Chapter 1

Biochemical Kinetics

1.1 Interconversion

Consider a chemical reaction in which substance is converted between an active form, A and an inactive form B (and vice versa).

$$A \underset{k^-}{\overset{k^+}{\rightleftharpoons}} B$$

where k^+, k^- are the forward and reverse reaction rates. We will let A and B denote the chemical concentrations of the two forms, and assume that the reaction is well-mixed (so we can neglect spatial distribution). We also assume that the total amount of A and B is a known constant,

$$A + B = C,$$

and that initially $A(0) = A_0$.

Exercise 1.1.1 (Simple chemical interconversion)

(a) Show that this system leads to a single equation

$$\frac{dA}{dt} = k^+ C - (k^+ + k^-)A \tag{1.1}$$

(b) Define dimensionless variables representing the fractions in active and inactive form and a dimensionless time t^* as follows:

$$a = A/C, \quad b = B/C, t^* = t/\tau,$$

where τ is to be selected. Rewrite the equations using these dimensionless variables. Show that the specific choice of timescale

$$\tau = \frac{1}{k^+ + k^-}$$

simplifies the equation (by reducing the number of independent parameters that appear in it).

(c) After dropping the *'s, show that you arrive at the new (dimensionless) equation:

$$\frac{da}{dt} = a_S - a \tag{1.2}$$

What is the meaning of the new parameter a_S in terms of the original parameters in the model?

(d) Any one of many methods will lead to an explicit analytic solution of the simple ordinary differential equation (1.2). (For example, the substitution $x(t) = a_S - a(t)$). Use your favorite method to find that solution.

Solution to 1.1.1:

(a)

(b)

(c)

(d)

1.2 Michaelis-Menten Kinetics

We now consider an enzyme catalyzed reaction

$$E + S \underset{k^-}{\overset{k^+}{\rightleftharpoons}} ES \rightarrow EP \overset{k_2}{\rightarrow} E + P$$

The first step, an association between the substrate and enzyme to form a complex ES is reversible, with forward and reverse rates k^+, k^-, respectively. The last two steps are assumed to be very fast, since the enzyme catalyzes the reaction.

We will define variables that represent the concentrations of intermediates as follows. Let

e= concentration of the enzyme E,
s= concentration of the substrate S,
x= concentration of the complex ES,
p= concentration of the product P,

If substrate is neither added nor removed during the reaction, then the following conservation statements hold:

$$e_T = e + x = e(0)$$

$$S_T = s + x + p = s(0)$$

The **Law of Mass Action** states that the rate of a chemical reaction is proportional to the product of its reactants. This then allows us to formulate differential equations for the chemical concentrations as follows:

$$\frac{ds}{dt} = k^- x - k^+ es \tag{1.3a}$$
$$\frac{dx}{dt} = k^+ es - k^- x - k_2 x \tag{1.3b}$$
$$\frac{dp}{dt} = k_2 x \tag{1.3c}$$

Exercise 1.2.2 (QSS and Michaelian kinetics)

(a) Use one of the conservation statements to eliminate e from the equations for s and x, and show that you arrive at

$$\frac{ds}{dt} = (k^- + k^+ s)x - k^+ e_T s \tag{1.4a}$$
$$\frac{dx}{dt} = k^+ e_T s - (k^+ s + k^- + k_2)x \tag{1.4b}$$

(b) Consider the following scaling of the problem. Let $s = s^* s_T, x = x^* e_T, t = t * \tau$ where starred variables are dimensionless concentrations and τ is dimensionless time. Here we have selected the total enzyme and substrate concentrations as the scales for E, S, respectively. The choice of timescale will emerge from the arguments below. Rewrite the model in terms of the new (dimensionless) variables.

(c) Consider the case where the total enzyme concentration is much lower than the total substrate concentration i.e. $e_T << s_T$. Chose the "slow" timescale $\tau = 1/(k^+ e_T)$. Explain why this is a slow timescale. Then simplify the equations. Show that (after dropping the stars) you arrive at dimensionless equations

$$\frac{ds}{dt} = \left(\frac{k^-}{k^+ s_T} + s\right) x - s \tag{1.5a}$$
$$\epsilon \frac{dx}{dt} = s - \left(s + \frac{k^- + k_2}{k^+ s_T} x\right) \tag{1.5b}$$

What is the parameter ϵ in terms of the original parameters? Show that ϵ is small.

