

Single Channel Characteristics of a High Conductance Anion Channel in “Sarcoballs”

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ABSTRACT Previously undescribed high conductance single anion channels from frog skeletal muscle sarcoplasmic reticulum (SR) were studied in native membrane using the “sarcoball” technique (Stein and Palade, 1988). Excised inside-out patches recorded in symmetrical 200 mM TrisCl show the conductance of the channel's predominant state was 505 ± 25 pS ($n = 35$). From reversal potentials, the P_{O}/P_{K} ratio was 45. The slope conductance vs. Cl^{-} ion concentration curve saturates at 617 pS, with $K_{0.5}$ estimated at 77 mM. The steady-state open probability (P_{o}) vs. holding potential relationship produces a bell-shaped curve, with P_{o} values reaching a maximum near 1.0 at 0 mV, and falling off to 0.05 at ± 25 mV. Kinetic analysis of the voltage dependence reveals that while open time constants are decreased somewhat by increases in potential, the largest effect is an increase in long closed times. Despite the channel's high conductance, it maintains a moderate selectivity for smaller anions, but will not pass larger anions such as gluconate, as determined by reversal-potential shifts. At least two substates different from the main open level are distinguishable. These properties are unlike those described for mitochondrial voltage-dependent anion channels or skeletal muscle surface membrane Cl channels and since SR Ca channels are present in equally high density in sarcoball patches, we propose these sarcoball anion channels originate from the SR. Preliminary experiments recording currents from frog SR anion channels fused into liposomes indicate that either biochemical isolation and/or alterations in lipid environment greatly decrease the channel's voltage sensitivity. These results help underline the potential significance of using sarcoballs to study SR channels. The steep voltage sensitivity of the sarcoball anion channel suggests that it could be more actively involved in the regulation of Ca^{2+} transport by the SR.

INTRODUCTION

The sarcoplasmic reticulum (SR) of skeletal muscle is an intracellular membrane system that controls the free Ca^{2+} concentration of the muscle cell and therefore determines when the fiber will contract or relax. It is widely accepted that uptake of Ca^{2+} ions into the SR occurs via the Mg^{2+} -dependent Ca^{2+} ATPase or Ca pump (for

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review, see Inesi, 1985), and that Ca^{2+} ions are released through the Ca release channels (Smith et al., 1986; Stein and Palade, 1988). The assumed role for K and anion channels found in SR is that of passively balancing charge build-up during Ca^{2+} movements (McKinley and Meissner, 1978; Somlyo et al., 1981). Recent work by Fink and Stephenson (1987) and Abramcheck and Best (1989) on SR K channels in skinned skeletal muscle fibers, supports the idea that SR K channels can affect Ca^{2+} movements by demonstrating that purported modifiers of SR K channel conductance in vivo also modify the SR's ability to transport Ca^{2+} . It is also possible that SR anion channels may play a similar role as a pathway for counter-ion movement during release or uptake of Ca^{2+} , though no definitive functional role has been assigned to SR anion channels in excitation-contraction (E-C) coupling.

Although many data have been collected on SR Ca and K channels both using planar bilayer techniques (Miller, 1978; Coronado et al., 1980; Smith et al., 1986*a*, *b*), and measuring ion fluxes across isolated SR vesicles (for example McKinley and Meissner, 1978; Palade, 1987; for review, see Meissner, 1983) comparatively few data have been published on SR anion permeability or anion channels. Miller (1978) first reported observing single anion channels from isolated SR using the bilayer fusion method, and two other groups have also confirmed these observations (Smith et al., 1986*a*; Kasai et al., 1985), with both groups reporting data on a 100-pS (in 100 mM choline-Cl), voltage-independent Cl channel. Recently, Kasai and co-workers (Tanifuji et al., 1987) have published results of bilayer studies on a 200-pS anion channel (also in 100 mM choline-Cl) from isolated rabbit SR. This channel has a larger conductance than the previous examples of single SR anion channels, and is voltage sensitive as well.

Until now, biochemical isolation of SR has been a prerequisite for observing single SR channels, so there has been no way to assess potential damage from the isolation procedures. In addition, alterations in behavior of SR channels in bilayer work could arise from differences in lipid composition. Bilayer fusion studies use lipids or lipid ratios that are different from those in the native SR membrane, and cannot reproduce the lipid asymmetry found in native SR (Herbette et al., 1984). Changes in lipid composition have recently been shown to have effects on channel conductance (Bell and Miller, 1984) and kinetics (Coronado, 1987).

Recently, a preparation (termed "sarcoballs") was developed in our lab allowing patch-clamp studies to be done directly on SR channels in native internal membrane (Stein and Palade, 1988). This allows us to directly measure SR anion channels without the need for potentially harmful biochemical isolation, and should minimize effects from changes in lipid composition. In this communication, we report on the single channel properties of a high conductance anion channel observed in these sarcoballs. Comparison of its single channel properties with those of mitochondrial and muscle surface membrane anion channels suggests that this anion channel may originate in the SR. A preliminary account of this work has appeared in abstract form (Hals and Palade, 1988).

METHODS

Sarcoball Preparation

The procedure for sarcoball production has already been described (Stein and Palade, 1988). Briefly, one end of an individual fiber is dissected from a semitendinosus muscle of *Rana*

catesbeiana in frog Ringer's solution containing (in millimolar): 117 NaCl, 4.7 KCl, 1.2 CaCl₂, 5 MOPS (3-morpholino-propanesulfonic acid), pH adjusted to 7.2 and tonicity of 240 mosM. Fine forceps are then used to mechanically peel the sarcolemmal membrane off the fiber. Immediately after skinning, the skinned portion of the fiber is removed from the muscle and transferred to a 4-ml recording dish containing the desired recording solution. Sarcoballs, which appear as hemispheres of membrane (20–200 μm across) on the skinned fiber, are then visualized for patching under 400 X magnification on an inverted microscope.(M100PF; Swift Instruments Corp., Dover, MA).

Liposome Production

The technique used was a modification of that published by Tomlins and Williams (1987), with the modification that the SR proteins were not detergent solubilized before their addition to the liposomes. Briefly, isolated rabbit SR vesicles (fraction R3, Saito et al., 1984) were added to a liposome suspension (~5 mg SR protein was added to 25 mg of lipids suspended in 1 ml of 200 mM KCl). The liposome suspension is prepared by mixing phosphatidyl serine (PS) and phosphatidyl ethanolamine (PE) dissolved in chloroform (Avanti Polar Lipids, Inc., Birmingham, AL) in a 1:1 ratio. The chloroform is evaporated off with nitrogen gas, the recording solution is added, and the liposomes are formed by sonication for ~10 min. After mixing the liposomes and SR together, the suspension is then frozen and thawed several times to produce liposomes large enough to patch (5–25 μm diameters). Liposomes are then patched using the same methods as those used for sarcoball recording. The standard recording solution was (in millimolar): 100 CaCl₂ in the pipette and 200 KCl in the bath, with 0.5 mM Ca²⁺ added to the bath to aid seal formation. These solutions were used to enable rapid identification of the selectivity of any channel seen, based on the observed reversal potential. The same protocol was used to record frog SR channels in liposomes, and frog SR was isolated according to Volpe et al., (1988).

Electrophysiology

Electrodes were pulled from an intermediate hardness glass (Corning 7052; Friedrick and Dimmock, Millville, NJ) in two stages on a David Kopf puller (model 700C; David Kopf Instruments, Tujunga, CA), and then fire-polished to resistances between 5 and 25 MΩ; higher resistance electrodes were used to increase the probability of obtaining only a single anion channel in the patch. Electrode offsets were balanced before forming a giga-seal, and seal resistances typically ranged between 10 and 50 GΩ. Seals formed spontaneously under these conditions or with slight negative pressure. After seal formation, patches were excised to achieve the inside-out mode of the patch technique (Hamill et al., 1981) to allow exposure of the inner surface of the patch to various solutions by bath perfusion. Outside-out patches were obtained periodically to look for asymmetries in the channel's properties. All data presented here are from inside-out patches, except for Fig. 8.

Initial studies (Stein and Palade, 1988) suggest that the sarcoball Ca channels are oriented with the cytoplasmic surface of the channels facing the pipette solution, and the luminal surface facing the bath solution. For clarity, all potentials are expressed as pipette potentials with respect to a grounded bath. Therefore, the potential given is that at the cytoplasmic surface of the SR anion channels. Potentials were changed using a voltage divider that allowed step changes in DC voltage. Single channel currents were recorded with a patch clamp amplifier (model 8900; DAGAN Corp., Minneapolis, MN). The output of the amplifier was filtered at 5 kHz (–3 dB) using an 8-pole Bessel low-pass filter (model 902LPF; Frequency Devices, Haverhill, MA) and then stored on magnetic tape using a Racal Store 4DS FM tape recorder (Racal Dana Instruments Inc., Irvine, CA) for later analysis. The output of the filter was also viewed simultaneously on a Gould OS300 oscilloscope (Gould Inc., Cleve-

land, OH) and recorded on an Astro-Med single channel chart recorder (model Z-1000, Astro-Med, Inc., West Warwick, RI).

