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Tissue Distribution of High Molecular Weight Calmodulin-Binding Protein

Rajendra K. Sharma

**Department of Pathology and Saskatoon Cancer Center
College of Medicine
The University of Saskatchewan
Saskatoon, Saskatchewan, Canada S7N 0W0**

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Polyclonal antibodies raised against bovine heart high molecular weight calmodulin-binding protein were used to study the distribution of this protein in diverse bovine tissues. The high molecular weight calmodulin-binding protein, in addition to bovine heart, is also present in lung and brain at much lower levels, but not in skeletal muscle, spleen, kidney or uterus.

A number of hormones and neurotransmitters exert their effects on cellular functions by regulating cytosolic Ca^{2+} concentrations. Ca^{2+} may flow through Ca^{2+} channels in the plasma membrane and/or may be mobilized from intracellular storage sites due to the stimulation of specific cell surface membrane receptors. The mechanisms by which Ca^{2+} achieves its intracellular effects appear to be diverse. In many instances, the actions of Ca^{2+} are mediated by specific cytosolic Ca2+ receptor proteins such as calmodulin (1-4). Recently a number of enzymes and other proteins have been reported to be regulated by calmodulin. Although their biological roles are largely unknown, the molecular structures and the modes of interaction with calmodulin appear to be diverse. This suggests that the expression of diverse biological functions of calmodulin may depend on the properties of calmodulin-binding proteins. In this regard, it is important to elucidate the molecular properties and biological functions of calmodulin-binding proteins.

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I have reported a novel calmodulin-binding protein which was purified from bovine cardiac muscle (5). The distribution of this protein in various bovine tissues was compared on the basis of molecular mass and found that this protein may be cardiac-specific (5). In this study, we use the Western blotting technique to demonstrate the distribution of the high molecular weight calmodulin-binding protein (Mr=140,000) in various bovine tissues. The results suggest that, in addition to cardiac muscle, this calmodulin binding protein is also present in lung and brains, in very low concentration.

MATERIALS AND METHODS

Bovine brain calmodulin was prepared as described by Gopalakrishna and Anderson (6) and further purified by gel filtration (Sephacryl S-200) column chromatography. The column was pre-equilibrated with buffer A (20 mM Tris-HCl, 1 mM magnesium acetate, 1 mM imidazole, pH 7.0, 10 mM 2-mercaptoethanol) containing 0.01 mM Ca^{2+} and 0.1 M NaCl. Calmodulin-Sepharose 4B gel was prepared as described by Sharma et al. (7). The chicken gizzard caldesmon (8) was a generous gift from Dr. M.P. Walsh (University of Calgary). Peroxidase-conjugated goat anti-mouse IgG was purchased from Jackson Immunoresearch Laboratories Inc.

Preparation of tissue homogenate and total calmodulin-binding proteins for gel electrophoresis and immunoblotting: All steps were carried out at 4 °C. Fresh bovine tissues (heart, spleen, lung, skeletal muscle, uterus, and brain) were obtained from a local slaughterhouse and transferred to the laboratory in packed ice. All tissues were stored at -30°C until use. The crude homogenate and total calmodulin-binding protein samples were prepared from various tissues as described by Sharma (5).

Gel Electrophoresis - Polyacrylamide (12%) gel electrophoresis in the presence of SDS was carried out according to the procedure of Laemmli (9). Coomassie Blue was used to visualize the protein bands on the gel.

Immunoblotting Method - The immunoblotting procedure (5) was essentially as described by Towbin et al. (10).

Preparation of polyclonal antibody: New Zealand white rabbits were immunized with purified high molecular weight calmodulin-binding protein (5). Antibody titer was checked using the Elisa test (11). Antiserum was precipitated with solid ammonium sulfate to 40% saturation and precipitated proteins were sedimented at 10,000 x g for 30 min. The antibody was further purified by gel filtration on a Sephacryl S-200 column equilibrated with 20 mM Tris-Hcl, pH 7.2 containing 150 mM NaCl.

