Molecular Cloning and Expression of Serum Calcium-decreasing Factor (Caldecrin)*

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We previously reported on the purification of a serum calcium-decreasing factor, referred to as caldecrin, from porcine pancreas, that is thought to be a serine protease (Tomomura, A., Fukugisha, T., Noda, T., Noikura, T., and Saheki, T. (1992) FEBS Lett. 301, 277-281). In the present study, we purified caldecrin from rat pancreas and determined its primary structure by cDNA cloning. The predicted caldecrin protein is presumed to be synthesized as a preproenzyme of 268 amino acids with a signal peptide of 16 amino acids and an activation peptide of 13 amino acids, and is, with the exception of a central region, almost identical to the reported rat pancreatic elastase IV sequence. The caldecrin gene is selectively expressed in the pancreas, as judged by Northern blot analysis. After expression in BMT-10 cells, immunoreactive caldecrin was found in the culture supernatant, and analysis of expressed in the pancreas, as judged by Northern blot analysis. After expression in BMT-10 cells, immunoreactive caldecrin was found in the culture supernatant, and it inhibited the parathyroid hormone-stimulated 45Ca release from cultured fetal long bones. Catalytic site mutants were synthesized in a baculovirus system, and recombinant mutants also decreased the serum calcium level of mice. These data implicate caldecrin, a protease closely related to elastase IV, in the regulation of blood calcium levels.

It is well known that blood calcium is regulated by several calcitropic hormones, e.g. calcitonin, parathyroid hormone (PTH), and 1,25-dihydroxy vitamin D₃, synthesized by the thyroid, parathyroid gland, and kidney, respectively. However, the involvement of other organs in the regulation of blood calcium level is not clearly understood. It has been reported that some gastric factors reduce blood calcium levels and that vagally mediated hypocalcemia is induced by hypothalamic stimulation (1-3). Another etiologic organ is the pancreas. Acute pancreatitis causes hypocalcemia, which suggests that pancreas tissue contains hypocalcemic factors (4, 5). Among pancreatic hormones, glucagon and amylin are reported to decrease bones. Mutant caldecrins, which lacked protease activity, also decreased the serum calcium level of mice.

EXPERIMENTAL PROCEDURES

In Vivo Assay: SCDA—The SCDA of rat caldecrin was measured as described previously (9). Male BALB/c mice weighing 20-25 g were used, and the caldecrin fraction (200 µg/20 g body weight) was injected into a tail vein of mice previously starved for 18-20 h. Blood was taken for determination of serum calcium levels 4 h after the injection. The calcium concentration was measured by the o-cresolphthalein complex method (30). For inactivation of the protease activity of purified caldecrin, rat caldecrin at a concentration of 1.25 mg/mL was treated for 30 min at 4°C with 1 mM PMSF, diluted at least 100-fold with phosphate-buffered saline, and used for the in vivo assay. All assays were performed using at least 5 mice for each concentration of test material. Statistical significance was analyzed by Student’s t test.

In Vitro Assay: Bone Resorption Measured in Mice—Fetal Long Bone Cultures—Organ cultures of long bones from fetal mice were prepared.
as described by Sato et al. (31). 125I-\textit{Ca} (370 GBq/g calcium, 370 KBq/mouse; DuPont NEN) was injected into the abdomen of pregnant mice (ICR strain) at the 15th day of gestation, and the mice were killed the following day. The shafts of the radius and the ulna of the fetuses were dissected and cultured in serum free α-minimum essential medium (Life Technologies, Inc.). After 24 h of pre-culture, the bones were cultured in 72 h in α-minimum essential medium containing 0.2% bovine serum albumin (Seikagaku Kogyo, Tokyo, Japan), caldecrin fraction, and PTH (10 nM). All assays were performed in quadruplicate using bones from four fetuses. Bioactivity of caldecrin was measured by the inhibition of \(^{125}\text{I}\)-Ca release into the medium from PTH-stimulated bones and expressed as percent inhibition of PTH-stimulated \(^{125}\text{I}\)-Ca release.

