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Biochimica et Biophysica Acta 1235 (1995) 115–125

BBA Biochimica et Biophysica Acta

The high-conductance channel of porin-less yeast mitochondria

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Received 13 June 1994; revised 14 September 1994; accepted 2 December 1994

Abstract

Patch-clamp and planar bilayer experiments on porin-less yeast mitochondria have allowed the characterization of a cationic channel activated at matrix-side positive (unphysiological) potentials. In voltage-pulse experiments, inactivation was a faster process than activation and the time constant for inactivation was more steeply dependent on voltage than the one for activation. The channel exhibited various conductance states whose occupancy depended on the applied transmembrane potential. In bilayer experiments, the presence of the pCOx-IV leader peptide induced fast gating in a voltage-dependent manner. A comparison with previously described activities suggests that the pore may coincide with the peptide-sensitive channel (PSC) (Thieffry et al. (1988) EMBO J. 7, 1449–1454) as well as with two other activities (Dihanich et al. (1989) Eur. J. Biochem. 181, 703–708; Tedeschi et al. (1987) J. Membr. Biol. 97, 21–29) assigned to the mitochondrial outer membrane. The possible relationship of this channel to the mitochondrial megachannel is discussed.

Keywords: Porin-less mitochondrion; Peptide-sensitive channel; Permeability transition; Patch clamp; (Yeast)

1. Introduction

The investigation of mitochondrial channels has developed into a major subfield of bioenergetics. While the outer membrane porin (also called voltage dependent anion channel, VDAC, because of its properties) (revs.: [1–4]) remains by far the most studied and best known, a host of other conductances, attributed to inner membrane channels, have been described (revs.: [3–7]). The researchers in the field now face the task of establishing a correlation between the channels they observe in electrophysiological experiments and the permeation pathways identified in much previous, non-electrophysiological work. This has been recently achieved in the case of the 'mitochondrial megachannel' (MMC) [8,9] which has been identified [10–13] with the Ca^{2+}-activated, cyclosporin A-inhibited 'permeability transition pore' (PTP) (revs. [14,15]). A comparison of the electrophysiological properties of this pore with those of VDAC, along with other considerations, has led to the proposal [16–21] that it might be formed by a couple of cooperating porin molecules, possibly associated with the adenine nucleotide carrier and a third protein in a supramolecular complex. Partly to verify this model, we have begun an investigation of both wild-type and VDAC-less yeast mitochondria, using the patch-clamp and the planar bilayer techniques.

The electrophysiology of yeast mitochondria is of intrinsic interest, because of the rather detailed knowledge of the genetics of this organism, which should be a great asset to the work combining electrophysiology and molecular biology, like that recently performed on VDAC [22]. Most of the electrophysiological work done so far on mitochondria has utilized mammalian organelles. In the case of yeast, besides the porin, two channels have been described; both have been assigned to the outer membrane. Dihanich et al. [23] have reported on a slightly cationic ($P_a/P_c = 0.6$) [24] channel with a main conductance of about 2 nS in 1 M KCl, isolated from the outer membrane fraction of VDAC-less yeast mitochondria and observed using the

Abbreviations: BSA, bovine serum albumin; EM, electron microscopy; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MMC, mitochondrial megachannel; pCOx-IV, pre-cytochrome oxidase subunit IV; P_a (P_c), permeability to anions (cations); PMSF, phenylmethanesulfonyl fluoride; PSC, peptide-sensitive channel; PTP, permeability transition pore; VDAC, voltage-dependent anion channel (mitochondrial porin).

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SSDI 0005-2736(94)00306-8

planar lipid bilayer technique. The pore reportedly exhibited faster kinetics than the porin [24], and did not possess a noticeable voltage-dependence up to 100 mV.

The French groups of Thieffry and Henry have studied [25–28], by the tip-dip and planar bilayer methods and by patch-clamping giant proteoliposomes, a cationic pore, whose most remarkable property is that of interacting with leader peptide sequences (hence its acronym, PSC, standing for 'peptide-sensitive channel'). In 150 mM NaCl, the activity consisted mainly in transitions between levels separated by approx. 330 pS (500 pS in 150 mM KCl), with several substates. Addition of leader peptides produced, at voltages positive on the side of peptide addition, a transition to flickering kinetics, proposed to be associated with the passage of the leader peptides through the pore. The channel was reported to remain nearly always in the open state at low applied voltages, with both positive and negative bath potentials favoring fluctuations among different conductance levels and predominance by the lowest one(s). A similar pore has been observed in mammalian mitochondria [26,27,29–32].

This paper describes the properties of the most prominent channel observed by patch-clamping VDAC-less yeast mitochondria, which is also the only one with a size approaching that of VDAC or the MMC. Its properties suggest that it corresponds to the PSC, which is thus observed for the first time in situ. A part of this work has been previously presented in abstracts [33,34].

2. Materials and methods

2.1. Mitochondria

Mitochondria were prepared from *Saccharomyces cerevisiae* wild-type (D273-10B) and VDAC-less (HR-125-2A) [35] strains according to published procedures [36], purified by Nycodenz gradient centrifugation, resuspended in 600 mM sorbitol, 20 mM Hepes/K^+ (pH 7.4), frozen in the presence of 10 mg/ml BSA and stored at $-80°$ C until needed. Western blot analysis, carried out with anti-VDAC polyclonal antibodies, confirmed that the porin was absent from the VDAC-less mitochondria (not shown). Inner and outer membrane and 'contact site fractions' were prepared as described in [37,38]. The vesicles obtained by these procedures are mainly right-side-out [39]. All steps in these preparations were carried out in the presence of 1 mM PMSF.

