Incorporation of ionic channels from yeast plasma membranes into black lipid membranes

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ABSTRACT Recently, patch-clamping of yeast protoplasts has revealed the presence of plasma membrane K^+ channels (Gustin, M. C., B. Martinac, Y. Saimi, M. R. Culberston, and C. Kung. 1986. *Science (Wash. DC).* 233:1195–1197). In this work we show that fusion of purified plasma membranes into planar bilayers allows the study of the yeast channels. The main cationic conductances detected were of 64 and 116 pS, however, larger and smaller conductances have been observed. The two main conductances were sensitive to the K^+ channels blockers tetraethylammonium (TEA^+) and Ba^{2+}. Biionic experiments indicated that both conductances were K^+ selective.

INTRODUCTION

Ionic channels play a major role in the ionic permeability of biological membranes, and are present in all cells tested so far. In yeast, the membrane potential is set by a H^+-ATPase (1, 2), and K^+ ions participate in its regulation (2, 3). For some time it has been known that K^+ movements through the yeast plasma membrane occur by means of two K^+ carriers (4, 5) whose activity depends on an adequate cellular energy supply. In addition, patch-clamping of yeast protoplasts has recently revealed the presence of K^+-selective depolarization-activated channels (6), ATP-sensitive K^+ channels (7), and pressure-sensitive channels (8, 9).

With the purpose of extending the study of the yeast plasma membrane channels, we have begun a series of studies at a physiological level by measuring ion fluxes in intact cells and recording channel activity in model membrane systems. Here, we report the incorporation of K^+ channels from plasma membrane fragments of yeast into black lipid membranes by the fusion method (10). Part of this work has been presented in abstract form (11, 12).

MATERIALS AND METHODS

Plasma membrane fragments from a commercially obtained wild type strain of *Saccharomyces cerevisiae* (La Azteca S. A., Mexico City), and the wild type strain XT3000.3A were prepared as previously described (13) by a modification of the method of Franzusoff and Cirillo (14). This plasma membrane preparation shows approximately a 10% contamination by mitochondrial membranes as assessed by measurements of the sensitivity of ATPase activities to the inhibitors vanadate and oligomycin, previously reported from our laboratory (13). No vacuolar contamination was detected, because all ATPase activity was sensitive either to vanadate or oligomycin.

Black lipid membranes were formed from a 20 mg/ml solution of PE/PS 1:1, or diphytanoyl phosphatidyl choline (DiphyPC) (Avanti Polar Lipids, Birmingham, AL) in *n*-decane on a 0.2–0.25-mm diameter hole in a Saran Wrap plastic partition separating two Teflon compartments, each of ~0.5 ml capacity, by the technique of Müller and Rudin (15). Unless otherwise indicated, the *trans* side electrolyte solution contained 100 mM KCl, 0.1 mM $CaCl_2$, and 10 mM Hepes-KOH, pH 8.0; the *cis* side solution was the same, except that the KCl concentration was 300 mM. In the biionic experiments the *cis* side solution was replaced by 300 mM NaCl, 0.1 mM CaCl, and 10 mM Hepes-NaOH, pH 8.0. Channel incorporation was observed after the addition of 9–90 μg protein/ml to the *cis* side of the bilayer. All experiments were done at 20–22°C.

Voltage pulses were applied with a stimulator (model SD9; Grass Instrument Co., Quincy, MA) to the *cis* side. Single channel currents were measured under voltage clamping with a current to voltage converter with a 10 GΩ resistor in the feedback loop, at a bandwidth of 1 KHz. The current output of the converter was stored in a modified digital audio processor connected to a VCR (16, 17).

Unless otherwise indicated, recordings were filtered at 800 Hz (−3db point, eight-pole Bessel filter, model 744PL-3, Frequency Devices Inc., Haverhill, MA), and digitized at 2 KHz. Channel analysis was performed with the *p*-clamp 4.0 program, in the semiautomatic mode. Transitions lasting <4 digital points were discarded by the program for constructing the amplitude histograms. All reagents were of analytical grade. Solutions were filtered with 0.22-μm filters (Millipore/Continental Water Systems, Bedford, MA). The data for this work were obtained from 25 h of recordings from 81 bilayers.

RESULTS AND DISCUSSION

Before adding the yeast plasma membranes to the *cis* compartment, we routinely applied a control pulse of ±100 mV to the lipid bilayer. Membranes showing noise were discarded, and the chamber was washed again. After the control pulse, plasma membranes sonicated in 700 mM sucrose were added, and usually channel currents were observed within 1–90 min.