Perfusion of the bath solution was achieved using a modification of a technique by Dr. D.G. Lang (personal communication), where the patch electrode is moved from the recording chamber to a smaller 0.25-ml perfusion chamber. Bath perfusion is accomplished by gravity flow through PE tubing to the perfusion chamber and the excess solution is removed by aspiration. Solution changes required ~30–60 s. In all experiments, the Ag-AgCl wire of the patch electrode and the Ag-AgCl plug for the reference electrode were exposed to the same solution. The pool for the reference electrode was electrically connected to the bath solution (for recording or perfusion) by a 3 M KCl agar bridge. Perfusion experiments for selectivity measurements were all performed using the same protocol. Due to their low permeability in the channel, measurements for K^+ , Ca^{2+} , gluconate, and MOPS were performed using additional protocols (see Results). The patches were initially formed in symmetrical 200 mM TrisCl (for exceptions, see Table I) and the control current-voltage relationship was recorded. The pipette was then moved to the bath perfusion chamber, and the bath solution was exchanged to one containing 200 mM of the test anion. Cation concentrations were also 200 mM (400 mM in the case of divalent anions) and the cation species were identical throughout each experiment.

Solutions

The standard conditions for sarcoball recording were to record initially in symmetrical solutions of (in millimolar) 200 Tris-hydroxymethyl-aminomethane hydrochloride (TrisCl) at pH 7.0. The bath recording solution also contained 200 TrisCl with an additional 2 CaMOPS added to aid seal formation. Free Ca^{2+} concentrations in the pipette were not controlled but were in the range of 1–4 μ M, and did not affect channel activity. Changes in free Ca^{2+} concentration on either side of the patch were shown to have no effect on the channel properties (see Results). In some selectivity experiments 200 KCl or 200 tetraethylammonium chloride (TEACl) was used instead of TrisCl, where indicated in Results. This was done since some anions were unavailable as Tris salts, and it was necessary to keep the same cation on both sides of the patch to reduce any effects of cation permeability. Methanesulfonate and propionate were acquired from Aldrich Chemical Co., Milwaukee, WI. Tris base and MOPS acid were purchased from Calbiochem Behring, Corp., La Jolla, CA. All other chemicals were obtained from Sigma Chemical Co., St. Louis, MO.

Analysis

Single channel amplitudes were measured either manually from the chart paper records or by computer. Data to be analyzed was played back through the Frequency Devices filter at 0.5 kHz (–3 dB) and digitized at 500- μ s intervals using a Data Translation DT2801A board and a PC's Limited IBM PC AT "clone". The "Analysis" single channel analysis program developed by Dr. Hubert Affolter (courtesy of Dr. R. Coronado) was used to determine channel amplitudes by the following protocol: single channel amplitudes that fell into a user-defined window were obtained by subtracting the mean closed current from the mean open current. The open probability was calculated from the amplitude histogram by dividing the area of the "open" distribution into the summed area for both "open" and "closed" distributions. In all cases, open probability measurements were taken from single channel patches (i.e., where no multiple levels of current were ever observed during the entire experiment). The open time, closed time, and amplitude histograms were also obtained using the Analysis program or the IPROC2 single channel analysis program (Sachs et al., 1982), and were only taken from patches containing a single channel.

The following form of the Boltzmann equation was used to calculate the effective gating

charge for the voltage sensor of the channels:

$$P_o(V) = 1 / \{1 + \exp[(zF/RT)(V - V_0)]\} \quad (1)$$

where z = the effective gating charge, V_0 = the midpoint potential, and $RT/F = 25.4$ mV at 22°C.

RESULTS

Previously undescribed high conductance single anion channels from frog skeletal muscle SR were studied in native membrane using the "sarcoball" technique (Stein and Palade, 1988). As will be shown, visual inspection of the channel gating revealed the presence of a main open state, and two smaller substates (S1 and S2). We will first present the basic properties of the main open state, and then separately discuss the properties of the two substates.

Main Open State

Fig. 1 A shows an example of anion channel activity from several channels in a sarcoball patch in symmetrical 200 mM TrisCl. Frequently, long open events to the full amplitude level (at any potential) are interrupted by very brief, flickery closures. The current/voltage relationship for these currents is shown in Fig. 1 B, and gives a slope conductance of 528 pS. In patches exposed to symmetrical 200 mM TrisCl, the conductance of the channel's predominant state was 505 ± 25 pS ($n = 35$). Fig. 1 C also shows data from a different patch that illustrates the selectivity of the sarcoball anion channels for Cl^- over K^+ ions. The reversal potential closely follows that predicted for a perfectly selective Cl channel as calculated by the Goldman-Hodgkin-Katz (GHK) equation (dashed line). Solving the GHK equation with these values gives an estimated $P_{\text{Cl}}/P_{\text{K}}$ of 45 for the sarcoball anion channel. For comparison, similar selectivity data have also been plotted in Fig. 1 C for the mitochondrial voltage-dependent anion channel (VDAC).

Conductance

The slope conductance vs. Cl^- ion concentration relationship is shown in Fig. 2 A. It saturates at 617 pS, with an apparent K_m of 77 mM (calculated by a "best" fit with the Michaelis-Menten equation). The decrease in conductance at higher ion concentrations (above 500 mM Cl^- , in this case) is indicative of a multi-ion pore, or a channel with more than one binding site for ions in the pore (Hille, 1984). Fig. 2 B shows the same sarcoball conductance vs. Cl^- ion concentration curve on an expanded scale to include a similar plot for VDAC (taken from Colombini, 1986), illustrating the difference in conductance between the two channels.

Voltage Dependence

In symmetrical Cl^- solutions, the sarcoball anion channel's main open state has a steep voltage dependence with a high open probability centered around 0 mV. Fig. 3 A shows an example of the voltage dependence of currents from a single channel patch in symmetrical 200 mM TrisCl. As the potential is increased in either direction, the channel enters a longer-lived closed state(s). At potentials as small as +30

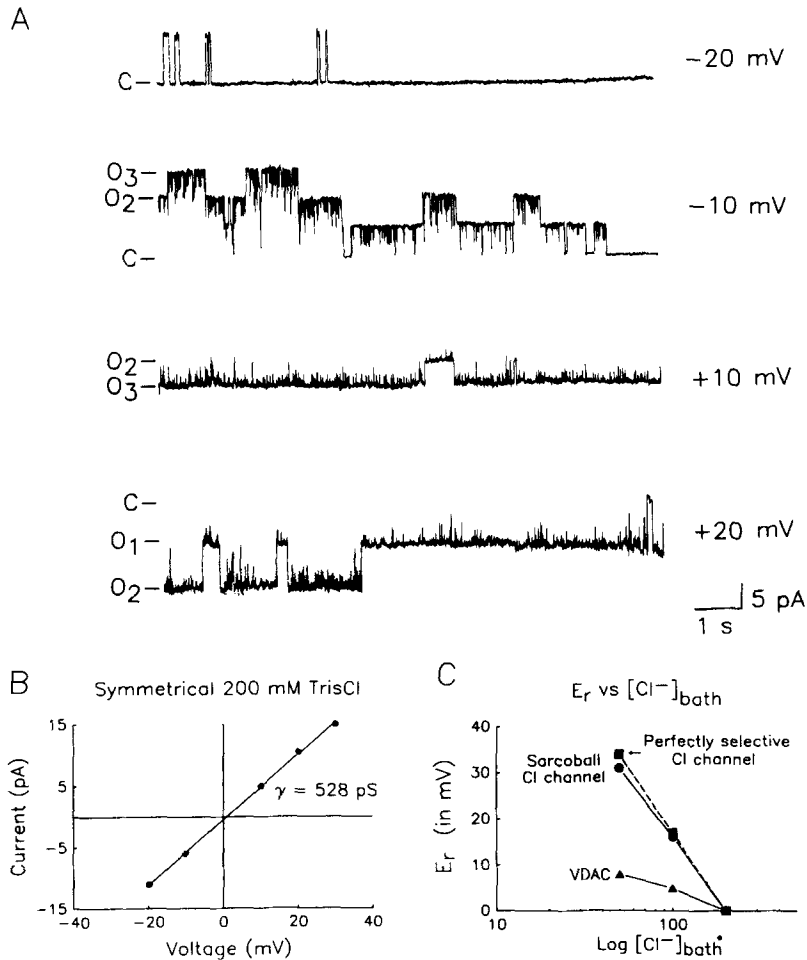


FIGURE 1. *A* shows a typical example of sarcoball anion channel activity from a multichannel patch, recorded in symmetrical 200 mM TrisCl. All records shown are from excised, inside-out patches. Usually more than one anion channel is obtained per patch. *C* denotes the zero current level, and O_1 , O_2 , etc., denote the open level for one or two channels, respectively. Note that fast closures are frequent, and substate levels are present. All current records shown were filtered at 0.5 kHz (-3 dB), digitized with a sampling frequency of $500 \mu\text{s}$ (2 kHz), and played back onto a Gould model 2400S pen recorder with an effective bandwidth of 90 Hz (-3 dB). *B* shows the current/voltage relationship for these traces, and gives a slope conductance of 528 pS for this example. *C* illustrates the anion/cation selectivity of the channels by plotting reversal potential shift against concentration of Cl^- in the bath. Patches were formed in symmetrical 200 mM KCl, and the bath solution was then perfused with 100 or 50 mM KCl. Similar data were calculated for VDAC from the GHK equation using a $P_{\text{Cl}}/P_{\text{K}} = 2$ (Colombini, 1980*b*). The dashed line was plotted for the same concentrations of KCl by using the Nernst equation for Cl^- ions.

or -20 mV, the channel remained completely closed; only transient events were observed immediately after switching the potential to these levels. Thus, the channels have a relatively narrow voltage range (~ 30 – 50 mV) in which any steady-state activity is observed. The steady-state open probability vs. holding potential relationship for these records (Fig. 3 B) observed produces a slightly asymmetric bell-shaped curve, with P_o values reaching a maximum near 1.0 at 0 mV, and falling off to 0.05 by -20 or $+30$ mV.