Purification of Rat Caldecrin from Rat Pancreas—Rat caldecrin was purified from acetic acid powder of rat pancreas according to the protocol described previously (9). The powder was stirred with 0.1 M Tris-HCl buffer (pH 7.5) in 2% NaCl for 1 h at 4 °C, and the extract was then centrifuged at 10,000 \(\times g\) for 30 min. The supernatant was fractionated with acetic (30–60%), dialyzed against water, and, next fractionated with saturated ammonium sulfate (45–60%), followed by dialysis against 50 mM sodium acetate buffer (pH 5.5). The dialyzed material was applied to a Q Sepharose Fast Flow column (Pharmacia Biotech Inc.; 4.5 cm \(\times\) 16 cm) equilibrated with the above buffer. After having been washed, the absorbed material was eluted by a stepwise gradient of NaCl from 0.1 to 0.5 M in the starting buffer. The second peak, which eluted after starting buffer, was collected, dialyzed against 0.2 M sodium acetate buffer (pH 6.8), and chromatographed through a Superdex 75 FPLC column (Pharmacia) equilibrated with the above-mentioned buffer. Single main peaks that eluted at molecular masses between 22 and 15 kDa were combined. Caldecrin in each fraction was measured by the in vitro assay using fetal long bone cultures and Western blotting using anti-porcine caldecrin antibody.

Immunoscreening of a Pancreatic cDNA Library—Rat pancreas gt11 cDNA library (Clontech) was immunoscreened with rabbit polyclonal anti-porcine caldecrin antibody as the primary antibody. All other staining steps were performed with a picBlue immunoscreening kit (Stratagene) according to the manufacturer's protocol.

Site-Directed Mutagenesis of Rat Caldecrin cDNA Using a Transformer System—The rat caldecrin cDNA from positive clones (rat pancreas cDNA library, pRPC) by the method of Huynh et al. (32). Briefly, BNN103 cells were infected with the gt11 clone. All clones that grew at 30 °C but lysed the cells at 42 °C were cultured at 30 °C overnight, at 42 °C for 2 min, and at 37 °C for 2 h. After centrifugation, the precipitates were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and Western blotting.

The cDNA library was rescreened with an EcoRI/PstI fragment of pRPC18 used as a probe. Positive clones were subcloned into pUC19, and both strands of inserted DNA were sequenced by use of Sequenase (version 2) (U.S. Biochemical Corp.) and an automatic DNA sequencer (Applied Biosystems).

Northern Blot Analysis—Total RNA was prepared from rat brain, lung, liver, pancreas, kidney, and blood by the acid guanidinium thiocyanate-phenol-chloroform method (33). For Northern blot analysis, RNA (17 \(\mu\)g) was denatured at 50 °C for 60 min with glyoxal/dimethyl sulfoxide/sodium phosphate buffer, subjected to 1% agarose gel electrophoresis, and transferred onto a nitrocellulose filter (Schleicher & Schuell). After the RNAs had been linked to the filter by a UV linker, the filter was pre-hybridized for 18 h at 37 °C in 3 \(\times\) SSC (1 M NaCl, 0.15 M Na acetate, pH 7.0), 10 °C Denhardt’s solution, 0.1% SDS, 0.2 mg/ml bovine serum albumin, 5 \(\times\), and 0.1% SDS at 37 °C for 2 h. The probe was then added to the filter, and the RNAs were hybridized overnight. The filter was washed three times with 2 \(\times\) SSC containing 0.1% SDS at room temperature for 5 min and twice with 0.1% SSC, 0.1% SDS at 58 °C for 30 min, and then exposed to x-ray film at −80 °C for 24 h.

Protein Analysis—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were performed on 20% acrylamide gel. The samples were denatured in electrophoresis buffer containing 5% mercaptoethanol for 10 min and subjected to SDS-PAGE (11). After the gel was stained with Coomassie blue and destained with water, the gels were dried and loaded onto a gel for western blotting. The antibodies used were goat anti-porcine caldecrin antibody (1:1,000 dilution). The antibody was detected with an enhanced chemiluminescence (ECL) detection kit (Amersham Corp.), and the bands were visualized on autoradiographs.
experimental Procedures," which had been originally developed for porcine caldecrin (9). Treatment of the purified rat caldecrin with 100 mg/kg body weight (Fig. 2). These results are very similar to those obtained with porcine caldecrin (9). Treatment of rat caldecrin with PMSF had no effect on SCDA, strongly suggesting that the SCDA of the rat caldecrin does not depend on a functional catalytic site of its protease moiety.

