Two types of rate-determining step in chemical and biochemical processes

Shiroki YAGISAWA

Faculty of Pharmaceutical Sciences, University of Nagasaki, Bunkyo Machi 1-14, Nagasaki 852, Japan

Close examination of the concept of the rate-determining step (RDS) shows that there are two types of RDS depending on the definition of 'rate'. One is represented by the highest peak of the free-energy diagram of consecutive reactions and holds true where the rate is defined in terms of the concentration of the first reactant. The other is represented by the peak showing the maximum free-energy difference, where the free-energy difference is the height of a peak measured from the bottom of any preceding troughs, where the definition of the rate is in terms of the total reactant concentration including intermediates. There are no criteria *a priori* for selecting one of them.

INTRODUCTION

The rate-determining step (RDS) is an important concept in the study of reaction mechanisms. Interpretation of structure–reactivity relationships, substrate-specificities of enzymes, kinetic isotope effects and various phenomena in metabolic processes requires a knowledge of the RDS of the processes. However, the concept of the RDS is frequently not specified or understood clearly, and failure to analyse the concept adequately often leads to confusion in the description of reaction mechanisms (Jencks, 1969). A common thesis that the RDS is the slowest step of multi-step reactions (Frost & Pearson, 1961), which is the case only under limited conditions, is often used outside the limit. Another thesis that the RDS is the step represented by the highest point in the free-energy diagram (Jencks, 1969), which is correct in principle, has not been effective for describing reactions involving stable intermediates. No rational way has been proposed for describing the RDS of processes involving stable intermediates. For these reasons, a comprehensive understanding of the concept is necessary and will be helpful in complicated discussions of reaction mechanisms.

THEORY

Let us consider a reaction:

and define the RDS as the step a change of which rate constant results in a significant, nearly proportional, change in the rate of the overall reaction defined as:

$$v = d[P]/dt \quad (2)$$

Furthermore, let us suppose that the change of rate constant occurs as a result of a change in the free energy of the corresponding transition state. This supposition means that the rate constants for the forward and reverse

reactions of a step change simultaneously in the same ratio. This is necessary to simplify discussion.

The attainment of a steady state is presumed for all the intermediates in the following discussion. When we consider reactions measured under conditions where the concentration of the first reactant, X_1 , is kept constant by a continuous supply, such as in some metabolic processes, or by regeneration, as in the case of enzymic reactions, arrival at a steady state can be expected for any set of k values. In this sense we can take the following discussion as applicable to any set of k values. However, when we consider the usual experimental procedures, some reactions may reach a steady state immediately or after a certain lag phase but others may not reach one at all. The presence of a lag phase causes no problem for the discussion, but reactions not reaching a steady state lie outside the limit of the present approach.

It also needs to be remembered that the overall rate is expected to be measured at a specified concentration of the reactant or to be corrected for concentration-dependence. In other words, when the rate is expressed as:

$$v = k_{\text{obs.}}[X] \quad (3)$$

where $[X]$ is the concentration of the reactant, it is not the value of v but that of $k_{\text{obs.}}$ that is of primary importance for the definition of the RDS. The value of $k_{\text{obs.}}$ depends on $[X]$.

A presupposition of the present theory is that $[X]$ is often tacitly used to refer to $\Sigma[X_i]$, the total reactant concentration, and not necessarily the concentration of the first reactant X_1 . This may be the case in qualitative discussions of RDS for ordinary reactions that are started with a constant amount of X_1 and not monitored for a decrease of X_1 , and it is obviously true in the discussions of enzyme reactions where the rates expressed as $k_{\text{obs.}}[E]_0$ where $[E]_0$ is the total enzyme concentration are usually considered. The two expressions of the rate:

$$v = k_{\text{obs.}}[X_1] \quad (4)$$

$$v = k'_{\text{obs.}} \Sigma[X_i] \quad (5)$$

lead to quite different conceptions of RDS, which will be called RDS of the first type and the second type respectively. In the present discussion of enzymic reactions, only the interconversion of enzyme species will be considered, the substrate concentration being taken as a constant involved in rate constants. Circulation of the reactants in enzymic reactions causes no difference in the following discussion as compared with linear reactions.

