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Tubulin-blocked state of VDAC studied by polymer and ATP partitioning

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Abstract

Recently reported functional interaction between voltage-dependent anion channel of the outer mitochondrial membrane, VDAC, and dimeric tubulin is observed as a reversible channel blockage. Using partitioning of poly-(ethylene glycol)s of different molecular weights and reversal potential measurements, we probe the size and ion selectivity of the fully open and tubulin-blocked states of VDAC reconstituted into planar lipid bilayers. While the effective radius of the channel decreases by only a factor of 1.34 ± 0.15 , the selectivity reverses from initially anionic to cationic. Directly measuring ATP partitioning we demonstrate that these changes prohibit ATP from entering the channel in its tubulin-blocked state.

Keywords

voltage-dependent anion channel; selectivity; PEG; mitochondria; microtubules; planar lipid bilayers

1. Introduction

One of the most abundant proteins in the cytosol of the majority of eukaryotic cells, dimeric tubulin, was recently found to be a potent inhibitor of voltage-dependent anion channel of the outer mitochondrial membrane, VDAC [1, 2]. The tubulin-VDAC interaction is seen as reversible transitions of the channel, reconstituted into planar lipid membranes, between its open and tubulin-blocked states. The inhibition is highly voltage-sensitive and, depending on the potential across the mitochondrial outer membrane might require micro-molar to nano-molar concentrations of tubulin. Experiments with isolated mitochondria suggest that VDAC-tubulin interaction is functionally important in regulation of mitochondrial respiration [2, 3]. The tubulin-blocked state is still highly ion-conductive (about 40% of the open state conductance in 1 M KCl), which may imply that VDAC inhibition by tubulin is limited by the value of this residual conductance. It is believed, however, that the major role of VDAC is regulation of ATP/ADP exchange [4–6] and not the flux of small ions, so what is really important is the effect of tubulin blockage on the nucleotide transport.

There is a long list of different compounds affecting VDAC voltage-gating (see [4, 5, 7]) where polyanions such as König's polyanion and dextran sulphate are the most potent

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inhibitors of VDAC [8, 9]. In particular, König's polyanion was shown to inhibit adenine nucleotide transport in isolated mitochondria [8] and cells [10]. However, the regulatory action of tubulin was recognized only very recently [2, 11]. To understand the functional significance of the VDAC-tubulin interaction, we investigate the major biophysical properties of the tubulin-blocked state.

In the present study we apply three approaches to assess functional features of the blocked state. We first estimate the change in the characteristic radius of VDAC upon its blockage by tubulin using polymer partitioning into the channel [12, 13] in both states. An exhaustive reference list concerning this approach can be found in a recent publication from our laboratory [14]. The essence of the approach is to analyze penetration of differently sized poly(ethylene glycol)s, PEGs, into the channel water-filled pore by measuring its conductance in the presence of these polymers. The channel conductance responds differently to PEGs of different molecular weight, with polymers that are small enough to partition into the pore reducing its conductance in a weight-dependent manner. Based on the characteristic molecular weight of polymer that separates partitioning from exclusion, we conclude that the effective cross-sectional area of the channel is reduced by a factor of two as a result of the blockage.

Second, we analyze the blockage-induced change in the channel small-ion selectivity at salt concentrations close to physiological. We show that selectivity of the channel reverses its sign: from predominantly anionic selectivity in the open state it shifts to cationic selectivity in the tubulin-blocked one.

Third, we estimate ATP partitioning into both open and tubulin-blocked channel. We find that while in the open state the addition of ATP reduces channel conductance, it does not change the conductance of the tubulin-blocked state. We conclude that ATP electrostatically and, at least partially sterically, is excluded from the tubulin-blocked state of VDAC.

2. Materials and methods

The procedure of VDAC reconstitution into lipid bilayers was previously described [2, 15]. Bilayers were formed from diphytanoyl phosphatidylcholine (Avanti Polar Lipids, Inc. Alabaster, AL). If not stated otherwise, 1 M KCl aqueous solutions buffered with 5 mM Hepes at pH 7.4 were used. Potential is defined as positive when it is greater at the side of VDAC addition (*cis*-side). After VDAC channels were inserted, tubulin was added to both sides of the membrane under constant stirring for 2 min. Consecutively, poly(ethylene glycol)s of various molecular weights (Sigma) were added to the final concentration of 15% (w/w). Solution of ATP (Sigma) in 1 M KCl and pH adjusted to 7.4 was introduced to the chamber compartments by perfusion. Currents were recorded and analyzed as previously described [2] (see also Supplemental material).

