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Ionic permeability of the mitochondrial outer membrane

Oscar Moran [1], Marina Sciancalepore [1], Gabriella Sandri [2], Enrico Panfili [2], Roberto Bassi [3], Cristina Ballarin [4], and M. Catia Sorgato [4]

[1] Laboratorio di Neurofisiologia, Scuola Internazionale Superiore di Studi Avanzati, Trieste, Italy
[2] Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, Trieste, Italy
[3] Dipartimento di Biologia, Università di Padova, Padova, Italy
[4] Dipartimento di Chimica Biologica e Centro del CNR per lo Studio della Fisiologia Mitocondriale, Università di Padova, Via Trieste 75, I-35121 Padova, Italy

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Abstract. The ionic permeability of the outer mitochondrial membrane (OMM) was studied with the patch clamp technique. Electrical recording of intact mitochondria (hence of the outer membrane (OM)), derived from mouse liver, showed the presence of currents corresponding to low conductances (< 50 pS), as well as of four distinct conductances of 99 pS, 152 pS, 220 pS and 307 pS (in 150 mM KCl). The latter were voltage gated, being open preferentially at positive (pipette) potentials. Very similar currents were found by patch clamping liposomes containing the isolated OM derived from rat brain mitochondria. Here a conductance of approximately 530 pS, resembling in its electrical characteristics a conductance already attributed to mitochondrial contact sites (Moran et al. 1990), was also detected. Immunoblot assays of mitochondria and of the isolated OM with antibodies against the outer membrane voltage-dependent anion channel (VDAC) (Colombini 1979), showed the presence of the anion channel in each case. However, the typical electrical behaviour displayed by such a channel in planar bilayers could not be detected under our experimental conditions. From this study, the permeability of the OMM appears different from what has been reported hitherto, yet is more in line with that multifarious and dynamic structure which apparently should belong to it, at least within the framework of mitochondrial biogenesis (Pfanner and Neupert 1990).

Key words: Mitochondria – Ionic channels – Mitochondrial outer membrane – Patch clamp

Introduction

Until recently, most of the studies on mitochondrial permeability have been devoted to the understanding of the transport mechanisms in the inner membrane. The characterization of several highly specific carriers has thus been achieved. The permeability of the OMM started to receive some attention only after the finding that the VDAC resides in it (Colombini 1979; De Pinto et al. 1987; Freitag et al. 1982; Lindén et al. 1982; Roos et al. 1982; Schein et al. 1976; Zalman et al. 1980). Through multiple investigations, mainly of the purified protein, in planar bilayers (Colombini 1979; De Pinto et al. 1985, 1987; Freitag et al. 1982; Roos et al. 1982; Schein et al. 1976), the VDAC was found to have a state of maximal conductance (of approx. 4 nS in 1 M KCl), which shifted however to lower conducting states (closed states) at voltages higher than 20 to 30 mV (Benz 1990; Colombini 1986). In the closed state, the selectivity changed from slightly anionic to cationic. Such modulation of the ion permeability of the VDAC by voltage, which would become effective at the contact sites between the two mitochondrial membranes (Hackenbrock 1968), led to the proposal that the OM may in fact control the metabolism of energised mitochondria, and the activity of peripheral kinases, through inhibition of adenine nucleotides and phosphocreatine transport across the VDAC at the contact sites (Benz et al. 1988, 1990). A reevaluation of the role of the OM has also come from studies of mitochondrial biogenesis, given the unquestionable involvement of such a membrane in the transport of macromolecules to and from the organelle (Pfanner and Neupert 1990).

Recently, the patch clamp technique has been applied to integral mitochondria (Kinnally et al. 1987, 1989b; Tedeschi et al. 1987, 1989), and high intensity currents have been recorded. The lack, under these conditions, of single channel events, was judged an expected phenomenon, as in *Neurospora crassa* (Freitag et al. 1982) and liver (De Pinto et al. 1987) mitochondria, the expression of the VDAC is such that a few thousand molecules are likely to be present in a membrane area equal to that of a pipette patch. However, in contrast to the behaviour of the VDAC in planar bilayers (Colombini 1979; De Pinto et al. 1987; Freitag et al. 1982; Roos et al. 1982; Schein et al. 1976), the OM conductance decreased only with increasing positive (pipette) potentials. An entity similar to the VDAC was also found when mitochondrial

Offprint requests to: M. C. Sorgato

membranes were studied with the "tip-dip" technique (Février et al. 1990; Thieffry et al. 1988).

During an electrophysiological investigation of proteoliposomes containing a mitochondrial fraction particularly enriched in contact sites, we too failed to detect the VDAC (Moran et al. 1990). This result, combined with the increasing importance attributed to the OM, prompted us to investigate more closely the electrical properties of the OMM with the patch clamp technique, by use of two strategies. One was the electrical recording of OM patches of integral mitochondria. The other consisted of the electrical recording of patches obtained from liposomes containing either the isolated OM or both mitochondrial membranes.

This paper reports the findings of such a study, which, for the first time, shows that apart from low conductances which seem to be a ubiquitous in mitochondrial membranes, four distinct and voltage dependent conductances are present in the OM, either when part of integral mitochondria, or after its isolation.