Current fluctuations at three positive voltages in PE/

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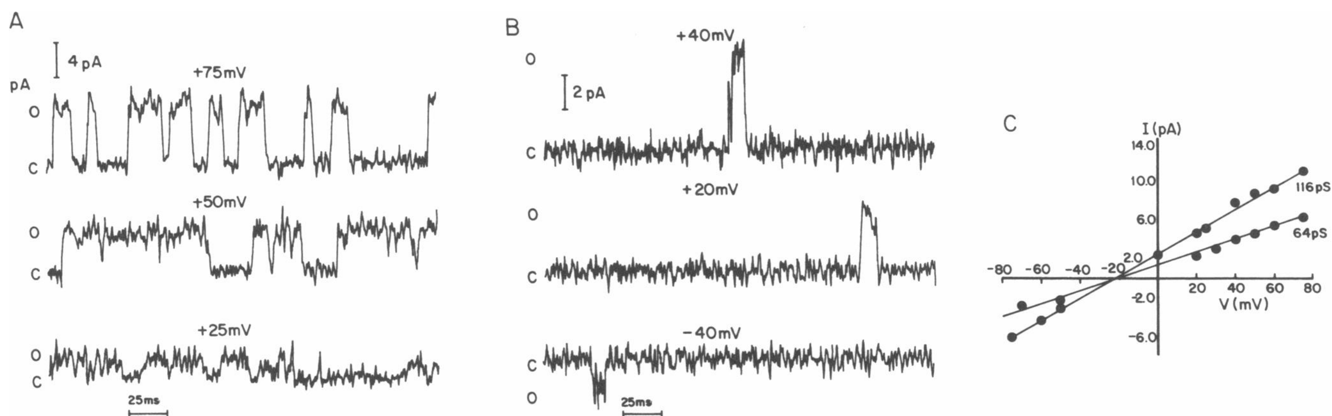

FIGURE 1 (*A*) Current records of a 64-pS channel in a PE/PS bilayer. The solutions were 300 mM KCl, 0.1 mM $CaCl_2$, 10 mM Hepes-KOH, pH 8.0, *cis;* and 100 mM KCl, 0.1 mM $CaCl_2$, 10 mM Hepes-KOH, *trans*. No activity was detected at *cis*-negative voltages. (*B*) Records from a bilayer in which the 116-pS channel was incorporated. Activity could be seen at both positive and negative voltages. Conditions were the same as in *A*. (*C*) I-V plots from data as in *A* and *B*. Each point represents the average of the transitions obtained from four bilayers. Standard deviations did not exceed 10% of the average values.

PS membranes are shown in Fig. 1 *A*. The currents most likely originate from the activity of plasma membrane channels, and not from channels coming from membranes of intracellular origin. This assumption is based on the degree of purity of the membrane preparations (see Materials and Methods). On the other hand, the only channels which have been described in yeast are from plasma membrane origin (see below) and a 435-pS cationic channel from the tonoplast. This later channel requires millimolar Ca^{2+} to be active (18), which excludes its detection under our conditions. In the experiment illustrated in Fig. 1 *A*, the single channel activity was only observed at positive voltages. This behavior is consistent with that observed in patch clamped protoplasts (6), in