Results from kinetic analysis of the voltage dependence are shown in Fig. 4. Long records (10–15 min at each potential) from a single channel patch were examined at

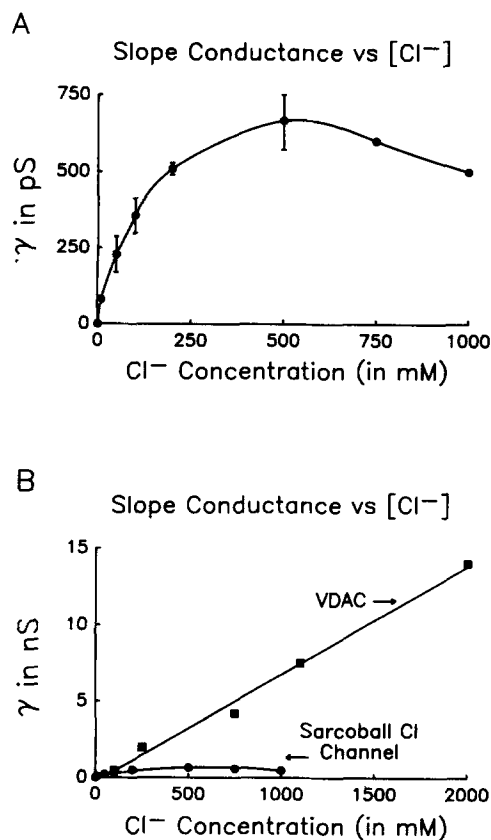


FIGURE 2. A shows the plot of slope conductance vs. KCl concentration for sarcoball anion channels. The data points were obtained by forming sarcoballs in symmetrical solutions of the desired concentration of TrisCl. Error bars not shown are smaller than the size of the data point. The apparent K_m from the curve is 77 mM. B shows the same data on scale expanded to include the same relationship for VDAC (taken from Colombini, 1986). Note the magnitude and linearity of the increase in VDAC conductance vs. Cl concentration as compared with the same curve for sarcoball anion channels.

both positive and negative voltages. Examples of the raw data are shown in Fig. 4 A. The analysis supports the symmetrical nature of the voltage dependence; no significant differences were found comparing the effects of positive or negative changes in potential.

Two open times (17 and 324 ms) and two closed times (4.6 ms and a faster one beyond the recorded frequency limits) were distinguishable at $+10$ mV, as seen in Fig. 4 B. At $+15$ mV there was an increase in the open time from 17 to 42 ms, but the 4.5-ms closed time remained unchanged. However, the appearance of long

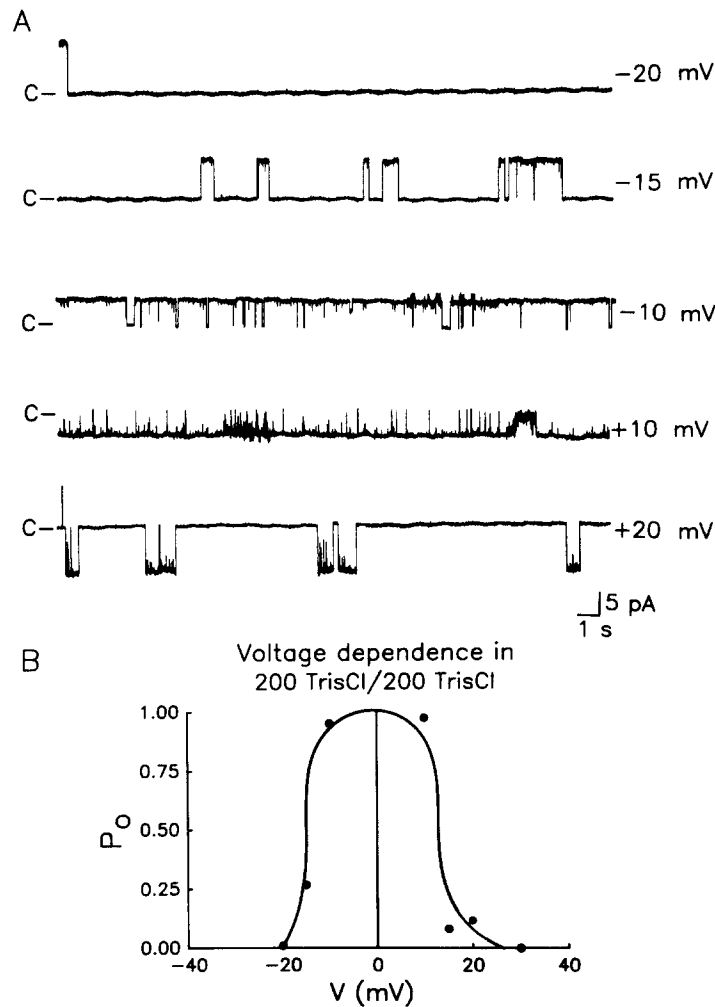


FIGURE 3. Voltage dependence of the sarcoball anion channels. *A* shows an example of records used to construct the plot in *B*. These records are from a single channel, excised patch in symmetrical 200 mM TrisCl. *C* is the closed current level, and voltages are as labeled. *B* shows a plot of P_o vs. holding potential. As mentioned in the Methods, polarities refer to potential inside the pipette. This plot was produced from the above traces for a single anion channel (i.e., no multiple current levels were ever observed). P_o was calculated using the "Analysis" program using the following protocol: 1-min segments of current records (filtered at 0.5 kHz [-3 dB]), beginning immediately after a potential change, were digitized (sampling frequency = 500 μ s), and P_o was obtained as stated in Methods.

closed times, from a third closed state, were observed. This long closed state is easily seen in the raw records (Fig. 4 *A*), but even with 10–15 min of data at each potential, not enough of these events were obtained for formal analysis. A rough estimate from about 50 events gives a tau of \sim 850 ms, at +15 or -15 mV. Thus, the major effects of changes in voltage are the appearance of a long closed state and an increase in the shorter open time.

