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Effective Rate Constants and General Isotope Effect Equations for Steady State Enzymatic Reactions with Multiple Isotope-Sensitive Steps[1]

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In the theoretical treatment of apparent isotope effects corresponding to one-step perturbations, the nonisotopic rate constants are commonly collected as commitments. Such a collection is not possible where a multistep perturbation occurs. However, the multistep perturbations can be treated if the nonisotopic rate constants are collected as effective rate constants, each of which is defined as the rate constant of a kinetic step times the overall equilibrium constant of the steps preceding that kinetic step. If all of the steps are isotope-sensitive, the isotopic equation, written as a ratio of the apparent isotope effect to the overall rate, will be simply a sum of the ratios of intrinsic isotope effect to effective rate constant from all of the steps. Isotopic equations for reactions in which not all of the steps are isotope-sensitive are then obtained by setting the intrinsic kinetic isotope effects and the equilibrium isotope effects of the isotope-insensitive steps to equal one. Designed for treatment of isotope effects derived from single isotopic labelings, the method can also be extended to isotope effects from multiple isotopic labelings. Perturbation of kinetic barriers by equilibrium isotope effects is also discussed.

Isotope effects have been extensively used as probes of enzyme kinetics (*1, 2*) and mechanisms (*3–6*), and, more recently, to investigate quantum mechanical tunneling in hydrogen transfer reactions (*7, 8*). Depending on how many kinetic steps are sensitive to an isotopic substitution, an isotope effect may fall into one of the categories: the isotope effects corresponding to one-step perturbations and those corresponding to multistep perturbations (*3*). Early studies have been mainly focused on the isotope effects corresponding to the one-step perturbations and general isotopic equations for treatment of such isotope effects are well formulated (*5*). In contrast, few experiments (see Ref. (*9*)) and no theoretical analysis have been performed on the isotope effects corresponding to multistep perturbations, although many isotope effects can be categorized in this type.

In the formulation of general isotopic equations of one-step perturbations, the rate constants are commonly collected as commitments (*5*), which allows expression of a simple relationship between the apparent and the intrinsic isotope effects. When two or more steps are isotope-sensitive, such a collection can no longer be

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carried out since a commitment to one isotope-sensitive step cannot be simultaneously a commitment to another. However, when the rate constants are grouped as "effective rate constants," an isotopic equation can be easily formulated whether the apparent isotope effect corresponds to a one-step or a multistep perturbation.

EFFECTIVE RATE CONSTANTS AND GENERAL ISOTOPIC EQUATIONS

Effective rate constants and general isotopic equations of V/K systems. For a simple four-step, linear reaction mechanism,

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} I_1 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} I_2 \underset{k_6}{\overset{k_5}{\rightleftharpoons}} I_3 \xrightarrow{k_7} E + P, \qquad [1]$$

under the V/K condition, it can be shown that

$$\frac{1}{(V/K)} = \frac{1}{k_1} + \frac{1}{k_3K_1} + \frac{1}{k_5K_1K_3} + \frac{1}{k_7K_1K_3K_5}, \qquad [2]$$

where $K_1 = k_1/k_2$, $K_3 = k_3/k_4$, and $K_5 = k_5/k_6$. There are four kinetic terms in Eq. [2]. Each of the kinetic terms corresponds to an energy barrier of a kinetic step in the mechanism (*10*) and, thus, determines the *effective* rate of the transformation of that step. Defining the denominator of a kinetic term as an effective rate constant, r_i, corresponding to step i, we may rewrite Eq. [2] as

$$\frac{1}{(V/K)} = \frac{1}{r_1} + \frac{1}{r_2} + \frac{1}{r_3} + \frac{1}{r_4}. \qquad [3]$$

In general, it can be shown that

$$\frac{1}{(V/K)} = \sum_{i=1}^{n} \frac{1}{r_i}, \qquad [4]$$

where n is the total number of steps in the reaction. An effective rate constant of a step in a V/K system is the forward rate constant of that step times the overall equilibrium constant of the steps preceding that step,[2] or

$$r_i = k_jK_{(j-2)}K_{(j-4)}K_{(j-6)} \cdots \qquad [5]$$

where $j = 2i - 1$.

The definition of effective rate constants leads to a simple way for formulation of general isotopic equations. If all of the four steps in the reaction given by Eq.

