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Short communication

ON THE ROLE OF BALL AND CHAIN INTERACTIONS IN RECOVERY FROM THE INACTIVATION OF THE SHAKER POTASSIUM CHANNEL [#]

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Abstract: We describe a new factor in the recovery from inactivation in the ball and chain model. We propose a model in which the tension from the chain may help pull the ball away from its binding site, reducing the duration of the inactivation period. A corresponding model was built and analysed.

Key words: Ion channels, Inactivation, Recovery from inactivation, Ball and chain model

INTRODUCTION

The ball and chain model of inactivation (Fig. 1) was proposed by Armstrong and Bezanilla [1]. Originally, the model was applied to sodium channels to explain how they inactivate after opening. More recent studies showed that the inactivation particle in the sodium channel is not exactly a ball on a chain, but rather a "hinged lid" [2]. However, this discovery did not decrease the significance of the ball and chain model, because the mechanism was found to apply to the shaker potassium channel [3-5].

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Abbreviations used: kT – Boltzmann constant times environment temperature, RHS – right-hand side

The potassium channel ball and chain consists of a hydrophilic series of residues in the chain (60 residues) and a hydrophobic series of residues which form a ball (20 residues). It is assumed that the ball wanders in the space near the pore, and can occlude it to inactivate it, which is an event with a nonzero probability.

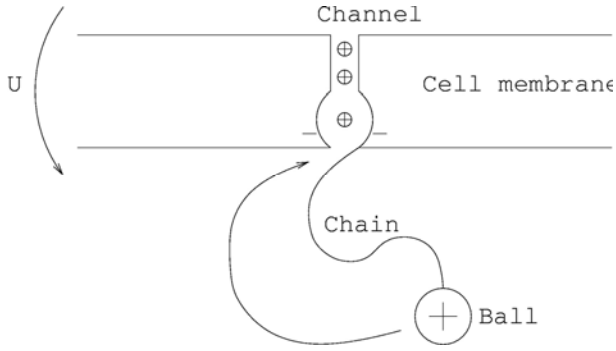

Fig. 1. The ball and chain model of channel inactivation. The ball wandering in the space around the channel's pore can occlude it and stop the ion flow.

As can be seen, the ball and chain model provides an intuitive idea of the inactivation process, but does not reveal much about the possible mechanism of recovery from inactivation. This problem was the subject of various studies taking experimental approaches. For example, Demo and Yellen [6] investigated the influence of transmembrane voltage and K^+ ion concentration on the recovery from inactivation. They found that increased K^+ concentration speeds up the recovery from inactivation. Voltage reversal was also found to speed up the inactivation. To explain this behaviour, they proposed a mechanism where the K^+ ions enter the channel from the extracellular side and push the inactivating particle from its binding site.

The model of Demo and Yellen is quite intuitive and simple. More recent studies have altered this point of view. Gomez-Lagunas and Armstrong [7] discussed how potassium channel recovery from inactivation can be sped up by an increase in the concentration of non-permeant ions. On the other hand, it is also stated that there is an additional inactivation state that is insensitive to ionic concentration changes [7].

These findings complicate the model, and indicate that ionic concentrations and transmembrane voltage cannot be the only factors involved in the general process of recovery from inactivation. This appears to be the case. Looking at the results of Zagotta *et al.* [4], it can be seen that in a channel inactivated by an artificial inactivation particle, the duration of inactivation (before the channel recovers from inactivation) is longer than under natural conditions. This finding is the main basis of this paper. We postulate that the chain plays an inportant role in the recovery from inactivation, namely that the motion of the chain can drag the inactivation particle out of its binding site.

