Biochimica et Biophysica Acta 982 (1989) 1–8
Elsevier

BBAMEM 74416

The high-affinity calcium binding protein of sarcoplasmic reticulum. Tissue distribution, and homology with calregulin

Larry Fliegel [1], Kimberly Burns [1], Michal Opas [2] and Marek Michalak [1]

[1] *Heritage Cardiovascular Disease Group Department of Pediatrics and Biochemistry University of Alberta, Edmonton Alberta and* [2] *Department of Anatomy, University of Toronto Toronto Ontario (Canada)*

(Received 3 January 1989)

Key words Calcium binding protein, Sarcoplasmic reticulum, Endoplasmic reticulum Calregulin homology

The 55-kDa high-affinity calcium binding protein (HACBP) was first identified and isolated from skeletal muscle sarcoplasmic reticulum (SR) Using polyclonal antibodies raised against the HACBP isolated from skeletal muscle we have identified this protein in cardiac and smooth muscle as well as in non-muscle cells. Although the 55-kDa protein has a size, properties and localization similar to that of calsequestrin, the two proteins are immunologically distinct. The NH_2-terminal sequence of uterine HACBP is also completely different from that of calsequestrin but it is identical to that of rabbit liver calregulin, a recently identified calcium binding protein Indirect immunofluorescence staining of frozen sections and culture cells from a variety of tissues shows that the 55-kDa protein localizes predominantly to junctional SR and T-tubule areas in skeletal muscle, to SR in smooth and cardiac muscle cells, and to ER in a variety of non-muscle cells These data show that the protein is present in a wide variety of tissues and suggest that it is a protein common for both sarcoplasmic and endoplasmic reticulum membranes.

Introduction

The sarcoplasmic reticulum (SR) membrane plays a key role in the regulation of the contraction-relaxation cycle of skeletal muscle [1–4] The SR ($Ca^{2+} + Mg^{2+}$)-dependent ATPase removes calcium from the cytosol causing a decrease in free calcium concentration and initiating relaxation Calsequestrin [5], a major calcium binding protein in SR membranes, acts to store calcium within the SR membrane lowering the free calcium concentration within the SR, and thereby lowering the calcium gradient against which the ($Ca^{2+} + Mg^{2+}$)-dependent ATPase must pump

Investigations on the protein composition of the skeletal muscle SR have revealed the presence of a high affinity calcium binding protein (HACBP) of apparent molecular weight of 55000, which was first identified and purified by Ostwald and MacLennan [6] In the presence of 100 mM KCl, it binds calcium with a much higher affinity than calsequestrin (K_d 2.5–4 μM vs 1 mM) but with a lower capacity (1 mol of calcium per mol of protein vs 40) [5,6] The HACBP is a minor component of the SR membrane localized to the lumen of the SR membranes [7] The HACBP is present in light, intermediate and heavy SR fractions (free and junctional SR) and it may be increased in fractions enriched in transverse tubules [7]

In this study, we have identified the HACBP in cardiac, smooth muscle and in non-muscle cells Using immunofluorescence microscopy techniques the HACBP has been localized to SR in skeletal muscle and to ER membranes in non-muscle tissues Our results also show that calregulin a calcium binding protein of unknown function recently identified in bovine liver [8] is probably identical to the HACBP A preliminary report of this work has appeared elsewhere [9].

Experimental procedures

Materials

DEAE-Sepharose was purchased from Pharmacia. Affi-Gel Blue Affi-Gel 10, gel electrophoresis reagents and molecular weight markers were from Bio-Rad

Abbreviations SR, sarcoplasmic reticulum, HACBP, high-affinity calcium binding protein ER, endoplasmic reticulum EDTA, ethylenediaminetetraacetic acid, EGTA, ethyleneglycol bis(β-aminoethyl ether)tetraacetic acid, PBS, phosphate-buffered saline, SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis Pipes 1,4-piperazinediethanesulfonic acid.

