Volume 330, number 2, 201–205 FEBS 12967 September 1993

The mitochondrial permeability transition pore may comprise VDAC molecules

I. Binary structure and voltage dependence of the pore

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Received 16 July 1993

Electrophysiological records suggest that the pore responsible for the mitochondrial Ca^{2+}-dependent permeability transition (PTP), identified as the mitochondrial megachannel (MMC) observed in patch-clamp experiments, may comprise two cooperating porin (VDAC) molecules. We have re-investigated the voltage dependence of the megachannel, which favors the closed state(s) at negative (physiological) transmembrane potentials. This behavior confirms that MMC corresponds to the permeabilization pore. As detailed in the accompanying paper [(1993) FEBS Lett. 330, 206–210] this voltage dependence resembles that of VDAC. Alpidem, a ligand of the mitochondrial benzodiazepine receptor, which reportedly comprises VDAC, the adenine nucleotide carrier and a third component, elicited currents from silent mitoplast patches, suggesting that the benzodiazepine receptor may be identical to the PTP/MMC.

Permeability transition; Patch clamp; VDAC; Mitochondrial megachannel; Mitochondrial benzodiazepine receptor; Rat liver mitochondria

1. INTRODUCTION

It has been known since the early sixties that the inner membrane of Ca^{2+}-loaded liver or heart mitochondria loses its impermeability. This 'permeability transition' (PT), is due to the opening of a pore (PTP) with a diameter of about 3 nm (for review see [1]). While much evidence supports the identification of the PTP with the mitochondrial megachannel (MMC) observed in patch-clamp experiments [2–5], its molecular identity remains to be determined. The clues available so far consisted mainly in the effects on the PT by compounds (ADP, atractyloside, bongkrekate) known to interact with the adenine nucleotide carrier (ADC) [1,6–9], which have led to the proposal that the PTP may be formed by the ADC itself [10]. This possibility seems now less likely ([9,11], this paper), but it is hard to believe that the interactions mentioned above are due to a mere coincidence. It seems therefore likely that the ADC is one of the components of the PTP.

Other evidence suggests that the MMC/PTP may comprise VDAC as the pore-forming component. The size of the full-conductance MMC/PTP is twice that of VDAC, and its most common substate is the 'half-conductance' level [5]. The behavior of the channel (see below) suggested to us that the largest transitions might reflect the cooperative gating of two components. Haworth and Hunter have shown that each PTP contains two Ca^{2+}- and two ADP-binding sites, with strong cooperativity effects [7,12]. Cross-linking studies have suggested that a portion of VDAC forms dimers in the outer membrane of yeast mitochondria [13], as is also the case in complexes with Triton [14]. The ADC is known to function as a dimer [15]. Evidence has been obtained for the presence of complexes formed by the ADC and VDAC, residing in the contact sites and possibly involved in their formation [16,17]. The peripheral benzodiazepine receptor (mBzR) [18] has been reported to consist of VDAC, the ADC and an 18-kDa protein [19]. Recently, benzodiazepines have been reported to affect the activity of mitochondrial channels [20]. The PT is unleashed by depolarization [21,22], a suggestive finding since VDAC in planar membranes bathed in high-salt media adopts the fully open state only at potentials close to zero [23]. Both the VDAC [24] and the MMC [25] exhibit a wealth of subconductance states. Support for the idea that VDAC might contribute to form the PTP also comes from the near coincidence of the estimates of the sizes of the PTP, from experiments monitoring the osmotic effects of solutes [26], and of VDAC, from ultrastructural studies [27].

These considerations have prompted us to begin an

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

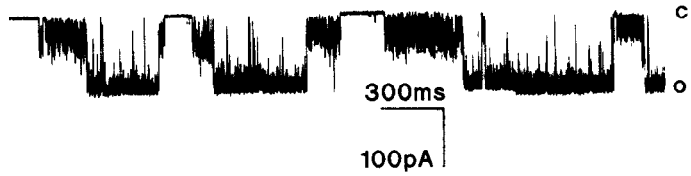

Fig. 1. Representative current record from a mitoplast, illustrating the 'half-size' state and cooperation between two full-size megachannels. (A) MMC activity (4.35 nS) in symmetrical 0.5 M KCl. Gating events often involve passage through a flickering half-size conductance (2.2 nS), corresponding to that of the fully open VDAC in this medium. V, −30 mV; sampling frequency, 5 kHz; filter, 7 kHz.

investigation of the issue, by carrying out a comparative study of the electrophysiological properties of the VDAC and of the MMC/PTP, and of the effects of a mBzR ligand on the conductance of mitoplast patches. This paper mainly deals with the voltage dependence of the permeabilization pore and the effects of Alpidem, while its companion paper [11] reports on the investigation of the properties of purified VDAC. The results are consistent with the hypothesis that a VDAC dimer may be the channel-forming component of the mitochondrial permeability transition pore. Part of this work has already been communicated at meetings [28,29].

