Published in final edited form as:

Sci Signal. ; 4(191): tr8. doi:10.1126/scisignal.2001988.

Obtaining and Estimating Kinetic Parameters from the Literature

Susana R. Neves*

Department of Pharmacology and Systems Therapeutics and systems Biology Center New York, Mount Sinai School of Medicine, New York, NY 10029, USA

Abstract

This Teaching Resource provides lecture notes, slides, and a student assignment for a lecture on strategies for the development of mathematical models. Many biological processes can be represented mathematically as systems of ordinary differential equations (ODEs). Simulations with these mathematical models can provide mechanistic insight into the underlying biology of the system. A prerequisite for running simulations, however, is the identification of kinetic parameters that correspond closely with the biological reality. This lecture presents an overview of the steps required for the development of kinetic ODE models and describes experimental methods that can yield kinetic parameters and concentrations of reactants, which are essential for the development of kinetic models. Strategies are provided to extract necessary parameters from published data. The homework assignment requires students to find parameters appropriate for a well-studied biological regulatory system, convert these parameters into appropriate units, and interpret how different values of these parameters may lead to different biological behaviors.

Keywords

K_D ; K_m ; V_{max} ; Michaelis-Menten; enzyme

Summary of Lecture

Computational modeling is a useful predictive tool to obtain mechanistic insight into signaling processes. Several computational studies have yielded information on how the cell

Copyright 2008 by the American Association for the Advancement of Science; all rights reserved.

*Corresponding author. susana.neves@mssm.edu.

Educational Details

Learning Resource Type: Lecture, assignment, digital presentation

Context: Graduate

Intended Users: Teacher, learner

Intended Educational Use: Learn, plan, teach

Discipline: Biostatistics; bioinformatics, genomics, and proteomics

Technical Details

Format: PowerPoint (.ppt)

Size: 4.5 MB *Requirements:* Microsoft PowerPoint *Format:* MATLAB

Supplementary Materials

<http://stke.sciencemag.org/cgi/content/full/sigtrans;4/191/tr8/DC1>

Slides

Answer key

Citation: S. R. Neves, Obtaining and estimating kinetic parameters from the literature. *Sci. Signal.* **4**, tr8 (2011).

uses complex networks of interacting pathways and feedback mechanisms to regulate multiple cellular functions (1). Computational approaches have been widely used to produce predictive models that have recapitulated the biological system of interest, and have provided nonintuitive hypotheses that have been experimentally validated (1, 2). The construction of such kinetic signaling models depends on the specification of reactions and their corresponding parameters; thus, it relies on the availability of experimental data on kinetic rate constants and the concentrations of cellular components. Often, these values are not available and they must be estimated from time-course and dose-response experiments.

One of the initial stages in the assembly of a kinetic model is to establish the reaction scheme of all of the components of interest within the network (Slides 2 and 3). This may involve a wire diagram listing all of the components within the pathway or subnetwork of interest, their appropriate subcellular location, and their connections displayed as arrows (for stimulatory connections), plungers (for inhibitory connections), or lines (for neutral connections, such as scaffolding reactions). Next, the connectivity diagram must be converted into chemical reactions, which are mathematically represented by the law of mass action (see the lecture “An Introduction to Dynamical Systems”). Michaelis-Menten approximations can also be used to describe enzymatic reactions. Once these reactions have been written out, the experimental data needed for the reaction parameters are collected. These data include not only parameters, such as kinetic rate constants and initial concentrations of components, but also input:output relations (Slide 3), which are experimental data (ideally in the experimental system of interest) that are used to constrain the unknown parameters within the model. These types of data include time courses and dose-response curves of output responses to varying inputs.

Input:output relations are as essential to the development of a kinetic model as are kinetic parameters, because they are the guiding data used to constrain the model (3). These types of data are especially valuable during the parameterization of values that lack direct experimental measurements. The availability of input:output relations can also determine the modularity of the model. For example, in a model of stimulation of the β -adrenergic receptor (β AR), a time course of changes in the concentration of the second-messenger cyclic adenosine monophosphate (cAMP) could define the input of the module to be isoproterenol (a β AR agonist) and the output to be cAMP. This computational module will include all of the reactions involved in the binding of isoproterenol to the β AR, receptor activation, activation of the heterotrimeric guanine nucleotide-binding protein (G protein) G_s , activation of adenylyl cyclase (AC), and production and degradation of cAMP (Slide 3). Even though not all of the parameters for each of these reactions may have been experimentally determined, and despite the use of some estimated parameters in the model, if the simulation results of a module recapitulate an experimentally defined input:output relation, then the module can be considered to be accurate (Slide 4) (1). There may be other types of data available, such as input:output relations in the presence or absence of additional perturbations. For example, a time-course analysis of cAMP concentrations in response to stimulation of cells with isoproterenol in the presence or absence of a phosphodiesterase (PDE) inhibitor can be helpful in determining the contribution of phosphodiesterase activity in regulating the amount of cAMP. If additional experimental data are available, overlapping modules, such as those with isoproterenol as the input but