Correspondence M Michalak, Heritage Cardiovascular Disease Group, Department of Pediatrics, 417A Heritage Medical Research Bldg., Edmonton, Alberta Canada, T6G 2R7

Nitrocellulose and polyvinylidene difluoride membranes were obtained from Millipore Horseradish peroxidase linked rabbit anti-goat antibody and goat anti-rabbit antibody were from Bio-Can Scientific Ltd and Boehringer Mannheim, respectively Pipes was from Sigma Dispase was purchased from Collaborative Research Trypsin-EDTA, fetal calf serum, penicillin, fungizone, streptomycin, and alpha minimal essential medium were purchased from Gibco Bovine hearts were obtained from a local slaughterhouse Uteri from adult female rabbits were obtained immediately after term, and were a generous gift of Dr G D Lopaschuk, University of Alberta Purified bovine liver calregulin and rabbit anti-bovine calregulin antibody were a generous gift of Dr D M Waisman, University of Calgary

Preparation of membrane fractions

Rabbit skeletal muscle SR vesicles and cardiac SR vesicles were isolated as described by Meissner [10] and Pegg and Michalak [11], respectively Uterine microsomes were isolated by homogenizing the rabbit uterus with a Polytron PT-10 in a buffer containing 120 mM NaCl 10 mM imidazole-HCl (pH 7 4), 0 5 mM phenylmethylsulfonyl fluoride (PMSF) The homogenate was centrifuged at $1600 \times g$ for 10 min and the supernatant was saved The pellet was rehomogenized in the same buffer and centrifuged as above and both supernatants were combined These were centrifuged at $10000 \times g$ for 15 min and pellets were discarded The supernatant was pelleted at $100000 \times g$ for 1 h and the microsomes were suspended in 250 mM sucrose, 10 mM Tris (pH 8 0), frozen in liquid N_2 and stored at $-70°C$

Partial purification of HACBP

HACBP was isolated from bovine hearts, rabbit skeletal muscle, and rabbit uterus by selective ammonium sulfate precipitation, followed by fractionation by DEAE-Sepharose This procedure was identical to that previously described for the purification of calsequestrin [12] and was developed in the laboratory of Dr K P Campbell, University of Iowa This method involves direct extraction of the HACBP from whole muscle homogenates and eliminates the need to prepare a SP fraction Membrane-bound proteins are solubilized by this homogenization procedure [12] At the final stage of purification an ammonium sulfate precipitate containing both HACBP and calsequestrin was dissolved in 100 mM potassium phosphate buffer (pH 7 1), containing 1 mM EGTA, dialyzed against the same buffer for approximately 20 h, and applied to a $2 5 \times 8$ cm column of DEAE-Sepharose equilibrated with 100 mM potassium phosphate (pH 7 1), containing 1 mM EGTA and 50 mM NaCl The column was washed with initial buffer and protein fractions (approx 5 ml/fraction) were eluted with a linear gradient of 0 05 to 1 0 M NaCl [12] The fractions enriched in HACBP (200–250 mM NaCl) were identified by immunostaining with anti-HACBP antibody, and pooled

SDS-PAGE, transfer of proteins to nitrocellulose, and reaction with antibodies

SDS-PAGE (10% acrylamide) was carried out according to Laemmli [13] The gels were stained with Coomassie blue or with the cationic carbocyanine dye 'Stains-All' [14] Densitometric scans of SDS-PAGE were carried out on a Beckman DU-65 spectrophotometer Protein samples were transferred to nitrocellulose or to polyvinylidene difluoride membranes [17] The standards used were Bio-Rad low range molecular weight proteins, phosphorylase *b* (97400), bovine serum albumin (66200), ovalbumin (42700), carbonic anhydrase (31000), soybean trypsin inhibitor (21500), and lysozyme (14400), or Bio-Rad prestained markers; phosphorylase *b* (135000), bovine serum albumin (75000), ovalbumin (50000), carbonic anhydrase (39000), soybean trypsin inhibitor (27000), lysozyme (17000) Immunoblotting was carried out in the presence of 1% milk powder Protein bands reactive with antibodies were visualized by developing a peroxidase reaction

Preparation of antibodies

For immunization HACBP was purified from skeletal muscle SR vesicles by the procedure described by Ostwald and MacLennan [6] Calsequestrin was purified from skeletal and cardiac muscle homogenates by the ammonium sulfate precipitation method [12]. Antibodies against HACBP and skeletal muscle calsequestrin were raised in mature goats by the procedures described earlier [7] Antibody against cardiac calsequestrin was raised in rabbits as described earlier [7]

Immunofluorescence microscopy

The tissues dissected from newborn rabbits were either fixed and processed for cryomicrotomy as described [16], or used to establish primary cultures For tissue culture the tissues were finely chopped, incubated at 37°C for 30 min in Dispase, followed by 20–30 min incubation with trypsin-EDTA Cell suspensions were decanted from over larger pieces of undigested tissues, centrifuged, incubated with pure fetal calf serum for 10–15 min, and plated onto collagen-coated cover slips in alpha minimal essential medium supplemented with 10% total fetal calf serum and containing 100 IU penicillin, 0 25 μg fungizone and 100 μg streptomycin per ml

