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Rapid report

Interaction of mitochondrial voltage-dependent anion channel from rat brain with plasminogen protein leads to partial closure of the channel

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Abstract

Voltage-dependent anion channel (VDAC) is reported to be the receptor for plasminogen kringle5. In this paper, the interaction of VDAC from rat brain mitochondria with plasminogen protein has been investigated through bilayer electrophysiological studies. We report for the first time that interaction of plasminogen with VDAC leads to partial closure of the channel on a lipid bilayer. This could be a mechanism of modulation of VDAC gating in a cellular system.
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Keywords: Rat brain VDAC; Plasminogen protein; BLM; Electrophysiology; Gating; Regulation

Voltage-dependent anion channel (VDAC) is an abundant protein in the outer mitochondrial membrane, which forms large voltage-gated pores in planar lipid bilayer, and acts as the pathway for the movement of metabolites like ATP in and out of the mitochondria by passive diffusion [1,2]. VDAC has been found to play a crucial role in apoptotic cell death [3,4]. Recently it has been reported that VDAC plays a role in cellular communication, especially synaptic communication [5]. Although some data are available on modulation of VDAC, a lot more is necessary to understand its gating mechanism. We have demonstrated previously that VDAC can be phosphorylated in vitro by the catalytic subunit of protein kinase A [6] and it reduces the channel current (magnitude) as per bilayer electrophysiological data [7]. Recent studies suggest that VDAC is the receptor for plasminogen [8]. Human plasminogen (pg) contains structural domains called kringles as obtained by proteolytic cleavage (kringles 1–5). Out of these, K5 is reported to interact with VDAC [8]. In the present work, we demonstrate, for the first time, through bilayer electrophysiological experiments that plasminogen protein leads to closure of VDAC channel.

VDAC purified from rat brain mitochondria using the method of De Pinto et al. [9], was reconstituted into the planar lipid bilayer membrane (BLM) made up of Plant Phosphatidyl choline and cholesterol (obtained from Avanti Polar Lipids, Birmingham, AL, USA) according to the method of Roos et al. [10]. Multichannel membrane recording of VDAC was performed (on BLM) in symmetric bath solution containing 500 mM KCl, 10 mM Hepes (pH7.4), and 5 mM $MgCl_2$ (obtained from Sigma Chemical Co., St. Louis, MO). Recombinant human plasminogen protein (a gift from Dr. Girish Sahni, Institute of Microbial Technology, Chandigarh, India) was obtained by overexpressing the protein and its purity was confirmed by getting a single band in SDS-PAGE. To see the effect of interaction of plasminogen protein with VDAC, a final concentration of 0.128 μg/ml of purified human plasminogen protein was added to the cis chamber, the solution was stirred for 30 min and current traces were recorded at different clamping potentials (-50 to $+50$ mV). In order to find out if plasminogen interaction has any effect on the channel conductance, the abovementioned electrophysiological recordings of native and plasminogen bound VDAC were compared.

When the channel was treated with plasminogen, the channel current is reduced significantly as demonstrated by continuous current traces, as well as their representative

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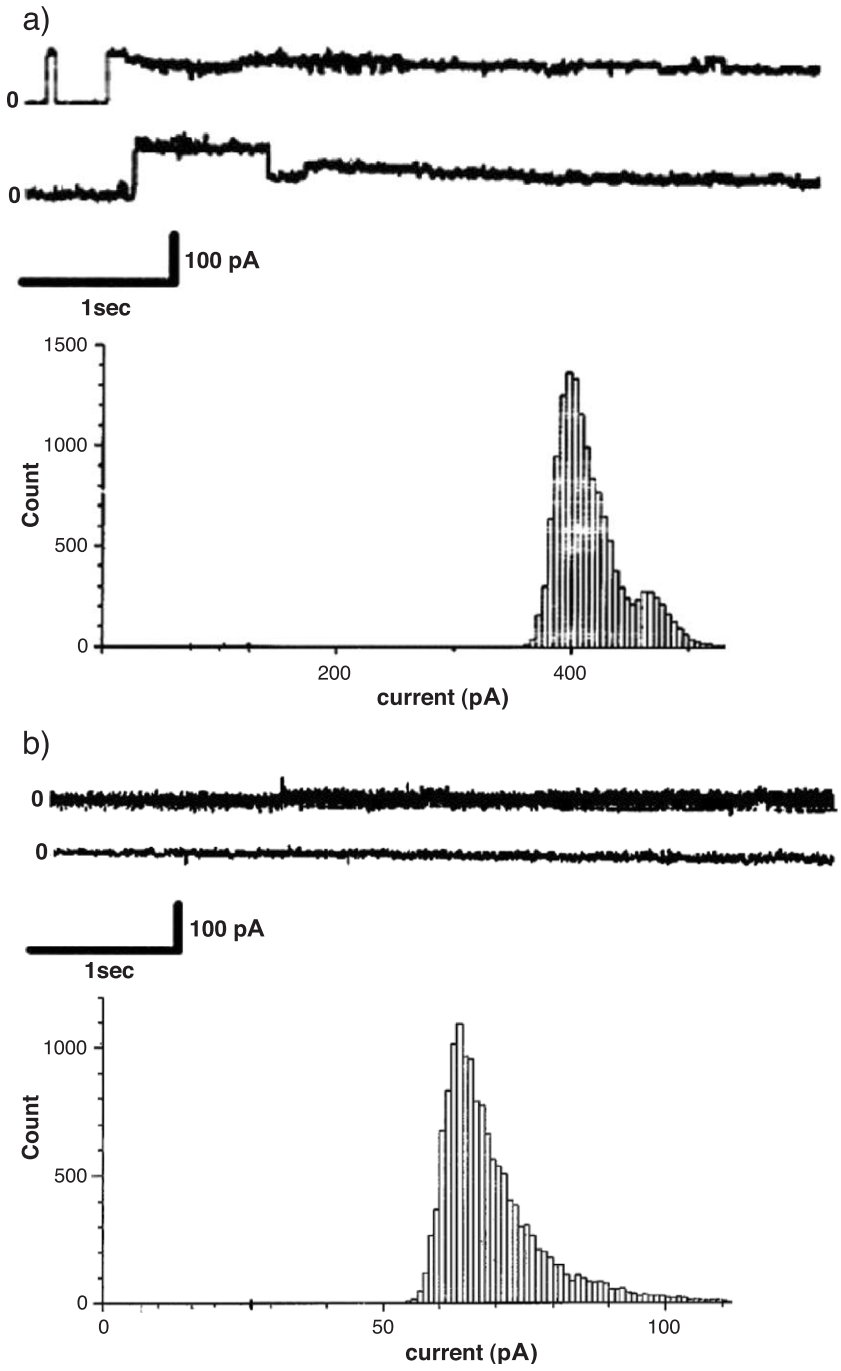

