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# ESTIMATION OF UPPER BOUNDS FOR THE RATES OF ENZYMATIC REACTIONS

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A general methodology that allows the estimation of maximum rates of enzymatic reactions is described. For a typical mechanism of an enzymatic reaction, the rate is a function of kinetic parameters which are unknown but required to obey certain constraints. Specifically, the ratio of forward to backward rate constants must be consistent with the equilibrium constant, and the rate of each bimolecular reaction-step must be less than the rate of collision of the two reactant species. If additional information is available on the reaction rate, more constraints can be introduced. By maximizing the rate expression with respect to the kinetic parameters, subject to all applicable constraints, a first-principles upper bound is obtained for the reaction rate. If the reaction rate is actually known, the methodology can alternatively estimate an extremum for the concentration of the enzyme, a substrate, or a product. Simple thermodynamic arguments could also provide bounds for concentrations or the direction (but not the magnitude) of the rate, by examining only the overall transformation of reactants to products and completely ignoring the mechanism. The collision-limit treatment proposed here exploits basic internal characteristics of enzymatic reaction mechanisms to predict better bounds for the concentrations and the thermodynamically allowable maximum magnitude of the rate.
KEYWORDS Enzymatic reactions Upper bounds Rates.

## 1. INTRODUCTION

Enzymatic reaction mechanisms involve attachment of the reactants (substrates) to the enzyme, transformation of the reactants to products, and finally detachment of the products. Because the mechanisms must involve the attachment of the reactants on the enzyme, it is frequently mentioned in the literature that the rate of a single-reactant irreversible enzymatic reaction has an upper bound, equal to the rate of encounters or collisions‡ between the enzyme and the substrate (Hammes and Schimmel, 1970; Fersht, 1977; Hiromi, 1979):

$$r_{max} = k e_{total}[A] \tag{1}$$

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‡ The term *collision* customarily refers to gases, while the term *encounter* is more appropriate for liquids; here, we will use the two terms interchangeably. We will avoid the term *diffusion*, which is often used for this situation in the literature, because it may give the erroneous impression that there are macroscopic concentration gradients in the system.

where $e_{total}$ is the concentration of the enzyme (excluding any amounts that are deactivated or inhibited), $[A]$ is the concentration of the substrate, and $k$ is the bimolecular collision parameter. Maximum values for $k$ are reported as roughly $10^9\,M^{-1}\,s^{-1}$.

Naturally, most enzymatic reactions cannot attain this rate, because they are limited by additional substrates, dissociation of the products from the enzyme, and, most importantly, slow intramolecular rearrangements within one or more substrate-enzyme complexes. Equation (1) yields nonetheless a valid (conservative) upper bound for all enzymatic reaction rates. This upper bound is a theoretical and unattainable maximum for most enzymatic reactions, but it could be achieved by the most efficient enzymes under optimal conditions. This maximum has been used in the evaluation of enzyme efficiency (Albery and Knowles, 1976), defined as the ratio of the observed rate to the maximum collision rate of Eq. (1). It was demonstrated that there are enzymes which do attain the upper bound, i.e., their intramolecular rearrangements are so efficient that rates are limited only by collisions.

Theoretical maximum rates are conceptually similar to theoretical maximum yields of pathways. The maximum yields are often unattainable, because their derivation neglects important mechanisms regulating the flux through various pathways. However, they provide a sound upper limit and a useful reference point in the analysis of real processes and proposed improvements to them.

### *Deficiencies of the Simple Upper-Bound Expression*

In all of the previous work, the maximum rate is simply viewed as equal to the rate of encounter of the enzyme with a substrate. Thus, the maximum rate is essentially derived from only one particular step of the reaction mechanism. Additional constraints, stemming from a more detailed picture of the reaction mechanism or from the reversibility of the reaction, are not employed. An enzymatic reaction with many substrates and products should theoretically have a slower rate, because the enzyme must encounter each of the substrates (often in a predefined sequence) before the reaction can be completed. The effect of multiple substrates and products on the reaction rate is not reflected in Eq. (1).

An enzymatic reaction that approaches equilibrium becomes progressively slower and its rate becomes zero at equilibrium. Equation (1) does not take into account the displacement of the reaction from equilibrium. The equation predicts for a reaction that is very close to equilibrium the same high rate it would predict for an irreversible reaction. In fact, Eq. (1) neglects thermodynamics to the point that it would provide a high maximum rate even for reactions that are thermodynamically *infeasible*.

### *Predictions from Thermodynamics*

Simple thermodynamic analysis alone (specifically, a comparison between the mass-action ratio and the equilibrium constant) can predict whether a reaction is feasible or not. Unfortunately, it can say nothing about the maximum permissible

rate at which the enzymatic reaction could take place. Thus, it suffers from a shortcoming similar to the pure collision-limit estimation of Eq. (1). While it provides the correct answer about the feasibility of the enzymatic reaction, it provides no means to quantify the fact that as the reaction approaches equilibrium (and becomes less favored thermodynamically) it must take place at slower rates.

Figure 1(a) shows the thermodynamic rate-prediction as a function of the thermodynamic driving force, i.e., the displacement of the reaction from equilibrium. This displacement is represented by $1-Q/K_e$ where $Q$ is the mass-action ratio and $K_e$ is the equilibrium constant of the enzymatic reaction. When $1-Q/K_e>0$ the reaction is feasible. When $1-Q/K_e<0$ the reaction is infeasible; in fact, the reverse reaction takes place, causing the negative rate depicted in the Figure 1(a). The collision-limit of Eq. (1), taken literally, produces a flat rate profile, shown in Figure 1(b); the rate value depends only on the substrate that was used in Eq. (1).

A simple combination of thermodynamic and collision-limit considerations is shown in Figure 2(a). It is achieved by calculating separately the maximum rate for the reverse reaction, and using that rate when the forward reaction is infeasible. This is not a satisfactory solution because the reaction takes place in both (forward and backward) directions at all times; it does not merely switch from one direction to the other at one particular point. Only the *net* rate happends to change sign when the mass-action ratio is equal to the equilibrium constant (i.e., when the driving force goes through zero). If the reaction mechanism is fixed, a single rate expression should be valid on both sides of the equilibrium. The expression should predict positive rate when, overall, the forward reaction is thermodynamically favored and negative rate when the backward reaction is favored. However, the transition from one region to the

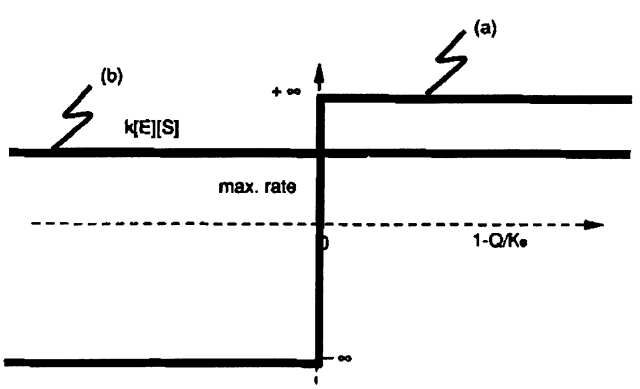


FIGURE 1 The maximum rate of an enzymatic reaction as a function of the displacement from equilibrium, $1-Q/K_e$, where $Q$ is the mass action ratio and $K_e$ is the equilibrium constant. The horizontal axis is assumed to denote variations in $K_e$ rather than concentrations. (a) Simple thermodynamic arguments provide the sign of the rate, but no information about its magnitude; in effect, the maximum rate is infinitely large. (b) The collision limit between the enzyme $E$ and one substrate $S$ yields a fixed maximum rate, independent from thermodynamic requirements.

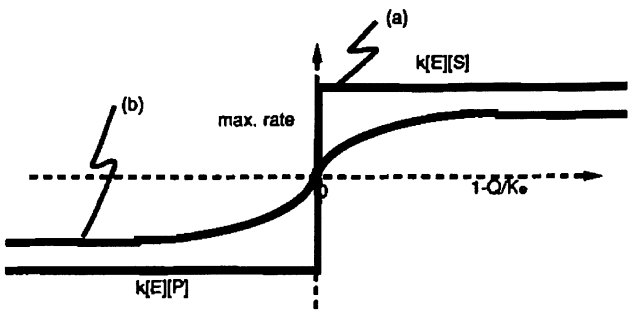


FIGURE 2 The maximum rate of an enzymatic reaction as a function of the displacement from equilibrium. (a) A simple combination of thermodynamics and collision limits (between the enzyme *E* and a substrate *S* or a product *P*) yields a maximum rate with a finite jump at equilibrium. (b) The maximum rate curve should make a smooth transition at equilibrium, while far from equilibrium it should still take into account the presence of other substrates and products which restrict the maximum rate of reaction.

other should be smooth. Figure 2(b) shows a rate curve that has the desired characteristics.

