

# Incorporation of a Voltage Sensitive Pore from Guinea Pig Heart Mitochondria into Black Lipid Membranes and Characterization of Electrical Properties

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A pore from guinea pig heart mitochondria has been incorporated into BLM's (Black lipid membranes) in a highly oriented manner and its electrical properties studied. The pore shows multistate behaviour, the distribution of the pore between different conducting states being very sensitive to voltage. This has been proven by computation of single-pore experiments. Highest single pore conductance was 4.5 nSi in 1 M KCl, independent of voltage and with no detectable preference for cations or anions. The pore from guinea pig heart mitochondria reacts more sensitively to voltage than pores of mitochondria from other tissues so far incorporated into BLM's.

The outer membrane of mitochondria and Gram-negative bacteria is largely unspecifically permeant to molecules up to a defined molecular weight. For the outer membrane of *E. coli* an upper limit for permeation of 550 daltons has been reported [1], for the outer membrane of liver mitochondria values between 5000 and 12000 daltons [2, 3].

Pore-forming proteins from the outer membrane of rat-liver mitochondria have been characterized and incorporated into BLM's by different groups [4, 5].

The electrical properties of the newly incorporated channel are similar to those of channels from other sources which have also been incorporated into BLM's (rat liver mitochondria [4, 6], mitochondria from *Neurospora crassa* [7, 8], mitochondria from *Paramecium* sp. [9], outer membrane of *E. coli* [10, 11], outer membrane of *Salmonella typhimurium* [12], outer membrane of *Pseudomonas aeruginosa* [13]). These findings make it possible to place the new channel into a group of substances, the so called "Porins", following the nomenclature suggested by Nakae [1], or VDAC (Voltage dependent anion channel) described first by Colombini [4, 7, 9].

## Materials and Methods

BLM's have been produced in teflon-holes according to Mueller and Rudin [14] with a diameter

of 0.12 mm (for single channel experiments) and 1.0 mm (for macroscopic measurements). Thinning of the membrane was monitored by observing the interference-colors *via* a magnifying-glass and measuring the membrane-capacity. BLM's found to have a resistance greater than  $5 \times 10^7$  ohm/cm<sup>2</sup> were used for further experiments.

Asolectin (Type II-S from Sigma chemicals GmbH München) as a 1% solution in *n*-decane was used for membrane formation. All measurements were performed at room temperature.

Guinea-pig mitochondria were isolated in a slightly modified version of the procedure developed by Pande and Blanchaer [15].

Mitochondria were suspended in 1% Triton-x-100 (1 mg protein/ml final concentration as determined after Lowry) by vortexing for 1 min and subsequent sonication for 1 min (Econo-clean cleaning bath). Then aliquots of this solution (5–30 microliter) were added to the front-compartment (cis-compartment, 5 ml total volume). Addition of Triton-x-100 alone in the concentrations mentioned above did not affect membrane-conductance.

Processing of single pore recordings was done with a HP-1000 computer system.

## Results

The smallest entity of conductance, which we were able to incorporate into BLM's was 4.5 nSi in 1 M KCl. Figure 1 shows the incorporation of 3 pores into a BLM. Variation of the electrolyte-concentration between 0.1 M and 1.0 M KCl did not lead to a detectable saturation of single-channel conductance

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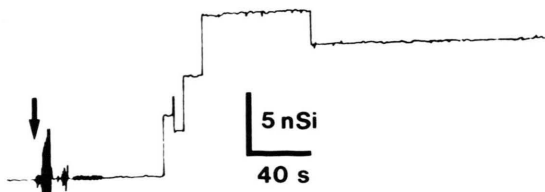


Fig. 1. A typical recording of incorporation of three pores into a BLM is shown. The arrow indicates addition of 10 microliter mitochondrial suspension to the cis compartment followed by stirring. Membrane potential ( $U_m$ ): +10 mV; membrane lipid: 1% asolectin in *n*-decane; electrolyte: 1 M KCl.

