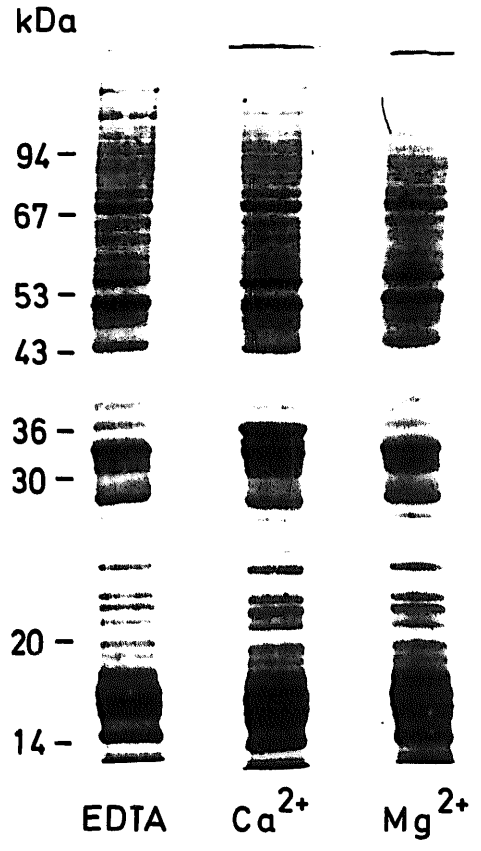

Fig. 1. Ca^{2+}-dependent cytoplasmic membrane-binding proteins. SDS-PAGE in a 12% slab gel of Ehrlich ascites tumor cell membranes prepared in the presence of 1 mM EDTA (lane 1), 1 mM Ca^{2+} (lane 2) or 1 mM Mg^{2+} (lane 3). Molecular mass standards: 94, 67, 53, 43, 36, 30, 20 and 14 kDa.

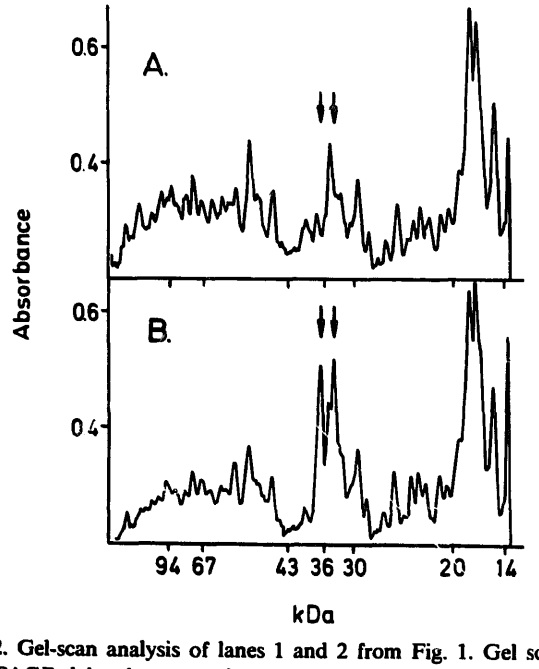

Fig. 2. Gel-scan analysis of lanes 1 and 2 from Fig. 1. Gel scan of SDS-PAGE slab gel representing proteins from Ehrlich ascites tumor cell membranes prepared in the presence of 1 mM EDTA (A) and 1 mM Ca^{2+} (B). The arrows indicate the position of the additional protein peaks appearing in the membranes prepared in the presence of Ca^{2+}.

