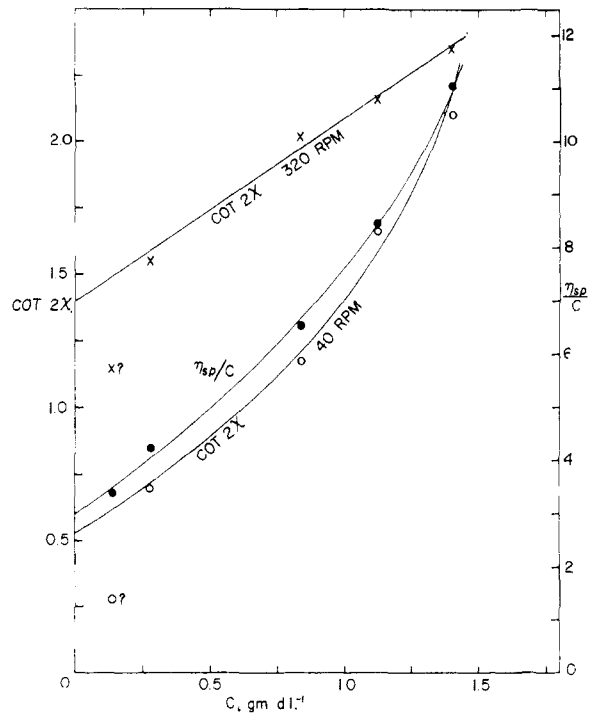

Fig. 11.—Comparison of cot 2χ–C and (η_{sp}/C)–C plots of rabbit actomyosin solution. Data were taken from ref. 26.

a high degree of rigidity. It is therefore of interest to test the applicability of the proposed equations to these macromolecular systems. As an example we will choose von Muralt and Edsall's pioneer work on actomyosin.[26] In Fig. 11 are plotted cot 2χ against the concentration (assuming the protein contains 16% N) at two rates of shear (the lowest and the highest measurable RPM). Also included in the figure is the (η_{sp}/c)–c curve, the data of which were measured in an Ostwald viscometer presumably at very low rate of shear. The close similarity in the shape of both flow birefringence (at low r.p.m.) and viscosity is self-explanatory. According to the original paper, the lower R.P.M. corresponded to about $D = 10$ sec.$^{-1}$ and α $(= D/\Theta)$ was close to 1. Although the actual rate of shear in the viscometer was not mentioned, the listed values seemed also to lie close to the Newtonian region. Thus the two curves were comparable at about the same range of rates of shear. The apparently straight line obtained at a high r.p.m. was mostly certainly due to the sharp drop in $k'[\eta]$ in eq. 5, since this protein exhibits extremely strong non-Newtonian viscosity at higher rates of shear.

Bovine plasma albumin is another extreme case for illustration. Due to its low asymmetry this protein can only be oriented in a highly viscous medium and at a very high concentration. Edsall and Foster[27] had reported an estimated length of 190–200 Å. for a 4.48% (w./v.) solution in 88.45% (w./w.) glycerol. At this concentration the ratio of $(\eta_{sp}/c)/[\eta]$ was estimated to be about 1.4,[28] using the viscosity data in aqueous solutions. Using eq. 12 one finds a calculated value of $\eta_0\Theta_0/T$ of 42 rather than the published value of 30. Consequently the estimated length of the ellipsoid should be close to 170 Å., which is in better agreement with the currently accepted value of 150 Å. It seems highly desirable to reinvestigate this protein in several more concentrated solutions so that any scattering in experimental points can be smoothed out. In fact, with our proposed equations it will be possible to extend the lower limit of flow birefringence technique, thus enabling us to study many proteins of very low asymmetry. Likewise, for those having very high asymmetry it is of pertinent importance to determine the extent of concentration dependence. These studies would certainly prove or disprove the general applicability of our proposed equations.

Acknowledgments.—The author wishes to express his gratitude to Dr. E. R. Blout and Professor P. Doty for their kindness in providing the polypeptide sample. He would also like to thank Dr. E. Passaglia for many invaluable discussions on the manuscript.

(26) A. von Muralt and J. T. Edsall, *J. Biol. Chem.*, **89**, 375, 351 (1930); J. T. Edsall, *Trans. Faraday Soc.*, **26**, 837 (1930).

(27) J. T. Edsall and J. F. Foster, THIS JOURNAL, **70**, 1860 (1948).

(28) J. L. Oncley, G. Scatchard and A. Brown, *J. Phys. Colloid Chem.*, **51**, 184 (1947).