with the activation of either cAMP-dependent protein kinase (PKA) or CREB as the output, may also be used. Input:output curves can also be used as editing tools that assist in improving the model topology. The inability to qualitatively match simulation results with input:output curves is always informative, because it may highlight the need to reconsider the network topology used and could require either the inclusion of additional reactions or the exclusion of irrelevant ones.

Concentrations of Cellular Components

The cellular concentrations can be obtained from studies that use methods such as purifications, quantitative Western blotting, and quantitative radioligand-binding assays (Slide 5). In purification studies, a series of fractionation steps and measurements of the specific activity of the protein of interest are done in parallel (Slides 6 and 7). The specific activity is defined as an enzymatic conversion of substrate into product or the specific binding of the protein of interest to a labeled interactor (compound or protein). Most published studies summarize the results from each purification step in a table format. The information in the table is used to back-calculate the cellular concentration. Slides 6 and 7 illustrate sample calculations from published data (4, 5). Such purification approaches from native sources were popular in the precloning era of research, and often multiple gene products were pooled under one specific activity. For example, what was once named “glucagon-sensitive adenylyl cyclase” (6), we now know as the gene products AC5 and AC6, and possibly AC8 (Slide 8). Thus, care must be taken to track the evolution of nomenclature over time. A caveat of estimating concentrations from purification studies of highly heterogeneous tissue, such as the brain or kidney, is that it is possible to underestimate the concentration if the protein of interest is not broadly present throughout the tissue.

Quantitative Western blotting techniques can also be used, provided that antibodies against the protein of interest are available. The concentration of the protein of interest can be extrapolated by creating a standard curve using a purified tagged form of the protein. This tagged version of the protein is overexpressed in a heterologous system and isolated to homogeneity using affinity purification. Known amounts of purified protein are resolved by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) together with various amounts of cellular lysate containing unknown amounts of the protein of interest (Slides 9 and 10). After Western blotting analysis with an antibody that recognizes both the tagged and endogenous forms of the protein of interest, the intensities of the resulting bands of the lysate can be used to infer the cellular concentration of the protein of interest (7, 8). For receptors, and other membrane proteins, concentrations can be quantified in experiments using radiolabeled, high-affinity agonists or antagonists that bind specifically to the protein of interest (Slide 11) (9). This method enables the quantification of membrane proteins of low abundance. Radioligand-binding assays can be used in intact tissue, which allows the measurement of concentrations in situ, when appropriate unit conversions are applied (Slide 12).

Reaction Rates

Next, we review binding and enzymatic reactions and define important concepts, such as the dissociation constant, K_D (Slides 13 to 16). Reaction rate constants can be obtained from the primary experimental literature. Structure and function studies are a source of kinetic data, because most of these studies involve systematic mutagenesis of amino acid residues followed by measurement of the resulting effects on the specific activity of the protein of interest. The dissociation constant K_D for binding reactions can be obtained from saturation binding studies (Slide 17), which we illustrate with a published example (10). In these studies, a protein of interest is incubated with increasing amounts of a labeled interactor (another protein or small molecule) until a maximal concentration of the complex is formed and the system is saturated. The maximum number of binding sites ($B_{\max}$) can also be obtained from radioligand-binding studies. K_D values are also measured from surface plasmon resonance studies, in which an immobilized protein on a surface interacts with a mobile binding partner protein. The change in the reflectivity of the immobilized surface correlates with the binding events, enabling a K_D value to be calculated (Slide 18) (11). K_D describes equilibrium or steady-state conditions. K_D is defined as the ratio of dissociation rate (k_{off}) to the association rate (k_{on}) and at steady-state conditions, it is equal to the ratio of product to reactants (Slide 19). These rates can be explicitly measured through dissociation binding experiments, which track the amount of released labeled reactant from a preformed complex, and association binding experiments, which track the incorporation of a labeled protein into the complex over time (Slide 20). It is possible that the K_D has been experimentally determined but the reaction rates are not available. This is problematic, because multiple combinations of k_{off} and k_{on} values will give rise to the same K_D value and, thus, the same steady-state value of the resulting complex, but the time that evolves while it reaches equilibrium will vary (Slide 21).