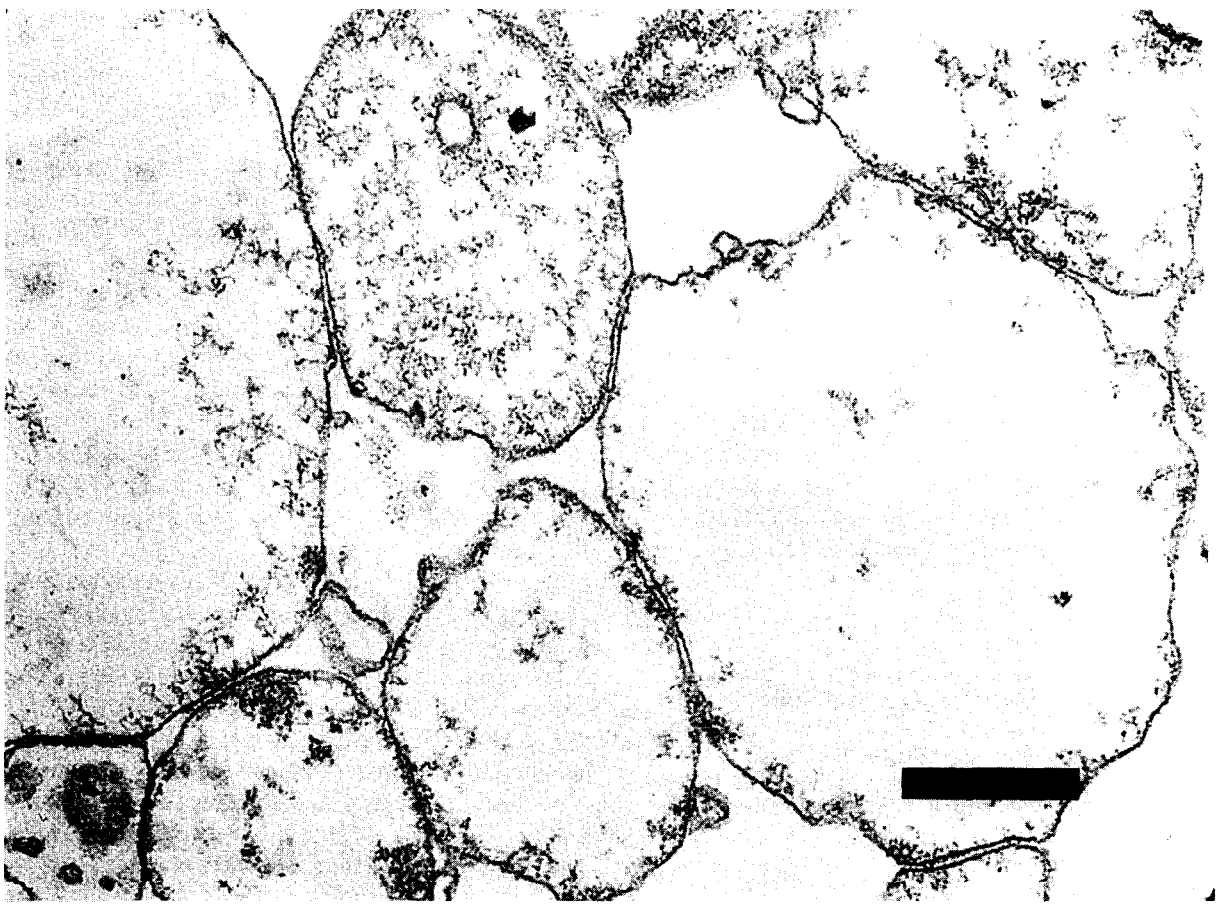


Fig. 1. EM image of VDAC-less mitoplasts under the conditions of patch-clamp studies. See Materials and methods for the procedure followed. Bar: 0.4 μm.

2.2. Electron microscopy (Fig. 1)

The mitochondria were incubated for 5 min at room temperature in the medium used for patch-clamp experiments (see below), and pelleted in a table-top centrifuge. The pellet was fixed in 2.5% glutaraldehyde (0.1 M cacodylate buffer), post-fixed with 1% osmic acid in the same buffer, dehydrated and included in Epon 812. Thin sections were observed using a Philips EM 301.

2.3. Patch-clamp experiments

Experiments and data analysis were conducted essentially as previously described [9,12]. Instantaneous patch $I-V$ relationships were obtained by manually turning the V_{hold} knob on the EPC7 patch-clamp unit at a rate of about 1 mV/s. The current and voltage signals recorded on magnetic tape were sampled at 30 or 50 Hz and plotted in XY fashion by computer routines. In selectivity experiments, different KCl concentrations were present on the two sides of the membrane, and P_K/P_{Cl} ratios were calculated from the measured reversal potentials using the Goldman-Hodgkin-Katz equation.

The VDAC-less yeast mitochondria we utilized are larger and more suitable for patch-clamping than either the wild-type ones or rat liver mitochondria. The size difference was evident in both iso- and hypotonic media: it might be related to the need of a larger outer surface to compensate permeability deficiencies due to the lack of porin [40,41]. KCl concentrations much higher than 100 mM induced shriveling of the mitochondria, making them unfit objects for patch-clamp experiments. Unless otherwise specified the experimental medium was therefore symmetrical 100 mM KCl, 100 mM sorbitol, 0.1 mM $CaCl_2$, 20 mM Hepes/K^-, pH 7.2 (this medium contained about 117 mM K^+ and 100 mM Cl^-). Seals of acceptable resistance were obtained in about 70% of attempts, and channel activity was observed in nearly every case. In some experiments excised patches were obtained from the pipette-attached mitoplast by slicing it open using the edge of a glass fragment sitting on the bottom of the patch-clamp chamber.

2.4. Bilayer experiments

Use was made of a home-made apparatus, using established techniques [42]. Briefly, purified azolectin (Sigma) membranes were prepared by painting a decane solution across a hole of approx. 300 μm diameter in a Teflon film partition separating two Teflon chambers. In a few experiments, bovine brain phosphatidylethanolamine (Avanti) was used, without any evident alteration of the channel properties. The *trans* side always contained the standard medium used also for patch-clamp experiments. The *cis* side initially contained (in order to facilitate vesicle fusion) a medium with a 2- or 3-fold higher KCl and sorbitol concentration, which was replaced with the standard medium during the experiment. The contents of the chambers could be stirred.