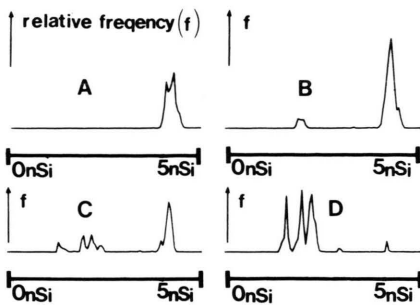


Fig. 2. Distribution of different open states of the pore in steady state conductance (B and D) and during the first 10 seconds after changing the membrane potential from 0 mV to a given value. Relative frequency of events was calculated by dividing the conductance range of 5 nSi into intervals of 50 pSi and counting the number of events in a given time period (total record length, 60 s, was 512 points) divided by the number of selected records. A:  $U_m$ : -10 mV, first 10 s after voltage jump. Number of records ( $n$ ) = 17. B:  $U_m$ : -10 mV, steady state conductance (s 30 to s 60 are taken for calculation).  $n$  = 17. A second conducting state with a conductivity of 2.0 nSi is indicated. C:  $U_m$ : -25 mV, first 10 s after voltage jump.  $n$  = 16. At least 5 different states of conductivity can be discerned (1.4 nSi; 2.0 nSi; 2.2 nSi and 2.4 nSi for the lower conducting states). D:  $U_m$ : -25 mV, steady state conductance (s 30 to s 60).  $n$  = 16. Number of counted open states with lower conductivity far exceed the number of counted total open states. Membrane lipid: 1% Asolectin in *n*-decane; electrolyte: 1 M KCl.

(0.49 nSi in 0.1 M KCl). The pore did not show just simple fluctuations between one closed and one open state, but instead a number of open states, which could be discerned through differences in conductivity (Fig. 2). Single pore-conductance was calculated from the initial step of current increase during incorporation of a pore into a BLM and from the initial current through the pore directly after applying a voltage-step of membrane potential rising from 0 mV to a defined value. Figure 3 shows single pore recordings at different membrane potentials.

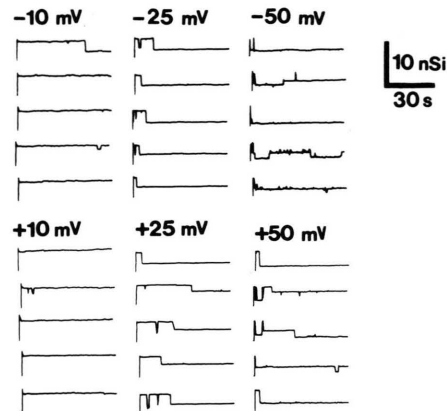


Fig. 3. Single Pore recordings are shown. Applied membrane potential is given above each row of recordings. Every record starts with a voltage-jump from 0 mV to the given value. Different open states with different conductivity can be clearly discerned. Membrane-lipid: 1% Asolectin in *n*-decane; electrolyte: 1 M KCl.

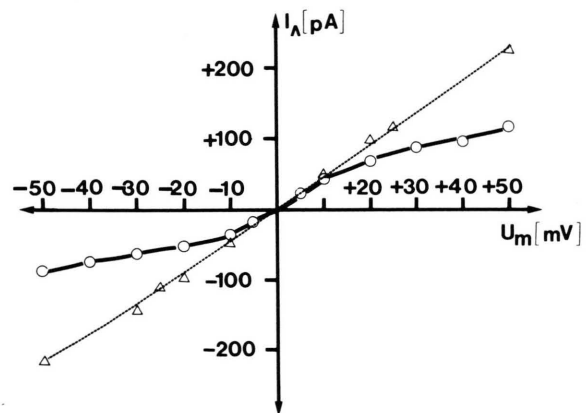


Fig. 4.  $\Delta$ : Initial single-pore current ( $I_{\lambda}$ ) as a function of membrane potential ( $U_m$ ). Graph was calculated with a least square fit (slope: 4.52; interception: +0.56 pA). Membrane lipid: 1% asolectin in *n*-decane; electrolyte: 1 M KCl.  $\circ$ : Average single pore current under steady state conditions, calculated from graph of Fig. 5.

