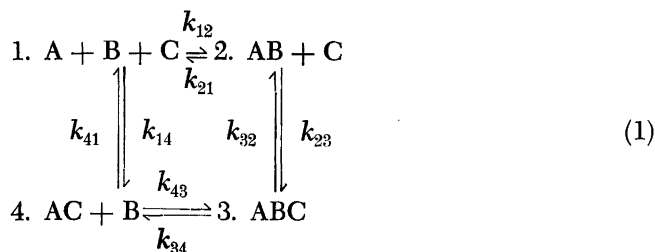# Interpretation of Relaxation Times

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When a reaction system is displaced from equilibrium it returns to equilibrium in the quickest possible way. If the displacements from equilibrium are small the kinetics are characterized by a number of relaxation times which is equal to the number of independent concentration variables. These relaxation times depend upon the various rate constants and equilibrium concentrations and are to be identified with single steps only in special cases. The expressions for the two relaxation times for a simple system of general interest in connection with enzyme kinetics are given and simplified for various special cases.

For a complicated reaction system the approach to equilibrium is described by a very complicated equation, if indeed the simultaneous rate equations can be integrated. However, in connection with the development of relaxation methods for studying very fast reactions Eigen[1-4] has pointed out that if the initial displacement of the various concentration variables from their equilibrium values is small, their subsequent change with time may be expressed as a sum of exponential terms. Each term is characterized by relaxation time τ (the time for that term to fall to $1/e$ of its initial value), and the number of relaxation times is equal to the number of independent concentration variables. The general theory for the calculation of relaxation times has been given[5,6], and these methods have been applied to enzymatic reactions[7,8]. However, the subject is sufficiently complicated so that it appears desirable to discuss the application of these ideas to a relatively simple system of general interest.

$$
\begin{array}{ccc}
& k_{12} & \\
1.\ A + B + C & \underset{k_{21}}{\rightleftharpoons} & 2.\ AB + C \\
k_{41} \Big\Updownarrow k_{14} & & k_{32} \Big\Updownarrow k_{23} \\
& k_{43} & \\
4.\ AC + B & \rightleftharpoons & 3.\ ABC \\
& k_{34} &
\end{array}
\tag{1}
$$

The system can exist in four states and the rate constants indicate the initial and final states for each reaction. In this mechanism, A could represent enzyme, B coenzyme and C hydrogen ion; or A could represent antibody, B hapten with C not representing anything, that is A and AC would be isomers. The time variation of any concentration variable after an initial small displacement from equilibrium (as by a sudden temperature change[9]) would be given by

$$c_i - \bar{c}_i = \Delta c_i = K_{1i}e^{-t/\tau_1} + K_{2i}e^{-t/\tau_2} + K_{3i}e^{-t/\tau_3} \tag{2}$$

where $\bar{c}_i$ is the equilibrium concentration.

The expressions for the relaxation times for system 1 would be obtained as follows: There are 6 concentration variables and 3 conservation relations so that there are 3 independent concentration variables, 3 independent rate equations and 3 relaxation times. Any three independent rate equations for the system are written down. These rate equations are linearized by replacing each concentration (X) by (X) + Δ(X), where (X) is the equilibrium concentration and Δ(X) is the displacement from equilibrium. When products of concentrations are multiplied out, terms involving Δ^2 are ignored because the displacements from equilibrium are small. In this way the three differential equations may be reduced to the form

$$\frac{d\Delta(A)}{dt} = a_{11}\Delta(A) + a_{12}\Delta(AB) + a_{13}\Delta(ABC) \tag{3}$$

$$\frac{d\Delta(AB)}{dt} = a_{21}\Delta(A) + a_{22}\Delta(AB) + a_{23}\Delta(ABC)$$

$$\frac{d\Delta(ABC)}{dt} = a_{31}\Delta(A) + a_{32}\Delta(AB) + a_{33}\Delta(ABC)$$

According to the theory of linear differential equations the three relaxation times are obtained by solving the determinant
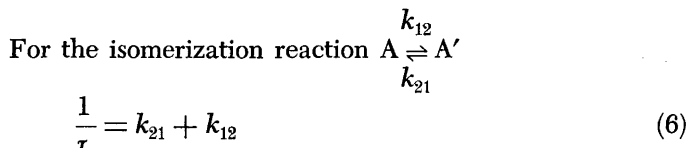
$$\begin{vmatrix} a_{11} + \dfrac{1}{\tau} & a_{12} & a_{13} \\[2mm] a_{21} & a_{22} + \dfrac{1}{\tau} & a_{23} \\[2mm] a_{31} & a_{32} & a_{33} + \dfrac{1}{\tau} \end{vmatrix} = 0 \tag{4}$$

