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Gating kinetics of potassium channel in rat dorsal root ganglion neurons analyzed with fractal model

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Abstract

The kinetics of ion channels have been widely modeled as a Markov process. In these models it is assumed that the channel protein has a small number of discrete conformational states and kinetic rate constants connecting these states are constant. To study the gating kinetics of voltage-dependent K^+ channel in rat dorsal root ganglion neurons, K^+ channel current were recorded using cell-attached patch-clamp technique. The K^+ channel characteristic of kinetics were found to be statistically self-similar at different time scales as predicted by the fractal model. The fractal dimension *D* for the closed times and for the open times depend on the pipette potential. For the open and closed times of kinetic setpoint, it was found dependent on the applied pipette potential, which indicated that the ion channel gating kinetics had nonlinear kinetic properties. Thus, the open and closed durations, which had the voltage dependence of the gating of this ion channel, were well described by the fractal model.

Keywords: Ion channel; Single channel recording; Potassium channel; Dorsal root ganglion neurons; Fractal model

1. Introduction

Ions can cross the hydrophobic lipid bilayer through ion channels in the cell membrane. Ion channels are large proteins located in the membranes of cells. They act as molecular transducers. They form pores that allow ionic current on the order of pA to pass across the membrane. The channels open and close in a stochastic manner dependent on the transmembrane voltage ('voltage-gated channels'), mechanical forces ('mechanosensitive channels'), or on the presence of other molecules such as neurotransmitters ('ligand-gated channels') [1]. Patch-clamp technique is widely used in biophysics and biochemistry.

Traditional paradigms of ion channels: It was implicitly assumed that ion channel proteins have a few stable conformational states such as closed, open and inactivated. This approach assumed that the switching can be represented by a Markov process. That is, it was assumed that the probability to switch states depends only on the state of the channel and not on the history of previous states and not on the duration of the time that a channel has remained in a state [2,3]. This assumption has

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given rise to a lively discussion [4–6]. On the one hand, the information about the validity of this condition can provide valuable insight into the system under investigation; on the other hand, conclusions drawn from a model that does not fit the process that has produced the data are very likely to lead to erroneous results. Thus, it is not evident from experimental data whether the system actually has the Markov process. The idea of testing of the Markov condition in ion channel recordings was put forward recently by Timmer and Klein [7]. Non-Markovian character of ionic current fluctuations in membrane channels was characterized [8]. Fractal analysis of a voltage-dependent potassium channel from culture mouse hippocampal neurons was reported [9].

This paper is organized as follows: firstly we briefly review the fractal model of ion channel kinetics; secondly Methods introduction and Results. In the end we draw conclusion.

2. Fractal model of ion channel kinetics

The word 'fractal' was coined by Benoit Mandelbrot in the late 1970s, but objects now defined as fractal in form have been known to artists and mathematicians for centuries. Mandelbrot's definition—'a set whose hausdorff dimension is not integer'—is clear in mathematical terms. In addition, related concepts are those of self-similarity and sub-divisibility. The length of the coastline of Britain or the length of the perimeter of the Koch curve increases as we measure it at finer spatial resolution. In a fractal model of ion channel kinetics, the kinetic rate constants increase as we measure the current through the channel at finer temporal resolution. That is, the faster we can look, the faster we see the channel fluctuating open and closed. Namely, the effective kinetic rate constant for opening or closing, k_{eff}, will depend on the time scale t_{eff} at which it is measured so that $k_{\mathrm{eff}}=At_{\mathrm{eff}}^{1-D}$. This effective kinetic rate constant summarizes the information about the many different kinetic processes that happen over many different time scales. It depends on only two parameters: the fractal dimension D, which determines the relative contribution of processes at short and long time scales, and the kinetic setpoint A, which determines if all the processes happen slowly or rapidly [10].

In the fractal model

$$\mathrm{C}\underset{k_{\mathrm{c}}}{\overset{k_{\mathrm{o}}}{\rightleftharpoons}}\mathrm{O}$$

the kinetic rate constant k_{o} or k_{c} for leaving the closed or open states is actually a function of the time scale t, at which they are observed such that $k_{\mathrm{o}}=At^{1-D}$ and $k_{\mathrm{c}}=A't^{1-D'}$, where A, A' are kinetic setpoints, and D, D' are the fractal dimensions.