Methods

Preparation of the OM and contact site fractions from non-synaptosomal rat brain mitochondria

Isolation and biochemical characterization of membrane fractions were accomplished as described by Moran et al. (1990), except that nagarse (a broad spectrum bacterial endopeptidase) was omitted during mitochondria isolation and phenylmethylsulfonyl fluoride (1 mM) was present throughout the preparation. However, no difference in the electrical behaviour was found in the membrane fractions prepared in this way or in rat brain mitochondria isolated according to Rehncrona et al. (1979).

Preparation of mouse liver and guinea pig non-synaptosomal brain mitochondria and of mouse liver mitoplasts

Liver mitochondria were prepared following the method described by Sorgato et al. (1989) for cuprizone-untreated mouse liver mitochondria, while guinea pig brain mitochondria were obtained according to Nicholls (1978). Mitochondrial fractions were suspended in 250 mM mannitol, 50 mM Hepes-KOH (pH 7.2) and 270 mM sucrose, 5 mM N-*tris*[hydroxymethyl]methyl-2-aminoethanesulfonic acid (pH 7), respectively. Untreated mouse liver mitoplasts (OM-free mitochondria) were prepared according to Sorgato et al. (1989).

Preparation of giant proteoliposomes

Liposomes were prepared and stored according to the method described by Criado and Keller (1987). Large proteoliposomes containing the isolated membrane fractions were also prepared according to the above reference. If mitochondria or mitoplasts had to be incorporated into liposomes, 20 µl of these suspensions (containing 50–150 µg total protein) were first mixed with liposomes, in a 1 to 10 ratio (v/v), and passed through three freeze-thaw cycles in a dry-ice/ethanol bath. Usually, 20–40 µl of such a preparation were then again mixed with liposomes and the method to obtain large vesicles was followed.

Experimental conditions of patch clamping and electrical recording

For giant liposomes, the conditions were as detailed by Moran et al. (1990), with bath and pipette solution containing 150 mM KCl, 0.1 mM $CaCl_2$, 20 mM Hepes-KOH (pH 7.2). With liposomes, patch seal resistances were higher than 20 GΩ. The activity of the patches was independent of their configuration (Hamill et al. 1981). When mitochondria were used, a few µl of the mitochondria suspension, put on the recording chamber, were diluted with the experimental solution (namely 150 mM KCl, 0.1 mM $CaCl_2$, 20 mM Hepes-KOH (pH 7.2)) and left for about 10 min before starting the electrical recording. With mitochondria, seal resistances varied from patch to patch (see Results). The low resistance values given in the text were subtracted from the resistance of the pipette. Patch clamping of untreated mouse liver mitoplasts was carried out as in Sorgato et al. (1989). Pipettes, pulled from Kimax-51 glass capillaries (Kimble Products), were coated with Silgard (Corning) and fire polished before use. Experiments were run at 18–22 °C.

Electrical recordings were performed by the patch clamp technique (Hamill et al. 1981), with an EPC-7 amplifier (List Medical Instruments). Data, stored on magnetic tapes, were filtered with a 4-pole Bessel filter (Ithaco, 4302), at a cut-off frequency of 1–5 kHz, and then transferred to an Atari (1040ST) microcomputer using a 12 bit analog to digital converter (VR-ST Instrutech). Sampling intervals were between 200 and 500 µs. Records were analysed with an Atari version of the TAC program (Instrutech).

Western blotting

A *Tris*-Tricine sodium dodecyl sulfate slab gel (Schägger and von Jagow 1987) (with a 10–16% linear gradient of polyacrylamide and with dithiotreitol present at a final concentration of 100 mM) was initially used to fractionate *(i)* mitochondria *(ii)* the fractions enriched in the OMM or contact sites. The gel was then blotted onto a nitrocellulose sheet (of 0.45 µm pore width) (Towbin et al. 1979) and assayed using a polyclonal antibody raised in rabbits against purified ox heart VDAC (De Pinto et al. 1985, 1989), diluted 1:1 000. The antibody binding was detected by using peroxidase-coupled anti-rabbit IgG (Burnette 1981; Hoyer-Hansen et al. 1985).

Protein determination

Protein concentration was determined according to Lowry et al. (1951).

Materials

L-α-phosphatidylcholine (L-α-lecithin), type II S, used to prepare liposomes, the affinity purified goat anti-rabbit IgG horseradish peroxidase conjugate, and the molecular weight standards (MW-SDS-70L kit), were purchased from Sigma. Nitrocellulose, acrylamide and polyacrylamide (electrophoresis grade) were obtained from Millipore Corporation (Badford, USA) and Kodak (USA), respectively. Nagarse was obtained from Serva (Heidelberg, Germany).

Results

Electrical recording of liposomes containing the isolated OMM

The OM was isolated from non-synaptosomal rat brain mitochondria, biochemically characterized, and inserted into liposomes (see Materials and methods).

