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Electrophysiological Characterization of Contact Sites in Brain Mitochondria*

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From morphological and biochemical studies it has been recognized that the regions where the outer and inner membranes of mitochondria come in close contact (contact sites) can be the route mechanism through which mitochondria interact directly with the cytoplasm. We have studied these regions electrophysiologically with the patch clamp technique, with the aim of understanding if this direct interaction is mediated by high conductance ion channels similar to the channel already detected in the inner membrane of mitochondria (Sorgato M. C., Keller, B. U., and Stühmer, W. (1987) *Nature* 330, 498–500).

Contact sites isolated from rat brain mitochondria were thus incorporated into liposomes subsequently enlarged sufficiently to be patch clamped. This study shows that these particular fractions contain ion channels with conductances ranging from approximately 5 picosiemens to 1 nanosiemens (in symmetrical 150 mM KCl). Most of these channels are not voltage-dependent and can be open at physiological potentials sustained by respiring mitochondria.

The lack of voltage sensitivity seems not to be the outcome of methodological artifacts, as voltage-gated channels are detected in giant liposomes containing either the outer mitochondrial membrane or a partially purified fraction of the inner mitochondrial membrane.

These data therefore indicate that channels present in mitochondrial contact sites have properties which render them amenable to perform several of the functions hypothesized for these regions, particularly that of translocating macromolecules from the cytoplasm to the matrix of mitochondria.

The role of most of the highly conductive ion channels found in the plasma membrane of a variety of excitable and non-excitable cells is now firmly established (1). Certainly less is known of the function of several ion channels discovered in intracellular membranes. The reason lies primarily in the fact that, with the exception of sarcoplasmic reticulum (2), these intracellular membrane channels were not sought until recently. There were basically two causes for this. First was the implied notion that such water-filled pores would unlikely be found in intracellular membranes, particularly in those involved in bioenergetics. Second, because it was difficult to apply electrophysiological techniques to cellular organelles. Both of these obstacles have now been removed by different means, and high conductance single channels have been recorded in several membranes, such as plant vacuoles and chloroplasts (3, 4), mitochondria (5–8), and synaptic vesicles (9, 10), to name a few. The roles of the majority of these channels are, however, still ill defined.

By applying the patch clamp technique to mouse liver mitoplasts, vesicles composed of the inner membrane of mitochondria (IMM),[1] we have identified a channel of 107-pS conductance (in 150 mM KCl), called the IMM channel (6, 11). Detection of the same channel also in mouse heart mitoplasts and in liposomes containing a partially purified fraction of the inner membrane of ox heart mitochondria (11) has led us to conclude that the 107-pS channel is a physiological component of the IMM.

The high conductance property suggests that the channel is a water-filled pore (1). Such characteristic therefore restricts the range of its possible roles, especially in view of the well established function of the IMM in energy transduction. Also, the peculiar voltage dependence displayed, which would close the channel at the high negative membrane potential sustained by respiring mitochondria, renders the properties of the channel difficult to reconcile with the bioenergetic function of the organelle.

These findings have therefore prompted us to investigate electrophysiologically the so-called contact sites, regions where the outer and inner membrane of mitochondria are tightly juxtaposed, and which appear, from biochemical (Ref. 12 and references therein) and light and electron microscopy studies (13–16), to connect either directly contiguous mitochondria or the cytoplasm to the matrix of mitochondria. Among the various roles ascribed to the contact sites in both excitable and nonexcitable tissues, that of involvement in macromolecular transport is particularly appealing (for a review, see Ref. 17). Indeed several of the hypothesized features of such import machinery belong to a water-filled pore (18). As the 107-pS IMM channel fits ideally in such context, the study of contact sites could provide us with an answer for its possible role.

We have studied the electrical properties of the contact sites by patch clamping giant liposomes containing these regions isolated from rat brain. This paper shows that voltage-independent channels of conductance ranging from approximately 5 pS to 1 nS (in symmetrical 150 mM KCl) are present in this membrane fraction. Using the same method of incor-

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[1] The abbreviations used are: IMM, inner mitochondrial membrane; VDAC, voltage-dependent anion channel; S, siemens; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Ω, ohm.

poration, it is demonstrated that channels are also found in the isolated outer membrane of rat brain mitochondria and in a partially purified fraction of the inner membrane derived from ox heart mitochondria. However, most of the channels of the latter fractions show voltage-dependent gating.

The lack of voltage sensitivity and their particular location in mitochondria thus render the contact site channels ideally suited for being part of a direct connection device between the inside and outside of the organelle, possibly for the purpose of translocation of macromolecules.

EXPERIMENTAL PROCEDURES

Methods

Preparation of the Outer Membrane and Contact Site Fractions from Nonsynaptosomal Rat Brain Mitochondria—Rat brain mitochondria were prepared according to Rehncrona *et al.* (19) and subfractioned according to Sandri *et al.* (20). Basically, the latter method consisted in disrupting mitochondria by osmotic lysis, shrinkage, and sonication, followed by centrifugation of the material on reverse discontinuous sucrose density gradient (*d*: 1.17, 1.15, 1.13). The outer membrane and contact site fractions were then characterized on the basis of the preferential distribution of marker enzymes for the different regions of mitochondria (20, 21). Marker enzyme assays were as described in Ref. 20.