MARCUS HOOK, PENNSYLVANIA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

Kinetics of the Reversible Michaelis–Menten Mechanism and the Applicability of the Steady-state Approximation[1]

BY WILMER G. MILLER[2] AND ROBERT A. ALBERTY

RECEIVED MAY 12, 1958

The exact analytical solutions to the rate equations for the reversible Michaelis–Menten mechanism are derived for the case that $k_1 = k_4$. The steady-state approximation is shown to be a good approximation if $s_0 \gg e_0$ or if $(e_0 + s_0) \ll (k_2 + k_3)/k_1$. A perturbation solution is developed for the case that $k_1 \neq k_4$ and the applicability of the steady-state approximation for this case is discussed.

Introduction

Enzyme kinetic data frequently can be represented by the reversible Michaelis–Menten mechanism

$$\underset{e_0 - x}{E} + \underset{s_0 - p - x}{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \underset{x}{X} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \underset{e_0 - x}{E} + \underset{p}{P} \qquad (1)$$

where E represents enzyme,[3] S and P represent substrates, and X is the intermediate. The total molar concentration of enzyme is represented by e_0 and the initial concentration of substrate by s_0. The concentrations of the intermediate and product

(1) This research was supported by grants from the National Science Foundation and from the Research Committee of the Graduate School of the University of Wisconsin from funds supplied by the Wisconsin Alumni Research Foundation.

(2) Socony-Mobil Fellow (1957–1958).

(3) The symbol E actually represents the enzymatic site rather than the enzyme but generally the number of sites per molecule is unknown. Letting E represent the enzyme rather than the enzymatic site so that e_0 represents the total molar concentration of the enzyme increases the rate constants by a factor equal to the number of sites per molecule, assuming no site–site interaction. The mechanism is not restricted to enzyme catalysis but might be applied to heterogeneous catalysis, with E representing the sites on the surface of the solid.

at any time are represented by x and p, respectively. The rate equations are therefore

$$\dot{x} = k_1 s_0 e_0 + (k_4 - k_1)e_0 p - [k_1 e_0 + k_1 s_0 + (k_4 - k_1)p + k_2 + k_3]x + k_1 x^2 \quad (2)$$

$$\dot{p} = (k_3 + k_4 p)x - k_4 e_0 p \quad (3)$$

The steady-state rate equation for this mechanism, derived by Haldane[4] for the case that $s_0 >> e_0$, is

$$\dot{p} = \frac{k_3 e_0 s/K_S - k_2 e_0 p/K_P}{1 + s/K_S + p/K_P} \quad (4)$$

where K_S and K_P are the Michaelis constants for the substrate and product, respectively, with $K_S = (k_2 + k_3)/k_1$ and $K_P = (k_2 + k_3)/k_4$. Chance[5] has obtained differential analyzer solutions for the case that $k_4 = 0$, and Yang[6] has discussed a solution for this case using a reversion method. Various approximate solutions have been discussed for the transient phase of the reaction when $k_4 = 0$.[7-10] In deriving these approximate solutions, the product concentration was considered to be negligible during the transient phase. However the complete analytical solution of equations 2 and 3 has not been obtained.

In the present paper the exact solution for the reversible Michaelis–Menten mechanism is derived for the case that $k_1 = k_4$ and a perturbation solution is developed for the case that $k_1 \neq k_4$.

Exact Solutions when $k_1 = k_4$.—When $k_1 = k_4$, rate equations 2 and 3 become, respectively

$$\dot{x} = k_1 e_0 s_0 - (k_1 s_0 + k_1 e_0 + k_2 + k_3)x + k_1 x^2 \quad (5)$$

$$\dot{p} = (k_3 + k_4 p)x - k_4 e_0 p \quad (6)$$

Equation 5 is immediately integrable and upon applying the initial condition $t = 0$, $x = 0$ and rearranging gives

$$x = \frac{2k_1 e_0 s_0 (1 - e^{-dt})}{d - b + (d + b)e^{-dt}} \quad (7)$$

where

$$a = k_1 \quad (8)$$

$$b = -(k_1 e_0 + k_1 s_0 + k_2 + k_3) \quad (9)$$

$$c = k_1 e_0 s_0 \quad (10)$$

$$d = +(b^2 - 4ac)^{1/2} = [(k_1 e_0 + k_1 s_0 + k_2 + k_3)^2 - 4k_1^2 e_0 s_0]^{1/2} \quad (11)$$

If equation 7 is substituted into equation 6, the resulting differential equation is linear in p but has non-constant coefficients. This equation, however, can be integrated by the method of variation of parameters and upon applying the initial condition $t = 0$, $p = 0$ and simplifying the following equation is obtained.