Current records from a liposome excised patch are shown in Fig. 1 A and B, where the most common conductances are of 97, 156 and 244 pS (Fig. 1 C). At variance with what is shown in Fig. 1 B, these conductances were not routinely found in the same patch. All three conductances were found to be sensitive to voltage. This is macroscopically evident from Fig. 2 A, where ramps of ±70 mV were imposed on an excised patch. Under these conditions, a higher number of events is elicited at positive potentials. The non-linear potential effect on the activity of the OM protein-containing liposomes is further supported by the data of Fig. 2 B and C, where the time distribution of total activity (measured at any current level), and the probability of appearance of each conductance value reported in Fig. 1 C, are indeed shown to be higher at positive voltages.

In a previous study of mitochondrial electrophysiology, we reported that proteoliposomes formed with a mitochondrial fraction enriched in contact sites had, among others, a conductance of 550±20 pS, which was voltage independent (Moran et al. 1990). Figure 3 shows that a very similar conductance is present in the OM-containing liposomes. Here again, this conductance is voltage independent (data not shown).

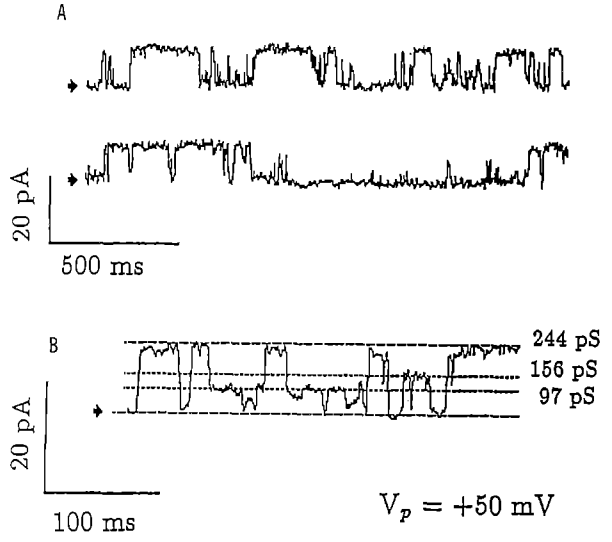
The same conductances represented in Figs. 1 and 3 were also observed in liposomes in which mitochondria or mitoplasts (from mouse liver or guinea pig brain) had been incorporated (data not shown). As the incorporation was carried out by freezing and thawing, this result demonstrates that this latter step is not harmful to the channel-forming proteins of the OM.

Ionic selectiviy of an excised patch was measured under asymmetrical conditions (50 mM KCl in the pipette, 150 mM KCl in the bath) and by imposing a voltage ramp protocol similar to that of Fig. 2 A (data not shown). The reversal value of 8.5 mV indicated that the patch permeability was slightly cationic (the permeability ratio being 2 to 1 for potassium to chloride).

Electrical recording of integral mitochondria

The electrophysiological study of the mitochondrial OM was also carried out with integral liver mitochondria, isolated from untreated mice.

The patch clamping of the OM in situ turned out to be more troublesome than in proteoliposomes. We could



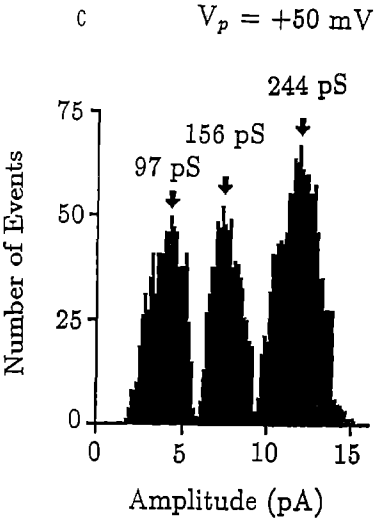


Fig. 1 A–C. Conductances of 97 pS, 156 pS and 244 pS detected in liposomes containing the OMM from rat brain. Electrical recording was performed in a liposome excised patch, in the presence of symmetrical 150 mM KCl. The sign of the potential (Vp) always refers to that of the pipette. Records were low-pass filtered at 1 kHz. The arrow at the beginning of traces indicates the zero current level, after subtraction of leakage. This latter operation was considered correct as the leakage current was found to be linearly dependent on the applied voltage. **A** Current records at 50 mV. **B** A section of the experiment shown in **A** on amplified scales, to emphasize the different conductance levels. **C** Amplitude histogram constructed from the same records. The mean values of the conductances (7 patches) are 97.6±19 pS (mean±SD), 157±14 pS and 235±23 pS respectively. These conductances were found in 80% of active patches examined