For further use, fractions were diluted to a final sucrose concentration of 0.7 M and centrifuged for 1 h at 250,000 × *g*. Pellets were then suspended in 0.25 M mannitol, 0.05 M Hepes-KOH (pH 7.2) and stored in small (microliter range) aliquots at −80 °C.

Preparation of the Inner Mitochondrial Membrane Fraction—This consisted in a fraction containing the membraneous part of mitochondrial ATP synthase. It was extracted with urea (22) from ox heart ATP synthase isolated according to Ref. 23, and inserted in asolectin liposomes according to Lippe *et al.* (24). The pH of the resulting proteoliposomes was always brought to 7.0 before further treatments.

Preparation of Giant Proteoliposomes—Small aliquots (2–20 μl containing 2–50 μg of protein) of contact site and outer membrane fractions or liposomes containing the inner membrane fraction were mixed with 180 μl of asolectin liposomes and centrifuged as described (25). The resulting proteoliposomes were then enlarged to 5–50-μm diameter vesicles by dehydration-rehydration steps (26).

Protein Determination—Protein concentration of the fractions to be incorporated in giant liposomes was determined according to Ref. 27. Contact site and outer membrane proteins were pretreated with trichloroacetic acid in order to extract them from brain phospholipids (28).

Experimental Conditions of Patch Clamping and Electrical Recording—Giant liposomes were usually perfused with the experimental buffer before being patch clamped. Bath and pipette solution contained 150 mM KCl, 0.1 mM $CaCl_2$, and 20 mM Hepes-KOH (pH 7.2). Single channel currents were measured using the patch clamp technique (29) and were recorded with an EPC-7 amplifier (List Medical Instruments). Data were stored on a video tape for further analysis, using a modified pulse code modulator (30). Pipettes pulled from Kimax-51 glass capillaries (Kimble Products) were coated with Silgard (Corning) and fire polished before use. When filled with experimental solution, their resistance was usually between 8 and 15 MΩ. Seal resistances were higher than 20 GΩ. Experiments were run either in cell (liposome)-attached or excised patches (29). In either case patches were very stable, frequently lasting for longer than 1 h. The sign of the voltage given throughout always refers to that of the pipette. We preferred this to the physiological convention, as for most experiments with proteoliposomes it was not possible to know if the channels retained the *in vivo* polarity. All experiments were run at room temperature.

Data Analysis—Data stored on magnetic tapes were filtered with a 4-pole Bessel filter (Ithaco, 4302) at a cut-off frequency of 1–3 KHz and then transferred to an Atari (1040ST) microcomputer using a 12 bits analog to digital converter (M2 LAB Instrutech). Sampling intervals were between 100 and 200 μs. Acquired data were digitally filtered with a Gaussian filter (31) at a cut-off frequency of 0.5–1 KHz. Records were analyzed with an Atari version (Instrutech) of the TAC program (32). Amplitude histograms were constructed using the mean variance method (33).

Materials

Soybean phospholipids (asolectin) used to incorporate the inner mitochondrial membrane fraction were obtained from Sigma and partially purified by washing with acetone followed by extraction into ethyl ether. L-α-Phosphatidylcholine (L-α-lecithin), type II S, used to form giant liposomes, was also from Sigma.

RESULTS

The patch clamping of large liposomes containing contact sites from rat brain mitochondria shows the presence of channels of very different conductance values. To expedite their exposition, channels are grouped in order of increasing value.

Contact Sites from Rat Brain Mitochondria Have Ion Channels of Low Conductance Value (<100 pS)—On the basis of current amplitude analyses, low conductance channels can be divided into two subgroups, with conductance of 6 and 12 pS (Fig. 1*A*) and 21, 28, and 33 pS (Fig. 1*B*), respectively. The appropriateness of this division is substantiated by the observation that the two populations of channels are rarely found associated with each other. The probability of finding the low conductance channels not associated with higher conductance channels (see later) is rather low (in 3 out of 21 patches), thus precluding the accurate analysis of their open probability at different voltages. On the other hand, the mean open time of the two populations (see legend of Fig. 1) is the same at either