RESULTS AND DISCUSSION

The specificity of anti-high molecular weight calmodulin-binding protein antibody was tested by Western blotting technique using the following purified proteins: high molecular weight calmodulin binding proteins, caldesmon, myosin, myosin light chain kinase, tropomyosin, calmodulin-dependent phosphodiesterase and calmodulin-dependent

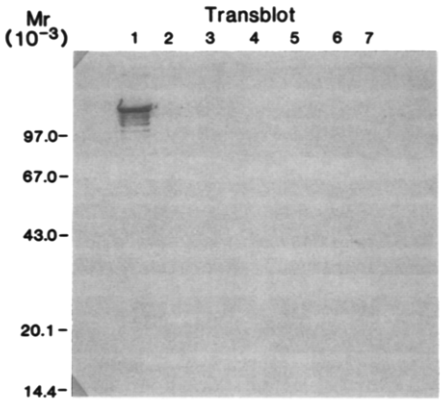

Fig. 1. **Transblot showing specificity of high molecular weight calmodulin-binding protein antibodies. The following purified proteins (10 μg of each) were subjected to 10% SDS-PAGE and transblotted onto a nitrocellulose membrane as described under "Materials and Methods". Lanes 1, high molecular weight calmodulin-binding protein; 2, caldesmon; 3, myosin; 4, myosin light chain kinase; 5, tropomyosin; 6, calmodulin-dependent phosphodiesterase and 7, calmodulin-dependent phosphatase.**

phosphatase (Fig 1). Only the high molecular weight calmodulin-binding protein was detected on the immunoblot. The immunoblot (Fig 1) reveals the presence of small amounts of proteolytic fragments of high molecular weight calmodulin-binding protein which is highly susceptible to proteolysis (12).

The presence of high molecular weight calmodulin-binding protein was reexamined by SDS-PAGE and Western blotting technique in the total calmodulin-binding protein fraction from various bovine tissues: spleen, skeletal muscle, lung, brain, heart, uterus and kidney (Fig 2). A protein of apparent molecular weight 140,000 daltons was not observed in any of the tissues examined except heart (Fig 2A), but it is apparent from the immunoblot (Fig 2B) that high molecular weight calmodulin-binding protein (140,000) may also be present in lung and brain. Since the total calmodulin-binding protein fraction was prepared in an identical manner from each tissue and assuming that the anti-high molecular weight calmodulin-binding protein antibody cross-reacted equally with high molecular weight calmodulin-binding protein from each tissue, we conclude that, in addition to heart, the lung and brain also contain this protein but at much lower levels. We have also examined the distribution of this protein in crude

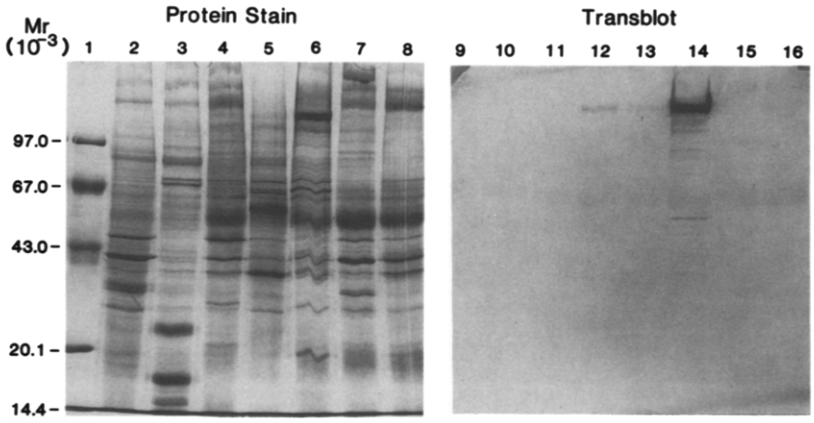

Fig. 2. **The distribution of high molecular weight calmodulin-binding protein in the total calmodulin-binding protein fraction in diverse bovine tissues. Total calmodulin-binding protein fraction was prepared from various bovine tissues as described under "Materials and Methods". Total calmodulin-binding protein fractions (20 μg of each) were subjected to SDS-PAGE and either stained with Coomassie Blue (Protein Stain) or immunoblotted (Transblot). Lanes: 1, molecular weight standards; 2, spleen; 3, skeletal muscle; 4, lung; 5, brain; 6, heart; 7, uterus; 8, kidney; 9-16, respective transblot of lanes 1-8.**

bovine tissue extracts. A crude extract of each tissue examined was prepared as described under "Materials and Methods". The crude extracts were subjected to SDS-PAGE and immunoblotting. Again high molecular weight calmodulin-binding protein (140,000) was apparent only in heart, lung and brain (data not shown) and the relative contents were the same as described by immunoblotting of the total calmodulin-binding protein fraction (Fig 2B).

The physiological significance of the high molecular weight calmodulin-binding protein is not known at present. Although present in highest amount in cardiac muscle, this protein of 140,000 daltons was also detected in the lung and brain, but not in skeletal muscle, spleen, kidney or uterus.

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