$$p = \frac{2k_3 s_0 \left\{ d\left[1 - e^{-\frac{k_1 e_0 (2k_1 s_0 + b - d)t}{b - d}}\right] - \frac{k_1 e_0 (2k_1 s_0 + b - d)}{b - d}[1 - e^{-dt}] \right\}}{(k_2 + k_3)[d - b + (d + b)e^{-dt}]} \quad (12)$$

If $s_0 >> e_0$, $d = -b$ and equations 7 and 12 become

$$x = \frac{k_1 e_0 s_0}{k_1 s_0 + k_2 + k_3}[1 - e^{-(k_1 s_0 + k_2 + k_3)t}] \quad (13)$$

and

$$p = \frac{k_3 s_0}{k_2 + k_3}\left\{\left[1 - e^{-\frac{k_1(k_2 + k_3)e_0 t}{k_1 s_0 + k_2 + k}}\right] - \frac{k_1(k_2 + k_3)e_0}{(k_1 s_0 + k_2 + k_3)^2}[1 - e^{-(k_1 s_0 + k_2 + k_3)t}]\right\} \quad (14)$$

Since equations 7 and 12 involve no approximations in their derivation, they give the concentration of intermediate and product exactly over the entire course of the reaction, including both the transient and steady-state phases. Before discussing the nature of these equations the significance of the restriction that $k_1 = k_4$ will be discussed.

The condition that $k_1 = k_4$ is exactly the restriction that the Michaelis constants for the substrate and product must be equal. Enzyme reactions that can be represented by mechanism 1 under given conditions and which have equilibrium constants in the neighborhood of unity have been studied in both the forward and reverse directions.[11,12] For the fumarase, enolase and phosphoglucose isomerase reactions[13] $K_S = K_P$ at a pH which depends upon the buffer and ionic strength.

For enzyme reactions where the equilibrium constant is much greater than unity, *i.e.*, the reaction goes "to completion," data for the Michaelis constants of both the reactant and product are difficult to find in the literature. This in part appears to be due to a common practice which has developed among enzyme kineticists that if a reaction goes essentially to completion, it is assumed that $k_4 = 0$. Obviously in this case a Michaelis constant for the product is meaningless. As has been pointed out,[14] *the condition for a reaction following mechanism 1 going to completion is that $k_1 k_3 >> k_2 k_4$ and this puts no condition on k_4.* If $k_1 = k_4$, $k_3 >> k_2$ would be sufficient to cause the reaction to go essentially to completion. In many cases product inhibition could be explained by using mechanism 1 rather than letting $k_4 = 0$ and adding a separate reaction to account for the inhibition. Product inhibition constants often have values very close to the Michaelis constant for the substrate,[15] thus suggesting that if mechanism 1 were used to interpret product inhibition calculated values of k_1 and k_4 would be nearly equal. The restriction that has been used in deriving the equations is thus one that is met under particular conditions in some enzyme systems and may even be applicable to enzymatic reactions which essentially go to completion.

It is of interest to compare equations 7, 12, 13 and 14 with approximate equations obtained by others for the case where $k_4 = 0$. Equation 7 is identical (if $\bar{s}$, the average substrate concentration, is taken to be s_0) with the equation derived by

(4) J. B. S. Haldane, "Enzymes," Longmans, Green and Co., London, 1930, p. 81.

(5) B. Chance, *J. Biol. Chem.*, **151**, 553 (1943).

(6) C. Yang, *Arch. Biochem. Biophys.*, **51**, 419 (1953).

(7) H. Gutfreund, *Disc. Faraday Soc.*, **20**, 167 (1955).

(8) K. J. Laidler, *Can. J. Chem.*, **33**, 1614 (1955).

(9) M. F. Morales and D. E. Goldman, THIS JOURNAL, **77**, 6069 (1955).

(10) P. A. T. Swoboda, *Biochim. et Biophys. Acta*, **23**, 70 (1957).

(11) R. M. Bock and R. A. Alberty, THIS JOURNAL, **75**, 1921 (1953).

(12) F. Wold and C. E. Ballou, *J. Biol. Chem.*, **227**, 313 (1957).

(13) K. K. Tsuboi, J. Estrada and P. B. Hudson, *ibid.*, **231**, 19 (1958).

(14) R. A. Alberty in P. D. Boyer, H. Lardy and K. Myrbäck, "The Enzymes," 2nd Ed., Academic Press, New York, N. Y., 1958.

(15) R. J. Foster and C. Niemann, *Proc. Natl. Acad. Sci.*, U. S., **39**, 999 (1953); H. T. Huang and C. Niemann, THIS JOURNAL, **73**, 1541 (1951).

Laidler[8] for the appearance of intermediate during the transient state. This similarity is expected since the initial appearance of intermediate should be independent of the value of k_4. Whereas Laidler's expression is only good for the transient state, equation 7 is exact for the entire course of the reaction if $k_1 = k_4$ and x asymptotically approaches the steady-state or equilibrium value.

