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Molecular Cloning and Characterization of Calumenin in Rabbit Skeletal Sarcoplasmic Reticulum

Dai Hyun Jung and Do Han Kim

Kwangju Institute of Science and Technology (K-JIST), Kwangju, Republic of Korea

Calumenin was previously identified as a high affinity Ca^{2+} binding protein in mouse cardiac sarcoplasmic reticulum (SR). For the present study, a 48 kDa skeletal homologue of calumenin was identified by sucrose-density gradient of rabbit skeletal SR membranes, concanavalin A treatment, 2D-gel electrophoresis, $^{45}Ca^{2+}$ overlay, Stains-all staining, and MALDI-TOF analysis. We attempted to clone the skeletal calumenin by RT-PCR based on mouse cardiac and human calumenin sequences. The deduced amino acid sequence (315 residues) of the skeletal calumenin showed high identity to mouse cardiac calumenin (90%). As seen in the cardiac calumenin, the deduced sequence contains a 19 amino acid N-terminal signal sequence and a HDEF C-terminal sequence, a putative retrieval signal to ER. Also, the skeletal calumenin contains one N-glycosylation site, three PKC phosphorylation sites, eight casein kinase 2 phosphorylation sites, and 6 EF-hand domains. GST-calumenin showed a conformational change and increased mobility in the presence of Ca^{2+} in SDS-PAGE. Three calumenin interacting proteins (ryanodine receptor 1, glycogen phosphorylase, and phosphofructo kinase) were identified by pull-down assay with GST-calumenin and solubilized SR. All the interactions were Ca^{2+} dependent. The present results suggest that calumenin plays an important role in Ca^{2+} homeostasis of muscle cells.