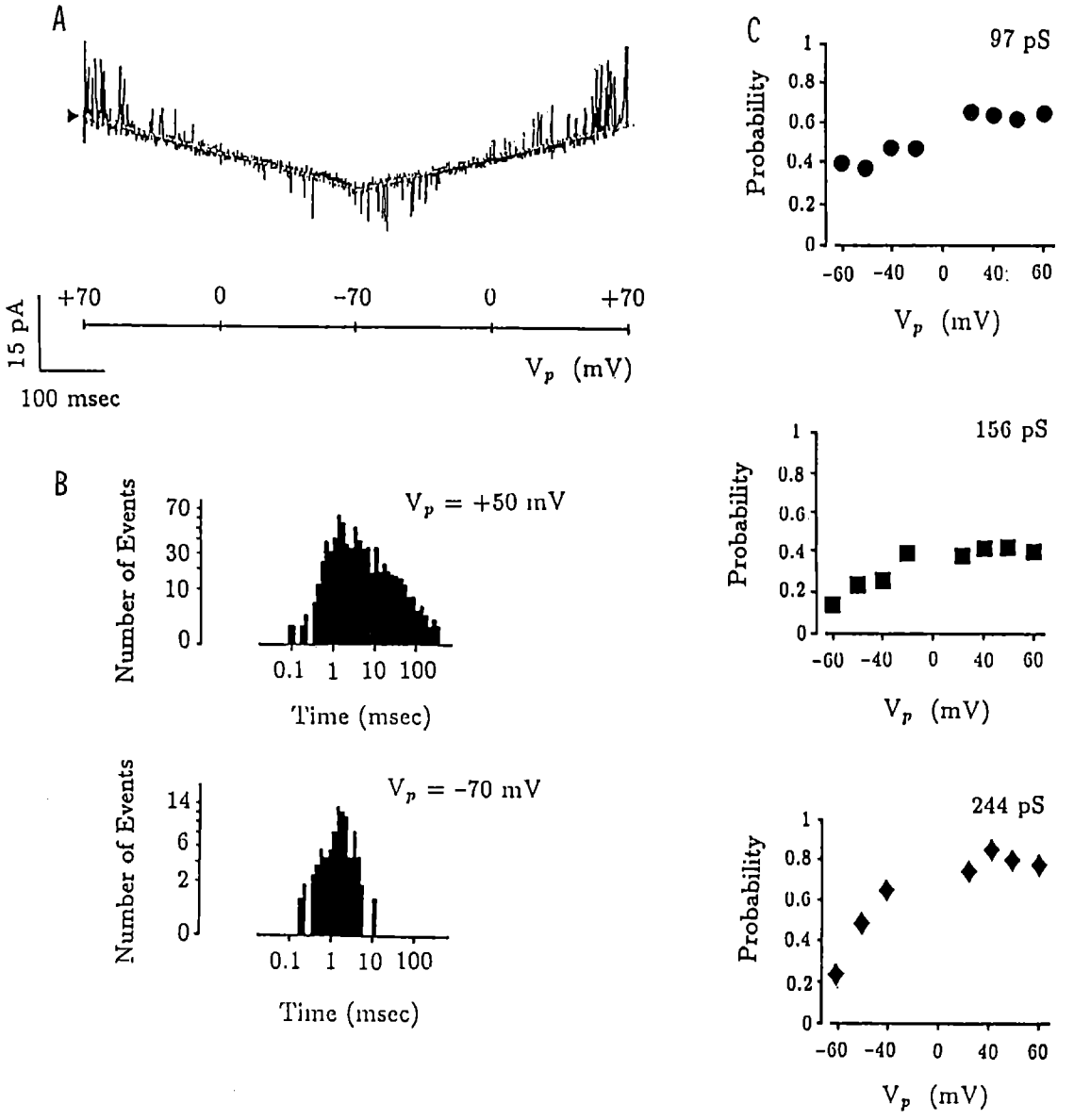


Fig. 2A–C. Voltage sensitivity of the OMM-containing liposomes. Experimental details are as described in the legend to Fig. 1. All data were collected from excised patches. **A** Current recorded upon application of two successive ramps of pipette potentials of ± 70 mV. Each ramp lasted 400 ms. In this case, the arrow indicates the closed state. **B** Time distribution of the total activity (total open time) measured at 50 mV and at -70 mV. At 50 mV, the time distribution was fitted by a double exponential function, yielding time constants of 3.6 ms and 27.9 ms. At -70 mV, the total activity time distribution was fitted by a single exponential, with a time constant of 2.1 ms. **C** Probability of the 97 pS, 156 pS and 244 pS conductance events as a function of the applied voltage

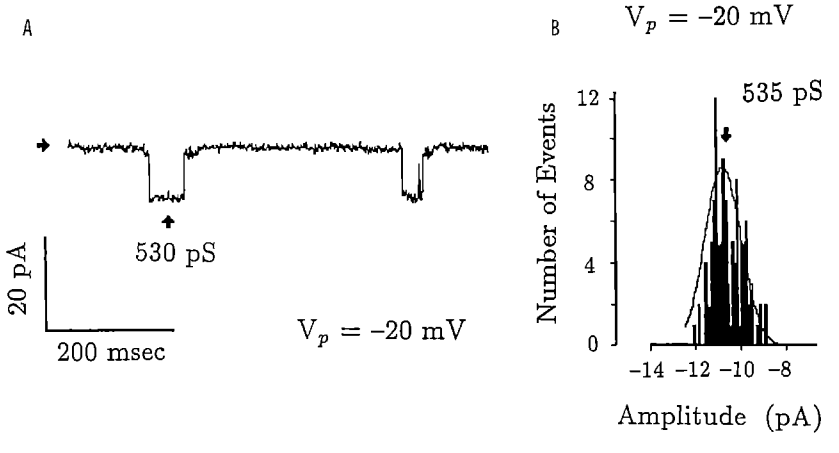


Fig. 3A, B. A 535 pS conductance detected in the OMM-containing liposomes. Experimental details and symbols are as in the legend to Fig. 1, except for the configuration of the patch, which in this case was a liposome-attached patch. **A** Current record taken at -20 mV with openings of 530 pS. **B** Amplitude histogram constructed from records of the same patch of **A**, showing a mean conductance of 535 ± 37 pS. This conductance was observed in 7 out of the 16 active patches examined

