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Molecular Cloning of the High Affinity Calcium-binding Protein (Calreticulin) of Skeletal Muscle Sarcoplasmic Reticulum*

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A cDNA clone encoding the high affinity Ca^{2+}-binding protein (HACBP) of rabbit skeletal muscle sarcoplasmic reticulum was isolated and sequenced. The cDNA encoded a protein of 418 amino acids, but a comparison of the deduced amino acid sequence with the NH_2-terminal amino acid sequence of the purified protein indicates that a 17-residue NH_2-terminal signal sequence was removed during synthesis. This was confirmed by studies of *in vitro* translation of mRNA encoding the protein. Structural predictions did not reveal any potential transmembrane segments in the protein. The COOH-terminal sequence of the high affinity Ca^{2+}-binding protein, Lys-Asp-Glu-Leu, is the same as that proposed to be an endoplasmic reticulum retention signal (Munro, S., and Pelham, H. R. B. (1987) *Cell* 48, 899–907). All of these characteristics suggest that the protein is localized in the lumen of the sarcoplasmic reticulum.

The mature protein of M_r 46,567 contains 109 acidic and 52 basic amino acids. Structural predictions suggest that the first half of the molecule forms a globular domain of 8 anti-parallel β-strands with a helix-turn-helix motif at the extreme NH_2 terminus. The next one-third of the sequence is proline-rich. This segment can be subdivided into a charged region which contains a 17-amino acid repeat, followed by a proline, serine, and threonine-rich segment extending from Pro-246 to Thr-316. Thirty-seven acidic residues are clustered within 56 amino acids at the COOH terminus of the protein. Although the protein binds 1 mol of Ca^{2+}/mol with high affinity, no "EF-hand" consensus sequence was observed in the protein. The acidic COOH terminus, however, could account for the low affinity, high capacity Ca^{2+} binding observed in the protein.

In agreement with other involved laboratories, we have chosen the name calreticulin for the protein.

The sarcoplasmic reticulum is an intracellular membrane system responsible for the regulation of Ca^{2+} concentrations within muscle fibers (1). The membrane is composed of several integral and peripheral proteins of which the Ca^{2+}-ATPase (2–4), calsequestrin (5–7), the Ca^{2+} release channel (8–11), the 53- and 160-kDa glycoproteins (12–14), the 165-kDa Ca^{2+} and low density lipoprotein-binding protein (15), and phospholamban (16–18) have now been purified and cloned.

The high affinity Ca^{2+}-binding protein (HACBP)[1] was discovered in studies of the Ca^{2+} binding properties of detergent extracts of sarcoplasmic reticulum (19). Two soluble proteins in these extracts, calsequestrin (5) and the HACBP (20), could be separated on DEAE-cellulose. Calsequestrin was found to bind 43 mol of Ca^{2+}/mol with low affinity; the HACBP bound 25 mol of Ca^{2+}/mol with low affinity and 1 mol of Ca^{2+}/mol with high affinity (19, 20). Calsequestrin is present in these membranes in severalfold higher concentration than the HACBP (21). Several lines of evidence support the view that the HACBP is located in the lumen of the sarcoplasmic reticulum (21). Unlike calsequestrin, binding of Ca^{2+} to the HACBP does not induce conformational changes that can be detected by changes in circular dichroism or UV absorption spectra (22). During differentiation of myoblasts to myotubes, the synthesis of calsequestrin and the HACBP seem to be coordinated but their synthesis is not coordinated with the synthesis of the Ca^{2+}-ATPase (23).

Calsequestrin acts to lower lumenal free Ca^{2+} to enhance the function of the Ca^{2+} pump and to localize lumenal Ca^{2+} near the junctional face of the terminal cisternae (24). The function of the HACBP, however, is still unknown. Recently, we have reported that the protein is present in cardiac and smooth muscle tissues as well as in non-muscle cells (25). Indirect immunofluorescence staining of frozen sections and cultured cells from a variety of tissues showed that the HACBP is localized predominantly to endoplasmic reticulum membranes (25), suggesting that the protein is common for both sarcoplasmic and endoplasmic reticulum membranes, and may be more important in non-muscle cells than in muscle cells. Our results (25) also showed that the HACBP is probably identical to calregulin, a Ca^{2+}-binding protein recently identified in bovine liver (26). Koch and his colleagues (27–29) have recently identified a group of proteins that make up the matrix component of the endoplasmic reticulum and one of these (CRP 55) also resembles the HACBP. The protein is luminally located, binds Ca^{2+} with low capacity, and has the same molecular mass. They have suggested that

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J05138.