2. MATERIALS AND METHODS

The mitochondrial megachannel was studied in patch-clamp experiments on mitoplasts, conducted essentially as previously described [2,5,25]. Voltage scans were obtained by manually turning the V_{hold} knob of the EPC-7 control unit. 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. Voltages quoted are those of the bath electrode, zero being conventionally assigned to the pipette electrode.

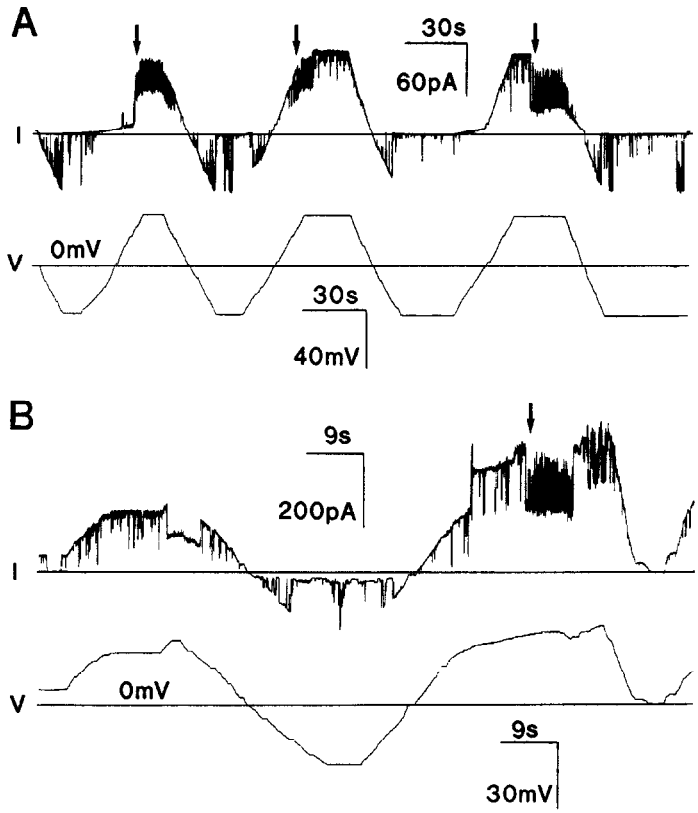

Fig. 2. Voltage dependence of the megachannel in mitoplast patches under voltage scan conditions. Current (I) and voltage (V) tracings. The horizontal solid lines indicate the zero current or potential levels. The arrows point to periods of intense 'flickering' between conductance states. Lower conductance channels were also present. Sampling frequency, 50 Hz. Medium: (A) 0.15 M KCl, 0.1 mM $CaCl_2$, 20 mM HEPES, pH 7.2; (B) the same, but [KCl] was 0.5 M.