In the Markov model k_{o} and k_{c} are constant, so that the transition probabilities between different states depend only on the current state of the ion channel. In fractal model, the transition probabilities depend both on the current state of the channel and on how long it has been in that state. As we will see below, $1\leqslant D<2$. Thus, as t increases, k_{o} and k_{c} decrease. That is, in the fractal model, the longer the channel resides in any state, the less likely it is per unit time to exit that state. We will show below that this is equivalent to the fractal scaling relationship $k_{\mathrm{eff}}=At_{\mathrm{eff}}^{1-D}$.

In the present study we will derive the closed time durations. The equations for the open times are analogous with $k_{\mathrm{o}}(t)$ replaced by $k_{\mathrm{c}}(t)$. If $p(t)$ be the probability for which the channel remains closed for the duration $[0, t]$, then $p(t+\Delta t)$ be the probability for which the channel remains closed for duration $t+\Delta t$, thus $p(t+\Delta t)=p(t)(1-k_{\mathrm{o}}\Delta t)$, $p(t)=\exp((-A/(2-D))t^{2-D})$ and closed time probability density function can be shown as

$$f(t)=-\frac{\mathrm{d}p(t)}{\mathrm{d}t}=At^{1-D}\exp\left(-\frac{A}{2-D}t^{2-D}\right)$$

Since we expect to observe more rapid behavior at shorter time scales, k_{o} must be a nonincreasing function of t, so that the fractal scaling law $k_{\mathrm{o}}=At^{1-D}$ requries $D\geqslant 1$. The restriction that $p(0)=1$ requries that $D<2$. Thus, the fractal dimension D is restricted to the range $1\leqslant D<2$.

Let the smallest time interval that can be resolved define the effective time scale t_{eff}. We can only detect channel closing of duration $t>t_{\mathrm{eff}}$. Thus, the channel kinetics can be meaningfully

measured as the effective kinetic rate constant, k_{eff}, which follows the conditional probability that a channel that has been closed for at least a duration of t_{eff} is bound to open. Hence we can find that

$$k_{\text{eff}}(t_{\text{eff}}) = f(t_{\text{eff}})/p(t_{\text{eff}}) = At_{\text{eff}}^{1-D}.$$

This effective kinetic rate constant summarizes the information about the processes that happen at many different time scales. The fractal dimension D determines the relative contribution of various processes at different time scales and the kinetic setpoint A determines whether all the processes take place slowly or rapidly. The method of the fractal analysis for ion single channel was referred [10,11].

3. Methods

3.1. Isolation of dorsal root ganglion neurons

Two- to three-week-old Sprague–Dawley rats, irrespective of sex, were decapitated, and the thoracic and lumbar segments of vertebrate column were dissected and longitudinally divided into two halves along the median lines on both dorsal and ventral sides. The DRGs together with dorsal and ventral roots and attached spinal nerves were taken out from the inner side of each half of the dissected vertebrate and transferred into Dulbecco's Modified Eagle's Medium (DMEM, Sigma) at pH 7.4. After the removal of attached nerves and surrounding connective tissues, the DRGs were minced with iridectomy scissors and incubated with enzymes including trysin (type III, Sigma) 0.5 mg/ml, collagenase (type IA, Sigma) 1.0 mg/ml and DNase (type IV, Sigma) 0.1 mg/ml in 5 ml DMEM at 35 °C in a shaking bath for 40 min. To stop the enzymatic digestion, 1.25 mg/ml soybean trypsin inhibitor (type II-S1, Sigma) was added. The isolated neurons were transferred into a 35-mm culture dish and kept still for at least 30 min. All experiments were performed at room temperature (20–30 °C) [12–16].

3.2. Solutions and electrophysiology

Single channel recording was carried out at room temperature using an EPC-9 patch-clamp amplifier (Germany). The potassium ion channel current was recorded using the cell-attached patch-clamp technique.