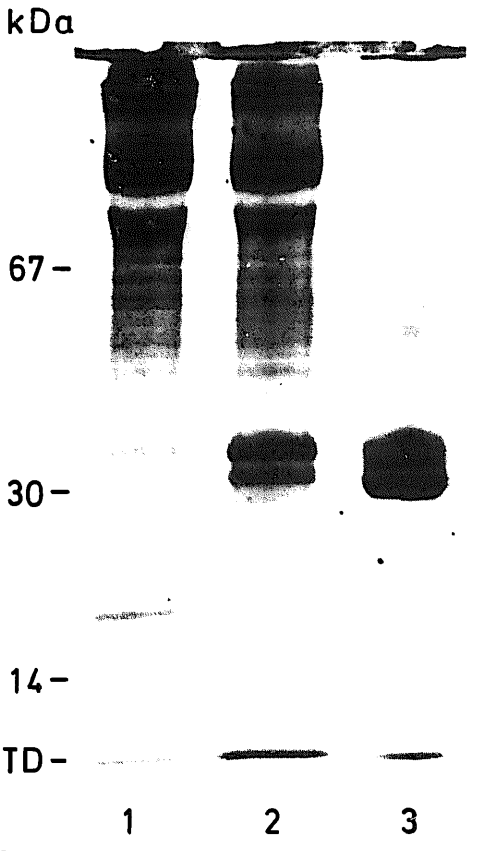

Fig. 3. Ca^{2+}-dependent membrane-binding proteins. SDS-PAGE in a 12% mini slab gel of inside-out vesicles of the human red cell membrane (lane 1) and inside-out vesicles incubated with Ehrlich ascites tumor cell cytoplasm in the presence of 1 mM Ca^{2+} (lane 2). Lane 3 represents the proteins released from the vesicles by EGTA after preincubation of the vesicles with Ca^{2+}-containing cytoplasm (crude preparation).

components with higher molecular weights are also observed in this heavily loaded gel.

The Ca^{2+}-binding protein isolated from Ehrlich ascites cell cytoplasm by Ca^{2+}-dependent affinity binding to inside-out vesicles of the human red cell membrane (crude preparation) tends to aggregate when dialyzed against distilled water. SDS-PAGE (I) and IEF (II) were therefore performed on the crude preparation liberated from inside-out vesicles (A), the protein that stayed in solution during dialysis (B), and the precipitate protein (C), as shown in Fig. 4. SDS-PAGE of the crude preparation shows three peaks which can be assigned masses of 33, 34 and 37 kDa (IA). The soluble protein fraction contains 33 and 34 kDa peptides, but is devoid of the 37 kDa protein (IB). The 37 kDa protein is found in the precipitate, which also contains some 34 kDa protein (IC). The IEF scan of the crude preparation shows protein peaks with the isoelectric points (p*I*) of 5.4, 5.8, 6.1, 7.3 and 7.8 (IIA). The soluble protein scan (IIB) is devoid of the p*I* 7.3 and 7.8 proteins, as compared to the crude preparation, but has retained the other proteins. The p*I* 7.3 and 7.8 proteins are found in the precipitate together with p*I* 5.4 and 5.8 proteins