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[2] The effective rate constant of the first step is always the microscopic rate constant itself ($r_1 = k_1$), since no equilibrium exists before step 1.

[1] are perturbed by an isotopic substitution, the apparent isotope effect will be governed by the isotopic equation[3]

$$\frac{^{p}(V/K)}{(V/K)} = \frac{^{p}k_1}{k_1} + \frac{^{p}k_3{}^{p}K_1}{k_3K_1} + \frac{^{p}k_5{}^{p}K_1{}^{p}K_3}{k_5K_1K_3} + \frac{^{p}k_7{}^{p}K_1{}^{p}K_3{}^{p}K_5}{k_7K_1K_3K_5}, = \frac{^{p}r_1}{r_1} + \frac{^{p}r_2}{r_2} + \frac{^{p}r_3}{r_3} + \frac{^{p}r_4}{r_4} \quad [6]$$

in which the superscript p represents D (deuterium), T (tritium), 13 (^{13}C), 15 (^{15}N), 18 (^{18}O), etc., and ^{p}k is an intrinsic kinetic isotope effect ($^{D}k = k/k_D$, for example) and ^{p}K an equilibrium isotope effect. In general, an isotopic equation for a n step V/K system can be expressed as

$$\frac{^{p}(V/K)}{(V/K)} = \sum_{i=1}^{n} \frac{^{p}r_i}{r_i}. \quad [7]$$

It should be noted that although an isotope effect is always a ratio of the nonisotopic parameter over the isotopic one, such as $^{p}(V/K) = (V/K)/(V/K)_p$, $^{p}r_1 = r_1/(r_1)_p$, $^{p}k_1 = k_1/(k_1)_p$, and so on, an isotopic equation may take different forms provided that it defines the correct relationship between the apparent isotope effect and the intrinsic isotope effects. For example, Eq. [7] can also be written in the form of the apparent isotope effect: $^{p}(V/K) = (V/K) \sum_{i=1}^{n} \frac{^{p}r_i}{r_i}$. But the inherent relationship between the apparent isotope effect and the intrinsic isotope effects does not change.

Verification of the general isotopic equation. Equation [6] can be justified by noting the fact that it can be transformed into the rate equation for the isotopic substrate only. For example, for reaction [1], the overall rate with a carbon-13 labeled substrate under V/K conditions can be expressed according to Eq. [2] as

$$\frac{1}{(V/K)_{13}} = \frac{1}{(k_1)_{13}} + \frac{1}{(k_3)_{13}(K_1)_{13}} + \frac{1}{(k_5)_{13}(K_1)_{13}(K_3)_{13}} + \frac{1}{(k_7)_{13}(K_1)_{13}(K_3)_{13}(K_5)_{13}}. \quad [8]$$

This equation is equivalent to

$$\frac{(V/K)}{(V/K)_{13}(V/K)} = \frac{k_1}{(k_1)_{13}k_1} + \dots \quad [9]$$

However, since $(V/K)/(V/K)_{13} = {}^{13}(V/K)$, $k_1/(k_1)_{13} = {}^{13}k_1$, etc., Eq. [9] may be expressed as

$$\frac{^{13}(V/K)}{(V/K)} = \frac{^{13}k_1}{k_1} + \frac{^{13}k_3{}^{13}K_1}{k_3K_1} + \frac{^{13}k_5{}^{13}K_1{}^{13}K_3}{k_5K_1K_3} + \frac{^{13}k_7{}^{13}K_1{}^{13}K_3{}^{13}K_5}{k_7K_1K_3K_5}. \quad [10]$$

This equation is essentially identical to Eq. [6].

While the above argument is mathematically correct, it is curious, however, how physically the rate expression for an isotopic substrate alone correlates to the

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[3] Nonisotopic parameters will not be subscripted in this paper.

