Volume 330, number 2, 206–210 FEBS 12968 September 1993

The mitochondrial permeability transition pore may comprise VDAC molecules

II. The electrophysiological properties of VDAC are compatible with those of the mitochondrial megachannel

Ildikó Szabó[a], Vito De Pinto[b], Mario Zoratti[a,*]

[a]*CNR Unit for the Physiology of Mitochondria, Department of Exp. Biomedical Sciences, Via Trieste 75, 35121 Padova, Italy*
[b]*Department of Pharmaco-Biology, CNR Unit for Mitochondria and Bioenergetics, Bari, Italy*

Received 16 July 1993

The electrophysiological properties of isolated mitochondrial porin (VDAC), reconstituted in planar bilayers or proteoliposomes, resemble those of the mitochondrial megachannel believed to be the permeability transition pore. In particular, a correspondence was found with regard to the voltage dependence: VDAC was driven to closed states by potentials of either sign, but the effect was not symmetrical; voltages negative in the compartment to which VDAC was added were more effective. The results are consistent with the hypothesis that the PTP may consist of two cooperating VDAC channels, plus presumably an adenine nucleotide carrier dimer and a third component known to be part of the mitochondrial benzodiazepine receptor.

Permeability transition; Patch-clamp; VDAC; Porin; Mitochondrial channel; Rat liver mitochondria

1. INTRODUCTION

As detailed in the accompanying paper [1], the properties of the mitochondrial megachannel (MMC), thought to coincide with the permeability transition pore (PTP) [2–5], suggest that it might be formed by a pair of VDAC molecules, along with other components. We report here on an electrophysiological study of the isolated rat liver mitochondria VDAC, and compare the results to those obtained with the MMC/PTP.

2. MATERIALS AND METHODS

Rat liver VDAC was prepared [6] as a solution (4.5–6 μg protein/ml by the Lowry method) in 3% Triton. As documented in Fig. 1, the preparation did not contain any contaminants visible in gel electrophoresis patterns, even after storage at −80°C for several months. The electrophysiological properties of VDAC were investigated using the planar bilayer technique [7], employing phosphatidylethanolamine (Avanti Polar Lipids) membranes, and by patch-clamping giant porin-doped azolectin liposomes. Incorporation of VDAC into the liposomes was achieved by filling the patch pipette with a suspension obtained by diluting the VDAC preparation 1000- to 5000-fold in the experimental medium. Azolectin (Sigma Type II) was purified by acetone precipitation. Appropriate controls were carried out to exclude artifacts due to residual Triton, in both experimental approaches. In the case of the bilayer experiments, each membrane was observed at various voltages for a few minutes prior to the addition of VDAC, to exclude the possibility of channel-like artifacts. Data were recorded on tape and analyzed off-line using the Axon's pClamp 5.5.1 program set. Experiments were conducted in symmetrical 0.5 M or 0.15 M KCl, 0.1 mM $CaCl_2$, 20 mM HEPES-K^+, pH 7.2. All experiments on VDAC-doped liposomes were conducted in low-salt medium. Voltages quoted are those of the compartment to which VDAC had been added (*cis* compartment or pipette interior).

*Corresponding author. Fax: (39) (49) 828 6576.

Abbreviations: pS, picoSiemens; nS, nanoSiemens; MMC, mitochondrial megachannel; PT, permeability transition; PTP, permeability transition pore; VDAC, voltage-dependent anion channel (mitochondrial porin); ADC, adenine nucleotide carrier; mBzR, mitochondrial benzodiazepine receptor; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

3. RESULTS

The behavior of VDAC in planar bilayers bathed in high-salt solutions was largely in agreement with the published reports (for review see [8]): VDAC mainly exhibited less-than-full size gating events, and closed under the influence of an applied potential of either sign above 20–30 mV. The activity was generally 'slow', but at times the channel flickered at a high frequency between states (not shown). We found the voltage dependence of VDAC to be a function of the salt concentration of the medium: the response to a voltage pulse of a given size became less marked as [KCl] decreased. A quantitative study of this behavior will be published elsewhere (Szabó et al., in preparation). Because of this property, and because the half-size state of the MMC is more evident at high salt concentrations, many of the experiments were conducted in 0.5 M KCl. We confirmed that

 Published by Elsevier Science Publishers B.V.