The model

It has yet to be established in realistic modeling how the chain could interact with the ball. The chain is composed of 60 hydrophilic amino acid residues. The first idea was that the chain could drag the ball out of its binding site by means of inertial interactions. However, the time scale of velocity relaxation for inertial processes is actually too small, which we can see by solving Newton's equation of motion for the damping medium:

$$m\frac{dv}{dt} = -6\pi r\eta v \quad (1);$$

$$v = \exp\left(-\frac{6\pi r\eta}{m}t\right) = \exp\left(-\frac{t}{\tau}\right) \quad (2)$$

For an average amino acid with a radius of 1.6 Å and average mass of 2 10^{-25} kg [9], and an intracellular fluid viscosity of $\eta = 5$ cps [8], we obtain $\tau = 13$ ps. This is very small, especially compared with the time scale of the measured recovery from inactivation (milliseconds) and the diffusion time ($\tau_D = \frac{D}{L^2}$, where L is the chain length, 200 Å [9]), which is of the order from micro to milliseconds, depending on whether we consider a single amino acid residue, $D \approx 10^{-5}$ cm^2/s, or the ball, $D \approx 10^{-9}$ cm^2/s [10]).

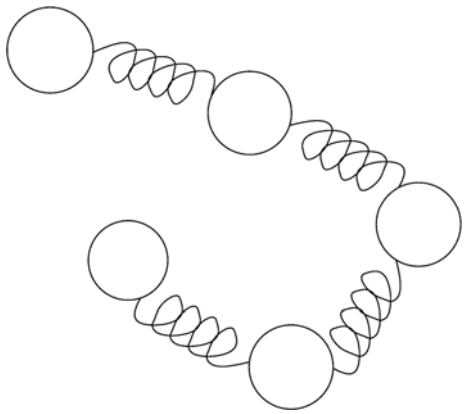

Fig. 2. The Rouse model of a polymer. Subsequent subunits are held together by spring-like connections.

Another possibility is that the chain fluctuations themselves drag the ball out of its binding site. A similar idea, involving entropic forces, was recently presented by Bezrukov *et al.* [11]. The natural way to investigate this is to do the simulation of the Brownian dynamics of the chain residues. Since we are not interested in the folding pattern of the chain, but only in the magnitude of fluctuations near the ball, we do not need a detailed simulation of a peptide; we can simplify the Brownian dynamics to the simulation of a Rouse model of

a polymeric chain [12, 13] (Fig. 2). In this model, we cannot obtain exact configurations of the chain, because we do not model the bond type exactly. Instead, the cumulative force induced by fluctuations can easily be measured: it is proportional to the elongation of the bond between two subunits.
The algorithm used in such a problem is of the Ermak and McCammon type [14], which allows the calculation of the displacements of amino acid subunits based on the equation:

$$\Delta r_i = \sum_{j=1}^{3N} \frac{\partial D_{ij}}{\partial r_j} \Delta t + \frac{1}{kT} \sum_{j=1}^{3N} D_{ij} F_j \Delta t + R_i(D_{ij}, \Delta t) \qquad (3)$$

where the index i goes through all the interacting subunit coordinates, F is an external force acting on the Brownian subunit, and R is a gaussian force whose variance depends upon the diffusion tensor D_{ij} and the time step. The first derivative of the diffusion tensor with respect to displacement disappears for most of the commonly used diffusion tensors [14].
The diffusion tensor in general should include the hydrodynamic interactions between particles (taking the form of the Oseen tensor or Rotne-Prager tensor) [14, 15]. Unfortunately this leads to a large increase in the computational effort to calculate the random force.

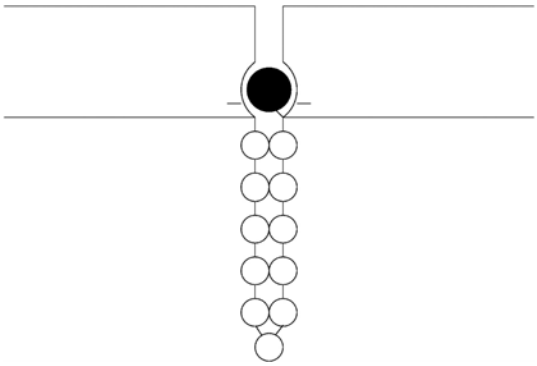

Fig. 3. The initial condition for the chain in the Brownian dynamics simulation. The black circle denotes the ball, the white circles correspond to the chain subunits.