Note that Eq. (1) implies that only one substrate affects the maximum rate. In reality, each substrate and product reduces the maximum rate, because it inserts additional steps in the chain of the enzymatic reaction mechanism, slowing the reaction down—especially since the extra steps are bimolecular and prone to the collision-limitations suggested by Eq. (1). Thus, as shown in Figure 2(b), the rate should be lower than the simple prediction of Eq. (1), even very far from equilibrium.

*The work presented here*

In this work the estimation of maximum rates is extended to multi-substrate, multi-product reactions, reversible or irreversible, in a thermodynamically consistent way. The result is a general methodology that allows the estimation of an upper bound for the rate of any enzymatic reaction (Mavrovouniotis, 1988). This technique presented here assumes a typical fast mechanism for the reaction, with constraints imposed on the kinetic parameters. The constraints require that kinetic parameters be consistent with the equilibrium constant, and that the rate of each bimolecular step be smaller than the collision rate of the species participating in the step. The kinetic parameters are then determined so that the constraints are satisfied and the rate is maximized.

If the actual reaction rate is known, the method can be inverted and used to estimate a bound on the concentration of a reactant or a product. Without the technique presented in this paper, concentration bounds could be estimated through exclusively thermodynamic arguments (i.e., comparison of the mass-action ratio to the equilibrium constant), using the sign of the reaction rate and neglecting its magnitude. An upper bound for a reactant concentration can also be estimated from Eq. (1), regardless of the proximity of the reaction to equilibrium. With the technique presented here, however, the concentration bounds will account for both thermodynamic and collision limitations, and they will always be tighter. The comparison of actual reaction rates to upper bounds,

carried out by Albery and Knowles (1976), using only Eq. (1), demonstrated that there exist enzymes which attain the upper bound; the technique proposed here tightens the upper bounds, producing more realistic results. Simple thermodynamic analysis examines only the reactants and products of a transformation. The presented approach is, conceptually, an extension of thermodynamic analysis to account for the character of enzymatic-reaction mechanisms. However, it depends only on general characteristics and not specific details of the actual reaction mechanism.

Following a more precise statement of the problem addressed, along with necessary assumptions, the problem is recast in terms of dimensionless quantities, and solved for several reaction schemes. Typical values for collision parameters are then estimated, along with ranges for the dimensionless quantities. Simple numerical examples are finally discussed in order to demonstrate the principles involved and present other potential applications and future directions.

*Significance*

The significance of this method lies in that it extends the thermodynamic analysis of enzymatic reactions to account for constraints imposed by the nature of enzymatic reaction mechanisms. Since collision limitations are derived from *general* enzymatic reaction mechanisms, the results do *not* depend on any characteristics of the particular enzyme.

The technique allows the estimation of bounds for parameters related to reaction kinetics, such as rates and concentrations, for which actual experimental data are often not available. It is equally applicable to enzymatic steps of **intracellular** biochemical pathways and **extracellular** enzymatic transformations. Although it is implied that the enzyme catalyzing the reaction is a known, existing enzyme, the method can also be used when an enzyme is *sought* to carry out a certain transformation; if the method determines that, under given conditions, the transformation cannot take place with the desired rate, then it can be *a priori* concluded that no suitable enzyme exists, i.e., the transformation is not a single feasible enzymatic reaction.

## 2. PROBLEM AND ASSUMPTIONS

For a given enzymatic reaction, it is assumed that the following are known:

- The order of substrate binding,
- the order of product release from the enzyme,
- the equilibrium constant $K_e$,
- the enzyme concentration $e_{\text{total}}$, and
- the substrate and product concentrations.

It is further assumed that the conditions of the reaction correspond to an aqueous solution with no macroscopic concentration gradients, and that the enzyme works alone, instead of being part of a multi-enzyme complex. Under these assumptions, the objective is to find the maximum rate of the reaction under steady-state conditions.

The reaction attains its maximum rate when its only limitation is the physicochemical collision of the species. The biochemical literature usually refers to this as "diffusion limitation", but the term "collision limitation" will be used in this paper, because "diffusion" implies macroscopic concentration gradients which are not considered here. The steps of dissociation, intramolecular rearrangement, and rearrangement within an enzyme-substrate complex can take place instantaneously. This is justified since the characteristic rate constants can be as high as $10^{10}\,s^{-1}$ for dissociation (Fersht, 1977), $10^{12}\,s^{-1}$ for intramolecular rearrangement (Fersht, 1977), but only $10^{6}\,s^{-1}$ for a bimolecular collision. The last result can be obtained from Eq. (1) for $[A] = 1\,mM$ and $k = 10^{9}\,M^{-1}\,s^{-1}$.

An additional assumption is that the species that bind to the enzyme come from the bulk of the solution and not from another site on the enzyme. Thus, $H^+$, $OH^-$, and $H_2O$ should not participate in the reaction mechanism, as they may be supplied by other sites of the enzyme or a metabolite. This is not a restrictive assumption, but rather a guideline in the use of the methodology: Whenever such species do occur, they are assumed to bind instantaneously, and the corresponding steps must be excluded from the mechanism.

## 3. TREATMENT OF A TWO-REACTANT TWO-PRODUCT ORDERED MECHANISM

### *Derivation of the Rate Equation*

The methodology for obtaining a maximum rate is illustrated here for a particular reaction mechanism. An ordered mechanism with two reactants $A$ and $B$ and two products $P$ and $Q$ has the form indicated in Figure 3. The rate of the reaction at

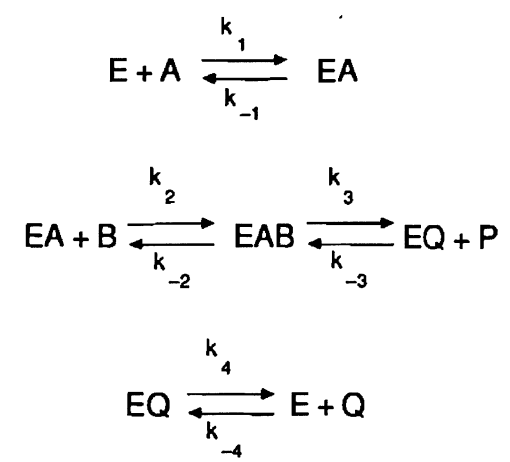


FIGURE 3 The general ordered mechanism for a two-reactant two-product enzymatic reaction.

steady state, $r$, can be determined from the system:

$$r = k_1[E][A] - k_{-1}[EA] \tag{2}$$

$$r = k_2[EA][B] - k_{-2}[EAB] \tag{3}$$

$$r = k_3[EAB] - k_{-3}[EQ][P] \tag{4}$$

$$r = k_4[EQ] - k_{-4}[E][Q] \tag{5}$$

$$e_{\text{total}} = [E] + [EA] + [EAB] + [EQ] \tag{6}$$

Since the concentrations of multi-species complexes will not be considered again, it is convenient to drop the brackets from concentrations. As a function of $A$, $B$, $P$, $Q$, $e_{\text{total}}$, and the kinetic parameters, the rate is expressed as:

$$r = e_{\text{total}}(k_1k_2k_3k_4AB - k_{-1}k_{-2}k_{-3}k_{-4}PQ)D^{-1} \tag{7}$$

where

$$\begin{aligned} D = {} & k_{-4}k_{-3}k_2BPQ + k_{-4}k_{-3}k_{-1}PQ + k_{-4}k_{-3}k_{-2}PQ + k_{-4}k_2k_3BQ \\ & + k_{-4}k_{-1}k_3Q + k_{-4}k_{-2}k_{-1}Q + k_{-3}k_1k_2ABP + k_{-3}k_{-2}k_1AP + k_{-3}k_{-2}k_{-1}P \\ & + k_1k_2k_4AB + k_1k_2k_3AB + k_2k_3k_4B + k_1k_3k_4A + k_{-2}k_1k_4A \\ & + k_{-1}k_3k_4 + k_{-2}k_{-1}k_4 \end{aligned} \tag{8}$$

*Introduction of Constraints*

There are two kinds of constraints that can be imposed on the kinetic parameters. The first is that the ratio of the forward rate constants to the backward rate constants must be equal to the equilibrium constant:

$$K_e = (k_1k_2k_3k_4)(k_{-1}k_{-2}k_{-3}k_{-4})^{-1} \tag{9}$$

The second limitation applies only to (forward or backward) bimolecular steps. Since a collision is required for the two reacting species to form a complex, the rate of each bimolecular step cannot exceed the rate of collision of the two species in the aqueous solution. In effect:

$$k_i \leq b_i \quad \text{for} \quad i = 1, 2, -3, -4 \tag{10}$$

where $b_i$ is the collision-determined upper bound for the rate constant $k_i$. Under the constraints (9) and (10), the objective is to find values for $k_i$ (for $i = \pm 1, \pm 2, \pm 3$, and $\pm 4$) so that the rate $r$ in Eqs. (7) and (8) is maximized.

