SOLVING THE RATE EQUATIONS FOR CONSECUTIVE REACTIONS

For a two-step association of enzyme and substrate,

$$E + S \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} X_1 \underset{k_{-2}}{\overset{k_2}{\longleftrightarrow}} X_2 \tag{1}$$

we can write two independent rate equations,

$$-\frac{d[E]}{dt} = k_1[E][S] - k_{-1}[X_1] \tag{2a}$$

$$-\frac{d[X_2]}{dt} = k_{-2}[X_2] - k_2[X_1] \tag{2b}$$

and two mass balance equations

$$[E]_o = [E] + [X_1] + [X_2] \tag{2c}$$

$$[S]_o = [S] + [X_1] + [X_2] \tag{2d}$$

We want to solve the set of coupled rate equations (eqs. 2a-b) to obtain explicit expressions for the concentration of each species as a function of time and expressions for the characteristic time, τ, for each step in terms of the rate constants. For an analytical solution, the rate equations must be linear in the concentrations. There can be no terms containing a product of concentrations. This is equivalent to requiring that each step of the mechanism be first order. In the mechanism of eq. (1) the step governed by k_1 is second order, which leads to a term with a product of concentrations, $[E][S]$, in eq. (2a). The difficulty can be worked around in several ways:

(1) require $[S]_o >> [E]_o$;

(2) require that all concentrations be near equilibrium.

Case 1. $[S]_o >> [E]_o$

For mixing (e.g., stopped flow) data, it is convenient to fix $[S]_o >> [E]_o$. The bimolecular step of eq. (1) then becomes pseudo-first order. We define the pseudo-first-order constant

$$k_1' = k_1 [S]_o \tag{3}$$

Introducing eqs. (2c) and (3) into eqs. (2a-b),

$$-\frac{d[E]}{dt} = (k_1' + k_{-1})[E] + k_{-1}[X_2] - k_{-1}[E]_o \tag{4a}$$

$$-\frac{d[X_2]}{dt} = (k_{-2} + k_2)[X_2] + k_2[E] - k_2[E]_o \tag{4b}$$

It is convenient to rewrite eqs. (4) as

$$\frac{d[E]}{dt} + a_{11}[E] + a_{12}[X_2] = C_1 \tag{5a}$$

$$a_{21}[E] + a_{22}[X_2] + \frac{d[X_2]}{dt} = C_2 \tag{5b}$$

where

$a_{11} = k_1 [S]_o + k_{-1}$
$a_{12} = k_{-1}$
$a_{21} = k_2$
$a_{22} = k_{-2} + k_2$
$C_1 = k_{-1}[E]_o$
$C_2 = k_2[E]_o$

For a set of simultaneous linear first-order differential equations, such as eqs. (5), solutions for the time dependence of the concentration of the species i are of the form

$$[X_i] = L_i + \sum_j A_{ij} e^{m_j t} \tag{6}$$

where

$L_i = constant$

$A_{ij} = f([E]_o, [S]_o; k_\pm)$

$m_j = f(k_\pm)$

$k_\pm = sequence\ k_{-j}\ to\ k_{+j}$

j = 1, number of reaction steps

The first problem is to evaluate the m_j in terms of the step constants k_{+j}, k_{-j}. To do this we introduce the trial solutions $[E] = Ae^{mt}$ and $[X_2] = Be^{mt}$, obtaining

$$mAe^{mt} + a_{11}Ae^{mt} + a_{12}Be^{mt} = C_1 \tag{7a}$$

$$a_{21}Ae^{mt} + a_{22}Be^{mt} + mBe^{mt} = C_2 \tag{7b}$$

In evaluating m, we are allowed to work with the corresponding system of homogeneous equations, obtained by setting C_1 and $C_2 = 0$. Doing this, and also dividing through by e^{mt} to eliminate this factor,