Isolation of Rat Caldecrin cDNA Clones—To isolate rat caldecrin cDNA clones, we screened a rat pancreas Agt11 cDNA library (8 × 10⁴ clones) with anti-porcine caldecrin antibody. After four rounds of immunoscreening, five immunoreactive clones were identified. Recombinant lysogens of these clones were then expressed and subjected to SDS-PAGE and Western blot analysis (data not shown). One of three positive clones, pRPC18, contained a cDNA insert of 634 base pairs (bp) in length. Sequence analysis revealed that the insert cDNA of pRPC18 involved an open reading frame predicting a protein having high homology with the elastase family. Since the clone did not contain the full-length sequence, the cDNA library was rescreened with an EcoRI/PstI fragment of pRPC18 as a probe. One positive clone of longer size than pRPC18, containing a cDNA insert of 0.9 kilobase pairs, was then isolated and sequenced.

Tissue-specific Expression of Rat Caldecrin—To estimate the size of the caldecrin mRNA and to analyze its expression pattern in the rat, we analyzed total RNA from various tissues (brain, lung, liver, kidney, and blood) by Northern blotting, using the full-length (899 bp) rat caldecrin cDNA as a probe. With pancreatic RNA, a single band corresponding to an mRNA of approximately 1.1–1.2 kilobases hybridized with the probe; whereas no hybridization band was observed with any other tissue (Fig. 3). This indicates that the rat caldecrin gene is selectively expressed in the pancreas.

Primary Structure of Rat Caldecrin—As shown in Fig. 4, the rat caldecrin cDNA contained a 899-bp insert with a 807-bp open reading frame extending from an ATG codon at nucleotide 14 to a TGA stop codon at position 820. A polyadenylation signal, AATAAA, and a poly(A) tail were found in the 3′-untranslated region of the cDNA. The deduced caldecrin polypeptide comprised 268 amino acids, including signal peptide, activation peptide, and mature form. The calculated molecular weight of full-length rat caldecrin was 29,374. The nucleotide sequence of rat caldecrin cDNA is very similar (99.3% homology) to that of rat elastase IV throughout the entire coding region (29), suggesting that both mRNAs are products of the same gene.

Amino Acid Sequencing of Rat and Porcine Caldecrin—To investigate whether the purified rat caldecrin corresponds to the protein predicted from the isolated cDNA, we sequenced the purified rat caldecrin. As given in Table I, part A, amino acid sequencing of rat caldecrin through cycles 1–15 showed that a valine appeared on the first cycle, and equivalent moles of glycine and valine on the second cycle, which was followed by pairs of amino acids for the next 7 cycles. The amino acid residue in the 1st cycle and those residues from cycles 10 to 15 corresponded to the amino acid sequences of the predicted mature form of rat caldecrin. Furthermore, pairs of amino acid residues from cycles 2 to 9 consisted of amino acid residues derived from the predicted activation peptide and mature form, but sequencing of the activation peptide was terminated at asparagine at position 5.

Next, purified rat caldecrin was digested in the presence of PMSF with a metalloendopeptidase that degrades specifically on the amino-terminal side of lysine residues. After separation of the resulting fragments by C18 reverse-phase HPLC, amino acid sequences of several major peptides were determined (Fig. 5A). All amino acid sequences of the six peptides examined
were identical to the corresponding parts of the amino acid sequences deduced from the rat caldecrin cDNA (Fig. 4). Then, to compare at the molecular level the caldecrins purified from rat and porcine pancreas, we also subjected porcine caldecrin to the same metalloendopeptidase cleavage protocol. Again, all sequences of the nine fragments obtained showed high homology with the deduced amino acid sequence of rat caldecrin (Figs. 4 and 5B). These results indicate that the caldecrins purified from rat and porcine pancreas are both members of the same elastase IV subfamily.