3. Results and discussion

The effect of PEG of three different molecular weights on VDAC open and tubulin-blocked states is shown in Figure 1. In the presence of PEG 106 (Fig. 1A) conductances of both states are reduced by polymer addition. This is related to polymer partitioning into the channel pore, which displaces ions and increases solution viscosity (Fig. 1, dotted lines *Open* and *Open (PEG)*). The conductance of the tubulin-blocked state is also notably decreased in the presence of PEG 106, but to a much lesser degree by PEG 400 (Fig. 1A, B, dotted lines *Blocked* and *Blocked (PEG)*), suggesting that it is too narrow for molecules of PEG 400 to partition significantly. In the case of PEG 10000 (Fig. 1C), the open and tubulin-blocked states show some increase of conductance, meaning that this polymer is

effectively excluded from both channel states. It is also noteworthy that PEG increases the interaction between tubulin and VDAC, leading to more frequent blockage events.

Figure 2 summarizes experiments on polymer partitioning interpolated to zero voltage. In both, open and tubulin-blocked states, the ratio increases from a value close to the ratio of solution conductivities as changed by polymer addition (interrupted straight line at about 0.6) to a value that is slightly above 1.0. The data-points for the tubulin-blocked state are shifted to the left, in the direction of smaller polymer molecular weights. This suggests that the characteristic size of the blocked state is smaller since it is able to exclude polymers more effectively.

To quantify the partitioning we use the approach [13] outlined in sufficient detail in a recent publication [14]. We apply the following empirical formula to fit the data:

$$g(w)/g_{noPEG} = g(w)_{max}/g_{noPEG} - \chi \exp(-(w/w_0)^\alpha), \quad (1)$$

where $g(w)/g_{noPEG}$ is the ratio of the channel conductance in the presence of PEG with molecular weight w to its conductance in polymer-free solution, and χ is the parameter characterizing the relative amplitude of the change in the channel conductance between the regimes of complete exclusion, $g(w)_{max}$, and full penetration, $g(w)_{min}$:

$$\chi = (g(w)_{max} - g(w)_{min})/g_{noPEG}. \quad (2)$$

Choosing $g(w)_{max}/g_{noPEG} = 1.07$ and $g(w)_{min}/g_{noPEG} = 0.54$ to account for the conductance behavior at the highest and lowest polymer molecular weights, we fit Eq. (1) to the experimental data to obtain the characteristic polymer weight w_0 which separates regimes of polymer penetration and exclusion, and parameter α which characterizes the sharpness of the transition between the two regimes.

As a result we arrive to the following values for the characteristic polymer molecular weight w_0 : 679 ± 47 for the open and 417 ± 31 for the tubulin-blocked state, with the values of α equal to 1.08 ± 0.1 and 1.15 ± 0.14 , correspondingly. Since the hydrodynamic radius of PEG in water scales as polymer molecular weight in power $3/5$ [16], we conclude that the ratio of the channel radius in the open state to that in the blocked state is 1.34 ± 0.15 ; the cross-sectional areas differ by a factor of 1.8 ± 0.2 . Thus, polymer partitioning suggests that the aperture of VDAC in the tubulin-blocked state is reduced by about a factor of two. The size of the largest polymer that partitions into the open state, PEG 1000, for the reasons that are not clear at the moment is smaller than that of PEG 3400 obtained by liposome swelling method [17]. PEG partitioning into the open state of VDAC described in the present study agrees with the earlier reported results [18] if one takes into account that the higher polymer concentration used in Ref. [18] drives the polymer partitioning curve to larger characteristic molecular weights [19]. It is also interesting to compare our data with experiments on the osmotically-driven transition of VDAC to its closed state [20] where the estimate for the volume of water expelled from the channel suggests a more profound structural change. Importantly, conductance distribution of the closed states of VDAC is very broad [9, 17, 21]; it depends on the magnitude and duration of applied voltage stimuli (e.g., slow triangular voltage wave versus steady state voltage). This is drastically different from the tubulin-induced blocked state described here.

It is believed that regulation of ATP/ADP fluxes is the major function of VDAC [4, 5]. Because at physiological salt concentrations ATP is a multi-charged anion, it is important to characterize the ionic selectivity of VDAC blocked state at the salt conditions close to those

of physiologically relevant. Here we used 150 mM vs. 50 mM gradient of KCl to determine the reversal potentials of the open and blocked states of the channel (Figure 3). As expected, the voltages corresponding to zero current at this KCl concentration gradient, the so-called reversal potentials, are different for the open and tubulin-blocked states. They are 15.5 mV and -13.7 mV, correspondingly, meaning that the channel selectivity is reversed from $t^+ = 0.2$, $t^- = 0.8$ (where t^+ and t^- are cationic and anionic transport numbers, see Supplemental material) in the open state favoring anions, to $t^+ = 0.75$, $t^- = 0.25$ in the tubulin-blocked state favoring cations.