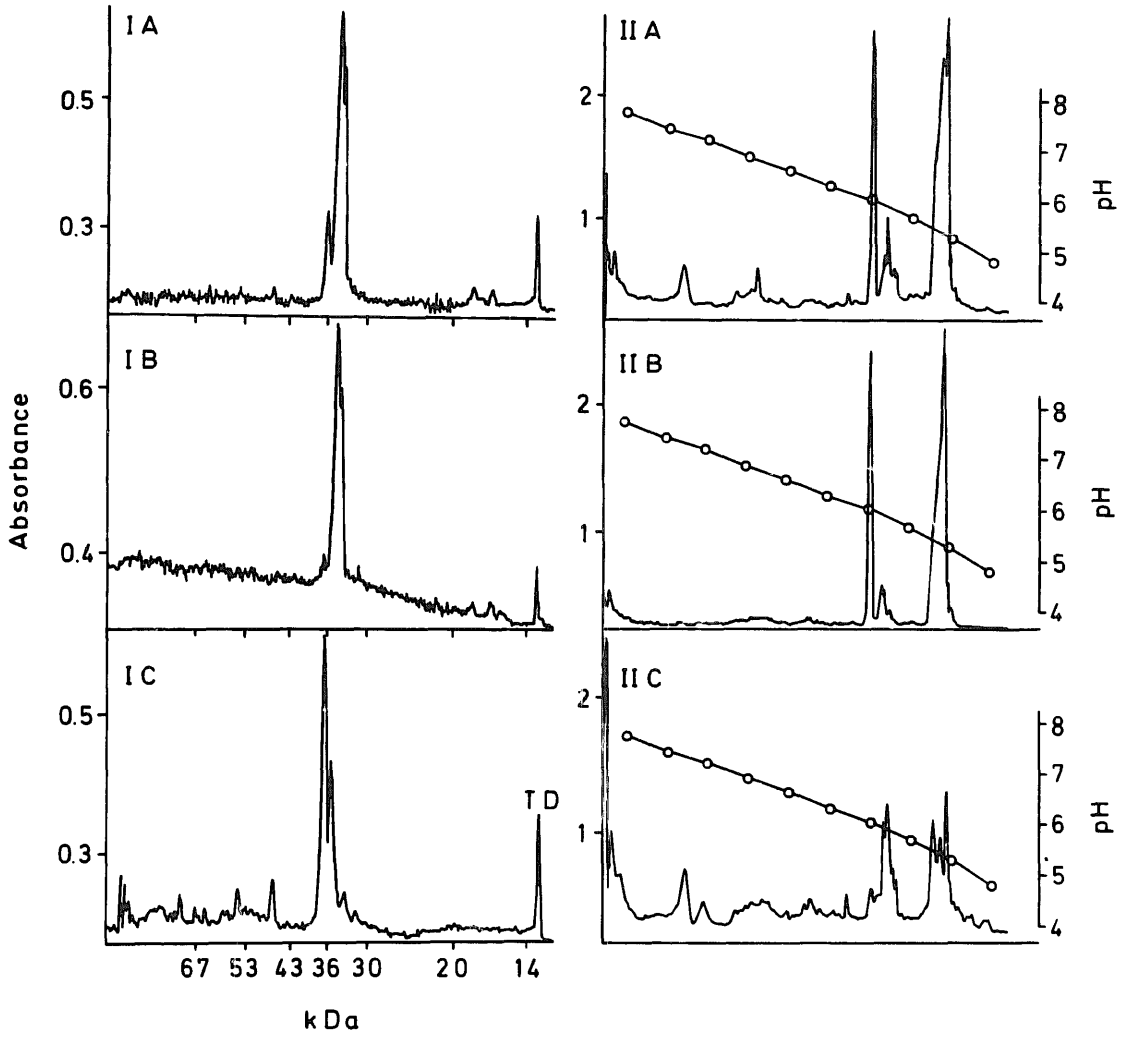

Fig. 4. Gel-scan analysis of Ca^{2+}-binding protein from Ehrlich ascites cell cytoplasm by SDS-PAGE (I) and IEF (II). (A) crude preparation isolated by membrane affinity; (B) proteins that stayed in solution after dialysis of the crude preparation; (C) precipitate proteins obtained from the dialysate. In I, the molecular weight standards are indicated as well as the tracking dye (TD). The pH profile in the IEF gels is indicated in II.

(IIC). The p*I* 6.1 protein is totally missing from the precipitate. The IEF gels of the crude and the precipitate proteins also showed insolubilized proteins which hardly entered the gel. It therefore appears that the 37 kDa protein aggregates during dialysis and that it has p*I* values of 7.3 and 7.8. The 34 kDa precipitated protein may represent the p*I* values of 5.4 and 5.8, also found in the soluble part. The soluble 33 kDa protein may represent the p*I* 6.1 protein (see Table I). A second-dimension electrophoresis of crude Ca^{2+}-binding protein and cytoplasm IEF gels showed that the Ca^{2+}-binding protein can be identified in the total cytoplasm (Fig. 5A and B).