Expression of Rat Caldecrin in BMT-10 Cells—To examine the functional properties of recombinant rat caldecrin, we prepared a construct of the rat caldecrin cDNA in the pCAGGS expression vector and introduced the latter by the DEAE-dextran method into BMT-10 cells. Upon SDS-PAGE and Western blot analysis of cells harvested after 2 days in culture, a 30-kDa

**Fig. 4.** Nucleotide and deduced amino acid sequences of rat caldecrin cDNA. The primary nucleotide sequence (upper row) and deduced amino acid sequence indicated by the single-letter code (second row) of rat caldecrin are shown. An open reading frame extends from the translation initiation site (ATG) to the termination site (TGA). This is followed by a 3'-untranslated region that ends with a polyadenylation signal (bold underlined) and the poly(A) tail. The vertical arrowheads indicate proteolytic processing sites. The deduced amino acid sequence is numbered sequentially from the amino terminus of the predicted active enzyme. The amino acid residues of the charge-relay system are circled (37), and the residues determining the substrate binding specificity are boxed (15). The corresponding amino acid sequences of fragments obtained by proteolytic cleavage of rat caldecrin are underlined with a dashed line. The amino acid sequences of fragments analyzed after proteolytic cleavage of porcine caldecrin are shown below the rat caldecrin sequences (third row). The deduced amino acid sequence of elastase IV (NBRF-PDB accession number JQ1473) is given in the bottom row. Asterisks indicate residues conserved between rat caldecrin and rat elastase IV.
beentransfected with the sense cDNA, whereasthe medium of reactivity was high in the medium of cultured cells that had secretory proteins. As shown in Fig. 6, caldecrin immunoreactive band was detected in lysates of cells bearing sense-strand cDNA but not in those containing the antisense cDNA or vector alone (Fig. 6). The culture medium of the transfected cells was also examined, since pancreas proteases of caldecrin immunoreactive band was detected in lysates of cells bearing rat and porcine caldecrin.

Caldecrins purified from rat and porcine caldecrin.

Amino-terminal sequences of purified rat caldecrin (A) and comparison of the amino-terminal sequences deduced from rat caldecrin cDNA with other related enzymes (B).

| A. | Purified rat caldecrin cycles: |
|----|-------------------------------|
|    | 1 2 3 4 5 6 7 8 9 10 15 |
| V (V/G) | (G/N) | (G/P) | (E/A) | (D/F) | (A/P) | (V/P) | (P/N) |
| 1   | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2   | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 3   | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 4   | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 5   | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 6   | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| 7   | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| 8   | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 9   | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| 10  | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| B. | Rat caldecrin |
|    | Rat proelastase IV |
|    | Rat proelastase IIb |
|    | Bovine chymotrypsin A |
|    | Bovine chymotrypsin B |
|    | Rat chymotrypsin B1 |
|    | Porcine chymotrypsin C |

Amino acid sequences were performed on the numbered peaks. The effluent was monitored at 229 nm. Amino acid sequences were performed on the numbered peaks, and residues are indicated by the single-letter code. Unidentified amino acid residues are denoted by "---".

Table I

| Amino-terminal sequences of purified rat caldecrin (A) and comparison of the amino-terminal sequences deduced from rat caldecrin cDNA with other related enzymes (B) |
|---|---|---|---|---|---|---|---|
| V (V/G) | (G/N) | (G/P) | (E/A) | (D/F) | (A/P) | (V/P) |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| BMT-10 cell transfected with either pCAGGS vector alone (V), rat caldecrin cDNA in the antisense orientation (R), or rat caldecrin cDNA in the sense orientation (N) were cultured for 1 or 2 days. The cell lysates obtained from 2-day cultures, and the media from 1- and 2-day-old cultures, were analyzed by Western blotting using anti-porcine caldecrin antibody. Molecular sizes of markers are indicated on the left.

cultured cells transfected with antisense cDNA or vector alone did not contain detectable amounts of antigen.

Recombinant rat caldecrin was also examined for hypocalcemic activity. Medium conditioned by the cells transfected with the caldecrin cDNA was used in the in vitro assay utilizing fetal long bone cultures, which cultures have been shown to be a valid model system to study porcine caldecrin activity (9). As shown in Fig. 7, medium containing recombinant rat caldecrin inhibited dose-dependently the PTH-stimulated release of \(^{45}\)Ca from the bone. The conditioned medium caused maximum inhibition at 20 \(\mu\)g/ml of assay medium and maintained this level of inhibition at higher doses. The conditioned medium of cultured cells transfected with antisense cDNA did not affect the PTH-stimulated release of calcium from the bone.