2.5. Voltage convention

In this paper we follow the voltage sign convention suggested by Silverstein et al. [43], i.e., the voltage reported refers to the matrix side of the mitochondrial membrane, zero being assigned to the cytoplasmic side by convention. In the case of bilayer experiments, the voltage reported is that of the compartment opposite to the side of addition of the vesicles (*trans* side). Currents are considered negative, and plotted downwards, when they correspond to a flow of positive charges from the cytoplasmic to the matrix side of the membrane (i.e., from the *cis* side to the *trans* side in the case of bilayer experiments).

3. Results

Fig. 1 shows a representative electron micrograph of porin-less mitochondria suspended in the medium used in patch-clamp experiments (see Materials and methods). In many such images, a two-membranes contour could be observed only occasionally. When present, the two membranes either covered only a small part of the contour of the mitochondrion, or enclosed a much smaller-than-average object. Presumably the outer membrane was largely disrupted upon exposure of the mitochondria to the hypoosmotic experimental medium, so that the targets of patch-clamp experiments were in effect mitoplasts.

Fig. 2 exemplifies the behavior observed when seals were established on these objects and currents were recorded in the cell-attached configuration. The total patch current decreased at negative potentials (Fig. 2A), a behavior observed, with some quantitative variability, in most experiments. In the example shown in Fig. 2A, complete pore closure occurred after a few seconds at -80 mV. The current-conducting channels can be better visualized in the example of Fig. 2B, which illustrates the behavior at positive and negative voltages under these conditions (application of a steady potential). The channels strongly preferred the open state at positive potentials, showing only extremely rare closures. The frequency of closures at negative potentials increased with increasing applied voltage. We observed considerable variations in this behavior from experiment to experiment: in some cases closures were rare also at negative potentials (see Figs. 2A and 3). We do not present therefore a quantitative analysis.

In the course of most experiments, a spontaneous, transient large-amplitude electrical event (Fig. 3) was followed by a marked apparent change in the behavior of the channels (Figs. 3 and 4). Simultaneously, the mitoplast disappeared, i.e., it was no longer visible under phase-contrast optics. The maximal observable patch conductance

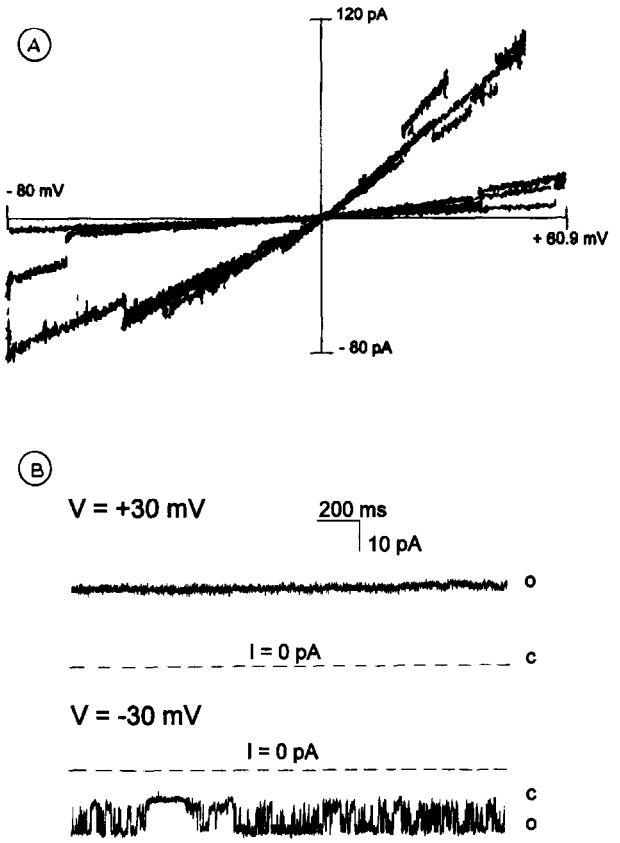


Fig. 2. Voltage dependence in the cell-attached configuration. (A) Instantaneous $I-V$ curves. See Materials and methods for experimental details. Both the pipette and the bath contained standard medium. The voltage was repeatedly scanned in the −80 to +60 mV range. The channels eventually closed while being held at negative potentials, and remained essentially closed during the subsequent scans. (B) Exemplificative current traces at +30 and −30 mV. Filter: 3 kHz. Digital sampling frequency: 2 kHz.

(i.e., the number of active channels) was invariably higher after this event. There was no evident correlation between the channel activity exhibited by the cell-attached patch and the occurrence of the current burst. Our interpretation of these observations, together with those described below,

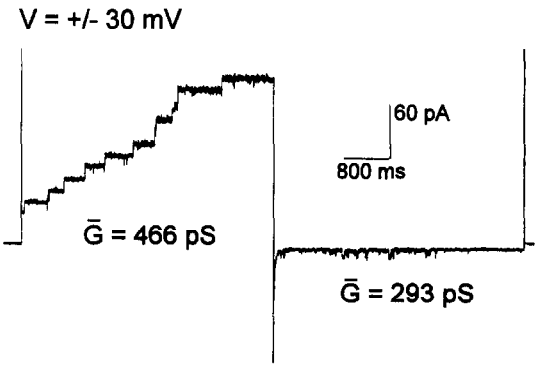


Fig. 4. The response to voltage jumps in the whole-cell configuration. Current trace. Voltage protocol: a positive potential (+30 mV in the example shown) was applied for 4 s, followed by 4 s at the opposite voltage (−30 mV in the example) and by 1 s at 0 mV. Capacitive current spikes indicate voltage changes. Filter: 3 kHz. Digital sampling rate: 1 kHz.

is that the electrical transient signaled the transition from the cell-attached to the whole-cell configuration, which led to the disappearance of the mitoplast because of the equilibration of the matrix space with the pipette medium.

The EM images of the targets of the patch-clamp experiments, and, more significantly, the apparent inversion in the polarity of the channel voltage dependence going from the cell-attached to the whole-cell configuration (see Fig. 4), suggest that the pores might be present in the inner membrane, or at contact sites. The PSC, with which we tentatively identify the channel in question (see below), has however been assigned to the outer membrane [27,31]. This point is discussed further in the Discussion section.