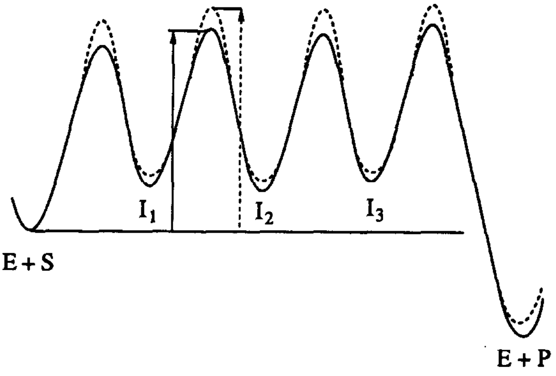


FIG. 1. Schematic free energy profiles for the reaction defined by Eq. [1] (see text) with carbon-12 (—) and carbon-13 (---) substrates. All four steps in the reaction are assumed to be sensitive to the carbon-13 labeling. The arrow with solid line represents the kinetic energy barrier for the second step of the reaction with the carbon-12 substrate ($(\Delta G_2^\ddagger)_{12}$) while the arrow with dashed line represents the barrier of the same step of the reaction with the carbon-13 substrate ($(\Delta G_2^\ddagger)_{13}$).

apparent isotope effect. Let's examine Eq. [8] more closely. In the terms of effective rate constants, Eq. [8] may be written as

$$\frac{1}{(V/K)_{13}} = \frac{1}{(r_1)_{13}} + \frac{1}{(r_2)_{13}} + \frac{1}{(r_3)_{13}} + \frac{1}{(r_4)_{13}}. \quad [11]$$

It has been noted that each of the kinetic terms on the right side of Eq. [11] represents an energy barrier of the corresponding kinetic step (*10*). In fact, the energies of steps 1, 2, 3, and 4 can be calculated, respectively, by

$$(\Delta G_1^\ddagger)_{13} = RT\ln\frac{1}{(r_1)_{13}} + C \quad [12a]$$

$$(\Delta G_2^\ddagger)_{13} = RT\ln\frac{1}{(r_2)_{13}} + C \quad [12b]$$

$$(\Delta G_3^\ddagger)_{13} = RT\ln\frac{1}{(r_3)_{13}} + C \quad [12c]$$

and

$$(\Delta G_4^\ddagger)_{13} = RT\ln\frac{1}{(r_4)_{13}} + C, \quad [12d]$$

where R is the gas constant, T the temperature, and C a constant. Further, each of the energy barriers can be decomposed into two energy terms: the energy barrier for the carbon-12 substrate and the energy difference between the barriers for the carbon-13 and carbon-12 substrates. For example, the second energy barrier (see Fig. 1) can be expressed as

$$\begin{aligned}(\Delta G_2^{\ddagger})_{13} &= RT\ln\frac{1}{r_2} + \left[RT\ln\frac{1}{(r_2)_{13}} - RT\ln\frac{1}{r_2}\right] + C \\ &= RT\ln\frac{1}{r_2} - RT\ln\frac{(r_2)_{13}}{r_2} + C \\ &= RT\ln\frac{1}{r_2} + RT\ln{}^{13}r_2 + C \\ &= RT\ln\frac{{}^{13}r_2}{r_2} + C.\end{aligned} \tag{13}$$

Comparing Eq. [13] with Eq. [12b], we arrive at

$$\frac{1}{(r_2)_{13}} = \frac{{}^{13}r_2}{r_2}. \tag{14}$$

This result is identical to that given by Eqs. [8] and [10].

Effective rate constants and general isotopic equations of V systems. The V system of the reaction defined by Eq. [1] is more complex than the V/K system. The physical connections between the effective rate constants and the corresponding steps are not as straightforward. It is therefore desirable to analyze the V system in some more detail.

A kinetic step of a V/K system can have only one energy barrier (*10*). In contrast, a kinetic step of a V system may have two or more energy terms. The reason for this discrepancy is as follows. In the V/K system of the reaction defined by Eq. [1], the enzyme complexes I_1, I_2, and I_3 do not exist in any significant amount compared to that of the free enzyme and substrate. As a result, the energy barriers of the four steps have only one common ground state, E + S (*10*). We define such a reaction as a *simple* reaction. The reaction of the V system, however, is more complex since the enzyme complexes I_1, I_2, and I_3 exist simultaneously in significant amounts. If the reaction of each of the three enzyme complexes is considered as a simple reaction, the reaction of the V system can be decomposed into three simple reactions (Fig. 2). The first reaction starts from I_1, the second from I_2, and the third from I_3, and the three rates are calculated (in the same way as for the V/K system), respectively, by

$$\frac{1}{V_1} = \frac{1}{k_3} + \frac{1}{k_5K_3} + \frac{1}{k_7K_3K_5} \tag{15a}$$

$$\frac{1}{V_2} = \frac{1}{k_5} + \frac{1}{k_7K_5} \tag{15b}$$

$$\frac{1}{V_3} = \frac{1}{k_7}. \tag{15c}$$

The energy barriers of each of the simple reactions also share only one common ground state, and the relative levels of the three ground states, corresponding to the three simple reactions, are determined by the equilibrium constants of the equilibriums connecting the three ground states (Fig. 2). Because of this complex-

