PARTIAL PURIFICATION OF A CALSEQUESTRIN-LIKE PROTEIN FROM SHARK (SQUALUS ACANTHIAS) SKELETAL MUSCLE

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Previous electrophysiologic and morphologic studies of cultured shark rectal gland cells in our laboratory suggest that calcium may regulate apical membrane chloride conductance through local actions in the apical cytoplasm of these cells. Calciosomes are vesicular organelles thought to comprise part of the intracellular depot of calcium in non-muscle cells which is responsive to messengers generated from phosphatidylinositol phospholipids. Calsequestrin (CS) is a calcium-binding protein which is found in large quantities in muscle sarcoplasmic reticulum. Immunocytochemical techniques have localized CS in calciosomes of several cell types including hepatocytes, pancreatic acinar cells and neurons (Volpe,P. et al., Proc. Natl. Acad. Sci. USA 85:1091-1095, 1988). In addition, CS-like proteins have also been found in the endoplasmic reticulum of non-muscle cells. The object of this work was to purify CS from shark skeletal muscle. Polyclonal antibodies will be raised against the purified protein for use in immunoblot and immunocytochemical studies designed to determine if rectal gland epithelial cells contain CS and whether it is concentrated in vesicular structures in the apical cytoplasm. Such a localization would provide strong support to our hypothesis that calcium mobilization near the apical plasma membrane has an important role in controlling the chloride conductance of this barrier.

White skeletal muscle from Squalus acanthias was homogenized in Hepes buffered sucrose, pH 7.4 and the cell debris pelleted. Sarcoplasmic reticulum (SR) vesicles were isolated from the supernatant by differential and sucrose density gradient centrifugation (Meissner, G., Biochim. et Biophys. Acta 241:51-68, 1975). Higher density vesicles contain Ca-Mg ATPase (MW 105 kd) and one other Ca-binding protein (MW 65 kd). In the high density shark SR vesicle fraction (Fraction #3) we have tentatively identified two similar proteins in SDS-PAGE gels using Stains-All, a cationic carbocyanic dye which selectively stains Ca-binding proteins dark purple (Campbell, K.P., et al., J. Biol. Chem. 258:11267-11273, 1983). The higher MW protein runs at 95 kd, consistent with it being Ca-ATPase. The second protein has a molecular weight of 52 kd and probabaly represents CS. Macer and Koch (J. Cell Sci. 91:61-70, 1988) have shown that permeabilization of SR vesicles with saponin selectively releases Cs into the supernatant. To determine whether the 52 kd protein is solubilized when SR vesicles are permeabilized, aliquots of Fraction #3 were treated with 0.01% saponin for 2.5, 5, 7.5 or 10 minutes in 10mM Tris buffer (pH 7.4) and then centrifuged at 100,000Xg for 50 minutes. The supernatant and pellet were analyzed separately by SDS-PAGE followed by Stains-All staining. Treating Fraction #3 with saponin for increasing periods of time resulted in loss of the majority of the 52 kd protein from the pellet and its concomitant appearance in the supernatant. On the basis of its enrichment in SR vesicles, its apparent molecular weight by SDS-PAGE, its characteristic staining with Stains-All and its solubilization with saponin, we tentatively conclude that the 52 kd protein is shark skeletal muscle CS. Biochemical characterization of this protein using phenyl-Sepharose chromatography and radiolabeled calcium binding is necessary to verify its homology to mammalian CS.
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