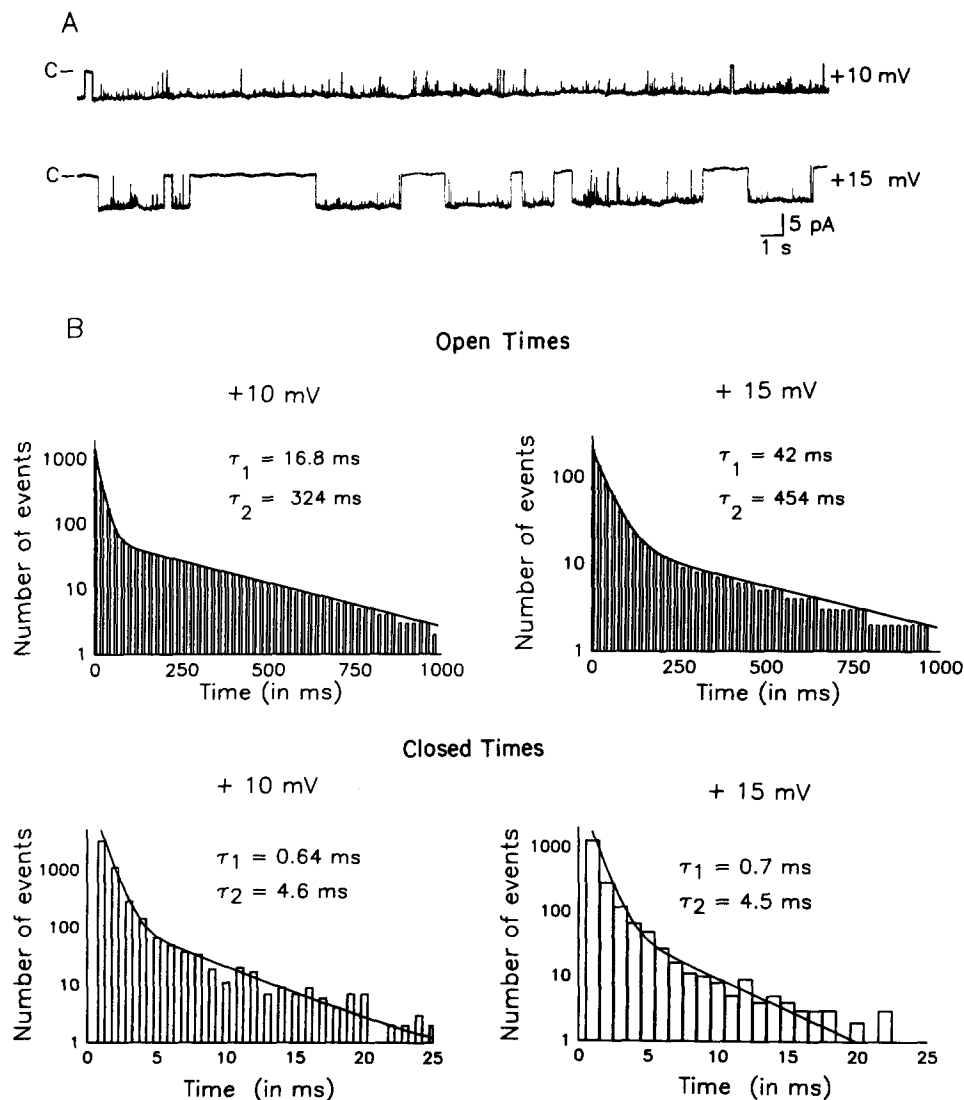


FIGURE 4. Kinetic analysis of the voltage dependence. *A* shows examples of the records used for the analysis. Visual inspection suggests that the only major difference is the appearance of a long-lived closed state. The open threshold detector was placed at a current level corresponding to approximately two-thirds of main open state current level. Likewise, the closed threshold detector was placed at a current level of approximately one-third of the main open state current. This was done to reduce the counting of frequency-clipped open or closed events. Open and closed time distributions for the +10 and +15 mV records are shown in *B*. Several open and closed states were observed, but the faster open and closed time constants were beyond the recorded frequency limits. Note that as potential is increased only +5 mV, there is a small increase in τ_1 from the open time distributions. As observed in the raw data (*A*), there is also a dramatic increase in long closed times. While not enough events were obtained for the long closed time distribution, a rough estimate from ~50 events gives a value of near 850 ms.

Selectivity

Table I provides a complete listing of permeability ratios for anions tested. Despite the channel's high conductance, it maintains a fair selectivity for smaller anions. Monovalent anions with higher atomic weight (and therefore smaller hydrated radii) pass more easily through the pore, and produce a selectivity sequence similar to a lyotropic series.

The larger anions gluconate and MOPS had almost no permeability in the sarcoball anion channels (Table I), and therefore measurement of reversal potentials under biionic conditions was no longer accurate (i.e., no true reversals could be observed, and the current/voltage relationships rectified strongly as the reversal potential was approached). Therefore, their permeability was tested by forming a patch in symmetrical 200 mM X-Cl and then by perfusing the bath with a solution containing 200 mM X-Cl and 200 mM X-test anion. The permeability ratios were then calculated by solving the GHK equation for the case with two potentially permeant ions present on both sides of the membrane (assuming cation X is impermeant; for the equation, see the legend of Table I). Given that anion channels in some preparations are capable of passing cations with anions (Franciolini and Nonner, 1987), we tested the selectivity of the two physiologically relevant intracellular cations, K^+ and Ca^{2+} . Neither of these two cations was significantly permeable in the channel.

Effect of Sulfate Ions

SO_4^{2-} was fairly permeant in the channel, but the channel's gating was changed when this ion carried current. Fig. 5 shows a patch with two anion channels where 100 mM SO_4^{2-} was added to the bath solution. In the control records (Fig. 5 A), the channels are open nearly all the time between 10 and 20 mV, as seen in the plot of P_o vs. voltage. After the addition of the SO_4^{2-} ions, the channels are only transiently open at these same potentials (Fig. 5 B) and a decrease in P_o at these potentials is observed in the plot of P_o vs. voltage. We suspect the increased charge on the divalent ion is the basis of the effect on sarcoball anion channel gating, because the addition of similar concentrations of phosphate ions also produced a decrease in open probability (data not shown).

No Ca^{2+} Sensitivity

Since changes in free Ca^{2+} ions are of such critical importance for SR function, and Ca^{2+} ions have been shown to regulate Cl^- conductances in other preparations (Rogawski et al., 1987; Thorn and Martin, 1987), we looked for effects on the channel properties using physiologically relevant changes in free Ca^{2+} ion concentration on both sides of the membrane. Neither altering Ca^{2+} ion levels from 5 μ M to 10 mM on the SR luminal surface, nor from nanomolar levels to 5 mM on the cytoplasmic surface had any observable effects on the channels (data not shown). High levels of free Ca^{2+} on either side (50–100 mM) only shifted the channel's voltage-dependence curve along the voltage axis by ~ 10 mV, and this shift was attributable to changes in the surface potential of the patch membrane (data not shown). Thus

TABLE I
Reversal Potential Shifts, Permeability Ratios, and Conductance

Test ion	Relative hydrated size	E_{rev}	n	P_{test}/P_{Cl}	γ_{test}
Monovalents					
		<i>mV</i>			<i>pS</i>
NO ₃	1.07	-10.7	2	1.53	450
SCN	1.16	-9.5	6	1.45	410
I	1.00	-8.5	2	1.39	450
Br	0.98	0.0	5	1.00	416
Cl	1.00	0.0	—	1.00	505
HCO ₃	1.72	1.2	6	0.96	377
Methane sulfonate	2.15	8.5	2	0.72	300
Aspartate	—	32.0	2	0.28	—
Propionate	2.13	33.5	2	0.27	—
Glutamate	2.82	40.0	2	0.21	—
Gluconate	—	*	3	0.04	—
MOPS	—	*	2	0.00	—
K	1.04	‡	8	0.02	—
Polyvalents					
Ca (+2)	1.50	—	2	0.00	—
SO ₄ (-2)	1.92	6.1	5	0.15	283
Oxalate (-2)	—	10.0	4	0.11	275
Phosphate [‡]	2.12	17.8	3	§	250
Pyrophosphate [‡]	—	8.0	2	§	400
Citrate (-3)	3.20	8.3	4	§	—
Phosphocreatine (-2)	—	27.0	4	0.03	—

Reversal potential shifts are with respect to the control "X"-Cl solution. Anion concentrations were 200 mM throughout and were bionic for the test anion and Cl⁻ (for exceptions, see below). The concentration of cation X was symmetrical 200 mM (400 mM in the case of divalent anions) during the experiments and was Tris except for SCN, gluconate, and I⁻ where the cation was K⁺, and for Br⁻ where the cation was TEA. Permeability ratios were calculated from: $E_{rev} = RT/zF \ln [(P_A/P_{Cl})([A]/[Cl])]$, where [A] denotes concentration of the anion A. Permeability ratios for divalent anions were calculated using a modification of the GHK equation (Lee and Tsien, 1984): $E_{rev} = RT/2F \ln [(P_1 - [observed\ monovalent]_i)/(4P_2 - [divalent]_o)]$. A value of 25.4 mV for RT/F at 22°C was used in all calculations.

*When the permeability of the ion tested was small (gluconate, MOPS), the permeabilities were measured by adding 200 mM of the test ion to the bath while maintaining a symmetrical 200 mM Cl⁻ gradient; cations used were Tris for MOPS, and K⁺ for gluconate. As before, the permeability ratios were calculated by solving the GHK equation; the following equation was used: $E_{rev} = RT/zF \ln [(P_{Cl}[Cl]_i + P_A[A]_i)/(P_{Cl}[Cl]_o + P_A[A]_o)]$, where z = the valence of the ion (-1). Ca²⁺ permeability was measured by forming a patch in symmetrical 200 mM TrisCl, and then determining the reversal potential after changing the bath solution to one with 200 mM TrisCl and 50 mM CaMOPS.

[‡]The following equation was used for the case of K⁺ only: $E_{rev} = RT/F \ln [(P_{Cl}[Cl]_i + P_K[K]_o)/(P_{Cl}[Cl]_o + P_K[K]_i)]$. Permeability to K⁺ was calculated using two different protocols. The first was to measure reversal potentials from patches formed in a 2:1 KCl gradient. The second was by measuring reversal potentials in a 4:1 KCl gradient, where control reversal potentials from each patch were determined in a 1:1 KCl solution, before the perfusion to the 4:1 KCl gradient.

[§]No permeability ratio is given for phosphate ions because at pH 7 there is a 60:40 mixture of the divalent and monovalent species, respectively. Therefore, at pH 7, the relative contribution to current from each species remains unknown. This may also mean that the conductance for the more permeant species is underestimated. The case is similar for pyrophosphate, as at pH 7 there is a 50:50 mixture of divalent and trivalent species. Citrate currents were unresolvable, indicating a low conductance for citrate currents. Since citrate, pyrophosphate, and phosphocreatine are trivalents, and the GHK equation is not applicable for trivalents, no permeability ratios for these anions are given.