For immunofluorescence microscopy cells on cover slips were fixed for 10 min in 3 7% formaldehyde in PBS extracted for 2 min with 0 1% Triton X-100 in a buffer containing 1 mM EGTA, 4% (w/v) poly(ethylene glycol) 8000 (PEG 8000), 100 mM Pipes (pH 6 9) Staining was carried out for 30 min at room temperature on cells or 6–8 μm frozen sections with the anti-

body against HACBP (diluted 1 50 in PBS) followed by 10 min wash in PBS and incubation for 30 min at room temperature with rabbit anti-goat FITC-conjugated IgGs (Nordic) diluted 1 30 in PBS For absorption experiments 35-μl aliquots of antibody against HACBP (diluted 1 50 in PBS) were incubated for 1 h at 37 °C with 12 μg of purified uterine HACBP, centrifuged and the supernatant used for immunofluorescence staining. As an additional control, the immunofluorescence procedure was performed with the omission of the antibody against HACBP After the final wash in PBS, the cover slips were mounted in Vinol 205S (St Lawrence Chemical, Toronto, Ontario) containing 0 25% 1,4-diazobicyclo[2,2]octane (Polysciences) and 0 002% *p*-phenylenediamine (Fisher Scientific) to prevent photobleaching A Zeiss Photomicroscope III equipped with an epifluorescence condenser and selective FITC filters was used for observation and photography Photographs were recorded on TMax-400 negatives (Kodak) pushed to 1600 ASA with TMax developer

Miscellaneous procedures

NH_2-terminal sequence analysis of HACBP was carried out using partially purified uterine HACBP (from the DEAE-Sepharose column, Fig. 2) separated on SDS-PAGE slab gels and transferred to poly(vinylidene difluoride) membranes [17] Automated protein sequence analysis [18] of the poly(vinylidene difluoride) membrane electroblotted protein was carried out on an Applied Biosystems Model 470A gas-liquid phase protein sequencer connected on-line to an Applied Biosystems Model 120 A HPLC, using the current protocols of Applied Biosystems for both instruments Chromatograms were recorded on a Spectra Physics Model 4270 recording integrator. All chemicals used for protein sequence analysis were from Applied Biosystems, Foster City, CA Two independent sequence analysis were performed with repetitive yields of about 93% Protein was determined by the method of Lowry et al [19], using bovine serum albumin as a standard

Results

Identification and isolation of HACBP

Fig 1 shows the protein composition and peroxidase staining of nitrocellulose sheets reacted with HACBP antibody All three tissues skeletal muscle SR, cardiac SR and uterine microsomes, contained a 55-kDa protein immunoreactive with the HACBP antibody raised against the skeletal muscle form of this protein (Fig 1B) The antibody against skeletal muscle calsequestrin, when tested against the same membrane fraction did not react with HACBP and reacted only with skeletal muscle SR (Fig. 1C) An additional series of high molecular weight proteins (> 130 kDa) crossreacted in skeletal muscle SR with the anti-calsequestrin antibody, however, their relation to calsequestrin is not known yet

For the partial purification of HACBP we adapted a procedure described for the purification of calsequestrin [12], which takes advantage of the high solubility of HACBP in ammonium sulfate solutions. We have shown earlier that HACBP was not precipitated by ammonium sulfate concentrations as high as 65% saturation [20] However, it could be precipitated together with calsequestrin by ammonium sulfate concentrations above 90% saturation at pH 5 0 A partially purified preparation of HACBP could then be obtained after DEAE-Sepharose chromatography of the ammonium sulfate precipitated protein fractions This method eliminates completely the need to prepare SR fractions prior to the isolation of the HACBP

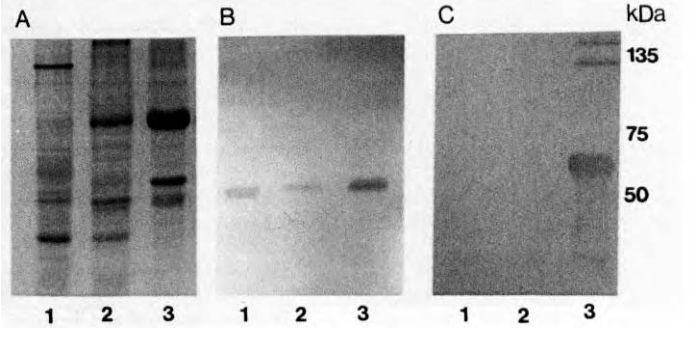

Fig. 1 SDS-PAGE and immunoblot analysis of uterine microsomes (1), cardiac muscle SR (2), and skeletal muscle SR (3) Membrane fractions were prepared as described under Experimental Procedures (A) Coomassie blue staining of polyvinylidene difluoride membrane [17], (B) immunoreactivity of SR proteins with antibody prepared against HACBP, and (C) immunoreactivity of SR proteins with antibody prepared against skeletal muscle calsequestrin