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[1] The abbreviations used are: HACBP, high affinity Ca^{2+}-binding protein; bp, base pairs; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

it is a major Ca^{2+}-binding protein of the endoplasmic reticulum (29). A search for the non-muscle analog of calsequestrin carried out in the laboratories of Drs. T. Pozzan (Institute of General Pathology, University of Padova) and J. Meldolesi (Department of Pharmacology, University of Milano) has shown that rat and rabbit liver and brain microsomes contain a soluble, luminal protein of identical size to HACBP that binds large quantities of Ca^{2+} with low affinity.[2] Since calsequestrin is absent from these endoplasmic reticulum preparations, it is conceivable that the HACBP may, indeed, represent the major Ca^{2+} sequestering protein in the endoplasmic reticulum.

In this article we have obtained a full-length cDNA clone encoding the HACBP and report the deduced amino acid sequence of the rabbit fast-twitch skeletal muscle clone. We show that the deduced sequence, starting at amino acid residue 18, corresponds to the NH_2-terminal protein sequence of the HACBP. We also show that the COOH-terminal sequence of the protein terminates with the residues Lys-Asp-Glu-Leu-COOH (KDEL-COOH), which are believed to be an important signal in the retention of peripheral endoplasmic reticulum membrane proteins (30, 31). Comparison of our sequence with that of CRP 55[3] also established the identity of the HACBP with CRP 55. Since the HACBP is a protein common to both sarcoplasmic and endoplasmic reticulum (Ref. 25 and this work), the name calreticulin is proposed for the protein.

EXPERIMENTAL PROCEDURES

Materials—Restriction endonuclease and DNA-modifying enzymes were obtained from Boehringer Mannheim and Bethesda Research Laboratories. The plasmids pTZ18R and 19R were from Pharmacia LKB Biotechnology Inc. [γ-^{35}S]ATP, [γ-^{32}P]ATP, and [α-^{32}P]ATP were obtained from Du Pont-New England Nuclear. [^{35}S]Methionine was from Amersham Corp. Ponceau S was from Sigma. Peroxidase-conjugated rabbit anti-goat IgG was from Bio-Rad. Nitrocellulose membrane filters and polyvinylidene difluoride membranes were from Schleicher and Schuell and Millipore, respectively. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and sequencing gels were prepared using reagents from Bio-Rad or Boehringer Mannheim. All chemicals were of the highest grade available.

Preparation of Polyclonal Antibodies—Polyclonal antibodies against the HACBP were produced in goat as described earlier (21). For affinity purification of the antibody, the HACBP was purified by ammonium sulfate precipitation (25), separated by preparative SDS-PAGE, and transferred to nitrocellulose (32). The nitrocellulose sheets were stained with Ponceau S, and the band corresponding to the HACBP was cut out and incubated with a 1:25 dilution of the antiserum in phosphate-buffered saline containing 1% milk powder. Bound antibodies were eluted by incubation of the nitrocellulose strips in 200 mM glycine, pH 2.8. After 2 min of incubation, the pH of the antibody solution was adjusted to 8.0 by the addition of 1 M Tris-HCl, pH 8.8. In some experiments, the antibody solution was dialyzed overnight against phosphate-buffered saline and stabilized by the addition of bovine serum albumin to a final concentration of 1 mg/ml.

Cell-free Translation—Cell-free translation of neonatal rabbit skeletal muscle mRNA was performed as described by Reithmeier *et al.* (33). Newly synthesized HACBP labeled with [^{35}S]methionine was immunoprecipitated with goat-anti rabbit HACBP. Immunoprecipitates were prepared as described previously (33), resolved by SDS-PAGE according to Laemmli (34), and exposed to an x-ray film at −70 °C.