Note that $k_i$ are not necessarily intrinsic reaction rate constants, since they may be limited by a physical process such as the collision of the species. Despite this partly phenomenological character of the $k_i$ parameters the equilibrium relation (9) must still be satisfied because the collision rate constants do not depend on the concentrations.

*Solution of the Optimization Problem*

The mathematical solution of the problem is simplified through the introduction of the parameters:

$$m_i = k_i/k_{-i} \quad \text{for} \quad i = 1, 2, 3, 4 \tag{11}$$

and substitution of the kinetic parameters $k_{-1}$, $k_{-2}$, $k_3$, and $k_4$ in the rate expression as follows:

$$k_{-1} = k_1/m_1 \tag{12}$$

$$k_{-2} = k_2/m_2 \tag{13}$$

$$k_3 = k_{-3}m_3 \tag{14}$$

$$k_4 = k_{-4}m_4 \tag{15}$$

The equilibrium constraint is satisfied by the substitution:

$$m_3 = K_e/(m_1m_2m_4) \tag{16}$$

The parameters $k_1$, $k_2$, $k_{-3}$, $k_{-4}$, $m_1$, $m_2$, and $m_4$ are all non-negative, while $k_1$, $k_2$, $k_{-3}$, and $k_{-4}$ are also upper-bounded by collision limits. Working with this set of seven independent variables, the derivatives of the reaction rate are determined as follows:

$$\frac{\partial r}{\partial k_1} = G(k_{-4}^2k_{-3}^2m_1^2k_2^2m_4K_e)(m_1m_2m_4BPQ + m_1m_4PQ + m_4K_eB + K_eBQ) \tag{17}$$

$$\frac{\partial r}{\partial k_2} = G(k_{-4}^2k_{-3}^2k_1^2m_1m_4K_e)(m_1m_2m_4PQ + K_eQ + m_1m_4K_eA + m_4K_e) \tag{18}$$

$$\frac{\partial r}{\partial k_{-3}} = G(k_{-4}^2k_1^2m_1^2k_2^2m_4^2K_e)(Q + m_1m_2m_4AB + m_1m_4A + m_4) \tag{19}$$

$$\frac{\partial r}{\partial k_{-4}} = G(k_{-3}^2k_1^2m_1^2k_2^2m_4K_e)(m_1m_2m_4ABP + m_1m_4AP + m_4P + K_eAB) \tag{20}$$

$$\begin{aligned}\frac{\partial r}{\partial m_1} = G(k_{-4}k_{-3}k_1k_2m_4K_e)(&-k_{-4}k_{-3}m_1^2k_2m_2m_4BPQ\\ &-k_{-4}k_{-3}m_1^2k_2m_4PQ + k_{-4}k_{-3}k_1K_eQ - k_{-3}k_1m_1^2k_2m_2m_4ABP\\ &-k_{-3}k_1m_1^2k_2m_4AP + k_{-4}k_{-3}k_1m_4K_e - k_{-4}k_1m_1^2k_2m_2m_4^2AB\\ &-k_{-4}k_1m_1^2k_2m_4^2A)\end{aligned} \tag{21}$$

$$\begin{aligned}\frac{\partial r}{\partial m_2} = -G(k_{-4}k_{-3}k_1m_1^2k_2m_4^2K_e)(&k_{-4}k_{-3}m_1k_2BPQ\\ &+k_{-4}k_{-3}k_1PQ + k_{-3}k_1m_1k_2ABP + k_{-4}k_1m_1k_2m_4AB)\end{aligned} \tag{22}$$

$$\begin{aligned}\frac{\partial r}{\partial m_4} = G(k_{-4}k_{-3}k_1m_1k_2K_e)(&k_{-4}k_{-3}m_1k_2K_eBQ\\ &+k_{-4}k_{-3}k_1K_eQ + k_{-3}k_1m_1k_2K_eAB - k_{-4}k_1m_1^2k_2m_2m_4^2AB\\ &-k_{-4}k_1m_1^2k_2m_4^2A - k_{-4}k_1m_1k_2m_4^2)\end{aligned} \tag{23}$$

where:

$$G = e_{\text{total}}(AB - PQK_e^{-1})D^{-2} \tag{24}$$

Since a positive driving force is required for the reaction to take place, $G$ must be positive. Consequently, Eqs. (17) to (20) yield:

$$\frac{\partial r}{\partial k_i} > 0, \quad \text{for} \quad i = 1, 2, -3, -4. \tag{25}$$

Thus, to yield the maximum possible rate, the four bimolecular step parameters $k_1$, $k_2$, $k_{-3}$, and $k_{-4}$ must be at their collision limits. Since

$$\frac{\partial r}{\partial m_2} < 0 \tag{26}$$

and $m_2 \geq 0$, in order to maximize the rate $r$, $m_2$ must be equal to 0. This directly implies that $k_{-2} \to \infty$ since it has already been concluded that $k_2$ is non-zero. Considering that the mechanism steps $-2$ and 3 compete for the same intermediate, the fact that $k_{-2} \to \infty$ implies that the concentration of that intermediate is zero, and the reaction can only take place if $k_3 \to \infty$. The only remaining independent variables are $m_1$ and $m_4$, which do not assume extreme values. They are obtained from the solution of the system of equations:

$$\frac{\partial r}{\partial m_1} = 0 \tag{27}$$

$$\frac{\partial r}{\partial m_4} = 0 \tag{28}$$

with $k_1$, $k_2$, $k_{-3}$, and $k_{-4}$ at their collision limits and $m_2 = 0$, as indicated above. The system cannot be solved analytically.

Returning to the original set of independent variables, the results can be summarized as follows. To maximize the rate as given in Eqs. (7) and (8): set $k_{-2} \to \infty$ and $k_3 \to \infty$ maintaining Eq. (9); set the parameters $k_1$, $k_2$, $k_{-3}$, and $k_{-4}$ to their collision-determined upper bounds; and obtain $k_{-1}$ and $k_4$ from the system:

$$k_{-1}k_{-4}^2k_{-3}k_2BK_eQ + k_{-1}^2k_{-4}^2k_{-3}K_3Q + k_{-1}k_{-4}k_{-3}k_1k_2ABK_e = k_4^2k_1^2k_2A + k_{-1}k_4^2k_2A + k_{-1}k_4^2k_1k_2 \tag{29}$$

$$k_4k_{-4}k_{-3}k_1k_2PQ + k_4k_{-3}k_1^2k_2AP + k_4^2k_1^2k_2A = k_{-1}^2k_{-4}^2k_{-3}K_eQ + k_{-1}^2k_4k_{-4}k_{-3}K_e \tag{30}$$

Under the assumptions stated, substitution of all these values for the kinetic parameters into the rate equation will yield the maximum rate.

## 4. NONDIMENSIONALIZATION

The algebraic expressions and the optimization procedure can be simplified if appropriate dimensionless parameters are introduced. In this section, the ordered

reaction mechanism, for any number of reactants and products, is described in terms of a set of dimensionless parameters. Then the example from the previous section is recast in a dimensionless form, and final results for other mechanisms are listed.

*Dimensionless Parameters for any Ordered Mechanism*

For a biochemical reaction with $n$ reactants and $m$ products:

$$\sum_{i=1}^{n} S_i \rightarrow \sum_{i=n+1}^{n+m} S_i \tag{31}$$

the general ordered mechanism is:

$$S_i + E_i \underset{k_{-i}}{\overset{k_i}{\rightleftarrows}} E_{i+1}, \qquad i = 1, \ldots, n \tag{32}$$

$$E_i \underset{k_{-i}}{\overset{k_i}{\rightleftarrows}} E_{i+1} + S_i, \qquad i = n+1, \ldots, n+m \tag{33}$$

For more compact notation, the symbol $E_{n+m+1}$ is allowed as an alternative token for $E_1$. Let $t_i$ be the characteristic time of step $i$, where $i = \pm 1, \ldots, \pm n$:

$$t_i = k_i^{-1} S_i^{-1}, \; t_{-i} = k_{-i}^{-1}, \quad \text{for} \quad i = 1, \ldots, n \tag{34}$$

$$t_i = k_i^{-1}, \; t_{-i} = k_{-i}^{-1} S_i^{-1}, \quad \text{for} \quad i = n+1, \ldots, n+m \tag{35}$$

The dimensionless parameters that will be used are:

$$u_i = t_i / t_1, \; i = 2, \ldots, n \quad \text{or} \quad i = -(n+m), \ldots, -(n+1) \tag{36}$$

$$h_i = t_{-i} / t_i, \; i = 1, \ldots, n \quad \text{or} \quad i = -(n+m), \ldots, -(n+1) \tag{37}$$

$$f = \frac{\prod_{i=n+1}^{n+m} S_i}{\prod_{i=1}^{n} S_i} K_e^{-1} \tag{38}$$

$$e_i = \frac{E_i}{\sum_{i=1}^{n+m-1} E_i} = E_i / e_{\text{total}} \tag{39}$$

$$r_{nd} = \frac{r t_1}{e_{\text{total}}(1-f)} \tag{40}$$

*Physical Significance of the Dimensionless Parameters*

Using $t_1$ as the global time scale, $u_i$ is simply the scaled characteristic time of step $i$. It can also be viewed as the "activity" of $S_i$ scaled by the activity of $S_1$. Two

compounds can have the same activity $u_i$, even with different concentrations, if their rate constants for reactions to enzyme species are inversely proportional to their concentrations. $h_i$ is the equilibrium constant of step $i$, with respect to enzyme species alone. At equilibrium, $h_i$ is equal to $e_{i+1}/e_i$. $f$ is the mass action ratio divided by the equilibrium constant; the dimensionless driving force for the reaction is $1-f$. $e_i$ is the concentration of enzyme species $E_i$ as a fraction of total enzyme. $r_{nd}$ is the dimensionless rate per unit driving force, per mole of total enzyme, per unit time $t_1$. It is essentially the inverse of the dimensionless "resistance" that the mechanism puts up against the driving force.

