Mathematical Biosciences 000 (2016) 1–9

Contents lists available at ScienceDirect

Mathematical Biosciences

journal homepage: www.elsevier.com/locate/mbs

On the validity and errors of the pseudo-first-order kinetics in ligand–receptor binding

Wylie Stroberg[a], Santiago Schnell[a,b,c,*]

[a] Department of Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI 48109, USA
[b] Department of Computational Medicine and Bioinformatics, University of Michigan Medical School, Ann Arbor, MI 48109, USA
[c] Brehm Center for Diabetes Research, University of Michigan Medical School, Ann Arbor, MI 48105, USA

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Pseudo-first-order kinetics
Ligand–receptor binding
Experimental design
Approximation validity
Rate constant estimation
Fitting procedure

ABSTRACT

The simple bimolecular ligand–receptor binding interaction is often linearized by assuming pseudo-first-order kinetics when one species is present in excess. Here, a phase-plane analysis allows the derivation of a new condition for the validity of pseudo-first-order kinetics that is independent of the initial receptor concentration. The validity of the derived condition is analyzed from two viewpoints. In the first, time courses of the exact and approximate solutions to the ligand–receptor rate equations are compared when all rate constants are known. The second viewpoint assesses the validity through the error induced when the approximate equation is used to estimate kinetic constants from data. Although these two interpretations of validity are often assumed to coincide, we show that they are distinct, and that large errors are possible in estimated kinetic constants, even when the linearized and exact rate equations provide nearly identical solutions.

1. Introduction

In biochemical kinetics, simplifying assumptions that decouple or reduce the order of rate equations for complex reaction mechanisms are ubiquitous. Aside from making theoretical analysis of complex reactions more tractable, order-reducing approximations can greatly simplify the interpretation of experimental data [1,2]. Experiments performed under conditions that allow for linearization have historically been the preferred method for estimating equilibrium and rate constants because they allow for the isolation of a subset of the interactions [3–5]. For this reason, when designing an experiment, it is essential to know the necessary conditions for the simplifying assumptions to be valid. Significant theoretical work has been directed at deriving rigorous bounds for the validity of simplifying assumptions [6–11], but this work often overlooks the manner in which the reduced models are used to interpret experimental results. In many cases, the simplified models are used to estimate equilibrium and rate constants from experimental data [12–14, for example]. Rarely is the validity of a simplifying assumption analyzed with this utility in mind. To examine how this viewpoint can affect the conditions for validity, we consider the simplest model for ligand–receptor binding with 1:1 stoichiometry [15].

Binding of ligand to cell surface receptors has been amenable to in vitro experimental investigation for the past four decades [16]. In the typical experimental approach, isolated membranes possessing free receptors are studied using ligands as pharmaceutical agents [17]. In the simplest model of such an experiment, the binding of a ligand L to a receptor R is a bimolecular reversible association reaction with 1:1 stoichiometry yielding a ligand–receptor intermediate complex C:

$$L + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C, \tag{1}$$

where k_1 and k_{-1} are, respectively, the association and dissociation rate constants of the ligand–receptor complex. This reaction scheme is mathematically described by a system of coupled nonlinear second-order differential equations. By applying the law of mass action to reaction (1), we obtain

$$\frac{\mathrm{d}[C]}{\mathrm{d}t} = -\frac{\mathrm{d}[R]}{\mathrm{d}t} = -\frac{\mathrm{d}[L]}{\mathrm{d}t} = k_1([R][L] - K_S[C]). \tag{2}$$

In this system the parameter $K_S = k_{-1}/k_1$ is the equilibrium constant [4,15] and the square brackets denote concentration. Since no catalytic processes are involved, the reaction is subject to the following conservation laws:

$$[R_0] = [R](t) + [C](t) \tag{3}$$

* Corresponding author at: Brehm Center 5132, 1000 Wall Street, Ann Arbor, MI 48105-1912, USA.
E-mail addresses: stroberg@umich.edu (W. Stroberg), schnells@umich.edu (S. Schnell).

http://dx.doi.org/10.1016/j.mbs.2016.09.010
0025-5564/

$$[L_0] = [L](t) + [C](t), \tag{4}$$

where $[R_0]$ and $[L_0]$ are the initial receptor and initial ligand concentrations. If the bimolecular reaction (1) is initiated far from the equilibrium and in the absence of ligand–receptor complex, the system (2) has the initial conditions at $t = 0$:

$$([L], [R], [C]) = ([L_0], [R_0], 0) \ . \tag{5}$$

We have expressed quantities in terms of concentration of species. These equations are frequently given in terms of binding site number, using the identity [15]

$$[C] = \left(\frac{n}{N_{AV}}\right)C, \tag{6}$$

where n is the cell density, N_{AV} is Avogadro's number, and C denotes the number of ligand-bound receptors per cell. We use the concentration formulation here for clarity and without loss of generality.

