CONDUCTANCE AND SELECTIVITY PROPERTIES OF A SUBSTATE OF THE RABBIT SARCOPLASMIC RETICULUM CHANNEL

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ABSTRACT In this paper I describe the occurrence and properties of a subconductance state of the cation-selective channel of the sarcoplasmic reticulum. The substate conductance is 60% of the major state conductance in every salt solution examined. When single channel conductance is plotted vs. ion concentration (for potassium or thallium salt solutions) the Michaelis-Menten constant is nearly the same for both conductance states, while the maximum conductance is reduced for the subconductance state. Both conductance states show anomalous conductance behavior in mixed potassium-thallium solutions that may be modeled in the same way. These results indicate that the ionic selectivity of the channel is the same for both open states.

INTRODUCTION

The cation-selective channel of the rabbit sarcoplasmic reticulum (SR) (rabbit SR channel) has been described as a two-state ion channel based on single channel conductance measurements in artificial bilayer membranes (1). A similar channel derived from frog SR has instead two open states (2). In this paper I report a subconductance state of the rabbit SR channel with a conductance ~60% that of the major conductance state for all ions examined. The binding constants for both conductance states are nearly identical (for potassium and for thallous ions), and the substate exhibits the same anomalous behavior as does the major state in mixed potassium-thallium salt solutions. These results suggest that the maximum conductance of an ion channel may be altered independently of ion binding.

MATERIALS AND METHODS

Currents from single rabbit SR channels were recorded with a high gain current-to-voltage amplifier. These channels were inserted into artificial bilayer membranes composed of 10 mg/ml 90% phosphatidyl ethanolamine (PE) and 10% phosphatidylcholine (PC) in decane unless otherwise noted (lipids from Avanti Polar Lipids Inc. [Birmingham, AL], decane from Sigma Chemical Corp. [St. Louis, MO]). Channel currents appeared following addition of SR vesicles (prepared by the method of Miller and Rosenberg [3]) to the solution bathing one face of the bilayer (termed the *cis* face) in the presence of 1 mM Ca^{2+}. Channel insertion was stopped by the addition of excess EDTA to the bath containing the vesicles.

RESULTS AND DISCUSSION

In Fig. 1 single channel current records in symmetric solutions of potassium (*A*), sodium (*B*), and thallium (*C*) illustrate both conductance states of the channel. The same conductance behavior is seen also in symmetric ammonium, rubidium, and mixed ionic solutions (data not shown). In all these solutions, the smaller conductance state (which I will call the low state) has ~60% of the conductance of the larger state. The major conductance state (which I will call the high state) has been well described in the literature (1, 3).

The SR channel appears to be able to enter or leave the low state from either the closed or the high state. Transitions from high to low, and vice versa, without intervening

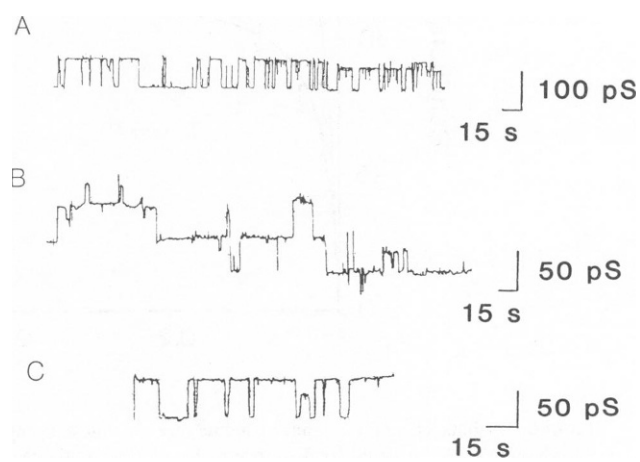

FIGURE 1 Single rabbit SR channel current steps in symmetric single salt solutions. Membranes were formed from PE-PC in decane (10 mg/ml) across 0.3 mm holes in polystyrene. (*A*) potassium, (*B*) sodium, (*C*) thallium (0.1 M acetate salt solutions).

