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MINI-REVIEW

Properties of Channels in the Mitochondrial Outer Membrane

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Abstract

Patch-clamping studies with native outer mitochondrial membranes show a complex behavior. In the range of potentials in which the polarity of the pipette is positive, the behavior resembles that of VDAC incorporated into bilayers. Accordingly, there is a decrease in conductance with voltage. An involvement of VDAC is also supported by responses of the patches to the presence of polyanion or treatment with succinic anhydride, both of which affect VDAC. In contrast, in the negative range of potential, the conductance of the patches generally increases with the magnitude of the voltage. The increase in conductance shows a biphasic time course which is consistent with a model in which channels are first activated (first phase) and then assembled into larger high-conductance channels (second phase). A variety of experiments support the notion that an assembly takes place. The time course of the conductance increase is consistent with formation of the second-phase channels from 6 $\pm$ 1 subunits.

Key Words: Mitochondrial outer membrane; mitochondrial channels; patch clamping.

Introduction

We have been patch-clamping the outer mitochondrial membrane of mouse liver mitochondria (Tedeschi *et al.*, 1987; Kinnally *et al.*, 1987, 1989). Some limited experiments were also carried out with fused vesicles prepared from outer mitochondrial membranes prepared from *Neurospora* with similar

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results. We originally used intact giant mitochondria of mice maintained on a diet containing cuprizone (Bowman and Tedeschi, 1983). More recently we have begun using mitochondria from normal mice with essentially the same results. In our experience, with young mice (17 to 24 days old) a significant portion of the isolated liver mitochondrial population is 3 to 4 μm in diameter, allowing for patch-clamping procedures.

The outer-membrane patches were of low resistance even at low ionic strength. This is not surprising considering the expected large concentration in the membrane of known channels such as the voltage-dependent anion-selective channel (VDAC or mitochondrial porin). In *Neurospora* VDAC corresponds to at least 20% of the total outer membrane protein (Freitag *et al.*, 1982). The concentration is comparable in rat liver mitochondria (De Pinto *et al.*, 1987) which are more closely related to the mouse liver mitochondria used in our experiments. These values suggest a VDAC membrane concentration in the range of a few thousands per square micrometer (Freitag *et al.*, 1982), approximately the surface area of our patches. We calculate similar concentrations from our patch resistances. In addition to these considerations, other outer-membrane channels are beginning to be reported (Kinnally *et al.*, 1987; Thieffry *et al.*, 1988 and private communication; Benz *et al.*, 1989).

Behavior Similar to VDAC

In our studies, in the positive range of potentials (in relation to the pipette) the patches decrease in conductance with the magnitude of the voltage (Tedeschi *et al.*, 1987), a behavior similar to that of VDAC in bilayers (e.g., Colombini, 1987). In agreement with the involvement of VDAC, the patches were found to respond appropriately to the presence of polyanions or treatment with succinic anhydride. The first has been shown to increase the voltage sensitivity of VDAC, and the latter, to decrease it (Colombini, 1987).

Evidence for Two Channels and an Assembly Process

In the negative range of potentials (in relation to the patch pipette) a similar decrease in conductance was occasionally observed. However, more frequently we found a voltage-dependent increase in conductance with time (Tedeschi *et al.*, 1987; Kinnally *et al.*, 1987, 1989). This effect is reversible and reproducible as shown when a second pulse at the same voltage is applied after a suitable delay (Fig. 1). The increase in conductance takes place in two phases. The first-phase occurs rapidly, whereas the second phase develops more slowly with time (see, for example, Fig. 2). As discussed later, the

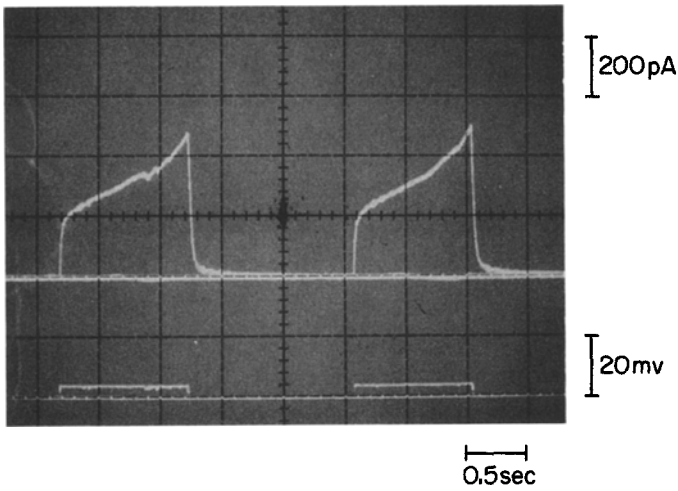


Fig. 1. Oscilloscope traces showing the reproducibility and reversibility of the conductance increase produced in mitochondrial outer membrane patches by identical pulses. The base line corresponds to zero voltage and current (from Tedeschi *et al.*, 1987).