*Dimensionless Maximum-Rate Result for the Example*

In terms of dimensionless parameters the two-reactant two-product system (Eqs. 2 to 6) can be written as:

$$r_{nd} = e_1 - e_2/h_1 \tag{41}$$

$$r_{nd} = u_2^{-1}(e_2 - e_3/h_2) \tag{42}$$

$$r_{nd} = u_{-3}^{-1}(e_3/h_{-3} - e_4) \tag{43}$$

$$r_{nd} = u_{-4}^{-1}(e_4/h_{-4} - e_1) \tag{44}$$

$$e_1 + e_2 + e_3 + e_4 = 1 \tag{45}$$

$$f = h_{-3}h_{-4}/h_1h_2 \tag{46}$$

TABLE I

Result of dimensionless maximization of the rate for an ordered mechanism with 1 reactant and 1 product

| Reaction: $S_1 \rightarrow S_2$ |
|---|
| Rate: $\frac{1}{r_{nd}} = u_{-2}f + 1$ |

TABLE II

Result of dimensionless maximization of the rate for an ordered mechanism with 1 reactant and 2 products

| Reaction: $S_1 \rightarrow S_2 + S_3$ |
|---|
| Rate: $\frac{1}{r_{nd}} = u_{-2}f + u_{-3}f + 2h_{-3}u_{-3} + 2h_{-3} + 1$ |
| With $h_{-3}$ from the equation: $h_{-3} = \left\{\frac{u_{-2}f}{u_{-3}+1}\right\}^{1/2}$ |

TABLE III

Result of dimensionless maximization of the rate for an ordered mechanism with 1 reactant and 3 products

| Reaction: $S_1 \rightarrow S_2 + S_3 + S_4$ |
|---|
| Rate: $\frac{1}{r_{nd}} = 2\frac{u_{-2}f}{h_{-3}h_{-4}} + \frac{u_{-2}f}{h_{-3}} + u_{-2}f + 2\frac{u_{-3}f}{h_{-4}} + u_{-3}f + u_{-4}f + h_{-3}u_{-3} + 1$ |
| With $h_{-3}$ and $h_{-4}$ from equations: $h_{-4}u_{-2}f + u_{-2}f = h_{-3}^2 h_{-4}u_{-3} + h_{-3}^2 h_{-4}^2 u_{-4} + h_{-3}^2 h_{-4}^2$ |
| $u_{-2}f + u_{-3}h_{-3}f = h_{-3}^2 h_{-4}^2 u_{-4} + h_{-3}^2 h_{-4}^2 + h_{-3}h_{-4}^2 u_{-4} + h_{-3}h_{-4}^2$ |

TABLE IV

Result of dimensionless maximization of the rate for an ordered mechanism with 2 reactants and 1 product

| Reaction: $S_1 + S_2 \rightarrow S_3$ |
|---|
| Rate: $\frac{1}{r_{nd}} = u_{-3}f + \frac{2u_2}{h_1} + u_2 + 1$ |
| With $h_1$ from the equation: $h_1 = \left(\frac{u_2}{u_{-3}f + f}\right)^{1/2}$ |

The maximum rate for the reaction, in terms of dimensionless parameters, can be expressed as:

$$r_{nd} = (u_{-3}h_1 f h_{-4}^{-1} + u_{-3}f h_{-4}^{-1} + u_2 h_{-4}h_1^{-1} + u_2 h_1^{-1} + u_{-4}h_1 f + u_{-3}f + h_1 f + u_{-4}f + u_2 + u_{-4}h_{-4} + h_{-4} + 1)^{-1} \quad (47)$$

where $u_2$, $u_{-3}$, and $u_{-4}$ are collision limited and $h_1$ and $h_{-4}$ are determined from

TABLE V

Result of dimensionless maximization of the rate for an ordered mechanism with 2 reactants and 2 products

| Reaction: $S_1 + S_2 \rightarrow S_3 + S_4$ |
|---|
| Rate: $\frac{1}{r_{nd}} = \frac{u_{-3}h_1 f}{h_{-4}} + 2\frac{u_{-3}f}{h_{-4}} + u_{-3}f + u_{-4}f + \frac{h_{-4}u_2}{h_1} + 2\frac{u_2}{h_1} + u_2 + 1$ |
| With $h_1$ and $h_{-4}$ from the equations: $u_{-3}h_1^2 f + h_{-4}u_{-4}h_1^2 f + h_{-4}h_1^2 f = h_{-4}^2 u_2 + h_{-4}u_2$ |
| $u_{-3}h_1^2 f + u_{-3}h_1 f = h_{-4}^2 u_2 + h_{-4}^2 u_{-4}h_1 + h_{-4}^2 h_1$ |

TABLE VI

Result of dimensionless maximization of the rate for an ordered mechanism with 2 reactants and 3 products

Reaction: $S_1 + S_2 \rightarrow S_3 + S_4 + S_5$

Rate:

$$\frac{1}{r_{nd}} = 2\frac{u_{-3}h_1 f}{h_{-5}h_{-4}} + 2\frac{u_{-4}h_1 f}{h_{-5}} + u_{-5}h_1 f + h_1 f + 2\frac{u_{-3}f}{h_{-5}h_{-4}} + \frac{u_{-3}f}{h_{-4}} + u_{-3}f + 2\frac{u_{-4}f}{h_{-5}} + u_{-4}f + u_{-5}f + \frac{h_{-5}h_{-4}u_2}{h_1} + u_2 + h_{-4}u_{-4} + 1$$

With $h_1$, $h_{-4}$, and $h_{-5}$ from the equations:

$$u_{-3}h_1^2 f + h_{-4}u_{-4}h_1^2 f + h_{-5}u_{-5}h_{-4}h_1^2 f + h_{-5}h_{-4}h_1^2 f = h_{-5}^2 h_{-4}^2 u_2 + h_{-5}^2 h_{-4} u_2 + h_{-5}h_{-4}^2 u_2$$

$$u_{-3}h_1^2 f + h_{-5}u_{-3}h_1 f + u_{-3}h_1 f = h_{-5}^2 h_{-4}^2 u_2 + h_{-5}h_{-4}^2 u_{-4}h_1 + h_{-5}^2 u_{-5}h_{-4}^2 h_1 + h_{-5}^2 h_{-4}^2 h_1$$

$$h_{-4}u_{-4}h_1^2 f + h_{-4}u_{-4}h_1 f - h_{-5}u_{-3}h_1 f = h_{-5}^2 h_{-4} u_2 + h_{-5}^2 u_{-5}h_{-4}h_1 + h_{-5}^2 h_{-4}h_1 - h_{-5}h_{-4}^2 u_{-4}h_1$$

the following system of equations:

$$u_{-3}h_1^2 f + u_{-4}h_1^2 h_{-4} f + h_1^2 h_{-4} f = h_{-4}^2 u_2 + h_{-4}u_2 \tag{48}$$

$$u_{-3}h_1^2 f + u_{-3}h_1 f = u_2 h_{-4}^2 - u_{-4}h_1 h_{-4}^2 - h_1 h_{-4}^2 \tag{49}$$

*Results for Other Ordered Mechanisms*

Similarly to the above example, the optimization results for all ordered reaction schemes that involve three or fewer reactants and three or fewer products have been obtained, and are listed in Tables I to IX. The results have been, whenever possible, simplified algebraically.