closures, as shown in Fig. 1 *A*, are commonly seen. If the low state and the high state seen in Fig. 1 *A* were each due to separate populations of channels, overlapping openings to a level of 160% of the high state should occur, yet none were seen in this experiment. If the high state were instead due to overlapping openings from channels of 40 and 60% of the high state, openings to 40% of the high state should occur. These were not observed. This indicates that the observed transitions are from one open state to another and are not due to the existence of two channel types. Steplike

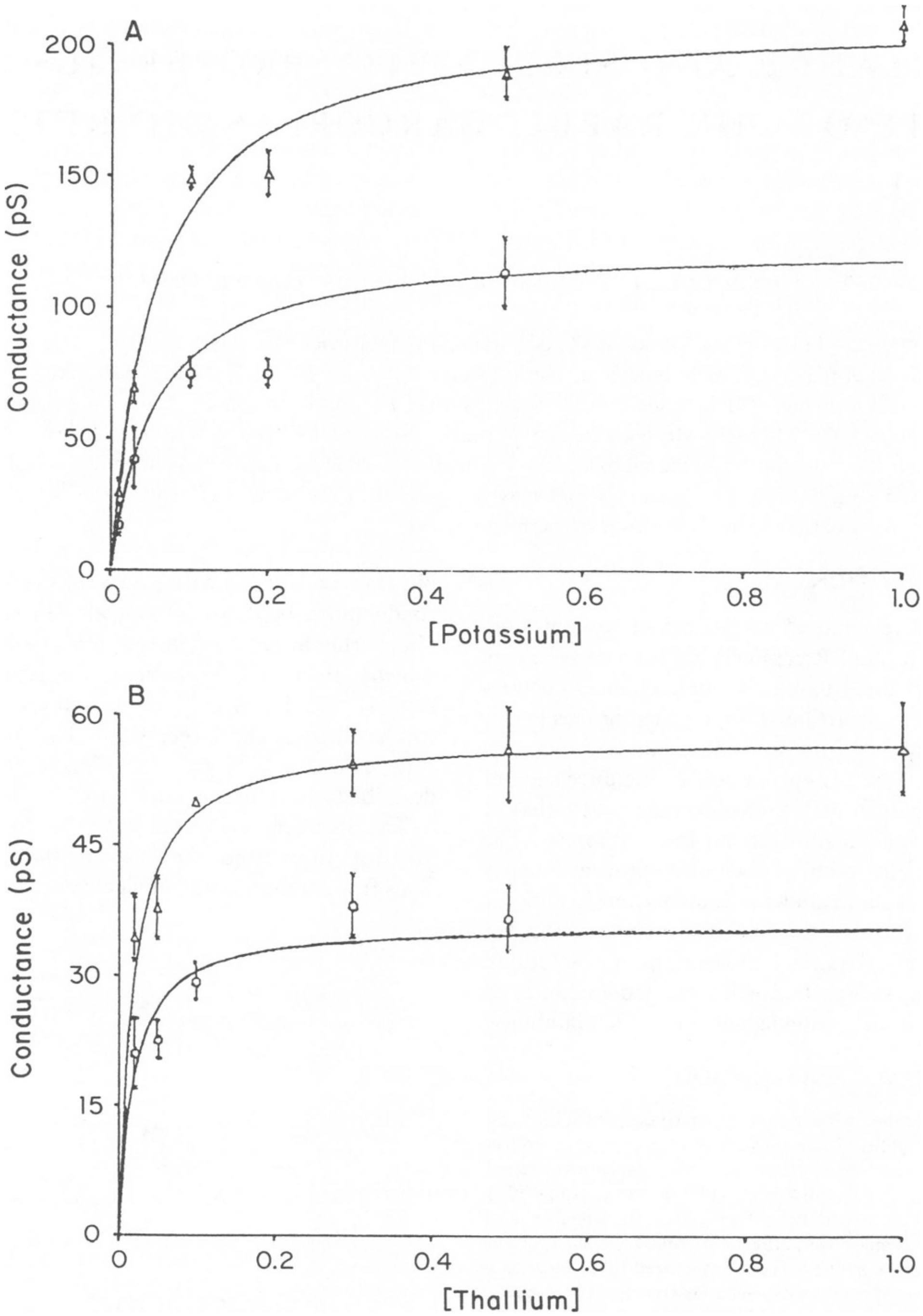

FIGURE 2 Plots of single channel conductance vs. ion concentration in symmetric single salt solutions for both high and low state conductances. The reported conductance is the average single channel conductance determined from many single openings at ±20 mV (in some cases, where the current at 20 mV was too small for reliable measurements, values at ±40 mV were also included). The high state values are plotted with triangles, the low state values with octagons. The maximum channel conductances used to plot the theoretical curves are: for potassium, 212 pS (high state) and 127 (low state); for thallium, 57.5 pS (high state) and 36 (low state). Both potassium curves are drawn using a Michaelis-Menten constant (K_m) of 55 mM, whereas both thallium curves are drawn with a K_m of 18 mM. (The K_m regression fits for potassium were within 3 mM of each other, whereas the thallium K_m regression fits were within 4 mM.)

changes in channel current to levels other than the high or the low state are very rarely seen (4).

The low state has been observed in membranes of various compositions, including negatively charged (phosphatidylserine containing) and cholesterol containing membranes, in addition to the neutral membranes used for the channel currents shown in Fig. 1. This conductance state has been observed in channels derived from all SR vesicle preparations examined.