Screening and Sequencing of cDNA Clones—The λgt11 cDNA expression libraries were constructed from poly(A)$^+$ RNA of rabbit slow-twitch (soleus) and fast-twitch (psoas) muscle as described previously (35). The slow-twitch muscle λgt11 expression library was screened with affinity-purified polyclonal antibodies against rabbit fast-twitch HACBP (36). Screening of this library resulted in the isolation of a 637-bp cDNA clone (bases 1117–1754 in Fig. 2) which encoded the COOH-terminal sequence of the protein (amino acid residues 374–401 of the full-length clone described in Fig. 2). This cDNA was isolated from the vector, labeled with ^{32}P, and used to screen the fast-twitch muscle λgt11 cDNA library. This resulted in the isolation of cDNA clone 2 of 2048 bp which was derived from an incompletely processed mRNA and contained an unexcised intron. This clone contained nucleotides −5 to 1754 in Fig. 2, encoding all of the mature protein and five-sixths of 2 amino acids in the signal sequence. An exon/intron boundary clearly lies between bases −6 and −5 in Fig. 2. In order to isolate full-length cDNA, a restriction fragment from clone 2 encoding the NH_2-terminal protein sequence of the HACBP was isolated and used to probe a neonatal rabbit skeletal muscle cDNA library in the vector pcDX described previously (3). This resulted in the isolation of a cDNA clone (clone 3) of 1865 bp encoding the complete amino acid sequence of HACBP, including the signal sequence. This cDNA was subcloned into the plasmids pTZ18R and 19R and characterized by restriction endonuclease mapping. Single-stranded DNA templates of cDNA fragments subcloned into pTZ18R and 19R were sequenced by the dideoxy method of Sanger *et al.* (37).

Northern Blot Analysis—Total and poly(A)$^+$ RNAs were isolated from various rabbit tissues (38) and 10-μg samples of each RNA were separated by electrophoresis in a denaturing formaldehyde, 0.8% agarose gel (39), stained with ethidium bromide, and blotted onto nitrocellulose or nylon membranes (40). Hybridization was with ^{32}P-labeled, nick-translated cDNA fragments as described earlier (40). Identical results were obtained with cDNA fragments from the 5′ or 3′ ends of the clone.

Protein Structure Analysis—The amino acid sequence of the HACBP was analyzed for hydropathy and secondary structure (41–43) using Protein Works and theoretical isoelectric point using the IBI Pustell sequence analysis programs. Computer resources used to carry out our studies were also provided by the BIONET® National Computer Resource for Molecular Biology (44), whose funding is provided by the Biomedical Research Technology Program, Division of Research Resources, National Institutes of Health.

Miscellaneous—All recombinant techniques were conducted according to standard protocols (39). NH_2-terminal sequence analysis of the HACBP was carried out using partially purified rabbit uterine and skeletal muscle HACBP (25) separated on SDS-PAGE (34) and transferred to polyvinylidene difluoride membranes (45). Automated protein sequence analysis was carried out as described earlier (46) on an Applied Biosystems Model 470A gas-liquid phase protein sequenator connected on-line to an Applied Biosystems Model 120A HPLC, using the current protocols of Applied Biosystems for both instruments. All chemicals used for protein sequence analysis were from Applied Biosystems, Foster City, CA.

RESULTS

Fig. 1 shows the restriction map and sequencing strategy for the cDNA encoding the HACBP. The nucleotide and deduced amino acid sequences of the cDNA are described in Fig. 2. Our longest clone, clone 3, contained 1865 bp of which 1254 encoded a protein of 418 amino acids. The 5′ untranslated region in clone 3 is 60 bp long and the 3′ untranslated sequence is 551 bp long. The 3′ untranslated region of the HACBP cDNA has a long poly(A) tail 14 bp downstream from the probable polyadenylation signal sequence ATTAAA. This sequence is identical to the polyadenylation signal se-

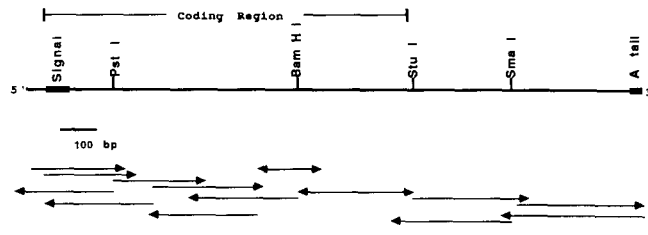

FIG. 1. **Restriction endonuclease map and sequencing strategy of a cDNA clone encoding rabbit skeletal muscle HACBP.** A full-length cDNA clone encoding the HACBP was obtained as described under "Experimental Procedures." *Darkened lines* represent the signal sequence at the 5′ end and a polyadenylation signal at the 3′ end. *Arrows* indicate the direction and extent of sequencing.

[2] S. Treves, J. Meldolesi, and T. Pozzan, personal communication.
[3] G. L. E. Koch, personal communication.

