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# Modulation of the voltage-dependent anion channel of mitochondria by elaidic acid

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ABSTRACT

Dietary *trans* fatty acids (TFAs) are known to increase the risk of cardiovascular diseases by altering plasma lipid profile and activating various inflammatory signaling pathways. Here we show that elaidic acid (EA), the most abundant TFA in diet, alters the electrophysiological properties of voltage-dependent anion channel (VDAC) of mitochondria. Purified bovine brain VDAC, when incorporated in the planar lipid bilayer (PLB) composed of 1,2-diphytanoyl-sn-glycero-3 phosphatidyl choline (DPhPC) and EA in a 9 to 1 ratio (wt/wt), exhibited complete closing events at different voltages. The closing events were observed at even −10 mV, a voltage at which VDAC usually remains fully open all the time. Additionally, the voltage sensitivity of VDAC was lost in presence of EA; the channel conductance did not decrease with increasing voltages. In identical experimental conditions, membrane containing oleic acid (OA), the *cis* isomer of EA did not produce any such effect. We propose that EA possibly exerts its adverse effect by modulating VDAC.

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## 1. Introduction

*Trans* fatty acids (TFAs) are unsaturated fatty acids with at least one carbon-carbon double bond in the *trans* configuration [1]. TFAs are not synthesized in the body but are found abundantly in processed food, meat, dairy products, various hydrogenated oils and margarines [1,2]. Consumption of TFAs has been shown to increase the risk of coronary heart diseases [2–4]. TFAs alter the plasma lipid profile by increasing low-density lipoprotein-cholesterol and decreasing high-density lipoprotein-cholesterol [5,6]. TFAs inhibit the metabolic conversion of linoleic acid to arachidonic acid and polyunsaturated fatty acid (PUFA), increasing the risk for coronary heart diseases [7,8]. Consumption of TFAs has also been linked to the development of insulin resistance [9]. TFAs increase the influx of $Ca^{2+}$ into endothelial cells, thus accelerating calcification processes in the vascular wall [10]. Studies showed that TFAs get incorporated into the plasma membrane and alter membrane-biophysical properties such as fluidity, by altering phospholipid composition [11,12].

Voltage-dependent anion channel (VDAC) is one of the major resident proteins of the outer membrane of the mitochondria. It forms the primary passage for ions and metabolites between mitochondria and cytosol. VDAC has a β-barrel structure with a α-helix at the N-terminus. Movement of the helix is believed to be associated with opening and closing, i.e. gating of the channel [13]. VDAC is also involved in programmed cell death [14]. Many pro-apoptotic and anti-apoptotic proteins interact with VDAC and modulate its activity [14]. It is well known that composition of membrane lipid affects the properties of ion channels [15]. VDAC is known to be modulated by several lipids. Phosphatidylglycerol significantly enhances VDAC oligomerization in the membrane, whereas cardiolipin (CL) disrupts VDAC supramolecular assemblies [16]. Phosphatidylethanolamine (PE) and CL induce voltage asymmetry in VDAC current-voltage characteristics [17,18]. It has been shown that properties of gramicidin and alamethicin channels vary with the lipid composition of the bilayer [19–21]. The effects of PUFAs on voltage-gated ion channels have been reported [18,22–24]. Different theories ranging from the effects of PUFAs on membrane fluidity to specific binding with the channel proteins have been proposed [25]. Lipophilic PUFAs may bind to the lipid bilayer or at the bilayer/channel interface or at the hydrophobic pockets in the channel protein itself. Interaction of PUFAs with the voltage sensor may change the voltage dependency of the channel.

In the present work we demonstrate the modulation of VDAC by EA. VDAC, isolated from bovine brain, behaved differently when EA was incorporated in the planar lipid bilayer (PLB). Altered

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properties of VDAC have been correlated with the known apoptosis-inducing effect of EA.

## 2. Materials and methods

### 2.1. Chemicals

Hydroxylapatite, protein molecular weight marker and other chemicals for gel electrophoresis were obtained from Bio-Rad Laboratories, Inc. (USA). Celite-545 was procured form SRL (India). Triton X-100, HEPES and other chemicals were purchased from Sigma-Aldrich. 1,2-diphantanoyl-sn-glycero-3 phosphatidyl choline (DPhPC), elaidic acid (EA) and oleic acid (OA) were purchased from Avanti Polar Lipids (Alabaster, USA).

### 2.2. Purification of VDAC

VDAC was purified from bovine brain, following the method described by De pinto et al. [26]. Bovine brain was collected from a local slaughter house. Tissues were chopped into small pieces and then homogenized in buffer containing 250 mM Sucrose, 10 mM Tris (pH 7.4). Homogenate was centrifuged at 600*g* for 10 min at 4 °C. The supernatant was centrifuged further at 5000*g* for 15 min. The mitochondrial pellet was incubated for 15 min in hypotonic buffer containing 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, followed by centrifugation at 42,500*g* for 10 min. The pellet was re-suspended in hypotonic buffer, supplemented with 3% Triton X-100 at a final protein concentration of 5 mg/ml and centrifuged at 45,000*g* for 45 min. The supernatant was passed through a dry hydroxylapatite/celite column. The eluate containing purified VDAC was used for bilayer recording.