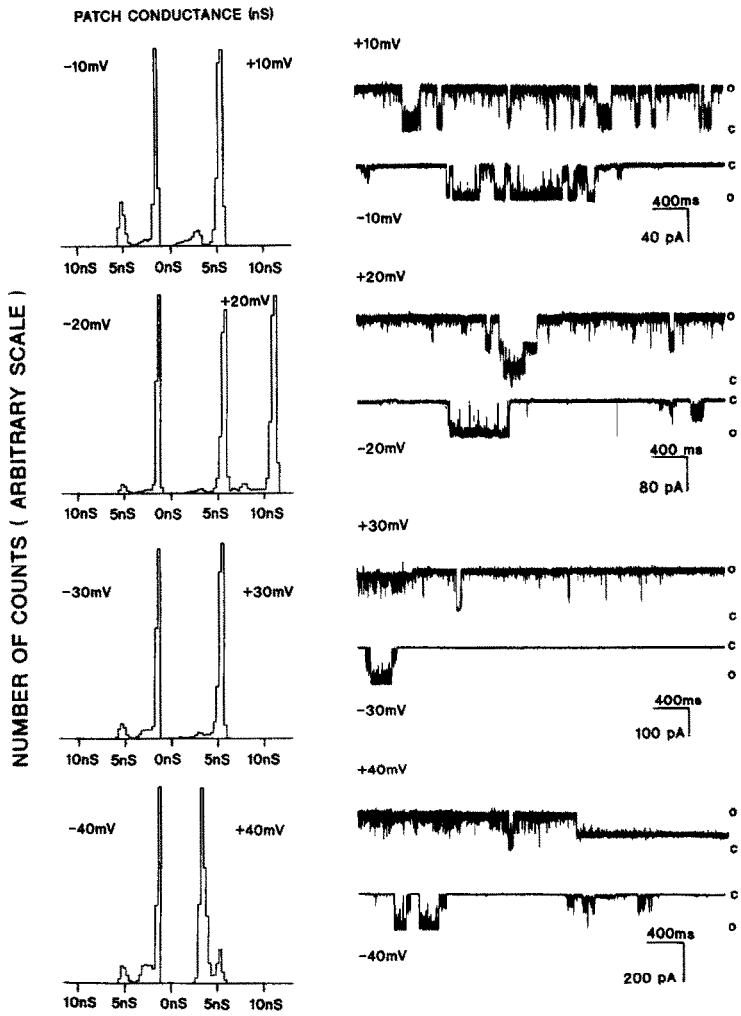

Fig. 3. The voltage dependence of the mitochondrial megachannel under steady applied potentials. A representative patch-clamp experiment on a mitoplast. Medium, symmetrical 0.5 M KCl, 0.1 mM $CaCl_2$, 20 mM HEPES, pH 7.2; sampling frequency, 5 kHz; filter, 7 kHz. The potential was switched from zero to the desired value, and held for about 40 s. Each pulse was followed by an approx. 1-s period at zero voltage. Two megachannels were active during the pulse at +20 mV. Left side: all-point patch conductance histograms obtained by dividing the current amplitude histograms resulting from each voltage pulse by the applied voltage. Conductances plotted to the right (left) of zero were measured at positive (negative) applied potentials. Right side: representative current traces.

3. RESULTS

The PTP appears in patch-clamp experiments as a relatively 'slow' channel with a conductance of about 4.3 nS in 500 mM KCl (Fig. 1). One of its characteristics is the wealth of substates, the most important of which is one of half-size which often appears as an intermediate stage, with 'flickering' fast transitions to the closed state, during openings and closings (Fig. 1 and [5]). As will be detailed elsewhere, the probability of the channel operating in the 'half-conductance state' increased steeply with medium [KCl]. Fig. 1 qualitatively illustrates the fact that the 'open channel noise' was higher in the half-conductance level than in the full-conductance state. Along with statistical considerations on the gating pattern, this indicates that the currents are not conducted by two independent channels. Rather, it appears that when both 'halves' of the channel are conducting, they reciprocally stabilize the open conformation. This behavior is consistent with a model envisioning a cooperative interaction of two porin molecules. The channels on rare occasions displayed more extended cooperation, resulting in gating steps in the range of a few nS in 150 mM KCl (not shown).

When subjected to 1- or 2-s pulses of voltage with alternating polarity, separated by 100 ms 'rest' intervals at zero mV, the PTP/MMC behaves in a characteristic manner: at negative voltages it tends to occupy almost exclusively the maximum conductance state, while at increasingly positive potentials it spends an increasing fraction of time in lower levels [25]. In previous work, we have employed almost exclusively this 'pulsed' protocol. A somewhat different picture of the voltage dependence emerged from experiments involving slow

