

Chapter 1 Single-enzyme kinetics

§ 1.1 Kinetics of enzyme-catalysed reactions

Under conditions of constant temperature and pressure, the change in the Gibbs free energy ΔG determines the direction and the extent in which a reaction may occur, and life processes are no exception to this rule. For a simple process that converts S into P,

$$\Delta G = \Delta G^\circ + RT \ln \frac{[P]}{[S]} \quad (\text{Eq. 1.1})$$

When $\Delta G < 0$, this process may proceed in the forward direction (S is converted into P), when $\Delta G > 0$ the reaction proceeds backward (P is converted into S), and when $\Delta G = 0$, there is no net progress and the reaction is in equilibrium. In other words, when [S] and [P] have their equilibrium values, $\Delta G = 0$, and the equilibrium constant K_{eq} is:

$$K_{eq} = \frac{[P]_{eq}}{[S]_{eq}} = e^{-\frac{\Delta G^\circ}{RT}} \quad (\text{Eq. 1.2})$$

Although ΔG determines the direction of the reaction, the rate is a different matter. In living cells the rate of a reaction largely is determined by the enzyme that catalyses the reaction. According to biochemistry, the conversion of S to P, catalysed by enzyme E, under initial conditions (no P present) could proceed as:



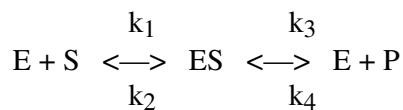
The rate of this reaction is given by the well-known Michaelis-Menten expression:

$$v = \frac{V_{\max}[S]}{[S] + K_M} \quad (\text{Eq. 1.3})$$

In this expression V_{\max} is the maximal rate of the reaction when [S] is increased, and the Michaelis constant K_M is the concentration of S where the rate is exactly half of V_{\max} .

Intermezzo 1: derivation of Michaelis-Menten equation

The reaction scheme is:



Odd-numbered kinetic constants (k_1 and k_3) apply to the forward direction (left to right), even-numbered kinetic constants (k_2 and k_4) to the backward reaction.

First assumption: steady state for ES $\frac{d[ES]}{dt} = 0$

$$k_1 [E] [S] + k_4 [E] [P] = k_2 [ES] + k_3 [ES]$$

$$(k_1 [S] + k_4 [P]) [E] = (k_2 + k_3) [ES]$$

With total enzyme concentration $e = [E] + [ES]$

$$\frac{[ES]}{e} = \frac{(k_1[S] + k_4[P])}{(k_1[S] + k_4[P]) + (k_2 + k_3)}$$

and

$$\frac{[E]}{e} = \frac{(k_2 + k_3)}{(k_1[S] + k_4[P]) + (k_2 + k_3)}$$

The rate of the reaction is:

$$v = \frac{d[P]}{dt} = -\frac{d[S]}{dt} = k_1 [E] [S] - k_2 [ES] = k_3 [ES] - k_4 [E] [P]$$

(so $S \rightarrow P$ is the forward direction). Putting in the expressions for $[E]$ and $[ES]$:

$$v = \frac{(k_1 k_3 [S] - k_2 k_4 [P])e}{(k_1[S] + k_4[P]) + (k_2 + k_3)} \quad (\text{Eq. 1.4})$$

In this expression the maximal forward rate is achieved when $[S] \rightarrow \infty$. The rate then is

$$V_{\max}(\text{forward}) = V_S = k_3 e$$

The maximal backward rate is approached when $[P] \rightarrow \infty$. The rate expression predicts that $v \rightarrow -k_2 e$. However, V_P is defined as positive number:

$$V_{\max}(\text{backward}) = V_P = k_2 e$$

Michaelis constants (K_M 's) can be defined both for S (K_S) and for P (K_P):

$$K_S = \frac{k_2 + k_3}{k_1} \quad K_P = \frac{k_2 + k_3}{k_4} \quad (\text{Eq. 1.5})$$

Introducing V_S , K_S , V_P and K_P into Eq. 1.4, this turns into:

$$v = \frac{V_S \frac{[S]}{K_S} - V_P \frac{[P]}{K_P}}{1 + \frac{[S]}{K_S} + \frac{[P]}{K_P}} \quad (\text{Eq. 1.6})$$

This expression relates the rate v to the concentrations of S and P. The relation between these three variables contains four parameters: K_S , V_S , K_P and V_P .