Single pore conductivity was independent of magnitude and polarity of the applied membrane potential in the range of  $\pm 50$  mV (Fig. 4).

On the other hand, the fluctuations between different levels of conductance occurring during and after inactivation of membrane current were shown to be highly dependent on membrane potential (see Fig. 2). Using these data for the measurement of single pore conductance would indicate a voltage dependence of single pore conductance.

By establishing an electrolyte concentration gradient across the membrane, no preference for

cations or anions could be observed within experimental error. A computed reversal potential of +4 mV for single pore current could be observed for a gradient of 0.1 M KCl cis-side, 0.2 M KCl trans-side. With the assay used only stronger preferences of the pore can be detected with certainty for either cations or anions.

By incorporating many pores into a BLM and applying a stepwise increase of membrane potential

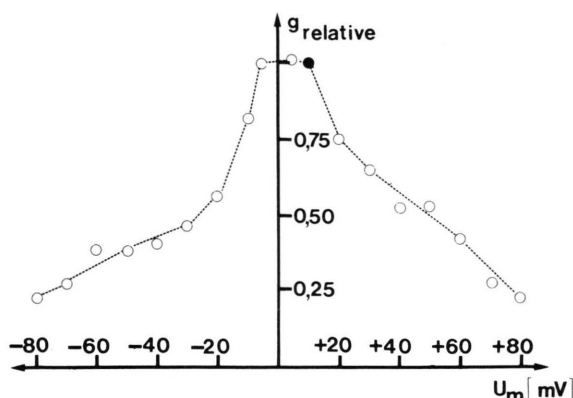


Fig. 5. Membrane steady state conductance of membranes with many pores incorporated ( $g_{\text{relative}}$ ) is plotted against membrane potential ( $U_m$ ). Averages from 5 different experiments. As reference point for the conductivity the value for  $U_m + 10$  mV (●) was taken and given the dimensionless value 1.0, to make the different experiments with different absolute conductivity levels comparable. Membrane lipid: 1% asolectin in *n*-decane; electrolyte: 1 M KCl.

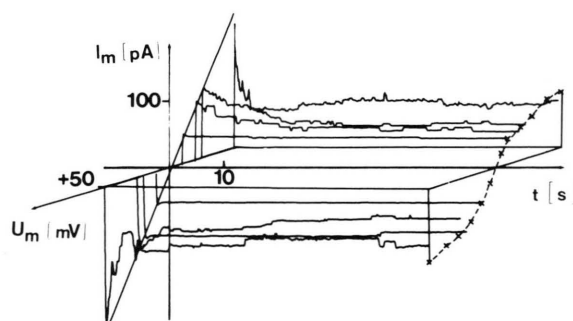


Fig. 6. 3-d plot of averaged single-pore recordings. At the point  $t = 0$  membrane potential was changed from 0 mV to a defined value. Currents at negative applied potentials are directed upwards due to recording situation (potential applying electrode in the *cis*-compartment, recording electrode in the *trans*-compartment). Note that currents at  $t = 0$  show linear relationship with voltage. x: steady state membrane current calculated from Fig. 4. Number of recordings taken for calculating averages for different membrane potentials: +10 mV:  $n = 17$ ; +20 mV:  $n = 8$ ; +25 mV:  $n = 13$ ; +50 mV:  $n = 7$ ; -10 mV: 17; -20 mV:  $n = 8$ ; 25 mV:  $n = 16$ ; -50 mV:  $n = 8$ . Membrane lipid: 1% asolectin in *n*-decane; electrolyte: 1 M KCl.

from 0 mV to a given voltage, inactivation of membrane current could be observed, dependent on the magnitude and polarity of the applied voltage. The steady state current after inactivation apparently depends also on magnitude and polarity of the applied voltage (Fig. 5). With respect to the polarity of the applied membrane potential, the asymmetry of inactivation and of steady state current can be confirmed by summation of single channel experiments (Fig. 6).