For the additional condition that $s_0 >> e_0$, equations derived by Laidler[8] and by Swoboda[10] for the transient state appearance of intermediate are identical with equation 13 which is again to be expected as the value of k_4 is unimportant during the transient state. Gutfreund[7] and Swoboda[10] have also derived an expression for the appearance of product, which may be compared with equation 14. The second term in the equations of Gutfreund and Swoboda is identical with the second term in equation 14 while the first term in their equation can be obtained from our first term by expanding the exponential term in a power series and keeping only the first two terms. An extrapolation of the linear (early steady state) region to obtain k_1, as Gutfreund has suggested, must be made with considerable caution.

Terms involving e^{-dt} in equations 7 and 12 are responsible for the initial rapid rise in x while terms arising from the first exponential term of equation 12 can be identified with the "steady state." The applicability of the steady-state approximation depends essentially on the relative values of d and $[k_1e_0(2k_1s_0 + b - d)]/(b - d)$, and the larger the relative value of d becomes, the better the degree of approximation of $\dot{x} = 0$. There are several ways in which the relative values of these quantities can be changed, one of which is to change the ratio of e_0 to s_0. If $s_0 >> e_0$, equation 12 simplifies to equation 14, and the ratio of $k_1s_0 + k_2 + k_3$ to $k_1(k_2 + k_3)e_0/(k_1s_0 + k_2 + k_3)$ will always be greater than s_0/e_0. The equation for the concentration of product as a function of time derived using the steady state approximation is given by[16]

$$p = \frac{k_3 s_0}{k_2 + k_3}\left[1 - e^{-\frac{k_1(k_2 + k_3)e_0 t}{k_1 s_0 + k_2 + k_3}}\right] \qquad (15)$$

which is identical with equation 14 except for the transient term. Equation 15 is that of a pseudo first-order reaction and a plot of $-(1/e_0t)\ln(1 - p/p_{eq})$ *vs.* t gives a straight line with zero slope; therefore any difference between the steady-state value of product and the actual value would be seen easily in this type of plot.

Figure 1 illustrates the effect of varying the enzyme concentration while holding the substrate concentration constant. The values for the rate constants are those calculated from steady-state kinetic data for the fumarase system at 25°, *p*H 6.81, 0.1 ionic strength in tris-(hydroxymethyl)-aminomethane acetate buffer containing 0.09 M NaCl.[17] The initial substrate concentration, 10^{-4} M, was chosen to be approximately equal to the Michaelis constant, since this is in the range of substrate concentrations where initial velocity studies usually are made. As the enzyme concentration is reduced, the value of $-(1/e_0t)\ln(1 - p/p_{eq})$ approaches the steady-state value more closely at smaller and smaller extents of reaction. When the enzyme concentration is 10^{-4} times the substrate concentration (curve D), the steady-state value is within 1% of the true value when the product concentration has reached only 0.3% of its equilibrium value. But even at this concentration the fumarase reaction would occur too rapidly for ordinary initial velocity measurements to be made. As a rule experiments to measure "initial velocities" are arranged so that the reaction will be about 5% to its equilibrium value in five minutes or longer. In the case of fumarase this corresponds to an enzyme concentration of about 5×10^{-10} M. Using this concentration of enzyme and equations 13 and 14, a simple calculation shows that at 10^{-3} seconds x is *within 0.001% of its steady-state value* while the product concentration has *reached only about 0.001% of its equilibrium value.* This completely justifies the use of the steady-state approximation in the study of the fumarase reaction under the condition that the Michaelis constants are equal.

Figure 2 illustrates the effect of varying the substrate concentration while holding the enzyme concentration constant. Comparing curve A in Fig. 1 with curve A in Fig. 2, it is evident that the steady-state assumption becomes a better approximation as both the enzyme and substrate concentrations become much smaller than the Michaelis constant, even if $e_0 \geq s_0$. The values for $-(1/e_0t)\ln(1 - p/p_{eq})$ in A and C have been multiplied by appropriate constants in order to make their steady-state values coincide with the steady-state value of B. The effect on the appearance of intermediate of changing the initial substrate and enzyme concentrations is shown in Fig. 3.

It is thus seen that the steady-state approximation becomes better as e_0/s_0 becomes small or as $e_0 + s_0$ becomes smaller than $(k_2 + k_3)/k_1$. The latter condition is sufficient even though $s_0 = e_0$ or even $e_0 > s_0$. It is of interest that the steady-state approximation can be applicable even though the concentration of substrate is not much larger than the concentration of enzyme.

