ILLUSTRATION OF MODEL CREATION ON EXAMPLE OF APPROXIMATIONS TO THE STEADY STATE CURRENT OF CHEMICAL CYCLIC PROCESSES

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Abstract: *An approach for creation of biophysically based models for the steady state current of cyclic processes is investigated. When the process (like chemical reactions) can be described by a system of linear ordinary differential equations, an analytic expression for its steady state exists. The analytic expression is especially simple for the current of single cycle processes. In biologic context, concentrations of many substances change in a very restricted (patho)physiological range. This allows neglecting some terms of the analytic expression and thus obtaining biophysically based models that are both simple and adequate for description of currents produced by enzymes, pumps or transporters. The approximations obtained could be reduced to the existing empirical models. A clear way of expanding a specific empirical model for obtaining the desired quality and range of validity is also represented. The described approach is general and can be useful for creating biophysically based models of other types of processes.*

KEYWORDS: MATHEMATICAL MODEL, CYCLIC PROCESSES, STEADY STATE CURRENT, ENZYME, TRANSPORTER, PUMP

1. Introduction

To construct an empirical model of some process one has to fit the parameters of a predefined function to some available experimental data [1]. One could construct many functions that are close to each other for some range of data. The choice of a suitable function is complex and requires taking into account specific information. The range of possibilities spans from a lucky guess to a detailed study of the underlying processes. A comparison of various alternative models may illustrate the advantages and the drawbacks of different approaches. The purpose of this paper is to illustrate important aspects of model creation on the example of the most studied chemical cyclic process – the Na+/K+ ATPase (NKA).

2. Analysis of existing NKA models

2.1. Empirical models

Cyclic processes are abundant in biologic environment. Actions of enzymes, transporters, pumps are all cyclic process. All of them collect some resources, perform the appropriate actions, release the products and then they are ready to start again thus forming a cycle. Typically, one is interested in the cumulative effect of many cycles. As a result, the steady state current or at least the average turning rate is used to characterize the cycle. The first great success was description of the steady state enzyme reaction rate – v, as a function of substrate concentration – [S], performed by Michaelis and Menten (1913) [2] and represented by eq. (1).

$$v = \frac{d[P]}{dt} = \frac{[S]*Vmax}{[S]+Km} \quad (1)$$

where Vmax is the maximal reaction rate, [P] is the product concentration, 1/Km is the substrate's affinity.

The Michaelis – Menten relation becomes a basis for many empirical models of protein kinetics. The models of DiFrancesco and Noble [3] eq. (2) and of Luo and Rudy [4] eq. (3) are those for NKA. The Luo and Rudy model has become the standard model of choice for NKA [5-12]. Empirical equations like eq. (2) and (3) are simple, easy to use and to calculate. Unfortunately it is not clear when the approximation is valid, and how to modify the equation to expand the range of validity.

$$Ipmp = P_0 \frac{[Ko]}{[Ko]+pko} \frac{[Nai]}{[Nai]+pnai} \quad (2)$$

$$Ipmp = P_0 \frac{[Ko]}{[Ko]+pko} \frac{1}{1+\left(\frac{pnai}{[Nai]}\right)^{1.5}} * \\ * \frac{1}{1+0.1245e^{-0.1\frac{FV}{RT}}+0.0365\sigma e^{-\frac{FV}{RT}}} \quad (3)$$

where P_0 is the maximal current, $\sigma = \frac{1}{7}(e^{\frac{[Nao]}{67.3}} - 1)$, [Ko], [Nai] and [Nao] are external potassium, internal and external sodium concentrations, respectively; pko and pnai are corresponding affinities; V is membrane potential; F and R are the Faraday and universal gas constants, T is the absolute temperature.