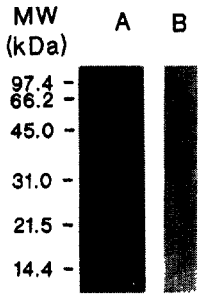

Fig. 1. SDS gel electrophoresis of the rat liver VDAC preparation used. Lane A, 2 μg protein, Coomassie-blue staining; lane B, 0.75 μg protein, silver staining.

acidic pH values enhance the voltage sensitivity of VDAC [9]. Thus an acid pulse at a given voltage led to channel closure, a behavior qualitatively similar to that exhibited by the PTP [5].

In the majority of the planar bilayer experiments with isolated VDAC we observed a definite asymmetry of the response to long-voltage pulses, as exemplified in Fig. 2. On average, the channels both responded more rapidly and closed more extensively when the applied voltage was negative in the cis compartment, indicating that the molecules inserted in one preferential direction. A more detailed study of this behavior is under way. It should be noted that even though the voltage dependence of VDAC in bilayers is often spoken of as being symmetrical about zero, asymmetry is actually often observed (see for example Fig. 4 in [10] and Fig. 6 in [11], and the brief discussion in [8]).

VDAC inserted into azolectin liposomes also behaved as a 'slow' channel (Fig. 3A), but often it showed more lively activity, as in the example of Fig. 3B. As expected, it operated in a variety of conductance levels besides the full-conductance one of approx. 650 pS in 150 mM KCl (Fig. 3A). Large conductance steps, presumably arising from cooperative gating, were often observed (Fig. 3A; see also Fig. 4). 1.0–1.3 nS activity (Fig. 3B), the preferred size of the MMC, was encountered in about 20% of the experiments. Some of the most common conductance variations corresponded to transitions often seen in mitoplasts (e.g. 530–550, 450–470, 310–350, 220–250 and 110–130 pS). When subjected to the voltage pulse protocol, for comparison purposes, VDAC in liposomes responded in a manner reminescent of MMC behavior [12]. Voltage pulses of the size normally used in experiments on the MMC (10–50 mV), in 0.15 M KCl, had only little effect, or resulted in the disappearance of shallow substate levels at negative potentials. Higher voltages however produced a pattern