Approximate Solution for $k_1 \neq k_4$.—The following discussion will be restricted to the case where $s_0 >> e_0$. In this case $s_0 = s + p$ and rate equations 2 and 3 simplify to

$$\dot{x} = k_1s_0e_0 + (k_4 - k_1)e_0\dot{p} - [k_1s_0 + k_2 + k_3 + (k_4 - k_1)p]x \qquad (16)$$

$$\dot{p} = (k_3 + k_4p)x - k_4e_0p \qquad (17)$$

Equations 16 and 17 are non-linear and have no known analytical solutions. To investigate the nature of x as a function of time equation 16 is first differentiated with respect to time, to obtain

$$\ddot{x} = (k_4 - k_1)e_0\dot{p} + [k_1s_0 + k_2 + k_3 + (k_4 - k_1)p]\dot{x} + (k_4 - k_1)x\dot{p} \qquad (18)$$

If x were to go through a maximum $\dot{x} = 0$ and $\ddot{x} < 0$ at the maximum. Since $\dot{p} \geq 0$, the only way that x can go through a maximum is for $k_1 > k_4$.[18]

(16) R. A. Alberty, W. G. Miller and H. F. Fisher, THIS JOURNAL, **79**, 3973 (1957).

(17) C. Frieden, R. Wolfe, Jr., and R. A. Alberty, *ibid.*, **79**, 1524 (1957).

(18) M. F. Morales, in P. D. Boyer, H. Lardy and K. Myrbäck, "The Enzymes," 2nd Ed., Academic Press, New York, N. Y., 1958.

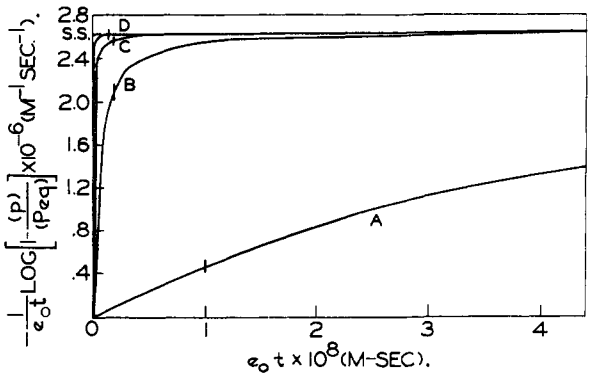

Fig. 1.—Kinetic plots at various enzyme concentrations with $s_0 = 10^{-4}$ *M*: A, $e_0 = 10^{-4}$ *M*; B, $e_0 = 10^{-6}$ *M*; C, $e_0 = 10^{-7}$ *M*; D, $e_0 = 10^{-8}$ *M*. Values used for the rate constants are $k_1 = k_4 = 7.9 \times 10^8\ M^{-1}$ min.$^{-1}$, $k_2 = 5.5 \times 10^4$ min.$^{-1}$ and $k_3 = 1.24 \times 10^4$ min.$^{-1}$. The steady-state value is labelled s.s. The value of $e_0 t$ at which the product concentration is one per cent. of its equilibrium value is marked by a short vertical line.

Thus as the ratio of k_4 to k_1 changes, an envelope of curves for x *vs.* t is obtained, with all curves for $k_1 > k_4$ going through a maximum and with no curves for $k_4 \geqslant k_1$ having a maximum, but instead approaching their maximum value asymptotically at infinite time.[19] If x goes through a maximum for the forward reaction, it cannot for the reverse reaction. A logical way to obtain solutions for $k_1 \neq k_4$ is to consider the desired solutions as perturbations[20] of the solution for $k_1 = k_4$. The desired solutions are then

$$x = x_0 + (k_4 - k_1)x_1 + (k_4 - k_1)^2 x_2 + \cdots = \sum_{n=0}^{\infty} x_n (k_4 - k_1)^n \quad (19)$$

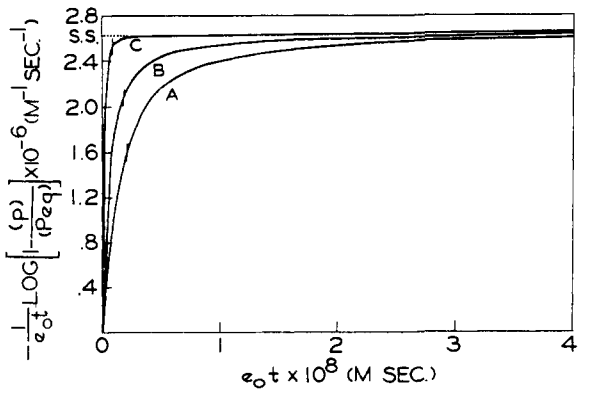

Fig. 2.—Kinetic plots at various substrate concentrations with $e_0 = 10^{-6}$ *M*: A, $s_0 = 10^{-6}$ *M*; B, $s_0 = 10^{-4}$ *M*; C, $s_0 = 10^{-3}$ *M*. The values of rate constants are the same as for Fig. 1. The steady-state value is labelled s.s. The value of $e_0 t$ at which the product concentration is one per cent. of its equilibrium value is marked by a short vertical line.