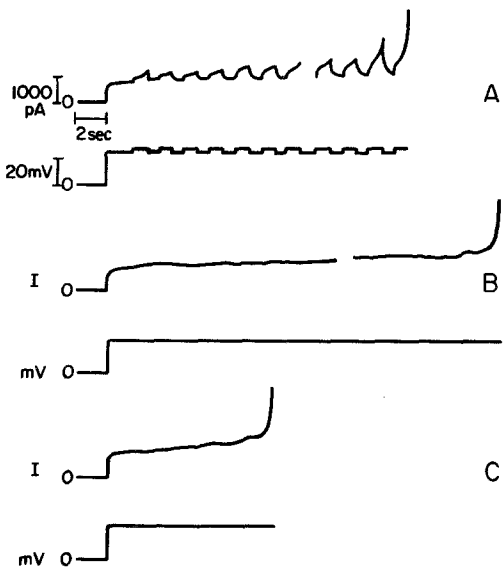


Fig. 2. Effects on current recordings of small subthreshold voltage pulses superimposed on a threshold pulse (from Kinnally *et al.*, 1987). (A) A 20-mV pulse with 2-mV pulses superimposed at regular intervals; (B) 20-mV pulse alone; (C) 22-mV pulse alone.

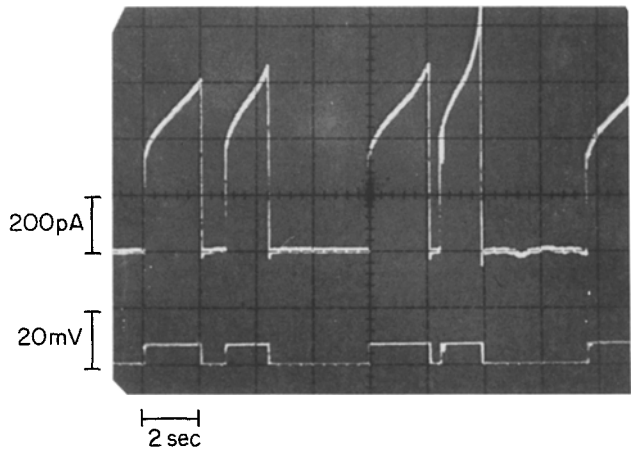


Fig. 3. Oscilloscope traces of outer-membrane patch currents (upper record) evoked by 7.5-mV pulses delivered in pairs (lower record). The time between pulses is varied. The base lines correspond to zero current and voltage (data from Kinnally *et al.*, 1987).

kinetics of the conductance increases suggest two kinds of channels corresponding to the two phases, a first-phase channel activated by voltage that is a precursor of the second type, a higher-conductance multimeric channel (Kinnally *et al.*, 1989).

The results of a variety of experiments also indicate that the second phase corresponds to an assembly from subunits (Kinnally *et al.*, 1987, 1989), which is then followed by a disassembly when the voltage pulse is terminated. Small voltage pulses superimposed on a larger minimal threshold voltage produce a progressively larger increase in conductance, as shown in Fig. 2, consistent with an assembly process. Similarly, a second pulse above threshold applied a short time after the original pulse induces a larger, faster response, which is consistent with reconstitution of a channel from a partially disassembled state (Fig. 3). The magnitude of this effect is inversely related to the time between pulses (Figs. 3 and 4). The time course of the disassembly derived from two pulse experiments is shown in Fig. 4. The disassembly occurs with a $t_{1/2}$ of approximately 250 msec. This is much longer than the time needed to return to the original conductance state after the pulse is terminated, which takes place in less than 50 msec (Kinnally *et al.*, 1989). These observations suggest that the partial disassembly collapses the second-phase channel. In addition to these considerations, there are additional observations supporting an assembly process. The striking temperature dependence of the second-phase process is very similar to that shown by other channel systems which require assembly such as *S. aureus* α-toxin (Ikigai and Nakae, 1984) or alamecithin (Boheim *et al.*, 1980). Finally