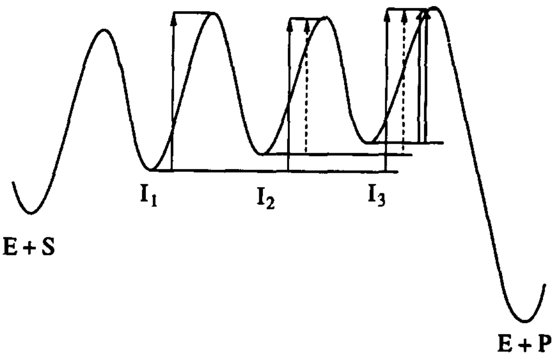


FIG. 2. The energy barriers involved in the three simple reactions of the V system (see text). The arrows with solid line represent the energy barriers of the reaction of I_1, those with dashed line represent the barriers of reaction of I_2, and the one with double lines represents that of the reaction of I_3.

ity, a single step involved in the V system may have two or more energy terms. As shown in Fig. 2, the first step has one energy term, but the second step has two and the third has three. Therefore, the rate of the whole V system may be written as

$$\frac{1}{V} = \left(\frac{1}{k_3}\right)_2 + \left(\frac{1}{k_5} + \frac{1}{k_5K_3}\right)_3 + \left(\frac{1}{k_7} + \frac{1}{k_7K_5} + \frac{1}{k_7K_3K_5}\right)_4, \qquad [16]$$

with the subscripts to the parentheses being designated to the steps in the complex reaction.

If all of the steps in the V system are isotope-sensitive, the apparent isotope effect will be given by

$$\frac{{}^{\mathrm{p}}V}{V} = \left(\frac{{}^{\mathrm{p}}k_3}{k_3}\right)_2 + \left(\frac{{}^{\mathrm{p}}k_5{}^{\mathrm{p}}K_3}{k_5K_3} + \frac{{}^{\mathrm{p}}k_5}{k_5}\right)_3 + \left(\frac{{}^{\mathrm{p}}k_7{}^{\mathrm{p}}K_3{}^{\mathrm{p}}K_5}{k_7K_3K_5} + \frac{{}^{\mathrm{p}}k_7{}^{\mathrm{p}}K_5}{k_7K_5} + \frac{{}^{\mathrm{p}}k_7}{k_7}\right)_4. \qquad [17]$$

The isotopic equation can be verified in the same way as used for the verification of the V/K system. The reaction defined by Eq. [1] has only one irreversible sequence. But the same rules apply to reactions with more irreversible sequences.

Perturbation of kinetic energy barriers by an equilibrium isotope effect. The kinetic aspects of intrinsic kinetic isotope effects have been extensively discussed (*11, 12*). In contrast, the kinetic properties of equilibrium isotope effects have not been systematically analyzed. The analysis of multistep perturbations given above provides a unique opportunity for a discussion on the kinetic consequences of equilibrium isotope effects.

Common to both the V and the V/K systems, an equilibrium isotope effect will not perturb the step where the isotope effect is derived from. Whereas, all of the steps following that step will be perturbed. For example, in the V/K system of reaction [1], as shown by Eq. [6], ${}^{\mathrm{p}}K_1$ does not perturb the first step from which it physically arises, but it perturbs steps 2, 3, and 4. A pictorial representation of