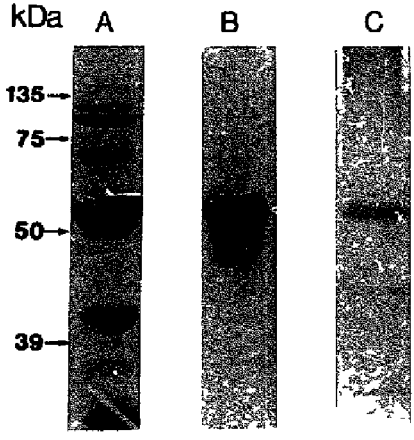

Fig 2 Identification of the HACBP in DEAE-Sepharose fractions of ammonium sulfate extract of rabbit uterine muscle Ammonium sulphate extraction and DEAE-Sepharose chromatography were carried out as described under Experimental Procedures (A) Protein composition of HACBP containing fraction (Coomassie blue staining) (B) Identification of HACBP containing fraction by immunostaining (nitrocellulose membranes) (C) Staining of the HACBP containing fraction with 'Stains-All'

Fig 2 shows the partially purified HACBP from the rabbit uterus by ammonium sulfate precipitation and DEAE-Sepharose chromatography The peak HACBP containing fractions eluted at 200–250 mM NaCl The HACBP appeared to be present in relatively large amounts in uterus The uterine protein was strongly immunoreactive with the anti-skeletal muscle antibody indicating significant homology between the two proteins With the enriched uterine HACBP fractions, we observed that the protein stained blue with the cationic carbocyanine dye 'Stains-All' (Fig 2C) A smaller molecular weight protein also stained blue (Fig 2C) and likely represents a degradation product of the purified HACBP Using the same procedure the HACBP has also been partially purified from the bovine heart and rabbit skeletal muscle (data not shown) Similar to the uterine HACBP, the cardiac and skeletal muscle HACBP stained blue with 'Stains-All' (data not shown) The bovine cardiac and skeletal muscle HACBP were of the same apparent molecular weight as the uterine protein and eluted from DEAE-Sepharose prior to cardiac and skeletal muscle calsequestrin (200–250 mM NaCl vs 300–350 mM NaCl)

Intracellular localization of HACBP

Longitudinal frozen sections of newborn rabbit gracilis muscle consistently displayed a striated, double-banded pattern of staining with the antibody against HACBP (Fig 3A) HACBP-positive bands comprising a doublet were found closely apposed to both sides of a Z-line and occupy a total length of 1.1 ± 0.2 μm with sarcomere length of $2.9\ \mu m \pm 0.1\ \mu m$ Narrow unstained bands separating the two HACBP-positive bands in the doublets corresponded to Z-lines as visualized with phase contrast microscopy (Fig 3D) Absorption of the antiserum with partially purified HACBP abolished the specific staining (Fig 3B) Omission of the HACBP antiserum from the staining procedure abolished staining completely (Fig. 3C) Staining of frozen sections of newborn rabbit intestine (Fig 3G–I) showed heavy intracellular labelling of intestine epithelium and underlying ir smooth muscle cells (Fig. 3G) Heavy staining of liver parenchyma was also observed with anti-HACBP antibody (Fig 3M) Absorption of the specific antiserum with the antigen as well as the omission of the specific antiserum from the staining procedure abolished the staining of both intestine (Fig 3H, I) and liver (Fig 3N, O) frozen sections

To facilitate intracellular localization of HACBP, we have stained spread cells from primary cultures of a variety of tissues from newborn rabbits In spread cells, HACBP localized exclusively to a perinuclear system of membranes corresponding to ER in smooth (Fig 4A) and cardiac muscle (Fig 4B), fibroblasts (Fig 4B), intestine epithelium (Fig 4C), and cardiac endothelium (not shown)