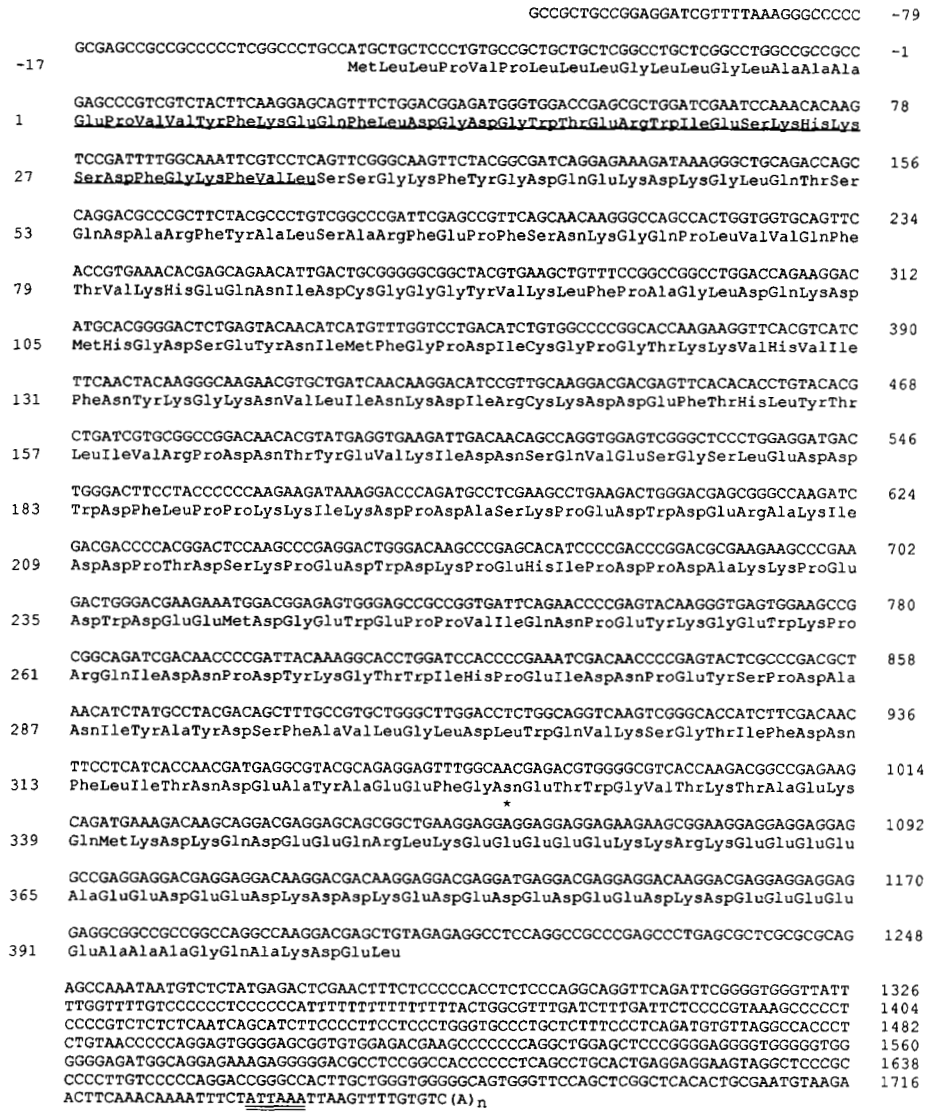

FIG. 2. **Nucleotide and predicted amino acid sequences of rabbit skeletal muscle HACBP.** Amino acid residues are *numbered negatively* within the signal sequence with amino acid residue 1 corresponding to the first residue of the mature processed protein. Amino acid sequences obtained by protein sequencing are *underlined*. A possible *N*-glycosylation site is indicated by an *asterisk*. The polyadenylation signal is *double underlined*.

quence in the cDNA encoding the 53,000-dalton sarcoplasmic reticulum glycoprotein (13).

The codon for the initiator methionine was identified on the basis of several criteria. The mature protein isolated from either rabbit uterus or rabbit skeletal muscle begins with the sequence Glu-Pro-Val-Val-Tyr-Phe- (25). We have assigned the glutamate in this sequence as amino acid residue 1 in Fig. 2 and the codon for it as nucleotides 1–3. An in-frame stop codon, TAA, is found at position −90 to −88 relative to the glutamate codon. The only methionine codon between nucleotide −90 and −1 lies at position −51 to −49 coding for amino acid residue −17. The sequence surrounding this codon, CTGCCATGC, fits the consensus sequence (CCG(A)CCATGG) for a eukaryotic initiator site (47).