If a channel were to begin to flicker between open and closed states, filtering by the amplifier might give the appearance of a lower conductance state, when in fact a new kinetic state of the channel is observed. The apparent conductance level would then be a function of the flickering rate and the amplifier cut-off frequency. In my experiments, the same low conductance state is seen in membranes with different lipid compositions and in the presence of various different ionic solutions. Either variation might be expected to affect the flickering rate. Although current records were filtered at various frequencies between 10 and 500 Hz in different experiments, the same two conductance levels were observed in all experiments. These observations suggest that the low state described here is a subconductance state, not a different kinetic state, of the rabbit SR channel.

The fraction of the open time spent in the substate is highly variable, but in experiments in two different salt solutions (comprising 12 membranes in all) <20% of the channel open time was spent in the low state. The average time spent in the high open state was greater than the average time spent in the low open state (as determined either by fitting an exponential to the open time durations or by dividing the total time spent in either open state by the number of transitions from that state [4]). For example channels in PE-decane bilayers bathed in symmetric 0.1 M potassium acetate averaged 7.9 s in the high open state and 3.3 s in the low state at +30 mV applied potential. At −10 mV, the average durations were 5.8 s and 1.7 s in the high and low states, respectively (potential is with respect to the pool opposite to the one to which vesicles are added). Current records occasionally appear noisier in the low state than in the high state, which might reflect the differing kinetic properties of the two states.

For a channel modeled as a sequence of energy barriers and wells the transition to a subconductance state might come about by altered ion binding, altered maximum conductance, or both. Thus, the ratio of the open state conductances might be expected to differ for each ion species, because a subconductance state need not have the same ion selectivity as the major conductance state. That