Identification of NH_2-terminal sequence of HACBP

To obtain information about the primary protein structure of HACBP, we sequenced the NH_2-terminal portion of uterine HACBP The following eight NH_2-terminal residues were obtained from two independent analysis NH_2-Glu-Pro-Val-Val-Tyr-Phe-Lys-Glu-(EPVVYFKE) The sequence exactly matches the NH_2-terminal sequence of rabbit liver calreguln as reported by Khanna et al [21]

Homology of calreguln to HACBP

In order to further determine the relationship between the HACBP and calreguln, we tested the two proteins for cross-reactivity with their respective antibodies Rabbit anti-bovine calreguln antibody cross-reacted with uterine and skeletal muscle HACBP (Fig 5A) Our polyclonal antibody against rabbit skeletal muscle HACBP, however, cross-reacted only very weekly with bovine liver calreguln (Fig 5B)

Discussion

We have identified and localized HACBP in skeletal, cardiac and smooth muscle as well as in a variety of non-muscle cells The HACBP is present in a crude microsomal fraction of the uterus and in cardiac SR vesicles The rabbit uterus, a tissue highly enriched in

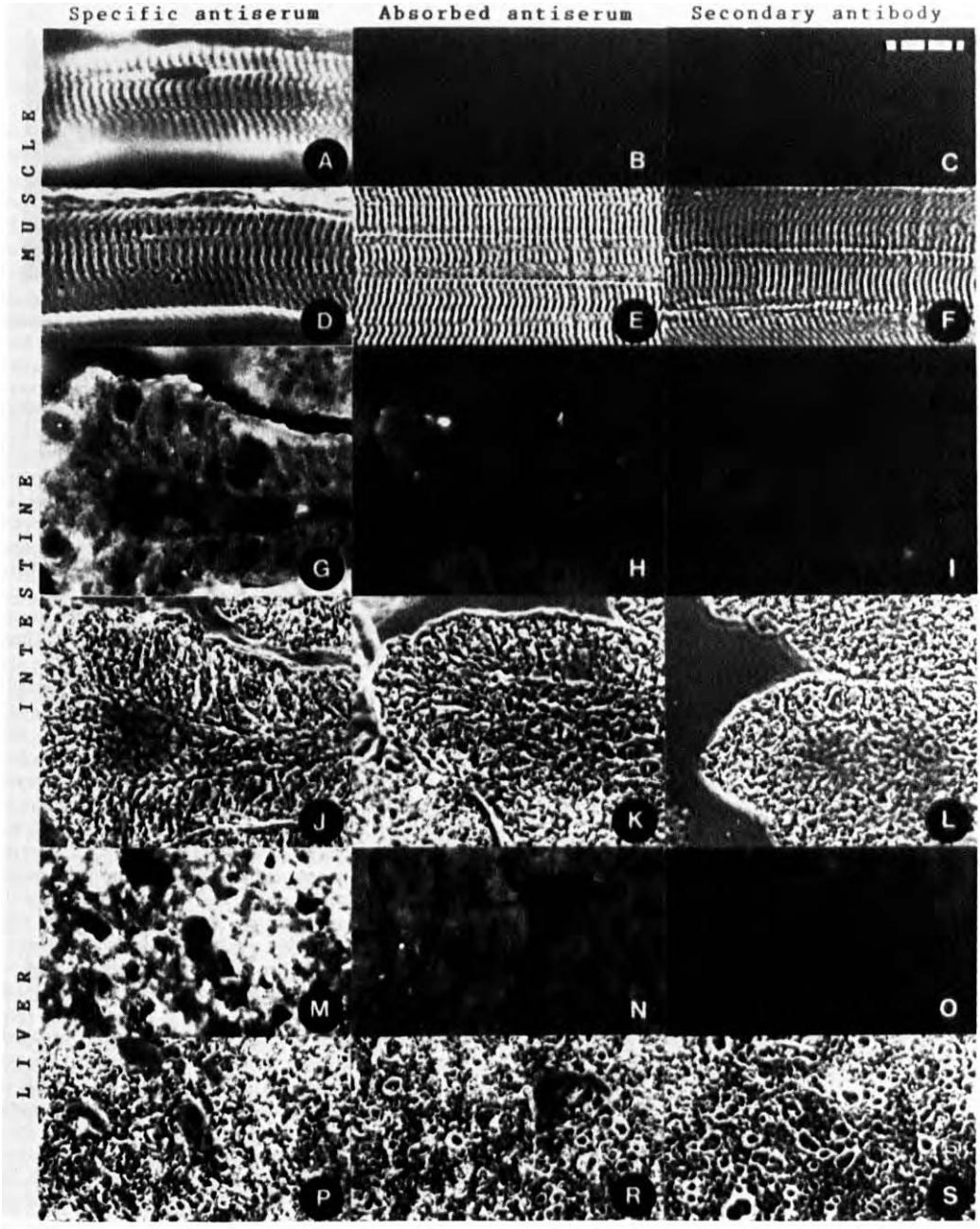

Fig 3 Localization of the HACBP in frozen sections of skeletal muscle (gracilis) (A–F), intestine (G–L) and liver (M–S) of newborn rabbit by immunofluorescence microscopy In longitudinal sections of muscle (A) HACBP is present in striated pattern consisting of double bands closely apposed to both sides of each of Z-lines (D) Absorption of the antibody with HACBP abolishes the specific staining (B) Omission of the antibody against HACBP resulted in no staining (C) (E) and (F) are phase contrast images of (B) and (C), respectively HACBP is present intracellular in the intestine epithelium and underlying it smooth muscles (G) as well as in liver hepatocytes (M) (J) and (P) are phase contrast images of (G) and (M), respectively Absorption of the antibody with HACBP abolishes the specific staining in both liver (H, K) and intestine (M, P). Secondary antibody gives no staining when used without the specific antibody (intestine, I, L, liver, O, S) Scale division = 10 μm.

