On the Application of the Steady State to Kinetic Isotope Effects

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Abstract: On the basis of theoretical calculation it was shown that the steady-state approximation applied to the concentration of an intermediate product of a complex reaction can lead to false results of kinetic isotope effects when the intrinsic isotope effect is large and the partitioning factor is much larger than unity. The method of correct measurements is proposed for such cases. It implies detection of apparent isotope effect at very small fraction of reaction (less than 1%) and observation of its dependence on the reaction progress.

Kinetic isotope effects have been proven as a useful probe of reaction mechanisms.[1] Facile determination of experimental values is now possible due to the development of different methods of measurement.[2] Most data are obtained by the competitive method which yields isotope ratios for reacting species measured at a given stage of reaction. For an elementary reaction the isotope ratios can be corrected for the extent of reaction f, giving an isotope effect; e.g., for the reaction

$$A_1 \xrightarrow{k} A_3 \tag{1}$$

$$\alpha = k_L/k_H = \ln(1-f)/\ln(1-Rf/S) \tag{2}$$

where $f = A_3/A_1^0$, $R = A_{3H}/A_{3L}$, and $S = A_{1H}^0/A_{1L}^0$. Subscripts L and H correspond to light and heavy isotopes, respectively. Equation 2 is frequently used in the form

$$\alpha = k_L/k_H = S/R \tag{3}$$

which is valid for small values of f. Approximation 3 can be successfully applied up to about 10% of the conversion. In the case of complex reactions the situation is less clear. A large number of processes of chemical and biochemical importance can be described by reaction 4 assuming a steady state[3] for the concentration of the intermediate A_2. It is known from chemical

$$A_1 \underset{k_2}{\overset{k_1}{\rightleftarrows}} A_2 \xrightarrow{k_3} A_3 \tag{4}$$
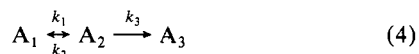
kinetics that the experimental reaction rate for such reaction is given by

$$k_e = \frac{k_1 k_3}{k_2 + k_3} \tag{5}$$

Equation 5 was adopted for the interpretation of kinetic isotope effects on complex reactions by simply substituting k by k_e in eq 2 and 3. Then after rearrangements

$$\alpha_e = k_{eL}/k_{eH} = \frac{\alpha_1}{\alpha_2} \frac{\alpha_3 + x\alpha_2}{1 + x} \tag{6}$$

where $x = k_{3L}/k_{2L}$.

The above approximation is in common use. If the first step of the reaction is binding of the substrate to an enzyme or a catalyst, as is the case for most biochemical reactions, then one can assume no isotope fractionation in the first step ($\alpha_1 = \alpha_2 = 1$) and rewrite eq 6 in the form

$$\alpha_e = \frac{\alpha_3 + x}{1 + x} \tag{7}$$

This equation has been frequently used[4] for the analysis of the magnitude of the intrinsic isotope effect α_3 and the partitioning factor x.

Table I. The Input Parameters for Lines in Figures; $k_{1L} = 1$

line no.	k_{2L}/k_{1L}	k_{3L}/k_{1L}	α_1	α_2	α_3
1	1	100	1.05	1	1
2	100	1	1.05	1	1
3	1	100	1	1.05	1
4	100	1	1	1.05	1
5	1	100	1	1	1.05
6	100	1	1	1	1.05
7	0.01	100	1	1	1.05
8	1	100	1	1	1.02
9	100	100	1	1	1.05
10	1	30	1	1	1.05

In this paper I am going to show that the steady-state approximation can sometimes lead to false interpretation of kinetic isotope effects even if it is valid for reaction rates. For this purpose let us consider the full expression for the concentration of A_3 of reaction 4. The solution of differential equations gives[5]

$$\gamma = A_3/A_1^0 = \frac{\lambda_2(1 - e^{-\lambda_3 k_1 t}) - \lambda_3(1 - e^{-\lambda_2 k_1 t})}{\lambda_2 - \lambda_3} \tag{8}$$

where

$$\lambda_2 = \frac{k_1 + k_2 + k_3 + \{(k_1 + k_2 + k_3)^2 - 4k_1 k_3\}^{1/2}}{2k_1}$$

$$\lambda_3 = \frac{k_1 + k_2 + k_3 - \{(k_1 + k_2 + k_3)^2 - 4k_1 k_3\}^{1/2}}{2k_1}$$

In order to compare the real fractionation with the one predicted by the steady state we have to use eq 3 as there is no apparent rate constant for the "full" model. As was pointed above in the range 0–0.1 for the extent of reaction, the ratio S/R is a good approximation of isotope effect. The expression for this ratio is obtained by combining eq 3 with eq 8 written for two isotopic species.

$$\alpha_e = \frac{S}{R} = \frac{A_{1H}^0 A_{3L}}{A_{1L}^0 A_{3H}} = \frac{\gamma_L}{\gamma_H} =$$
$$\frac{\lambda_{2L}(1 - e^{-\lambda_{3L} k_{1L} t}) - \lambda_{3L}(1 - e^{-\lambda_{2L} k_{1L} t})}{\lambda_{2H}(1 - e^{-\lambda_{3H} k_{1H} t}) - \lambda_{3H}(1 - e^{-\lambda_{2H} k_{1H} t})} \frac{\lambda_{2H} - \lambda_{3H}}{\lambda_{2L} - \lambda_{3L}} \tag{9}$$

(1) Melander, L.; Saunders, W. H., Jr. "Reaction Rates of Isotopic Molecules"; Wiley: New York, 1980.
(2) Paneth, P., submitted for publication.
(3) Bodenstein, M. *Z. Phis. Chem.* **1913**, *85*, 329.
(4) Cleland, W. W.; O'Leary, M. H.; Northrop, D. E., Eds. "Isotope Effects on Enzyme-Catalyzed Reactions"; Univeristy Park Press: Baltimore, 1977.
(5) Sievert, H. R.; Tenney, P. N.; Xermeulen, T. U.S. Atomic Energy Comm. UCRL 10575, 1962.

