Fractal Behaviour of Mitochondrial Chloride Channels

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Abstract. *Mitochondrial chloride channels are integral membrane proteins located in the inner membrane of mitochondria. These ion channels contribute to the mitochondrial membrane potential which is necessary to drive the production of "energy-containing" molecules – ATP. The method of ion channel reconstitution into artificial lipid membrane allows the measurement of ionic current flowing through a single ion channel. Analysis of single-channel current provides means for the insight into the conformational changes of the protein structure. We analysed the gating kinetics at different time scales. The transition from open (conductive) state into closed (non-conductive) state is characterized by a defined number of kinetic rate constants. In the contrary, the transition from closed to open state is better described with continuum set of kinetic rate constants, which is typical for fractal behaviour.*

Keywords: Membrane Protein, Single-channel Current, Gating Kinetics, Fractal Behaviour

1. Introduction

Ion channels are membrane proteins that provide hydrophilic pathway for ions transport across the hydrophobic lipid membrane. Due to thermal fluctuations the ion channels undergo frequent conformational changes leading to transitions between two functionally different states – open (conductive) state and closed (non-conductive) state. Transitions between open and closed states are called *channel gating* [1]. Method of ion channel reconstitution into artificial lipid membrane (BLM method) is commonly used to obtain ionic current from a single protein. This current is in order of picoamperes. The analysis of the duration of the sequential transitions (dwell time) gives us the information about the kinetic of conformational changes. Each type of ion channels is characterized by a specific number of open and closed conformational states and each of these states has a typical kinetic rate constant. The gating kinetics of ion channels has been commonly described as Markov process, which means that the kinetic rate constants are independent of the time spent in the current state and of the previous transitions [2]. However, these assumptions are not always valid when one studies the conformation changes of a protein. A novel approach was developed by Liebovitch and co-workers that sees the ion channel as a protein having a continuum of conformational states leading to complex behaviour over several orders of time scale what is a typical fractal property [3]. The method is based on analysis of dwell times in a chosen (open or closed) state; these are visualized in histogram with bins having exponentially growing width (logarithmic histogram).

2. Subject and Methods

Our aim was to characterize the gating kinetics of the mitochondrial chloride channel. Hearts were excised from anesthetized and decapitated rats and homogenized. The crude mitochondrial fraction was obtained in several steps of differential centrifugation and finally purified on Percoll density gradient. Purified mitochondria were sonicated at 30 kHz for 2 minutes to obtain submitochondrial vesicles. The vesicles containing chloride channels were incorporated into artificial lipid membrane that was formed by application of solution of

synthetic phospholipids in n-decane onto an orifice in a septum separating two compartments of the measurement chamber. Single-channel current was measured (Fig.1). The signal was amplified with ratio 100 mV/pA, filtered by analogue low-pass 8-pole Bessel filter (cut-off frequency 1 kHz) and digitized with sampling frequency 5 kHz. We measured the single-channel currents in non-physiological gradient of potassium chloride (1 mol.l^{-1}/ 50 mmol.l^{-1}) to have good signal-to-noise ratio so that the noise fluctuations would not affect the measured gating of the channels. The solutions were buffered with 10 mmol.l^{-1} HEPES and Tris to 7.4 pH. The dwell times of open and closed states, respectively, were detected in pClamp 10.0 software (Axon Instruments, Inc.). This information was further processed in program developed in Matlab 2012b.

Fig. 1. Representative single-channel current. A typical current trace is in general characterized by step transitions between two levels corresponding to open (conducting) state and to closed (non-conducting) state.

3. Results

We analyzed separately the time that the channel spent in open state and in closed state (dwell time). By plotting number of dwell times $N(t)$ lying in interval $(t;\ t + \Delta t)$ we created histograms for open dwell times and closed dwell times, respectively. Width of the interval Δt increases exponentially (Δt = 1, 2, 4, 8 ... ms) for this reason the number of events $N(t)$ is normalized by Δt (Fig.2). Histogram of open dwell times has approximately linear trend in semi-log plot what is characteristic of exp(-kt) distribution. Obtained kinetic rate constant for shorter dwell times (1 ÷ 20 ms) was k = 220 Hz while for longer dwell times it was k = 109 Hz. On the contrary, histogram of closed dwell times has linear trend in log-log plot which is typical for continuum set of kinetic rate constants. In this case the rate constants were uniformly distributed within the range 0,1 ÷ 1 kHz.