Fig. 4 illustrates the response of the patch current to voltage pulses under whole cell conditions: a number of channels opened progressively in response to transitions to potentials positive on the matrix (and pipette) side, and closed, more rapidly, in response to pulses to negative potentials. The curves obtained by averaging several such responses to voltage jumps could be fitted by single expo-

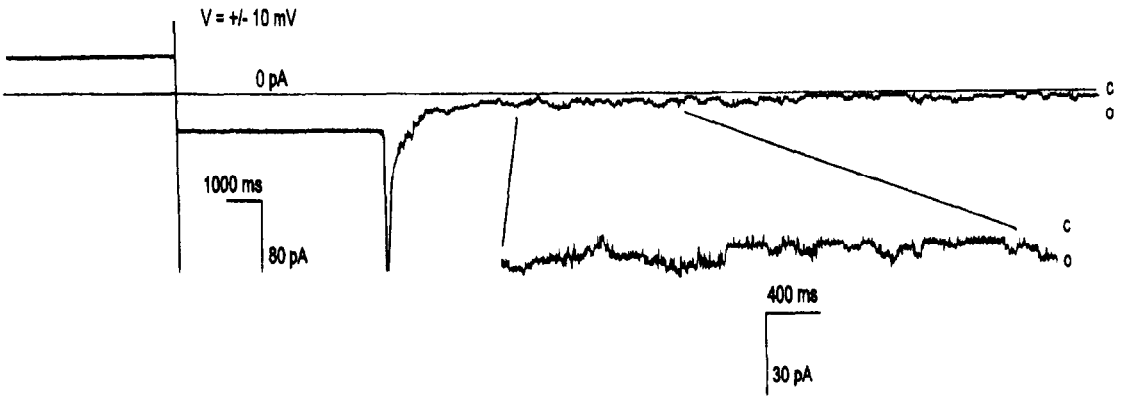


Fig. 3. A transition from the cell-attached to the whole-cell configuration. Current trace. Applied voltage: +10 and −10 mV. Filter: 3 kHz. Digital sampling rate: 1 kHz. (Inset) Amplification of a segment of the trace, as indicated.

nentials (an example is shown in Fig. 5A). The time constants from two experiments are plotted semilogarithmically as a function of the final jump voltage in Fig. 5B. The relaxation process is characterized by higher time constants for activation than for inactivation, i.e., channel inactivation is a faster process than activation (in contrast to the properties of VDAC). The voltage dependence of the time constants also differs markedly for the two processes. The fits shown in Fig. 5B correspond to e-fold decreases of the time constant every 55 mV for the activation process, and every 13 mV for inactivation. The voltage dependence of the channels is also reflected in the patch current/voltage relationships, exemplified in Fig. 6. It should be noticed that the behavior of the channels at negative voltages was different in the cell-attached and whole-cell configurations. In the former case, the channels in many experiments exhibited frequent transitions between the open and closed state(s) (Fig. 2B), but no inactivation (i.e., long-lasting closures) was observed. In bilayer experiments, the channels either behaved as in patch-clamp 'whole cell' experiments (Fig. 7A), or, more rarely, closed in response to voltage steps of either sign (Fig. 7B). In rare cases, there was no definite voltage dependence (Fig. 7C).

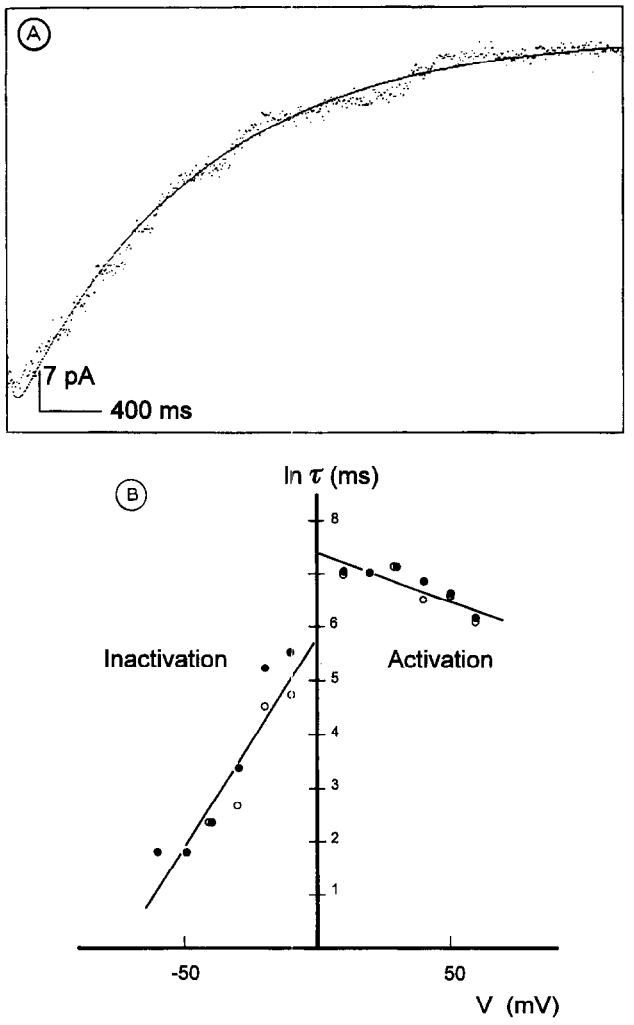


Fig. 5. Time constants of the activation and inactivation process in whole-cell as a function of voltage. (A) An example of activation in response to a voltage transition from zero to +30 mV in whole-cell (average of current traces of the type shown in Fig. 4). The plot is fitted with an exponential with a time constant of 1200 ms. (B) A semilogarithmic plot of time constants obtained from fits like the one shown in Fig. 5A vs. the jump voltage. The data refer to the activation process at positive voltages, and to inactivation at negative voltages. Data from two experiments, distinguished by different symbols. For each point, 5–20 traces were averaged and fitted.