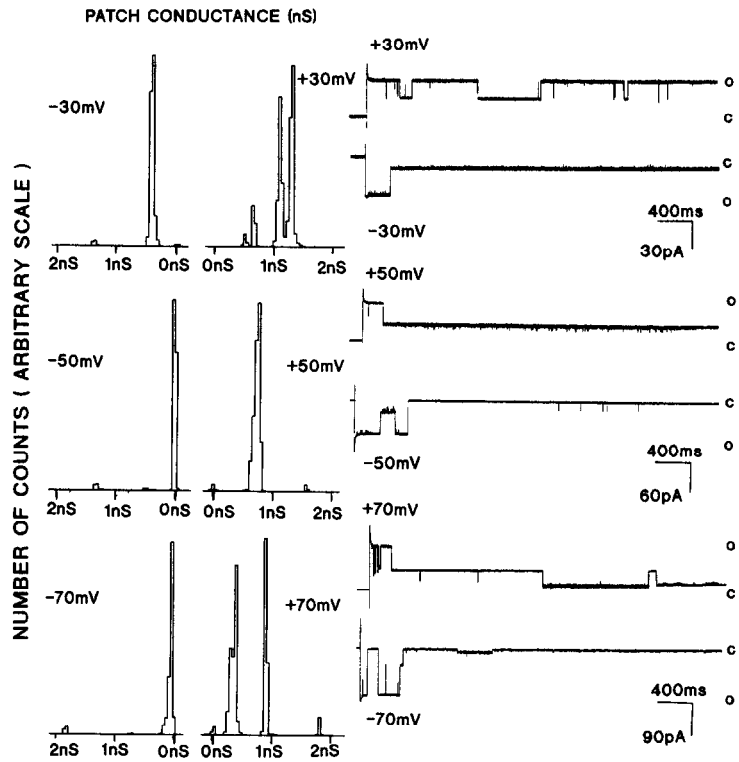

Fig. 2. Asymmetrical voltage dependence of VDAC in a planar bilayer. Part of a representative experiment in the 0.5 M KCl medium. Sampling frequency, 5 kHz; filter, 3 kHz. Left side: all-point conductance histograms obtained by dividing the current amplitude histograms by the applied voltage. The histograms were constructed from current records of the same length (about 10 s) for each couple of potentials of equal magnitude and opposite sign. Right side: current records covering the voltage jumps and the initial part of the intervals used to construct the corresponding histograms. The peaks following the onset of voltage are due to the capacitance-charging currents.

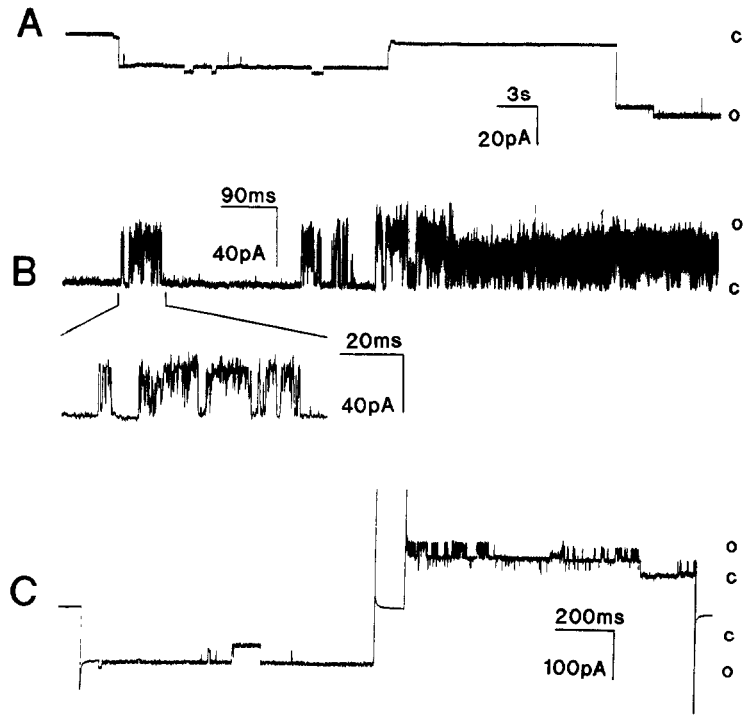

Fig. 3. Current records from VDAC-doped azolectin liposomes. Representative record from patch-clamp experiments in symmetrical 0.15 M KCl medium. (A) 'Slow' activity; V, −20 mV. Major conductance steps (from the left): 0.8, 0.65 and 1.7 nS; sampling frequency, 1 kHz; filter, 3 kHz. (B) 'Fast' activity; V, 30 mV. Sampling frequency, 10 kHz; filter, 7 kHz. The inset shows, on an expanded time scale, a burst with a conductance of 1.3 nS. (C) The behavior of VDAC under voltage pulse conditions. V, ± 100 mV; sizes of the conductance steps, 250–350 pS. Sampling frequency, 5 kHz; filter, 7 kHz.

similar to the one observed with mitoplast membranes: at negative voltages high and stable conductance levels predominated, while at positive voltages, frequent transitions to partially closed states took place (Fig. 3C).

The response of VDAC in liposomes to 'slow' voltage changes was studied in symmetrical 150 mM KCl, the conditions of most experiments on mitoplasts. As mentioned, under these conditions the VDAC was not strongly influenced by voltage. A voltage dependence could be observed in about $\frac{1}{3}$ of the experiments ($n = 15$). Also in this experimental system VDAC showed a tendency to behave asymmetrically, as exemplified in Fig. 4.

A series of determinations of channel selectivity (not shown) led us to the conclusion that both the MMC (in mitoplasts) and VDAC in liposomes possess only a limited, practically indistinguishable, ability to discriminate between K^+ and Cl^- (see also [3]).

