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An automated method for generating analogic signals that embody the Markov kinetics of model ionic channels

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Abstract

In this work we present an automated method for generating electrical signals which reflect the kinetics of ionic channels that have custom-tailored intermediate sub-states and intermediate reaction constants. The concept of our virtual single-channel waveform generator makes use of two software platforms, one for the numerical generation of single channel traces stemming from a pre-defined model and another for the digital-to-analog conversion of such numerical generated single channel traces. This technique of continuous generation and recording of the activity of a model ionic channel provides an efficient protocol to teach neophytes in the field of single-channel electrophysiology about its major phenomenological facets. Random analogic signals generated by using our technique can be successfully employed in a number of applications, such us: assisted learning of the single-molecule kinetic investigation via electrical recordings, impedance spectroscopy, the evaluation of linear frequency response of neurons and the study of stochastic resonance of ion channels.

Keywords: Markov kinetics; Digital-to-analog conversion; Ion channels; Impedance

1. Introduction

Almost two decades ago, one of the most exciting venues was opened for both electrophysiologists and molecular biophysicists, with the introduction of a technique aimed at studying single ionic channels. That is, the 'patch-clamp' technique enabled scientists for the first time to detect and record ion fluxes through individual ionic channels and therefore helped the investigation of the functional behavior of ionic channels at the single-molecule resolution (Sakmann and Neher, 1984). Due to the large amount of literature to date, it is sufficient to say here that ionic channels are proteins that form aqueous holes in membranes, through which selected ions move passively down their electrochemical gradients, and they are central to many properties of cell membranes. Although tremendously complex in structure, the ionic channels functions can be safely described by mostly two features: they open and close in response to stimuli during a process called gating and, when open, they select between different species of ions, in a process called selectivity (Miller, 2000; Kuyucak et al., 2001; Chung and Kuyucak, 2002; MacKinnon, 2003). The gating property of channel proteins points to the fact that a limited number of stable conformations of the ionic channels do exist and reaction rates of transitions among them are controlled by a variety of factors, such as: light, potential difference, allosteric interactions of ionic channel proteins with specific agonists. Conformational changes of ionic channels associated with their transitions among intermediate states are represented by kinetic diagrams consisting of several states and the transition probabilities between those states, and by energy level models consisting of energy minima, fixed energy barriers and pathways between such states. The lifetime of each intermediate state is usually modeled as a memoryless random variable that does not depend on the age of that particular intermediate. The system can be treated as a Markov process with discrete states in continuous time. The equation which describes the evolution of the system is the differential form of the Chapman–Kolmogorov equation, which is called the master equation (Van Kampen, 1992). A large and consistent deal of literature has been published over the