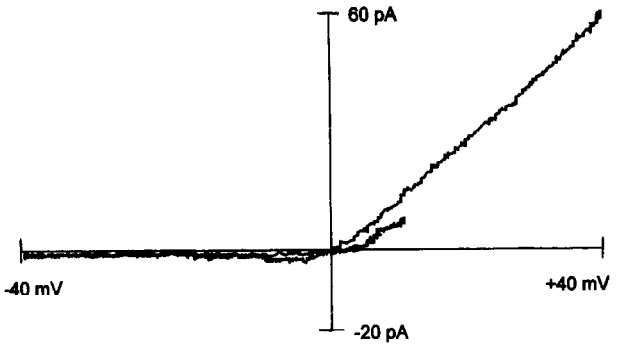


Fig. 6. Instantaneous $I-V$ relationship in whole-cell. Total patch current/voltage curves recorded in symmetrical standard medium. For experimental details see Materials and methods.

Remarkably, the mean size of the conductance steps due to channel gating varied depending on the applied voltage. This can be observed in Fig. 4: the opening steps at +30 mV measure, on average, 466 pS, while the gating steps at −30 mV are in the order of 300 pS. Fig. 8 illustrates the voltage dependence of the average size of the gating steps at various potentials in a typical patch-clamp whole-cell experiment utilizing the voltage pulse protocol. The mean conductance of the pores was a function of the applied potential (Fig. 8A), while at any given potential the conductance histogram showed a considerable spread (an example in Fig. 8B). These results are best interpreted as arising from the presence of several conductance substates, whose occupancy is a function of the potential. While they could be observed in patch-clamp experiments as well (see Fig. 2B), substates were more evident in records from planar bilayer experiments (see Fig. 7A,C). That the various observed conductances represented different conductance states of the same channels, rather than activity by different channels, can also be deduced from their common voltage dependence and kinetic properties. It should be mentioned that the behavior outlined above (Fig. 8) was not invariably observed. In a minority of the experiments, especially when using the planar bilayer approach, the channels seemed to remain 'locked' in a given conductance state, most often close to 200 pS, and gated only between this level and the closed state, irrespective of voltage (not shown).

Fig. 9 exemplifies the dependence of the patch conductance on voltage at steady-state, in the cell-attached and whole-cell configurations. The vast majority of the current

was conducted by the channels under discussion, and the curves reflect the dependence of both the channel open probability and the single-channel average conductance on voltage. For comparison purposes, the dependence of the steady-state conductance of a representative rat liver mitoplast patch on voltage is also shown. In this case the current was conducted essentially by two MMC channels: the shape of the plot results from the tendency of these channels to inactivate at negative potentials, and to spend an increasing proportion of time in substates as the voltage is increased in the positive range [9,17].

Determinations of current reversal potentials under asymmetric medium conditions indicated that the yeast channels are cationic, selecting for K^+ over Cl^-. The extent of the preference for K^+ varied somewhat from experiment to experiment. In several patch-clamp experiments, $I-V$ curves of the type shown in Fig. 6, obtained with higher KCl on the cytoplasmic side, intersected the voltage axis between zero and 10 mV, with associated P_K/P_{Cl} ratios of 1 to 3. In bilayer experiments, P_K/P_{Cl} ratios of 4 to 6 were consistently measured. Fig. 10 shows one representative bilayer experiment, in which the channel did not change its conductance as a function of voltage (see above).

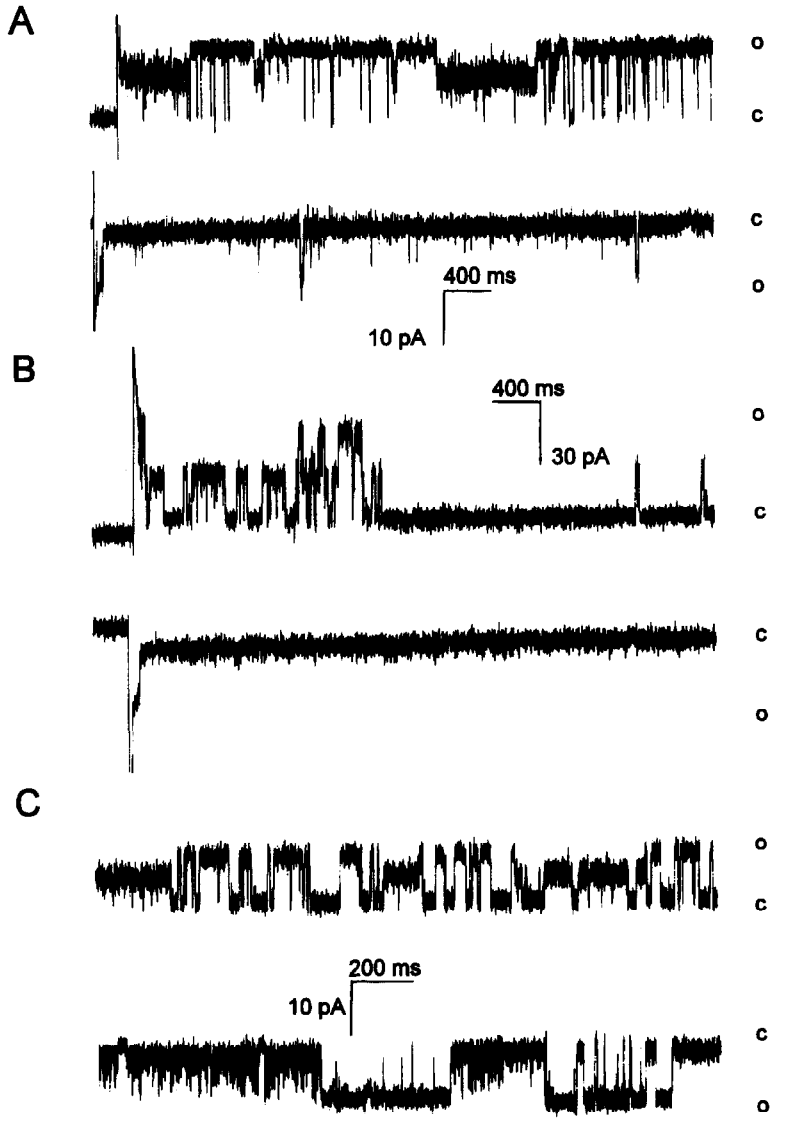
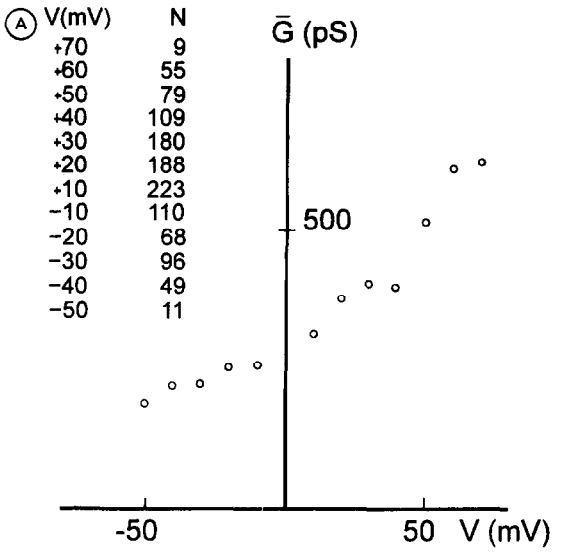