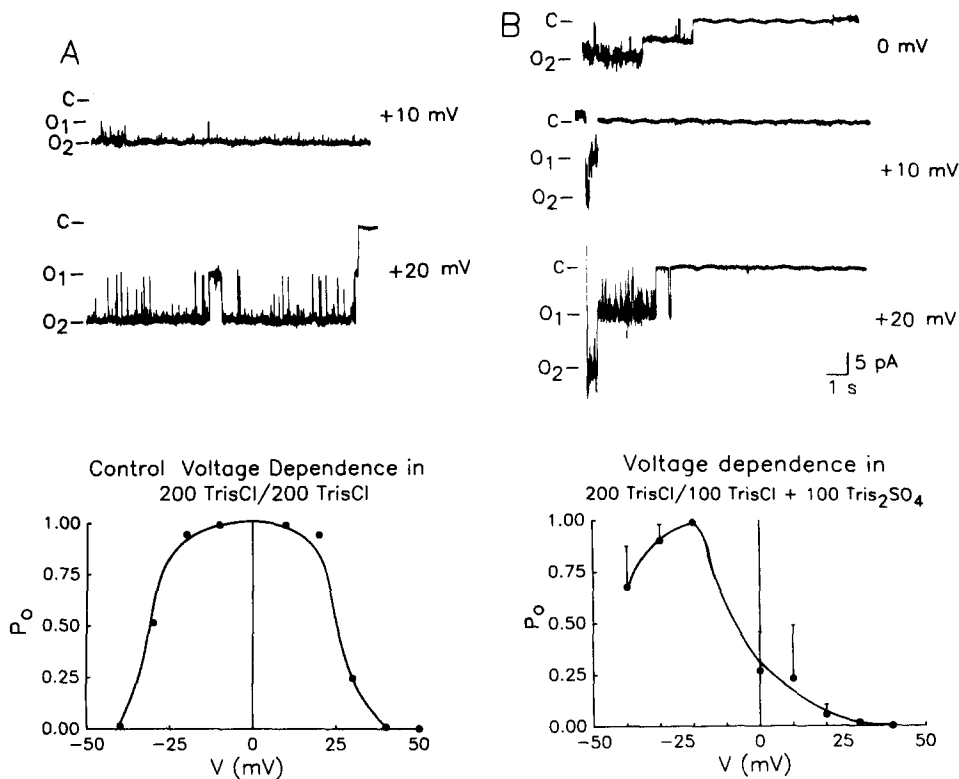


FIGURE 5. Effect of 100 mM SO_4^{2-} ions added to the bath solution. These data are from a two channel patch initially in symmetrical 200 mM TrisCl; the addition of SO_4^{2-} ions gives a final bath concentration of 100 mM TrisCl + 100 mM Tris₂SO₄. The control traces are shown in A, along with the plot of P_o vs. voltage. The P_o was determined by holding the patch at 0 mV (the reversal potential) and stepping to the test potential for 1 min. The P_o for the test potential segment was then measured using the protocol given in the Methods. B shows records 1–2 min after the addition of SO_4^{2-} ions to the bath solution, along with the corresponding plot of P_o vs. voltage. The time spent open at these same potentials is reduced so much that only transient events are observed. The P_o values for events in the presence of SO_4^{2-} were calculated as the mean of five separate "trials." Each trial consisted of holding the potential at -10 mV (the reversal potential) for 500 ms and stepping to the test potential, also for 500 ms. The P_o of the test potential was then measured.

activity of the Ca pump or Ca release channels can apparently only influence these SR anion channels by changes in SR membrane potential.

Anion Channels in Liposomes Lack Voltage Dependence

The difference in permeability and blocking action of SO_4^{2-} and other discrepancies between the sarcoball anion channels and bilayer studies on rabbit SR anion channels (Tanifuji et al., 1987) could be due to effects of isolation and changes in the lipid environment in bilayer experiments, or to species differences. Preliminary

results of experiments attempting to distinguish between these possibilities suggest species differences are less of a factor. We have recorded high conductance SR anion channels from isolated SR vesicles of rabbit skeletal muscle incorporated into liposomes (Fig. 6; see also Hals and Palade, 1987). These rabbit SR anion channels

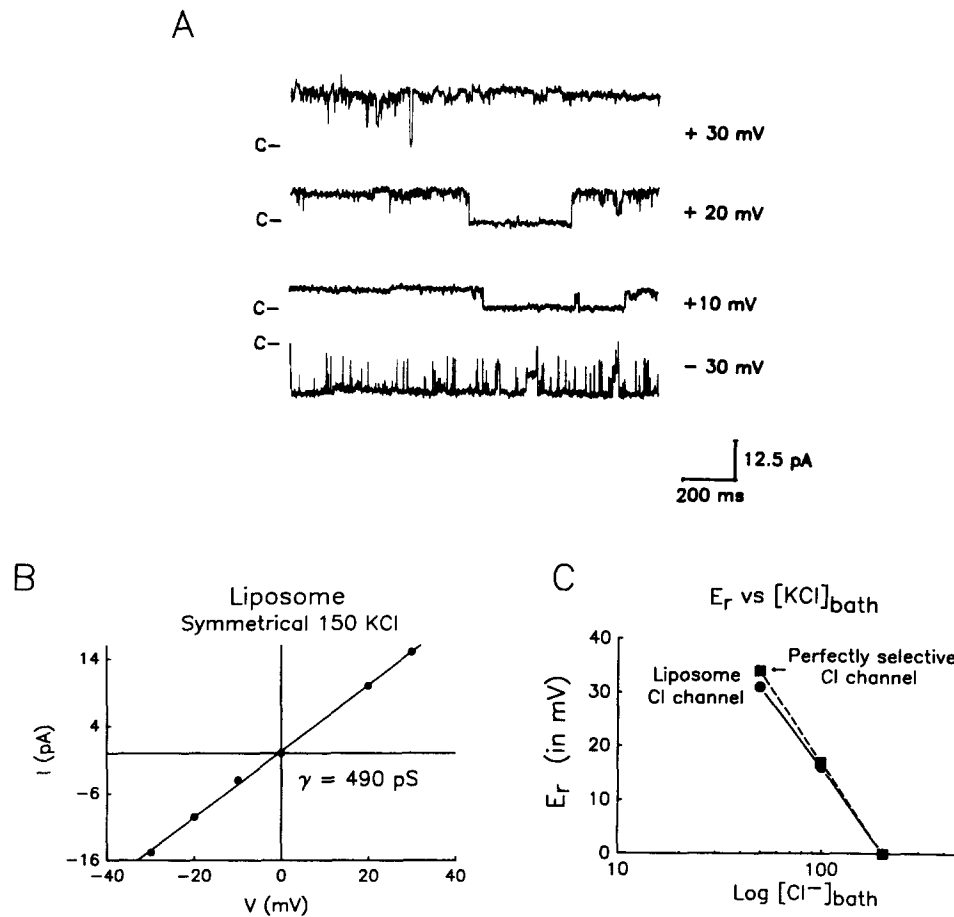


FIGURE 6. High conductance anion channels recorded from liposomes fused with isolated rabbit skeletal muscle SR. The liposomes are prepared as described in the Methods. These data are from an excised single channel patch recorded in symmetrical 150 mM KCl. Notice that substate levels are numerous, and that the events are not voltage dependent. *B* gives the current/voltage relationship for these events and a slope conductance of 490 pS is obtained. *C* shows that the liposome anion channels are selective for Cl^- over K^+ ions. Solving the GHK equation for these conditions gives a $P_{\text{Cl}}/P_{\text{K}}$ of 22.

were very similar to the ones observed in sarcoballs, but they displayed no voltage dependence (Fig. 6 *A*). Like the sarcoball anion channels, the rabbit SR channels were permeable to SO_4^{2-} ions and the channel's open probability was dramatically lowered when SO_4^{2-} was added to the bath solution (SR luminal surface, data not

shown). Fig. 7 demonstrates that when isolated frog SR was added to the liposomes instead of rabbit SR, similar results were obtained. There were large conductance channels that were not voltage dependent, but were still Cl^- selective (Fig. 7 *B*). In addition, SO_4^{2-} ions added to the bath solution (SR luminal surface) affected channel gating (data not shown).

Substates

Although substate levels were copious, only two substates were consistent enough to permit further characterization. While both substates were seen in almost every

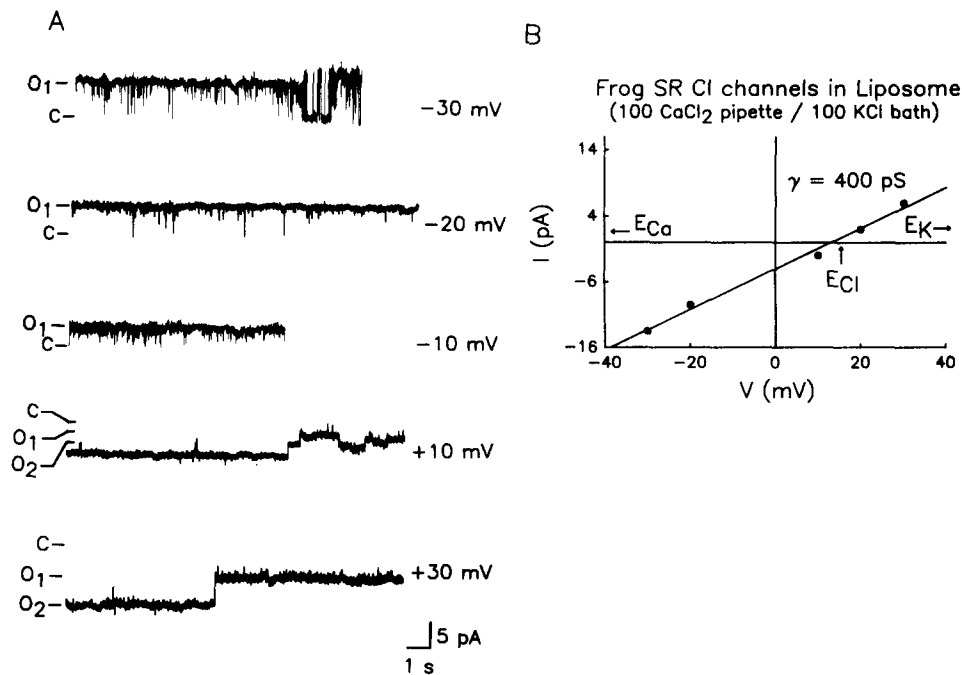


FIGURE 7. High conductance anion channels from isolated frog SR recorded in liposomes. *A* demonstrates that similar high conductance, anion-selective, voltage-independent events are observed in liposomes made using isolated frog SR instead of rabbit SR. These records are from a patch with at least two anion channels, recorded with 100 mM CaCl_2 in the pipette and 100 mM KCl in the bath. These conditions were used so events from Ca^{2+} , K^+ , and Cl^- currents could easily be distinguished. *B* shows the current/voltage relationship for the records. Note the high slope conductance and anion selectivity. *C* shows that a change in gating is also induced in these frog liposome anion channels when 100 mM Tris_2SO_4 is added to the bath solution.

patch, neither substate was active nearly as often as the main open level (except for the S1 substate in "cell-attached" recording mode, see next paragraph).