TABLE VII

Result of dimensionless maximization of the rate for an ordered mechanism with 3 reactants and 1 product

Reaction: $S_1 + S_2 + S_3 \rightarrow S_4$

Rate:

$$\frac{1}{r_{nd}} = h_2 u_2 f + u_{-4}f + 2\frac{u_3}{h_1 h_2} + \frac{u_3}{h_2} + u_3 + 2\frac{u_2}{h_1} + u_2 + 1$$

With $h_1$ and $h_2$ from the equations:

$$h_1 h_2^2 u_2 f + u_{-4}h_1^2 h_2^2 f + h_1^2 h_2^2 f = h_1 u_3 + u_3$$

$$u_{-4}h_1^2 h_2 f + h_1^2 h_2 f - h_1 h_2^2 u_2 f = h_2 u_2 - h_1 u_3$$

TABLE VIII

Result of dimensionless maximization of the rate for an ordered mechanism with 3 reactants and 2 products

Reaction: $S_1 + S_2 + S_3 \rightarrow S_4 + S_5$

Rate:

$$\frac{1}{r_{nd}} = h_2u_2f + \frac{u_{-4}f}{h_{-5}} + u_{-4}f + u_{-5}f + 2\frac{h_{-5}u_3}{h_1h_2} + 2\frac{u_3}{h_1h_2} + \frac{u_3}{h_2} + u_3 + 2\frac{h_{-5}u_2}{h_1} + 2\frac{u_2}{h_1} + u_2 + h_{-5}u_{-5} + h_{-5} + 1$$

With $h_1$, $h_2$, and $h_{-5}$ from the equations:

$$u_{-4}h_1^2h_2f + h_{-5}u_{-5}h_1^2h_2f + h_{-5}h_1^2h_2f - h_{-5}h_1h_2^2u_2f = h_{-5}^2h_2u_2 + h_{-5}h_2u_2 - h_{-5}h_1u_3$$

$$h_{-5}h_1h_2^2u_2f + u_{-4}h_1^2h_2^2f + h_{-5}u_{-5}h_1^2h_2^2f + h_{-5}h_1^2h_2^2f = h_{-5}h_1u_3 + h_{-5}^2u_3 + h_{-5}u_3$$

$$u_{-4}h_1^2h_2^2f + u_{-4}h_1^2h_2f + u_{-4}h_1h_2f = h_{-5}^2u_3 + h_{-5}^2h_2u_2 + h_{-5}^2u_{-5}h_1h_2 + h_{-5}^2h_1h_2$$

TABLE IX

Result of dimensionless maximization of the rate for an ordered mechanism with 3 reactants and 3 products

Reaction: $S_1 + S_2 + S_3 \rightarrow S_4 + S_5 + S_6$

Rate:

$$\frac{1}{r_{nd}} = h_2u_2f + \frac{u_{-4}f}{h_{-6}h_{-5}} + \frac{u_{-4}f}{h_{-5}} + u_{-4}f + \frac{u_{-5}f}{h_{-6}} + u_{-5}f + u_{-6}f + 2\frac{h_{-6}h_{-5}u_3}{h_1h_2} + 2\frac{h_{-6}u_3}{h_1h_2} + 2\frac{u_3}{h_1h_2} + \frac{u_3}{h_2} + u_3 + 2\frac{h_{-6}h_{-5}u_2}{h_1} + 2\frac{h_{-6}u_2}{h_1} + 2\frac{u_2}{h_1} + u_2 + h_{-5}u_{-5} + h_{-6}u_{-6}h_{-5} + h_{-6}h_{-5} + h_{-6}u_{-6} + h_{-6} + 1$$

With $h_1$, $h_2$, $h_{-5}$, and $h_{-6}$ from the equations:

$$u_{-4}h_1^2h_2f + h_{-5}u_{-5}h_1^2h_2f + h_{-6}u_{-6}h_{-5}h_1^2h_2f + h_{-6}h_{-5}h_1^2h_2f - h_{-6}h_{-5}h_1h_2^2u_2f = h_{-6}^2h_{-5}^2h_2u_2 + h_{-6}^2h_{-5}h_2u_2 + h_{-6}h_{-5}h_2u_2 - h_{-6}h_{-5}h_1u_3$$

$$h_{-6}h_{-5}h_1h_2^2u_2f + u_{-4}h_1^2h_2^2f + h_{-5}u_{-5}h_1^2h_2^2f + h_{-6}u_{-6}h_{-5}h_1^2h_2^2f + h_{-6}h_{-5}h_1^2h_2^2f = h_{-6}h_{-5}h_1u_3 + h_{-6}^2h_{-5}^2u_3 + h_{-6}^2h_{-5}u_3 + h_{-6}h_{-5}u_3$$

$$u_{-4}h_1^2h_2^2f + u_{-4}h_1^2h_2f + h_{-6}u_{-4}h_1h_2f + u_{-4}h_1h_2f = h_{-6}^2h_{-5}^2u_3 + h_{-6}^2h_{-5}^2h_2u_2 + h_{-6}h_{-5}^2u_{-5}h_1h_2 + h_{-6}^2u_{-6}h_{-5}^2h_1h_2 + h_{-6}^2h_{-5}^2h_1h_2$$

$$h_{-5}u_{-5}h_1^2h_2^2f + h_{-5}u_{-5}h_1^2h_2f + h_{-5}u_{-5}h_1h_2f - h_{-6}u_{-4}h_1h_2f = h_{-6}^2h_{-5}u_3 + h_{-6}^2h_{-5}h_2u_2 + h_{-6}^2u_{-6}h_{-5}h_1h_2 + h_{-6}^2h_{-5}h_1h_2 - h_{-6}h_{-5}^2u_{-5}h_1h_2$$

## 5. ASYMPTOTIC BEHAVIOR

The dimensionless quantities allow easier examination of limiting cases that facilitate the understanding of the maximum-rate concepts. These cases are particularly useful in light of the complexity of the general analytic expressions.

*Effect of f in a Simplified System*

As an example, the effect of $f$ (the mass action ratio divided by the equilibrium constant) on the maximum rate of a bioreaction with one reactant and three products will be examined. To reduce the number of independent parameters, the analysis is restricted, by assuming that $u_i = 1$ for $i = -2, -3, -4$. This is the case when the products have approximately equal molecular weights and the concentration of each product is roughly 60% of the concentration of the reactant. This restriction simplifies the equations for the maximum rate (Table III) to:

$$r_{nd} = (fh_{-3}^{-1}h_{-4}^{-1} + fh_{-3}^{-1} + fh_{-4}^{-1} + 3f + 2h_{-3}h_{-4} + h_{-3} + 2h_{-4} + 1)^{-1} \tag{50}$$

with $h_{-3}$ and $h_{-4}$ being the solution of the following system, simplified through some algebraic manipulation:

$$f = 2h_{-4}^2 h_{-3} \tag{51}$$

$$2h_{-4}(1 + h_{-4}) = h_{-3}(1 + 2h_{-4}) \tag{52}$$

*Reaction Very Far from Equilibrium*

To find the asymptotic behavior when $f \ll 1$, Eqs. (51) and (52) are solved for $h_{-3}$ and $h_{-4}$, and substituted in Eq. (50) to yield $r_{nd}$. As a first approximation, only terms of order $f^{1/3}$ are retained:

$$h_{-3} \approx (2f)^{1/3}, \qquad h_{-4} \approx (f/4)^{1/3} \tag{53, 54}$$

$$r_{nd}^{-1} = 1 + 3\,(2f)^{1/3} \tag{55}$$

This result does not offer sufficient accuracy, because, even for quite small values of $f$, terms of $f^{1/3}$ can be comparable to 1. To ensure the applicability of the approximation, it is wise to obtain the next term in the asymptotic expansion by retaining terms of order $f^{2/3}$ as well:

$$h_{-3} = (2f)^{1/3} - \tfrac{1}{3}\,(2f)^{2/3} \tag{56}$$

$$h_{-4} = (f/4)^{1/3} + \tfrac{1}{12}\,(2f)^{2/3} \tag{57}$$

$$r_{nd}^{-1} = 1 + 3\,(2f)^{1/3} + \tfrac{5}{2}\,(2f)^{2/3} \tag{58}$$

Application of these forms shows that even for a reaction removed 100-fold from equilibrium ($f = 0.01$, i.e., the mass action ratio is 100 times smaller than the equilibrium constant) the maximum rate is less than half the rate expected for an irreversible reaction ($f = 0$). Hence, the fact that the reaction is *not fully irreversible* exerts a significant effect on the maximum rate.