(19) The same condition applies to general equation 2 where there is no restriction on the relative concentrations of enzyme and substrate.

(20) R. Courant and D. Hilbert, "Methods of Mathematical Physics, I," Interscience Publishers, New York, N. Y., 1953.

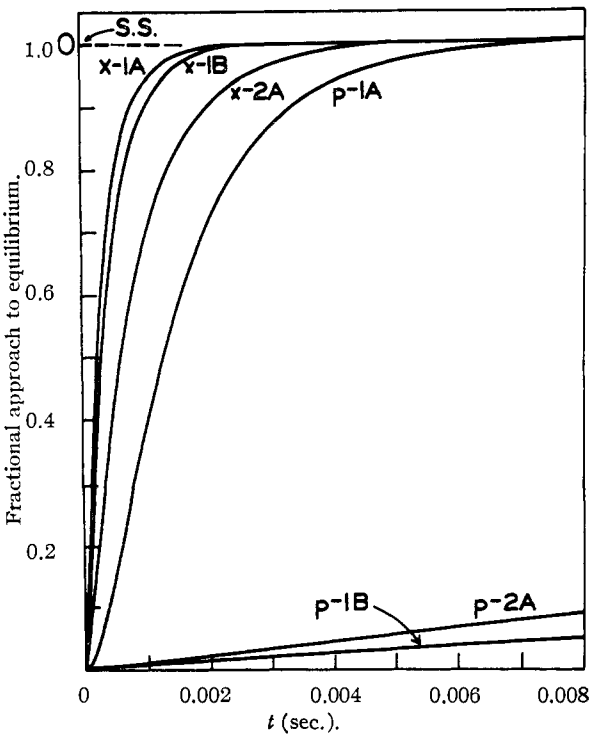

Fig. 3.—Fractional approach to equilibrium for both intermediate (x) and product (p) for the values of the rate constants given for Fig. 1. Curves marked 1A and 1B were calculated using the same enzyme and substrate concentrations as for curves A and B in Fig. 1; similarly curves 2A correspond with curve A in Fig. 2. The steady-state value is labelled s.s. Equations 7 and 12 were used in making the calculations.

$$p = p_0 + (k_4 - k_1)p_1 + (k_4 - k_1)^2 p_2 + \cdots = \sum_{n=0}^{\infty} p_n (k_4 - k_1)^n \quad (20)$$

with x_0 and p_0 being the solutions of x and p, respectively, when $k_1 = k_4$. The quantities x_1 and p_1 are the first-order perturbation terms, x_2 and p_2 the second-order perturbation terms, etc. The expression for x_0 is given by equation 13

$$x_0 = \frac{k_1 e_0 s_0}{\beta} [1 - e^{-\beta t}] \quad (21)$$

where $\beta = k_1 s_0 + k_2 + k_3$ and the equation for p_0 is

$$p_0 = \frac{k_1 k_3 s_0}{k_4(k_2 + k_3)} [1 - e^{-\alpha t}] - \frac{k_1 k_3 s_0 e_0}{\beta^2} [1 - e^{-\beta t}] \quad (22)$$

where $\alpha = [k_4(k_2 + k_3)e_0]/(k_1 s_0 + k_2 + k_3)$. Equation 22 differs from equation 14 in that in the derivation of equation 12, k_4 is allowed to keep its identity wherever possible.

To obtain x and p as functions of time equations 19 and 20 and their first derivatives are substituted into equations 16 and 17 and the coefficient of each power of $(k_4 - k_1)$ is equated to zero. Thus, considering equation 19, the coefficient of the $(k_4 - k_1)$ term will be a first-order differential equation in x_1, the coefficient of $(k_4 - k_1)^2$ involves x_2, etc. The coefficient of $(k_4 - k_1)^n$ gives

$$\dot{x}_n + \beta x_n = e_0 p_{n-1} - \sum_{m=0}^{n-1} p_{n-1-m}\, x_m \quad (n \neq 0) \quad (23)$$

$$\dot{p}_n + k_4(e_0 - x_0)p_n = k_3x_n + k_4\sum_{m=1}^{n} p_{n-m}x_m \quad (n \neq 0) \tag{24}$$