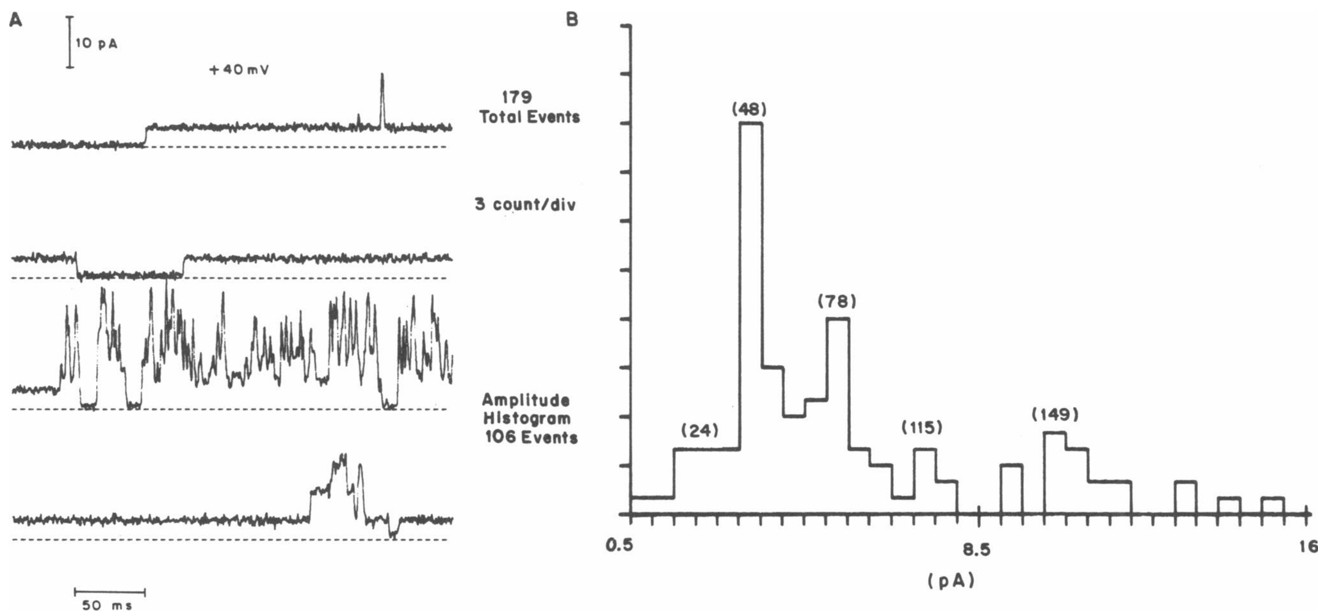

FIGURE 2 Appearance of several current levels after fusion of plasma membrane vesicles into a PE/PS bilayer. Experimental conditions were as in Fig. 1. (*A*) Current fluctuations at 40 mV. Traces are not consecutive; they were chosen to exemplify the appearance of several current amplitudes. Note the burstlike behavior in the third trace from top to bottom. (*B*) Current amplitude histogram of the record in *A*. Histogram peaks are marked with their corresponding conductances. Note the existence of several conductance levels yielding a skewed distribution.

which only depolarizing voltages elicited currents. Nevertheless, in many bilayers it was possible to record currents at both positive and negative voltages, which allowed us to construct more complete I-V curves, as shown in Fig. 1 *C*. Asymmetric voltage responses of yeast channels seemed to be dependent on the lipid composition of the planar bilayers (see below).

Two main conductances of 64 and 116 pS were obtained from the slope of the curves in Fig. 1 *C*. These conductance values are close to those found for pressure-sensitive channels (40 and 75 pS) that are present in yeast protoplasts (8, 9). Because little is known about the yeast pressure-sensitive channels, we cannot compare them yet with our data. The differences between the conductances we found and those reported in patch-clamp studies of yeast protoplasts for K^+ channels (13 pS [6] and 17 pS [7]) could have several explanations. (*a*) In the patch-clamp study (6) the solutions employed were at pH 7.2, and in this study we initially choose to work at pH 8.0. This choice was made because studies with intact cells carried out in our laboratory have shown that K^+ efflux is greater at pH 8.0 than at lower pH values (unpublished observations), and there are reports of K^+ currents that are reduced very steeply by lowering the pH from 8.0 to 5.0 (19). (*b*) The difference between the conductance values could also be due to variations in the growth conditions employed. Experimental conditions are known to have striking effects on the properties of yeast transport systems (20). (*c*) It is possible that in the bilayer experiments some channel modifications can occur during membrane purification. (*d*) The selective incorporation of high-conductance channels into the bilayers. In this respect, it must be mentioned that in general, regardless of the amount of protein added to the *cis* compartment, several conductance levels were observed in each experiment. This rather common phenomenon is exemplified in Fig. 2 *A*. The amplitude histogram (Fig. 2 *B*) shows that in this bilayer, the 116 pS conductance was found along with other conductances less frequently incorporated in other experiments. The point is further documented in

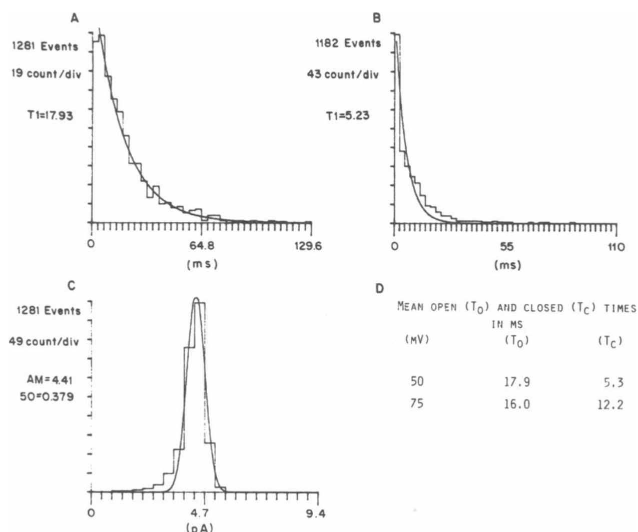

FIGURE 3 Kinetic behavior of a single 64-pS channel. Experimental conditions are the same as in Fig. 1. (*A*) Open dwell-time histogram. (*B*) Closed dwell-time histogram. Both the open and closed histograms were adjusted with a single exponential. (*C*) Current amplitude histogram of the record at 50 mV. (*D*) Mean open and closed times at two voltages calculated from the time constants of histograms, as in *A* and *B*.