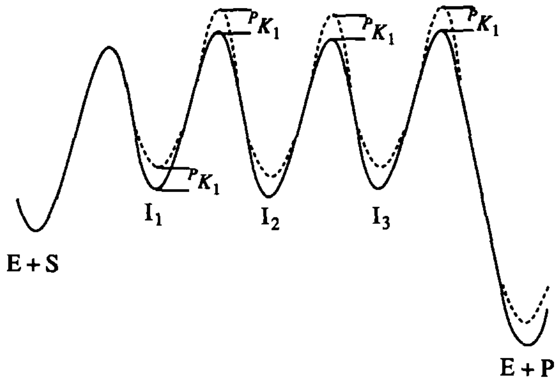


FIG. 3. The free energy profiles of reactions with nonisotopic (—) and isotopic (---) substrates. Except for the equilibrium isotope effect derived from step 1, all of the intrinsic isotope effects are equal to one. As shown, the equilibrium isotope effect derived from step 1 cannot perturb step 1 but inevitably perturbs steps 2, 3, and 4.

the effect of $^{P}K_1$ on these kinetic barriers is given by Fig. 3. The same pattern applies to $^{P}K_3$ and $^{P}K_5$. Similarly, in the V system (Eq. [17]), $^{P}K_3$, derived from step 2, perturbs steps 3 and 4 but not step 2. $^{P}K_5$, derived from step 3, perturbs only step 4. However, for the V system, the degree to which a step can be perturbed by an equilibrium isotope effect depends on the magnitudes of the equilibrium constants of preceding steps. For example, if the second step is the only isotope-sensitive step and the third step is rate-limiting, Eq. [17] becomes

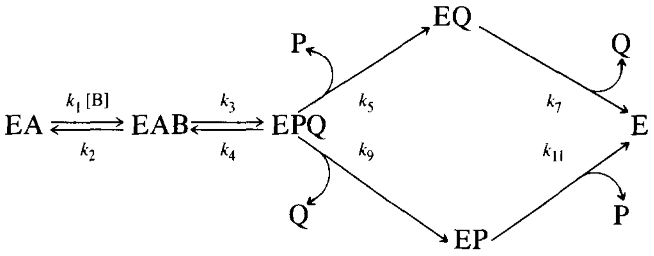
$$\frac{^{P}V}{V} = \left(\frac{^{P}K_3}{k_5K_3} + \frac{1}{k_5}\right)_3 \qquad [18a]$$

or

$$^{P}V = \frac{^{P}K_3 + K_3}{1 + K_3}. \qquad [18b]$$

Equations [18a] and [18b] are derived based on a mechanism in which all of the steps are reversible. Nevertheless, the expression of $^{P}K_3$ can be attenuated by K_3 significantly. Assume $^{P}K_3 = 1.2$, and $K_3 = 1$, indicative of a quite reversible step. The value of Eq. [18b] will be 1.1 rather than 1.2, which is a 50% attenuation of the intrinsic equilibrium isotope effect. Of course, if K_3 is large so that step 2 becomes irreversible, the equilibrium isotope effect will not perturb the overall rate at all. The reason that this phenomenon may happen in V systems is that a step in a V system may have two or more energy barriers, as discussed previously, and which barrier is dominant in that step depends on the equilibrium constants of the preceding steps. A kinetic step of a V/K system has only one energy term and, therefore, there cannot be such a dependence.

Isotopic equations of random bisubstrate reactions. All of the discussion as presented so far has been devoted to linear, ordered, reaction mechanisms. For random mechanisms, the general forms of Eqs. [7] and [10] are reserved in the isotopic equations, but the behavior of the intrinsic isotope effects involved in the branches is different from those within the linear segment. The random mechanism given by Eq. [15] from Cleland (*13*) will be discussed here as an example. The mechanism is rewritten below:



[19]

where A and B are substrates and P and Q are products. For the *V*/*K* system, the kinetic equation is given by

$$\frac{1}{(V/K)} = \frac{1}{r_1} + \frac{1}{r_2} + \frac{1}{r_3}, \qquad [20]$$

where r_1 and r_2 are the same as those in Eq. [3], but

$$\frac{1}{r_3} = \frac{1}{(k_5 + k_9)K_1K_3}. \qquad [21]$$

If all of the steps in this mechanism are isotope-sensitive, the isotopic equation of the *V*/*K* system is given by

$$\frac{{}^{\mathrm{P}}(V/K)}{(V/K)} = \frac{{}^{\mathrm{P}}r_1}{r_1} + \frac{{}^{\mathrm{P}}r_2}{r_2} + \frac{{}^{\mathrm{P}}r_3}{r_3}, \qquad [22]$$