According to the proposed model [1, 2], blockage of VDAC by tubulin is interpreted as penetration of one of the tubulin negatively charged C-terminal tails (CTT) into the VDAC net positive pore. Experimental evidence supporting this model is that tubulin with truncated CTT does not block VDAC [2]. The negatively charged tail shifts the balance of charges in the VDAC pore towards net negative charge, which explains the slight cationic selectivity of the tubulin-blocked state. It should be noted that this selectivity reversal is analogous to that observed at channel transition to the voltage-induced closed states [9, 21], but, similarly to conductance, is well defined and does not show the variability inherent for voltage-induced closed states. Comparison of these properties of voltage-induced closed states and tubulin-blocked state favor CTT permeation block model over the tubulin enhanced voltage gating.

A bulk of recent research on “wide” channels (see Refs. [22-24] and references therein) demonstrates that their ionic selectivity is mostly of electrostatic origin. Therefore, change in the channel selectivity should be much more pronounced for the multi-charged ATP than for singly-charged chloride anion. Taken together with the additional steric hindrance in the blocked state, our findings suggest that the tubulin-blocked state should be virtually impermeant for ATP.

To support this assertion, we performed direct measurements of ATP partitioning into VDAC, following approach described earlier [15, 25]. It was shown that ATP added to the membrane-bathing solution of 1 M NaCl decreased its specific conductivity, but, because of ATP binding to the VDAC pore, it reduced the channel conductance to a larger degree [15]. To check if this would be the case with the tubulin-blocked state, we measured channel conductance in the presence of both tubulin and ATP. The results shown in Figure 4 demonstrate that a measurable conductance decrease is observed only for the open state of the channel. The effect is somewhat smaller than that reported earlier for VDAC of a different origin (*Neurospora crassa*) [15], but is in a qualitative agreement with the earlier findings. Symmetric addition of 100 mM ATP decreases channel conductance in the open state by about 10% but, within the accuracy of our measurements, does not change the conductance of the tubulin-blocked state. This suggests that ATP is excluded from the blocked state, thus supporting our arguments based on the changes in channel radius and small-ion selectivity.

4. Conclusions

We find that blockage of VDAC by tubulin decreases its aperture approximately by a factor of two as probed by differently sized PEGs (Figs. 1 and 2). Even more importantly, the blockage reverses channel anionic selectivity for small singly-charged ions to cationic (Fig. 3). For the multi-charged and bulkier ATP molecule direct assessment of its effect on the channel conductance in the two states (Fig. 4) shows that, within the accuracy of our measurements, ATP does not partition into the blocked state. Combined together, these arguments allow us to conclude that the tubulin-blocked state of VDAC is virtually impermeant for ATP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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List of Abbreviations

VDAC	voltage-dependent anion channel
PEG	poly-(ethylene glycol)

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Figure 1.

Traces of ion current through single VDAC channels in the presence of 10 nM tubulin as changed by addition of PEG 106, PEG 400, and PEG 10000. Left traces: channel blockage by tubulin in polymer-free solution; right traces: effect of PEG addition on the channel conductive states. Membrane-bathing solution contains 1 M KCl with 5 mM HEPES, pH 7.4. The dashed lines at the bottom indicate zero current. The records were digitally filtered at 1 kHz using Bessel algorithm.

Figure 2.

The relative changes in VDAC conductance induced by addition of 15% (w/w) PEG of different molecular weight. The ratio of channel conductance in the presence of a particular PEG to its conductance in polymer-free solution is plotted as a function of PEG molecular weight. Triangles and circles correspond to the open and tubulin-blocked states of the VDAC, respectively. The dotted line at 0.6 corresponds to the ratio of bulk solution conductivities with and without polymers. Solid lines through the experimental data represent the best fits using Eq. (1).

Figure 3.

Current-voltage relationships for a single channel in the open (triangles) and tubulin-blocked (circles) states in a membrane separating 150 mM (cis) and 50 mM (trans) KCl solutions (2 mM HEPES, pH 7.4). Blockage of VDAC by tubulin reverses the original anionic selectivity of the channel to the cationic one as is evidenced by the change in the sign of the reversal potential.

Figure 4. Effect of addition of 100 mM ATP on VDAC conductance in open and tubulin-blocked states. The upper three groups of data-points show that addition of ATP reduces conductance of the open state due to ATP partitioning into the channel [15, 25]. Three lower groups of data-points demonstrate that tubulin-blocked state of VDAC is not affected by ATP suggesting that this state is impermeable for ATP.