The Hummel and Dreyer method was used to investigate whether the mixture of Ca^{2+}-dependent membrane-binding proteins in the crude preparation has the ability to bind Ca^{2+}. Fig. 6 shows that Ca^{2+} binds to the proteins, as a peak of radioactivity coincides with the protein peak as they pass through a column equilibrated with the ^{45}Ca buffer. The removal of ^{45}Ca from the solvent results in a region of decreased radioactivity in the eluent. Two test proteins were used: calmodulin, which showed a similar binding of ^{45}Ca, and bovine serum albumin, which did not bind ^{45}Ca, showing that the binding was specific for the Ca^{2+}-binding proteins.

The binding of tritium-labelled Ca^{2+}-binding protein to inside-out vesicles was studied as a function of varying free Ca^{2+} concentrations. Fig. 7 shows that the Ca^{2+}-binding protein bound calcium-dependently to the

TABLE I

*Molecular masses and p*I *of Ehrlich ascites cell cytoplasmic calcium-binding proteins*

Crude preparation (kDa)	Soluble fraction (kDa)	Precipitate (kDa)	p*I*
33	33	–	6.1
34	34	34	5.4, 5.8
37	–	37	7.3, 7.8

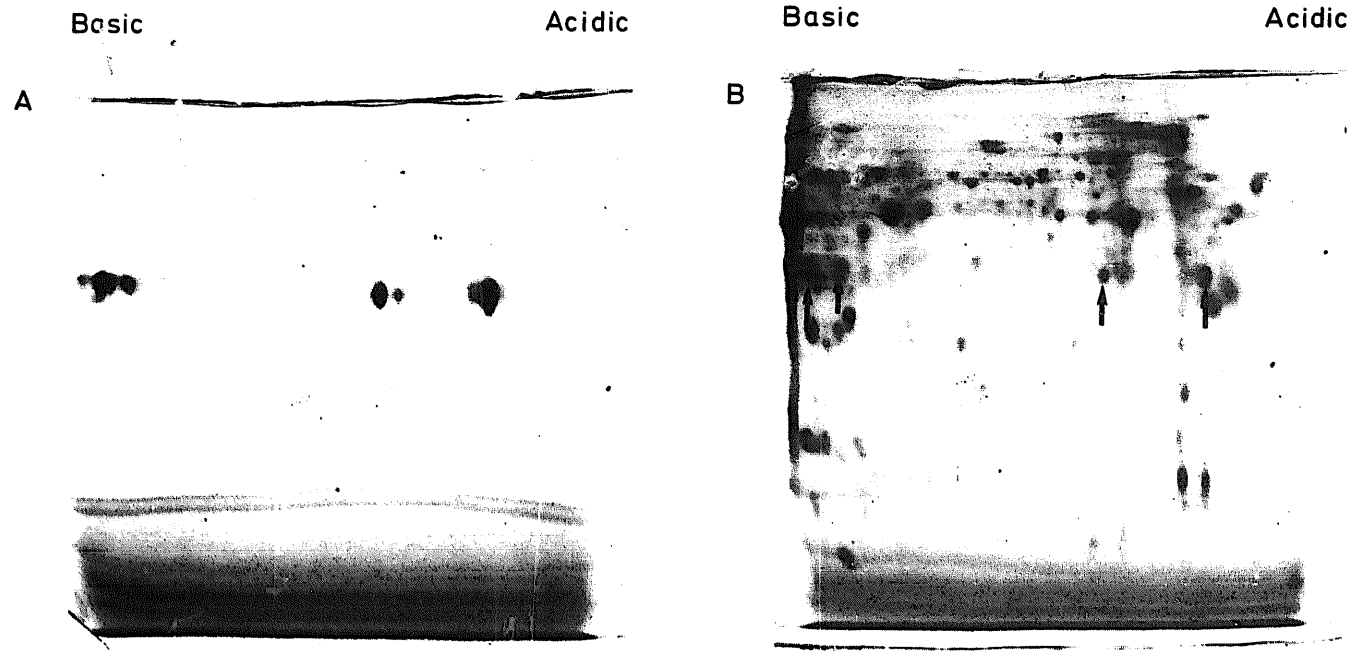

Fig. 5. Second-dimension SDS-PAGE of the IEF gel of crude Ca^{2+}-binding protein (A) and Ehrlich ascites cell cytoplasm (B). The arrows in (B) indicate the position of the Ca^{2+}-binding proteins.