The luminal localization of the HACBP (21) suggests that it should be made with a signal sequence that would allow it to enter the sarcoplasmic reticulum. In this respect, the NH_2-terminal sequence Met-Leu-Leu-Pro-Val-Pro-Leu-Leu-Leu-Gly-Leu-Leu-Gly-Leu-Ala-Ala-Ala- upstream of the glutamate between positions −17 and −1, is a reasonable signal sequence. It contains a hydrophobic stretch of amino acids but it is atypical in that it lacks a basic residue near its NH_2 terminus. The cleavage point between Ala and Glu is typical since a small amino acid such as Gly or Ala precedes the cleavage site in most signal sequences (48). From these observations we conclude that the initiator methionine is at the position defined in Fig. 2, that the HACBP is made with a signal sequence and that the signal sequence is 17 amino acids in length.

To confirm this point, we have carried out *in vitro* translation of mRNA encoding the HACBP. Fig. 3 shows that the [^{35}S]methionine-labeled protein immunoprecipitated by the HACBP antibody (this antibody was described in Ref. 23) is, indeed, synthesized as a higher molecular weight component than the mature HACBP. The precursor form of HACBP had an apparent mass of 57,000 Da, about 2,000 Da larger than the mature form of the protein and corresponding to the size of the predicted signal sequence.

Structural Analysis—The results of structural analysis of the deduced amino acid sequence of the HACBP are presented in Fig. 4. The hydropathy plot shows that the NH_2-terminal signal sequence is hydrophobic but that there are no long hydrophobic segments capable of spanning the membrane bilayer. The NH_2-terminal half of the molecule (residues 1–186) is predicted to have a globular structure. The mature protein sequence begins with a helix-turn-helix motif (residues 1–74) which does not contain a consensus "EF-hand" sequence. This is followed by a sequence predicted to form 8

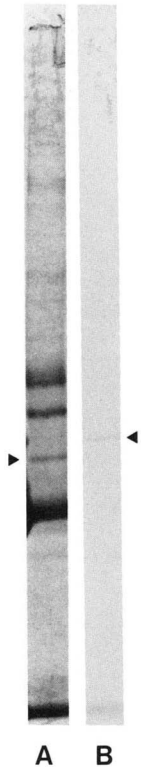

FIG. 3. **Cell-free translation of skeletal muscle mRNA.** Cell-free translation was carried out in the rabbit reticulocate lysate as described under "Experimental Procedures." [^{35}S]Methionine-labeled translation products were immunoprecipitated with goat anti-rabbit HACBP antibody and separated on SDS-PAGE (34). *A*, Coomassie Brilliant Blue staining of the mature form of the HACBP immunoprecipitated and separated on SDS-PAGE; *B*, autoradiograph of the cell-free translated [^{35}S]methionine-labeled precursor of the HACBP. The electrophoretic mobilities of mature and precursor proteins are indicated by *arrows*.

anti-parallel β-strands connected by protein loops. Two regions of short α-helices are predicted at residues 98–103 and 149–154. The sequence from residue 187–285 contains an abundance of prolines. The first part of this region (to residue 246) is also highly charged. This region contains the sequence PXXIXDPDAXKPEDWDE which occurs at residues 188–204 and repeats from residues 222 to 238. This is followed by a proline-, serine-, and threonine-rich sequence (residues 246–316) predicted to have a high-turn potential. The COOH-terminal 20% of the protein is highly acidic. Although predicted to form an α-helical structure, this region is likely in an extended conformation due to charge repulsion. In the last 56 residues, 37 are acidic while 10 are basic.

After subtraction of a 17-amino acid signal sequence, the mature protein would be comprised of 401 amino acids with a M_r of 46,567. This is considerably smaller than that measured through gel mobility (19–20).

Sequence Identity with Other Proteins—A comparison of the deduced amino acid sequence of the HACBP with all sequences available in the BIONET® National Computer Resource for Molecular Biology (44) revealed some degree of sequence homology between the acidic COOH terminus of HACBP and acidic sequences in other proteins and between HACBP and other proteins containing the KDEL sequence. These identities are probably fortuitous, however, and do not represent true homology among these proteins. In a previous study (25), we showed sequence identity between rabbit HACBP and rabbit liver calregulin. In a personal communication with Dr. G. L. E. Koch (MRC Laboratory of Molecular Biology, Cambridge)[3] we established that there is also sequence identity between rat CRP55 and HACBP.