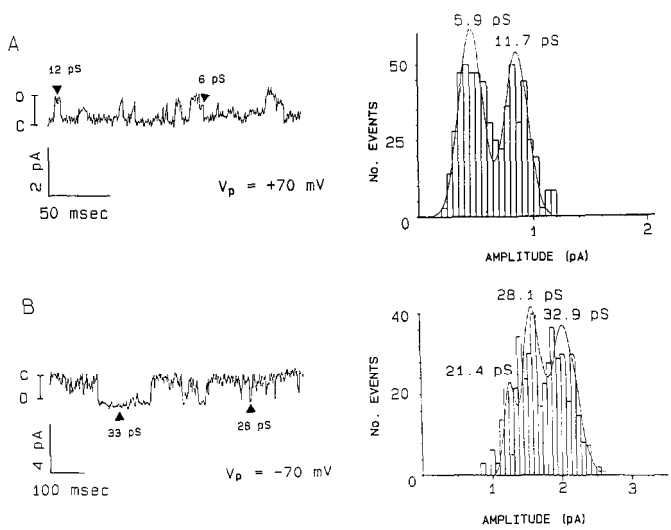

FIG. 1. **Low conductance ion channels detected in liposomes containing mitochondrial contact sites.** Contact sites were isolated from rat brain mitochondria and inserted into liposomes as described under "Experimental Procedures." Liposomes were then enlarged by a dehydration-rehydration step. Electrical recording was performed in the liposome attached configuration, in the presence of symmetrical 150 mM KCl. The sign of the potential (V_p) always refers to that of the pipette. Records were filtered at 1 KHz. *C* (zero current) and *O* at the side of traces indicate the *closed* and *open* state of the channels. Each amplitude histogram was constructed according to Patlak (33), from data of a single patch. *A*, *left panel*: current records at 70 mV, showing channels of 6 and 12 pS. *Right panel*: amplitude histogram of records taken at 70 mV, resulting in two peaks of conductance of 5.9 ± 2 pS (mean ± S.D.) and 11.7 ± 1.5 pS. These two conductances are statistically different ($p \ll 0.001$). Channel mean open time, $<t_o>$, is 17.2 ms. *B*, *left panel*: currents recorded at −70 mV, showing channels of 28 and 33 pS. *Right panel*: amplitude histogram of records taken at −70 mV, resulting in three peaks of conductance of 21.4 ± 2.5, 28.1 ± 1.9, and 32.9 ± 1.6 pS. Here also the three conductances are statistically different ($p \ll 0.001$). $<t_o>$ is 68.8 ms. $<t_o>$ value and not time constants of open times are given, because of the small number of events that occurred. This precluded the possibility of having a statistically significant fitting for the calculation of the time constant value.

+70 or −70 mV, suggesting that they are probably voltage insensitive.

On much rarer occasions, it was possible to detect conductances up to approximately 90 pS. However, due to their low frequency of appearance we could not analyze them in detail.

Contact Sites from Rat Brain Mitochondria Have Ion Channels of 475- and 550-pS Conductance—Contact sites frequently showed single channel conductances much higher than shown hitherto. Fig. 2 shows channels of 475 and 550 pS. These channels display open and closed times of many seconds (Fig. 2*A* and upper part of *B*), but we also observed that either state could last for much longer time periods (up to many minutes). Apparently, the transition from one long-lasting state to the other is independent of the applied voltage (data not shown).

During the long events short openings take place. This can be appreciated especially in the *lower panel* of Fig. 2*B*, where parts of the upper record is shown on a faster time scale.

Interestingly, both the 475- and 550-pS channels appear most frequently in groups of 2–4 unities.

The 475-pS Channel Has Two Substates of 245 and 373 pS—The electrical behavior of the 475-pS channel is singularly characteristic. Indeed, apart from the behavior afore described (Fig. 2*A*), it frequently occurs that the channel has partial closures (flickering) of different amplitude, thus giving rise to substates of 373- or 245-pS conductance (Fig. 3). As far as we could detect, this flickering occurs only upon opening

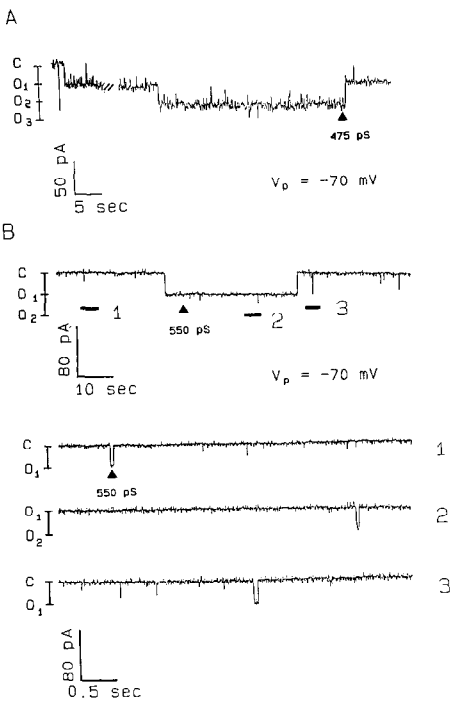

FIG. 2. **Ion channels of 475- and 550-pS conductance detected in liposomes containing mitochondrial contact sites.** All records were taken at −70 mV. O_1, O_2, and O_3 indicate the subsequent openings of channels. Other experimental details and symbols are as described in the legend to Fig. 1. *A*, record taken from an excised patch, showing the opening and closing of three channels of 475 pS. The interruption on the left of the trace indicates a time interval of 90 s. *B*, *upper panel*: record taken from a liposome attached patch, showing the opening and closing of two 550 ±20-pS channels. *Lower panel*: expansion on a faster time scale of selected parts of the upper record (marked with *bars*). The 475-pS channel was observed in 8 patches (out of the 21 active patches examined) with a mean conductance value of 472 ± 18 pS. The 550-pS channel was observed in 7 (out of the 21 active patches examined) with a mean conductance value of 545 ± 8 pS.