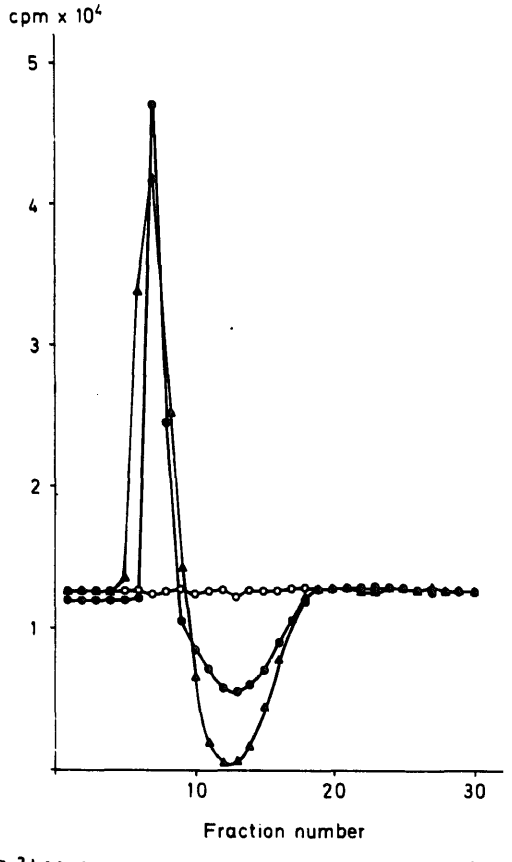

Fig. 6. Ca^{2+}-binding by the Hummel-Dreyer method. Ca^{2+}-binding to the Ca^{2+}-dependent membrane-binding proteins from the cytoplasm of the Ehrlich ascites cell as measured by equilibrium dialysis on a Sephadex column (▲); calmodulin (●); BSA (○).

vesicles, with half-maximum binding at $3.4 \cdot 10^{-4}$ M Ca^{2+}. Tagged protein bound to the vesicles could be exchanged with the unlabelled protein and was displaced in a concentration-dependent manner by the unlabelled Ca^{2+}-binding protein (results not shown).

In order to calculate the average number of Ca^{2+}-binding sites (n) of the calcium-binding proteins, a Hill plot of the data was made according to the equation

$$\log \frac{Q/Q_{max}}{1-Q/Q_{max}} = n \log Ca - \log K$$

where Q represents the fractional calcium-dependent membrane-bound Ca^{2+}-binding protein. Fig. 8 shows the Hill plot and the slope of the figure gives $n = 4.8$, which represents an average number of calcium-binding sites on the 33 and 34 kDa proteins.

In order to investigate whether the Ca^{2+}-binding protein might be related to other known proteins of the same molecular weight and Ca^{2+} dependency known to be substrates of the protein tyrosine kinase, phosphorylation studies were performed. Intact Ehrlich ascites tumor cells were incubated for 30 min at 37 °C in saline containing inorganic ^{32}P. Ca^{2+}-binding proteins were subsequently isolated from the cell cytoplasm by the membrane-affinity method. SDS-PAGE of crude Ca^{2+}-binding protein on minigels and subsequent autoradiography were performed. Fig. 9 shows that no incorporation of ^{32}P took place in the Ca^{2+}-binding proteins. Trace amounts of several other proteins of larger molec-