Once all of the kinetic rates for the binding reactions are collected, the reaction rate for any given component in the model should reflect all the reactions that produce or consume the component (Slide 22). For example, the simplified reaction scheme of a ligand-dependent activation of a G protein– coupled receptor consists of four reactions: (i) Ligand (L) binds to the receptor (R); (ii) the receptor binds to the G protein (G); (iii) the ligand binds to a precoupled form of the receptor (RG); and (iv) the ligand-receptor complex (LR) binds to the G protein. For the sake of clarity, we will ignore any events downstream of the formation of the active receptor–G protein complex (LRG). The rate of production of LR depends on two reactions: production ($L + R \leftrightarrow LR$) and consumption ($LR + G \leftrightarrow LRG$) (Slide 22).

For enzymatic reactions represented with the Michealis-Menten approximation, the K_m and $V_{\max}$ values are needed. Enzymatic studies involve in vitro reactions with a known amount of purified enzyme, varying initial amounts of excess substrate, and a detectable product. Product formation is monitored as a function of time for each reaction. The initial rate of product formation is then plotted as a function of the initial concentration of substrate. From this plot, the K_m and $V_{\max}$ values can be extracted. The $V_{\max}$ is the maximal rate of the reaction and is defined by the k_{cat} (or the turnover number) and the total enzyme concentration. The K_m is the concentration of substrate that gives rise to half the $V_{\max}$ (Slide 23). After reviewing the Michaelis-Menten equations (Slides 24 to 33), the lecture describes,

using an example from the literature (12), how to convert between $V_{\max}$ and k_{cat} values if the enzyme concentration is known (Slides 34 and 35).

Parameter Estimation

For those kinetic parameters that lack experimental values, the use of input:output curves can guide their estimation. Unknown parameters can be estimated with available software packages such as MATLAB. Starting-point values can be used during the process of constraining the model. These values should be within physiological ranges: K_D for complexes in the high nanomolar to micromolar range; initial concentrations in the nanomolar range; and k_{cat} in the range of 0.1 to 10 s^{-1} (Slides 36 to 39). The type of experimental data used to constrain the model is from input:output relations described previously. Once all parameters have been obtained, parameter-sensitivity analysis is performed. Sensitivity analysis consists of varying parameters and monitoring the effect(s) on model output. This process identifies those parameters that have the greatest consequence on the output of the model and require the most accuracy. In the homework assignment, the students are asked to use data from a purification study to determine cellular concentration. They are also asked to convert kinetic parameter values to units that can be used in kinetic models, and to create a reaction scheme.

Problem Set

One of the regulatory reactions described in the lecture notes (Slide 3) is the degradation of cAMP by PDEs. PDEs can also degrade cyclic GMP, but with quantitatively different efficiency. This question concerns how the kinetic parameters describing PDE activity, and the regulation of this enzyme, influence the ability of different PDE subtypes to modulate cAMP and cGMP-dependent cellular signaling.

Question 1. Determine the cellular concentration of PDE1A in brain tissue based on the data in Table 1.

Question 2. PDE1A activity is regulated. (2a) Specify the mode of regulation, and write the appropriate reaction scheme of enzymatic/binding events to reflect such regulation. (2b) Hypothetically, if the mode of regulation involves a decrease in the K_m , what would happen to the rate of the reaction?

Question 3. PDE1A has K_m value of 2 μM for cGMP, and 40 μM for cAMP. Determine the fraction of $V_{\max}$ that would be obtained at the following substrate concentrations:

- a. $[S] = 1 \text{ } \mu\text{M}$
- b. $[S] = 4 \text{ } \mu\text{M}$
- c. $[S] = 20 \text{ } \mu\text{M}$.

Question 4. Determine the k_{cat} values of the PDE1A subtype for both cAMP and cGMP as substrates. The $V_{\max}$ for PDE1A1 is 166.0 $\mu\text{mol min}^{-1} \text{ mg}^{-1}$ (cAMP) and 93 $\mu\text{mol}^{-1} \text{ min}^{-1} \text{ mg}^{-1}$ (cGMP). Assume that 2 mg of enzyme was used for each reaction. Note: Convert the

concentration value into μM units. Units for kinetic parameters should be in μM (K_m or K_D) and s^{-1} (k_{cat}). Be mindful of any nomenclature changes that may have occurred over time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Special thanks to E. Sobie and A. W. Boran for critical reading. **Funding:** This work was supported by Systems Biology Center of New York (PSDGM071558) and NIH (5R01DK087650).