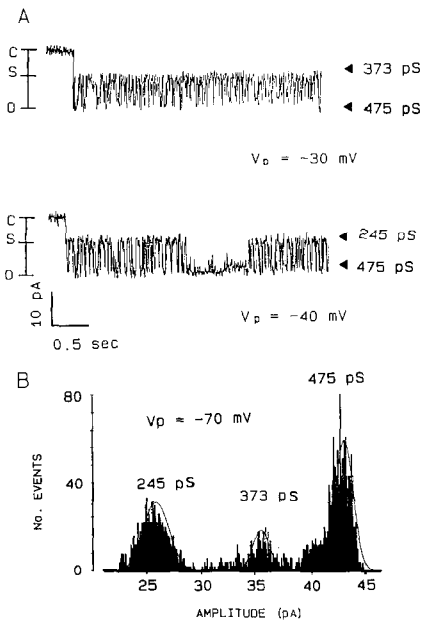

FIG. 3. **Substates of the 475-pS channel detected in mitochondrial contact sites.** All data were acquired from a liposome excised patch. *S*: sublevel opening. Other symbols and experimental details are as described in the legend to Fig. 1. *A*, records taken at −30 and −40 mV showing the opening of a 475-pS channel (C to O transition) with partial closures (O to S transitions) that give rise to sublevels of 373 pS (*upper record*) or 245 pS (*lower record*). *B*, amplitude histogram of records taken at −70 mV. The first two gaussians correspond to sublevels of 245.4 ± 6.3 and 373.7 ± 12.1 pS conductance. The third to the maximal conductance state of 474.9 ± 10.1 pS.

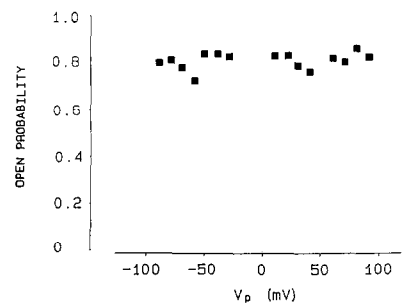

FIG. 4. **Voltage independence of channels found in mitochondrial contact sites.** The open probability of the 475-pS channel was measured at different voltages. Data were collected independently of the state of the channel (maximally open or at subconductance states (Fig. 3)), during the time periods in which the closed time was less than 20 s. The mean value is 0.8. Data were acquired from liposomes excised patches.

of the 475-pS channel. This corroborates our conclusion that the phenomenon is due to different open states of one type of channel (the 475-pS one).

High Conductance Channels Are Voltage-independent—Fig. 4 relates the open probability of the 475-pS channel (including its substates of 245 and 373 pS) to the applied voltage (from −90 to + 90 mV). There is clearly no voltage dependence. Also the 550-pS channel is insensitive to voltage (data not shown).

Contact Sites from Rat Brain Mitochondria Have Ion Channels of Very High Conductance Value (Up to Approximately 1 nS)—Records of Fig. 5 show that very high conductance channels can be found in the contact site fractions. As an example, channels of 660 and 970 pS are shown, but conductances of intermediate value were also observed. In line with

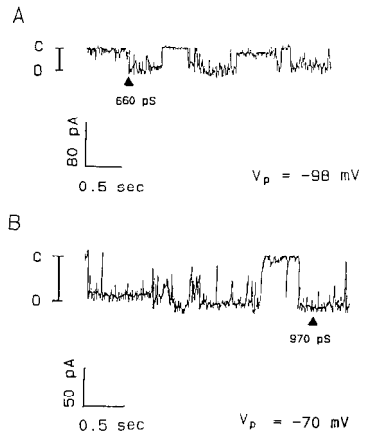

FIG. 5. **Very high conductance ion channels detected in liposomes containing mitochondrial contact sites.** Records taken from liposome attached patches showing channels of 660 ± 25 pS (*A*) and 970 ± 22 pS (*B*). Very high conductive channels (up to approximately 1 nS) were observed in 6 patches (out of the 21 examined). Experimental details and symbols are as described in the legend to Fig. 1.

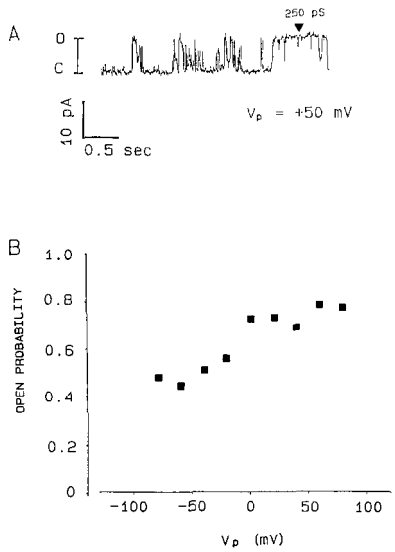

FIG. 6. **A voltage-dependent ion channel of 250-pS conductance detected in liposomes containing the outer mitochondrial membrane.** The outer membrane was isolated from rat brain mitochondria and inserted into liposomes with the same methods used for contact sites (see "Experimental Procedures"). All data were obtained from a liposome excised patch. Symbols and other experimental details are as described in the legend to Fig. 1. *A*, record showing a 250 ± 8 pS channel. *B*, open probability at different voltages of the 250-pS channel.

the other channels, also these very high conductance channels are voltage-independent (data not shown).