*Reaction Near Equilibrium*

At the other end of the spectrum, for $f \to 1$ Eqs. (50), (51), and (52) after similar solution and substitution yield:

$$h_{-3} \approx 1 - \frac{1-f}{2+2^{1/2}}, \qquad h_{-4} \approx \frac{1}{2^{1/2}} - \frac{1-f}{4} \tag{59, 60}$$

$$r_{nd} \approx (2^{1/2}3 + (3 + 2^{-1/2})(1+f))^{-1} \approx (7.95 + 3.71f)^{-1} \tag{61}$$

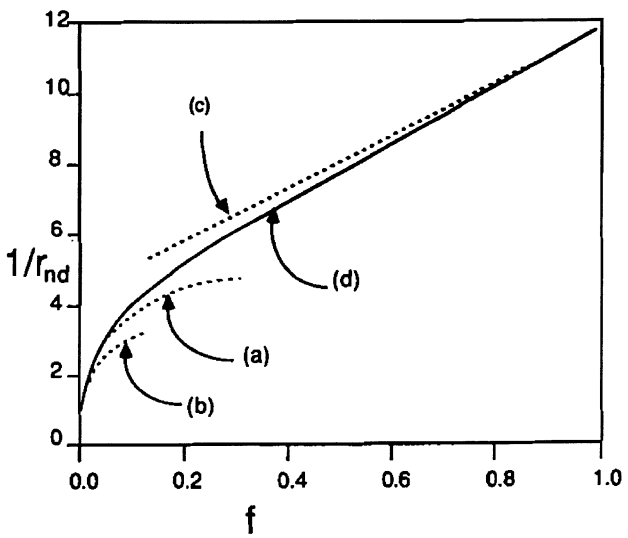


FIGURE 4 General numerical solution and analytic asymptotic solutions for one-reactant three-product enzymatic reactions, for $u_1 = 1$, $u_2 = 1$, and $u_3 = 1$. (a) Asymptotic solution for $f \to 0$, retaining terms of order $f^{1/3}$. (b) Asymptotic solution for $f \to 0$, retaining terms of order $f^{2/3}$. (c) Asymptotic solution for $f \to 1$. (d) General solution for any value of $f$ between 0 and 1.

For $f \to 1$, $r_{nd}$ varies approximately linearly with $f$, and no severe effect of $f$ on the rate is observed. Figure 4 shows the numerical solution of the example, for any value of $f$, along with the two asymptotic cases that were examined.

It should be noted that $r_{nd}$ is already expressed as rate per unit driving force $(1-f)$, so the effect of $f$ examined here is in addition to the effect expected solely from the reduction of the driving force. To account for the overall effect of $f$ on the rate, when the reaction is near equilibrium, Eq. (61) must be combined with Eq. (40) which relates $r_{nd}$ to $r$. For the example examined here, the maximum rate is proportional to $(1-f)/(7.95+3.71f)$. In the region close to equilibrium, i.e., as $f \to 1$, this function becomes zero. Thus, in agreement with the expectations set forth in the introduction of the paper, the rate smoothly approaches zero as the reaction approaches equilibrium.

## 6. ESTIMATION OF COLLISION PARAMETERS

Collision-determined upper bounds for the kinetic parameters, along with other ranges useful in the methodology, are estimated in this section. These kinetic parameters are necessary in the application of the presented technique. The collision limit $k$ for a bimolecular mechanism-step between a substrate $S$ (of small molecular weight) and an enzyme $E$:

$$E + S \xrightarrow{k} ES \tag{62}$$

is given by (Hiromi, 1979):

$$k = \rho_S \rho_E k_0 \tag{63}$$

where $\rho_S$ and $\rho_E$ are steric factors, smaller than one, and $k_0$ the encounter constant.

*Steric Factor for the Substrate*

The steric factor $\rho_S$ ranges from as small as roughly 0.01 (for large molecules) to as large as comparable to 1 (for small molecules). The participation of water, $H^+$, or $OH^-$ in reactions will be neglected, because, as discussed in Section 2, these molecules do not necessarily originate from the bulk of the solution. Other molecules occurring in biochemical reactions are generally complicated enough to have unreactive orientations, leading to $\rho_S$ significantly smaller than 1. A conservatively high value will be nevertheless assumed for $\rho_S$. Specifically, it is assumed that $\rho_S = 1/3$, signifying that one third of the possible orientations of $S$ are suitable for binding.

*Steric Factor for the Enzyme*

Since the active site of an enzyme is the area surrounding only a small number of bonds, it is modelled here as a circle of diameter 8 Å, or $8 \times 10^{-10}$ m. The point of interest here is essentially the inaccuracy allowed in the collisions. The value assumed above implies that, if the substrate misses the exact binding spot by more than 4 Å, the collision will be ineffective. In this sense, estimation of $\rho_E$ as the area of the active site divided by the total area of the enzyme (Hiromi, 1979) yields

$$\rho_E \approx (2 \times 10^{-10}\,\text{m}/r_E)^2 \tag{64}$$

where $r_E$ is the enzyme radius.

*Approximate Rate of Encounter*

For unchanged molecules, the rate of encounter $k_0$ is given by (Gutfreund, 1972, Hiromi, 1979):

$$k_0 = 4\pi N(D_E + D_S)(r_E + r_S), \text{ in } \text{M}^{-1}\,\text{s}^{-1} \tag{65}$$

where $N$ is Avogadro's number ($6 \times 10^{26}\,\text{kmol}^{-1}$), $D_E$ and $D_S$ are the diffusion coefficients of the enzyme and the substrate in $\text{m}^2/\text{s}$, and $r_E$ and $r_S$ are molecular radii of the enzyme and the substrate in m. Since $E$ is a big molecule and $S$ a small molecule, $D_S$ is much larger than $D_E$, and $r_E$ is much larger than $r_S$. This allows the elimination of the effect of $D_E$ and $r_S$, through the introduction of a correction constant factor equal to 1.25:

$$(D_E + D_S)(r_E + r_S) \approx 1.25\, D_S r_E \tag{66}$$

Substitution of Eq. (66) in Eq. (65) then yields:

$$k_0 \approx 5\pi N D_S r_E \tag{67}$$

For the diffusion coefficient of the substrate, data from the CRC Handbook of

Chemistry and Physics (Weast and Astle, 1985) yield the approximation:

$$D_S \approx 7.3 \times 10^{-9} M_S^{-0.45}, \quad \text{at} \quad 25°C \tag{68}$$

where $M_S$ is the molecular weight of $S$. The elevation of $D_E$ with temperature is roughly 2% to 3% per degree. The enzyme radius is related to the enzyme diffusivity through the Stokes-Einstein relation:

$$D_E = RT/6\pi N r_E \eta \tag{69}$$

For viscosity $\eta$ equal to that of the solvent (water), and using $D_E$ data from the literature (Hiromi, 1979), Eq. (69) yields:

$$r_E \approx 4.5 \times 10^{-11} M_E^{0.38} \tag{70}$$

where $M_E$ is the molecular weight of $E$.
Through substitution of Eqs. (68) and (70) into Eq. (67), $k_0$ can be expressed as an empirical function of the molecular weights $M_E$ and $M_S$:

$$k_0 \approx 3 \times 10^9 M_S^{-0.45} M_E^{0.38}, \quad \text{in} \quad \mathrm{M^{-1}\,s^{-1}} \tag{71}$$

*Resulting Collision Parameter and Relevant Assumptions*

Substitution of Eqs. (64) and (71) into Eq. (63), under the stated assumption that $\rho_S = 1/3$, yields an expression of $k$ as an empirical function of the molecular weights $M_E$ and $M_S$:

$$k \approx 2 \times 10^{10} M_S^{-0.45} M_E^{-0.38}, \quad \text{in} \quad \mathrm{M^{-1}\,s^{-1}} \tag{72}$$

This relation is valid if one assumes that there are no electrostatic effects, the viscosity of the solution is equal to that of water, and the temperature is 25°C. Rough ranges and typical values for some of the parameters discussed, based on the above result and data from Ingraham *et al.* (1983), are shown in Table X. The correlation between $k_i$ and $k_1$, due to the fact that the molecular weight $M_E$ is the same, was taken into account for the estimation of the range of $u_i$ in Table X.

TABLE X

Ranges for parameters pertinent in the maximum-rate methodology

| Quantity | Minimum | Maximum | Typical value |
|---|---|---|---|
| $S_i$ | $5 \times 10^{-6}$ M | $5 \times 10^{-3}$ M | $10^{-4}$ M |
| $M_S$ | 20 | 800 | 200 |
| $M_E$ | $10^4$ | $10^6$ | $10^5$ |
| collision $k_i$ | $6 \times 10^6\ \mathrm{M^{-1}\,s^{-1}}$ | $2 \times 10^8\ \mathrm{M^{-1}\,s^{-1}}$ | $2 \times 10^7\ \mathrm{M^{-1}\,s^{-1}}$ |
| $t_i = 1/k_i S_i$ | $3 \times 10^{-2}$ s | $10^{-6}$ s | $4 \times 10^{-4}$ s |
| $u_i = t_i/t_1$ | $2 \times 10^{-4}$ | $6 \times 10^3$ | 1 |
| $f$ | $10^{-12}$ | 0.99 | 0.01 |
| $e_{\text{total}}$ | $2 \times 10^{-8}$ M | $10^{-4}$ M | $10^{-6}$ M |
| $r/e_{\text{total}}$ | $2\ \mathrm{s^{-1}}$ | $1000\ \mathrm{s^{-1}}$ | $100\ \mathrm{s^{-1}}$ |
| $r_{nd}$ | $10^2$ | $10^9$ | $3 \times 10^5$ |

The numbers are only meant to be used in the investigation of realistic parameter values. The bounds are *not* absolute and the "typical values" are just order-of-magnitude estimates and not statistical averages of observed values.