where

$${}^{\mathrm{P}}r_3 = {}^{\mathrm{P}}(k_5 + k_9){}^{\mathrm{P}}K_1{}^{\mathrm{P}}K_3 \qquad [23]$$

in which ${}^{\mathrm{P}}(k_5 + k_9)$ is the ratio of the sum of the rate constants, $(k_5 + k_9)$, of the lighter isotope reaction over that of the heavier isotope reaction, and it is given by

$${}^{\mathrm{P}}(k_5 + k_9) = \frac{\frac{k_5}{k_9}{}^{\mathrm{P}}k_5}{\frac{k_5}{k_9} + {}^{\mathrm{P}}k_5} + \frac{\frac{k_9}{k_5}{}^{\mathrm{P}}k_9}{\frac{k_9}{k_5} + {}^{\mathrm{P}}k_9}. \qquad [24]$$

This equation indicates that the extent to which an intrinsic isotope effect on a step after the branching point can express in the apparent isotope effect depends on the relative values of k_5 and k_9, the rate constants which determine the relative fluxes of the branches. The larger the rate constant is, the higher the expression of the intrinsic isotope effect on that rate constant will be.

In the V system of the random reaction, the kinetic equation may be written as

$$\frac{1}{V}=\left(\frac{1}{k_3}\right)_2+\left(\frac{1}{(k_5+k_9)K_3}+\frac{1}{(k_5+k_9)}\right)_3+\left(\frac{1}{(k_5+k_9)k_7/k_5}+\frac{1}{(k_5+k_9)k_{11}/k_9}\right)_4 \qquad [25]$$

and the general isotopic equation is then given by

$$\frac{{}^{\mathrm{p}}(V)}{V}=\left(\frac{{}^{\mathrm{p}}k_3}{k_3}\right)_2+\left(\frac{{}^{\mathrm{p}}(k_5+k_9){}^{\mathrm{p}}K_3}{(k_5+k_9)K_3}+\frac{{}^{\mathrm{p}}(k_5+k_9)}{(k_5+k_9)}\right)_3$$

$$+\left(\frac{{}^{\mathrm{p}}(k_5+k_9){}^{\mathrm{p}}k_7/{}^{\mathrm{p}}k_5}{(k_5+k_9)k_7/k_5}+\frac{{}^{\mathrm{p}}(k_5+k_9){}^{\mathrm{p}}k_{11}/{}^{\mathrm{p}}k_9}{(k_5+k_9)k_{11}/k_9}\right)_4. \qquad [26]$$

APPLICATION OF THE GENERAL ISOTOPIC EQUATIONS

Generally, the procedure for deriving an isotopic equation may be separated into two steps: (a) deriving a general isotopic equation assuming all of the steps are isotope-sensitive and (b) reducing the general isotopic equation for situations where not all of the steps are isotope-sensitive. Of course one can combine the two steps together once he or she becomes familiar with the method.

Application of the general isotopic equations for reactions involving single isotopic labelings. Given a general isotopic equation for a kinetic mechanism, isotopic equations for different multistep perturbations can be readily derived. For example, for the V/K system given by the reaction [1], if k_3, k_4, k_5, and k_6, namely, steps 2 and 3, are isotope-sensitive, Eq. [6] reduces to

$$\frac{{}^{\mathrm{p}}(V/K)}{(V/K)}=\frac{1}{k_1}+\frac{{}^{\mathrm{p}}k_3}{k_3K_1}+\frac{{}^{\mathrm{p}}k_5{}^{\mathrm{p}}K_3}{k_5K_1K_3}+\frac{{}^{\mathrm{p}}K_3{}^{\mathrm{p}}K_5}{k_7K_1K_3K_5}. \qquad [27]$$

Dividing both sides of Eq. [27] by $1/(V/K)$, dropping the common term k_1, and rearranging, we obtain

$${}^{\mathrm{p}}(V/K)=\frac{{}^{\mathrm{p}}k_3+\dfrac{k_3}{k_2}+\dfrac{k_4}{k_5}{}^{\mathrm{p}}k_5{}^{\mathrm{p}}K_3+\dfrac{k_4k_6}{k_7k_5}{}^{\mathrm{p}}K_3{}^{\mathrm{p}}K_5}{1+\dfrac{k_3}{k_2}+\dfrac{k_4}{k_5}+\dfrac{k_4k_6}{k_7k_5}}. \qquad [28]$$

