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Reconstitution of the solubilized cardiac sarcoplasmic reticulum potassium channel

Identification of a putative M_r ~80 kDa polypeptide constituent

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Recent evidence has indicated that potassium ion movement through sarcoplasmic reticulum (SR) K^+ channels is an important countercurrent for Ca^{2+} release from SR. We used Chaps-solubilized SR vesicles and sucrose density gradient centrifugation to identify components of the canine cardiac SR K^+ channel. To overcome the difficulty of the absence of a high-affinity specific ligand, we have successfully applied the planar lipid bilayer reconstitution technique to identify and functionally assay for the solubilized SR K^+ channel. We found that Chaps solubilization of the channel did not change the protein's functional properties. The cardiac SR K^+ channel sediments as a 15–20S protein complex. A polypeptide of M_r ~80 kDa was found to specifically comigrate with the 15–20S gradient fractions and might be a major constituent of the cardiac SR K^+ channel.

Potassium channel; Sarcoplasmic reticulum; Protein purification; Lipid bilayer; Cardiac muscle; Chaps

1. INTRODUCTION

Cardiac and skeletal muscle contraction is initiated by a rapid release of Ca^{2+} ions through a high-conductance channel present in the sarcoplasmic reticulum (SR) [1–3]. Skeletal and cardiac muscle SR also contains ion-specific channels through which K^+, Na^+, H^+, and Cl^- fluxes ocuur [4]. The monovalent ion countermovement through these conducting pathways is believed to minimize charge buildup across the SR during rapid Ca^{2+} release and possibly during Ca^{2+} uptake by the SR [5–7]. Recent evidence indicates that most of the K^+ flux in skeletal SR vesicles occurs via SR K^+ channels and that this ion movement represents an important countercurrent for SR Ca^{2+} release during muscle contraction [8,9]. Further, the K^+ conductance for cardiac SR K^+ channels appears to be regulated by Ca^{2+} levels in the SR lumen [10]. SR K^+ channels from mammalian cardiac and skeletal muscle show substantial differences in their gating and permeation properties, indicating that they are comprised of different, although probably related, proteins [9,11–14]. Further biochemical characterization of these channels requires their purification, which has been impeded so far by the lack of a high-affinity specific ligand. Using ryanodine as a specific probe, Lai et al. [2] and Anderson et al. [3] have purified the SR Ca^{2+} release channel from skeletal and cardiac muscle, respectively, using Chaps-solubilized SR vesicles and sucrose density gradient centrifugation. Using an identical procedure, we now report the functional reconstitution of the cardiac SR K^+ channel from Chaps-solubilized SR vesicles following sedimentation through a linear sucrose gradient. We found that the cardiac SR K^+ channel sediments as a 15–20S protein complex and that Chaps solubilization of the channel did not change the protein's functional properties. A polypeptide of M_r ~ 80 kDa was found to specifically co-migrate with the 15–20S gradient fractions and might be a major constituent of the SR K^+ channel. Part of this work has been reported in abstract form [15].

Abbreviations: Chaps, 3-[(3cholamidopropyl)dimethylammonio]-1-propanesulfonate.

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2. MATERIALS AND METHODS

Phospholipids were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL) and Chaps from Boehringer Mannheim (Indianapolis, IN). SDS gel molecular weight standards were obtained from Sigma. All other reagents were of reagent grade.

Cardiac SR membranes were prepared from canine ventricular tissue as previously described [3,16]. Membranes were solubilized in 1.5% Chaps in the presence of 5 mg/ml phosphatidylcholine and cen-