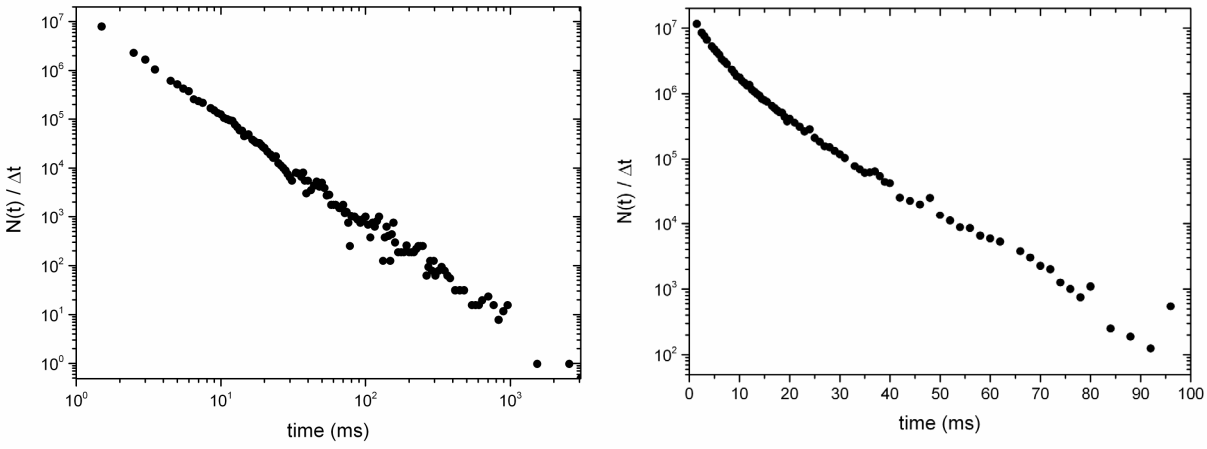

Fig. 2. Histograms for closed (left) and open (right) dwell times. Data on the left have linear behaviour in log-log plot while data on the right are approximately linear in semi-log plot. The (left) histogram has linear trend in log-log plot, which is a characteristic for fractal behaviour and the (right) histogram has linear trend in semi-log plot, which is considered as characteristic of Markov behaviour.

4. Discussion and Conclusions

We described the gating kinetics of mitochondrial chloride channels. Despite the role that these channels have in mitochondrial physiology and pathophysiology, their molecular identity remains unknown. So far, the only way to approach to their identity is to characterize these channels from electrophysiological measurements. The method used here provides a glimpse into the physical properties and function of the ion channel. It gives us information about the energy barriers separating different conformations of the channel and their possible time-dependence. Not the least, structurally similar ion channels, originating from one ion channel family, may display similar behaviour of gating kinetics, as this is connected to the structural features of the protein. Unfortunately, this analysis was applied only on a very limited number of ion channel types. From these, one was chloride channel located in the plasma membrane of skeletal muscle cells [4]. The behaviour of this channel was different from what we described, indicating that these channels are not identical. Nevertheless, similar description of the gating kinetics was presented in [2], where the authors studied voltage-dependent potassium channels. The non-Markov behaviour of other type of ion channels was confirmed also in case of calcium-activated potassium channel [5]. However, the fractal behaviour is not a general feature of ion channels; in some cases the Markov model is more suitable for the description of gating kinetics [6].

The open dwell times of mitochondrial chloride channels can be described by either two kinetic rate constants (109 and 220 Hz) or by one constant, the value of which is in this limited range. On the other side, the closed dwell times clearly displayed a broad range of kinetic rate constants spreading through several orders of magnitude. The Markov or fractal character of the ion channel kinetics can be determined from the dwell time histograms with varying bin width. According to the theory described in [3], the histogram that has linear trend in log-log plot is characteristic for fractal behaviour and when the histogram has linear trend in semi-log plot, it is considered as characteristic of Markov behaviour. This behaviour can be explained by a static approach or by a dynamic approach. From the static point of view, the channel possesses a set of closed states, each having a kinetic rate constant slightly different (smaller) from the previous one [7]. The dynamic explanation is that the ion channel has only one closed state whose kinetic rate constant is changing in time. From the molecular view, this can be considered as if the protein was settling down in the membrane to an ultimate conformation from which it will switch to open state with very low probability unless it is overcome by some other stimulus (e.g. application of voltage) [8].

Acknowledgements

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