Affinity purification of the skeletal muscle ryanodine receptor using a gluthatione-S-transferase-FKBP12 fusion protein

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Calcium is the most ubiquitous second messenger. In numerous cell types, the calcium release channel ryanodine receptor (RyR) plays a central role in calcium signalling. This channel is associated with a plethora of proteins in the cell, and constitutes the core of a supra-macromolecular complex which integrates cell signals and translates them into a calcium signal. In the heart and in the skeletal muscle, RyRs associated with their co-/accessory proteins are central to the excitation-contraction process which is still not fully understood at the molecular level. A better understanding of this critical step and, by extension, of the calcium signalling in many cell types requires better knowledge of the mechanisms which determine the ryanodine receptor function, *via* improved structure-function studies. The most precise studies of ryanodine receptor function are based on single channel recordings after incorporation of the RyR into planar lipid bilayers. Unfortunately, purity of the channel used in those experiments has not been adequate to determine RyR's intrinsic properties. Most studies have been done with microsomal vesicles enriched in RyR. The so called purified receptor contains significant contamination by several proteins. When RyR has been exogenously expressed in cells which don't express it endogenously, the extent of the channel's association with other proteins is not known. Determination of the channel's intrinsic properties and of the precise influence of its numerous co-/accessory proteins requires functional studies performed with pure RyR.

FKBP12 is a small protein which associates tightly with the RyR (Mackrill *et al.,* 2001). We have expressed a GST-FKBP12 fusion protein and used it to perform affinity purification of the RyR from rabbit skeletal muscle isolated after animals had been euthanased by captive bolt. The procedure has been described with few modifications (Mackrill *et al.,* 2001). Heavy sarcoplasmic vesicles were solubilised with 3-[(3-cholamidopropyl)-dimethylamino]-1-propanesulfonate in the presence of phosphatidylcholine. Non solubilised proteins were eliminated by ultracentrifugation; supernatant was then incubated with GST-FKBP12 fusion proteins immobilised on agarose beads. Unbound and unspecifically bound material was removed by successive washes. Purity of the purified ryanodine receptor and yield of the protein were estimated by silver nitrate staining of SDS-PAGE and colorimetric assay, respectively. Attempt to release RyR from the beads-fusion protein complex by the drug rapamycin failed. The ryanodine receptor-fusion protein complex was released from the beads by glutathione, and the excess of fusion protein eliminated by gel filtration. The RyR was purified virtually to homogeneity, the main usual contaminants (calsequestrin and Ca^{2+}-ATPase) not being detected on silver nitrate. Yield of the purified RyR from the heavy sarcoplamic vesicles was estimated close to 60%.

Intrinsic functional properties of the purified protein will be studied using single channel recordings and ^{3}H-ryanodine binding assays. We are also expressing some RyR's co-/accessory proteins as fusion proteins with tags allowing their affinity purification. Addition of those purified co-/accessory proteins to the buffer bathing the purified channel should allow us to explore their regulatory effects on the receptor.

Mackrill, J.J., O'Driscoll, S., Lai, S.F.A., & McCarthy, T.V. (2001) *Biochemical and Biophysical Research Communications* **285,** 5257.