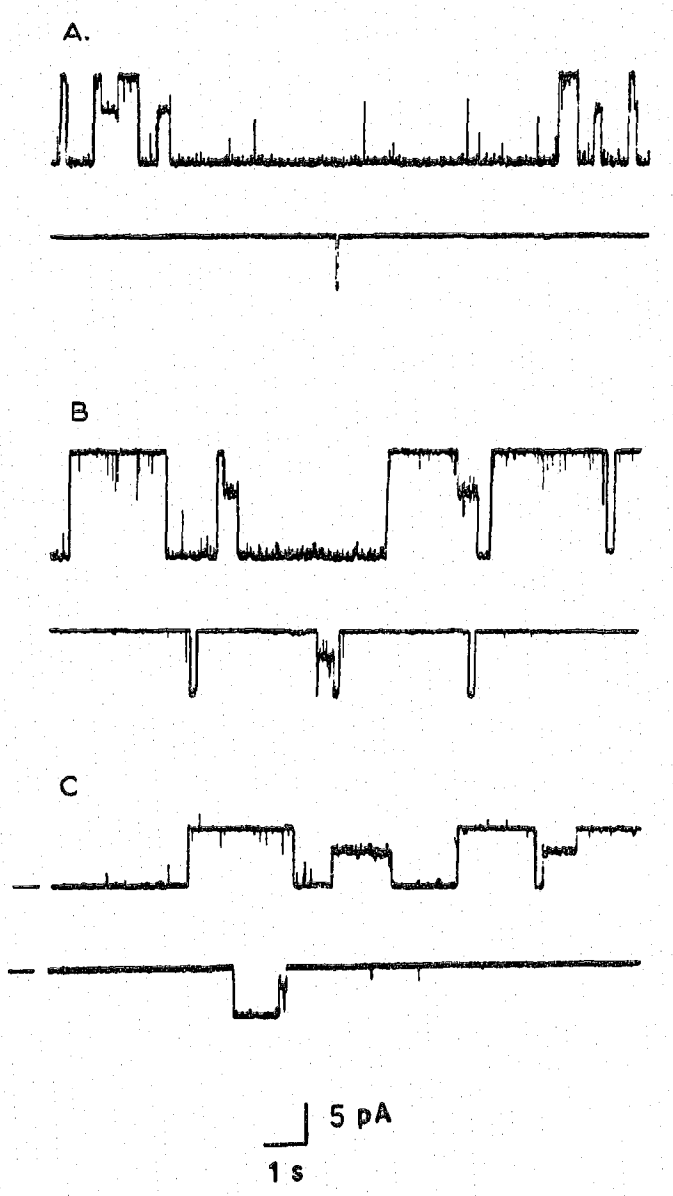

Fig. 1. Single-channel recordings obtained from Chaps-solubilized SR-K^+ channel proteins incorporated into a planar lipid bilayer membrane. Single-channel currents, shown as upward deflections for positive holding potentials and downward for negative potentials, were recorded either in (A) symmetric 100 mM KAc, 10 mM histidine, pH 7.1 solution or (B) symmetric 500 mM KAc, 10 mM histidine, pH 7.1 solution at the voltages 60 mV (A and B, upper trace) and −40 mV (A and B, lower trace). The probability of open channel events increased with increasing positive holding potentials. Both the conductance and the probability of open channel events increased with increasing K^+ concentration. The principal SR K^+ channel substate (O_1) can also be observed. (C) Single-channel recordings under the same conditions as (B), but with the native SR K^+ channel at 33 mV (upper trace) and −27 mV (lower trace).

trifuged through a 5–20% linear sucrose gradient using a Beckman SW 28 rotor as previously described [2,3]. Eighteen to twenty fractions of ~2.0 ml each were collected from the gradient and tested for K^+ channel activity by reconstitution into planar lipid bilayers as described below. An apparent sedimentation coefficient for the solubilized SR K^+ channel was obtained as previously described [2,3] using the globular enzyme sedimentation markers β-galactosidase, catalase and yeast alcohol dehydrogenase ($S_{20,w}$ of 16, 11.2 and 7.6, respectively).