Cells were isolated and maintained in Hank's balanced salt solution with the following composition (mM) NaCl 150, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, D-glucose 10, its osmolarity was adjusted to 340 mOsm with sucrose and pH was adjusted to 7.4 with KOH. Cell-attached bath solution contained (in mM) NaCl 5, KCl 150, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, $CdCl_2$ 0.2, D-glucose 10, pH 7.4. The patch-pipette (internal) solution contained (in mM) NaCl 5, KCl 150, $MgCl_2$ 1, HEPES 10, EGTA 11, its osmolarity was adjusted to 340 mOsm with sucrose and pH was adjusted to 7.4 with KOH. The pipettes had resistances of 8–12 MΩ after backfilling with an internal solution.

Single channel current was recorded with EPC-9 (Germany). Current signals were filtered with a cutoff frequency of 1 kHz (eight-pole Bessel) and sampled at 5 kHz. For single channel analyses, we used data from patches in which only one K^+ channel was present.

4. Results

At depolarizing pipette potentials of −30, −40, −50, −60 mV, the K^+ channel current were recorded in cell-attached configuration of patch-clamp method. Fig. 1 shows single channel current trace at $V_p = -60$ and −50 mV.

4.1. Determining the fractal feature of K^+ single channels in DRGs

The open and closed duration times had been measured from the single channel records to construct histograms at $V_p = -40$ mV. The histogram for 2240 closed durations was shown in Fig. 2. Also the histogram for 2576 open durations was shown in Fig. 2. We constructed closed time duration of bin sizes of 1, 2, 4, 8, 16, 32, 64, 128 ms and open time duration of bin sizes of 1, 2, 4, 8 ms. Only histograms with monotonically decreasing numbers for both the closed and open durations in the first four bins were used for further analysis. The lines on each histogram were

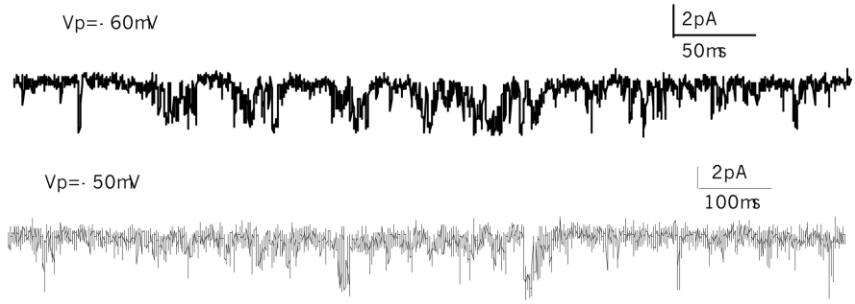

Fig. 1. Single channel current trace at $V_p = -60$ and -50 mV.

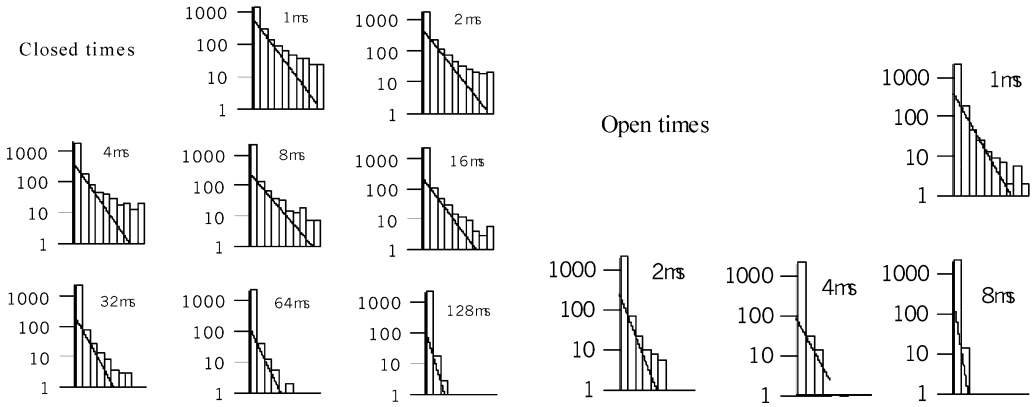

Fig. 2. Semilogarithmic histogram for 2240 closed time duration and for 2576 open time duration was shown. We constructed closed time duration of bin sizes of 1, 2, 4, 8, 16, 32, 64, 128 ms and open time duration of bin sizes of 1, 2, 4, 8 ms at $V_p = -40$ mV.