Ca^{2+}-binding Sites—The HACBP has a single high affinity Ca^{2+}-binding site (20). As a consequence, we anticipated that an EF-hand type Ca^{2+}-binding site (49) would be detected in the primary sequence of HACBP. No evidence of an EF-hand sequence was observed, however. The closest homology to an EF-hand sequence was found between residues 237 and 248. This sequence was inadequate in that residue 248 was valine rather than a residue with an oxygen containing side chain and in that this sequence is not bounded by predicted helices. The location of the high affinity Ca^{2+}-binding site is, as yet unknown.

The sequence between residues 342 and 391 is very acidic, 32 out of 40 residues in this sequence are acidic. Since the HACBP has been shown to bind about 25 mol of Ca^{2+}/mol with low affinity (19), it is probable that this sequence is responsible for the low affinity Ca^{2+} binding.

Northern Blot Analysis and Tissue Distribution of HACBP mRNA—cDNA fragments encoding either NH_2- or COOH-terminal regions of the HACBP hybridized to the same RNA species in Northern blots from a variety of tissues. Fig. 5 shows Northern blot analysis with probes from the 5′ end of the clone. The cDNA hybridized to mRNAs of 1.9 kilobases in all the tissues tested; liver, kidney, brain, cardiac muscle, and fast- and slow-twitch rabbit skeletal muscle. With longer periods of exposure, a second band of approximately 3.75 kilobases could be visualized on the autoradiograms. In all cases, the probe hybridized to a much greater extent with the poly(A)$^+$ RNA in comparison with total RNA, suggesting that the binding was specific to a mRNA species and was not due to nonspecific binding to a ribosomal subunit.

DISCUSSION

We have cloned and sequenced cDNA encoding the HACBP, one of several Ca^{2+}-binding proteins present in the lumen of the sarcoplasmic reticulum (1, 24). The molecular weight of the mature HACBP determined here from the deduced amino acid sequence (M_r 46,567) is less than that previously estimated by SDS-PAGE either in the Weber-Osborn system (50) or the Laemmli system (34) (M_r 55,000) (19–21). The discrepancy in M_r is not likely due to glycosylation. Although one potential glycosylation site was found in the HACBP (residue 326) the protein isolated from skeletal muscle sarcoplasmic reticulum membranes is not glycosylated and does not bind concanavalin A (21). Bovine liver calregulin, which is identical to the HACBP (25), can be isolated using concanavalin A-Sepharose affinity chromatography, suggesting that the bovine liver protein is glycosylated (51, 52). Other proteins have been reported to move with anomalous mobilities in SDS-PAGE. For example, calsequestrin (24), moves with anomalous mobility in a Laemmli gel but its mobility in Weber-Osborn gels is identical to its M_r (21). The secondary structure of the HACBP and/or anomalous binding of SDS to the protein may be responsible for the reduced mobility of this protein in SDS-PAGE in comparison to the predicted molecular weight.

Previous work has shown that the HACBP is a Ca^{2+}-binding protein (19, 20). The amino acid sequence of HACBP confirms that the mature protein is acidic with a net charge of −57 at neutral pH and a pI of 4.14. HACBP binds 1 mol of Ca^{2+}/mol of protein with an affinity comparable to that of an EF-hand-like Ca^{2+}-binding site and 25 mol of Ca^{2+}/mol with low affinity (19, 20). We failed to detect any EF-hand-like sequence in the deduced amino acid sequence of the protein. Further studies, perhaps site-directed mutagenesis, will be required to