Table I

Cardiac SR K^+ channel

	Native membrane	Solubilized membrane
γ_{O_2} at 500 mM [K^+] (pS)	221 ± 7	223 ± 8
γ_{O_1} at 500 mM [K^+] (pS)	127.3 ± 6.7	126.2 ± 8.9
$\gamma_{O_1}/\gamma_{O_2}$	0.57 ± 0.03	0.56 ± 0.04
ΔH_{O_2} (kcal/mol)	4.2 ± 1.0	4.5
$K_{d\ Cs,cis}$ (+30) (mM)	8.8	9.0
$K_{d\ Cs,trans}$ (−30) (mM)	13.1	14.4
$\delta_{Cs,cis}$	0.26	0.25
$\delta_{Cs,trans}$	0.27	0.26

Bilayers composed of a mixture of PE/PS = 1:1 or PE alone in decane (20 mg/ml) were formed across a ~0.3 mm hole separating symmetric K^+-acetate, 10 mM histidine, pH 7.1 solutions. Each of the sucrose gradient fractions was then tested for the presence of functional SR K^+ channels by adding small aliquots (0.5–2.0 μl) to the *cis* chamber solution (3 ml) of the planar lipid bilayer apparatus [2]. Following incorporation of the SR K^+ channel into the bilayer, the chambers were perfused as specified in the text (Figs. 1 and 2). Current signals were digitized and stored, low-pass filtered at 100 Hz (8-pole Bessel) and analyzed as described previously [2,14].

3. RESULTS

Fig. 1 compares single SR K^+ channel recordings obtained from Chaps-solubilized SR (1A and 1B) with those from native SR membranes (1C). For both the solubilized and native SR K^+ channels, two major conductance states cl aracteristic of this channel were observed. Tne *I/V* relation of the fully-open state (O_2) and substate (O_1) of the channel from solubilized membranes were ohmic (−80 to 100 mV, data not shown) and closely approximated those of the channel from native membranes (Fig. 1C and Table I).

We also examined the concentration dependence of conductance (Fig. 2), activation enthalpy for permeation, and Cs^+ blockade. We found that concentration dependence of K^+ conductance and temperature dependence of K^+ permeation, both for the fully open and the major substate of the K^+ channel [17], were essentially identical for the SR K^+ channel obtained from native and solubilized membranes (Table I). Similar values for the Cs^+ dissociation constant and fractional electrical distance were obtained from SR K^+ channels obtained from both sources (Table I). We also found that Cs^+ blockade of the reconstituted solubilized cardiac SR K^+ channel was competitive with K^+, as observed for SR K^+ channels from native membrane vesicles (data not shown).

We determined the relative concentration of SR K^+ channel in different gradient fractions by recording the frequency of appearance of SR K^+ unitary currents following addition of each fraction to the *cis* chamber. SR

K^+ channel fusion events were most frequently observed with fractions 10 and 11 of the gradient (Fig. 3A), suggesting an apparent sedimentation coefficient of 15–20S for the cardiac SR K^+ channel. Analysis of the protein composition of gradient fractions by SDS-PAGE indicated the presence of several major polypeptides in the 15–20S region that was enriched in K^+ channel activity (Fig. 3B). Most prominent among these was an M_r ~80 kDa band, which appeared to comigrate specifically with the K^+ channel-containing fractions.

4. DISCUSSION

The K^+ channel of muscle SR is believed to mediate cation countermovement resulting from Ca^{2+} efflux through Ca^{2+} release channels during excitation–contraction coupling. Recently the cardiac and skeletal muscle SR Ca^{2+} release channels have been purified [2,3] and their cDNAs cloned and sequenced [18]. To more fully understand all of the processes governing SR function during EC coupling, we also need to know the protein composition and structure of the SR K^+ channel. In contrast to the SR Ca^{2+} release channel, however, the absence of a high-affinity ligand for the SR K^+ channel hindered attempts to biochemically characterize the SR K^+ channel protein. In this study, we have applied the planar lipid bilayer reconstitution technique to identify and functionally assay for detergent-solubilized SR K^+ channels. This technique might prove to be useful for identifying and purifying other ion channels as well.