Fig. 1. VDAC channel current traces in the absence and presence of plasminogen protein. Continuous current traces of rat brain VDAC at +40 mV and its corresponding all point histogram (a) native VDAC (b) plasminogen-treated VDAC. The medium consisted of 500 mM KCl, 10 mM Hepes and 5 mM $MgCl_2$ (pH7.4). 0 represents base line.

amplitude histograms in Fig. 1. Results show that after addition of plasminogen, there is a significant shift in the current peak of the histogram in both positive as well as negative potentials. For example, at +40 mV, current peak shifts from 410 to 65 pA due to plasminogen treatment. It was observed that when plasminogen was added to the cis chamber, there is a significant decrease in the channel conductance through the entire range of potential as shown in Fig. 2.

Our results on both current and conductance, as described in the previous paragraph, show that the interaction of plasminogen protein with VDAC leads to a decrease in the current flow through the channel, suggesting that plasminogen interaction leads to partial closure of the VDAC in a planar lipid bilayer. It is important to know if this finding also holds in a cellular situation. If so, then there are some important consequences, especially for regulation of metabolite transport across mitochondrial

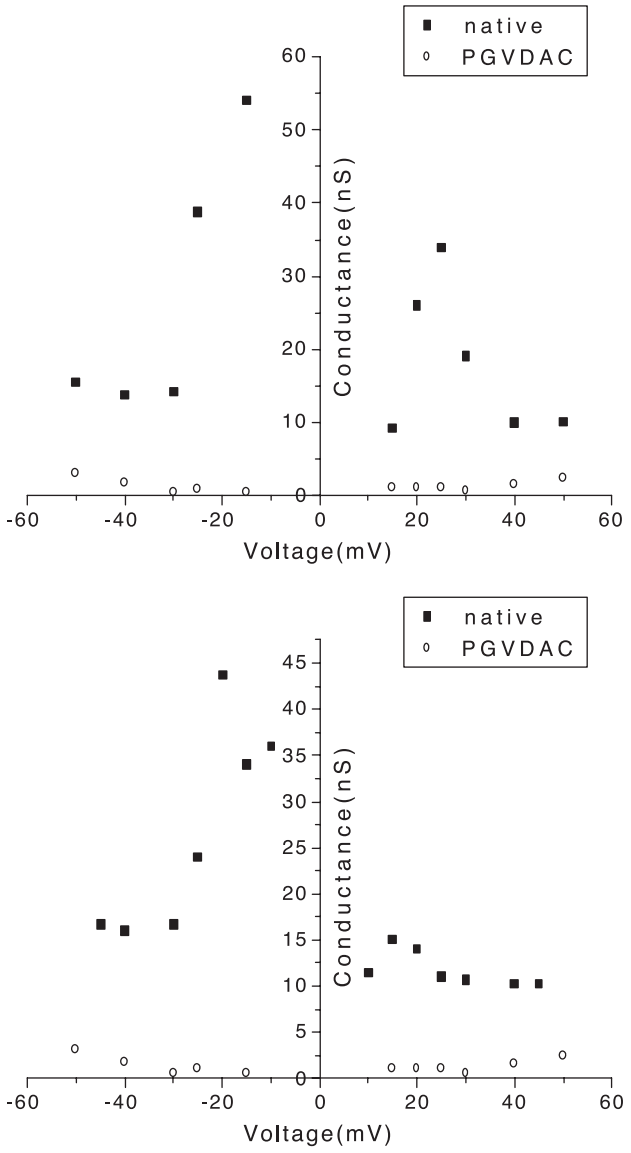

Fig. 2. Conductance of native and plasminogen-treated VDAC. Conductance values plotted against BLM potential (mV) for two representative experiments.

membrane by plasminogen protein. It has been reported that deficiency of plasminogen in brain tissue makes it resistant to neurodegenerative diseases and vice versa [11]. Our results suggest that interaction of plasminogen with VDAC leads to partial closure of the channel, hence inhibition in the transport of ATP and other metabolites across the mitochondrial membrane. van der Heiden et al. [12] proposed a model of mitochondrial outer membrane (MOM) permeabilization, which involves a regulation of VDAC channels [12,13]. According to them, VDAC channel closure prevents the efficient exchange of ATP and ADP between the cytosol and the mitochondrial matrix. Loss of the outer membrane permeability due to VDAC closure might result in the accumulation of the products of mitochondrial activity within the intermembrane space, generation of an osmotic gradient, and matrix swelling followed by the rupture of the outer membrane. Could this disturbance in transport through VDAC due to its interaction with plasminogen protein be a mechanism of brain cell death? The functional implications of such an event as mentioned above are many and need to be verified in vivo.

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