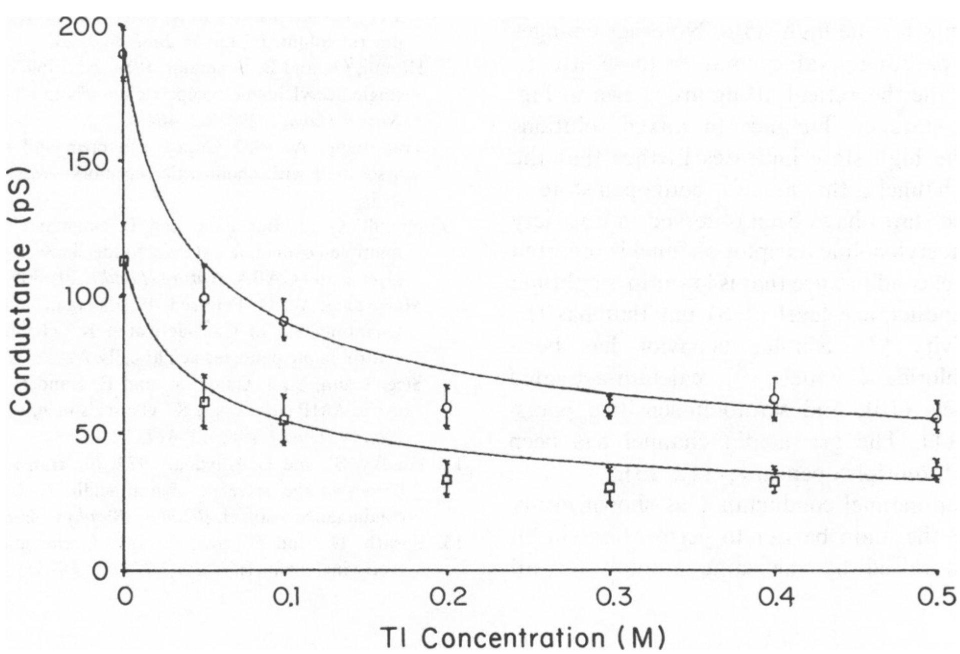

FIGURE 3 Plot of single SR channel conductance in mixed K^+ and Tl^+ solutions, showing fit of the model of Fox and Ciani (6) to both open states. Experimental points are plotted with octagons (high state) and squares (low state). The total of the K^+ and Tl^+ concentrations was 0.5 M in all solutions. The values on the abscissa are the Tl^+ concentrations in molar units. Thus, the points to the extreme left are the channel conductances for both states in 0.5 M K^+, whereas the points to the right of these show channel conductances in symmetric 0.45 M K^+-0.05 M Tl^+. The parameters of the model were fitted to the high state conductances; the curve for the low state uses this same fit, but with maximum channel conductances being 60% of the high state values. There are seven free parameters for mixed K^+-Tl^+ solutions: the channel maximum conductance for K^+ when Tl^+ occupies the external site, the channel maximum conductance for Tl^+ when the external site is free of Tl^+, the ratios, for K^+ and for Tl^+, of ion exit rates to the *cis* and the *trans* pools, the binding constants for both ions to the external site, and the electrical distance from one membrane face to the internal site. The values used for ion binding constants to the internal site, for the maximum channel conductances for K^+ when the external site is free of Tl^+, and for Tl^+ when the external site is occupied by Tl^+ were determined by results from single cation solution experiments (4, 6).

the ions tested all show the same ratio of low to high state conductance, however, might indicate that the channel selectivity is the same for both states. In Fig. 2, SR channel conductance is plotted as a function of ion concentration for potassium (A) and for thallium (B). These data have been fitted by a simple saturation curve (a rectangular hyperbola) using the same binding constant for both high and low state conductance. The maximum channel conductance for the low state is 60% of that for the high state. Thus it appears that the two open states differ only in their maximum conductance to ions and not in their binding affinity. The high state values are the binding constants and maximum conductances previously reported for the rabbit SR channel (1, 5).

The SR channel high open state exhibits anomalous conductance behavior in mixed thallous-potassium ion solutions (5). This behavior can be modeled, in a single internal site Eyring rate theory model of the SR channel, by postulating the existence of external ion binding sites that alter the channel conductance when occupied by thallous ion (4, 6).

The low state of the SR channel also shows anomalous conductance behavior in the presence of thallous ion, which mirrors that of the high state and which is also fitted by the model. In fitting the model to the low state conductance values, the maximum channel conductances were reduced to 60% of the values for the high state. No other changes were made in the parameter values used for these fittings. These results and the theoretical fitting are shown in Fig. 3. That the low state conductance in mixed solutions mimics that of the high state indicates further that the selectivity of the channel is the same for both open states.

Subconductance states have been observed in a variety of channels. The acetylcholine receptor channel is reported to exhibit a sublevel conductance that is lower in amplitude than the main conductance level (7, 8) but that has the same ion selectivity (7). Similar behavior has been observed with chloride channels (9), calcium-activated potassium channels (10), and serotonin-sensitive potassium channels (11). The gramicidin channel has been reported to exhibit multiple open states (12, 13).

An alteration in channel conductance as shown in my data is possible if the main barrier to permeation (in an Eyring model) is raised by the same amount for all permeating ions. This might be accounted for by a conformational change in the protein that alters the electrostatic component of the energy barrier for all ions but does not affect the contribution of other parameters, such as ion size or mass, to that barrier.

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