Fig. 7. Examples of the different observed responses to applied voltage jumps in bilayer experiments. Current traces. In each of the three panels the top trace corresponds to the application of a positive potential. The signal was filtered at 3 kHz and sampled at 2 kHz (A and B) or 5 kHz (C). In (A) and (B), the capacitive current spikes at the beginning of the traces signal the application of the voltage pulse (from zero mV). Notice the presence of conductance substates in (A) and (C). (A) Assymmetrical response: the application of negative voltages induces long lasting closure. V: +40 and −40 mV. Symmetrical standard medium. (B) Approximately symmetrical (VDAC-like) voltage dependence. The channels inactivated upon application of both positive and negative voltages. V: +40 and −40 mV. Symmetrical standard medium. (C) No clear cut voltage dependence. V: +30 and − 30 mV. The *cis* side contained 190 mM KCl.



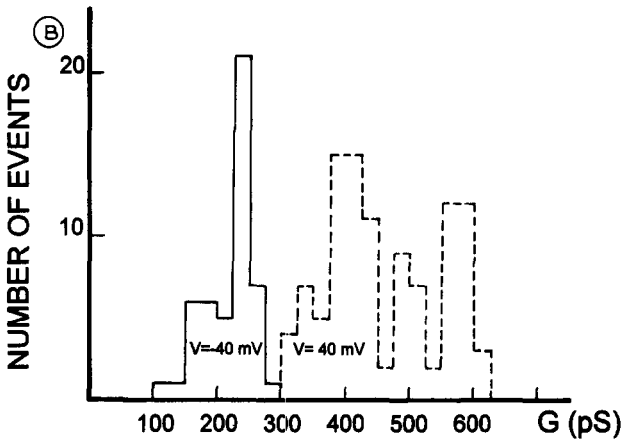


Fig. 8. Voltage dependence of pore conductance. (A) The average single-channel conductance ($G = I/V$) determined in voltage pulse experiments in the whole-cell configuration (See Fig. 4 for a current trace) is plotted as a function of the voltage prevailing after the jump. Data from three separate patches. The numbers listed refer to the number of measurements averaged for each voltage. (B) Conductance distribution histograms for the data plotted in (A) at $V = -40$ (all bins below 300 pS) and $V = +40$ (all bins above 300 pS). Bin size: 25 pS.

The properties described above suggested that the channel might be the one studied, using different experimental approaches, by Thieffry, Henry and collaborators [25–32]. We sought to confirm this identification by checking whether the addition of the pCOx-IV leader peptide (kindly provided by Prof. Henry) induced the change in channel behavior described by those authors [25–28,30]. We could not reach a definite conclusion in patch-clamp experiment, because the leader peptide had a strong destabilizing effect on the seal at concentrations as low as 1 μM, which were not sufficient to induce a clear change in the kinetic properties of the channel. Higher concentrations could instead be used in planar bilayer experiments, in which the

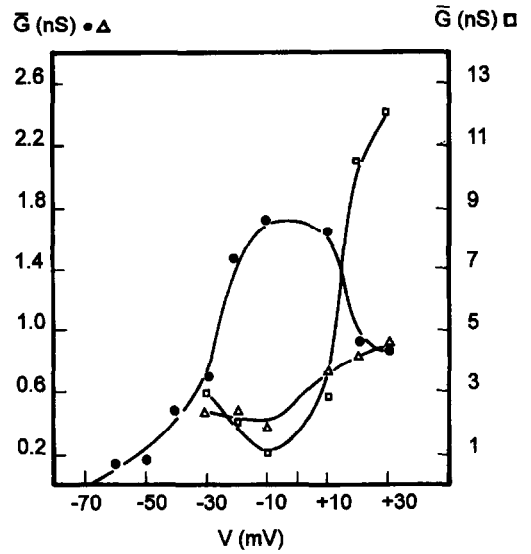


Fig. 9. Voltage dependence of the channel at steady-state in patch-clamp experiments. The conductance of a patch in the cell-attached configuration (△; left-hand scale) and of the membrane in the whole-cell configuration (□; right-hand scale) (standard symmetrical medium) is plotted as a function of the applied voltage. The conductance of a cell-attached patch of a rat liver mitoplast in the same medium is also shown (●; left-hand scale) for comparison purposes. The current flowing in the circuit was averaged over the final one minute of a three-minute period at each voltage, and was divided by the applied voltage to obtain the average conductance. The vast majority of the current was conducted by the yeast channel under discussion or, in the case of the experiment on a rat liver mitoplast, by the 'mitochondrial megachannel'. Representative experiments are shown.

expected effect was indeed observable (Fig. 11). The strong similarity of this behavior with that described by the French investigators strongly supports the identification of