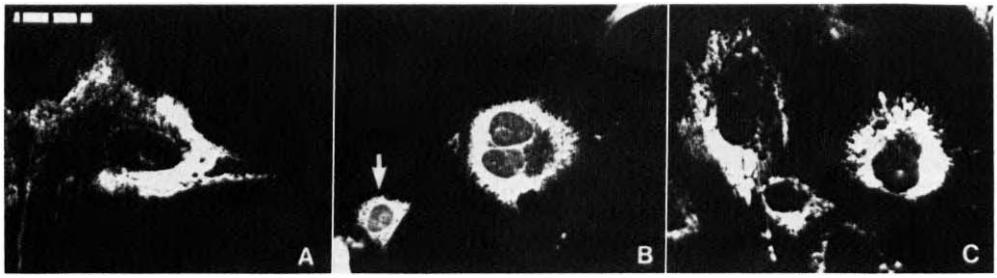

Fig 4 Immunofluorescence localization of the HACBP in cultured smooth (A) and cardiac (B) muscle cells, and intestine epithelial cells (C) Arrow in (B) shows the distribution of the HACBP in a fibroblast. In all these cells HACBP is present in a perinuclear system of membranes comprising ER Scale division = 40 μm

smooth muscle [22], contains high amounts of the HACBP The molecular weight of the HACBP from cardiac, skeletal and smooth muscle estimated by SDS-PAGE in Laemmli gel system [13] is approx 55 000

HACBP and calsequestrin are similar in size and localization, and both bind Ca^{2+} but in different amounts and with different affinities [5,6] The present results show that these two proteins are clearly distinct although present in the same membrane systems Polyclonal antibodies against either skeletal or cardiac muscle calsequestrin do not recognize HACBP and antibodies against the HACBP do not recognize either the skeletal or cardiac form of calsequestrin In addition, the HACBP from skeletal muscle migrates slightly faster on Laemmli SDS-PAGE than calsequestrin (55 kDa vs 63 kDa), and the skeletal and cardiac forms of the HACBP can be separated from calsequestrin by DEAE-Sepharose chromatography Another protein

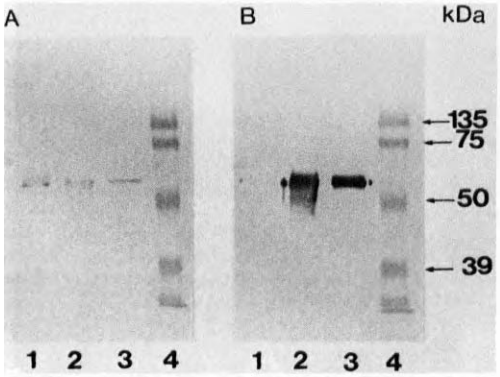

Fig 5 Immunoblotting of HACBP and calreguhn with polyclonal antibodies against calreguhn (A) and HACBP (B) SDS-PAGE was carried out in 10% acrylamide slab gel according to the procedure of Laemmli [13] Lane 1 bovine liver calreguhn, lane 2, rabbit uterine HACBP, lane 3, rabbit skeletal muscle HACBP, lane 4, prestained molecular weight markers 5 μg of protein was applied on each lane