## 7. NUMERICAL EXAMPLES

In this section, simple examples of the application of this methodology, focusing on a section of the glycolytic pathway shown in Figure 5, are shown.

### *Parameters for Small Molecules*

Estimates for the necessary parameters of the metabolites involved are shown in Table XI. The concentrations for the pathway intermediates in Table XI were taken to be equal to their concentrations in human erythrocytes, provided by Lehninger (1975).

### *Parameters for Enzymes*

The molecular weights of enzymes are often not known, and the molecular weight of each enzyme in the pathway is assumed here to be equal to 40,000, which is probably an underestimate for most enzymes and will result in higher values for the maximum rates. This follows a general strategy: When only a rough range for a parameter is known, the parameter is assumed to have the value that leads to a conservative overestimate of the maximum rate.

The equilibrium constant of each step (Table XII) can be estimated from Gibbs Free-Energy values provided by Lehninger (1975).

### *Results*

Table XII shows the maximum-rate calculations for the analyzed steps of the pathway. To obtain values for the maximum rate itself, values for enzyme concentrations are required. Since these are not known, upper bounds for the specific activities of the enzymes ($r/e_{total}$) were calculated, in units of moles of substrate per mole of enzyme per second. Some other interesting results are worth mentioning:

(a) If the order in which reactants bind to the enzyme is reversed the maximum rate estimate changes by less than 15%. Similarly, if the order in which products

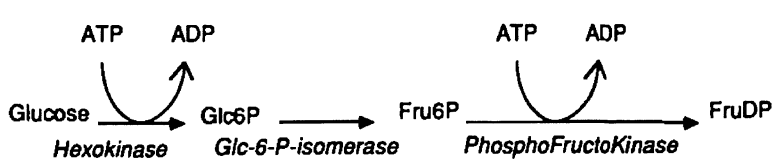


FIGURE 5 A section of the glycolytic pathway.

TABLE XI

Estimation of important parameters for intermediates of the glycolytic pathway

| Intermediate | Mol. weight | Collision $k_i$ ($10^7\ M^{-1}\ s^{-1}$) | Concentration ($\mu M$) | $[k_i S_i]^{-1} = t_i$ ($\mu s$) |
|---|---|---|---|---|
| ATP | 503 | 2.2 | 1850 | 24 |
| ADP | 424 | 2.3 | 138 | 320 |
| Glucose | 180 | 3.5 | 5000 | 6 |
| Glc6P | 258 | 2.9 | 83 | 420 |
| Fru6P | 258 | 2.9 | 14 | 2500 |
| FruDP | 336 | 2.6 | 31 | 1200 |

dissociate is reversed, the rate upper-bound is not severely affected. Considering the other uncertainties in the estimation, the binding order is not expected to have a significant effect on the maximum rate.

(b) If the reaction catalyzed by *hexokinase* is assumed to be irreversible, the constraint relating the kinetic parameters to the equilibrium constant is removed, and a simplified algebraic expression is obtained for the dimensionless maximum rate:

$$r_{nd} = (1 + u_2)^{-1} \tag{73}$$

Application of Eq. (73) yields $r/e_{total} = 3.2 \times 10^4$. This result does not deviate significantly from the result obtained without the irreversibility assumption ($r/e_{total} = 2.9 \times 10^4$). In effect it is quite reasonable to assume that the reaction is irreversible.

Using the results of the calculations, whenever enzyme concentrations are given, the maximum rate for each step and the overall maximum rate for the pathway can be calculated. If, on the other hand, the overall pathway rate is given, the minimum required concentration for each enzyme can be calculated, and possible bottlenecks, where high enzyme concentrations are required, can be predicted.

To compare the simple approach of Eq. (1) to the presented method, one can apply Eq. (1) to Glucose-6P Isomerase. The ratio $r/e_{total}$ is equal to $k[S]$, whose inverse has already been calculated, for glucose-6-phosphate, in the last column of Table XI. Thus, Eq. (1) yields $r/e_{total} = 24 \times 10^2$. The proposed new technique,

TABLE XII

Estimation of maximum-rate parameters for some steps of the glycolytic pathway

| Step | $K_e$ | $f$ | $r_{nd}$ | $r/e_{total}$ ($s^{-1}$) |
|---|---|---|---|---|
| Hexokinase | $8.6 \times 10^2$ | $1.4 \times 10^{-6}$ | 0.17 | $2.9 \times 10^4$ |
| Glucose-6P-isomerase | $5.0 \times 10^{-1}$ | $3.4 \times 10^{-1}$ | 0.65 | $5.3 \times 10^2$ |
| Phosphofructokinase | $3.1 \times 10^2$ | $5.3 \times 10^{-4}$ | 0.89 | $3.6 \times 10^2$ |

however, yields an upper bound ($5.3 \times 10^2$ from Table XII) which is considerably tighter. This is generally true for reactions that are not very far from equilibrium. It occurs because, as suggested in the Introduction and Figure 2(b), the method takes into account thermodynamic limitations; for reactions that approach equilibrium, the method predicts a rate upper-bound approaching zero. Equation (1) has no provisions for thermodynamics and equilibrium.

One might observe that Phosphofructokinase (PFK) has a maximum rate close to that of Glucose-6P Isomerase (Table XII), even though PFK is not as close to equilibrium. However, PFK involves more substrates and products which introduce collision limitations in more steps of the mechanism. As mentioned in the Introduction, these limitations in the mechanism compound and lead to a tighter rate upper-bound for the whole reaction.

A comparison of actual reaction rates to upper bounds has been carried out by Albery and Knowles (1976), using only Eq. (1). They demonstrated that there exist enzymes which attain the upper bound. Since the technique presented here tightens the upper bounds, its results are even more realistic.

## 8. EXTREMA FOR OTHER PARAMETERS

Although the problem was stated as maximization of the rate, the same procedures and formulae also answer the following optimization questions:

(a) What is a lower bound for the substrate concentration $S_i$ that can achieve a desired or observed rate $r$, for given concentrations of the remaining substrates and products $S_k$, $k \neq i$, and given enzyme concentration $e_{\text{total}}$?

(b) What is a lower bound for the enzyme concentration $e_{\text{total}}$ that can achieve a given rate $r$, for given substrate and product concentrations $S_i$?

(c) What is an upper bound for the concentration for a product $S_p$ that can accommodate a given rate $r$, for given concentrations of the remaining products and substrates $S_k$, $k \neq p$, and given enzyme concentration $e_{\text{total}}$? This upper bound for the concentration will normally be quite high, and thus useless, but when the reaction is close to equilibrium reasonable concentrations *will* be obtained.

(d) What is a lower bound for the equilibrium constant $K_e$ that allows a given rate $r$, for given substrate and product concentrations $S_k$, and given enzyme concentration $e_{\text{total}}$? In this case, an obvious minimum is the mass action ratio of the reaction, but this methodology will yield a tighter (i.e. larger) minimum.

Case (b) was already mentioned in the example. Cases (a) and (c) combined can yield both upper and lower bounds of an intermediate in a metabolic pathway, when the rate of the pathway and concentrations of all the other metabolites are given.

For all of these questions, the simple thermodynamic and collision arguments mentioned in the Introduction can provide some answers, but the methodology presented here will always provide better results, i.e., tighter bounds. This was demonstrated, in the example, for Glucose-6P isomerase.

## 9. EXPLOITATION OF ADDITIONAL CONSTRAINTS

Further narrowing of the bounds yielded by the methodology (i.e., increase of the lower bounds and decrease of the upper bounds) can be achieved through the exploitation of partial information on the rate of the reaction. Knowledge on specific activity, Michaelis constants, or reaction rate data under some particular conditions, introduces additional constraints in the maximization. With more constraints, the maximum rate can be reduced further and other parameter bounds accordingly tightened.

### *Constraints on the Maximum Enzyme Turnover*

As an illustration, consider the example two-reactant · two-product ordered mechanism, which was examined in detail in Sections 2 and 3 of this paper. The maximum enzyme turnover, $V_{max}$, of the enzyme can be derived from the general rate Eqs. (7) and (8), by setting $P = 0$, $Q = 0$, $A \to \infty$, and $B \to \infty$:

$$V_{max} = \frac{r}{e_{total}} = \frac{k_3 k_4}{k_3 + k_4} \tag{74}$$

Any equality or inequality constraint on $V_{max}$ yields, through this equation, an additional constraint involving the kinetic parameters. The constraint can be used in the maximization to yield tighter bounds.