If we omit the hydrodynamic interactions, and take a simple diagonal diffusion tensor, then it is possible to obtain a longer simulation time. As regards the obtained values of tension in the chain, we can expect that the values without hydrodynamic interactions are similar to or smaller than those in the full model, and thus they can be treated as a lower estimate [15].
The value of the diffusion coefficient for the diagonal diffusion tensor $D_{ij} = D\delta_{ij}$ was taken from Liebovitch *et al.* [16] and set at $D = 10^{-5}$ cm^2/s. The random force was gaussian with variance equal to:

$$\sigma^2 = 6D\Delta t \quad (4)$$

The external forces F in the Brownian dynamics simulation originate at the bond interactions. After [14], the bond strength (spring elasticity coefficient in the model) was set at:

$$\kappa = \frac{kT}{\delta^2} \quad (5)$$

where δ stands for the inter-residue displacement (assumed to be δ = 3.33 Å). The initial condition for the Brownian dynamics was set to a fully elongated chain in the direction perpendicular to the membrane surface with both ends fixed at the channel's pore (Fig. 3). The simulation time step was taken to equal 1 ps.

RESULTS AND DISCUSSION

Fig. 4 shows histograms of the tension between the chain and the ball. They were calculated for three lengths of chain: two residues, four residues and eight residues. A comparable simulation for the 60 residues of the real chain was computationally unfeasible; however, we did the calculations for short time scales, and the results are qualitatively similar. We can observe that when the chain gets longer, the maximum tension achieved in the fluctuations increases. This means that we observe a cooperative action of fluctuations acting on the whole chain.

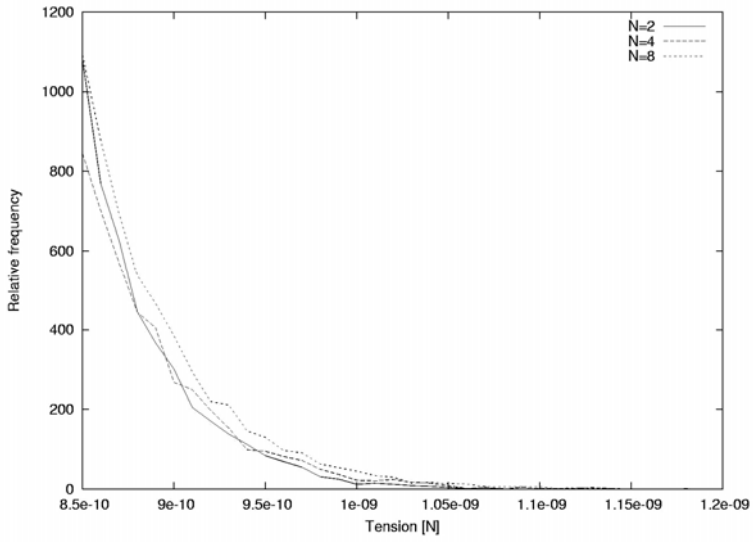

Fig. 4. Histograms of the tensions achieved in the chain in the simulation period of 1 ms (time step 1 ps; 10^6 iterations) for different chain lengths. Increasing the chain length increases the maximum tension achieved by the tail of this distribution. Note that the distribution is cut at lower tensions, and that we did not show tensions smaller than 8.5 nN.

The simulation was also verified for shorter time steps to check that the tensions do not arise from computational defects. The results confirmed that this effect is real.

The results can be compared to rough estimates of the electrostatic and hydrophobic forces that act in the ball-channel complex. These forces have not yet been measured precisely, and there is still a debate on which interactions stabilise the complex (as can be seen, for example in [5]).

The hydrophobic forces can be (over)estimated on the basis of the equation presented in Aldrich *et al.* [5], where it is stated that each $Å^2$ of the surface adds 25 cal/mol to the free energy. Checking the unburied surfaces of the hydrophobic amino acids [17] of the ball, we find that the maximum area that can contribute equals 387.9 $Å^2$. This corresponds to $6.74 \cdot 10^{-20}$ J ≈ 20 kT of free energy. Overestimating that the work needed to separate the hydrophobic surfaces to a distance of one water molecule equals the free energy difference between the bound and unbound states, we obtain the value of force needed for the separation as $F = 0.337$ nN. Bear in mind that the chain tension fluctuations obtain (instantaneous) values of force larger than 1 nN.