Steady-state conditions for the reaction give:

$$d[X_i]/dt = k_{(i-1)}[X_{i-1}] + k_{-i}[X_{i+1}] - k_{+i}[X_i] - k_{-(i-1)}[X_i] = 0 \quad (i = 2 \text{ to } n-1) \quad (6)$$

$$d[X_n]/dt = k_{+(n-1)}[X_{n-1}] - k_{+n}[X_n] - k_{-(n-1)}[X_n] = 0 \quad (7)$$

These give:

$$k_{+(i-1)}[X_{i-1}] - k_{-(i-1)}[X_i] = k_{+i}[X_i] - k_{-i}[X_{i+1}] \quad (i = 2 \text{ to } n-1) \quad (8)$$

$$k_{+(n-1)}[X_{n-1}] - k_{-(n-1)}[X_n] = k_{+n}[X_n] \quad (9)$$

Eqs. (8) and (9) together with eqn. (10) give eqn. (11):

$$k_{+n}[X_n] = v \quad (10)$$

$$k_{+i}[X_i] - k_{-i}[X_{i+1}] = v \quad (i = 1 \text{ to } n-1) \quad (11)$$

The solution of the simultaneous equation:

$$\begin{pmatrix} k_{+1} & -k_{-1} & & & \\ & k_{+2} & -k_{-2} & & \\ & & k_{+3} & -k_{-3} & \\ & & & \ddots & \\ & & & & k_{+(n-1)} & -k_{-(n-1)} \\ & & & & & k_{+n} \end{pmatrix} \begin{pmatrix} [X_1] \\ [X_2] \\ [X_3] \\ \vdots \\ [X_{n-1}] \\ [X_n] \end{pmatrix} = \begin{pmatrix} v \\ v \\ v \\ \vdots \\ v \\ v \end{pmatrix} \quad (12)$$

derived from eqns. (10) and (11), gives:

$$[X_i] = \left(\frac{1}{k_{+i}} + \frac{k_{-i}}{k_{+i}k_{+(i+1)}} + \dots + \frac{k_{-i}k_{-(i+1)}\dots k_{-(j-1)}}{k_{+i}k_{+(i+1)}\dots k_{+(j-1)}k_j} + \dots + \frac{k_{-i}k_{-(i+1)}\dots k_{-(n-1)}}{k_{+i}k_{+(i+1)}\dots k_{+(n-1)}k_{+n}} \right) \quad (i = 1 \text{ to } n) \quad (13)$$

An important fact to be noted here is that the value of each term in eqn. (13), when considered in its logarithmic form, is proportional (more exactly linearly related) to the height of a peak measured from the level of $[X_i]$ in the free-energy diagram as illustrated in Fig. 1, and then the maximum term in the equation is associated with the maximum free-energy difference measured by this method. This relationship between the terms in eqn. (13) and the free-energy differences is apparent, since in the free-energy diagram based on an equation such as $k = A \cdot \exp(-\Delta G^\ddagger/RT)$ the heights of barriers for forward and reverse reactions are proportional to $\log(1/k) + C$, then the free-energy difference (exemplified in Fig. 1) is equal to the summation (for the forward reactions) and the subtraction (for the reverse reactions) of the $\log(1/k)$ values except for additional constants, which is the value given as the logarithm of the terms in eqn. (13). The terms in eqn. (13) can be interpreted also as the reciprocal of the equilibrium constant (for example $[X_j]/[X_i]$)

multiplied by the rate constant (k_{+j}), which can be readily related to the free-energy diagram. A similar relationship for some special cases has been pointed out (Albery & Knowles, 1976).