References and Notes

1. Bhalla US, Iyengar R. Emergent properties of networks of biological signaling pathways. *Science*. 1999; 283:381–387. [PubMed: 9888852]
2. Bhalla US, Ram PT, Iyengar R. MAP kinase phosphatase as a locus of flexibility in a mitogen-activated protein kinase signaling network. *Science*. 2002; 297:1018–1023. [PubMed: 12169734]
3. Neves SR, Iyengar R. Modeling of signaling networks. *Bioessays*. 2002; 24:1110–1117. [PubMed: 12447976]
4. Maekawa M, Nakamura S, Hattori S. Purification of a novel ras GTPase-activating protein from rat brain. *J. Biol. Chem.* 1993; 268:22948–22952. [PubMed: 8226805]
5. Rascón A, Lindgren S, Stavenow L, Belfrage P, Andersson KE, Manganiello VC, Degerman E. Purification and properties of the cGMP-inhibited cAMP phosphodiesterase from bovine aortic smooth muscle. *Biochim. Biophys. Acta*. 1992; 1134:149–156. [PubMed: 1313303]
6. Pohl SL, Birnbaumer L, Rodbell M. The glucagon-sensitive adenylyl cyclase system in plasma membranes of rat liver. I. Properties. *J. Biol. Chem.* 1971; 246:1849–1856. [PubMed: 4993961]
7. Premont RT, Buku A, Iyengar R. The G alpha z gene product in human erythrocytes. Identification as a 41-kilodalton protein. *J. Biol. Chem.* 1989; 264:14960–14964. [PubMed: 2504714]
8. Fujioka A, Terai K, Itoh RE, Aoki K, Nakamura T, Kuroda S, Nishida E, Matsuda M. Dynamics of the Ras/ERK MAPK cascade as monitored by fluorescent probes. *J. Biol. Chem.* 2006; 281:8917–8926. [PubMed: 16418172]
9. Buxton IL, Brunton LL. Direct analysis of beta-adrenergic receptor subtypes on intact adult ventricular myocytes of the rat. *Circ. Res.* 1985; 56:126–132. [PubMed: 2857116]
10. Asanuma K, Yanagida-Asanuma E, Faul C, Tomino Y, Kim K, Mundel P. Synaptopodin orchestrates actin organization and cell motility via regulation of RhoA signalling. *Nat. Cell Biol.* 2006; 8:485–491. [PubMed: 16622418]
11. Chook YM, Gish GD, Kay CM, Pai EF, Pawson T. The Grb2-mSos1 complex binds phosphopeptides with higher affinity than Grb2. *J. Biol. Chem.* 1996; 271:30472–30478. [PubMed: 8940013]
12. Zhang B, Wang ZX, Zheng Y. Characterization of the interactions between the small GTPase Cdc42 and its GTPase-activating proteins and putative effectors. Comparison of kinetic properties of Cdc42 binding to the Cdc42-interactive domains. *J. Biol. Chem.* 1997; 272:21999–22007. [PubMed: 9268338]
13. Hansen RS, Beavo JA. Purification of two calcium/calmodulin-dependent forms of cyclic nucleotide phosphodiesterase by using conformation-specific monoclonal antibody chromatography. *Proc. Natl. Acad. Sci. U.S.A.* 1982; 79:2788–2792. [PubMed: 6283544]

Table 1

Purification of heart and brain calmodulin-dependent phosphodiesterase (13).

Fraction	Total protein, mg	Total activity, * units	Specific activity, units/mg	Yield, %	Purification, fold
Heart [†]					
Extract	16,000	240 (100)	0.015	100	1.0
DEAE-cellulose	1,200	102 (26)	0.085	43	5.7
Antibody-Ultrogel	0.080	22 (2.1)	270	9.0	18,000
Brain [‡]					
Extract	1,100	130 (46)	0.12	100	1.0
DEAE-cellulose	350	67 (11)	0.20	51	1.6
Antibody-Ultrogel	0.071	21 (1.4)	300	16	2,400

* Activity was determined by using 1 mM cAMP and saturating Ca²⁺/calmodulin as described. Activity in parentheses was determined in the presence of EGTA.

[†]Data are based on 800 g of bovine ventricle.

[‡]Data are based on 100 g of bovine cerebral cortex.