4. DISCUSSION

The observations related above are consistent with the hypothesis that a VDAC dimer may be the pore-forming component of the PTP. Even in an artificial membrane, without the (putative) partners which concur to the formation of the PTP, VDAC exhibited a behavior (kinetics, voltage dependence, pH dependence, selectivity) compatible with that of the MMC of the mitoplasts. Quantitative differences, e.g. in the size of potentials needed to elicit a response, ought to be considered in the light of reports that the properties of isolated/reconstituted VDAC may differ from those of the molecule in its native membrane [13,14]. Importantly, VDAC can exhibit cooperative behavior, resulting in conductance steps larger than expected for the monomer, including the PTP-like 1.3 nS.

VDAC is generally thought to populate only the outer mitochondrial membrane, while the PTP/MMC connects the mitochondrial matrix and the environment, since the PT gives rise to osmotic phenomena and the MMC is often observed in patch-clamp experiments along with the '107 pS' channel [15], thought to reside in the inner membrane. However, the complete absence of VDAC from the inner membrane is far from proven. On the contrary, the available evidence would indicate that some is present. For example, Pon et al. [16] isolated membrane fractions from yeast mitochondria, observing that 4.8% of the total porin was present in the inner membrane fraction. In the same study, immunoelectronmicroscopy vs. VDAC in the whole mitochondria produced 4.6% of counts on the inner membrane, a remarkable coincidence. It should be stressed that the

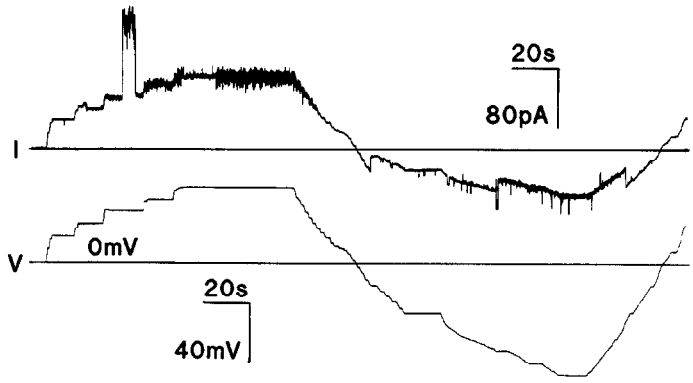

Fig. 4. Voltage depencence of VDAC in proteoliposomes. A representative patch-clamp experiment in symmetrical 0.15 M KCl medium. Current (I) and voltage (V) traces. The horizontal solid lines indicate the zero current and voltage levels. The average patch conductance is lower at negative potentials. The large event at left (at $V = 35$ mV) measures approx. 110 pA (3.1 nS) and may arise from the cooperative gating of 5 or more VDAC molecules. Sampling frequency, 50 Hz.

physiological transmembrane potential would by itself insure that the porin would remain closed.

Alternatively, the PTP might residue in contact sites. PTP-like conductances have been observed in contact site fractions [17]. EM photographs (not shown) indicate that our mitoplasts retain outer membrane fragments, and presumably contact sites, after osmotic shock. The presence of VDAC in mitoplasts prepared by this method has been reported [18], while other studies have indicated that exposure to Ca^{2+} leads to formation of contact sites [19,20]. Whether the induction of MMC activity by high Ca^{2+} might have something to do with this latter phenomenon ought to be considered. The PTP might well be preferentially localized at contact sites: mitochondria isolated from highly glycolytic tumor cells [21] or from glycogen-treated rats [22] are resistant to permeabilization upon Ca^{2+} loading. The same mitochondria show few contact sites [23,24]. An alternative explanation for these observations may be that the contact sites might act as a conduit for the passage of some VDAC molecules from the outer to the inner membrane.

In conclusion, we propose that the PTP consists of two cooperating VDAC molecules plus presumably two copies of the ADC and one or more copies of the 18 kDa protein which concurs to form the mBzR. These components would interact, giving rise to the observed cooperative behavior and to the various properties of the PTP. The large amount of available information on the permeability transition and on the presumed components of the pore should offer ample opportunities for a thorough verification of this proposal. It should be mentioned at any rate that the available data do not exclude the involvement of channels resembling, but not identical to, the VDAC. These might be, e.g. VDAC isoforms [25], or the peptide-sensitive channel described by the group of Thieffry and Henry (e.g. [26]).