### 2.3. SDS-PAGE

The purity of the protein was checked on SDS-PAGE. The eluted protein sample was resolved on 12% gel as per standard Lammeli's method [27], followed by silver staining. Molecular weight of the purified VDAC was compared using standard molecular weight marker (Bio-Rad).

### 2.4. Reconstitution of VDAC in planar lipid bilayer (PLB)

Purified VDAC was incorporated in PLB made up of DPhPC, as described before [28]. Briefly, DPhPC was dissolved in n-decane (20 mg/ml). The bilayer was created by painting lipid on the aperture (150 μm diameter) of polystyrene bilayer cuvette (Warner Instrument, USA). Both *cis* and *trans* chambers contained symmetric solutions of 1 M KCl, 5 M $MgCl_2$ and 10 mM HEPES (pH 7.4). The *cis* chamber was connected to the ground electrode while the *trans* chamber was connected to the recording electrode, attached to the head-stage of amplifier PC501A (Warner Instrument). VDAC was added to the *cis* chamber and mixed continuously with magnetic stirrer. The insertion of the channel in PLB was observed by monitoring membrane currents at different voltages. Signals were low-pass filtered at 1 kHz and digitized at 5 kHz. pClamp 9 (Molecular Devices, USA) was used for the data acquisition and analysis. To check the effect of EA and OA on VDAC, PLB was formed with the lipid mixture containing DPhPC and EA/OA in a 9:1 ratio (wt/wt), dissolved in n-decane.

## 3. Results

Bovine brain VDAC was isolated and purity of the protein was checked on SDS-PAGE, followed by silver staining. The molecular weight of the purified protein appeared to be around 33 kDa (Fig. 1A). The electrophysiological properties of purified VDAC were studied after reconstituting it in the PLB. Fig. 1B shows representative current-voltage (I-V) plot of VDAC, reconstituted in DPhPC membrane. At lower voltages the channel remains open but switches to lower conductance states at higher voltages which are the characteristic features of VDAC. In order to study the effect of EA, VDAC was incorporated in PLB, made up of DPhPC alone, DPhPC + OA (*cis*), or DPhPC + EA (*trans*). DPhPC is a saturated 16 C synthetic lipid with no double bond, whereas OA and EA are monounsaturated 18 C fatty acids; the double bond lies between 9th and 10th C. Incorporation of OA (10%) in the PLB did not alter the channel properties, whereas in presence of 10% EA, VDAC exhibited entirely different properties. Fig. 2 shows the comparison of VDAC current traces, recorded from the membranes with either *trans* or *cis* fatty acids as well as DPhPC alone. As shown in the figure, at −10 mV, VDAC adopted an open state and the closing events were not observed. However in presence of EA, VDAC exhibited brief closures and flickering to different sub-conductance states. Interestingly, in addition to the sub-conductance states, full closure of the channels (I = 0) were observed, which is quite unusual for VDAC. Same trend was observed at higher potentials. At −60 mV, in presence of EA the channels showed flickering to sub-conductance states and occasional full closure. Full closing events were observed at all holding potentials. When OA, the *cis* isomer of EA was incorporated in the membrane, VDAC showed its usual properties. At −60 mV, the single channel conductance of VDAC at different states range from 0.5 nS to 1.35 nS in DPhPC membrane. However, in presence of EA, in addition to the fully closed state, VDAC exhibited multiple higher conductance states ranging from 3.4 nS to 4.35 nS. It implies that EA abolished the voltage sensitivity of VDAC. VDAC did not switch to lower conductance states at higher potentials in presence of EA. This is clearly evident in the $G/G_0$ vs V plot (Fig. 3). Unlike DPhPC membrane, in presence of EA the conductance of VDAC did not reduce with increasing voltages. To understand the distribution of times in different conductance states, the current traces at −10 mV and +60 mV were idealized and the single channel events were detected (Fig. 4). Dwell time at different conductance states were plotted against current amplitudes. The number of dots at single level represents the number of times they appeared and time spent at that level (Fig. 4). At −10 mV, in DPhPC membrane VDAC remained open (conductance 4.3 nS) 100% of the time. However, the channel attained full closed state (blue) and different sub-states (red) in presence of elaidic acid (Fig. 4). The full closing event lasted 100–150 ms. The recording at +60 mV shows the increased frequency of different sub-states and complete closing events in presence of EA. The full closing events lasted for 80–110 ms.

## 4. Discussion

In order to understand the molecular basis of TFA-induced inflammation and apoptosis, the effect of EA on VDAC was studied. VDAC is a common target for multiple pro-apoptotic and anti-apoptotic signals. Dietary TFAs get inserted in the cell membrane and organelle membranes. Thus it is plausible to hypothesize that insertion of TFA in mitochondrial membrane would affect VDAC, which may in turn induce apoptosis.

Two major alterations in the properties of VDAC were observed upon inclusion of 10% EA in DPhPC membrane. VDAC showed flickering to sub-conductance states and full closing even at lower holding potentials. Full closing events were detected at all test voltages. Another remarkable change is the loss of voltage sensitivity. At higher voltages, VDAC generally stabilizes in a lower conductance state, which did not happen in the presence of EA. The effects are specific to *trans* fatty acid as the *cis* isomer i.e. OA did not

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