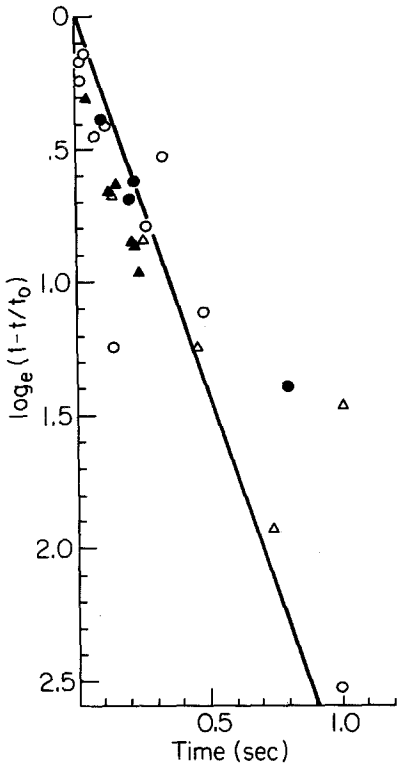
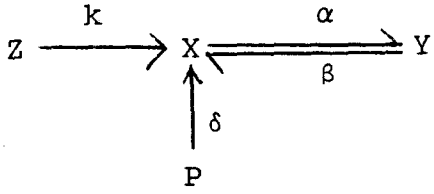


Fig. 4. Effect of the time interval between two identical pulses. The ordinate corresponds to $\log_e[1 - (t/t_0)]$, where t_0 and t correspond to the time to reach a conductance of 1000 pA for the first and second pulse, respectively. The abscissa corresponds to the time between the termination of the first pulse and the initiation of the second. Different symbols represent independent experiments. This graph was compiled from experiments similar to that represented in Fig. 3.

concanavalin A, which is presumed to favor aggregation, facilitates the increase in conductance.

The results are consistent with a model described by Schemes 1 and 2 (Kinnally *et al.*, 1989):



Scheme 1

$$n\ (Y) \underset{}{\overset{K_{eq}}{\rightleftharpoons}} (Y)_n$$

Scheme 2

In these schemes Z is a precursor of the activated channel X, Y is the subunit which instantaneously assembles to form $(Y)_n$, α, β, and k are rate constants, and n is the number of subunits needed to form the larger channel. We found it necessary to assume the presence of another precursor of X, P (omitted in the models of Fig. 5 for simplicity), present in large amounts and with a corresponding rate constant δ. Without this feature the model would predict the conductance to reach a maximal steady-state value, which we have never observed. In the model, this presumed precursor may actually correspond to new Z recruited into the patch electrophoretically. However, the actual mechanism is in question and may even correspond to an effect not described by this model, such as a cooperativity phenomenon. The experimental results (solid lines) at various voltage pulses and the computer simulation (crosses) are compared in Fig. 5. As shown, the data and the predictions of the models are in good agreement. The basic model shown by Schemes 1 and 2 is graphically shown in Fig. 6 in two forms. We currently favor model 1, since it is more likely from energetic consideration of the mechanism of assembly.

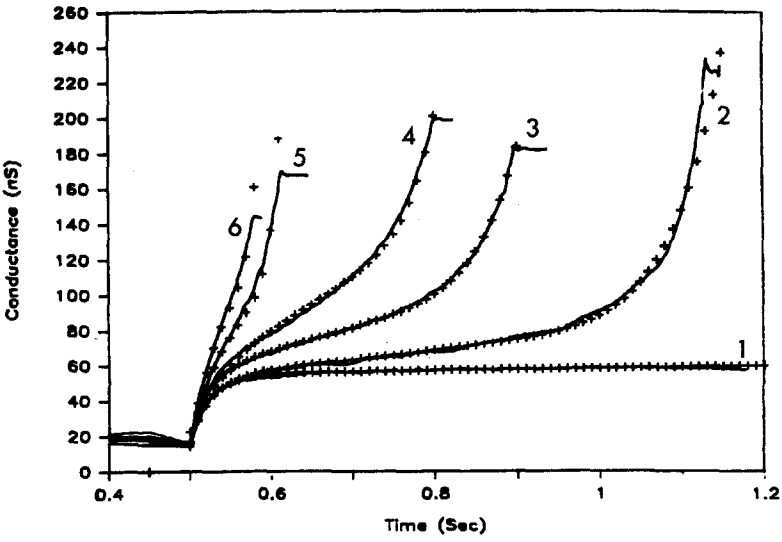


Fig. 5. Computer simulations (crosses) and experimental conductance changes (solid lines) for voltage pulses of 2, 2.5, 3.5, 3, 4, and 5 mV (curves 1–6) (from Kinnally *et al.*, 1989). The leveling off of the conductance is an artifact produced by saturation of the electronics.