The first substate, arbitrarily labeled S1, is a fast, flickery substate with a conductance approximately half of the main open state. Fig. 8 *A* shows examples of this substate at several different potentials from a sarcoball-attached patch. The current/voltage relationship is shown in Fig. 8 *B*, and the slope conductance from this

is 266 pS. This substate is present in most of the records observed from excised patches at low levels of activity, but primarily entered this “bursting” mode in certain sarcoball-attached patches (same as cell-attached mode). Compared with currents in excised patches (see Figs. 1 A and 8 D, for example), full closures of the main state occurred much less frequently when the S1 state was in this “bursting”

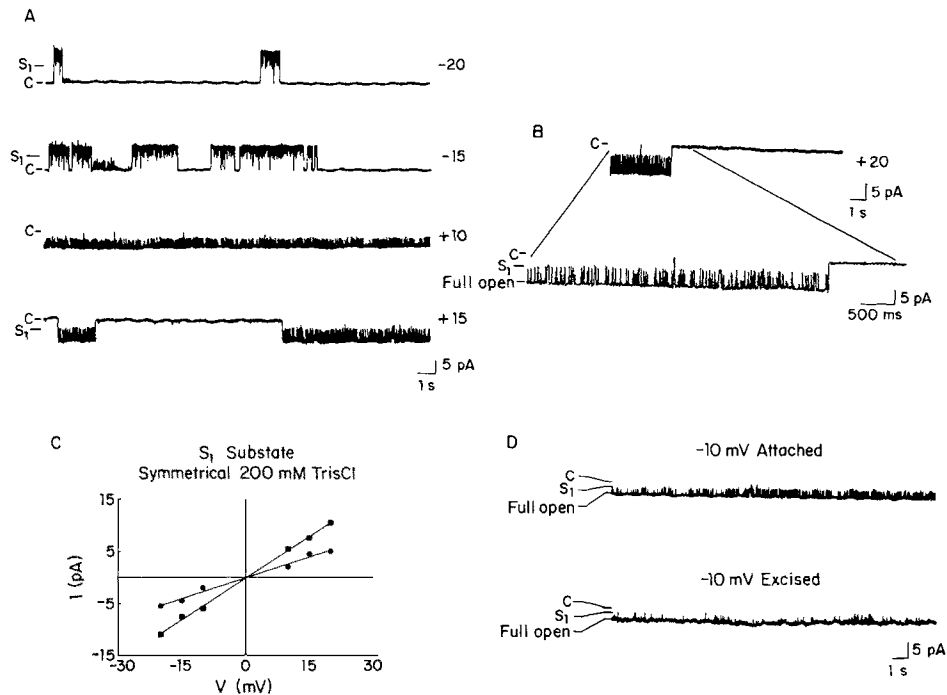


FIGURE 8. S1 substate of the sarcoball anion channels. *A* shows raw records at several potentials to illustrate the “bursting” mode of the S1 substate in a sarcoball-attached patch (equivalent to cell-attached mode). These records are from a single channel patch in symmetrical 200 mM TrisCl. Notice that the S1 substate only gates when the main state is open and that all S1 gating results in partial closures from the main open level. *B* shows an expanded trace from the same patch as in *A*, to show the substate events more clearly. *C* shows the current/voltage relationship for the S1 state from the above records. This line gives a slope conductance for the S1 state of 266 pS. For reference, the current/voltage relationship for the main open level ($\gamma_{\text{full open}} = 533$ pS) is shown as well (filled squares). *D* shows the effect of patch excision on the “bursting” mode of the S1 substate. These records are from a different single channel patch than those shown in *A*, and were made in symmetrical 200 mM TrisCl. Note the frequency of the S1 state when the patch is still attached to the sarcoball, and the decrease in S1 events which occurs immediately after excision of the patch.

mode. When patches with this bursting activity were excised, the bursting events immediately decreased to a low level (Fig. 8 D).

The mean open times of this state were calculated to be ~8 and 40 ms, and the mean closed time was 1.8 ms (data not shown). These values were calculated by taking the “open” state as the full open level of the channel, and the “closed” state

as the partial conductance level of the substate. While we never saw the S1 level at potentials at which the main state was closed, both the mean open time and mean closed times for this substate appear to be voltage independent (from 10 to 20 mV) in contrast to the steep voltage dependence of the main open state (Fig. 4 C).

The second substate (S2) is different from the S1 substate, in that instead of acting like a partial closure, it can open in addition to the main conductance level (Fig. 9 A) as well as open "independently" of the main level when the main open state has been shut down by its voltage dependence (Fig. 9 B). Fig. 9 C shows the current/voltage relationship for this substate, which gives a slope conductance of 125 pS. The expanded traces in Fig. 9 A show that simultaneous gating of the two events is easily observed (arrows denote these events), and is taken as evidence that the two states share a common gate. We estimated the probability of observing simultaneous gating of the main open state and S1 (in a 4-s record) to be ~1 in 150,000 (using a formula for calculating the probability of simultaneous gating events from two independent processes given the length of the record, the sampling frequency, and the total number of gating events [Busath and Szabo, 1988]). The fact that four such simultaneous events are seen in Fig. 9 A in <4 s strongly suggests that the simultaneous gating is not coincidental. Fig. 9 B also shows that, as with the S1 state, the voltage dependence of the S2 state is less steep than for the main open state. A less frequent observation is that the main open state can also apparently gate with S2 state in a nonadditive fashion (arrow).

DISCUSSION

It has long been known that isolated sarcoplasmic reticulum (SR) vesicles are permeable to Cl^- and other small anions (Kometani and Kasai, 1978; Garcia and Miller, 1984). Even though Miller first reported in 1978 on an anion channel in bilayer fusion studies of isolated SR vesicles, and SR K channels have been thoroughly studied (for review, see Miller, 1982), there remain very few data on single SR anion channels. This paper reports on the conductance, voltage-dependence, selectivity properties, and two substates of a previously undescribed anion channel in native SR membrane using the sarcoball preparation (Stein and Palade, 1988).

These Channels Are Likely to be SR Channels

Sarcoballs presumably form by spontaneous fusion of regions of SR membrane until a large (50–100 μm in diameter, Stein and Palade, 1988) bleb of membrane is produced. We know SR membrane contributes to the sarcoballs since SR Ca channels can be easily observed in sarcoball patches. These Ca channels have pharmacological properties (Stein and Palade, 1988, and unpublished observations) that have been described for SR Ca-release channels using planar bilayer techniques, namely: (a) 100-pS conductance for the predominant conductance in 50 mM Ca (gluconate)₂; (b) block by 2 μM ruthenium red; (c) increased open probability in response to 2 mM ATP or 10 mM caffeine; (d) limited selectivity for Ca^{2+} ions over K^+ ions, $P_{\text{Ca}}/P_{\text{K}} = 6.6$.

After the mechanical removal of the sarcolemma, the membranes remaining (SR, mitochondria, T tubule) are present in an approximate 75:18:7 ratio (Peachey, 1965). VDACs from outer mitochondrial membrane have been described in detail and their properties are very different from sarcoball anion channels. Compared