## Discussion

The large values for conductivity of the incorporated mitochondrial channel (4.5 nSi in 1 M KCl) and the fact that with increasing concentration of electrolyte the conductivity rises proportionally without saturation, lead us to assume a relatively large inner diameter of the pore. The specific conductance of 0.1 M KCl is 14 mSi/cm, that of 1 M KCl 110 mSi/cm at 25 °C. Assuming an equal conductivity within the pore and in the bulk medium gives a "specific conductivity" of the pore (conductivity of the pore/specific conductivity of the electrolyte) of  $3.5 \times 10^{-8}$  cm for 0.1 M KCl and  $4.1 \times 10^{-8}$  cm for 1.0 M KCl.

In addition, by making the very simplified assumption that the inner core of the pore is represented by a cylinder filled with bulk medium and that the length of this cylinder is 7.5 nm, one can calculate an inner diameter of 2.0 nm in 1 M KCl (calculation described in [13]).

Electron-microscopical studies of outer mitochondrial membranes showed pores with an inner diameter between 2.5 and 3.0 nm (Parsons, Williams and Chance [16]). Observations on outer membranes of plant mitochondria with x-ray diffraction indicate pores with an inner diameter between 1.8 and 2.0 nm (Manella [17]).

The pore described here did not show the features of channels highly selective for cations or anions, as for example the  $K^+$  channel from sarcoplasmic reticulum incorporated into BLM's by Miller [18] and coworkers (for review see [19]).

Other groups have been able to observe selectivity for ions ranging from anion-selectivity (VDAC, [9]) to cation-selectivity (Porin from *Pseudomonas aeruginosa*, [13]) with pores from other sources. The selectivity of single pore conductance was, however,

not always measured as defined above. Other groups also considered fluctuations between different open states.

The asymmetry of time- and potential-dependent inactivation of current indicates incorporation of the pore in a highly oriented manner. Potential-dependent inactivation of porines has been reported by a number of different authors [4–9, 11]. With some porines, however, no inactivation was observed [10, 12]. The pore we are dealing with shows one particular feature, it reacts very sensitively to potentials of some millivolts with partial inactivation. Only VDAC's [4, 7, 9] react in a similar way. With the other porines so far investigated, considerably higher potential steps are necessary [5, 6, 8, 11].

That the observed inactivation of current in BLM's with many incorporated pores is not due to different incorporated channels or some other unspecific effects can be proven by summation of single channel experiments. This shows clearly that inactivation and asymmetry of inactivation are due to fluctuations of the pore between different open states with different conductivity (see Figs. 2 and 6). These findings lend support to the speculation about a potential difference across the outer mitochondrial membrane regulating the conductance of the pores. An excess of negative charges located on the inner side of this membrane [20] can result in an intrinsic transmembrane potential. A Donnan

potential across the outer mitochondrial membrane is also imaginable. In the presence of a transmembrane potential, the studied pore from guinea-pig heart mitochondria likely regulates permeation across this membrane by changing inner diameter. Especially  $\text{Ca}^{2+}$ , even in small amounts, could affect the pore size within the outer membrane through saturation of negative charges located on the inner side of this membrane. Numerous cationic binding sites of phospholipid nature with preference for divalent cations are known to exist in cardiac submitochondrial particles [21].

General differences in the  $\text{Ca}^{2+}$ -transport system and ADP phosphorylation between liver mitochondria and heart mitochondria have been reported [22]. Our data show that also the pores in outer membrane of heart mitochondria show distinctly different properties than those from liver mitochondria. So far, the relevance of these findings for the mitochondrial functions in the heart remains open to speculation.

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