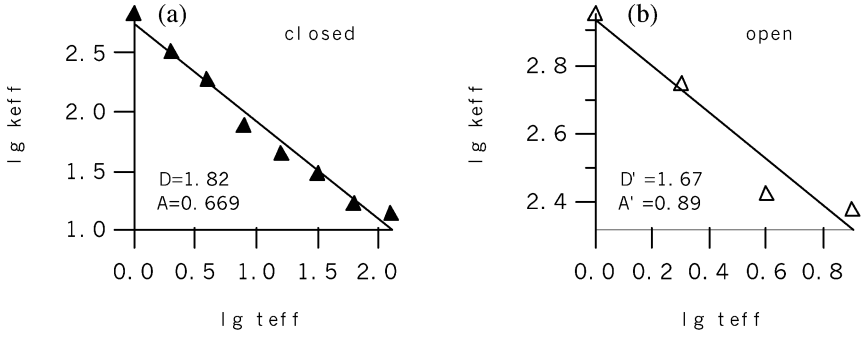

Fig. 3. Closed and open duration times fractal dimension and kinetic setpoint at $V_p = -40$ mV.

obtained from least squares fits using the second through fourth bins. The effective kinetic rate constant k_{eff} at the each bin size t_{eff} is the negative of the slopes of these lines. It was used semilogarithmic. The plots of $\log(k_{eff})$ vs. $\log(t_{eff})$ for both closed and open duration did not have the

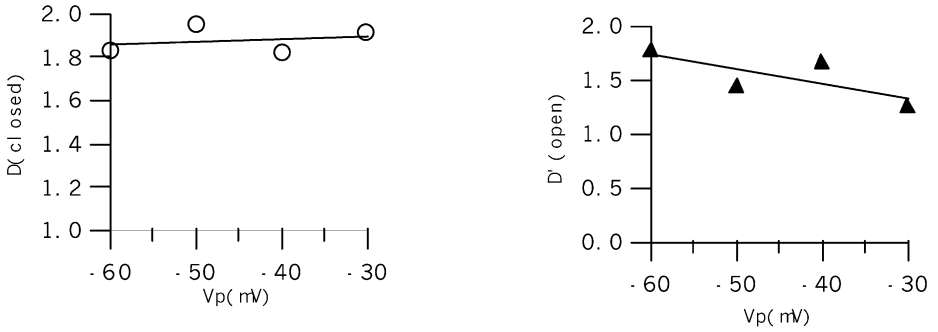

Fig. 4. The fractal dimension for both closed and open times varied with the pipette potential.

plateaus, which could indicate the existence of multiple discrete conformation as predicated by Markov models but instead were approximately straight lines, which indicated that these channels could be represented by a model using fractal kinetics. Fig. 3 shows closed and open duration times fractal dimension and kinetic setpoint at $V_p = -40$ mV.

4.2. Voltage dependence of the fractal dimensions

The experimental results showed the dependence of fractal dimension for closed duration and open duration on pipette potential. The fractal dimension for closed duration was nearly constant but there was a small change proportional to the absolute value of the pipette potential, but the fractal dimension for open duration was inversely proportional to the absolute value of the pipette potential. Under pipette potential of -30, -40, -50, -60 mV, the fractal dimension for closed duration was 1.916, 1.821, 1.95, 1.83, the fractal dimension for open duration was 1.26, 1.67, 1.45, 1.78, respectively. Fig. 4 shows the fractal dimension for both closed and open times that varied with the pipette potential.

4.3. Voltage dependence of the kinetic setpoints

The kinetic setpoints for both closed and open durations from single channel records of rat dorsal root ganglion neurons varied with the pipette potential. Under pipette potential of -30, -40, -50, -60 mV, the kinetic setpoint for closed duration was 0.818, 0.669, 0.6824, 0.549, the kinetic setpoint for open duration was 0.89, 0.88, 0.91, 0.85. Fig. 5 shows the kinetic setpoint for closed and open duration that varied with the pipette potential.

After the fractal dimension and kinetic setpoint were determined, we can compare fractal model with the experimental data. The closed and open durations were represented by the fractal model. For example, we considered probability density function $f(t) = At^{1-D}\exp((-A/(2-D))t^{2-D})$ fitting closed duration at $V_p = -40$ mV. Fig. 6 shows the fractal model fitting closed duration at $V_p = -40$ mV.