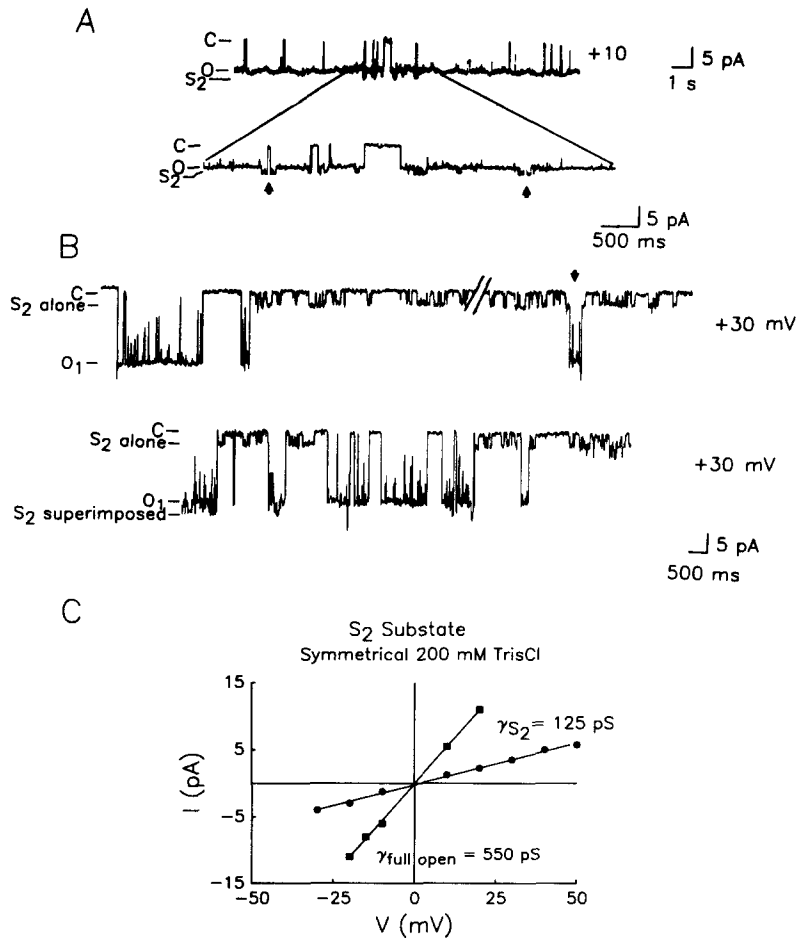


FIGURE 9. S2 substate of the sarcoball anion channel. *A* shows an example of S2 activity in a steady-state condition, at 10 mV. These records are from an excised patch containing a single sarcoball anion channel in symmetrical 200 mM TrisCl. Notice that the S2 state opens in addition to the full open level of the main open state, as if it were another channel. However, the expanded time scale for the same record shows two examples (arrows) of simultaneous gating of the two states. Given the frequency of gating of the sarcoball anion channels at this potential, it is statistically highly unlikely to observe simultaneous gating events with this frequency. These observations are taken as evidence that the two states share the same gate and are therefore part of the same channel protein. *B* shows examples of S2 substate gating (from a patch with two sarcoball anion channel proteins) at higher potentials, where only transient openings of the main state of the sarcoball anion channels can be observed. Note that in the top trace, the S2 state seems not to be shut down by the same level of voltage as the main state, that S2 only appears after the main level has already closed (inactivated), and that the S2 state continues to gate “independently” of the main open state. (The arrow indicates a rare event later in the record, where the channel opens to the main open state from the S2 state, but the total amplitude is only that of the full open level). Notice though, that in the bottom trace, the S2 level appears before the main state has inactivated. These records illustrate the complex relationship between the main and S2 states. *C* gives the current/voltage relationship for the S2 substate. For reference the equivalent data are provided for the main open level (filled squares) from the patch used in *A*.

with VDAC, sarcoball anion channels (*a*) have a lower slope conductance, particularly at higher Cl^- ion concentrations (Fig. 2 *B*), and saturate at a vastly lower conductance level; (*b*) are much more selective for anions vs. cations with $P_{\text{Cl}}/P_{\text{K}} = 45$ compared with only 2 for VDAC (Fig. 1 *C*); (*c*) only pass smaller anions (Table I) while VDAC can pass much larger anions, up to molecular weight 3,400 (Colombini, 1980*a*); and (*d*) close to a nonconducting state whereas the "closed" state for VDAC still conducts significant current (Schein et al., 1976).

Since the sarcolemmal membrane is mechanically removed, it is unlikely that surface membrane anion channels could be present in sarcoballs. Even if they were, their properties are sufficiently different from sarcoball anion channels (lower conductance [280 pS in 110 mM NaCl], lower anion/cation selectivity [$P_{\text{Cl}}/P_{\text{Na}} = 3.5$], and a very asymmetric voltage dependence, Woll et al., 1987).¹ T tubules constitute the next most likely source of contaminating membrane. T tubule anion channels for frog have not been described, but preliminary data on rabbit skeletal muscle T tubules (Coronado and Affolter, 1986), indicates that they have a much lower conductance than sarcoball anion channels.

The pronounced differences from VDAC, sarcolemmal and T tubule Cl channels, and the fact that the Cl and SR Ca-release channels appear to be the most abundant channels observed in sarcoballs, lead us to suggest that these sarcoball anion channels are from SR. Further evidence is provided by the observation of high conductance, Cl^- selective anion channels from purified SR vesicles incorporated into liposomes (Fig. 6; Hals and Palade, 1987) or planar bilayers (unpublished observations).

Voltage Dependence

The main open state of sarcoball anion channels is strongly voltage dependent in both the negative and positive direction with a high P_o centered around 0 mV (Fig. 3 *B*), as are many other high conductance anion channels (Schein et al., 1976; Blatz and Magelby, 1983; Coulombe et al., 1987). Using the Boltzmann equation (with $V_0 = \pm 15$ mV) to fit either the decrease in P_o vs. either positive or negative potential (from the data in Fig. 3) gives an equivalent gating charge for the voltage sensor of $z = 12$. This is much higher than for various other channels ($z = 6$ for Na and $z = 4.9$ for K channels in squid axons [Hodgkin and Huxley, 1952], $z = 2-3$ for Ca channels in snail neurons [Lux and Brown, 1984], and $z = 4.3$ for high conductance muscle surface anion channels [Woll et al., 1987]). However, an even steeper voltage dependence has been described for VDAC in the presence of large polyvalent anions (Mangan and Colombini, 1987).

Conductance and Selectivity

Many chloride/anion channels have been described from a variety of sources with conductances in the range of 200–450 pS, and $P_{\text{Cl}}/P_{\text{cation}}$ ratios of 1/5 (Blatz and

¹ While these authors found treatment with collagenase was necessary to facilitate patching of the surface membrane, P. Stein has developed a new surface membrane preparation that allows direct patching with no enzyme treatments (Stein and Palade, in press). Using this preparation, we find high conductance anion selective channels similar to those described in Woll et al. (1987), and distinctly different, in terms of conductance and voltage dependence, from those described here.

Magleby, 1983; Hanrahan et al., 1985; Kolb et al., 1985; Schneider et al., 1985; Coulombe et al., 1987; Kolb and Ubl, 1987; Thorn and Martin, 1987; Woll et al., 1987). The main open state of our channel fits this basic category, but is more conductive (505 ± 25 pS, $n = 35$), and yet *more* selective between anions and cations ($P_{\text{Cl}}/P_{\text{K}}$ ratio = 45, Fig. 1 C) than the channels reported above.

Ca^{2+} -precipitating anions, such as oxalate, phosphate, and pyrophosphate, have long been used in isolated SR vesicle studies to increase Ca^{2+} uptake into the vesicles (Hasselbach, 1964), and more recently to facilitate studies of Ca^{2+} -releasing agents (Palade, 1987). Since the participating anions are added outside the SR vesicles, some pathway must exist for their entry into the vesicles. Oxalate, phosphate, and pyrophosphate were all found to be permeant through the anion channel (Table I), but estimates of the channel density imply few of these anion channels exist per average isolated SR vesicle. We estimate from the average number of channels per patch, a density of ~ 1 channel per $6.94 \mu\text{m}^2$ of sealed patch membrane.² Since actual Cl^- activity inside frog skeletal muscle cells is low (Vaughan-Jones, 1982) we also measured the single channel conductance (of ~ 50 pS) in a symmetrical solution representing a first approximation to physiological conditions for permeant anions (in millimolar: 5 TrisCl [Macchia et al., 1978; Vaughan-Jones, 1982], 5 TrisPO₄ [Conway, 1957], and 10 TrisHCO₃ [Khuri et al., 1974], data not shown). This gives an average conductance of 90 pS per patch or 7.2 pS per square micrometer of sarcoball membrane. Thus, while the probability of a SR vesicle having an anion channel would be ~ 0.01 , the overall anion conductance of SR membrane would still be significant. This is a lower estimate than previously calculated from bilayer fusion studies using rabbit SR (1.4 anion channels/vesicle [Tanifuji et al., 1987]; 0.69 anion channels/vesicle [Morimoto and Kasai, 1986]). At present, this implies that either (a) there are other anion pathways in the SR membrane, or (b) the estimates of the anion channel density are less than the actual value in SR of an unaltered muscle cell, (either because the sarcoball anion channels are not uniformly distributed [i.e., are anchored in undisturbed SR membrane], functional, or active under our recording conditions). Although we tend to favor the latter conclusion (experiments with ryanodine activation of sarcoball SR Ca channels demonstrate that patches often contain many electrically silent channels, unpublished observations), there remains insufficient evidence at present to make any further conclusions at this point.

Gluconate is often used as an "impermeant" anion substitute although in most cases its actual permeability in anion channels has not been quantified.³ Our sarcoball anion channels do not significantly pass gluconate ions ($P_{\text{gluconate}}/P_{\text{Cl}} \leq 0.04$,

² We estimated the channel density from the average number of channels observed in patches made from pipettes of similar sizes (resistance, 20 M Ω). The average density was 1.8 ± 1.7 ($n = 61$) channels per patch. We used a rough area for "sealed patch membrane" of $12.5 \mu\text{m}^2$ for a pipette with a resistance of 20 M Ω from values for similar estimates (Sakmann and Neher, 1983). Thus, 1.8 channels/ $12.5 \mu\text{m}^2$ gives ~ 1 channel/ $6.94 \mu\text{m}^2$. Assuming an average SR vesicle diameter of 0.15 μm (Saito et al., 1984), we used an average vesicle surface area of $0.07 \mu\text{m}^2$ in our calculations.