(d) We now make a **quasi steady-state** assumption, which is essentially taking the asymptotic limit as $\epsilon \to 0$. In that case, Eqn. (1.5b) reduces to

$$\epsilon \frac{dx}{dt} \approx 0, \quad x \approx \frac{s}{s + \hat{K}_m}.$$

Plug this result in to Eqn. (1.5a) and show that you arrive at the **Michaelis Menten kinetics** equation,

$$\frac{ds}{dt} = -\hat{V}_{max} \frac{s}{s + \hat{K}_m}. \tag{1.6}$$

(e) Convert the dimensionless equation (1.6) to original dimension-carrying variables and find $\hat{V}_{max}, \hat{K}_m$ in terms of the original parameters.

Solution to 1.2.2:

(a)

(b)

(c)

(d)

(e)

In the above, we examined the reaction on a slow timescale. We can also look at what happens on a fast timescale by redefining the timescale. We do this below.

Exercise 1.2.3 (Slow time kinetics)

Define the short timescale $\tau = 1/(k^+ s_T)$ and carry out the following steps:

(a) Find the corresponding dimensionless system using Eqs. (1.4) with the new choice for timescale.

(b) Use the fact that $e_T << s_T$ to argue that on this timescale, the substrate concentration if roughly constant (in the asymptotic limit $\epsilon \to 0$.

(c) Determine how x behaves at $t \to \infty$ in this timescale.

(d) Convert to the original (dimension-carrying) variables and show that

$$\frac{dP}{dt} = V_{max} \frac{S}{K_m + S}. \tag{1.7}$$

(Find the values of V_{max}, K_m in terms of the original parameters.)

Solution to 1.2.3:

(a)

(b)

(c)

(d)

1.3 Dimerization and sigmoidal kinetics

Multi-step reactions, and reactions that involve dimerization can lead to a response that has a sigmoidal relationship between velocity and substrate concentration. For example, consider a population of cell-surface receptors that each have 2 sites for attachment of a ligand molecule. We could think of a scheme as follows to describe the formation of a complex consisting of a receptor bound to two ligands:

$$R + 2L \underset{k^-}{\overset{k^+}{\rightleftharpoons}} RL_2$$

In reality, it would be rare for three molecules to bind simultaneously, and likely, first one and then another ligand would attach to the receptor. However, as an *approximation* we could consider the above scheme. Using the notation r, ℓ, x for the concentration of receptors, ligand, and complexes of the type RL_2, and assuming that the total amount of receptor is constant ($r + x = T =$ constant), we can use the Law of Mass Action to arrive at

$$\frac{dx}{dt} = k^+ r\, \ell^2 - k^- x \tag{1.8}$$

Now suppose that receptors fill up rapidly, and consider a timescale τ that is much longer than the time taken for the initial transient (see our previous examples). Then a QSS assumption leads to

$$\frac{dx}{dt} \approx 0.$$

Define the new constant

$$k = \sqrt{\frac{k^-}{k^+}}.$$

Then the following result holds:

Exercise 1.3.4 (Fraction of bound receptors)
Show that the fraction of bound receptors at the QSS is given by:

$$\frac{x}{T} = \frac{\ell^2}{k^2 + \ell^2} \tag{1.9}$$

Solution to 1.3.4:

In some cases, the response of the cell will depend on the fraction of occupied receptors. For example, the receptor-ligand complex may be "internalized" - i.e. taken up into the interior of the cell, resulting in clearance of ligand from the medium. In that case, the rate of clearance would satisfy kinetics of the form:

$$\frac{d\ell}{dt} = \frac{\ell^2}{k^2 + \ell^2} \tag{1.10}$$

We refer to this as *sigmoidal kinetics.* An example closely related to this was discussed by Catherine Foley (PhD Thesis, McGill University, 2008). In her case, the ligand was a cytokine called G-CSF and the receptors were on the surface of immune cells called neutrophils.

In general, sigmoidal kinetics can arise in several ways, including (1) when there is dimerization or oligomerization (formation of complexes consisting of multiple molecules) (2) some kind of cooperativity in the process: for example, when a process is hard to initiate, but once initiated, proceeds much more rapidly.