In all high conductance channels (up to 1 nS), the distribution of their closed times was fitted by two exponentials, with a fast time constant of 300 ms and a slow time constant value higher than 40 s. The distribution of open times contains three exponentials, with fast, medium, and slow time constant values of 5 ms, 2 s, and 37 s, respectively.

An interesting outcome of all experiments with contact sites is the finding that the appearance of any type of channel is independent of the conformation of the patch (attached or excised).

The Lack of Voltage Dependence of the Contact Site Channels Is Not an Artifact Generated by the Methods Used—By patch clamping mitoplasts derived from mouse liver, Sorgato *et al.* (6) have shown the presence in the inner membrane of mitochondria of a 107-pS channel (in symmetrical 150 mM KCl), which was extremely voltage-sensitive. Although different from the IMM channel, the voltage-dependent anion channel (VDAC) found in the outer membrane of mitochondria (5) is also sensitive to the applied voltage. Therefore, even if not strictly axiomatically, one would expect the contact site channels to display a similar behavior. In contrast, we have found that such voltage-dependence is lacking (Fig. 4, and text). We have therefore considered whether (and which of) the methods used to obtain proteoliposomes suitable for patch clamping were the cause of protein modification(s), leading eventually to loss of voltage sensitivity of the channels.

Two lines of evidence argue against the possibility of methodological artifacts. First are the data of Fig. 6, which show the voltage dependence (Fig. 6*B*) of a 250-pS channel detected in liposomes containing the outer membrane of mitochondria (Fig. 6*A*). The outer membrane was isolated from rat brain mitochondria together with the contact sites (20, 21). This finding thus rules out the possibility that the isolation method is generally harmful with respect to voltage sensitivity. However, as neither the outer membrane nor the contact site channels have been studied before by applying our methodologies, it can still be argued that it is the formation and/or enlargement of proteoliposomes which perturbs the protein structure, in a different way depending on the type of protein. We have then patch clamped liposomes containing a partially purified fraction of the inner membrane of ox heart mitochondria, which is known to contain also the 107-pS IMM channel (11, 34) detected in mouse liver and heart mitoplasts (6, 11). Fig. 7 shows that indeed, after reconstitution into liposomes, such channel fully retains the conductance displayed in the native membrane (6, 11). Also, the voltage dependence, although less steep, is maintained, the isolation and reconstitution steps, or the different lipid environment,

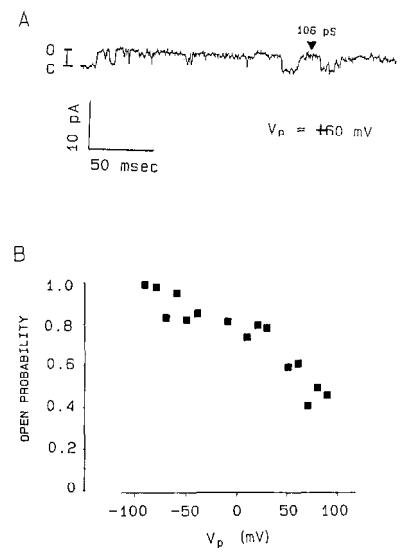

FIG. 7. **A voltage-dependent ion channel of 106-pS conductance detected in liposomes containing a partially purified inner mitochondrial membrane fraction.** The inner membrane fraction was isolated from ox heart mitochondria (see "Experimental Procedures"). Formation and enlargement of proteoliposomes were performed as with the contact sites and outer membrane fractions. All data were obtained from a liposome attached patch. Symbols and other experimental details are as described in the legend to Fig. 1. *A*, record showing a 106 ± 7 pS channel. This channel was observed in 3 patches (out of the 3 active patches analyzed), with a mean conductance of 107 ± 3 pS. *B*, open probability at different voltages of the 106-pS channel.