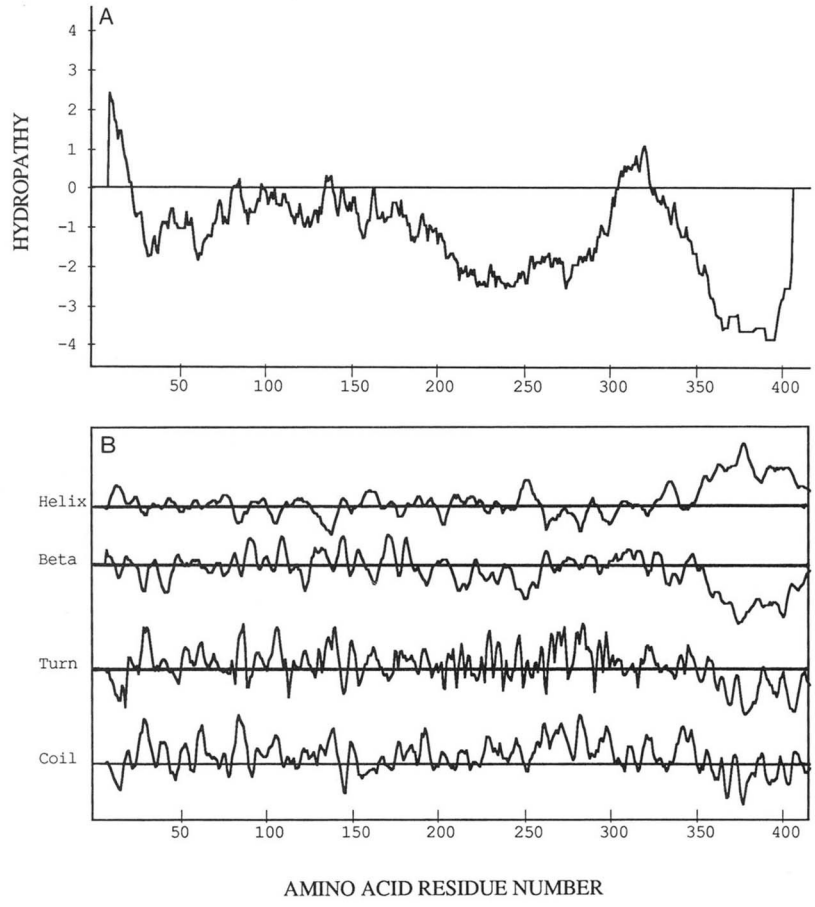

FIG. 4. **Secondary structure predictions for the HACBP.** The hydropathic character of the protein was evaluated using the procedure of Kyte and Doolittle (41) with a window of 21 amino acids (*A*). Probabilities of α-helix, β-sheet, β-turn, and random coil structures (42, 43) in the HACBP are shown in *B*.

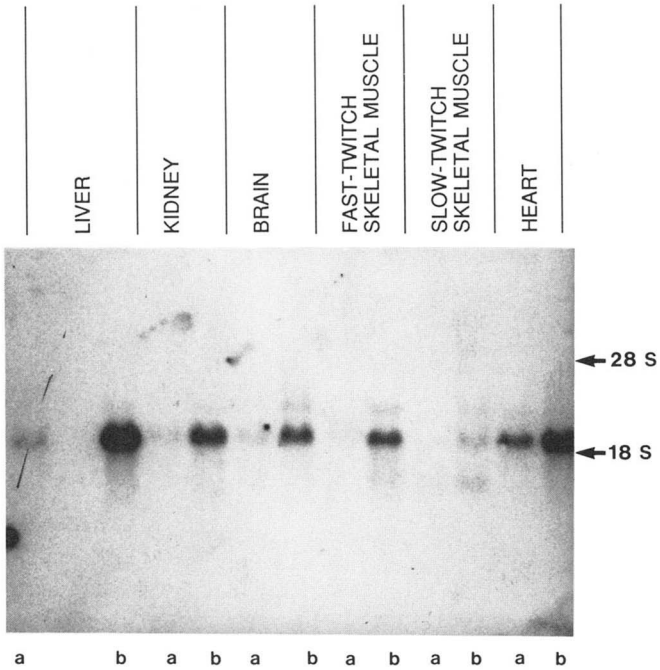

FIG. 5. **Northern blot analysis of RNA from rabbit tissues.** Total (*a*) and poly(A)$^+$ RNAs (*b*) from rabbit liver, kidney, brain, fast-twitch skeletal muscle (psoas), slow-twitch skeletal muscle (soleus), and cardiac muscle were isolated and fractionated on formaldehyde-agarose gels as described under "Experimental Procedures." The position of the 28 S and 18 S ribosomal subunits are indicated. The size of the mRNA hybridizing to the HACBP cDNA was estimated to be 1.9 kilobases. A second band at 3.75 kilobase could be visualized with longer periods of exposure.

identify the high affinity Ca^{2+}-binding site in the HACBP.

Secondary structure predictions for the protein suggest that it might have a "lollipop" shape. The NH_2-terminal half of the protein is a globular domain containing 8 anti-parallel β-strands. A helix-turn-helix motif present at the extreme NH_2 terminus is a potential Ca^{2+}-binding site. The acidic COOH-terminal region of the protein could represent a Ca^{2+}-binding domain. The sequence is reminiscent of the acidic structure found in calsequestrin (6, 7) and might represent a low affinity Ca^{2+}-binding region. This acidic sequence is likely in an extended conformation and may provide a site of interaction with other protein components.