2.2. ODE models

An approach to modeling the processes that is alternative to creation of empirical models is based on studying and description of the underlying chemical reactions. The chemical reactions are usually described as series of transitions between (often unknown) states. Let us denote the probability of the i-th state by $[T_i]$. Each transition between the states i and j, is described by a rate constant – $\alpha_{i,j}$. This results in a system of linear ordinary differential equations (ODE) that potentially fully describes the reaction – eq. (4).

$$\frac{d[T_i]}{dt} = \sum_j \alpha_{i,j} [T_j] \quad (4)$$

To perform direct numerical integration one must provide all the rate constants that describe transitions between the states.

For cyclic processes it is beneficial to distinguish forward rate constants ($\alpha_{i,j}$) and backward rate constants ($\beta_{i,j}$). For NKA, there are at least 30 rate constants (15 forward and 15 backward ones) [13]. However, only some of them have been estimated [14-18]. The most common approach used in this case is the reduction of the number of states in the cycle [13, 19, 20]. Initially, Chapman et al. [21] constructed a 6-state cycle; however, even in this case, some of the 12 rate constants had to be guessed [21]. Later, a complete set of rate constants was obtained for a 4-state NKA cycle only [13, 19, 22].

The rate constants have a close relation with the thermodynamic force that drives the reaction [23, 24]. For the rate constants that form a complete cycle one could write:

$$\frac{\alpha_{1,2}\alpha_{2,3}\dots\alpha_{n-1,n}\alpha_{n,1}}{\beta_{1,2}\beta_{2,3}\dots\beta_{n-1,n}\beta_{n,1}} = \exp(Y/RT) = e^{X} \quad (5)$$

where Y is the total, associated with the transported substances free energy. Further for clarity we would redefine X=Y/RT and would name X as total (driving) force.

To compensate simplifications, the reduction of the number of states in the cycle should be accompanied by more complex expressions used to define individual rate constants. Attempts were made to increase complexity of the 4-state NKA model as the simplified models faced problems to reflect inter-tissue and inter-species differences [19, 20].

2.3. Analytic models

In the steady state conditions a system of differential equations is transformed into a system of algebraic ones. This allows an exact analytic description of the steady state [23-25]. Some models are based on this observation [26-29].

The steady state current for a cyclic process looks like rational function with two terms above and n^2 terms below the line, where – n is the number of the cycle states. For a 3 – state cycle system the solution is like eq. (6):

$$Ist = \frac{\alpha_{1,2}\alpha_{2,3}\alpha_{3,1} - \beta_{1,2}\beta_{2,3}\beta_{3,1}}{\left(\alpha_{2,3}\alpha_{3,1}+\alpha_{3,1}\beta_{1,2}+\beta_{1,2}\beta_{2,3}\right)+\left(\alpha_{3,1}\alpha_{1,2}+\alpha_{1,2}\beta_{2,3}+\beta_{2,3}\beta_{3,1}\right)+\left(\alpha_{1,2}\alpha_{2,3}+\alpha_{2,3}\beta_{3,1}+\beta_{3,1}\beta_{1,2}\right)} \quad (6)$$

The general solution for an n-state cycle [23, 24] is a direct expansion of eq. (6):

$$Ist = \frac{\alpha_{1,2}\alpha_{2,3}\dots\alpha_{n-1,n}\alpha_{n,1} - \beta_{1,2}\beta_{2,3}\dots\beta_{n-1,n}\beta_{n,1}}{\left(\alpha_{2,3}\dots\alpha_{n-1,n}\alpha_{n,1}+\alpha_{3,4}\dots\alpha_{n,1}\beta_{1,2}+(n-2)\text{ other terms in a group}\right)+(n-1)\text{ other groups}} \quad (7)$$

Where, *Ist* as well as the rate constants have dimension of s^{-1}.

Unfortunately the rate constants in eq. (7) are still mainly unknown. The first approach to this problem is to reduce the number of states in the cycle to 2-6 and then to solve the system of ODE analytically. So obtained approximation is further transferred into an analytical function of concentrations and potential [26-28].