If only k_3 and k_4 are isotope-sensitive, Eq. [28] further reduces to

$${}^{\mathrm{p}}(V/K)=\frac{{}^{\mathrm{p}}k_3+\dfrac{k_3}{k_2}+\dfrac{k_4}{k_5}\left(1+\dfrac{k_6}{k_7}\right){}^{\mathrm{p}}K_3}{1+\dfrac{k_3}{k_2}+\dfrac{k_4}{k_5}\left(1+\dfrac{k_6}{k_7}\right)}=\frac{{}^{\mathrm{p}}k+C_{\mathrm{f}}+C_{\mathrm{r}}{}^{\mathrm{p}}K_{\mathrm{e}}}{1+C_{\mathrm{f}}+C_{\mathrm{r}}}, \qquad [29]$$

where ${}^{\mathrm{p}}k$ is the intrinsic kinetic isotope effect, ${}^{\mathrm{p}}K_{\mathrm{e}}$ the equilibrium isotope effect, and C_{f} and C_{r} are forward and reverse commitments [5], respectively.

For demonstration of the application for V systems, the reaction mechanism given by Eq. [5] from Northrop [12] will be used because it contains more than one irreversible sequence and is thus more general. This reaction mechanism is rewritten below:

$$E_1 + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E_2 \xrightarrow{k_3} E_3 \xrightarrow{k_5} E_4 \underset{k_8}{\overset{k_7}{\rightleftharpoons}} E_5 \xrightarrow{k_9} E_6 \xrightarrow{k_{11}} E_7 \xrightarrow{k_{13}} E_1 + P \qquad [30]$$

Using the method of effective rate constants, we have

$$\frac{1}{V} = \frac{1}{k_3} + \frac{1}{k_5} + \frac{1}{k_7} + \frac{1}{k_9 K_7} + \frac{1}{k_9} + \frac{1}{k_{11}} + \frac{1}{k_{13}}. \qquad [31]$$

If k_7, k_8, and k_9 are isotope-sensitive,

$${}^{\mathrm{P}}V = \frac{{}^{\mathrm{P}}k_7 + k_7\left(\frac{1}{k_3} + \frac{1}{k_5} + \frac{1}{k_{11}} + \frac{1}{k_{13}}\right) + \frac{k_7 + k_8{}^{\mathrm{P}}K_7{}^{\mathrm{P}}K_9}{k_9}}{1 + k_7\left(\frac{1}{k_3} + \frac{1}{k_5} + \frac{1}{k_9} + \frac{1}{k_{11}} + \frac{1}{k_{13}}\right) + \frac{k_8}{k_9}}. \qquad [32]$$

If only k_7 and k_8 are isotope-sensitive and the equilibrium isotope effect equals unity, Eq. [32] further reduces to

$${}^{\mathrm{P}}V = \frac{{}^{\mathrm{P}}k_7 + k_7\left(\frac{1}{k_3} + \frac{1}{k_5} + \frac{1}{k_9} + \frac{1}{k_{11}} + \frac{1}{k_{13}}\right) + \frac{k_8}{k_9}}{1 + k_7\left(\frac{1}{k_3} + \frac{1}{k_5} + \frac{1}{k_9} + \frac{1}{k_{11}} + \frac{1}{k_{13}}\right) + \frac{k_8}{k_9}} = \frac{{}^{\mathrm{P}}k_7 + R_{\mathrm{f}} + C_{\mathrm{r}}}{1 + R_{\mathrm{f}} + C_{\mathrm{r}}}, \qquad [33]$$

which is identical to Eq. [6] from Northrop (*12*) for the same reaction mechanism.