5. Discussion

Although Markov model was widely used to represent the ion channel kinetics, and interpreted the results from single channel and noise analysis experiments, the channels gating kinetics have been assumed to exist in a finite number of discrete states, the additional assumption that the transition rate constant among the states is independent both of time and of the previous channel activity defines the model as a time-homogeneous Markov chain model. However, it is known that the spontaneous fluctuations in the conformation of proteins involve many different processes that occur over many different time scales from 10^{-15} to 10^{3} s [9].

The present study results indicated that it was adapted to model the gating kinetics of K^+ channel in rat dorsal root ganglion neurons with fractal

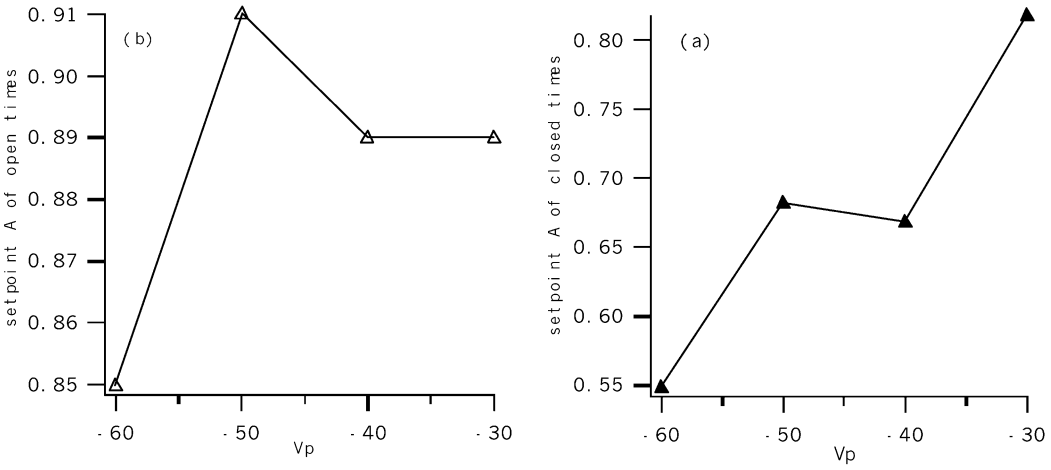

Fig. 5. The kinetic setpoint for closed and open duration varied with the pipette potential.

model. The results give evidence that the spontaneous fluctuation in the conformation of the ion channel protein may be a continuous process, which was well described by the fractal model.

Modeling gating kinetics of K^+ channel in rat dorsal root ganglion neurons with Markov process is based on two aspects. On the one hand, Markov model was chosen by the number of states and their connectivity was very difficult, on the other hand, the kinetics rate constants of Markov model was estimated, this was very complex. The fractal model depends on four parameters. Thus, the mathematical analysis needed to determine the parameters of the fractal model is much simpler than that required to determine the parameters of the Markov model.

The experimental results showed that the kinetic rate constants $k_o = At^{1-D}$ and $k_c = A't^{1-D'}$ were function of time t. Under depolarized (namely pipette potential was -30, -40, -50, -60 mV), the fractal dimension for both the closed and open durations varied with voltage. The kinetic setpoint for both the closed and open durations had complex connection with voltage.

It is very interesting to compare the results of this study with those of Liebovitch [9] and Liu

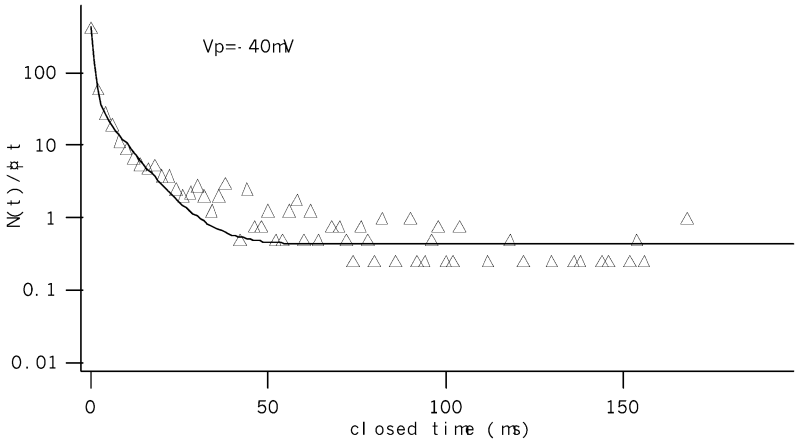

Fig. 6. The fractal model fitted closed duration at $V_p = -40$ mV.