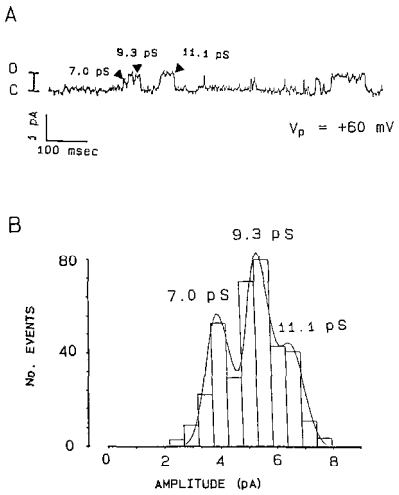

FIG. 8. **Low conductance ion channels detected in liposomes containing a partially purified inner mitochondrial membrane fraction.** The fraction was isolated from ox heart mitochondria. Data were obtained at 60 mV. Symbols and other experimental details are as in the legends of Figs. 1 and 7, except that the filter used was of 0.5 KHz. *A*, record showing channels of 7, 9, and 11 pS of conductance, which were present in 3 patches (out of 3). *B*, amplitude histogram showing three peaks of conductance, 7.0 ± 0.7, 9.3 ± 0.6, and 11.1 ± 0.7 pS. The three conductances are statistically different ($p << 0.001$). $<t_o>$ is 78.4 ms.

being possible causes of such slight modification.

In conclusion, all data favor the interpretation that the electrical characteristics of channels found in the contact site fraction are unlikely to be the outcome of gross methodological artifacts.

The Inner Membrane of Ox Heart Mitochondria Contains Very Low Conductance Ion Channels—Analysis of records from liposomes containing the IMM fraction provided us with another interesting piece of data. Here, channels very similar to two of the low conductance channels described for the contact sites are found (compare amplitude histograms of Figs. 1 and 8). Apart from conductance values, similarity lies also in the voltage independence of the mean open time (data not shown).

These findings are of particular importance because they demonstrate that very similar channels can be found in fractions isolated by completely different methods (see "Experimental Procedures") from mitochondria of different tissues (brain and heart).

DISCUSSION

To our knowledge, this is the first time that mitochondrial fractions, enriched in contact sites, have been studied by electrophysiological means and are found to contain ion channels. For the purpose of this discussion, the different conductances found can be roughly classified into at least two groups, one of low value (from 5 pS to approximately 100 pS) and one of high and very high value (from 475 pS to approximately 1 nS). It must be clear that the distinction between low and high conductance channels is meant on an electrophysiological basis. (Even the smaller channel described here would be regarded as a high turnover protein if compared to most enzymes and/or membrane carriers.)

With respect to the first group, it is important to recall that some of the low conductance channels are seen also in the IMM fraction (Fig. 8) and that they are found, although less frequently, when patch clamping liver mitoplasts or proteoliposomes containing the outer mitochondrial membrane (data not shown). Presumably they are too small to be involved in translocation of macromolecules. On the other hand, it has been shown that mitochondria can be connected to one another by specific junctions to form a network able to transmit $\Delta\Psi$ in the form of protons (13). It has also been proposed that, under some circumstances, other ions or small metabolites can go through the intermitochondrial contacts (35, 36). These would likely be made of channel-forming proteins connecting the matrix of adjacent organelles end to end. In this context, the low conductance channels of the contact sites are certainly suitable candidates for such a role.

How many channels of the second type are present in contact sites? An answer cannot be given with certainty. We are inclined to regard the 245- and 373-pS conductances as sublevels of the 475-pS channel, in view of the fact that the former appear only after the opening of the 475-pS channel. Nothing similar can instead be said for the 550-pS and larger channels, basically because these are almost always found associated with each other and with channels of lower conductance. With the aim of possibly recording from each type of channel separately, we tried to dilute the channels by incorporating less protein into liposomes (data not shown). Although under these circumstances, around 30% patches were silent (usually this percentage was around 5), the rest showed all types of high conductance channels. Even if inconclusive with respect to the real number of entitites present, this result might be taken to indicate a preferential aggregated state of these contact site channels.

Alternatively it is possible that, instead of being due to separate channels, the various conductances observed are the outcome of different aggregation states of protein-forming subunits. Such behavior, typical of the antibiotic alamethicin, has now been recognized also for several other membrane channels (37, 38).

Whatever the nature of the contact site protein composition, at this stage it cannot be distinguished whether the observed wide span of the higher conductances is *per se* physiologically important or the consequence of a disaggregation-aggregation phenomenon of some component of the contact sites, due to a different mobility of proteins in artificial membranes or to the reconstitution step of the contact site fractions in liposomes.

At variance with other mitochondrial channels described here and in Refs. 5–8 and 11, those of contact sites seem to lack voltage dependence. We have carefully controlled whether the methods used unspecifically provoke the loss of voltage dependence and have concluded that none are responsible of such effects (Figs. 6–8). On the other hand, undoubtedly these channels must be finely regulated *in vivo*. In view of our findings, such modulation, other than by voltage, must be due to a cytoplasmic and/or mitochondrial factor, or to a regulatory subunit, absent in our isolated material.

In previous experiments on integral mitochondria, undoubtedly containing contact sites, a 350-pS conductance channel has been observed by us and tentatively attributed to the VDAC (6). Now, in view of the results obtained with liposomes with the isolated outer membrane[2] but also of the data presented here (Fig. 3), we prefer to leave the question open.