It can be verified easily that at $[S] = K_S$ and $[P] = 0$, the half maximal forward rate is obtained, and at $[S] = 0$ and $[P] = K_P$, the half maximal backward rate. Special forms of Eq. 1.6 (such as Eq. 1.3 when $[P] = 0$) can be used under different conditions.

§ 1.2 The Haldane relationship

When the net rate is zero, equilibrium is reached. This occurs when the numerator of the rate expression (Eq. 1.6) is zero:

$$V_S \frac{[S]_{eq}}{K_S} - V_P \frac{[P]_{eq}}{K_P} = 0$$

From this follows the Haldane relation:

$$K_{eq} = \frac{[P]_{eq}}{[S]_{eq}} = \frac{V_S K_P}{V_P K_S} \quad (\text{Eq. 1.7})$$

The four parameters in the kinetic expression are mutually dependent. Sometimes it is useful to use this interdependence to eliminate e.g. V_P from the rate expression:

$$v = \frac{\frac{V_S}{K_S} \left([S] - \frac{[P]}{K_{eq}} \right)}{1 + \frac{[S]}{K_S} + \frac{[P]}{K_P}} \quad (\text{Eq. 1.8})$$

This form shows that of the four parameters in the kinetic expression, one (K_{eq}) is determined by the reaction (independent of the enzyme), while the three others (K_S , K_P and $V_{max} = V_S$) depend on the enzyme.

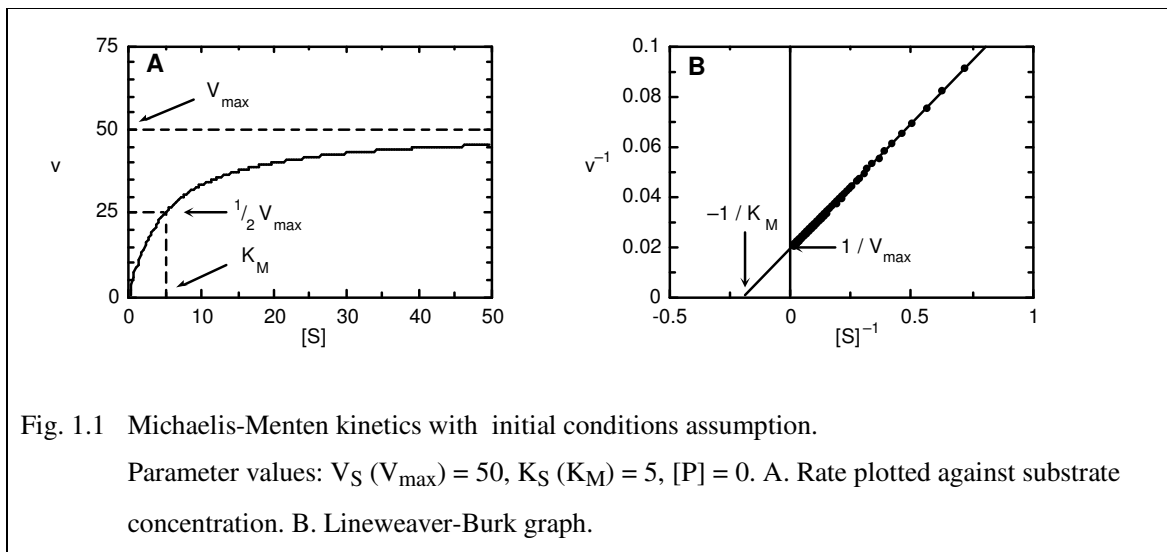
§ 1.3 Special conditions

When studying an isolated enzyme with the aim to determine the values of K_M 's and V_{max} 's, one can choose experimental conditions such that this is a relatively easy task. Some examples:

(a) *Initial conditions (forward reaction):* the second assumption is $[P] = 0$. The rate expression to be used is:

$$v = \frac{V_S \frac{[S]}{K_S}}{1 + \frac{[S]}{K_S}} \quad \text{or} \quad v = \frac{V_S [S]}{[S] + K_S} \quad \left(= \frac{V_{max} [S]}{[S] + K_M} \right) \quad (\text{Eq. 1.9})$$

From this familiar expression V_S and K_S can be estimated by a non-linear fitting procedure, or by a linear fit to e.g. a Lineweaver-Burk graph (see Fig. 1.1).