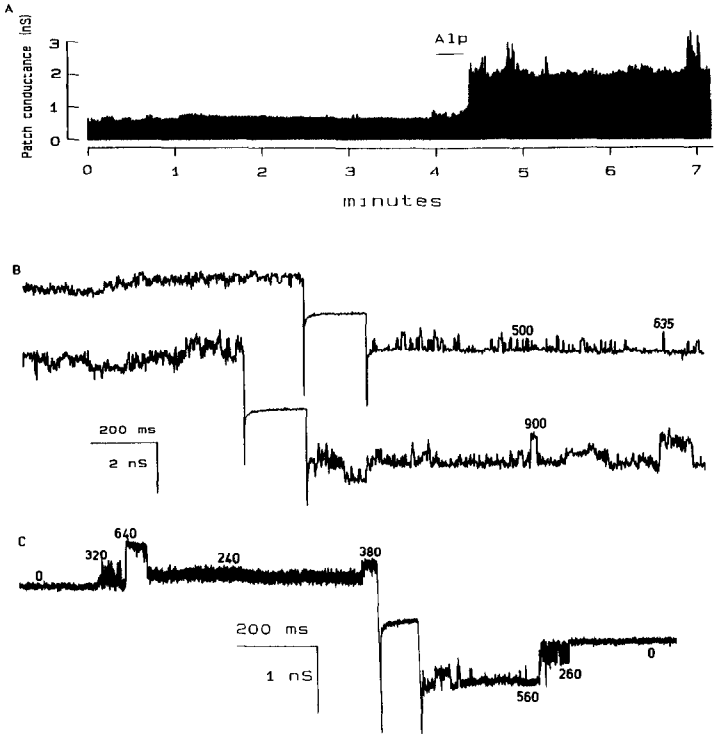

Fig. 4. The effect of Alpidem on silent mitoplast membranes. (A) Plot of the total (leaks not subtracted) patch conductance, averaged over 0.8-s intervals, vs. time. Where indicated, 2 μM Alpidem was added. The average conductance increase was close to 1.3 nS, corresponding to one full-conductance MMC/PTP. (B) Examples of current records after the addition of Alpidem. The short voltage pulse protocol (± 20 mV) was applied. Numbers refer to the conductance (pS) of the steps close to them. (C) An isolated burst of Alpidem-induced activity from another experiment. Voltage pulse protocol. V, ± 30 mV; sampling frequency, 10 kHz; filter, 5 kHz.

voltage scans (Fig. 2) or prolonged periods at a given potential (Fig. 3). At potentials close to zero, the mega-channel mainly occupied the fully open state. As the voltage was increased in the negative direction beyond about −20/30 mV, the MMC was driven into (a) long-lasting closed state(s). The process was 'slow': a few seconds were required, on average, for the channel to close. The voltage dependence was not symmetrical about zero: at positive potentials the channel showed a lower tendency to occupy the completely closed state(s) than at the corresponding negative potentials (see the traces in Fig. 2 and the conductance histograms of Fig. 3). Instead, in the positive range it often flickered rapidly between the fully open and partially or fully closed states. This behavior, pointed out by arrows in the current traces of Fig. 2, gives rise to the asymmetry observed in 'pulsed' experiments [25]. Both the 'pulsed' behavior and the 'slow' behavior were routinely observed in the same membrane patch by applying the two experimental protocols.

As part of this study, we have examined the effect of Alpidem, a benzodiazepine analog with nM affinity for the mBzR [30], on mitoplast membrane patches. Consistent with the presence of mBzRs, Alpidem (15 nM–2 μM) in 23 out of 30 experiments elicited high, flickering currents which were tentatively identified as being conducted by the VDAC/MMC on the basis of the size of the conductance steps (Fig. 4). No such currents developed in control experiments on azolectin liposomes (n = 5) and bacterial protoplasts (n = 4).

4. DISCUSSION

The identification of the MMC as the PTP is based on the similarity of the effects of a number of agents on the PT and on the channel observed in patch-clamp experiments. An apparent discrepancy concerned the voltage dependence of the permeabilization of mitochondria, due to pore opening, and of the MMC. Depolarization has been reported to be one of the causes of the former [21,22], while the MMC had been reported to remain in the fully open state open at negative (physiological) potentials [25,31]. The data presented above explain, at least in the case of our data, this discrepancy: the fully open state of the MMC is actually favored at low potentials of either sign. Physiological potentials bring about its closure, but the process is 'slow' at the potentials we employed: a few seconds usually intercur between application of the negative voltage and closure. Therefore, this behavior was missed in experiments involving the short-pulse protocol; however, it becomes noticeable if the length of the pulses is suitably increased

(not shown). Positive, i.e. unphysiological, potentials, which do not normally occur in experiments with suspensions of mitochondria, appeared to favor 'fast' partial closures of the MMC, resulting in a lower-than-maximal time-averaged conductance. The average conductance (or the conductance of an ensemble of channels) is therefore expected to decline on both sides of zero voltage, and more steeply at negative potentials (see Figs. 2 and 3). This behavior calls to mind that reported for VDAC-doped membranes [23]. The accompanying paper [11] shows that the analogy includes the asymmetry of the voltage dependence.

The observation that Alpidem elicits currents, most likely conducted by the PTP, from silent patches, together with the report of Kinnally et al. [20], suggests the identification of the PTP with the peripheral benzodiazepine receptor [18,19]. Finally, the electrophysiological recordings (Fig. 1) provide information on the spatial and functional organization of the pore, which seems likely to arise, in most cases, from the interaction of a porin dimer with a dimer of the ADC.

Acknowledgements: We thank Drs. S. Vicini and K. Krueger for suggesting the Alpidem experiments, Prof. G.F. Azzone for support, Profs. P. Bernardi, V. Petronilli, G. Bathori and D. Wolff for discussions, Mr. L. Pregnolato for technical help. This work was financed in part by the European Economic Community under the program 'Science'.

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