Another approach to the problem is also possible [29]. Instead of decreasing the number of states and complicating the individual rate constants, one could do just the opposite. That is to simplify individual rate constants by using the maximal number of states the transporter can occupy. Then, according to the mass action law, those rate constants would have a linear dependence on concentrations:

$$\alpha_k = \alpha'_k[Sb_k] \quad \beta_l = \beta'_l[Sr_l] \quad (8)$$

where α'_k and β'_l do not depend on concentrations, $[Sb_k]$ is concentration of the k-th binding substance, $[Sr_l]$ is concentration of the l-th released substance.

Eq. (8) could transform the terms under the line in eq. (7) into a multidimensional polynomial. Then, combining equations (5, 8 and 7) one could obtain for the current:

$$Ist = \frac{e^{X}-1}{Ae^{X}+B} \quad (9)$$

$$A = a_0\left(1 + \sum\frac{a_{sbk}([S])}{[Sb_k]} + \sum\frac{[Sr_l]}{a_{srl}([S])}\right)$$

$$B = b_0\left(1 + \sum\frac{b_{srl}([S])}{[Sr_l]} + \sum\frac{[Sb_k]}{b_{sbk}([S])}\right)$$

where a_0, b_0 have dimension of seconds, [S] represents concentrations of all the substances, $a_{sbk}([S])$, $a_{srl}([S])$, $b_{sbk}([S])$ and $b_{srl}([S])$ have dimension of concentration, X is the driving force [29]. For NKA the driving force would be:

$$X = \frac{dGatp}{RT} + \ln\left(\frac{[ATP]}{[ADP][P][H]}\right) - 3\ln\left(\frac{[Na_o]}{[Na_i]}\right) + 2\ln\left(\frac{[K_o]}{[K_i]}\right) + (3zna - 2zk)\frac{FV}{RT} \quad (10)$$

We see that many different models for a single underlying process could be created.

3. Discussion and recommendations

The steady state current depends on the driving force X, and concentrations of the related substances (eq. 9). Specific approximations would be appropriate in various conditions.

The processes where the driving force is close to zero would be reversible processes, where current could change direction. For them small changes in concentrations could cause large relative changes in the driving force X (eq. 10) and thus in the current. As a result, the effect of the driving force on the current could be significant for reversible processes and should be explicitly present in the model.

For irreversible processes the size of the driving force is significant ($|X|>>0$). Then the effect of the driving force on the current would be small (eq. 9). As a result it is often neglected like in the cases of empirical models described above (eq. 2, 3).

The driving force calculation should not be a problem. An explicit presence of the driving force in eq. (9) guarantees that the direction of the current would always be correct. So models that explicitly represent the effect of the driving force would have potentially larger range of validity.

In biologic environment, the concentrations of many substances change in very restricted ranges. This could be combined with the observation that the reaction rate in the Michaelis – Menten relation (eq. 1) has a weak sensitivity to the substrate concentration. The rate is 0.1Vmax when [S] = Km/9, and it is 0.9Vmax when [S] =

9Km. In other words an 81-fold increase in substrate concentration is required for increasing the rate from 10% to 90% of the limit [30]. So by neglecting the affinities to substances whose concentrations vary significantly less than 81 times, only a small error would be introduced in a model for the steady state current (eq. 9). If only substances, whose concentrations vary within narrow ranges, are used in the cycle, the current could often be described by a single parameter – its maximal turning rate. This is usually treated as an oversimplification. Therefore, in such cases, the models like those described by eq. (2) and eq. (3) are used, where contributions of the most significant terms are present and contribution of all other terms is neglected.

Concentration of some other substances could vary much more significantly (like 81-fold or similar). That could be intracellular Ca^{++}, pH or other signaling molecules. If that substance is part of the cycle, good reasons should be present to neglect its affinity. That affinity may have complex dependence on concentrations [29].