identify at least two technical reasons for this. The small diameter of mitochondria, 2–3 μm, which frequently provoked the suction of the organelle into the pipette, and what seemed to be an excessive elasticity of the OM. The latter made the formation of a satisfactory seal with the pipette difficult to achieve. Indeed, in the majority of cases, only low resistance patch seals could be obtained. On average, the resistance was only some 1.5 to 2.5 times higher than that of the pipette. With this type of seal, imposition of voltage pulses of different magnitude induced linear responses (Fig. 4).

Occasionally, better patch seals were formed, the resistance being between 1 and 10 GΩ. In a couple of trials, the patch was silent. However, when activity was present, despite the relatively low resistance value, the noise was sufficiently low to allow resolution of unitary currents.

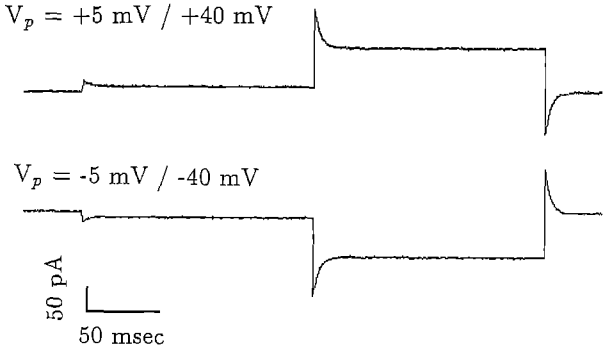


Fig. 4. Linear response to voltage pulses of the OM of integral mitochondria (low resistance seal). Liver mitochondria were suspended in the 150 mM KCl solution, the same medium as the pipette. Both recordings started with a holding potential of 0 mV. In the top trace, a 5 mV step was applied followed by a 40 mV step, before returning to 0 mV. In the lower trace, the same protocol was used but for the values of the pulses, which were of −5 mV and −40 mV, respectively. Pulses were imposed to a mitochondria-attached patch, under symmetrical conditions. Each pulse had a 150 ms duration. The patch resistance was of 350 MΩ, 8 times higher than that of the pipette. Also when patch clamping integral mitochondria, the sign of the potential (Vp) refers to that of the pipette

Under these circumstances, the activity of the patch was found to be markedly voltage dependent. In fact, at positive potential values (> 20 mV), an accurate analysis of all the current levels was frequently precluded because of superimposition of several single events. An example of this voltage sensitivity is the record obtained from a mitochondria-attached patch, shown in Fig. 5 A. At negative voltages, the patch activity was less and unitary current events could be analysed more easily.

Figure 5 B reports amplitude histograms of records taken at −30 mV, with peaks of conductances of 99 pS, 152 pS, 220 pS and 307 pS, respectively. It may be interesting to recall here that the 307 pS conductance is similar to that already described for integral liver mitochondria derived from cuprizone-treated mice (Sorgato et al. 1987), and to mention that, though not as frequently as in the native membrane, a conductance close to 307 pS was also found in proteoliposomes. The above currents were frequently, but not always, found associated in the same patch.

Figure 6 shows the outcome of pulses of ±10 mV and of ±40 mV applied to an attached patch. From this experiment, two observations can be made. One, that the baseline current (leak), measured at each applied potential, is proportional to the magnitude of the potential. The other that, also with this protocol, the activity of the patch is higher at positive potentials (see Fig. 5).

Low conductance channels can be found in the inner and outer mitochondrial membranes, either in situ or reconstituted

As reported for liposomes containing either the inner mitochondrial membrane or contact sites (Moran et al. 1990), the patch clamping of the OMM, in liposomes or in the integral organelle, also revealed the presence of low conductances. Examples are the traces shown in Fig. 7 A and B, recorded in proteoliposomes and intact mitochondria, respectively. Figure 7 C shows that similar currents are found by patch clamping untreated liver mitoplasts, as already shown by Kinnally et al. (1989 a).