Table 1. In many bilayers we have observed that the channel activity appears in bursts, as shown in the third trace of Fig. 2 *A*.

Because of the multiple conductance levels observed in each experiment, the kinetic analysis has been difficult to perform. However, in one experiment in which the only channel present was one of 64 pS, the open and closed dwell time histograms were adjusted with a single exponential, suggesting that there was only one open and one closed state, as shown in Fig. 3, *A–C*. The mean open and closed times for two voltages, given in Fig. 3 *D*, suggest that the channel is voltage dependent.

From the data on Fig. 1 *C*, a reversal potential of −22 mV was obtained for both the 64- and 116-pS conduc-

TABLE 1 Statistical information of channel records

PE/PS bilayers			DiphyPC bilayers		
Conductance	Reversal potential in 3:1 KCl gradient	% of bilayers	Conductance	Reversal potential in symmetrical KCl	% of bilayers
pS	*mV*		*pS*	*mV*	
65.3 ± 4.0	−21.4 ± 4.6	71.0	65.0 ± 4.0	−0.8 ± 3.7	60.0
112.0 ± 6.5	−21.8 ± 1.3	57.1	100.0 ± 9.3	−1.3 ± 1.6	80.0
41 ± 9.9	−22.6 ± 0	29.0	100.0 ± 9.3	−1.3 ± 1.6	80.0
170 ± 6.4	−21.6 ± 2.3	29.0	156.0 ± 8.5	0.9 ± 0.5	29.0
327 ± 19.0	−16.0 ± 9.1	29.0			

Conductances and reversal potentials were obtained from I-V plots from five to seven bilayers.