For $i = 1$, eqn. (13) gives:

$$v = [X_1] \left(\frac{1}{k_{+1}} + \frac{k_{-1}}{k_{+1}k_{+2}} + \frac{k_{-1}k_{-2}}{k_{+1}k_{+2}k_{+3}} + \dots + \frac{k_{-1}k_{-2}k_{-3}\dots k_{-(n-1)}}{k_{+1}k_{+2}k_{+3}\dots k_{+(n-1)}k_{+n}} \right) \quad (14)$$

This is an expression of v as a function of $[X_1]$ corresponding to eqn. (4). When one of the terms in the denominator of eqn. (14) is much larger than the sum of all others, the equation can be approximated by:

$$v = [X_1] \cdot \left(\frac{k_{+1}k_{+2}k_{+3}\dots k_{+(r-1)}k_{+r}}{k_{-1}k_{-2}k_{-3}\dots k_{-(r-1)}} \right) \quad (15)$$

where k_{+r} is the rate constant of the step corresponding to the highest peak of the energy diagram according to the preceding discussion of eqn. (13). When measured from the level of $[X_1]$, the maximum free-energy difference is always achieved at the peak showing the maximum free energy of the diagram. Eqn. (15) indicates that the

step represented by k_{+r} is the RDS in the present definition, since the value of v changes in proportion to k_{+r} , whereas the changes in other k values, cancelled in the denominator and the numerator, do not affect the value of v . This also indicates that the reaction proceeds as if the steps from X_1 to X_{r-1} are at equilibrium and only the final k_{+r} step is not at equilibrium. This situation has often been used as a condition for defining the r step as the RDS.

Summing up of the equations represented by eqn. (13)

Fig. 1. The value of a term in eqn. (13) depicted on the free-energy diagram

The arrow shows the free-energy difference represented by the third term in eqn. (13).

for $i = 1$ to n leads to eqn. (16), a second-type rate equation:

$$v = \left(\sum_{i=1}^n [X_i] \right) \left/ \sum_{i=1}^n \sum_{j=i}^n \frac{k_{-i} k_{-(i+1)} k_{-(i+2)} \dots k_{-(j-1)}}{k_{+i} k_{+(i+1)} k_{+(i+2)} \dots k_{+(j-1)} k_{+j}} \right. \quad (16)$$

The summation with respect to j in eqn. (16) is simply a condensed expression of eqn. (13) (excluding v) giving $1/k_{+j}$ ($= 1/k_{+i}$) for $j = i$. The double summation with respect to i and j gives $n(n+1)/2$ terms, each represented by one of the $n(n+1)/2$ energy differences given by the principle in Fig. 1 for varied i and j ($i \leq j$).

If one of the terms in the denominator is much larger than all of the others, i.e. if one of the energy differences on the energy diagram dominates all others, eqn. (16) can be approximated by:

$$v = \left(\sum_{i=1}^n [X_i] \right) \cdot \frac{k_{+p} k_{+(p+1)} k_{+(p+2)} \dots k_{+(q-1)} k_{+q}}{k_{-p} k_{-(p+1)} k_{-(p+2)} \dots k_{-(q-1)}} \quad (17)$$

where k_{+p} and k_{+q} are defined by the trough and the peak showing the largest difference on the energy diagram. With the same reasoning employed for eqn. (15), it is obvious that the step represented by k_{+q} in eqn. (17) is the RDS for the second-type definition of the rate, and X_p represents the predominant intermediate.

Fig. 2 illustrates the two types of RDS, each represented by the free-energy maximum and the maximum free-energy difference. They can be different steps for reactions in which an intermediate has a free-energy lower than the initial reactant, although they are the same for the reactions without stable intermediates.

The existence of two different RDSs for a single reaction might seem somewhat strange. An interpretation for this is that the k value variation in the process for determining the RDS affects $[X_i]$, and this effect is corrected only in the first-type definition. This can be seen by considering some simple cases.

DISCUSSION

RDS, a very convenient concept, is used with a variety of meanings. It is often used just as a synonym of the slowest step. The purpose of the present paper is not to criticize such uses of the term, but to help researchers using the term with its rigorous meaning through a comprehensive understanding of the concept. The dis-

inction of the two types of RDSs derived by the present theory is necessary and useful for discussing reaction mechanisms in terms of RDS.