### *Constraints on the Michaelis Constant*

To incorporate information on the Michaelis constant of reactant $B$, assume that $P = 0$, $Q = 0$, and $A \to \infty$, to obtain from the rate Eqs. (6) and (7):

$$\frac{r}{e_{total}} = \frac{k_1 k_2 k_3 k_4 B}{(k_3 + k_4) k_1 k_2 B + (k_{-2} + k_3) k_1 k_4} \tag{75}$$

From Eq. (75) it follows that $V_{max}$ is given by Eq. (74), and the Michaelis constant for $B$, $K_m$, by the equation:

$$K_m = \frac{(k_{-2} + k_3) k_4}{(k_3 + k_4) k_2} \tag{76}$$

Thus, as with $V_{max}$, it is possible to express constraints on $K_m$ as constraints among the kinetic parameters, and use them in the optimization.

### *Other Constraints*

Suppose that the *actual* rate is known under some particular concentrations of metabolites, and a maximum rate estimate is desired for some other concentrations. The rate datum is translated into a constraint among kinetic parameters through direct substitution of the related concentrations and the rate datum in Eqs. (7) and (8). Then the constrained maximization is performed using the new concentrations.

The introduction of any additional constraint, especially one stemming from a rate datum, further complicates analytical maximization, and may necessitate numerical application of the whole method. It is nevertheless useful when the data are available, and bounds significantly tighter than yielded by the original method are sought.

## 10. DISCUSSION

The results of the optimization show that in order to obtain the upper bound for the rate of an enzymatic reaction rate parameters for bimolecular steps should be set to their collision limits, while other rate parameters must take either extreme values (such as $+\infty$) or intermediate optimal values. The rate constraints derived by this methodology are always stronger than the collision limits previously known. Furthermore, they reflect thermodynamic considerations, as they predict a maximum rate that approaches zero as the reaction approaches equilibrium. The outlined maximum-rate methodology can alternatively be used to estimate ranges for other parameters affecting the rate of a biochemical reaction, rather than estimating the rate itself.

### *Handling Random-Order Mechanisms*

The restriction of the results to ordered mechanisms is not severe. For some reaction classes the order is known, as is the case with dehydrogenases, for which the coenzyme binds before the other substrate (Dixon and Webb, 1979). Otherwise, the mathematical and numerical analysis of random-order mechanisms can be carried out similarly, except that the expressions are much more complicated. A simpler solution is to assume the random-order maximum to be equal to the maximum among all possible ordered mechanisms. Since the binding of each substrate changes the conformation of the enzyme, even formally random-order mechanisms have a preferred order that accounts for most of the activity of the enzyme. For the purposes of maximum rate analysis, the preferred mechanism can be assumed as fully representative of the order of binding. The examples examined in this paper certainly indicate that the binding-order does not significantly affect the maximum rate. However, it is important to examine all ordered mechanisms when additional constraints stemming from $V_{max}$, Michaelis constants, or other data are exploited in the maximization, because the additional data may be very sensitive to binding order.

### *Applications*

Applying the methodology, biochemists can check whether a proposed pathway or reaction mechanism is thermodynamically feasible and consistent with the observed rates, while biochemical engineers can check whether a desired process performance is feasible. The methodology has already been used in the identification of rate-limiting steps of biochemical pathways (Mavrovouniotis, 1988). An assumption that a step is rate-limiting provides concentration ranges

for intermediates in the pathway. If, under these concentrations, the maximum rate estimate is below the observed rate, the candidate rate-limiting step must be rejected. With respect to the realistic value of the upper bound, i.e., its rough magnitude relative to actual rates, Albery and Knowles (1976) have already demonstrated that there exist enzymes which attain the upper bound.

*Multi-Enzyme Complexes*

When the enzyme catalyzing the reaction is part of a multi-enzyme complex, the effective concentrations of reactants and products may differ drastically from their bulk concentrations in the cell. The substrate and intermediate molecules may be transferred, within the complex, from one enzyme to the other, without visiting the bulk of the solution. Thus, the methodology cannot be applied to an enzyme belonging to a multi-enzyme complex. However, it can be applied to the complex as a whole, for which the assumptions of the methodology hold.

*Active Concentrations of Currency Metabolites*

Another problem where this methodology is pertinent to is the determination of the active concentration of a "currency" metabolite that participates in several bioreactions. Under some conditions a significant fraction of the total metabolite may be bound to enzymes, leaving only the remainder as reactive concentration. It would be interesting to employ the maximum rate methodology to determine the minimum complexed (inactivated) metabolite concentration for given $r$, $e_{total}$, and $S_i$ for a large set of reactions that involve the metabolite. Then one could estimate the remaining active concentration and compare it to the total concentration.

*Metabolic Efficiency and Evolution of Enzymes*

It would be very useful to examine the metabolic efficiency of enzymes, that is, their achieved rate divided by the predicted maximum. Based on this efficiency, enzymes could be classified as *almost perfected* when their efficiency is comparable to 1 (e.g. larger than 0.01 or 0.05), or *imperfect* when the efficiency is much smaller than 1. The level of this efficiency is expected to relate to two important factors:

(a) The intrinsic mechanistic difficulty of the reaction. Some reactions are so hard that the internal chemical barriers always overshadow physical collision factors.

(b) The metabolic importance of the bioreaction. There is stronger evolutionary pressure on enzymes that are more critical for the overall efficiency of a microorganism. Those enzymes are more likely to be *almost perfected.*

## 11. CONCLUSIONS

The novel methodology that was presented allows the estimation of a maximum rate for an enzymatic reaction, provided that the equilibrium constant and the

concentrations of the enzyme and the metabolites are known. It can estimate an extremum for *another* parameter if the actual rate of the reaction is known. The methodology is based solely on physicochemical considerations and can either be applied in the absence of kinetic data or exploit partial kinetic data to produce tighter bounds.

The approach presented here is consistent with the thermodynamics of the enzymatic reaction; it predicts a decrease in the maximum permissible rate as the reaction approaches equilibrium. Simple thermodynamic analysis examines only the reactants and products of a transformation. The presented approach is, conceptually, an extension of thermodynamic analysis to account for the character of enzymatic-reaction mechanisms. However, it depends only on general characteristics and not specific details of the actual reaction mechanism.

The method can be used to predict bounds for parameters when actual experimental data are not available, or to evaluate the feasibility of a postulated enzymatic transformation. Investigation of potential biological regularities involving enzyme efficiency may provide useful insights on the intrinsic reaction difficulty and the evolutionary perfection of enzymes.

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## NOMENCLATURE

| | |
|---|---|
| $\eta$ | solvent viscosity |
| $\rho_S$ | steric factor for a substrate colliding with an enzyme |
| $\rho_E$ | steric factor for an enzyme colliding with a substrate |
| $A$ | a reaction reactant, or concentration of reactant |
| $ADP$ | adenosine diphosphate |
| $ATP$ | adenosine triphosphate |
| $B$ | a reaction reactant, or concentration of reactant |
| $b_i$ | collision-determined upper bound for the kinetic parameter $k_i$ |
| $D$ | rate parameter defined in the paper by Eq. (8) |
| $D_E$ | enzyme diffusion coefficient |
| $D_S$ | substrate diffusion coefficient |
| $E_i$ | the $i^{th}$ enzyme complex (or its concentration) in a reaction mechanism |
| $e_i$ | dimensionless concentration of the $i^{th}$ enzyme complex |
| $e_{total}$ | total enzyme concentration |
| $f$ | the mass action ratio divided by the equilibrium constant |

| | |
|---|---|
| FruDP | fructose 1,6-diphosphate |
| Fru6P | fructose 6-phosphate |
| $G$ | rate parameter defined in the paper by Eq. (17) |
| Glc6P | glucose 6-phosphate |
| $h_i$ | ratio of the forward and backward time constants for the $i^{th}$ step |
| $k$ | collision rate constant of a single-reactant enzymatic reaction |
| $K_e$ | equilibrium constant |
| $k_i$ | rate constant for the $i^{th}$ step of a reaction mechanism |
| $K_m$ | Michaelis constant |
| $k_0$ | encounter constant for substrate-to-enzyme collisions |
| $M_E$ | enzyme molecular weight |
| $m_i$ | ratio of forward and backward rate constants for the $i^{th}$ step in a mechanism |
| $M_S$ | substrate molecular weight |
| $N$ | Avogadro's number ($6.02 \times 10^{26}$ kmol$^{-1}$) |
| $P$ | a reaction product, or concentration of product |
| $Q$ | a reaction product, or concentration of product |
| $R$ | ideal gas constant |
| $r$ | reaction rate |
| $r_{max}$ | maximum rate of a single-reactant irreversible enzymatic reaction |
| $r_{nd}$ | dimensionless reaction rate |
| $S_i$ | the $i^{th}$ metabolite (or its concentration) participating in a reaction |
| $T$ | temperature |
| $t_i$ | time constant for the $i^{th}$ step of a reaction mechanism |
| $u_i$ | dimensionless time constant for the $i^{th}$ step of a reaction mechanism |
| $V_{max}$ | maximum enzyme turnover |

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