(b) *Initial conditions (backward reaction)*: the second assumption is $[S] = 0$. The rate expression to be used is:

$$v = \frac{-v_p \frac{[P]}{K_p}}{1 + \frac{[P]}{K_p}} \quad \text{or} \quad v^* = \frac{V_p [P]}{[P] + K_p} \quad \left(= \frac{V_{\max} [P]}{[P] + K_M} \right) \quad (\text{Eq. 1.10})$$

(with $v^* = -v$).

Using initial conditions, the third assumption generally is made that the substrate (either S or P) is in large excess of the total amount of enzyme e . Then $[ES]$ ($[ES] \leq e$) can be neglected and $[S] = s$ or $[P] = p$ (the total concentrations added).

c) *Moiety-conserved conditions*. In this case the second assumption is $[S] + [ES] + [P] = q$ is constant. Again using the third assumption that substrates are in large excess above enzyme ($q \gg e$), $[ES]$ drops out of the conservation relation, and this turns into: $[S] + [P] = q$. This can be used to e.g. eliminate $[P]$ from the rate expression, yielding a hyperbolic relation between the two remaining variables v and $[S]$ (see § 3.4). Although it seems that introduction of q is adding an extra parameter, scrutiny of the rate equation (while keeping in mind that K_{eq} has a given value) reveals that only three parameters may be chosen independently. A few possible relations between v and $[S]$ are shown in Fig. 1.2.

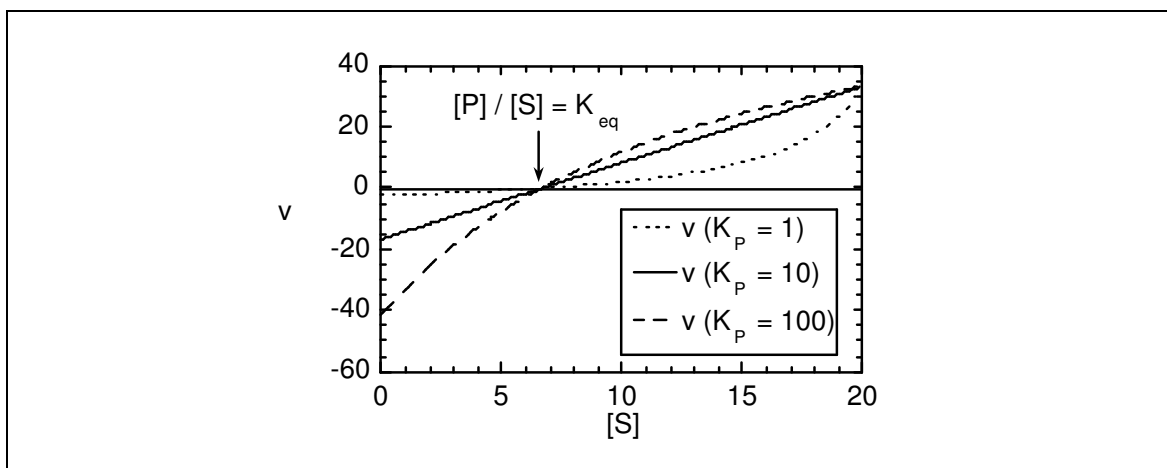


Fig. 1.2 Michaelis-Menten kinetics with moiety conservation ($[S] + [P] = \text{constant}$).

Parameter values: $V_S = 50$, $K_S = 10$, $K_{eq} = 2$, $[S] + [P] = 20$, K_P as indicated. Note that all three curves intersect the $[S]$ -axis ($v = 0$) at the equilibrium point. The curve is concave when $K_S > K_P$, linear when $K_S = K_P$ and convex when $K_S < K_P$.

One expects that initial conditions are extremely rare in cells (generally substrates and products are present); however, moiety conservation very often is a useful assumption in studying enzyme activity in the cell.