Use of two RDSs

There is no criterion *a priori* for selecting one of the two RDSs. For reactions without accumulation of intermediates, either of the two RDS can be used, since they give the same result. But, for reactions accompanied by accumulation of intermediates, the two types of RDS can be used depending on the types of reaction. The first-type RDS, with the principle of free-energy maximum, is effective for describing reactions in which $[X_i]$, the concentration of the initial reactant, is monitored or keeps constant, as in some metabolic processes. The first-type RDS can be applied even when the reactions are accompanied by the accumulation of intermediates, since in the derivation of the principle for the first-type RDS no limit was set to the amounts of the intermediates. This is an important result of the present theory.

The second-type RDS, with the principle of maximum free-energy difference, is effective for describing reactions in which the total reactant concentration is kept constant, as in enzymic reactions, or the concentration of the reactant is taken as $[X]_0 - [P]$, where $[X]_0$ is the initial reactant concentration, without distinguishing the intermediates from the reactant. The problem of identifying RDS on free-energy diagrams for enzymic reactions has been managed with the use of the first-type RDS by selecting a particular reactant as the predominant one and applying the principle of the free-energy maximum to the subsequent reaction steps (Hegazi, 1978). This procedure, however, is unsatisfactory in two ways. First, there is no rational way to designate the predominant reactant, which is not necessarily represented by the lowest free-energy trough. Secondly, the procedure is self-contradictory, since, as noted above, the principle of free-energy maximum can be applied in its original form even in the presence of stable intermediates. This type of problem can be solved easily by applying the second-type RDS, with the principle of maximum free-energy difference, taking into account the change of the free-energy profile by the substrate concentration. Sometimes, RDSs for kinetic parameters representing a part, rather than the whole, of sequential reactions such as $k_{cat.}$ and $k_{cat.}/K_m$ in enzymic reactions (Fersht, 1977) are also considered. These RDSs also can be discussed in terms of two types of RDS.

RDSs in the reverse reactions

The principle that the RDS is the same in the forward and the reverse reactions has been used in the discussion of reaction mechanisms. This holds for the first-type RDS, since there is only one maximum in a diagram, but it does not hold for the second-type RDS, because the free-energy difference is measured from a trough to a peak along the progress of the reaction, and the peak giving the maximum free-energy difference is not necessarily the same in the two directions. This, however, does not contradict the thermodynamic requirement that factors not affecting the equilibrium should change the rates of the forward and the reverse reactions in the same ratio, because the rates to be considered in this context are those of the first definition ($k[X_i]$ and $k[P]$), and then the thesis should be applied only to the first-type RDS.

Fig. 2. The two types of RDS illustrated on the free-energy diagram

X_p represents the predominant intermediate.

Fig. 3. The slowest-step principle illustrated on the free-energy diagrams

(a) The thesis does not hold for the reaction with a high-energy intermediate, but it holds if the steps for k_{-3} and k_{-4} are considered as a single step, k'_{-3} . (b) It holds for the second-type RDS of irreversible consecutive reactions.

Examination of the slowest-step principle

In the light of the present theory the thesis that the RDS is the slowest step can be examined more closely. The thesis fails when breakdown of a high-energy intermediate is the RDS. For example, the RDS for the free-energy diagram shown in Fig. 3(a) is the step represented by k_{-4} for both types of RDS definition, whereas the slowest step that is associated with the

largest barrier is that represented by k_{-3} . Therefore the thesis holds in the cases where reactions involve only intermediates with free energies similar to or lower than that of the starting material, or where only the conversion between them is considered, taking a series of steps involving high-energy intermediates as one step as shown in Fig. 3(a). In this case the thesis holds always for the second-type RDS and holds for the first-type RDS when the reaction does not involve intermediates with free energies lower than that for the starting material. Another case where the thesis holds is an irreversible consecutive reaction shown as:

Here, the thesis holds for the second type of RDS, but not for the first type, as shown in Fig. 3(b).

Useful equations in kinetic studies

Finally I call attention to eqn (19), a generalized form of eqn. (3), which can be derived from eqn. (13):

$$v = k_{\text{obs.}}[X_i] \quad (19)$$

Eqn. (19), in which the term $k_{\text{obs.}}$ can be related to the free-energy diagram according to the principle shown in Fig. 1 and involves only k values suffixed from i to n , but not those suffixed from 1 to $i-1$, will be useful in discussing reaction mechanisms. The equation holds regardless of the presence or the absence of RDS.

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