The processes in the cycle that we have studied so far may have nothing common with the regulation of the cycle. For example, in skeletal muscles, changes in concentration of cyclic adenosine mono phosphate (cAMP) activates the related kinase that in turn, modifies NKA to augment NKA current [31, 32]. Thus to obtain a realistic model, one may need to consider several cycles with possible ligand or voltage gated transitions between them.

A proper system of differential equations could potentially fully describe the chemical processes under study when the necessary states and rate constants are available. However obtaining the states and rate constants could be problematic. When all the substances have rather stable concentrations, the steady state current would be almost equal to its maximal turning rate. Finding many rate constants from what is essentially a single current value is an impossible task. To make situation even more complex external regulation by some signaling molecules or by membrane voltage is likely to be present in that case. To take information on the rate constants, one must perform measurements of the steady state current and of the concentrations of all of the involved substances with great precision. So obtaining rate constants to construct quantitative ODE model may be a very tricky task that is not always possible.

In such complicated cases, when creating quantitative ODE model is not possible, analytic approach that lead to eq. (9) provides a valuable alternative. Thus equation (9), turns out to be a more universal candidate for a quantitative model than a system of ODE. Nevertheless a system of ODE is a great starting point for an analysis of the underlying process [29].

4. Conclusion

Life is more complex than the steady state current of cyclic processes. Models for many processes have to be created. However creation of a useful quantitative model is not an easy task. Different types of models reveal different aspects of the underlying processes. Cyclic chemical processes are sufficiently well studied to illustrate many important aspects of model creation. I hope that the analysis represented here would be helpful in creation of new models.

5. References

1. Press, W.H., et al., *Numerical recipes in C: the art of scientific computing* 1992: Cambridge University Press.
2. Michaelis, L. and M.M.L. Menten, *The kinetics of invertin action.* FEBS letters, 2013. **587**(17): p. 2712-2720.
3. DiFrancesco, D. and D. Noble, *A model of cardiac electrical activity incorporating ionic pumps and concentration changes.* Philos Trans R Soc Lond B Biol Sci, 1985. **307**(1133): p. 353-98.
4. Luo, C.H. and Y. Rudy, *A dynamic model of the cardiac ventricular action potential. I. Simulations of ionic currents and concentration changes.* Circ Res, 1994. **74**(6): p. 1071-96.
5. Wallinga, W., et al., *Modelling action potentials and membrane currents of mammalian skeletal muscle fibres in coherence with potassium concentration changes in the T-tubular system.* Eur Biophys J, 1999. **28**(4): p. 317-29.
6. Pandit, S.V., et al., *A mathematical model of action potential heterogeneity in adult rat left ventricular myocytes.* Biophys J, 2001. **81**(6): p. 3029-51.
7. Bondarenko, V.E., et al., *Computer model of action potential of mouse ventricular myocytes.* Am J Physiol Heart Circ Physiol, 2004. **287**(3): p. H1378-403.
8. Hund, T.J. and Y. Rudy, *Rate dependence and regulation of action potential and calcium transient in a canine cardiac ventricular cell model.* Circulation, 2004. **110**(20): p. 3168-74.
9. Shannon, T.R., et al., *A mathematical treatment of integrated Ca dynamics within the ventricular myocyte.* Biophys J, 2004. **87**(5): p. 3351-71.
10. ten Tusscher, K.H., et al., *A model for human ventricular tissue.* Am J Physiol Heart Circ Physiol, 2004. **286**(4): p. H1573-89.
11. Fortune, E. and M.M. Lowery, *Effect of extracellular potassium accumulation on muscle fiber conduction velocity: a simulation study.* Ann Biomed Eng, 2009. **37**(10): p. 2105-17.
12. Grandi, E., et al., *Interplay of voltage and Ca-dependent inactivation of L-type Ca current.* Prog Biophys Mol Biol, 2010. **103**(1): p. 44-50.
13. Smith, N.P. and E.J. Crampin, *Development of models of active ion transport for whole-cell modelling: cardiac sodium-potassium pump as a case study.* Prog Biophys Mol Biol, 2004. **85**(2-3): p. 387-405.
14. Schulz, S. and H.J. Apell, *Investigation of ion binding to the cytoplasmic binding sites of the Na,K-pump.* Eur Biophys J, 1995. **23**(6): p. 413-21.
15. Schneeberger, A. and H.J. Apell, *Ion selectivity of the cytoplasmic binding sites of the Na,K-ATPase: I. Sodium binding is associated with a conformational rearrangement.* J Membr Biol, 1999. **168**(3): p. 221-8.
16. Holmgren, M., et al., *Three distinct and sequential steps in the release of sodium ions by the Na+/K+-ATPase.* Nature, 2000. **403**(6772): p. 898-901.
17. De Weer, P., D.C. Gadsby, and R.F. Rakowski, *Voltage dependence of the apparent affinity for external Na(+) of the backward-running sodium pump.* J Gen Physiol, 2001. **117**(4): p. 315-28.
18. Gadsby, D.C., et al., *The dynamic relationships between the three events that release individual Na(+) ions from the Na(+)/K(+)-ATPase.* Nat Commun, 2012. **3**: p. 669.
19. Garcia, A., et al., *Kinetic comparisons of heart and kidney Na+,K(+)-ATPases.* Biophys J, 2012. **103**(4): p. 677-88.
20. Lewalle, A., S.A. Niederer, and N.P. Smith, *Species-dependent adaptation of the cardiac Na+/K+ pump kinetics to the intracellular Na+ concentration.* J Physiol, 2014. **592**(24): p. 5355-71.
21. Chapman, J.B., E.A. Johnson, and J.M. Kootsey, *Electrical and biochemical properties of an enzyme model*