Because of the successfull use of Chaps in the solubilization and purification of the SR Ca^{2+} release channel,

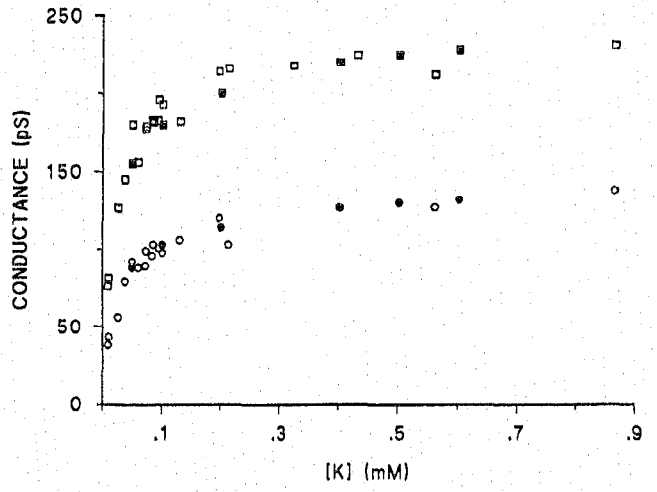

Fig. 2. Conductance-K^+ concentration relation. In symmetric KAc solution the relationship between O_2 and O_1 conductance states with increasing K^+ concentration was very similar for the SR K^+ channels obtained either from solubilized SR K^+ channels (filled squares and circles) or from native SR vesicles (empty squares and circles). The (O_1/O_2) conductance ratio obtained from both preparations was approximately 0.56.