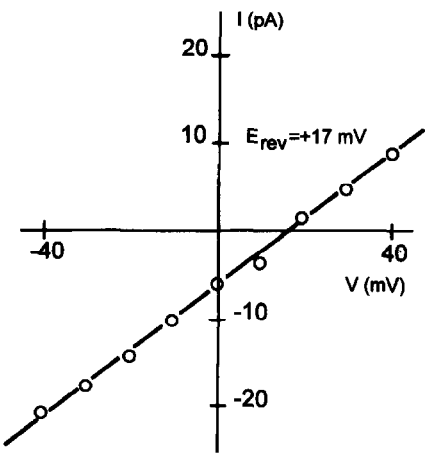


Fig. 10. Selectivity determinations. Plot of single channel amplitudes vs. the voltage applied in the trans side from a bilayer experiment with the inner membrane fraction of VDAC-less mitochondria. Each point is the average of 3–10 individual measurements. The points were fitted by linear regression. The trans compartment contained standard medium (K^+: 117 mM; Cl^-: 100 mM). The *cis* compartment contained 243 mM KCl, 15 mM Hepes/K^+ (total [K^+]: 256 mM), 400 mM sorbitol, 0.075 mM $CaCl_2$, pH 7.2. E_{rev}: 17 mV. P_K / P_{Cl}: 5.5.

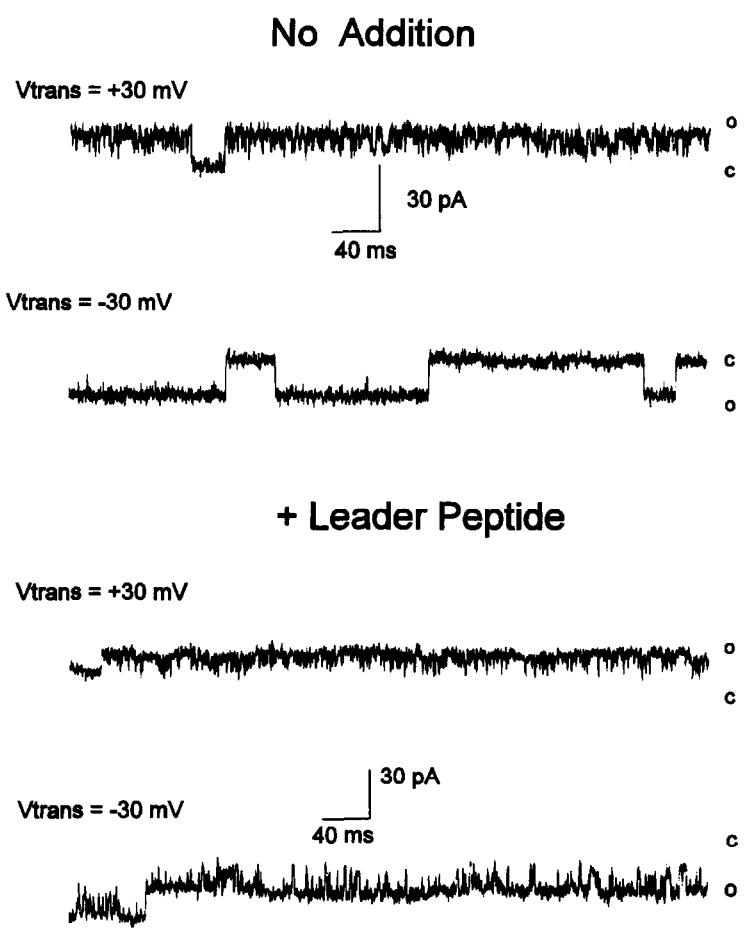


Fig. 11. Effect of a leader peptide on channel behavior. Bilayer experiment. Current traces recorded at $V = +30$ and -30 mV before (A) and after (B) the addition of 5 μM pCOx-IV leader peptide in the cis compartment. Filter: 3 kHz. Sampling rate: 5 kHz.

the channel with the PSC (compare, e.g., Fig. 11 with Fig. 6 of [26]).

Despite numerous attempts, in patch-clamp experiments we did not obtain any evidence of inhibition of the PSC by cyclosporin A, ADP or protons, all well-known inhibitors of the MMC and PTP [10,12–14]. We were also unable to observe swelling by either wild-type or VDAC-less yeast mitochondria in experiments based on the classical light-scattering assay, utilizing either Ca^{2+} and P_i or Ca^{2+} plus SH oxidizing agents.

4. Discussion

The properties illustrated above make it likely that the channel we observed coincides with the one studied by Henry, Thieffry and collaborators [25–32], using mainly the 'tip-dip' and bilayer techniques, in mammalian and in yeast mitochondrial membranes. The activities cover the same conductance range, and exhibit substates with a voltage-dependent occupancy pattern. Both are cationic, with similar selectivities for alkali cations over chloride. Both enter a fast gating mode in the presence of relatively high concentrations of leader peptide. The effect is voltage-dependent in the same manner. The major discrepancy between our observations and the French group's concerns the rectifying behavior we observed. The French colleagues reported instead, for the yeast channel in 'tip-dip' and bilayer experiments with 150 mM NaCl, an increased occupation of the lowest conductance levels with increasing voltages of either sign [25,26]. We have also observed this behavior in some bilayer experiments (Fig. 7B), but it was not the predominant one. Furthermore, the PSC has been reported to close completely only sporadically (e.g., [26]), while we routinely observed complete closures. The reasons for these discrepancies are not clear at present, but the differences might well be reconducible to a dependence of the properties of the channels on the composition of the membranes in which they are placed, or in the use (or lack of) detergents [26].

It is also likely that the channel in question may be the one described by Dihanich et al. [23]. These authors presented an amplitude histogram centered at 2.0 nS, obtained from bilayer experiments in 1 M KCl, at 10 mV. In 0.1 M KCl, the channel we studied often adopted the approx. 200 pS conductance state, especially at negative

potentials and in bilayer experiments. Dihanich et al.[23] reported that the channel exhibited no noticeable voltage dependence up to +100 and −100 mV. An explanation might be found in the fact that the PSC loses its voltage dependence upon proteolysis [26,27,29–31], or, again, in the use of detergents [26]. The Dihanich channel was also reported to be slightly cationic [23,24]. Schmid et al. [44] have described a 2.2 nS (1 M KCl) channel in pea mitochondria, which the authors tentatively identify with the PSC.