The present study has not provided clear hints as to the role of the 107-pS IMM channel (6, 11 and Fig. 7), which was not detected as such in the contact site fractions. However, given the possibility that proteins can be clustered in the contact site region (see above), and the evidence that the lipid environment is different from the inner membrane (21), we cannot exclude that the IMM channel is one of the channels described, being modified in conductance and voltage depend-

[2] O. Moran and M. C. Sorgato, manuscript in preparation.

ence by the interaction with other contiguous proteins or with different lipid molecules. If this were the case, then a tentative explanation for the awkward voltage dependence displayed by the IMM channel (6, 11, and Introduction) can be put forward. In fact such voltage dependence can be regarded as a safeguard mechanism that guarantees the closure of this highly conductive protein when present in regions other than contact sites. (A similar explanation for the closure of the IMM channel, at physiological potentials of mitochondria, has been postulated by Skulachev (36). In his hypothesis, the voltage gating is in line with the necessity of the channel to be closed when the connecting junctions between mitochondria are damaged. This would certainly be the case in mitoplasts and even more in proteoliposomes.)

As pointed out in the Introduction, there is increasingly growing evidence that contact sites play an important role in connecting the matrix of mitochondria with the cytoplasm. The microcompartment defined by juxtaposition of the two mitochondrial membranes has been thought, for example, to provide an ideal region (with the participation of VDAC (21, 39)) where mitochondrially synthesized ATP is preferentially trapped by external and intermembrane kinases (12, 21). Such a microcompartment also explains the route taken by cytoplasmically synthesized mitochondrial proteins to reach their final location within the organelle (17). To mediate such import, water-filled pores are the most likely candidates (18). Parenthetically, these same structures have been considered for protein translocation across other membrane systems (40, 41). Our finding that contact sites exhibit a high conductance is certainly in line with these hypotheses, demonstrating that the above mechanism is possible.

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REFERENCES

1. Hille, B. (1984) *Ionic Channels of Excitable Membranes*, Sinauer Associates Inc. Publishers, Sunderland, MA
2. Miller, C. (1978) *J. Membr. Biol.* **40,** 1–23
3. Hedrich, R., Flügge, U. I., and Fernandez, J. M. (1986) *FEBS Lett.* **204,** 228–232
4. Schoenknecht, G., Hedrich, R., Junge, W., and Raschke, K. (1988) *Nature* **336,** 589–592
5. Colombini, M. (1979) *Nature* **279,** 643–645
6. Sorgato, M. C., Keller, B. U., and Stühmer, W. (1987) *Nature* **330,** 498–500
7. Thieffry, M., Chich, J-F., Goldshmidt, D., and Henry, J-P. (1988) *EMBO J.* **7,** 1449–1454
8. Kinnally, K. W., Campo, M. L., and Tedeschi, H. (1989) *J. Bioener. Biomembr.* **21,** 497–506
9. Rahamimoff, R., DeRiemer, S. A., Sakmann, B., Stadler, H., and Yakir, N. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85,** 5310–5314
10. Thomas, L., Hartung, K., Langosch, D., Rehm, H., Bamberg, E., Franke, W. W., and Betz, H. (1988) *Science* **242,** 1050–1053
11. Sorgato, M. C., Moran, O., De Pinto, V., Keller, B. U., and Stühmer, W. (1989) *J. Bioener. Biomembr.* **21,** 485–496
12. Adams, V., Bosch, W., Schlegel, J., Wallimann, T., and Brdiczka, D. (1989) *Biochim. Biophys. Acta* **981,** 213–225
13. Amchenkova, A. A., Bakeeva, L. E., Chentsov, Y. S., Skulachev, V. P., and Zorov, D. B. (1988) *J. Cell Biol.* **107,** 481–495
14. Hackenbrock, C. R. (1968) *Proc. Natl. Acad. Sci. U. S. A.* **61,** 598–605
15. Biermans, W., Bernaert, I., De Bie, M., Nijs, B., and Jacob, W. (1989) *Biochim. Biophys. Acta* **974,** 74–80
16. Schwaiger, M., Herzog, V., and Neupert, W. (1987) *J. Cell Biol.* **105,** 235–246
17. Hartl, F-U., Pfanner, N., Nicholson, D. W., and Neupert, W. (1989) *Biochim. Biophys. Acta* **988,** 1–45
18. Pfanner, N., Hartl, F.-U., Guiard, B., and Neupert, W. (1987) *Eur. J. Biochem.* **169,** 289–293
19. Rehncrona, S., Mela, L., and Siejo, B. K. (1979) *Stroke* **10,** 437–446
20. Sandri, G., Siagri, M., and Panfili, E. (1988) *Cell Calcium* **9,** 159–165
21. Kottke, M., Adam, V., Riesinger, I., Bremm, G., Bosch, W., Brdiczka, D., Sandri, G., and Panfili, E. (1988) *Biochim. Biophys. Acta* **935,** 87–102
22. Galante, Y. M., Wong, S., and Hatefi, Y. (1981) *Arch. Biochem. Biophys.* **211,** 643–651
23. Stiggall, D. L., Galante, Y. M., and Hatefi, Y. (1979) *Methods Enzymol* **55,** 308–315
24. Lippe, G., Sala, F. D., and Sorgato, M. C. (1988) *J. Biol. Chem.* **263,** 18627–18634
25. Keller, B. U., Hedrich, R., Vaz, W. L. C., and Criado, M. (1988) *Pflügers Arch. Eur. J. Physiol.* **411,** 94–100
26. Criado, M., and Keller, B. U. (1987) *FEBS Lett.* **224,** 172–176
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275
28. Bensadoun, A., and Weinstein, D. (1976) *Anal. Biochem.* **70,** 241–250
29. Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981) *Pflügers Arch. Eur. J. Physiol.* **391,** 85–100
30. Benzanilla, F. (1985) *Biophys. J.* **47,** 437–441
31. Colquhoun, D., and Sigworth, F. J. (1983) in *Single-Channel Recording* (Sakmann, B., and Neher, E., eds) pp. 191–293, Plenum Press, New York
32. Sigworth, F. J. (1983) in *Single-Channel Recording* (Sakmann, B., and Neher, E., eds) pp. 301–321, Plenum Press, New York
33. Patlak, J. B. (1988) *J. Gen. Physiol.* **92,** 413–430
34. Stühmer, W., Keller, B., Lippe, G., and Sorgato, M. C. (1988) in *Hormones and Cell Regulation* (Nunez, J., Dumont, J. E., and Carafoli, E., eds) Vol. 165, pp. 89–99, Colloque INSERM/John Libbey Eurotext Ltd., London
35. Skulachev, V. P. (1988) *Membrane Bioenergetics*, Springer-Verlag, Berlin
36. Skulachev, V. P. (1990) *J. Membr. Biol.*, in press
37. Fox, J. A. (1987) *J. Membr. Biol.* **97,** 1–8
38. Meves, H., and Nagy, K. (1989) *Biochim. Biophys. Acta* **988,** 99–105
39. Roos, N., Benz, R., and Brdiczka, D. (1982) *Biochim. Biophys. Acta* **686,** 629–636
40. Singer, S. J., Maher, P. A., and Yaffe, M. P. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84,** 1015–1019
41. Pain, D., Kanwar, Y. S., and Blobel, G. (1988) *Nature* **331,** 232–237