§ 1.4 Irreversibility and product sensitivity

It is often thought that assuming initial conditions in the forward direction is a good approach to study irreversible reactions in the cell. "Irreversible" implies that K_{eq} is very large. Making K_{eq} very large turns Eq. 1.8 into:

$$v = \frac{\frac{V_S [S]}{K_S}}{1 + \frac{[S]}{K_S} + \frac{[P]}{K_P}} = \frac{V_S [S]}{[S] + K_S + [P] \frac{K_S}{K_P}} \quad (\text{Eq. 1.11})$$

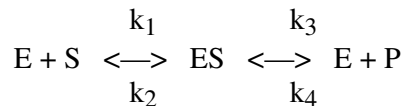
This expression differs from that valid under initial (forward) conditions (Eq. 1.9) in the presence of the term in the denominator containing $[P]$. This makes the rate *product sensitive*, an important feature of the kinetics, as we will see. To use initial conditions kinetics the reaction need not only be irreversible, but also product insensitive. For this K_P needs to be very large, which by itself helps irreversibility by increasing K_{eq} (see Eq. 7). One way of using "initial conditions" is to define an effective $[P]$ -dependent K_M for S :

$$K_S' = K_S \left(1 + \frac{[P]}{K_P} \right)$$

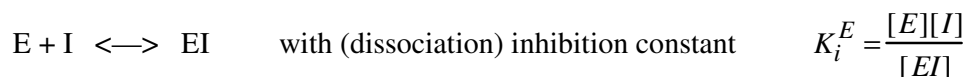
Which turns Eq. 1.11 into Eq. 1.9. However, to use this expression, $[P]$ should be constant under experimental conditions.

Intermezzo 2: reversible inhibition

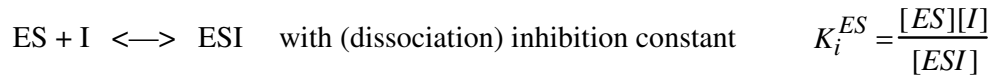
Again, the reaction scheme is:



with the addition that there is reversible binding of an inhibitor I to the enzyme:



And



EI and ESI are inactive forms: they cannot bind or release S or P.

In the derivation of kinetic expressions, the fourth assumption is made that these reactions are at equilibrium. An extension of the third assumption is that also the inhibitor is present in large excess with respect to the enzyme.

Using these assumptions it can be derived that the total enzyme concentration now is:

$$e = [E] + [EI] + [ES] + [ESI] = [E] \left(1 + \frac{[I]}{K_i^E} \right) + [ES] \left(1 + \frac{[I]}{K_i^{ES}} \right) \quad (\text{Eq. 1.12})$$

With this modification, a rate expression similar to Eq.1.6 can be derived, with (primed) parameters that depend on the inhibitor in the following manner:

$$V_S' = \frac{V_S}{\left(1 + \frac{[I]}{K_i^{ES}} \right)} \quad V_P' = \frac{V_P}{\left(1 + \frac{[I]}{K_i^{ES}} \right)} \quad (\text{Eq. 1.13})$$

$$K_S' = K_S \frac{\left(1 + \frac{[I]}{K_i^E} \right)}{\left(1 + \frac{[I]}{K_i^{ES}} \right)} \quad K_P' = K_P \frac{\left(1 + \frac{[I]}{K_i^E} \right)}{\left(1 + \frac{[I]}{K_i^{ES}} \right)} \quad (\text{Eq. 1.14})$$

When the inhibitor doesn't bind to E or to ES, the corresponding K_i is infinite.