Since the a's are functions of rate constants and equilibrium concentrations this would lead to very complicated expressions for the general case. However, considerable simplification results if the number of steps is smaller or some of the steps are so fast that certain substances can be considered to remain in equilibrium during a relaxation process. Also fast steps can be treated on the assumption that slow steps do not occur. Certain special cases derived from the general system can be treated separately.

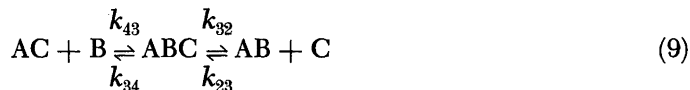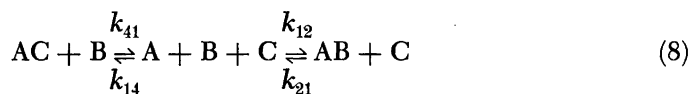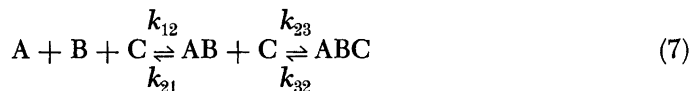*One step.* The simplest special case is one in which there is a single reaction[1-3].

$$A + B \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} AB$$

$$\frac{1}{\tau} = k_{21} + k_{12}\,[(\overline{A}) + (\overline{B})] \tag{5}$$
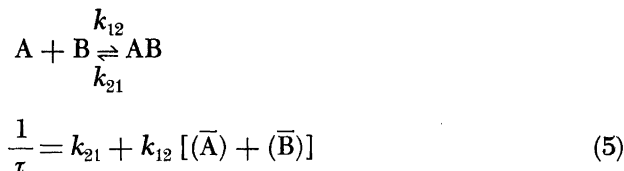
where $(\overline{A})$ and $(\overline{B})$ represent equilibrium concentrations.

For the isomerization reaction $A \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} A'$

$$\frac{1}{\tau} = k_{21} + k_{12} \tag{6}$$

so that changing a bimolecular step to a unimolecular step has the effect of replacing the sum of concentrations by 1.

Two steps. There are three qualitatively different sequences of two reactions in scheme 1.

$$A + B + C \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} AB + C \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} ABC \tag{7}$$

$$AC + B \underset{k_{14}}{\overset{k_{41}}{\rightleftharpoons}} A + B + C \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} AB + C \tag{8}$$

$$AC + B \underset{k_{34}}{\overset{k_{43}}{\rightleftharpoons}} ABC \underset{k_{23}}{\overset{k_{32}}{\rightleftharpoons}} AB + C \tag{9}$$

Each of these systems is characterized by two relaxation times which are obtained by solution of a quadratic equation. The complete expressions are quite complicated functions of the rate constants and equilibrium concentrations and allow for a possibility that would not allow the separate experimental determination of τ_1 and τ_2, that is, the possibility that these parameters are of the same magnitude. In general $1/\tau_1 + 1/\tau_2 = a_{11} + a_{22}$ so that if $\tau_2 \rangle\rangle \tau_1$ there will be a relatively simple expression for τ_1. Such expressions are given below. Another special case which simplifies the mathematics is that in which the square root term $(1-x)^{1/2}$ arising in the solution of the quadratic may be approximated with $1 - x/2$. In this way the following expressions are obtained for mechanism 7.

$$\frac{1}{\tau_1} = k_{21} + k_{12}\,[(\overline{A}) + (\overline{B})] + k_{32} + k_{23}\,[(\overline{AB}) + (\overline{C})] \tag{10}$$

$$\frac{1}{\tau_1\,\tau_2} = k_{21}\left\{k_{32} + k_{23}\,(\overline{AB})\right\} + k_{12}\,[(\overline{A}) + (\overline{B})]\left\{k_{32} + k_{23}\,[(\overline{AB}) + (\overline{C})]\right\} \tag{11}$$

These expressions indicate that studies of the dependencies of τ_1 and τ_2 on various concentration variables could yield the four independent rate constants in this mechanism. If τ_1 and τ_2 are not very different the complete expressions should be used to evaluate the rate constants. In any case the values of the various rate constants must be substituted into the quadratic expressions to make sure that the approximation used was satisfactory.