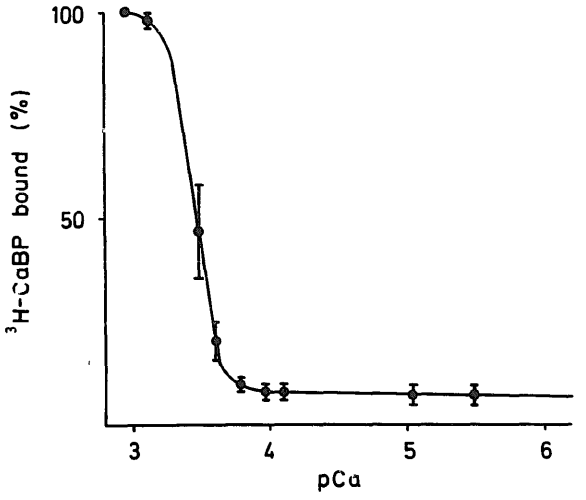

Fig. 7. Calcium dependency of ^{3}H-labelled Ca^{2+}-binding protein binding to inside-out vesicles of the human red cell membrane. 750 ng of tagged 33 and 34 kDa Ca^{2+}-binding proteins were incubated with 100 μg inside-out vesicles in 0.5 ml incubation medium containing 1 mM free Mg^{2+} and varying amounts of free Ca^{2+}. Ordinate, ^{3}H-labelled Ca^{2+}-binding protein bound (%); abscissa, p*Ca*.

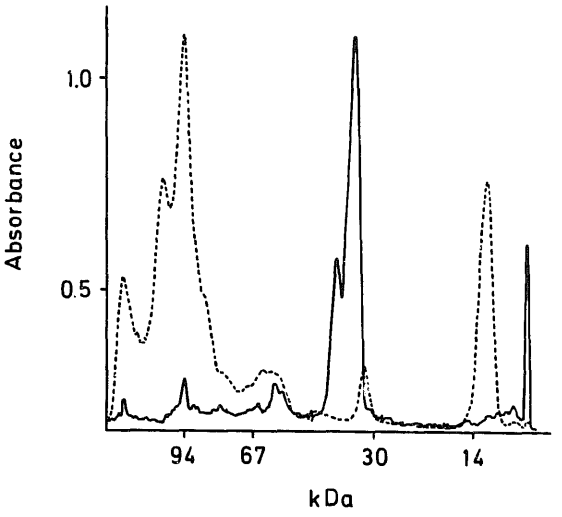

Fig. 9. Incorporation of ^{32}P into proteins isolated by calcium-dependent affinity to inside-out vesicles of the red cell membrane. Gel (full line) and autoradiographic (broken line) scan of mini SDS-PAGE of Ca^{2+}-binding protein isolated from the cytoplasm of Ehrlich ascites cell after subjection of the intact cell to phosphorylation.

ular weights and a single low-molecular-weight protein were found with high specific activity, but they were not further analyzed. A second-dimension electrophoresis of IEF gels and subsequent autoradiography showed that the minor activity peak close to the Ca^{2+}-binding protein peaks did not coincide with the Ca^{2+}-binding protein (data not shown). So the Ca^{2+}-binding protein seems to be different from calpactin I and II, lipocortin I and II, and lipomodulin, as well as other proteins known to be substrates of the protein tyrosine kinases [18–20].

Calmodulin antagonists coupled to Sepharose or Affi-Gel have been used as affinity material in order to purify calmodulin and other Ca^{2+}-binding proteins by

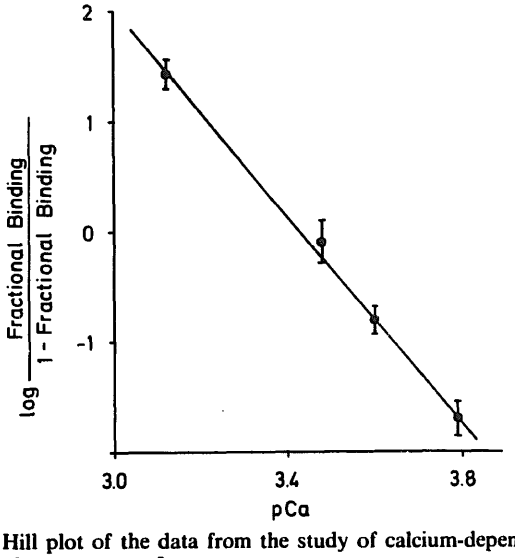

Fig. 8. A Hill plot of the data from the study of calcium-dependent binding of ^{3}H-labelled Ca^{2+}-binding protein to inside-out vesicles of the red cell membrane.