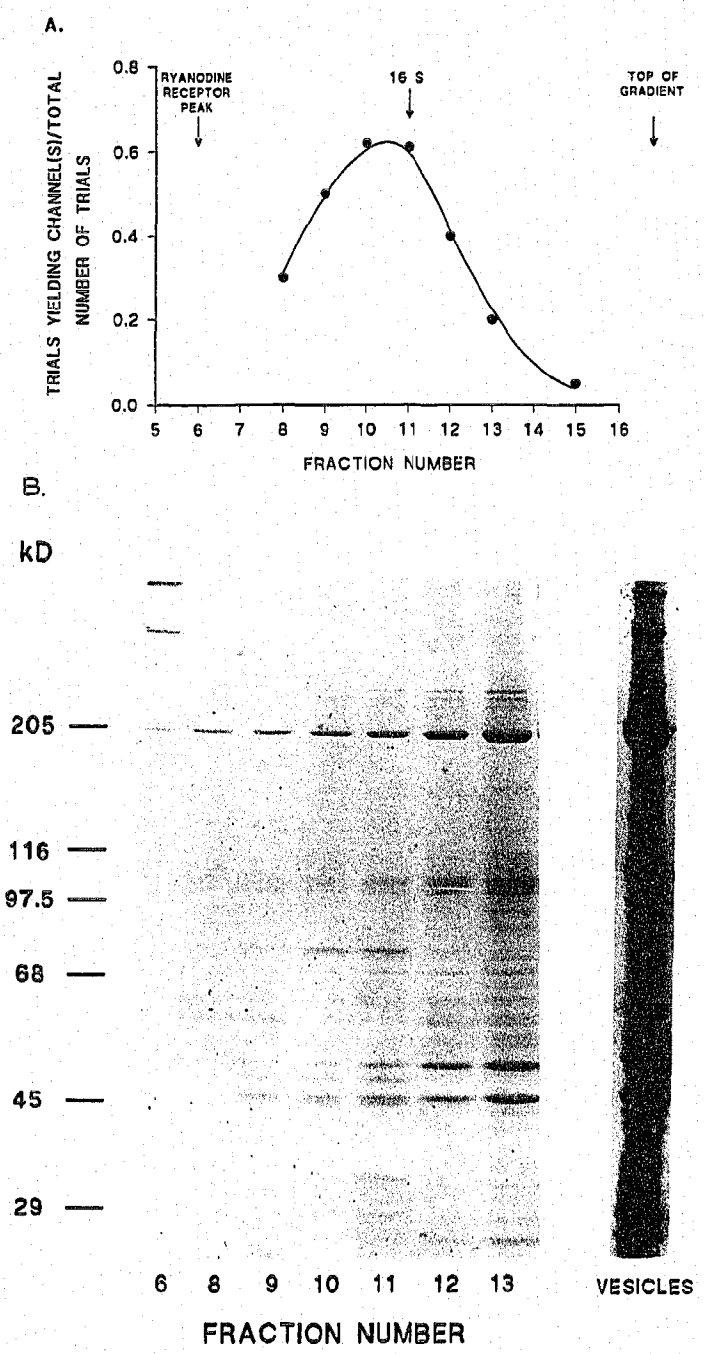

Fig. 3. (A) Ratio of successful experiments vs fraction number. Sucrose gradient fractions were collected from the bottom (fraction 1) to the top and were tested for SR K^+ channel activity by direct incorporation into a planar lipid bilayer. Ratio represents the number of successful experiments that resulted in reconstitution of one or more K^+ channels relative to the total number of experiments. Fractions 10 and 11 appeared to be more enriched in cardiac SR K^+ channel activity. Arrows indicate ryanodine receptor peak fraction and position of β-galactosidase markers ($S_{20,w}$ = 16). (B) Chaps-solubilized canine cardiac SR (2 mg/ml) was sedimented through a 5–20% linear sucrose gradient. Fractions were collected from the bottom of the gradient, and aliquots were electrophoresed on a 5–12% linear polyacrylamide gradient gel, then stained with 0.1% Coomassie brilliant blue R-250. An ~80 kDa band is enriched in fractions 10 and 11 compared with other fractions. The right column shows protein compositions of SR vesicles.

we employed a similar protocol to initiate the purification of the SR K^+ channel. Functional similarities between the reconstituted solubilized and native SR K^+ channel protein observed included similar values of single-channel conductance for the fully open state and the major substate, the relation of K^+ conductance to K^+ concentration, the activation enthalpy for permeation, and the parameters of Cs^+ blockade. Thus, it appears that Chaps solubilization and subsequent sedimentation through a linear sucrose gradient did not noticeably modify cardiac SR K^+ channel function, as was observed for the SR Ca^{2+} release channel. Detergent solubilization and partial characterization of the functional properties of reconstituted skeletal, but not cardiac, SR K^+ channels have been previously reported. Young et al. solubilized skeletal SR vesicles with deoxycholate and reconstituted a K^+ and Na^+ permeable channel into membrane vesicles by removal of detergent through dialysis [20]. Skeletal SR was also solubilized with cholate and reconstituted into liposomes [21]. Patch clamping the liposomes yielded single K^+ channel current which displayed values for unitary K^+ channel conductance, thermodynamics of gating, and cation selectivity similar to those obtained from K^+ channels following fusion of SR vesicles with planar lipid bilayers. However, a four-fold difference in the value of the apparent dissociation constant was observed for hexamethonium block [21]. Thus, several detergents can be used to purify and reconstitute functional SR K^+ channels. Our data further show that Chaps solubilization and subsequent partial purification by sucrose gradient centrifugation minimally alter the functional properties of the cardiac SR K^+ channel.

Comparison of the SR K^+ channel activity profile of gradient fractions in bilayer experiments (Fig. 3A) and the protein composition, as determined by SDS-PAGE (Fig. 3B), suggests that an M_r ~80 kDa polypeptide may be a major constituent of the cardiac SR K^+ channel. This M_r ~80 kDa was present mainly in the fractions corresponding to 15–20S and which were specifically enriched in K^+ channel activity. Identification of a putative M_r ~80 kDa component of the SR K^+ channel is of particular interest, since recent purification and cDNA cloning studies have revealed that proteins of 70–95 kDa are constituents of the A-type K^+ channel of rat brain (76–80 kDa) [22], Drosophila A channel (70 kDa) [23], and delayed rectifier of rat brain (95 kDa) [24]. Assuming that the cardiac SR K^+ channel is, as has been found for other intracellular ion channels [2,3,25–28], formed by four polypeptides of ~80 000 Da, a molecular weight of ~320 000 would be predicted for the oligomer. Since this molecular weight is somewhat less than expected for a protein with a sedimentation coefficient of 15–20S, the SR K^+ channel complex may be composed of more than four ~80 kDa polypeptides or contain additional protein constituents [29].

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