$$(m + a_{11})A + a_{12}B = 0 \tag{8a}$$

$$a_{21}A + (m + a_{22})B = 0 \tag{8b}$$

For non-trivial solutions, the determinant of the coefficients must be zero

$$\begin{vmatrix} m + a_{11} & a_{12} \\ a_{21} & m + a_{22} \end{vmatrix} = 0$$

Expanding

$$0 = (m + a_{11})(m + a_{22}) - a_{12}a_{21}$$

$$0 = m^2 + m(a_{11} + a_{22}) + a_{11}a_{22} - a_{12}a_{21} \tag{9}$$

Extracting the roots of eq. (9) by the quadratic formula

$$-m = \frac{1}{2}(p \pm q) \tag{10}$$

where

$$p = a_{11} + a_{22}$$

$$= k_1[S]_o + k_{-1} + k_{-2} + k_2$$

$$q = \sqrt{(a_{11} + a_{22})^2 - 4(a_{11}a_{22} - a_{12}a_{21})}$$

$$= \sqrt{p^2 - 4((k_1k_{-2} + k_1k_2)[S]_o + k_{-1}k_{-2})}$$

From eq. (6), it is seen that the reciprocal characteristic time for each step, $\frac{1}{\tau_j}$ can be identified with $-m_j$. Thus from eq. (10) we can write

$$\frac{1}{\tau_1} = \frac{1}{2}(p + q) \tag{11a}$$

$$\frac{1}{\tau_2} = \frac{1}{2}(p - q) \tag{11b}$$

From eqs. (11) one has the sums and products of the relaxation times

$$\frac{1}{\tau_1} + \frac{1}{\tau_2} = k_1[S]_o + k_{-1} + k_{-2} + k_2 \tag{12a}$$

$$\frac{1}{\tau_1\tau_2} = (k_1k_{-2} + k_1k_2)[S]_o + k_{-1}k_{-2} \tag{12b}$$

The expressions relating the characteristic time for a step to the rate constants and initial substrate concentration are used in interpreting pre-steady-state data from mixing experiments. The analysis could be carried further, to obtain general solutions of the rate equations, based on the values of m_j of eq. (10). This is not necessary in this context.

Case 2. Relaxation kinetics

We start again with the mechanism of eq. (1) and the rate equations (2a-b). In a relaxation experiment, concentrations $[E]$, $[S]$, etc., are established that differ only slightly from the equlibrium concentrations, $[\bar{E}]$, $[\bar{S}]$, etc. After a temperature jump that moves the system to a new equilibrium position, the reactant concentrations initially are at the values for the equilibrium before the jump and with time relax to the new equilibrium values. To describe this process, it is helpful to define substitutions for the concentration variables of eqs. (2a-b), by equating them to the new equilbrium value plus a concentration **difference** (*e*, *s*, etc.):

$$[E] = [\bar{E}] + e \tag{20}$$

$$[S] = [\bar{S}] + s$$

$$[X_1] = [\bar{X}_1] + x_1$$

$$[X_2] = [\bar{X}_2] + x_2$$

$$\frac{d[E]}{dt} = \frac{de}{dt}$$

$$\frac{d[X_2]}{dt} = \frac{dx_2}{dt}$$

The corresponding conservation equations, in terms of the differences from equilibrium, are

$$e + x_1 + x_2 = 0 \tag{21}$$

$$s = e$$

Introducing the definitions of eqs. (20) into eq. (2a)

$$-\frac{de}{dt} = k_1([\bar{E}] + e)([\bar{S}] + s) - k_{-1}([\bar{X}_1] + x_1)$$

$$= k_1[\bar{E}][\bar{S}] + k_1([\bar{E}]s + e[\bar{S}] + e\cdot s) - k_{-1}[\bar{X}_1] - k_{-1}x_1$$

Because at equilibrium the rates of the forward and reverse reactions for a step are equal, the terms $k_1[\bar{E}][\bar{S}]$ and $k_{-1}[\bar{X}_1]$ cancel in the above equation. We can drop the term $e\cdot s$ because as the product of differences it is neglibible. In accord with the conservation eqs. (21), s can be replaced with e, and -x_1, with ($e + x_2$).