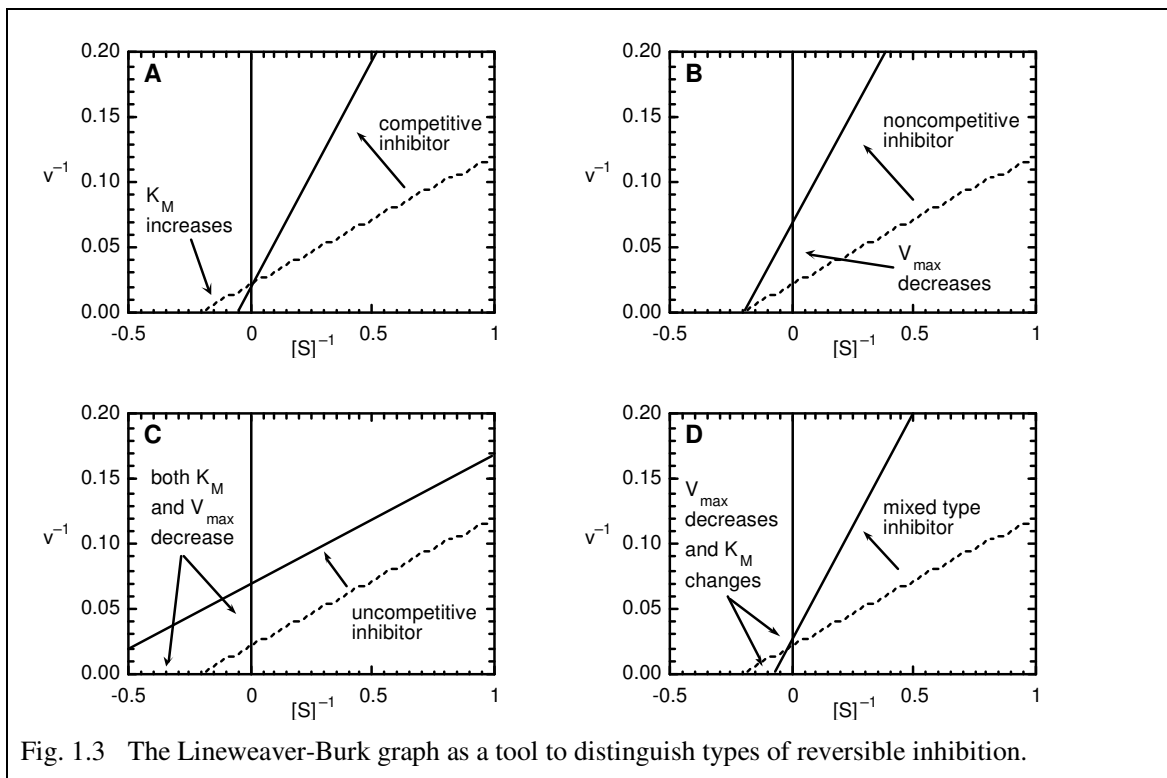


Fig. 1.3 The Lineweaver-Burk graph as a tool to distinguish types of reversible inhibition.

Initial conditions, with parameter values: $V_S = 50$, $K_S = 5$, $K_{eq} = 2$, $[P] = 0$, $[I] = 0.5$.

A. Competitive inhibition: $K_i^E = 0.2$, K_i^{ES} infinite. B. Noncompetitive inhibition: $K_i^E =$

$K_i^{ES} = 0.2$. C. Uncompetitive inhibition: K_i^E infinite, $K_i^{ES} = 0.2$ D. Mixed-type inhibition:

$K_i^E = 0.2$, $K_i^{ES} = 1$.

Special cases are:

(a) Exclusive binding of the inhibitor to E (*competitive* inhibition). Only K_S' and K_P' depend on $[I]$ (they increase with $[I]$). This occurs when inhibitor I competes with substrate (S, or P) for the same site on the enzyme.

(b) Binding of the inhibitor to E and ES with the same K_i (pure *non-competitive* inhibition). Only V_S' and V_P' depend on $[I]$ (they decrease with $[I]$).

(c) The inhibitor exclusively binds to ES (*uncompetitive* inhibition). V_S' , K_S' , V_P' and K_P' depend on $[I]$ (they all decrease with $[I]$).

(d) A fourth possibility is that the inhibitor binds to E and ES with different K_i (*mixed type* inhibition). All four parameters change.

Under initial conditions (e.g. $[P] = 0$) the four types of reversible inhibition are most easily distinguished in e.g. a Lineweaver-Burk graph (see Fig. 1.3).

In a completely analogous way one can think of different forms of (reversible) *activation* of an enzyme.

§ 1.5 Enzyme kinetics and the living cell

We have seen in what way one can describe the kinetics of the simplest enzyme-catalysed reaction. We will not discuss enzymes with more than a single substrate here, but in principle similar treatments of their kinetics have been developed. One should be aware that kinetic data about enzymes often have been determined under "initial conditions" of one kind or other, and kinetic behaviour of these enzymes inside the cell generally has to be understood under quite different conditions. Important considerations here are reversibility of the catalysed reaction (depending on K_{eq}), product sensitivity (depending on K_P) and moiety conservation.