Tedeschi and collaborators [45–48] have reported a strongly rectifying activity in patch-clamp experiments on giant liver mitochondria from cuprizone-fed mice and on vesicles of outer membrane from *Neurospora crassa* mitochondria. While single-channel data were not presented, the observed voltage dependence suggests that the current might have been conducted largely by PSC-like channels. Remarkably, in a small fraction of the experiments the current conducted declined sharply on both sides of zero applied voltage [45]. Moran et al. [49] have observed conductances of 99, 152, 220 and 307 pS (150 mM KCl), preferentially open at positive (pipette) potentials, in patch-clamp experiments on the outer membrane of mammalian mitochondria.

All the activities mentioned above were assigned by the authors to the outer membrane of mitochondria [23,31,45]. However, as mentioned above (see Results) the pores we observed presumably are situated in the inner membrane, or at contact sites. It seems likely that these may be only a minority of the total channel population. Consistent with this hypothesis, only one to several (maximally about 20) copies of the channels were detected in any given experiment. Thieffry et al. [26] have suggested that the PSC might in part reside at contact sites. The distribution of channel activities we found in bilayer experiments with membrane fractions is compatible with a preferential location of the channel in contact sites (Szabó et al., unpublished data). It should be stressed that the voltage dependence of these channels is such that physiological transmembrane potentials would favor the closed state(s). The difference in channel behavior in the cell-attached and whole-cell configurations (Figs. 2, 3 and 4) points to a role of matrix factors in controlling their gating properties.

While there is no doubt that the PSC is a separate molecular entity from VDAC, the distinction between the PSC and VDAC might not always be immediate in electrophysiological experiments with membranes in which both are present, since the range of conductance sizes of the PSC and of the porin are about the same and in some bilayer experiments the channel exhibited a VDAC-like tendency to close at potentials of either sign. The voltage-dependence exhibited by the channel in patch-clamp experiments nonetheless is clearly different from the closure induced by voltages of either sign reported for isolated and reconstituted VDAC [1,3,4,50] and for VDAC present in native membranes fused into proteoliposomes [51] or planar bilayers [52]. It should be noticed, however, that in at least one report [49] the authors could not detect in outer membranes an activity comparable to that of isolated VDAC in bilayers. The kinetics of the response of the PSC to voltage variations in patch-clamp whole-cell experiments, are also quite different from those of VDAC in reconstituted systems: for the PSC the inactivation process is much more rapid than the activation one (Fig. 5B), while the opposite is true for VDAC [1]. Again, however, a proper comparison ought to consider the properties of VDAC in its native environment, about which little is known.

Since the PSC is present also in mammalian mitochondria, the question arises of its possible involvement in the formation of the mitochondrial megachannel, perhaps instead of, or as a possible substitute for, VDAC (see Introduction; [17,18]). The highest conductance values displayed by the PSC are compatible with such an identification, if it is assumed that two PSC channels concur to form one MMC. On the other hand, the lower conductances displayed in the negative voltage range would not match the size of the MMC. Both the MMC and the PSC display substates. In response to a positive voltage pulse the PSC channels open (Figs. 2 and 4) and remain open. Higher positive voltages activate more channels and cause the single steps to be, on average, of higher conductance. In the case of the MMC, an analogous protocol results in maintained activity, with the frequent appearance of substates. The higher the positive voltage applied, the higher the percentage of time the MMC spends in lower-than-maximal conductance substates [9,18]. At negative voltages, the PSC inactivates rapidly in the whole-cell configuration (but not in cell-attached), and the single-channel events have, on average, a lower conductance than at positive voltages. The MMC inactivates slowly [18], and, when open, occupies preferentially the maximal conductance state. This difference in behavior is reflected in the diversity of the plots of steady-state open probability vs. voltage (Fig. 9). While the MMC is characterized by what appears to be a cooperative behavior of two composing units [12,17], patch-clamp experiments offered no evidence of cooperation in the case of the PSC. The MMC is essentially unselective [11], while the PSC is clearly, if weakly, cation-selective. Last but not least, in patch-clamp experiments the MMC is inhibited by cyclosporin A, ADP and protons [10–12], while none of these agents inhibited the PSC. Attempts to verify whether the pCOx-IV leader peptide affects the kinetics of the MMC, in patch-clamp experiments on rat liver mitoplasts, have so far failed because of the seal-destabilizing effect of the peptide. The considerations listed above seemingly deny an identification of the PSC as the, or as a constituent of, the MMC. However, caution is required in reaching this conclusion, since different species (yeast vs. rat liver) are being compared. The PSCs of yeast and mammals are similar, but not identical [25,26]. The available data suggest that yeast

mitochondria might not undergo the permeability transition. Work is under way in our lab to further investigate this point. It has been known for a long time that the susceptibility of mitochondria to Ca^{2+}-induced permeabilization varies strikingly depending on the species and organ of origin (e.g., [53–56]).

Despite much patch-clamp work on mammalian mitochondria, no channel has been identified as the PSC in that system. Even if distinct from the MMC, the PSC might sometimes have been mistaken for it.

VDAC-less mitochondria clearly are the system of choice for further studies on the PSC, for example for attempts at its identification in molecular terms and a verification of its role, if any, in protein import.

Acknowledgements

The preparation of the mitochondria and membrane fractions was carried out in the laboratory of Prof. G. Schatz (Biozentrum, Basel), whom we thank for hospitality and useful discussions. We also thank K. Suda for instruction in the preparation of mitochondria and submitochondrial fractions, J.-P. Henry for the generous gift of leader peptide, M. Fabbri for the EM work, L. Pregnolato for drawing some of the figures. We thank Dr. V. Petronilli for help with the swelling experiments. I. Szabó's stay in Basel was financed by an EMBO short-term fellowship, D. Wolff's stay in Padova by a CNR grant and by FONDECYT grant No. 1930909, G. Bathori's stay in Padova by an EEC grant (ERB-CIPA-CT-92-2259/3577). This work was financed in part by the European Economic Community under the 'Science' program.

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