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Open channel current noise analysis of S6 peptides from KvAP channel on bilayer lipid membrane shows bimodal power law scaling

Rajan Shrivastava [a,1], Chetan Malik [b,1], Subhendu Ghosh [a,c,*]

[a] Department of Biophysics, University of Delhi South Campus, New Delhi, 110021, India
[b] Department of Life Science, School of Sciences, Indira Gandhi National Open University, New Delhi, 110068, India
[c] Centre for Information Services and High Performance Computing (ZIH), Technical University Dresden, Germany

HIGHLIGHTS

- Power spectral density (PSD) of S6 single-channel open current traces has been analysed.
- Bimodal slope of PSD ($1/f$ and $1/f^2$) in ion channels has been reported.
- Two modes of non-equilibrium ion transport in S6 channel best describe the bimodal Power law scaling.

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ABSTRACT

Open channel current noise in synthetic peptide S6 of KvAP channel was investigated in a voltage clamp experiment on bilayer lipid membrane (BLM). It was observed that the power spectral density (PSD) of the component frequencies follows power law with different slopes in different frequency ranges. In order to know the origin of the slopes PSD analysis was done with signal filtering. It was found that the first slope in the noise profile follows $1/f$ pattern which exists at lower frequencies and has high amplitude current noise, while the second slope corresponds to $1/f^{2-3}$ pattern which exists at higher frequencies with low amplitude current noise. In addition, white noise was observed at very large frequencies. It was concluded that the plausible reason for the multiple power-law scaling is the existence of different modes of non-equilibrium ion transport through the S6 channel.

1. Introduction

Noise analysis is an important part of research in various fields, e.g. Physics, Geology, Environmental Sciences and Biology [1–3]. In physiology, a number of reports related to noise have come up over the last several years [4]. In general, Power law noise can be broadly classified into two categories, i.e. white noise ($1/f^0$) and coloured noise ($1/f^{1\text{-}3}$) [5]. The origin and importance of various types of noise like $1/f^0$, $1/f$, $1/f^2$ and $1/f^3$, have been reported by many authors [6–12]. Especially, $1/f$ noise has been discussed extensively [7,13–16]. Bak et al. invoked Self Organized Criticality (SOC) to explain $1/f$ noise in sandpile avalanche [6–8]. He discussed the idea of criticality in phase transition of a dynamical system without

* Corresponding author at: Department of Biophysics, University of Delhi South Campus, New Delhi, 110021, India. Tel.: +91 11 24113106x279; fax: +91 11 24115270.
E-mail address: profsubhendu@gmail.com (S. Ghosh).

[1] Both the authors contributed equally to this work.

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the need of any external intervention [7]. During the last couple of decades considerable effort has been made to understand the origin of power law noise in biological systems [17].

Lipid membranes (cellular and artificial) display fluctuation in its electrochemical properties to a good extent. In ion channels, which create passage across the cellular and organelle membranes, the fluctuations in electric current reflect the internal dynamics of the system. Over the years open channel noise analysis has been carried out in various ion channels, e.g. Voltage Dependent Anion Channel (VDAC), Maltoporin channel etc. [8,17]. The existence of colour noise, e.g. pink and brown noise has already been reported in the ion channel current [8,9,17]. However, there is a lot of controversy regarding the conceptual interpretation of the power law noise. In general, the origin of noise in ion channel current is related to ion transport and the fluctuation of their conformations. In the present work we have analysed the open channel current noise of the single channel formed by the S6 segment of KvAP on lipid bilayer membrane (BLM). This has been done keeping in view that BLM is a very good model which mimics the actual cell membrane. KvAP is a potassium channel from the bacterium *Aeropyrum pernix*. It is composed of six domains out of which S6 is found in the interior lining of the pore that permeates the ions [18]. S6 oligomerizes on the lipid bilayer to form a functional channel as shown by us previously [19]. Moreover, when various oligomers of S6 assemble to form multiple channels on the membrane their function becomes cooperative [20]. Here we report that the noise from an open ion-channel current could follow multiple noise patterns. We offer a physio-chemical explanation for the same in the context of S6 channel.