present in the SR membranes of skeletal and cardiac muscle and of size similar to HACBP on SDS-PAGE is the 53-kDa intrinsic glycoprotein [7,23] This protein is also different from HACBP The intrinsic glycoprotein is of slightly smaller apparent molecular weight in SDS-PAGE, and the HACBP antibody does not cross-react with purified 53-kDa glycoprotein Antibodies against this glycoprotein also do not react with HACBP (Fliegel. L , MacLennan, D H and Michalak, M , unpublished observations). The HACBP is also not glycosylated and does not bind any conconavalin A [7]. Thus, the HACBP is different from either calsequestrin or the intrinsic glycoprotein of the SR membranes Partially purified HACBP can be obtained from tissue homogenates after ammonium sulfate precipitation and DEAE-Sepharose This method, originally developed for the purification of cardiac and skeletal muscle calsequestrin [12], completely eliminates the need to prepare SR membranes prior to the isolation of the HACBP Partially purified preparations of uterine HACBP contain a protein band of 55-kDa, which stains blue with the carbocyanine dye 'Stains-All' confirming its Ca^{2+} binding properties, however, the staining with 'Stains-All' is relatively weak in comparison to that of calsequestrin [14] This may be an indication of its lower Ca^{2+} binding capacity compared to calsequestrin [5,6]

Our data show that the HACBP localizes predominantly to the area occupied by both junctional SR and T-tubule membranes in skeletal muscle and to SR in smooth and cardiac muscle cells, and to ER in a variety of non-muscle cells in vitro These data clearly show that the HACBP is present in a wide variety of tissues and suggest that it is a protein common for both SR and ER membranes It has been shown earlier that the HACBP is present in the isolated free and junctional SR vesicles, and it may be increased in fractions enriched in T-tubules [7] Although HACBP-positive staining is found closely apposed to both sides of a Z-line, the resolution of immunocytochemistry at an optical

microscopy level is not sufficient to distinguish whether antibody staining is localized in the junctional SR or in the T-tubule, or in both

In the course of these studies, we have made an unexpected discovery Based on NH_2-terminal amino acid sequence of rabbit uterus HACBP and the sequence recently reported for rabbit liver calreguhn [21], we conclude that calreguhn is either identical to the HACBP or it is at minimum homologous over a significant portion of the protein Calreguhn is a calcium binding protein of unknown function recently identified in bovine liver and in variety of other tissues [8,21] Similar to the HACBP [6] it binds 1 mol of calcium per mol of protein with an apparent $K_d = 0.05\ \mu M$ [24] The amino acid composition of both calreguhn [21] and the HACBP [25] are remarkably similar Calreguhn, like the HACBP, is associated with liver ER membranes [8] In order to confirm the identity of the two proteins, we tested polyclonal antibodies raised against the two proteins for cross-reactivity against one another The rabbit anti-bovine calreguhn antibody cross-reacted with purified HACBP from all three muscle tissues In contrast the goat anti-rabbit HACBP antibody reacted strongly with purified HACBP, but did not react well with purified bovine liver calreguhn (Fig 5) This may be due to differences in the protein from one species to another In fact, we noted in our study a decrease in the sensitivity of our anti-HACBP antibody, when used against bovine cardiac protein, in comparison to rabbit tissues Khanna et al. [21] also noted species differences between various forms of calreguhn It should not be ruled out, however, that only a limited portion of the two proteins primary sequences are identical

Using the polyclonal antibody against the HACBP, we have recently isolated a partial length cDNA clone encoding for the COOH-terminal of the HACBP from a rabbit skeletal muscle λgt11 expression library (Fliegel, L, Burns, K, MacLennan, D H, and Michalak, M, unpublished observations) The cDNA of the clone, hybridizes to mRNA of approx 2 5 kb in length, which is the expected size of mRNA coding for this protein, presuming 3′ and 5′ untranslated regions totalling about 1000 bp The COOH-terminal 28 amino-acid of the HACBP deduced from the cDNA clone is also rich in acidic residues, which is similar to the COOH-terminal of skeletal muscle calsequestrin [26] The function of these acidic residues is not known, but they may be involved in either calcium binding or may have some structural function such as anchoring the protein to the membrane The four COOH-terminal amino acids of the HACBP, -Lys-Asp-Glu-Leu-COOH deduced from our cDNA clone, correspond to the sequence described for other ER-associated calcium binding proteins, i e, endoplasmin (100 kDa) [27], protein disulphide isomerase (61 kDa) [28], as well as a family of stress-induced proteins [29] The terminal four amino-acids -Lys-Asp-Glu-Leu-COOH may be a part of a specific signal that causes retention of these proteins in the ER [30] It is possible that HACBP belongs to the same family of ER-associated, calcium binding proteins, but besides being abundant in non-muscle cells, it is also present in muscle tissues