Bioactivity of Mutant Caldecrins—To verify that the SCDA of caldecrin is not connected to its protease activity, we prepared recombinant wild-type and mutant caldecrins were synthesized by use of the baculovirus system, purified them by passage through a Mono Q column, and assayed them for SCDA. Sf9 cells transfected with recombinant wild-type or mutant caldecrin baculoviruses were synthesized and sequenced polypeptides with molecular masses of about 60 and 30 kDa in the medium. The 30-kDa polypeptide was found to be caldecrin by Western blotting. A single step ion-exchange procedure was sufficient to obtain almost pure recombinant caldecrins with immunoreactive band was detected in lysates of cells bearing sense-strand cDNA but not in those containing the antisense cDNA or vector alone (Fig. 6). The culture medium of the transfected cells was also examined, since pancreas proteases are secretory proteins. As shown in Fig. 6, caldecrin immunoreactivity was high in the medium of cultured cells that had been transfected with the sense cDNA, whereas the medium of

![Fig. 5. Separation and sequencing of proteolytic fragments of rat and porcine caldecrin. Caldecrins purified from rat (A) and porcine (B) pancreas were digested with metalloendopeptidase, applied onto a C18 reverse-phase HPLC column, and eluted with a linear gradient of acetonitrile (dashed line). The effluent was monitored at 229 nm. Amino acid sequences were performed on the numbered peaks, and residues are indicated by the single-letter code. Unidentified amino acid residues are denoted by "---".](http://www.jbc.org/)
tion 14, addition, three cysteine residues are present at the positions four disulfide bonds as observed in porcine elastase I (15). In allelastasefamilymembers,whichsuggeststheformationof 157,173,183,193, and214 of the matureproteinareconservedzyme. Eight cysteine residues present at positions 30, 46, 126, of a signal peptide, an activation peptide, and a mature en-
caldecrin cDNA encodes a serine protease zymogen consisting
This conclusion is based on the following findings. (i) The rat
analyzed protease of the chymotrypsin/elastase superfamily.
rat caldecrin is synthesized as a zymogen with a
indicates basal calcium release from bone.
90–95% purity as judged by SDS-PAGE. The purified wild-type
caldecrin showed chymotrypsin activity with a synthetic sub-
strate only after treatment with trypsin, indicating that it was
the proform of caldecrin (10). Fig. 8 shows that serum calcium
was decreased by the activated wild-type caldecrin but not by
the proform of caldecrin. Again, treatment of the activated
caldecrin with PMSF destroyed the chymotrypsin activity but
permitted retention of the SCDA. Mutations of His-45 and
Ser-187, which residues are required for serine protease activ-
ity, decreased the chymotrypsin activity to 0.6 and 0.11%,
respectively, of that of wild-type caldecrin. However, the acti-
vated mutant caldecrins (H45A and S187A) also decreased
serum calcium levels. These results suggest that the SCDA
of caldecrin has no connection with the protease activity of the
molecule.