Ca^{2+}-dependent binding. The calcimedins, a set of four proteins with Ca^{2+}-binding properties and molecular masses of 67, 35, 33 and 30 kDa can be isolated by affinity chromatography to fluphenazine-Sepharose [1].

When cytoplasmic extract was subjected to phenothiazine Affi-Gel affinity chromatography, calmodulin was eluted together with high-molecular-weight proteins, 88 and 100 kDa, and a single low-molecular-weight protein, 10 kDa [21]. The 10 kDa protein may be an analog to the subunit of calpactin, which normally constitutes a hetero tetramer $p36_2$, $p10_2$ [22], although calpactin has been identified as monomeric in the MAT-CA ascites tumor cell microvilli associated with the microfilaments [23].

The Ca^{2+}-binding proteins from the Ehrlich cell cytoplasm were not retained by Affi-Gel phenothiazine, indicating that, in this respect, these proteins may be different from other Ca^{2+}-binding proteins.

Discussion

The presence in Ehrlich ascites tumor cells of proteins that associate with cell membranes in a Ca^{2+}-dependent manner is clearly demonstrated. Association occurs to membranes from Ehrlich cells as well as to inside-out vesicles of human red blood cells.

The SDS-PAGE analysis (dissociating and denaturing conditions) shows that at least three polypeptides with slightly different molecular weights are present in the primary, crude preparation. By interpolation on a plot of log (standard molecular mass) versus travelling distance, the molecular masses were 33, 34 and 37 kDa. In the native state these may be combined in unknown proportions to form larger molecules. But comparison

of the two fractions obtained by dialyzing the crude preparation indicates that more than one native species is involved, because the 37 kDa peptide precipitates almost quantitatively accompanied by only trace amounts of 34 kDa polypeptides. We have tried to estimate the native molecular weight of the soluble fraction by chromatography on a Superose (Pharmacia) column, but the protein appeared as one peak with a K_D near 1, indicating adsorption to the gel matrix (data not shown).

The IEF analysis should be interpreted with more caution, because artificial changes in charge may occur in the presence of the high urea concentration. But comparison of the three fractions in Fig. 4 suggests that the 37 and 34 kDa peptides are associated mainly with p*I* values of 7.8, 7.3 and 5.8, and perhaps also 5.4. The soluble fraction is characterized by p*I* values of 6.1, 5.8 and 5.4.

From the isolation procedure of the proteins it can be concluded that they associate with membranes in the presence of Ca^{2+} and dissociate again when Ca^{2+} is removed. The proteins bind Ca^{2+}, as shown by their capacity to take up radioactivity from a buffer containing ^{45}Ca. The binding of Ca^{2+} to the proteins may therefore precede the binding of the proteins to the membranes. Application of ^{3}H-labelled 33 and 34 kDa protein to inside-out vesicles of the erythrocyte membrane demonstrates a calcium-dependent binding, K_d being $3.4 \cdot 10^{-4}$ M Ca^{2+}. A Hill plot of the data indicates 4.8 to be the average number of Ca^{2+}-binding sites on the 33 and 34 kDa proteins.

Many different Ca^{2+}-binding proteins with peptide molecular masses similar to those presented here are found present in many tissues. Most of these proteins have been described by Creutz et al. [2] under the collective term chromobindins. It appears that six of the chromobindins have molecular masses and p*I* values comparable to the Ca^{2+}-binding protein from Ehrlich cells. Some Ca^{2+}-binding proteins are substrates of protein kinases. This is not the case for the Ehrlich cell proteins, as these are not labelled with [^{32}P]phosphate under circumstances where numerous other cytoplasmic and membrane proteins display massive ^{32}P incorporation. Our proteins are therefore different from proteins assigned names Protein I, p36, lipocortins, and calpactins.