The homogeneous parts of both equations are the same as the corresponding homogeneous equations for x_0 and p_0, which have been integrated earlier.[21] Upon integration one obtains

$$x_n = e^{-\beta t}\int_0^t [e_0p_{n-1} - \sum_{m=0}^{n-1} p_{n-1-m}x_m]e^{\beta t}\,dt \quad n \neq 0 \tag{25}$$

$$p_n = e^{-\alpha t}\int_0^t \left[k_3x_n + k_4\sum_{m=1}^{n} p_{n-m}x_m\right]e^{\alpha t}\,dt \quad n \neq 0 \tag{26}$$

and thus the perturbation solutions are

$$x = x_0 + \sum_{n=1}^{\infty}\left\{(k_4 - k_1)^n e^{-\beta t}\int_0^t [e_0p_{n-1} - \sum_{m=0}^{n-1} p_{n-1-m}x_m]\,e^{\beta t}\,dt\right\} \tag{27}$$

$$p = p_0 + \sum_{n=1}^{\infty}\left\{(k_4 - k_1)^n e^{-\alpha t}\int_0^t \left[k_3x_n + k_4\sum_{m=1}^{n} p_{n-m}x_m\right]e^{\alpha t}\,dt\right\} \tag{28}$$

These equations all have the same initial condition that $x_n = p_n = 0$ when $t = 0$. Whether or not the above solutions can be used depends on the nature of their convergence. In principle they describe the course of x and p even for the case that $k_4 \to 0$.

For both the enolase reaction[12] and the fumarase reaction[11] the Michaelis constants for the substrate and product are within a factor of 10 of each other at most pH values. Thus the only perturbation terms that will be written out explicitly here will be those terms needed for the case that $0.1 \leqslant k_4/k_1 \leqslant 10$.

The first two perturbation terms for x and p derived from equations 25 and 26 are

$$x_1 = \frac{k_1k_3e_0s_0}{k_4\beta^2}[1 - e^{-\alpha t}] \tag{29}$$

$$x_2 = \frac{k_1k_3^2s_0e_0}{k_4^2\beta^3}[1 - e^{-\alpha t}] + \frac{2k_1^2k_3^2s_0^2e_0}{k_4^2(k_2 + k_3)\beta^3}\left[e^{-\alpha t} - e^{-2\alpha t} - \frac{k_4(2k_1s_0 + k_2 + k_3)(k_2 + k_3)e_0}{2k_1s_0\beta}te^{-\alpha t}\right] \tag{30}$$

$$p_1 = \frac{k_1k_3^2s_0}{k_4^2(k_2 + k_3)^2} - \frac{k_1k_3^2e_0s_0(2k_1s_0 + k_2 + k_3)}{k_4(k_2 + k_3)\beta^2}te^{-\alpha t} - \frac{k_1^2k_3^2s_0^2}{k_4^2(k_2 + k_3)^2\beta}e^{-2\alpha t} - \frac{k_1k_3^2s_0}{k_4^2(k_2 + k_3)\beta}e^{-\alpha t} \tag{31}$$

$$p_2 = \frac{k_1k_3^3s_0}{k_4^3(k_2 + k_3)^3}[1 - e^{-\alpha t}] - \frac{k_1k_3^3s_0e_0}{k_4^2(k_2 + k_3)^2\beta}\left[1 - \frac{2k_1^2s_0^2}{\beta^2}\right]te^{-\alpha t} - \frac{k_1k_3^3s_0e_0^2(2k_1s_0 + k_2 + k_3)^2}{2k_4(k_2 + k_3)\beta^4}t^2e^{-\alpha t} - \frac{2k_1^2k_3^3e_0s_0^2(2k_1s_0 + k_2 + k_3)}{k_4^2(k_2 + k_3)^2\beta^3}te^{-2\alpha t} + \frac{k_1^3k_3^3s_0^3}{k_4^3(k_2 + k_3)^3\beta^2}\left\{\left[1 - \frac{2(k_2 + k_3)}{k_1s_0}\right][e^{-2\alpha t} - e^{-\alpha t}] - \frac{3}{2}[e^{-3\alpha t} - e^{-\alpha t}]\right\} \tag{32}$$

Each higher perturbation expression contains many more terms than the one preceding it and even x_1 contains more terms than is shown in equation 29. Two criteria were used in discarding terms to obtain the above equations. For x_1 and x_2 all terms that had values less than the value of x_0 under the condition $s_0 >> e_0$ (or $k_1e_0 >> k_4e_0$) were discarded. Other terms went through maxima (or minima) and the values of these terms at their maxima (or minima) were compared with x_0 and were discarded if small compared to x_0. A similar procedure was used in discarding terms in p_1 and p_2.