[11]. In Liebovitch results, when the patch was hyperpolarized, the kinetic setpoint for both the open and closed durations of the K^+ channel from cultured mouse hippocampal neurons depended greatly on the voltage, but the fractal dimension was independent of the hyperpolarizing voltage [9]. In Liu results, when the patch was depolarized, the kinetic setpoint for both the open and closed durations of the K^+ channel from PC12 cell independent of the voltage, the fractal dimension depended on the voltage. The difference between our observations and those of Liebvitch's and Liu's may be caused, on the one hand, by different pipette potential, on the other hand, due to different structure of protein, or due to both, or due to pharmacon effect. We tend to believe that it is due to the protein structural difference between all kinds of K^+ channel. However, studies on ion channel structure and molecular dynamics, pharmacology, according to fractal dimension and kinetic setpoint to class all kinds of ion channel that should be the future direction of research.

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References

[1] B. Sakmann, E. Neher, Single-Channel Recording, Plenum Press, New York, 1983.
[2] B. Hille, Ionic Channels of Excitable Membranes, second ed., Sinauer Association, Sunderland, MA, 1992.
[3] D. Colquhoun, A.G. Hawkes, On the stochastic properties of single ion channels, Proc. R. Soc. Lond. B 211 (1981) 205–235.
[4] L.S. Liebovitch, P. Krekora. The physical basis of ion channel kinetics: the importance of dynamics. Proc. Inst. Math. Appl. Univ. Minn. (2001) 1–20.
[5] R. Horn, S.J. Korn, Model selection: reliability and bias, Biophys. J. 55 (1989) 379–381.
[6] L.S. Libovitch, Testing fractal and Markov models of ion channel kinetics, Biophys. J. 55 (1989) 373–377.
[7] J. Timmer, S. Klein, Testing the Markov condition in ion channel recordings, Phys. Rev. E 55 (1997) 3306–3311.
[8] A. Fulinski, Z. Grzywna, I. Mellor, Z. Siwy, P.N.R. Usherwood, Non-Markovian character of ionic current fluctuations in membrane channels, Phys. Rev. E 58 (1998) 919–924.
[9] L.S. Liebovitch, J. Michael Sullivan, Fractal analysis of a voltage-dependent potassium channel from culture mouse hippocampal neurons, Biophys. J. 52 (12) (1987) 979–988.
[10] L.S. Liebovitch, J. Fischbarg, J.P. Koniarek, Ion channel kinetics: a model based on fractal scaling rather than multistate Markov process, Math. Biosci. 84 (1987) 37–68.
[11] X.-M. Liu, M. Tao, X.-D. Han, Q. Fan, J.-R. Lin, Gating kinetics of potassium channel and effects of nerve growth factors in PC12 cells analyzed with fractal model, Acta Pharmacol. Sin. 22 (2001) 103–110.
[12] H.-Z. Hu, Z.-W. Li, Substance P potentiates ATP-activated currents in rat primary sensory neurons, Brain Res. 739 (1996) 163–168.
[13] H.-Z. Hu, Z.-W. Li, Modulation by adenosine of GABA-activated current in rat dorsal root ganglion neurons, J. Physiol. 501 (1997) 67–75.
[14] H.-Z. Hu, Z.-W. Li, J.-Q. Si, Evidence for the existence of substance P autoreceptor in the membrane of rat dorsal root ganglion neurons, Neuroscience 77 (2) (1997) 535–541.
[15] T.-H. Lan, G.-H. Li, Z.-W. Li, J.-R. Lin, Stimulatory effects of NMDA on intracellular Ca^{2+} nonlinear kinetic model in rat dorsal root ganglion neurons, Chin. J. Biomed. Eng. 1 (2002) 177–188.
[16] T.-H. Lan, J.-R. Lin, Rescaled range analysis applied to the study on intracellular Ca^{2+}, Pak. J. Biol. Sci. 6 (10) (2003) 925–931.