Among other calcium-binding proteins, Haigler et al. [24,25] have extracted a 33 kDa protein which inhibits phospholipase A_2, peak G, from human placenta, which may be identical to our 33 kDa protein. They share common properties, such as not being a substrate for kinase activity and having the same p*I* (6.0 vs. 6.1). Also, peak G binds Ca^{2+} and associates with liposomes in a Ca^{2+}-dependent manner. It is suggested to belong to the 'annexins', a name proposed by Geisow [26].

In conclusion, we have observed the presence in Ehrlich ascites tumor cells of Ca^{2+}-binding proteins that associate with membranes from Ehrlich cells and from human erythrocytes. The proteins contain peptides of 33–37 kDa and may therefore be related to earlier reported Ca^{2+}-binding proteins. Their presence is easily observable on two-dimensional PAGE of total cytoplasmic proteins and they may be isolated easily in reasonable amounts, which makes them appropriate objects for further study of their possible physiological role in cell regulation [27].

Acknowledgements

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References

1 Moore, P.M. (1986) Biochem. J. 238, 49–54.
2 Creutz, C.E., Dowling, L.G., Sando, J.J., Villar-Palasi, C., Whipple, J.H. and Zaks, W.J. (1983) J. Biol. Chem. 258, 14664–14674.
3 Shadle, P.J., Gerke, V. and Weber, K. (1985) J. Biol. Chem. 260, 16354–16360.
4 Südhof, T.C., Ebbecke, M., Walker, J.H., Fritsche, U. and Boustead, C. (1984) Biochemistry 23, 1103–1109.
5 Südhof, T.C., Walker, J.H. and Fritsche, U. (1985) J. Neurochem. 44, 1302–1307.
6 Glenney, J.R., Jr. (1985) FEBS Lett. 192, 79–82.
7 Feit, P.W., Hoffmann, E.K., Schødt, M., Kristensen, P. and Dunham, P.B. (1988) J. Membr. Biol. 103, 135–147.
8 Steck, T.L. and Kant, J.A. (1974) Methods Enzymol. 31A, 172–180.
9 Laemmli, U.K. (1970) Nature 227, 680–685.
10 O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007–4021.
11 Hummel, J.P. and Dreyer, W.J. (1962) Biochim. Biophys. Acta 63, 530–532.
12 Mathew, J.K., Krolak, J.M. and Dedman, J.R. (1986) J. Cell. Biochem. 32, 223–234.
13 Biocca, S., Calissano, P., Barra, D. and Fasella, P.M. (1978) Anal. Biochem. 87, 334–342.
14 Kumarasamy, R. and Symons, R.H. (1979) Anal. Biochem. 95, 359–363.
15 Kristensen, B.I. and Pape, L. (1982) Mol. Physiol. 2, 99–106.
16 Kristensen, B.I. (1986) Biochim. Biophys. Acta 862, 457–460.
17 Caldwell, P.C. (1970) in Calcium and Cellular Function (Cuthbert, A.W., ed.), pp. 10–16, MacMillan, New York.
18 Brugge, J.S. (1986) Cell 46, 149–150.
19 Hirata, F., Matsuda, K., Notsu, Y., Hattori, T. and Del Carmine, R. (1984) Proc. Natl. Acad. Sci. USA 81, 4717–4721.
20 Glenney, J.R., Jr. (1987) in Biochem. Soc. Trans. 622nd Meeting, Leicester, 15, 798–800.
21 Aabin, B. and Kristensen, B.I. (1987) Acta Physiol. Scand. 129, 18A.
22 Burgoyne, R.D. (1988) Nature 331, 20.
23 Liu, Y., Brew, K., Carraway, K.L. and Carraway, C.A.C. (1987) J. Cell. Biochem. 35, 185–204.
24 Haigler, H.T., Schlaepfer, D.D. and Burgess, W.H. (1987) J. Biol. Chem. 262, 6921–6930.
25 Schlaepfer, D.D. and Haigler, H.T. (1987) J. Biol. Chem. 262, 6931–6937.
26 Geisow, M.J. (1986) FEBS Lett. 203, 95–98.
27 Kristensen, B.I. and Kristensen, P. (1988) Acta Physiol. Scand. 132, 43A.