A comparison to electrostatic forces can be done if we know the potential profile of the channel. According to [18], near the channel mouth, one can expect 4 kT of electrostatic potential energy per charge (cf. Fig. 2C in [18]), i.e. 8 kT for the ball, which carries two charges. This potential falls to zero at a distance of 20 Å, giving an average force of 0.016 nN.

This is not a high value, and is in fact smaller than the value of hydrophobic interaction. Additionally, it should be taken into account that the recovery from inactivation has a weak dependency on the charge of the ball (as opposed to a dependency on hydrophobicity) [5].

A comparison can also be done by means of the bending energy concept. Taking the persistence length of a peptide chain as $L_p = 5$ Å [20], we can estimate the bending energy [12, 21] of the inactivated chain when it forms a circular loop:

$$E_B = \frac{2kTL_P L}{R^2} \approx 2kT \qquad (6)$$

Here, E_B is the binding energy, L is the chain length, and $R = L/2\pi$ is the radius of the loop formed by the inactivated chain. If the hydrophobic interactions dominate in the ball-channel binding and can reach at most 20 kT, we can see that the bending energy contribution is significant.

Apart from the bending energy, we can estimate the decrease in the entropy of the chain when entering the bound state. The entropy of the chain, expressed as a function of the chain's end-to-end vector R_E for a chain that is floating in space [12], is given as:

$$S = k \log\left[P(N, R_E) dR_E^3\right] + k \log\left[\int \Omega dR_E^3\right] \qquad (7)$$

The probability distribution gives the fraction of microstates available in the conformation versus the total number of microstates. For a free chain, this is given as:

$$P = \left(\frac{3}{2\pi Nb^2} \right)^{3/2} \exp\left(-\frac{3R_E^2}{2Nb^2} \right) \quad (8)$$

where N is the number of residues, and b is the Kuhn length, which for a wormlike chain is twice the persistence length L_P.

In our case, the chain is not floating in space, but exists in a half-space. The total number of microstates is thus reduced by 0.5, and the number of microstates for any particular radius R is also reduced by 0.5. Thus, the probability formula can be seen unchanged in the first approximation.

The last term on the RHS of the equation for entropy describes the entropy of the unbound state. The first term on the RHS describes the decrease in the entropy provided by the conformational constraint. In our case, the conformation which we need to inspect is where $R_E = 0$. Then, the exponential term (which is usually of interest in books devoted to polymer physics) reduces to 1, and the entropic contribution of the bound state to free energy equals:

$$TS = kT \log\left[\left(\frac{3}{2\pi Nb^2} \right)^{\frac{3}{2}} dR_E^3 \right] \quad (9)$$

The formula is very interesting, since $dR_E \rightarrow 0$. If this was the case, this term would grow to infinity. We cannot take this value for dR_E. What then is the meaning of this parameter? It specifies the possible deviations from the exact conformation in the system. The ball bond in the channel is not perfectly rigid, and we can safely assume that dR_E is in the range of 1 Å. The corresponding free energy change is 14 kT. This is again quite large, compared to the bond energy.

Summarizing, we can make the following statements. The experimental results in the literature imply a role for the chain in the recovery from inactivation process, i.e. the process is slower in the absence of the chain. The type of interaction has yet to be understood. In this paper, we showed that a simple inertial model is inappropriate, and we proposed some alterations to the existing model.

The proposed model for recovery from inactivation was based upon the Brownian motion of the chain and its interactions with the ball. The results obtained in this paper by means of a Brownian dynamics simulation indicate that the forces originating from this Brownian motion can reach values comparable to the values of the possible hydrophobic or electrostatic interactions between the ball and the channel. Estimates of the conformational entropy changes and bending energy further support this finding. This implies that these interactions cannot be omitted in the understanding of the recovery from inactivation process.