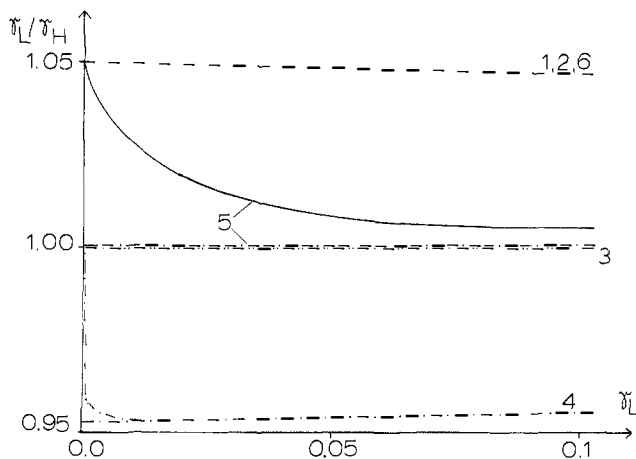[†]On leave from the Institute of Applied Radiation Chemistry, Technical University of Lodz, Poland.

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Figure 1. Dependence of apparent isotope effects derived by eq 9 and 11 on the reaction progress. See text.

Figure 2. Dependence of apparent isotope effects derived by eq 9 and 11 on the reaction progress. See text.

This expression can be compared with the analogous one which results from the steady-state approximation:

$$\gamma = 1 - e^{-k_e t} \tag{10}$$

and

$$\alpha_e = \frac{1 - e^{-k_{eL} t}}{1 - e^{-k_{eH} t}} \tag{11}$$

If the approximation can be applied, then time dependence of eq 9 and 11 should be the same whenever the condition $k_1 \ll k_2 + k_3$ (which led to the approximation) is met. It is convenient to look at the functions plotted against the extent of reaction rather than time. Some plots of both functions are shown in Figures 1 and 2. In order to normalize input data (Table I), k_{1L} was assumed to be equal to unity. Of course the actual value of k_{1L} does not change the results. It only affects the relation between the reaction progress and time.

From Figure 1 it can be seen that for many cases (lines 1, 2, 3, 6), the agreement between the full solution and an approximate one is satisfactory. Line 4 splits at a very small fraction of the reaction (less than 1%). The lower part corresponds to eq 11 and the upper to eq 9. This splitting is in fact a general phenomenon. However, in other cases the difference was too low to be noticed. It originates in different limits for these two functions as $t \rightarrow 0$. For eq 11 the limit is k_{eL}/k_{eH} while for eq 9 it is a $\alpha_1\alpha_3$. Thus the problem is whether this discrepancy can be seen, or in other words whether the difference appears in the time scale of our measurements or not. If it does, then our conclusions drawn on the basis of the steady-state approximation would be wrong. Line 5 shows such an unfavorable case when the two equations have completely different course in the range of the reaction progress available to our inspection. Unfortunately, this is the most im-
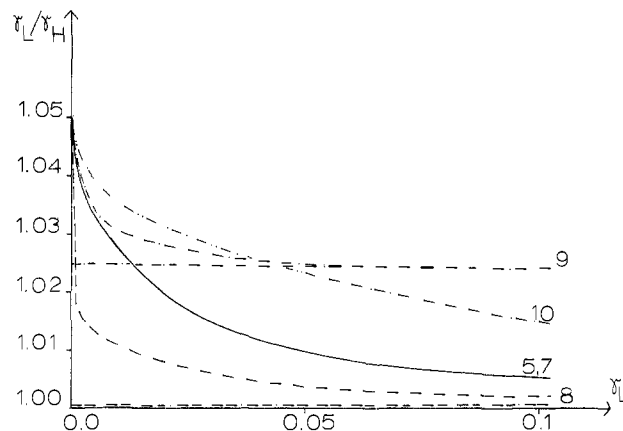
portant case when the intrinsic isotope effect is distinctly different from unity and the partitioning factor is large. More examples of such cases are illustrated in Figure 2. The lowest line in this figure corresponds, within the precision of drawing, to eq 11 for all lines but line 9. Lines 7 through 10 show the influence of some parameters on the difference between the time dependence of eq 9 and 11. Comparison of lines 8 and 5 indicates that this difference increases with the increase of isotope effect α_3. Lines 5 and 9 show that the larger k_3/k_2 ratio the more prominent the difference. From lines 5 and 10 it can be learned that this difference also increases with the increase of the intermediate concentration. The accumulation of the intermediate A_2 (expressed as A_2/A_1^0) at its maximum is less than 3% for the line 9 and less than 1% for all the other lines.

The conclusion which comes from the above discussion is that one has to be sure whether the steady state applies to an apparent isotope effect before analyzing results in terms of eq 6 or 7. And it should be kept in mind that the validity of the steady-state assumption with respect to reaction rates is not a sufficient condition for its application to the kinetic isotope effects. Therefore, the time dependence of an apparent isotope effect should be tested even for data obtained in the range 0–0.1 for f. If change of the apparent isotope effect with the extent of reaction is observed, the use of eq 6 or 7 leads to false results and one should try instead to fit data into the theoretical curve given by eq 9 in order to reveal the real values. It seems advantageous to work at an extremely low fraction of reaction, less than 1%. The extrapolation of such data gives the product $\alpha_1\alpha_3$. As α_1 is usually close to unity, this procedure leads directly to α_3. With regard to the partitioning factor, the curvature of a plot like the one discussed herein indicates that $x \gg 1$.