**DISCUSSION**

In this paper, we reported the primary structure of rat calde-
crin, a proteolytic enzyme from rat pancreas, and showed that
it is highly related or identical to elastase IV, a previously
analyzed protease of the chymotrypsin/elastase superfamily.
This conclusion is based on the following findings. (i) The rat
caldecrin cDNA encodes a serine protease zymogen consisting
of a signal peptide, an activation peptide, and a mature en-
ze. Eight cysteine residues present at positions 30, 46, 126,
157, 173, 183, 193, and 214 of the mature protein are con-
served in all elastase family members, which suggests the formation of
four disulfide bonds as observed in porcine elastase I (15). In
addition, three cysteine residues are present at the positions
–14, –13, and 112, indicating that a cysteine residue at posi-
tion –14 or –13 may form a disulfide bond with Cys-112. (ii)
The amino-terminal four residues of the mature form of pan-
creatic elastase, Val-Val-X-Gly, are highly conserved and these
residues follow an arginine residue. Table I, part A, corrobo-
rates that rat caldecrin is synthesized as a zymogen with a
signal peptide of 16 residues, and an activation peptide of 13
amino acids. Moreover, these data suggest that a cysteine (at
position –13) of the activation peptide forms a disulfide link
with an internal cysteine, i.e. 112, and that the activation
peptide is associated with the enzyme after proenzyme activa-
tion, a situation analogous to that seen with both elastase II
and chymotrypsin processing, as given in Table I, part B (11–
13, 19, 22, 29, 37). (iii) The nucleotide sequences of rat caldecrin
and rat elastase IV genes are 99.3% identical, but the deduced
amino acid sequences display only 90.3% identity (Fig. 4).
These results strongly suggest that rat caldecrin could be an
isoform of elastase IV generated by some unusual splicing
mechanism. Alternatively, and in our view more likely, how-
ever, the differences between our protein sequence and the
elastase IV sequence published by Kang et al. (29) result from
a frameshift reading error in the corresponding region of
the cDNA by the latter group (see Fig. 4). Unfortunately, the
elastase IV protein has so far not been investigated. Further
studies will be required to resolve this problem. In any event,
a stretch of the deduced amino acid sequence of rat caldecrin
cDNA, which is quite different from that of elastase IV, was
identical to the peptide sequence of a fragment obtained from
the purified rat caldecrin and had a high homology with the
respective peptide sequence of purified porcine caldecrin,
indicating that the caldecrin gene is really translated. More-
over, the deduced amino acid sequence of the human caldecrin
cDNA that we recently cloned displays, within the region of
sequence divergence between caldecrin and elastase IV, a
very high identity with the rat caldecrin analyzed in a study to
be published elsewhere.2

The amino acid residues characteristic of the serine protease
catalytic triad (38) are retained at positions His-45, Asp-92,
and Ser-187 of the deduced amino acid sequence. The other key
amino acid residues, Gly-209 and Val-221, thought to contrib-
ute to the substrate specificity of such enzymes, may enlarge
the substrate binding pocket to accommodate more bulky
amino acid side chains (19). The presence of an activation
peptide similar to that found in chymotrypsin and elastase II
suggests a chymotrypsin-like substrate preference and indi-
cates that caldecrin may be an evolutionary link between chy-
motrypsin and elastase I. Anionic elastase possessing chymo-
trypsin activity has not been found in rat or porcine pancreas.
The partial amino acid sequence of anionic chymotrypsin C has
been reported, but its cDNA sequence has not. The partial
amino acid sequence around an essential histidine of porcine

2 A. Tomomura, M. Akiyama, H. Itoh, I. Yoshino, M. Tomomura, T.
Noikura, and T. Saheki, manuscript in preparation.
chymotrypsin C (39) is not identical to that of porcine caldecrin.

So far, many proteases with elastolytic activity have been isolated from various tissues. Thus, careful consideration should be given to this nomenclature, in particular to elastase numbering. Rat and porcine caldecrins might be classified as elastase IV family members based on the current nomenclature rules, although the SCDA of these proteins apparently is not connected with protease activity. Yoneda et al. (40) have reported partial purification of a factor in a porcine pancreatic extract (PX) that decreases the blood calcium level, and they also showed that PX prevents progression of hypercalcemia and cachexia in mice inoculated with a carcinoma cell line (41). Amino-terminal sequencing of PX indicates that it belongs to the elastase IIB family, and recombinant elastase IIB displays hypocalcemic activity that is dependent on its protease activity (42). The reasons for this discrepancy between their results and ours are presently unclear. In any case, all presently available data show that same hypocalcemic factor(s) exists in the pancreas, and their structural analysis uncovers a novel regulatory function of these proteases. However, rat and porcine caldecrins treated with PMSF and even two kinds of the catalytic site mutants of rat caldecrin still displayed SCDA, suggesting that caldecrin may possess functional residues for SCDA that differ from those involved in the protease activity. The proform of caldecrin does not possess SCDA but acquires this property as well as protease activity after activation by trypsin treatment. These results suggest that the residues responsible for SCDA require a trypsin-induced conformational change to be come exposed at the outer surface of the molecule. The SCDA of caldecrin correlates with a decrease in serum hydroxyproline levels and with the inhibition of PTH-stimulated bone resorption (9), suggesting that caldecrin suppresses osteoclastic activity through an as yet unknown mechanism. All these data indicate that caldecrin, a protease closely related or identical to elastase IV, is a multifunctional protein that may be implicated in the regulation of blood calcium levels.

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Molecular Cloning and Expression of Serum Calcium-decreasing Factor (Caldecrin)
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