The sequence connecting the NH_2-terminal globular domain and the acidic COOH-terminal region is enriched in proline residues. A portion of this sequence (residues 187–284) contains a number of prolines spaced every 4 or 5 amino acids. This sequence could contain a repeating, rigid turn structure separating the globular head of the protein from the acidic tail.

Recently Koch and collaborators (27–29) have described a group of 5 proteins which constitute the lumenal material of the endoplasmic reticulum. One of these proteins, CRP 55, has characteristics identical to HACBP. The estimated M_r of both proteins is the same, the protein is very acidic and it binds Ca^{2+} with low affinity. The presence of a high affinity Ca^{2+}-binding site in CRP 55 has not been reported. In a personal communication[3] with Dr. G. L. E. Koch we have found that the rabbit HACBP and the rat CRP 55 have virtually identical amino acid sequences, as deduced from analysis of cloned cDNAs. The differences that are observed in our sequences are probably species related. Thus it is clear that HACBP, calregulin, and CRP 55 are the same protein

and that this protein is an acidic, Ca^{2+}-binding protein common to both the sarcoplasmic and endoplasmic reticulum. In discussions involving Drs. G. L. E. Koch and D. M. Waisman (Department of Medical Biochemistry, University of Calgary) we have agreed that the protein should be named calreticulin in order to eliminate confusion regarding the identity of this protein in the future.

A most interesting feature of the sequence of the HACBP is the presence of the Lys-Asp-Glu-Leu (KDEL) sequence at the COOH-terminal end. A number of peripheral membrane proteins in the endoplasmic reticulum contain the KDEL sequence at the COOH terminus (30, 31, 53). This includes some heat shock proteins and some glucose-regulated proteins which also contain relatively large numbers of acidic amino acids residues within their COOH termini (30, 53). The function of these acidic sequences is unknown, but they could be involved not only in Ca^{2+} binding, but also in protein retention in the endoplasmic reticulum and in intracellular localization. Munro and Pelham (30) have shown that the KDEL sequence may be responsible for retention of newly synthesized proteins within the lumen of the endoplasmic reticulum. When examining the subcellular localization of expressed endoplasmic reticulum proteins they noted that deletion or extension of this sequence allowed the protein to enter the secretory pathway and to be secreted from the cell (30). As HACBP contains the KDEL sequence, it is probably salvaged as an endoplasmic/sarcoplasmic reticulum protein (30). This is in line with our earlier observation that HACBP is localized to the lumen of the sarcoplasmic reticulum (21).

A number of sarcoplasmic reticulum proteins have recently been cloned and/or identified (2–18, 54) including calsequestrin, the 53- and 160-kDa glycoproteins, the 165-kDa Ca^{2+} and low density lipoprotein binding protein, and the 3,3′-5-triiodo-L-thyronine (T_3)-binding protein, all of which are considered to be peripheral membrane proteins. Only the HACBP and T_3-binding protein contain the KDEL retention sequence, allowing them to be salvaged by the mechanism proposed by Munro and Pelham (30, 31). The mechanism responsible for maintaining the intracellular location of proteins without this sequence is not yet known (53), but may involve a specific receptor as suggested for calsequestrin (55).

Although our studies have revealed valuable information about the structure of HACBP, the function of this protein remains unknown. Its localization in both sarcoplasmic and endoplasmic reticulum membranes in a variety of tissues (25) suggests a possible role in protein synthesis and modification. Alternatively it may play a role in Ca^{2+} storage in non-muscle tissue similar to that proposed for calsequestrin in muscle tissues. Drs. S. Treves, J. Meldolesi, and T. Pozzan[2] have shown that antibodies raised against the luminal Ca^{2+}-binding protein from rat liver microsomes appear to bind to structures referred to as calciosomes (56). Thus, the protein may bind Ca^{2+} in a more specialized compartment than was suspected in earlier studies (25, 29). The synthesis of CRP 55 is induced in non-muscle cells in response to a Ca^{2+} overload brought about by the presence of a Ca^{2+} ionophore in the growth medium. This supports a role of CRP 55 (HACBP) as a Ca^{2+} sequestering agent within non-muscle cells. This stress-induced synthetic response could also be related to its potential role in the refolding of proteins in response to stress. Heat shock proteins (hsp70 family) were shown recently to be involved in refolding of newly synthesized proteins (57, 58). Future investigation into the role of HACBP in Ca^{2+} binding and in protein modification should provide new information on the role of this interesting protein in endoplasmic and sarcoplasmic reticulum membranes.

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