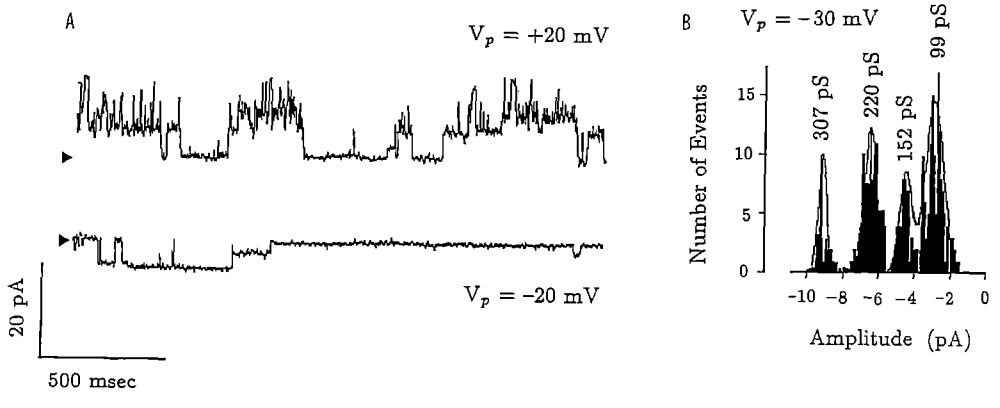


Fig. 5 A, B. Voltage dependent conductances of 99 pS, 152 pS, 220 pS and 307 pS detected in the OM of integral mitochondria (high resistance seal). Experimental details are as in the legend to Fig. 4, except that the patch seal was of 1.5 GΩ. Symbols are as in the legend to Fig. 1. **A** Recordings at 20 mV and −20 mV. **B** Amplitude histogram of records taken at −30 mV. The resulting peaks of conductance were of 99 ± 17 pS, 152 ± 11 pS, 220 ± 11 pS and 307 ± 9 pS. Each of these conductances was present in 7 out of 10 patches

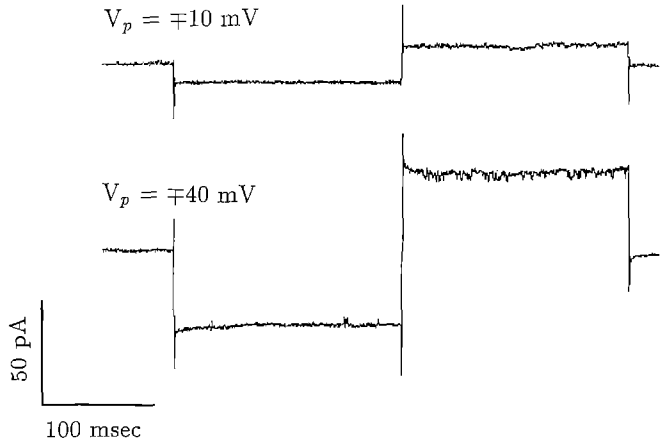


Fig. 6. Non-linear response to voltage pulses of the OM of integral mitochondria (high resistance seal). Both recordings started with a holding potential of 0 mV. In the top trace, a −10 mV step was applied followed by one of 10 mV, before returning to 0 mV. In the lower trace, a step of −40 mV was followed by one of 40 mV, before returning to 0 mV. Each pulse had a 200 ms duration. The resistance of the patch (mitochondria-attached) was of 1 GΩ. For other experimental details see the legend to Fig. 4

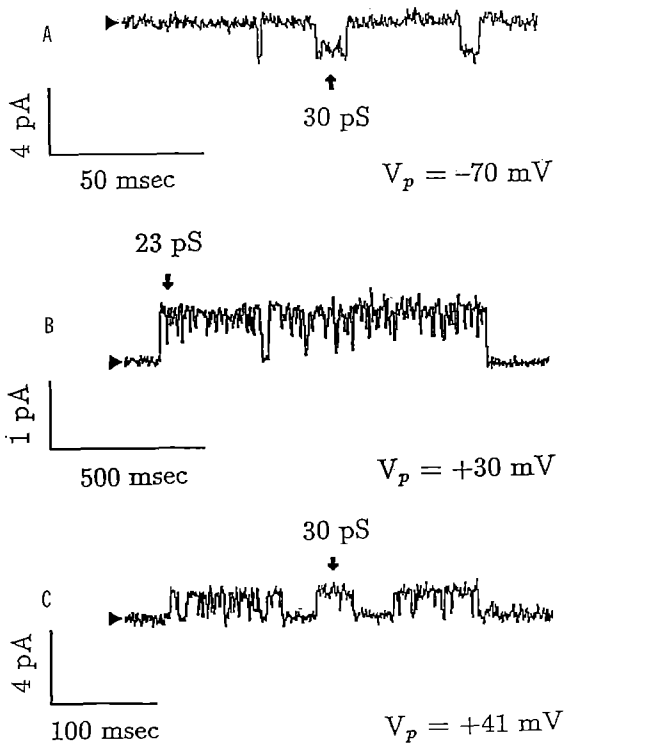


Fig. 7 A–C. Low conductances are common to both mitochondrial membranes. Current records were obtained from: **A** An OMM-containing liposome (attached-patch), at −70 mV. **B** A mitochondria-attached patch, at 30 mV. **C** A mitoplast-attached patch, at 41 mV. Experimental details and symbols are as in the legends to Figs. 1 and 4

Discussion

The only previous studies on integral liver mitochondria with the patch clamp technique (Kinnally et al. 1987, 1989 b; Tedeschi et al. 1987, 1989) yielded a rather different picture than that reported here, for the ionic permeability of the OM. Basically, no single channels but only high intensity currents were recorded. This was explained on the basis of the high density of the VDAC in the OMM (De Pinto et al. 1987), which would have also accounted for the low patch resistances obtainable. However, as currents had a different behaviour, depending on the sign of the imposed voltage, not all data were fully explicable merely on the basis of the information provided by the study of the OM, or of the isolated VDAC, with planar membranes (see Introduction). The possible existence of another type of channel, beside the VDAC (but presumably present as densely as the VDAC), was then put forward.