The simulation shows some new options for experimentation: shortening the chain should decrease the probability of obtaining high tension in the chain, and therefore would slow down the recovery from inactivation.

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REFERENCES

1. Armstrong, C.M. and Bezanilla F. Inactivation of the sodium channel, part II - gating current experiments. **J. Gen. Physiol.** 70 (1977) 567-590.
2. Goldin, A.L. Mechanisms of sodium channel inactivation. **Curr. Op. Neurobiol.** 13 (2003) 284-290.
3. Hoshi T., Zagotta, W.N. and Aldrich, R.W. Biophysical and molecular mechanisms of shaker potassium channel inactivation. **Science** 250 (1990) 533-538.
4. Zagotta, W.N., Hoshi, T. and Aldrich, R.W. Restoration of inactivation in mutants of shaker potassium channels by a peptide derived from ShB. **Science** 250 (1990) 568-571.
5. Murrell-Lagnado, R.D. and Aldrich, R.W. Interactions of amino terminal domains of shaker K channels with a pore blocking site studied with synthetic peptides. **J. Gen. Physiol.** 102 (1993) 949-975.
6. Demo, S.D. and Yellen, G. The inactivation gate of the shaker K^+ channel behaves like an open-channel blocker. **Neuron** 7 (1991) 743-753.
7. Gomez-Lagunas, F. and Armstrong, C.M. The Relation between ion permeation and recovery from inactivation of ShakerB K^+ channels. **Biophys. J.** 67 (1994) 1806-1815.
8. Haak, R.A., Kleinhans, F.W. and Ochs, S. The viscosity of mammalian nerve axoplasm measured by electron spin resonance. **J. Physiol.** 263 (1976) 115-137.
9. Collins, R.M.W. Physics of potassium ion channel inactivation in neurons. **Am. J. Undergr. Res.** 3 (2004) 27-35.
10. Małysiak, K., Borys, P. and Grzywna, Z.J. On the ball and chain model by simple and hyperbolic diffusion - an analytical approach. **Acta Phys. Pol. B** 38 (2007) 1865-1879.
11. Krasilnikov, O.V., Rodrigues, C.G. and Bezrukov, S.M. Single polymer molecules in a protein nanopore in the limit of a strong polymer-pore attraction. **Phys. Rev. Lett.** 97 (2006) 018301-1 – 018301-4, DOI:10.1103/ PhysRevLett.97.018301.
12. Rubinstein, M. and Colby, R.H. **Polymer Physics,** Oxford University Press, New York, 2003, 49-331.
13. Gedde, U.W. **Polymer Physics,** Kuwler Academic Publishers, Dordrecht, 2001, 107-108.

14. Ermak, D.L. and McCammon, J.A. Brownian dynamics with hydrodynamic interactions. **J. Chem. Phys.** 69 (1978) 1352-1360.
15. Dickinson, E. Brownian dynamics with hydrodynamic interactions: The application to protein diffusional problems. **Chem. Soc. Rev.** 14 (1985) 421-443.
16. Liebovitch, L.S., Selector, L.Y. and Kline, R.P. Statistical properties predicted by the ball and chain model of channel inactivation. **Biophys. J.** 63 (1992) 1579-1585.
17. Rose, G.D., Geselowitz, A.R., Lesser, G.J., Lee, R.H. and Zehfus, M.H. Hydrophobicity of amino acid residues in globular proteins. **Science** 229 (1985) 834-838.
18. Chung, S.H., Allen, T.W., Hoyles, M. and Kuyucak, S. Permeation of ions across the potassium channel: Brownian dynamics studies. **Biophys. J.** 77 (1999) 2517-2533.
19. Buscaglia, M., Lapidus, L.J., Eaton, W.A. and Hofrichter, J. Effects of denaturants on the dynamics of loop formation in polypeptides. **Biophys. J.** 91 (2006) 276-288.
20. Marko, J.F. and Siggia, E.D. Stretching DNA. **Macromolecules** 28 (1995) 8759-8770.