If one of the steps is equilibrated much faster than the other, these expressions take on simpler forms. If $k_{21} + k_{12}\,[(\bar{A}) + (\bar{B})] \rangle\rangle k_{32} + k_{23}\,[(\overline{AB}) + (\bar{C})]$, τ_1 is given by equation 5, and τ_2 by:

$$\frac{1}{\tau_2} = k_{32} + k_{23}\left\{(\overline{AB}) + \frac{(\bar{C})\,[(\bar{A}) + (\bar{B})]}{K_{21} + (\bar{A}) + (\bar{B})}\right\} \tag{12}$$

where $K_{21} = k_{21}/k_{12}$. Equation 12 may be derived directly, as discussed below, without solving a quadratic equation.

A further special case of interest occurs when B is buffered. For example, B might be hydrogen ion in a buffered solution and the rates of proton gain by A and loss by AB may be fast compared with other steps in the mechanism. In this case

$$\frac{1}{\tau_1} = k_{21} + k_{12}\,(B) \tag{13}$$

$$\frac{1}{\tau_2} = k_{32} + k_{23}\left[(\overline{AB}) + \frac{(\bar{B})\,(\bar{C})}{K_{21} + (\bar{B})}\right] \tag{14}$$

Terms in $[(\bar{A}) + (\bar{B})]$ are replaced with $(\bar{B})$ because $(\bar{B})$ does not change during the reaction, and the bimolecular reaction $A + B$ becomes a pseudo-unimolecular reaction with a rate constant proportional to $(\bar{B})$.

If the second step is fast $k_{21} + k_{12}\,[(\bar{A}) + (\bar{B})]\langle\langle k_{32} + k_{23}\,[(\overline{AB}) + (\bar{C})]$

$$\frac{1}{\tau_1} = k_{32} + k_{23}\,[(\overline{AB}) + (\bar{C})] \tag{15}$$

$$\frac{1}{\tau_2} = k_{12}\,[(\bar{A}) + (\bar{B})] + k_{21}\frac{[K_{32} + (\overline{AB})]}{K_{32} + (\overline{AB}) + (\bar{C})} \tag{16}$$

where $K_{32} = k_{32}/k_{23}$.

If in addition C is buffered

$$\frac{1}{\tau_1} = k_{32} + k_{23}\,(\bar{C}) \tag{17}$$

$$\frac{1}{\tau_2} = k_{12}\,[(\bar{A}) + (\bar{B})] + \frac{k_{21}K_{32}}{K_{32} + (\bar{C})} \tag{18}$$

If AB in mechanism 7 is in a steady state one of the rate equations is eliminated $(\mathrm{d}\,(AB)/\mathrm{d}t = 0)$, and the expression for the steady state relaxation time becomes

$$\frac{1}{\tau} = \frac{k_{21}\,k_{32} + k_{12}\,k_{23}\,\{(\overline{C})\,[(\overline{A}) + (\overline{B})] + (\overline{A})\,(\overline{B})\,\}}{k_{21} + k_{23}\,(C)} \tag{19}$$

If in addition C is buffered, the term in (A)(B) in the numerator is eliminated.

The mechanism

$$A + B \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} AB \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} AB' \tag{20}$$

may be considered to be a special case of mechanism 7. In the expressions for τ_1 and τ_2 terms in $[(\overline{AB}) + (\overline{C})]$ are replaced by 1 since the bimolecular reaction of AB with C has been replaced by unimolecular reaction. The term in $k_{23}(\overline{AB})$ vanishes. The relaxation times (*cf.* Ref. 4) given by expanding the square root term are

$$\frac{1}{\tau_1} = k_{21} + k_{23} + k_{32} + k_{12}\,[(\overline{A}) + (\overline{B})] \tag{21}$$

$$\frac{1}{\tau_1\,\tau_2} = k_{21}\,k_{32} + k_{12}\,(k_{23} + k_{32})\,[(\overline{A}) + (\overline{B})] \tag{22}$$

In another special case one of the reactants in the bimolecular reaction undergoes isomerization rather than the product, as in reaction 20.