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years on membrane channel kinetics based on discrete-state Markov models (Colquhoun and Sigworth, 1983; Horn and Lange, 1983; Ball and Sansom, 1989; Ball and Rice, 1992; Colquhoun and Hawkes, 1995; Tieleman et al., 2001). Although historically single protein dynamics (e.g., ion channels) was observed via a powerful blend of single-channel isolation techniques and electrophysiology protocols, aimed at current measurement through them under voltage-clamp conditions, alternative protocols with the same aim in mind were designed recently. That is, measurements based on confocal fluorescence microscopy, near field optics, two photon excitation, and single-photon detection have paved the way to studying enzymatic reactions in aqueous solution with single proteins (Nie et al., 1994; Xie and Trautman, 1998; Bai et al., 1999; Xie and Lu, 1999; Edman and Rigler, 2000). More recent advances in single molecule fluorescence methods have revealed the kinetic behavior of fluorescently labeled ionic channels under near native conditions (Schutz et al., 2000; Ishii and Yanagida, 2000; Ha, 2001; Harms et al., 2001; Borisenko et al., 2003). Recently, we have extended the use of electrical recording on single protein channels to visualize and investigate individual covalent and noncovalent interactions. For instance, the reversible reactions of organoarsenic (iii) compounds with the thiol group of a single cysteine residue projecting into the lumen of the α-hemolysin (αHL) pore have been examined (Shin et al., 2002). Moreover, we were successful in employing the electrical detection to monitor a multistep reaction at the single-molecule level for the first time (Luchian et al., 2003). As it is suggested by its ubiquitousness for a large variety of biomolecular processes, the in-depth analysis of single molecule kinetics is crucial for both the microscopic and macroscopic understanding of molecular dynamics. We evidenced in this introduction that over the decades, a rich pool of experimental and theoretical techniques has accumulated, aimed at unraveling of how single molecules behave. In this work we layout the principles of a relatively simple, yet powerful and portable method for generating electrical signals which reflect the kinetics of single- or multiple-ionic channels, having custom-tailored intermediate sub-states and intermediate reaction constants. Once generated, such signals can be easily visualized on the oscilloscope, can be adjusted interactively for the amount of white-noise present, and can be fed into an automatic acquisition system to simulate an electrophysiology experiment, providing thus a good way of learning the basics of single-channel electrophysiology recordings. The implementation of our technique of recording the activity of a model ionic channel provides a very handy and clear protocol to teach neophytes in the field of single-channel electrophysiology about its major phenomenological aspects. For instance, by adding to one's desire various types of noise (e.g., white and power-line noise) and substates, the close-to-original complexity of the generated traces is retained, and methods which address the extraction of interesting parameters about the studied kinetics can be easily and costlessly tackled. Our method provides a much simpler and cost-effective alternative to generating noisy signals with perfectly adjustable parameters and belonging to various classes of distributions, which can be used in applications where user-defined noisy signals are crucial for understanding novel paradigms on modern biophysics, such as stochastic resonance (Benzi et al., 1981; Douglass et al., 1993; Gammaitoni et al., 1998; Goychuk and Hanggi, 2000).

2. Materials and methods

At the core of our virtual single-channel waveform generator lay two software platforms, one for the numerical generation of single channel traces stemming from a pre-defined model (QuB, The Research Foundation State University of New York, USA) and another for the digital-to-analog conversion (D/A) of such numerical generated single channel traces (LabView, National Instruments Inc., USA). Once focused on a desired model for a single-channel kinetics scheme, including here the number of sub-states, their individual conductances as well as reaction constants among sub-states, records of various lengths for the digitized version of the electrical current mediated by the channel can be easily generated via the QuB software. Depending on the value of the intermediate reaction constants, an appropriate sampling frequency of the to-be generated traces should be chosen. At this stage, numerical traces can be generated either as ideal ones, with no Gaussian noise superimposed, or with noise of given variance; however, Gaussian noise can be added at any time during the D/A conversion procedure. By using a special designed read/write routine, numerical data generated by the QuB software is translated into the LabView format and then fed into a PCI-compatible device for the D/A conversion task. Although more expensive and equally sophisticated D/A cards do exist, we relied on a VIA AC97 Audio Controller soundcard (VIA Technologies Inc., Taiwan) for this task. Among others, a good resources link about soundcards and their usefulness as both A/D and D/A converters can be found at http://www.reed-electronics.com/tmworld/article/CA187380.html. Before use, we calibrated the D/A system in the sense that a sinusoidal signal with an amplitude expressed in arbitrary units and known frequency generated as a digital waveform in LabView, was monitored at the analog output of the soundcard with a digital oscilloscope (200 MHz Quad 9304 CM, Le Croy, USA), and the absolute value of the analog signal amplitude was measured. By repeating this procedure for several arbitrary units values of the digital waveform amplitude, we were able to predict the real value of the analog signal generated by the D/A card when the amplitude of the input digital wave was expressed in arbitrary units. The D/A sampling frequency of soundcard and the resolution per channel were set to 22050 Hz and 16 bits, respectively. In choosing the arbitrary values of the digital signals used for the D/A task, we kept in mind that such amplitudes should be confined to the region where the analog output