Acknowledgements: We thank Prof. G.F. Azzone for support, Profs. G. Bathori and D. Wolff for discussions, Dr. A. Nicolli for technical help. This work was financed in part by the European Economic Community under the program 'Science'.

REFERENCES

[1] Szabó, I. and Zoratti, M. (1993) FEBS Lett. 330, 201–205.
[2] Szabó, I. and Zoratti, M. (1991) J. Biol. Chem. 266, 3376–3379.
[3] Szabó, I. and Zoratti, M. (1992) J. Bioenerg. Biomembr. 24, 111–117.
[4] Bernardi, P., Vassanelli, S., Veronese, P., Colonna, R., Szabó, I. and Zoratti, M. (1992) J. Biol. Chem. 267, 2934– 2939.
[5] Szabó, I., Bernardi, P. and Zoratti, M. (1992) J. Biol. Chem. 267, 2940–2946.
[6] De Pinto, V., Prezioso, G. and Palmieri, F. (1987) Biochim. Biophys. Acta 905, 499–502.
[7] Alvarez, O. (1986) in: Ion Channel Reconstitution (Miller, C., Ed.) pp. 115–130. Plenum Press, New York.
[8] Sorgato, M.C. and Moran, O. (1993) Crit. Rev. Biochem. Mol. Biol. 18, 127–171.
[9] Ermishkin, L.N. and Mirzabekov, T.A. (1990) Biochim. Biophys. Acta 1021, 161–168.
[10] Doring, C. and Colombini, M. (1984) Biophys. J. 45, 44–46.
[11] Colombini, M. (1989) J. Membr. Biol. 111, 103–111.
[12] Petronilli, V., Szabó, I. and Zoratti, M. (1989) FEBS Lett. 259, 137–143.
[13] Moran, O., Sciancalepore, M., Sandri, G., Panfili, E., Bassi, R., Ballarin, C. and Sorgato, M.C. (1992) Eur. Biophys. J. 20, 311–319.
[14] Bàthori, G., Sahin-Tòth, M., Fonyò, A. and Ligeti, E. (1993) Biochim. Biophys. Acta 1145, 168–176.
[15] Sorgato, M.C., Keller, B.U. and Stuehmer, W. (1987) Nature 330, 498–500.
[16] Pon, L., Moll, T., Vestweber, D., Marshallay, B. and Schatz, G. (1989) J. Cell Biol. 109, 2603–2616.
[17] Moran, O., Sandri, G., Panfili, E., Stuehmer, W. and Sorgato, M.C. (1990) J. Biol. Chem. 265, 908–913.
[18] Schwaiger, M., Herzog, V. and Neupert, W. (1987) J. Cell Biol. 105, 235–246.
[19] Brdiczka, D. (1991) Biochim. Biophys. Acta 1071, 291–312.
[20] vanVenetje, R. and Verkleij, A.J. (1982) Biochim. Biophys. Acta 692, 397–405.
[21] McIntyre, H.J. and Bygrave, F.L. (1974) Arch. Biochem. Biophys. 165, 744–748.

[22] Prpic, V., Spencer, T.L. and Bygrave, F.L. (1978) Biochem. J. 176, 705–714.
[23] Nelson, B.D. and Kabir, F. (1985) Biochim. Biophys. Acta 841, 195–200.
[24] Denis-Pouxviel, C., Riesinger, I., Buehler, C., Brdiczka, D. and Murat, J.-C. (1987) Biochim. Biophys. Acta 902, 335–348.
[25] Pavlica, R., Hesler, C.B., Lipfert, L., Hirshfield, I.N. and Haldar, D. (1990) Biochim. Biophys. Acta 1022, 115–125.
[26] Thieffry, M., Neyton, J., Pelleschi, M., Fevre, F. and Henry, J.-P. (1992) Biophys. J. 63, 333–339.