Application of the general isotopic equations for reactions involving multiple isotopic labelings. The method, designed primarily for treatment of reactions with single isotopic labelings, can be easily extended to those involving double isotopic labelings. For the reaction mechanism described by Eq. [1], assume k_3, k_4, k_5, and k_6 are sensitive to carbon-13 labeling whereas k_5, and k_6 are sensitive to deuterium labeling. If hydrogen substrate is used, the carbon-13 isotope effect equation for the V/K system will be

$$\frac{{}^{13}(V/K)}{(V/K)} = \frac{1}{k_1} + \frac{{}^{13}k_3}{k_3 K_1} + \frac{{}^{13}k_5{}^{13}K_3}{k_5 K_1 K_3} + \frac{{}^{13}K_3{}^{13}K_5}{k_7 K_1 K_3 K_5}. \qquad [34]$$

By analogy, if deuterium substrate is used, the equation must be

$$\frac{{}^{13}(V/K)_{\mathrm{D}}}{(V/K)_{\mathrm{D}}} = \frac{1}{k_1} + \frac{{}^{13}k_3}{k_3 K_1} + \frac{{}^{13}(k_5)_{\mathrm{D}}{}^{13}K_3}{(k_5)_{\mathrm{D}} K_1 K_3} + \frac{{}^{13}K_3{}^{13}(K_5)_{\mathrm{D}}}{k_7 K_1 K_3 (K_5)_{\mathrm{D}}}, \qquad [35]$$

where ${}^{13}(V/K)_{\mathrm{D}}$ is the apparent double isotope effect and ${}^{13}(k_5)_{\mathrm{D}}$ and ${}^{13}(K_5)_{\mathrm{D}}$ are the intrinsic kinetic double isotope effect and equilibrium double isotope effect, respectively. If the rule of the geometric mean (*14*) holds, namely, ${}^{13}(k_5)_D = {}^{13}k_5$ and ${}^{13}(K_5)_D = {}^{13}K_5$, and since $1/(k_5)_D = {}^{D}k_5/k_5$ and $1/(K_5)_D = {}^{D}K_5/K_5$, Eq. [35] may be rewritten

$$\frac{{}^{13}(V/K)_D}{(V/K)_D} = \frac{1}{k_1} + \frac{{}^{13}k_3}{k_3K_1} + \frac{{}^{D}k_5{}^{13}k_5{}^{13}K_3}{k_5K_1K_3} + \frac{{}^{D}K_5{}^{13}K_3{}^{13}K_5}{k_7K_1K_3K_5}. \qquad [36]$$

Since

$$\frac{1}{(V/K)_D} = \frac{1}{k_1} + \frac{1}{k_3K_1} + \frac{{}^{D}k_5}{k_5K_1K_3} + \frac{{}^{D}K_5}{k_7K_1K_3K_5}, \qquad [37]$$

the apparent double isotope effect is then given by

$${}^{13}(V/K)_D = \frac{\dfrac{1}{k_1} + \dfrac{{}^{13}k_3}{k_3K_1} + \dfrac{{}^{D}k_5{}^{13}k_5{}^{13}K_3}{k_5K_1K_3} + \dfrac{{}^{D}K_5{}^{13}K_3{}^{13}K_5}{k_7K_1K_3K_5}}{\dfrac{1}{k_1} + \dfrac{1}{k_3K_1} + \dfrac{{}^{D}k_5}{k_5K_1K_3} + \dfrac{{}^{D}K_5}{k_7K_1K_3K_5}}. \qquad [38]$$

Equation [38] may be further rearranged depending on which intrinsic isotope effect is to be emphasized. For example, if ${}^{13}k_3$ is to be emphasized, Eq. [38] becomes

$${}^{13}(V/K)_D = \frac{{}^{13}k_3 + \dfrac{k_3}{k_2} + \dfrac{k_4}{k_5}{}^{D}k_5{}^{13}k_5{}^{13}K_3 + \dfrac{k_4k_6}{k_5k_7}{}^{D}K_5{}^{13}K_3{}^{13}K_5}{1 + \dfrac{k_3}{k_2} + \dfrac{k_4}{k_5}{}^{D}K_5 + \dfrac{k_4k_6}{k_5k_7}{}^{D}k_5}. \qquad [39]$$

V systems with double isotopic labelings are generally not useful in practice and will not be discussed.

CONCLUSION

Derivation of an isotopic equation is often tedious and time-consuming. However, by using the method described in this paper, an isotopic equation may be readily derived whether the isotope effect corresponds to a one-step or multistep perturbation or whether it corresponds to a single or multiple isotopic labeling. That such a general approach can be formulated is the result of the introduction of the concept of effective rate constants, which is not only useful for the derivation itself but also provides insights into the physical basis of the isotopic equations.

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