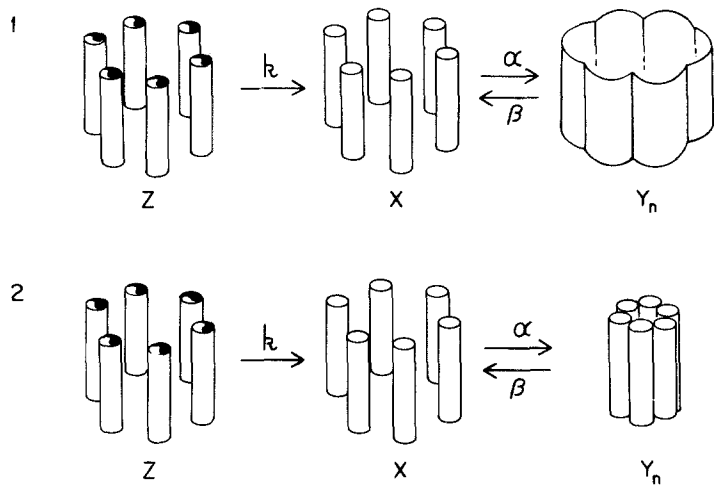


Fig. 6. Assembly models consistent with the conductance changes observed (Kinnally *et al.*, 1989). X corresponds to the first-phase channel and Y_n to the second-phase channel (see text). *Model 1.* The X channels formed by the activation of Z assemble into the Y_n channel so that the external walls of X become the external walls of Y_n. *Model 2.* The X channels assemble so that the Y_n channel is lined by the external walls of X.

However, both would explain the data equally well. Figure 6 represents n as 6. Using computer fits (Kinnally *et al.*, 1989), we have found that the data are most closely approximated by values of n of 6 ± 1. In our experiments, the kinetics of the second phase resemble the early kinetics of the conductance changes in planar lipid bilayers after the addition of *S. aureus* α-toxin (Belmonte *et al.*, 1987). The pore structure formed by the *S. aureus* toxin has been estimated from electron-microscope images to correspond to a hexamer (Füssle *et al.*, 1981). Oligomeric protein channels are commonly formed by three to six subunits (e.g., see Makowski *et al.*, 1977; Catterall, 1988).

Possible Role of Modulator Proteins

All the data collected in our experiments can be explained assuming one of the two models of Fig. 6. However, the various constants differ from patch to patch even in the same preparation (Kinnally *et al.*, 1989). We propose that endogenous modulators, possibly similar to the protein isolated from mitochondria by Holden and Colombini (1988), have a role in these processes. The modulator protein increases the voltage-induced rate and extent of closing of VDAC. In addition, synthetic polyanions have been shown to alter the rate and extent of closing of VDAC in a similar manner (Mangan and Colombini, 1987; Colombini, 1987).

We have presented arguments for a different amount of VDAC modulator in individual outer membrane patches (Tedeschi *et al.*, 1987; Kinnally *et al.*, 1989). This idea is based on our results which show a differential response of the individual patches to the synthetic polyanion poly[methacrylate, maleate, styrene (1 : 2 : 3)]. Presumably, some already contain modulator, and they already respond maximally. In addition, the polyanion seems to enhance the increase in conductance induced by negative potentials (see Fig. 7 of Tedeschi *et al.*, 1987), suggesting that the same or a similar modulator may favor the formation of the high-conductance channels as well.

Is VDAC a Component of the Two Channels?

VDAC represents the major component of the outer mitochondrial membrane and, as already mentioned, contributes about 20% of the total protein in that membrane in both *Neurospora* or in rat liver mitochondria. Since the function of the remaining protein is largely unknown, it is entirely possible that other proteins have channel activity, as indicated by recent reports (e.g., Benz *et al.*, 1989; Thieffry *et al.*, 1988 and private communication). An argument can also be made in favor of a role of VDAC in forming the two channels discussed here. For example, VDAC tends to aggregate in groups of six in the mitochondrial outer membrane (Mannella, 1987). Furthermore, it has a known affinity for concanavilin A, which favors the assembly of our high-conductance channel (Kinnally *et al.*, 1987).

Further Research

Study with mutants and reconstituted systems will undoubtedly answer some of the questions posed by this work. For example, yeast with altered VDAC, and strains totally deficient in VDAC, have been engineered (e.g., Blachly-Dyson *et al.*, 1989). A variety of new studies are beginning to appear, most recently using liposomes and patch-clamp techniques, suggesting the presence of channels other than VDAC in the outer mitochondrial membrane.

Acknowledgments

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