of the sodium pump. J Membr Biol, 1983. **74**(2): p. 139-53.
22. Oka, C., C.Y. Cha, and A. Noma, *Characterization of the cardiac Na+/K+ pump by development of a comprehensive and mechanistic model.* J Theor Biol, 2010. **265**(1): p. 68-77.
23. Hill, T.L., *Free energy transduction in biology: the steady-state kinetic and thermodynamic formalism*. 1977, New York: Academic Press. xii, 229 p.
24. Hill, T.L., *Free energy transduction and biochemical cycle kinetics*. 1989, New York: Springer-Verlag. 119 p.
25. King, E.L. and C. Altman, *A Schematic Method of Deriving the Rate Laws for Enzyme-Catalyzed Reactions.* The Journal of Physical Chemistry, 1956. **60**(10): p. 1375-1378.
26. Hernandez, J., J. Fischbarg, and L.S. Liebovitch, *Kinetic model of the effects of electrogenic enzymes on the membrane potential.* J Theor Biol, 1989. **137**(1): p. 113-25.
27. Gadsby, D.C. and M. Nakao, *Steady-state current-voltage relationship of the Na/K pump in guinea pig ventricular myocytes.* J Gen Physiol, 1989. **94**(3): p. 511-37.
28. Sagar, A. and R.F. Rakowski, *Access channel model for the voltage dependence of the forward-running Na+/K+ pump.* J Gen Physiol, 1994. **103**(5): p. 869-93.
29. Dimitrov, A., *An approach to expand description of the pump and co-transporter steady-state current.* Journal of theoretical biology, 2017. **412**: p. 94-99.
30. Cornish-Bowden, A., *Fundamentals of enzyme kinetics.* 2012, Weinheim, Germany: Wiley-Blackwell.
31. Clausen, T., *The sodium pump keeps us going.* Ann N Y Acad Sci, 2003. **986**: p. 595-602.
32. Clausen, T., *Quantification of Na+,K+ pumps and their transport rate in skeletal muscle: functional significance.* J Gen Physiol, 2013. **142**(4): p. 327-45.