³ Two exceptions are for the alveolar epithelial Cl channels, which have a published $P_{\text{gluconate}}/P_{\text{Cl}} = 0.4$ (Schneider et al., 1985), and for urinary bladder epithelial anion channels, with $P_{\text{gluconate}}/P_{\text{Cl}} = 0.07$ (Hanrahan et al., 1985).

Table I). In fact, we were never able to observe single channel events carried by gluconate.

The table also shows permeability for four anions commonly used in either skinned or cut fiber experiments: methanesulfonate (Endo and Nakajima, 1973; Stephenson, 1985), propionate (Volpe and Stephenson, 1986), aspartate (Palade and Vergara, 1982), and glutamate (Kovacs et al., 1979; Irving et al., 1987). All are permeant in the channel to some degree, however, the permeability ratios may be misleading. We were not able to resolve currents from the two amino acids or propionate, indicating that while permeant in the channel, the actual conductance of these anion substitutes through the sarcoball anion channels may be small. Assuming that anion movements are necessary to balance charge build-up in Ca^{2+} release and uptake, these results imply that using such anion substitutes could affect results in studies of Ca^{2+} release. In addition, effects on SR anion channels could be contributing to shifts in threshold for contraction (Hodgkin and Horowicz, 1960; Gomolla et al., 1983; Dulhunty, 1988) described for intracellular addition of various anions to skeletal muscle fibers.

Addition of Cl^- ions to Cl^- deficient media bathing skinned fibers (Endo and Nakajima, 1973) or isolated SR vesicles (Kasai and Miyamoto, 1976) produces a release of Ca^{2+} from the SR (" Cl^- -induced Ca^{2+} release"). Skinned fiber experiments imply that Cl^- -induced Ca^{2+} release is a result of depolarization of sealed-off T tubules, and not a direct effect on SR potential (Donaldson, 1985). While Donaldson's data strongly argue against a role for SR potential in Cl^- -induced Ca^{2+} release in skinned fibers, the cause of the Ca^{2+} release from isolated SR vesicles remains debatable; some investigators suggest the results are from nonspecific lysis of the SR vesicles due to osmotic shock (Meissner and McKinley, 1976) while others do not (Kasai and Miyamoto, 1976). It is possible that a decreased voltage dependence of anion channels in isolated SR contributes to differences between the results on Cl^- -induced releases in skinned fibers and isolated SR.

Comparison with Other SR Anion Channels

There is considerable variability in the few reports describing properties of single SR anion/ Cl^- channels. Smaller conductance (in 100 mM cholineCl) voltage-independent anion channels have been observed in planar bilayers after fusion of SR vesicles isolated from rabbit skeletal muscle (100 pS: Miller, 1978; Smith et al., 1986a). A 200-pS, voltage-dependent anion channel has also been described, again from similar isolated rabbit SR vesicles fused into planer bilayers (Tanifuji et al., 1987). Finally, high conductance voltage-independent anion channels (500 pS in 150 mM KCl) have been described in liposomes (Hals and Palade, 1987) made using a similar preparation of lipids and SR vesicles.

Sarcoball anion channels have some features in common with the 200-pS bilayer channels: both are highly conductive, have a similar selectivity sequence for halide ions, and are affected by changes in voltage and by SO_4^{2-} ions so that the total current passed is reduced. There are also many differences between the two channels. Bilayer anion channels (*a*) exhibit smaller conductance, saturating at 350 pS with a $K_{0.5}$ of 265 mM; *b*) are only slightly shut down at higher positive potentials (+ 50

mV); (c) are less permeable to SO_4^{2-} ions ($P_{\text{SO}_4}/P_{\text{Cl}} = 0.15$ for sarcoball channels, Table I, and 0.04 for bilayer channels, Tanifuji et al., 1987). In addition, SO_4^{2-} ions reduced the single channel amplitude and did not affect channel gating of the bilayer channel. SO_4^{2-} ions only reduced conductance in SR bilayer anion channels when added to the cytoplasmic surface (Tanifuji et al., 1987) and SO_4^{2-} ions have effects when applied to either side on sarcoball anion channels.

The differences between our results and those of Tanifuji et al. (1987) could be due to species differences between the two preparations (frog vs. rabbit) or to effects of biochemical isolation and changes in the lipid environment that are inherent in bilayer/liposome studies. Preliminary data addressing this issue suggest that some of the differences are due to the effects of isolation and/or changes in lipid composition, since we record high conductance (400–500 pS in 200 mM KCl), anion-selective, voltage-independent channels in liposomes made from either rabbit or frog muscle SR vesicles (Figs. 6 and 7). In contrast, observations of smaller conductance anion channels (100 pS in 200 mM KCl, data not shown) in bilayers or liposomes were rare. Further, under our recording conditions, we never observed smaller conductance Cl channels in any of the sarcoball patches. Thus, it is possible that the channel observed by Tanifuji et al. (1987) may actually be an “altered” form of the high conductance channels. Until more data are available, these conclusions remain speculative.

Substates

Many high conductance anion channels have frequent gating to a variety of substate levels (Blatz and Magleby, 1983; Schwarze and Kolb, 1984; Kolb et al., 1985; Boltina et al., 1987; Coulombe et al., 1987; Thorn and Martin, 1987; Woll et al., 1987). Some channels have even been described by a co-channel mechanism (Krouse et al., 1986). Several substate levels are also seen in sarcoball anion channel gating. Substates can arise from true stable states of altered conductance, or they can also be artifacts produced from short periods of extremely rapid gating that are unresolvable due to bandwidth limitations (for example, see Blatz and Magleby, 1986). The fact that P_o of the S1 state can be altered by patch excision (Fig. 8), argues against a bandwidth explanation, and suggests that there may be an as yet unidentified substance in the sarcoballs that is somehow interacting with the anion channels to produce this effect. The high frequency of simultaneous gating events support the idea that the S2 state may be a separate pore, but part of the *same* channel protein. This makes the S2 state less of a substate, and more of a co-channel (Krouse et al., 1986).

It is interesting to note that the S1 conductance level is about half of the main open level, and that the S2 conductance is about one-quarter of the main conductance level. The possibility exists that the sarcoball anion channels are composed of four to five “unit conductance channels” (Hymel et al., 1988; Ma and Coronado, 1988) of 125 pS, and the substates observed are just transient disruptions in the “normal” collective gating of the smaller channels. This could also potentially explain the results of Tanifuji et al. (1987); their channel could be composed of proteins with only two functional “unit conductance channels.”

Is There a Possible Role for This Channel in E-C Coupling?

K channels have been assumed to be present in the SR membrane as passive pathways by which counter-ions move, minimizing potential development during Ca^{2+} release or uptake (Garcia and Miller, 1984). Recent work by Fink and Stephenson (1987) offers evidence to support this hypothesis. Their study of drug effects putatively mediated by SR K channels in skinned skeletal muscle fibers, suggests that altering K^+ movements across SR can modify Ca^{2+} fluxes. It is equally possible that SR anion channels could play a similar role as SR K channels.

The steepness of the channel's voltage-sensitivity raises the possibility that this channel potentially could play a more active role in E-C coupling. The SR is currently viewed as a leaky membrane, with a gradient across the SR existing only for Ca^{2+} ions. The SR potential has been estimated to be 0 mV at rest (Somlyo et al., 1977), and Ca^{2+} release has been estimated to generate at most 7 mV (SR lumen = ground) in the presence of counter-ion movements (Oetliker, 1982; Garcia and Miller, 1984). Additionally, electron probe analysis has shown that during a tetanus, insufficient counter-ion movements by K^+ and Mg^{2+} occur to fully compensate the charge deficit created by Ca^{2+} release (Somlyo et al., 1981). Contributions from certain SR-permeable anions (bicarbonate and phosphate) could not be assessed in that study for technical reasons, and could be more significant during the shorter time course of a twitch. If the SR potential remains near 0 mV throughout contraction and relaxation in the muscle cell, then the anion channel's voltage dependence may not be physiologically significant. However, if the SR potential is only +8 to +10 mV away from 0 mV (SR lumen = ground), then a change of +7 mV during Ca^{2+} release could drastically decrease the P_o of the anion channels, and thus the anion permeability of the SR. The reduction in counter-ion flow would decrease the driving force for Ca^{2+} release and perhaps aid pump-mediated Ca^{2+} uptake due to the electrogenic nature of the pump (Morimoto and Kasai, 1986). This could contribute to the decline in rate of SR Ca^{2+} release observed during maintained voltage clamp pulses (Melzer et al., 1984). The lower steady-state rate of Ca^{2+} release that follows could be supported by K^+ and Mg^{2+} counter-ion flow. Thus, a possible function of the anion channels could be to shorten the time course of the Ca^{2+} transient and produce a faster twitch. Another physiological function would be to provide counter-ion movement during Ca^{2+} pumping (Kometani and Kasai, 1980), after the SR Ca^{2+} release channels have closed.

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Note added in proof: Rousseau et al. (1988, *European Biophysical Journal*, 16:143–151), have recently described data on a SR anion channel using planar bilayer techniques. They find results similar to Tanifuji et al. (1987).

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