2. Materials and methods

2.1. Bilayer electrophysiology and single-channel current recording

S6 segment of voltage dependent potassium channel (KvAP) derived from bacterium *Aeropyrum pernix*, was synthesized artificially by GenPro Biotech, India. Sequence of the peptide is as follows:-

LTGISALTLLIGTVSNMFQKIL

The peptide was incorporated in the bilayer membrane as described earlier [19,20]. Briefly, Cardiolipin (Avanti Polar lipids, U.S.A.) of stock solution 25 mg/ml in chloroform (w/v) and cholesterol (Avanti Polar lipids, U.S.A.) of 25 mg/ml in chloroform (w/v) were mixed in the ratio of 6:1 (v/v). The mixture was dried in nitrogen gas. An organic solvent *n*-decane (Sigma-aldrich, U.S.A.) (10 μl) was used to dissolve the dried Cardiolipin/cholesterol mixture. The mixture was used to form a bilayer film using a glass capillary. Experimental bathing solution consisted of buffer having salt concentration of 500 mM KCl, 5 mM $MgCl_2$, and 10 mM HEPES. The pH of the buffer was adjusted using Tris-Cl. The chamber for recording single channel activity is made up of polystyrene (Warner Instruments, U.S.A.). The cup has a hole of $\sim$150 μm diameter on which the lipid bilayer is painted. Both the chambers of the cup i.e. Cis and Trans are connected to an amplifier (Axopatch 200B, Axon Instruments) with the help of headstage (CV-203BU). The headstage contains two Ag/AgCl electrodes dipped in cis and trans polystyrene chambers containing 1 ml of buffer (pH = 7.4) on each side without salt bridge. The cis electrode was connected to the voltage supply, while the trans electrode acts as the ground. The current fluctuations detected by the electrode were sent to the amplifier through headstage which in turn sent it to the computer through an analogue to digital converter Digidata (1440A, Axon Instruments). S6 peptide having a concentration of 50 pg/ml (21 pmol/l) of the buffer was added to the cis chamber for single-channel current recording. The peptide solution was constantly stirred using magnetic force to speed up the process of insertion. As soon as insertion took place the stirring was stopped and the excess peptide was removed by perfusion. The single channel activity was recorded using the acquisition software CLAMPEX (PCLAMP 10.2., Axon Instruments) with externally applied voltage at a sampling frequency 10 kHz. Low pass Bessel filter of 2 kHz was used for filtration of external noise from the surrounding. The recording of current signal was done in 3 sets of experiments for each voltage from -80 to $+80$ mV at the interval of 10 mV. As a control measurement we recorded bilayer current before the incorporation of the S6 channel on BLM chamber. The experiments were performed at room temperature between 24 and 25 °C. The data were stored in the computer.

2.2. Noise analysis of electrophysiological data

Open and closed states in the single channel current recordings were identified as described in Ref. [19]. Data so obtained was analysed using the software CLAMPFIT (pCLAMP 10.2, Axon Instruments), Origin 5.0 (Origin lab Corp. U.S.A.) and Matlab 2014a (Mathwork, Inc. U.S.A.).

Noise analysis of S6 open channel current was done in Matlab 2014a on 0.4096 s recording (4096 data points) from the current vs. time data corresponding to open channel fluctuation at each voltage. Fast Fourier transform (FFT) was performed on 4096 data points with Matlab 'FFT' function which transforms data from the time domain to the frequency domain. To get the idea of power distribution with respect to frequency the coefficient of FFT were squared and plotted as power versus frequency in log–log scale, called power spectrum. The power spectra were characterized by their slopes that describe the type or "colour" of the noise. The slope of the power spectrum was calculated by least square fit of the spectrum (Matlab command 'fit') as the mean slope with 95% confidence interval and best linear fit was given. Similar analysis has been performed on bilayer currents (without ion channel) and S6 closed state currents. In order to understand the origin of noise, the open channel current signals were analysed in the following way. The low and high frequency components were