In our study with integral mitochondria, carried out in a medium containing 150 mM KCl, two types of patch seals were obtained. One of low resistance (between 15 and 350 MΩ, close to the value reported by Kinnally et al. (1987, 1989 b) and Tedeschi et al. (1987, 1989), if the difference in the (low) salt content of the medium used in the above references is accounted for), one of resistance in the GΩ range. The first type of seal was much more frequent. Indeed patch clamping of the OM in situ turned out to be far more difficult than that of the inner membrane (mitoplasts) (Sorgato et al. 1987, 1989) or of proteoliposomes (Moran et al. 1990 and this paper). In the latter cases, seals were always higher than 20 GΩ. Thus more data (single channel recordings) were obtained by patch clamping liposomes containing the isolated OM, or both mitochondrial membranes, than by examining the integral organelle. Nonetheless, with respect to the latter type of experimental approach, at variance with Kinnally et al. (1987, 1989 b) and Tedeschi et al. (1987, 1989), in the presence of low resistance seals the current was consistently found to be linearly dependent on the applied potential (Fig. 4). Parenthetically, the same linear response, with a low resistance seal, was obtained in few attempts with mitochondria suspended in a low salt medium (10 mM KCl) (data not shown).

In contrast, in the presence of 150 mM KCl, when seals were sufficiently good to allow the resolution of unitary currents (less than 10% of total patches), these were found (Figs. 5–7). Furthermore, with this type of seal, and with mitochondria in the attached configuration, the activity of the outer membrane was higher at positive (pipette) potentials (Figs. 5 A and 6), a result which is opposite to that previously reported (Kinnally et al. 1987, 1989 b; Tedeschi et al. 1987, 1989) with integral mitochondria, or with liposomes containing the OMM from *Neurospora crassa* (Tedeschi et al. 1987).

Four distinct conducting levels were frequently found in integral mitochondria and in the OM-containing liposomes (Figs. 1 and 5). The example reported in Fig. 1 for liposomes, i.e. the closure of the 244 pS conductance to the 97 pS level, could favour the hypothesis that the 97 pS conductance (plus perhaps the 156 pS one) were different levels of the larger conductive unit, the 244 pS. Unfortunately, however, at this stage, we do not feel it is possible to reach an unequivocal conclusion because, as reported in the Results section, with both whole mitochondria and liposomes, the three conductances were not always found simultaneously in the patch.

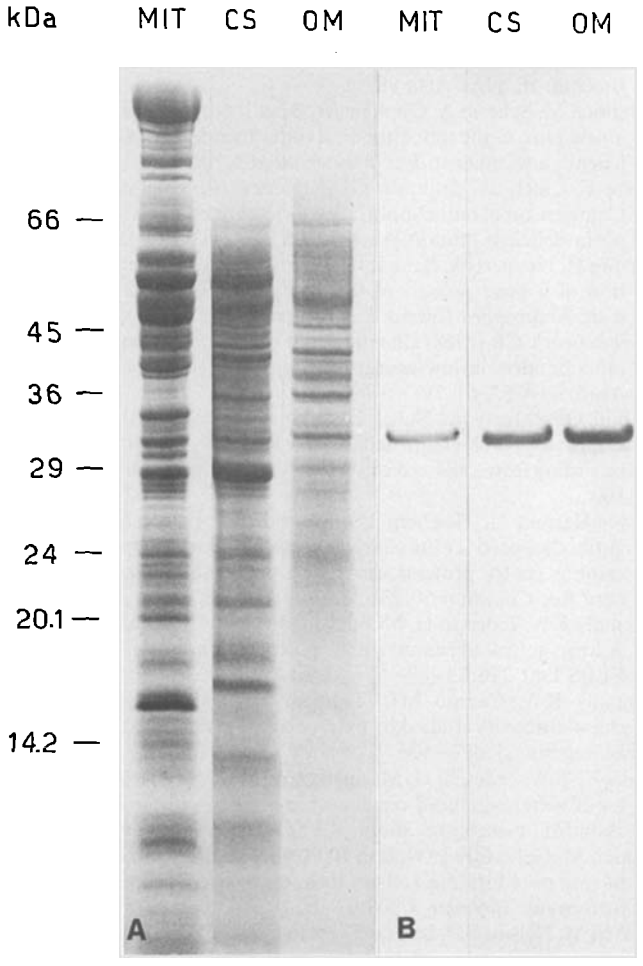


Fig. 8 A, B. Western-blot assays with polyclonal antibodies against ox-heart VDAC. **A** *Tris*-Tricine SDS PAGE (with a 10–16% polyacrylamide linear gradient) of whole liver mitochondria (400 μg) and the isolated rat brain contact site (300 μg) and OM (150 μg) fractions. The gel was fixed in methanol/water/acetic acid (2:2:0.4, v/v) with 10% trichloroacetic acid and stained with Coomassie brilliant blue. **B** Immunoblot assay of polypeptides obtained from an unstained gel equal to that shown in **A** and which was immediately transferred onto a nitrocellulose filter. The filter was assayed using polyclonal antibodies raised in rabbits against purified ox-heart VDAC and the immobilized antigens were visualized by use of peroxidase-coupled anti-rabbit immunoglobulins. Under the experimental conditions used to fractionate the polypeptides, the apparent molecular weight of the VDAC is 32 kDa