1.4 Competitive and noncompetitive enzyme inhibitors

A **competitive inhibitor** competes with substrate for the same active site on the enzyme, forming some no-functional complex, Z. We could analyze the entire system by rewriting the chemical balance equations for inhibitor as well as substrate.

$$E + S \underset{k^-}{\overset{k^+}{\rightleftharpoons}} ES \rightarrow EP \overset{k_2}{\rightarrow} E + P$$

$$E + I \rightleftharpoons Z$$

If we do so, we find that, effectively, the inhibitor reduces the forward reaction rate, i.e. leads to a lower apparent k^+, and thus, lower effective rate of binding of the true substrate. Hence, also, we find that such inhibitor reduces K_m but not V_{max}.

A **non-competitive inhibitor** "poisons" the enzyme, rendering some fraction of the enzyme molecules non-functional. This is equivalent to reducing the total (active) enzyme concentration, i.e. lowering e_T. As a result, V_{max} is decreased, but not K_m. We can distinguish between such cases by looking at data for enzyme catalysis and determining which of the parameters is affected as a result of an inhibitor.

Exercise 1.4.5 (Competitive and noncompetitive Inhibition)

Explain the assertions about the effect of inhibitors on K_m and V_{max} by recalling the way these parameters are related to the rate constants for the enzyme reaction.

Solution to 1.4.5:

1.5 Estimating reaction rates from data

Experimental measurements that determine reaction speed for various initial substrate concentrations can be used to estimate the values of the parameters of an enzyme catalyzed reaction. One simple way to do so is to transform the Michaelis-Menten relationship to a simpler (linear) relationship between the variables $1/S$ and $1/V$. The result is that ideally data points would fit on a straight line, called a **Linweaver Burke plot**. An advantage of this method is its relative simplicity. A disadvantage is that error in the data can be amplified by the transformation.

Nowadays, nonlinear data fitting routines can be used to estimate parameters very conveniently. Nevertheless, as an instructive simple problem, we consider the idea of the Linweaver Burke plot and a simple data-fitting exercise.

Exercise 1.5.6 (Determining parameters from data)

The following data was collected in an enzyme catalyzed reaction at three treatments: (1) The enzyme reacts with the substrate alone, (2) an inhibitor of some type is added to the enzyme-substrate reaction, (3) a different inhibitor is used with the original enzyme and substrate.

S	$(1)V$	$(2)V$	$(3)V$
1	0.191	0.153	0.106
5	0.414	0.364	0.221
15	0.484	0.471	0.274
30	0.521	0.521	0.287
45	0.514	0.516	0.296

In the table above, S stands for the substrate concentration and V for the observed speed of the reaction. The same five substrate concentrations have been here used in each of the three treatments.

Your task is to use this data to find the parameters V_{max} and K_m for the enzyme alone, and for the enzyme in the presence of inhibitor (2) and (3). Recall that for an enzyme-catalyzed reaction, the relationship between reaction speed and substrate concentration is

$$V = \frac{V_{max} S}{K_m + S}.$$

A Linweaver-Burke plot can reduce this job to fitting a straight line through some (transformed) data, making this job fairly easy. It is recommended to do the data analysis using software such as excel or other software of your choice.

Once you have estimated the values of the two parameters in each of the three cases, please determine which inhibitor is competitive and/or noncompetitive.

Solution to 1.5.6:

1.6 Other applications of related ideas

In this chapter we have seen examples of three types of kinetics, that we will denote linear, Michaelian, and sigmoidal. The response (in this case the speed of the reaction), is seen to depend either linearly or in a saturated way on the availability of reactant. In the sigmoidal case, the response curve has a change in curvature, starting out shallow, and accelerating to a steeper curve before reaching a plateau.

Similar ideas are used, for example, in ecology, to describe the response of a predator to prey density. A linear response implies that the rate of predation is proportional to prey density. This has been called a Type I response. Types II and III are analogous to the saturated enzyme kinetics. In Type II, a predator saturates when prey density p increases, so that the rate of predation, R is

$$R = V_{\mathrm{max}} \frac{p}{k + p} \tag{1.11}$$

In Type III, the rate is initially low, and accelerates before saturating.

$$R = V_{max}\frac{p^2}{\tilde{k}^2 + p^2} \tag{1.12}$$

The latter case could describe a situation where it is hard to find prey when it is in low abundance, and easier to do so once the prey density is sufficiently high.