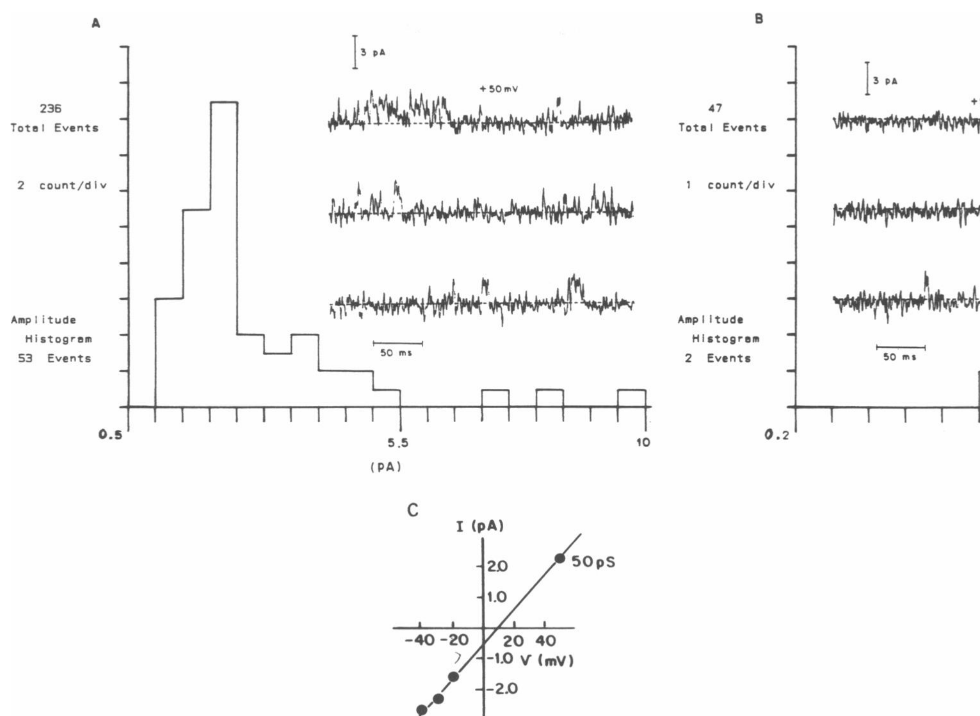

FIGURE 4 Channel behavior under biionic conditions in a DiphyPC bilayer. (*A*) Current transitions at 50 mV in the presence of the control 300 mM KCl buffers on both sides of the bilayer. The record was digitized at 2 KHz and filtered at 1 KHz. The analysis was performed with the pClamp 5.0 program in the semiautomatic mode. The amplitude histogram did not consider events with an open dwell time <4 digital points. A total of 236 events was taken from 45 s of record, and from these, a total of 53 were used to construct the histogram. (*B*) Current transitions at 50 mV from the same bilayer after the *cis* solution was replaced by a 300-mM NaCl buffer (see Material and Methods). Note that only 47 events were taken from 90 s of record, and from these only two were used by the program to construct the histogram. (That is, from a sample twice as long, there was a 96% reduction in the amount of events.) One of the two openings is shown in the trace. (*C*) I-V plot from data as in *B*. In this bilayer the slope conductance was 53 pS. A reversal potential of 11 mV was obtained taking into account the few openings seen at positive voltages and assuming a linear relation. Permeability ratios were calculated from the reversal potentials of three bilayers as in *C*, with the Goldman equation. An average of $(P_{K^+})/(P_{Na^+}) = 2.0$ was obtained.

tances (see also Table 1). The close correspondence between the measured reversal potentials and the theoretically expected Nernst potential for K^+ (−27 mV) indicates that the channels are cation selective. In two PE/PS bilayers, 10 mM TEA decreased the 64- and 116-pS slope conductances by an average 40%. $BaCl_2$ at 10 mM decreased the 64-pS conductance by 60% in one bilayer, and in two others, its addition at 16 and 75 mM markedly decreased the slope conductance of the incorporated channels. These results are consistent with K^+ channel characteristics. The presence of K^+-selective channels was first suggested in the patch-clamp study (6), where it was found that Na^+ was conducted poorly through the recorded channels. These results are consistent with the well-known high permeability to K^+ of the yeast plasma membrane (3).

To obtain further information about selectivity, we incorporated the channels into neutral bilayers formed with the synthetic lipid DiphyPC, which avoids uncertainties arising from surface potential effects. Two main conductances of 65 and 100 pS were obtained both in experiments performed under symmetric 300 mM KCl and using the usual 3:1 KCl gradient, as in the PE/PS experiments (not shown). The Na^+/K^+ permeability ratio was studied under biionic conditions (300 mM NaCl *cis*/300 mM KCl *trans*, see Fig. 4) and by measuring the change in the reversal potential produced by adding NaCl to the *cis* or *trans* side of bilayers containing the control KCl solutions. As shown in Fig. 4, replacement of *cis* K^+ for Na^+ always produced a marked decrease in the frequency of channel openings at *cis*-positive voltages (four bilayers). This result suggests that the incorporated yeast channels discriminate between Na^+ and K^+. However, as we always had to deal with several current amplitudes, in order to get some information about the relative permeabilities, it was assumed that the channels did not rectify, and the few positive-voltage transitions were taken into account. In this way, from data of three bilayers as that of Fig. 4 *C*, a minimum selectivity for K^+ consistent with a $(P_{K^+})/(P_{Na^+}) = 2$ was obtained for a

51-pS conductance. At this moment we cannot establish whether this conductance corresponds to the 65- or 100-pS transitions found in the control records. Finally, it must be mentioned that the assumption made to get an estimate of the permeability ratios has been previously used by other groups dealing with several conductance systems (21).

The main conductances found in DiphyPC bilayers (65 and 100 pS) were almost identical to those most commonly found in PE/PS bilayers. This result could indicate that the mouths of these channels are at a distance from the membrane interface, such that ionic concentrations near them are unaffected by the surface potential. However, further studies are needed before anything can be said concerning surface potential effects on conductance or channel gating. The two most frequent conductances found were sometimes incorporated into bilayers independently of each other. This point, along with the fact that under biionic conditions we only identified one clear conductance, suggests that the two conductances belong to different channels rather than to different substates of a single channel. Finally, it is worth mentioning that the asymmetric voltage response mentioned in relation to Fig. 1, has not been detected in neutral DiphyPC bilayers. Whether this indicates a selective incorporation of the channels or a lipid-induced behavior cannot be ascertained at this moment.

Incorporation of channels in model membranes has proved to be useful in the characterization of channels from cells and membrane sites not accessible to electrophysiological techniques. The yeast plasma membrane presents energy-dependent K^+ carriers along with K^+ and pressure-sensitive channels which endow these cells with a high potassium permeability (4–9). The present work shows that the recently described yeast plasma membrane channels (6, 9) are amenable to be studied in planar bilayers.

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