The system (2) can be solved, subject to the conservation laws [18]. Substituting (3) and (4) into (2) we obtain:

$$\frac{\mathrm{d}[C]}{\mathrm{d}t} = k_1(([R_0] - [C])([L_0] - [C]) - K_S[C]). \tag{7}$$

We can rewrite this expression by factoring as follows:

$$\frac{\mathrm{d}[C]}{\mathrm{d}t} = k_1((\lambda_+ - [C])(\lambda_- - [C])), \tag{8}$$

where

$$\lambda_\pm = \frac{(K_S + [R_0] + [L_0]) \pm \sqrt{(K_S + [R_0] + [L_0])^2 - 4[R_0][L_0]}}{2}. \tag{9}$$

This ordinary differential equation is readily solved subject to the initial conditions (5) as

$$[C](t) = \lambda_- \left(\frac{1 - \exp\left(-\frac{t}{t_C}\right)}{1 - \frac{\lambda_-}{\lambda_+}\exp\left(-\frac{t}{t_C}\right)}\right), \tag{10}$$

with

$$t_C = [k_1\sqrt{(K_S + [R_0] + [L_0])^2 - 4[R_0][L_0]}]^{-1}. \tag{11}$$

We note that $[C](t)$ increases monotonically with time and will approach λ_- from below. The quantity t_C is the timescale for significant change in $[C]$. In this particular case, t_C can be considered as the time required for the reaction to reach steady-state. Solutions for $[R](t)$ and $[L](t)$ can now be constructed by substituting (10) into conservation laws (3) and (4).

Although there is a closed form solution for the reacting species of the simple bimolecular ligand–receptor interaction, experimental biochemists prefer to determine the kinetic parameters of the ligand–receptor binding using graphical methods [15]. One of the graphical methods commonly used consists of plotting the solution of the ligand association assuming no ligand depletion on a logarithmic scale with respect to time. Both the association and dissociation rate constants can be determined using this linear graphical method [19]. Similarly, if one seeks to avoid inaccuracies due to logarithmic fitting, nonlinear regression can be used to fit the kinetic data to a single exponential. However, the use of both of these methods has the disadvantage of making an assumption with respect to the relative concentrations of ligand and binding sites [18].

In the ligand–receptor interaction with 1:1 stoichiometry and no ligand depletion it is generally thought that, if the initial ligand concentration is much higher than the initial receptor concentration, i.e.

$$[L_0] \gg [R_0], \tag{12}$$

the ligand concentration $[L]$ remains effectively constant during the course of the reaction, and only the receptor concentration $[R]$ changes appreciably with time [3,4,20,21]. Since kinetic order with respect to time is the same as with respect to $[R]$, reaction (1) is said to follow *pseudo-first-order* (PFO) kinetics if the $[R]$ dependence is of first order. The rates of second-order reactions in chemistry are frequently studied within PFO kinetics [22,23]. In the present case, the second-order reaction (1) becomes mathematically equivalent to a first-order reaction, reducing to

$$R \underset{k_{-1}}{\overset{k_\varphi}{\rightleftharpoons}} C, \tag{13}$$

where $k_\varphi \equiv k_1[L_0]$ is the pseudo rate constant. This procedure is also known as the method of flooding [5]. The solution of the governing equations for a reaction linearized by PFO kinetics (or flooding) is straightforward, and is widely employed to characterize kinetics and fit parameters with the aid of progress curves. An error is however present due to the fact that, in actuality, the concentration of the excess reactant does not remain constant [22].

In 1961, Silicio and Peterson [22] made numerical estimates for the fractional error in the observed PFO constant for second-order reactions. They found that the fractional error is less than 10% if the reactant concentration ratio, $[R_0]$: $[L_0]$ say, is tenfold. On the other hand, Corbett [24] found that simplified expressions with the PFO kinetics can yield more accurate data than is generally realized, even if only a twofold excess of one the reactants is employed. For ligand–receptor dynamics, Weiland and Molinoff [18] claim that the PFO simplification is acceptable if experimental conditions are such that less than 10% of the ligand is bound. These results indicate that the conditions whereby a second-order ligand–receptor reaction is reduced to first order remain to be well-established.

It is widely believed that second-order reactions can be studied by PFO kinetics using progress curves only when the excess concentration of one of the reactants is large [23,5, for example]. However, contrary to the widely established knowledge, Schnell and Mendoza [10] have found that the condition for the validity of the PFO in the single substrate, single enzyme reaction does not require an excess concentration of one of the reactant with respect to the other. In the present work, we derive the conditions for the validity of the PFO approximation in the simple ligand–receptor interaction. Additionally, we show two fundamentally different methods of assessing the validity of the approximation. The first compares the exact and approximate solutions to the rate equations under identical conditions. The second measures the veracity of parameters estimated by fitting the approximate model to data. Although these two measures of validity are generally assumed to coincide, we show that they are quantitatively and qualitatively distinct. In Section 2 the reduction of the ligand–receptor association by PFO kinetics is summarized followed by its dynamical analysis in Section 3. The new validity condition is derived in Section 4, and an analysis of the errors observed with the PFO kinetics is presented in Section 5. This is followed by a brief discussion (Section 6).

2. The governing equations of the ligand–receptor dynamics with no ligand depletion

In ligand–receptor dynamics with 1:1 stoichiometry and no ligand depletion, the second-order ligand–receptor interaction in reaction (1) is neglected when condition (12) holds; the reaction effectively becomes first order since the concentration of the reactant in excess is negligibly affected. This is equivalent to assuming that

$$[L_0] - [C] \approx [L_0]. \tag{14}$$

The alternative case, in which the depletion of the receptor is assumed to be negligible, is shown to be symmetric in Appendix A.