Thus the rate equations governing the relaxation process are

$$-\frac{de}{dt} = (k_1([\bar{E}] + [\bar{S}]) + k_{-1})e + k_{-1}x_2 \qquad (22a)$$

and by a similar treatment of eq. (2b),

$$-\frac{dx_2}{dt} = k_2 e + (k_{-2} + k_2)x_2 \qquad (22b)$$

These equations form a system of homogeneous linear first-order differential equations. We proceed as for Case (1), recasting eqs. (22) as

$$\frac{de}{dt} + a_{11}e + a_{12}x_2 = 0 \qquad (23a)$$

$$a_{21}e + a_{22}x_2 + \frac{dx_2}{dt} = 0 \qquad (23b)$$

The coefficients a_{ij} are defined as for Case (1), except ($[\bar{E}] + [\bar{S}]$) replaces $[S]_o$ in the expression for a_{11}.

Introducing the trial solutions $e = Ae^{mt}$ and $x_2 = Be^{mt}$, gives the characteristic equation

$$\begin{vmatrix} m + a_{11} & a_{12} \\ a_{21} & m + a_{22} \end{vmatrix} = 0$$

This is the same as for Case (1). Solving for *m* and noting that $1/\tau = -m$,

$$\frac{1}{\tau_1} = \frac{1}{2}(p + q) \tag{24a}$$

$$\frac{1}{\tau_2} = \frac{1}{2}(p - q) \tag{24b}$$

where

$$p = k_1([\bar{E}] + [\bar{S}]) + k_{-1} + k_{-2} + k_2$$

$$q = \sqrt{p^2 - 4((k_1 k_{-2} + k_1 k_2)([\bar{E}] + [\bar{S}]) + k_{-1} k_{-2})}$$

The sums and products of the relaxation times are

$$\frac{1}{\tau_1} + \frac{1}{\tau_2} = k_1([\bar{E}] + [\bar{S}]) + k_{-1} + k_{-2} + k_2 \tag{25a}$$

$$\frac{1}{\tau_1 \tau_2} = (k_1 k_{-2} + k_1 k_2)([\bar{E}] + [\bar{S}]) + k_{-1} k_{-2} \tag{25b}$$

The above formalism can be extended to the case of n > 2 consecutive reactions, described by the n differential equations

$$\frac{dx_i}{dt} + \sum_{j=1}^{n} a_{ij} x_j = 0 \qquad i = 1, n$$

for which the characteristic equation is

$$\begin{vmatrix} a_{11} - \frac{1}{\tau} & a_{12} & . & a_{1n} \\ a_{21} & a_{22} - \frac{1}{\tau} & . & a_{2n} \\ . & . & . & . \\ a_{n1} & a_{n2} & . & a_{nn} - \frac{1}{\tau} \end{vmatrix} = 0$$

The n relaxation times can be extracted by solving the determinant. Note that each relaxation time contains, in principle, a contribution from each rate constant of the mechanism. In practive, for a given τ_j, one or a few steps will dominante. It may be helpful to draw the analogy with the vibrations of the atoms of a molecule. The equations of motion are also a set of coupled first-order differential equations. For the atom motions, the eigenvalues are the frequencies of the normal modes of vibration.

An important special case of the two-step association of enzyme and substrate is where the first step is fast: k_1 and $k_{-1} >> k_2$ and k_{-2}; $\tau_1 << \tau_2$. By inspection of eq. (25a)

$$\frac{1}{\tau_1} = k_1([\bar{E}] + [\bar{S}]) + k_{-1} \tag{30a}$$

Substituting eq. (30a) into eq. (25b)

$$\frac{1}{\tau_2} = \frac{1}{k_1([\bar{E}] + [\bar{S}]) + k_{-1}}((k_1 k_{-2} + k_1 k_2)([\bar{E}] + [\bar{S}]) + k_{-1}k_{-2})$$

$$\frac{1}{\tau_2} = k_{-2} + \frac{k_2([\bar{E}] + [\bar{S}])}{([\bar{E}] + [\bar{S}]) + \dfrac{k_{-1}}{k_1}} \tag{30b}$$

$\dfrac{1}{\tau_1}$ is linear in reactant concentration. $\dfrac{1}{\tau_2}$ shows saturation behavior.

$$\frac{1}{\tau_2} = k_{-2} + \frac{k_2}{K_d}([\bar{E}] + [\bar{S}]) \qquad ([\bar{E}] + [\bar{S}]) << K_d$$

$$\frac{1}{\tau_2} = k_{-2} + k_2 \qquad ([\bar{E}] + [\bar{S}]) >> K_d$$