Acknowledgments

We would like to thank Dr D H MacLennan (University of Toronto) for a generous gift of antibody against the HACBP We thank Dr A O Jorgensen (University of Toronto) for providing us with frozen sections of rabbit gracilis muscle We would like to thank Dr D Waisman (University of Calgary), for a generous gift of calreguhn, and antibody against calreguhn We acknowledge the superb technical assistance of Ken Wlasichuk, and the excellent secretarial assistance of Colette Ethier This work was supported by the Alberta Heart and Stroke Foundation, the Medical Research Council of Canada, and the Alberta Heritage Foundation for Medical Research L F is a Postdoctoral Fellow of the Alberta Heritage Foundation for Medical Research M O is a Scholar of the Medical Research Council M M is a Scholar of the Medical Research Council and the Alberta Heritage Foundation for Medical Research

References

1 Fada, M Yamamoto T and Tonomura Y (1978) Physiol Rev 58 1–79
2 Ikemoto, N (1982) Annu Rev Physiol 44, 297–317
3 Martonosi A (1984) Physiol Rev 64 1240–1320
4 Michalak M (1985) in The Enzymes of Biological Membranes (Martonosi A N ed) Vol 3 pp 115–155 Plenum Press, New York and London
5 MacLennan D H Campbell K P and Reithmeier R A F (1983) in Calcium and Cell Function (Cheng, W Y ed) Vol 4 pp 151–173, Academic Press, New York
6 Ostwald T J and MacLennan, D H (1974) J Biol Chem 249 974–979
7 Michalak, M, Campbell, K P and MacLennan, D H (1980) J Biol Chem 255, 1317–1326
8 Waisman D M Salimath B P and Anderson M J (1985) J Biol Chem 260 1652–1660
9 Fliegel L Opas M and Michalak M (1988) Biophys J 53 338a
10 Meissner G (1984) J Biol Chem 259, 2365–2374
11 Pegg W and Michalak M (1987) Am J Physiol 252 H22–H31
12 Slupsky J R Ohnishi M Carpenter M R and Reithmeier R A F (1987) Biochemistry 26, 6539–6544
13 Laemmli U K (1970) Nature (London) 227, 680–685
14 Campbell, K P MacLennan D H and Jorgensen A O (1983) J Biol Chem 258, 11267–11273
15 Towbin H Staehelin T and Gordon, J (1979) Proc Natl Acad Sci USA 76, 4350–4354
16 Jorgensen A O Kalnins V and MacLennan, D H (1979) J Cell Biol 80, 372–384
17 Matsudaira, P (1987) J Biol Chem. 262, 10035–10038
18 Hewick, R M, Hunkapiller M W, Hood L E and Dreyer, W J (1981) J Biol Chem. 256, 7990–7997

19 Lowry O H Rosebrough N J Farr A L and Randall, R J (1951) J Biol Chem 193 265–275
20 Michalak, M and MacLennan, D H (1980) J Biol Chem 255 1327–1334
21 Khanna N C, Takuda M and Waisman, D M (1987) Biochem J 242 245–251
22 Daniel E E. Grover A K and Kwan C Y (1982) Fed Proc 41, 2898–2904
23 Campbell K P MacLennan D H, Jorgensen A O and Minuzer, M C (1983) J Biol Chem 258 1197–1204
24 Khanna, N C Tokuda M and Waisman, D M (1986) J Biol Chem 261 8883–8887
25 MacLennan, D H, Yip, C C, Iles, G H and Seeman, P (1972) Cold Spring Harbor Symp Quant Biol 37, 469–477
26 Fliegel, L Ohnishi M Carpenter, M R, Khanna, V K. Reithmeier, R A F and MacLennan, D H (1987) Proc Natl Acad. Sci USA 84, 1167–1171
27 Koch, G L E (1987) J Cell Sci 87, 491 492
28 Edman, J C Ellis, L, Blacker, R W, Roth, R.A and Rutter, W J (1985) Nature 317, 267–270
29 Munro S and Pelham, H R B (1986) Cell 46, 291–300
30 Munro, S and Pelham, H R B (1987) Cell 48, 899–907