Particular consideration should be focussed on the lack of detection, under our experimental conditions, of channels with the same conductance and voltage dependence of those described for the VDAC, i.e. the switching to lower conductance states at voltages higher than 20–30 mV (Benz 1990; Colombini 1986). Use of anti-VDAC antibodies revealed the expected presence of VDAC in both types of membrane preparations used in this study, as well as in the contact site fraction analysed in a previous study (Fig. 8). Thus, even if the lack of the VDAC electrical behaviour in proteoliposomes had to be ascribed to inactivation of this particular protein during the preparation and/or enlargement of the liposomes, this hypothesis must be ruled out in the light of the results with integral, untreated mitochondria. In contrast, retainment in liposomes of what can be shown in situ is further comforting proof that (at least some) mitochondrial proteins do not get grossly altered upon formation of large liposomes (see also Moran et al. 1990).

Additionally, it is appropriate to mention that, in some instances, Triton X-100 (at a final concentration of $2-3 \cdot 10^{-3}$%, v/v) was added during the recording of currents from the OM-containing liposomes. This control was performed in order to ascertain whether the detergent, present in some experiments with planar bilayers (Colombini 1979; De Pinto et al. 1985, 1987; Ross et al. 1982), was responsible for the particular behaviour of the VDAC. No change in the patch behaviour was, however, found (not shown).

Comparing our results with the past literature on the VDAC, at least two possible conclusions can be drawn from our work. One is that the VDAC is inactivated, or fails to be activated, under the patch clamp pipette. Although, to our knowledge, there is no report of such behaviour for other channels, this possibility cannot be ruled out at this stage. The alternative view somewhat follows that hypothesized by Kinnally and co-workers (1989 b), in that the VDAC has different properties in planar membranes than in situ. This is more likely, given the absence in artificial bilayers of the lipid and protein environment of the native membrane and/or because of the use of lipid solvents and of detergent-extracted proteins. If this is the case, then one of the conductances described could belong to the VDAC. Whatever the real explanation, our results demonstrate that conductances, other than that of the VDAC, are present in the OMM (Dihanich et al. 1989; Février et al. 1990; Tedeschi et al. 1989).

The role of these newly described channels is unknown. An appealing hypothesis is that they serve for protein transport, concurrently with those present in the inner membrane, as already predicted in (Blobel and Dobberstein 1975; Blobel 1980; Singer et al. 1987). Supporting this view is the finding that mitochondrial precursors are transported across the two membranes through a water-filled environment (Pfanner et al. 1987).

Finally, the variety of the many different conductances found in mitochondria requires some consideration. In contrast to most channels of the plasma membrane, which are usually found with the same characteristics in all patches, mitochondrial patches frequently present a range of superimposed levels of current (Février et al. 1990; Kinnally et al. 1989 a; Petronilli et al. 1989; Thieffry et al. 1988; this paper). Within this picture, we have tried to assign to the different mitochondrial membrane regions distinctive conductances, by studying them in situ and/or in liposomes (Moran et al. 1990; Sorgato et al. 1987, 1989, and this paper). Yet, the question that naturally springs to mind is what, if any, is the physiological significance of the very active patches, as they give the impression of being the outcome of aggregation states. Whether this is a physiological event due to the release of a modulator or, with respect to the OM, to a loss of interactions with the cytoskeleton (Lindén et al. 1989) or, conversely, is catalysed by the conformation of the patch under the pipette, is not known.

Interestingly, however, there is a common feature to both mitochondrial membranes and to the region of contact sites, i.e. the presence of the so called low conductances (Kinnally et al. 1989a; Moran et al. 1990; Sorgato et al. 1989, and Fig. 7). Unfortunately their behaviour, apart from the conductance value and voltage independence, cannot be easily determined because of their constant association with higher conductances. Yet, their ubiquity is highly suggestive of a common role in both the inner and outer mitochondrial membrane.

Note added. While this paper was under review, Wunder and Colombini reported that VDAC-like currents can be detected by patch clamping mitochondrial membranes-containing liposomes (1991, J Membr Biol 123:83–91). This result, however, does not contradict the main finding of our work carried out in intact mitochondria and in outer membrane containing-liposomes, i.e. that conductances with a behaviour different from the VDAC are present in the outer membrane of mitochondria. With respect to the lack of detection of the VDAC in our experiments, another possible explanation can be sought in the finding of Wunder and Colombini that the VDAC diffuses irreversibly out of the patch. The different lipid and protein composition of our systems, as well as the different origin of the mitochondria used could be additional reasons for the discrepancy in the results obtained in the two papers.

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