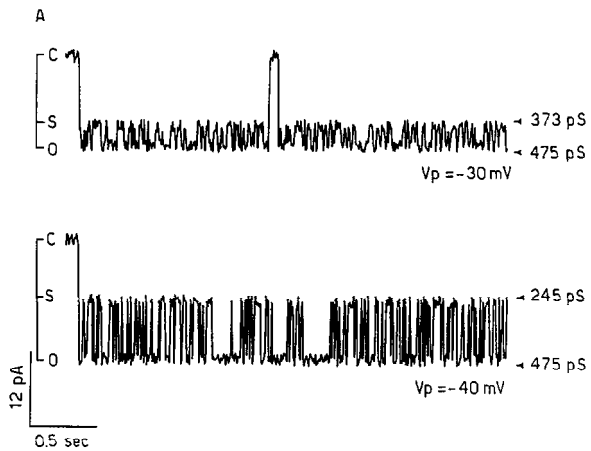
Additions and Corrections

Vol. 265 (1990) 908–913

Electrophysiological characterization of contact sites in brain mitochondria.

Oscar Moran, Gabriella Sandri, Enrico Panfili, Walter Stuhmer, and M. Catia Sorgato

Page 910: Fig. 3*A* should be replaced with the following figure:



Page 911, Fig. 5*A*: The correct unit of current amplitude is 140 pA.
Fig. 6*A*: The correct unit of current amplitude is 15 pA.
Fig. 7*A*: The correct unit of current amplitude is 20 pA.

Vol. 265 (1990) 3793–3802

Glucose oxidase from *Aspergillus niger*. Cloning, gene sequence, secretion from *Saccharomyces cerevisiae*, and kinetic analysis of a yeast-derived enzyme.

Katherine R. Frederick, James Tung, Richard S. Emerick, Frank R. Masiarz, Scott H. Chamberlain, Amit Vasavada, Steven Rosenberg, Sumita Chakraborty, Lawrence M. Schopfer, and Vincent Massey

Dr. Schopfer's name was misspelled. The correct spelling is shown above.

Vol. 265 (1990) 6961–6966

Molecular cloning and amino acid sequence of peptide-N^4-(N-acetyl-β-D-glucosaminyl)asparagine amidase from *Flavobacterium meningosepticum*.

Anthony L. Tarentino, Geraldine Quinones, Anne Trumble, Li-Ming Changchien, Barry Duceman, Frank Maley, and Thomas H. Plummer, Jr.

Page 6963, Fig. 3: The nucleotide sequence contains two typographical errors: 1) At nucleotide position 97, the codon for arginine should read CGC, not GGC; and 2) at nucleotide position 444, the codon for serine should be TCC and not TCG.

Electrophysiological characterization of contact sites in brain mitochondria.

O Moran, G Sandri, E Panfili, W Stühmer and M C Sorgato

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