$$AB' + C \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} AB + C \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} ABC \tag{23}$$
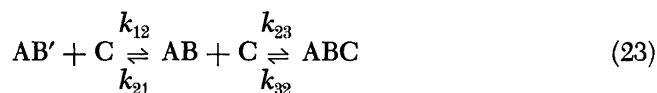
In this case the relaxation times given by expanding the square root term are

$$\frac{1}{\tau_1} = k_{21} + k_{12} + k_{32} + k_{23}\,[(\overline{AB}) + (\overline{C})] \tag{24}$$

$$\frac{1}{\tau_1\,\tau_2} = k_{32}\,(k_{12} + k_{21}) + k_{21}\,k_{23}(\overline{AB}) + k_{12}\,k_{23}\,[(\overline{AB}) + (\overline{C})] \tag{25}$$

Three steps. If one of the reactions in system 1 is eliminated there will still be three relaxation times, and the solution of a cubic equation will be required unless there is a big difference in the rates of equilibration of different steps. If two of the steps are fast it is just as easy to consider all four steps.

Four steps. We will consider the case where the reactions involving C are considerably faster than the reactions involving B, and that C is buffered. In considering the long relaxation time A and AC may be assumed to be in equilibrium, and the same will be true for AB and ABC. The relaxation process con-

sidered is simply a slow equilibration between the two equilibrium pairs A + AC and AB + ABC. This may be considered as an example of a simple bimolecular association with

$$\frac{1}{\tau} = k_{\text{dissoc}} + k_{\text{assoc}}\left[(\overline{A}) + (\overline{AC}) + (\overline{B})\right] \tag{26}$$

The sum $(\overline{A}) + (\overline{AC})$ is included in the concentration term since these two forms are in equilibrium and must be treated together. In the absence of C this expression must reduce to that for the upper line of scheme 1, namely equation 5, and at very high C concentrations the relaxation must be achieved by the lower path so that

$$\frac{1}{\tau} = k_{34} + k_{43}\left[(\overline{AC}) + (\overline{B})\right] \tag{27}$$

This is satisfied if the following forms are used for k_{dissoc} and k_{assoc}

$$k_{\text{dissoc}} = \frac{k_{21} + k_{34}(\overline{C})/K_{32}}{1 + (\overline{C})/K_{32}} \tag{28}$$

$$k_{\text{assoc}} = \frac{k_{12} + k_{43}(\overline{C})/K_{43}}{1 + (\overline{C})/K_{43}} \tag{29}$$

where $K_{32} = k_{32}/k_{23}$ and $K_{43} = k_{43}/k_{34}$. If C is not buffered more complicated expressions are obtained.

When certain reactants may be assumed to be in equilibrium the expression for the relaxation times may be derived directly without deriving the more general expression and simplifying. For instance in reaction 7, if the first step is in rapid equilibrium, one can write for the instantaneous equilibrium at any time during the slow relaxation

$$K_{21} = \frac{k_{21}}{k_{12}} = \frac{[(\overline{A}) + \varDelta(A)]\,[(\overline{B}) + \varDelta(B)]}{(\overline{AB}) + \varDelta(AB)} = \frac{[(\overline{A}) + \varDelta(A)]\,[(\overline{B}) + \varDelta(A)]}{(\overline{AB}) + \varDelta(AB)} \tag{30}$$

where the concentrations are expressed as final equilibrium values plus a displacement. This yields the following relation between $\varDelta(A)$ and $\varDelta(AB)$

$$\varDelta(A) = \varDelta(AB)\,\frac{K_{21}}{[(\overline{A}) + (\overline{B})]} \tag{31}$$

Using this relation, one can describe the slow relaxation by a single differential equation in $\varDelta(ABC) = -[\varDelta(A) + \varDelta(AB)]$:

$$\frac{\varDelta(ABC)}{dt} = k_{23}\left[(\overline{AB}) - \varDelta(ABC)\frac{(\overline{A}) + (\overline{B})}{K_{21} + (\overline{A}) + (\overline{B})}\right][(\overline{C}) - \varDelta(ABC)] - k_{32}[(\overline{ABC}) - \varDelta(ABC)] \tag{32}$$

This equation leads directly to equation 12 for τ_2. Expressions for the slow relaxation time in other cases where a fast step can be assumed to be in equilibrium can be derived in a similar way.

It has been impossible to give all the forms for the expressions for the relaxation times of these and related cases in this short paper, but the authors will be happy to supply the additional expressions in mimeographed form to those who are interested.

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