In Fig. 4 x/e_0 is plotted *vs.* t for two values of k_1/k_4: in A $k_1/k_4 = 10$ and in B $k_1/k_4 = 0.1$. The value of k_1 is the same as used in Fig. 1–3 and the values for k_2 and k_3 are such as to keep the Michaelis constant of the substrate the same as for Fig. 1–3 while still maintaining the equilibrium constant for the over-all reaction. To calculate the time course of x/e_0 as given by the steady-state approximation these equations were used

$$x/e_0 = \frac{k_1s_0 + (k_4 - k_1)p}{k_1s_0 + k_2 + k_3 + (k_4 - k_1)p} \tag{33}$$

$$\frac{(k_1 - k_4)p}{k_1k_3 + k_2k_4} - \frac{(k_2 + k_3)(k_1k_4s_0 + k_1k_3 + k_2k_4)}{(k_1k_3 + k_2k_4)^2}\ln\left[1 - \frac{p}{p_{eq}}\right] = e_0t \tag{34}$$

Equation 33 is obtained from equation 16 by setting $\dot{x} = 0$ and equation 34 is obtained by substituting equation 33 into equation 17 and integrating.[22] For curve A the value of x calculated from $x_0 + (k_4 - k_1)x_1 + (k_4 - k_1)^2x_2$ agrees very closely with the steady-state solution while in curve B the agreement is not so good (the third perturbation term is needed in this case). It appears that the steady-state approximation is very good for the fumarase reaction at least when $0.1 \leqslant k_1/k_4 \leqslant 10$. Furthermore the perturbation solution appears to converge very rapidly within this region.

The equilibrium values of x and p obtained from the perturbation solution are

$$x_{eq} = \frac{k_1e_0s_0}{\beta}\left\{1 + \left[\frac{(k_4 - k_1)k_3}{k_4\beta}\right] + \left[\frac{(k_4 - k_1)k_3}{k_4\beta}\right]^2 + \cdots\right\} = \frac{k_1e_0s_0}{\beta}\sum_{n=0}^{\infty}\left[\frac{(k_4 - k_1)k_3}{k_4\beta}\right]^n \tag{35}$$

$$p_{eq} = \frac{k_1k_3s_0}{k_4(k_2 + k_3)}\left\{1 + \left[\frac{(k_4 - k_1)k_3}{k_4(k_2 + k_3)}\right] + \left[\frac{(k_4 - k_1)k_3}{k_4(k_2 + k_3)}\right]^2 + \cdots\right\} = \frac{k_1k_3s_0}{k_4(k_2 + k_3)}\sum_{n=0}^{\infty}\left[\frac{(k_4 - k_1)k_3}{k_4(k_2 + k_3)}\right]^n \tag{36}[23]$$

If

$$\left|\frac{(k_4 - k_1)k_3}{k_4\beta}\right| < 1 \tag{37}$$

(21) Equation 24 is more easily integrated if equation 7 is used for x_0 rather than equation 13 in the homogeneous part of equation 24, and the condition $s_0 \gg e_0$ is applied after the homogeneous equation is integrated.

(22) R. A. Alberty, *Advances in Enzymology*, **17**, 42 (1956).

(23) The coefficient of the transient term in p_0 is always negligible compared to the coefficient of the other term when $s_0 \gg e_0$ and was neglected here.

and

$$\left|\frac{(k_4 - k_1)k_3}{k_4(k_2 + k_3)}\right| < 1 \qquad (38)$$

the summations in equations 35 and 36 converge and yield

$$x_{eq} = \frac{k_1k_4e_0s_0}{k_1k_4s_0 + k_1k_3 + k_2k_4} \qquad (39)$$

$$p_{eq} = \frac{k_1k_3s_0}{k_1k_3 + k_2k_4} \qquad (40)$$

Equations 39 and 40 are the correct equilibrium expressions when $s_0 >> e_0$. If $k_4 > k_1$ the inequalities 37 and 39 will always be satisfied and therefore the summations in equations 35 and 36 always converge to give the correct equilibrium expressions. However when $k_4 < k_1$ the inequalities will not *always* be satisfied and then the perturbation solution is not always applicable.

The first term in equation 35 is $(x_0)_{eq}$ and the first term in equation 36 is $(p_0)_{eq}$; the second terms are $(x_1)_{eq}$ and $(p_1)_{eq}$, etc. Thus by using particular values of the rate constants, s_0 and e_0 the number of perturbation terms needed to give any desired agreement with the correct value can be determined. In making the numerical calculations it appeared that this was also a good criterion for determining the number of perturbation terms needed to make the time course of the over-all reaction agree with the steady state solution.

Acknowledgments.—The authors are indebted to Miss Selma Hayman for many of the numerical calculations, to Dr. Wolfgang Wasow for suggesting the use of a perturbation solution and to Dr. Leonard Peller for suggestions concerning this research.

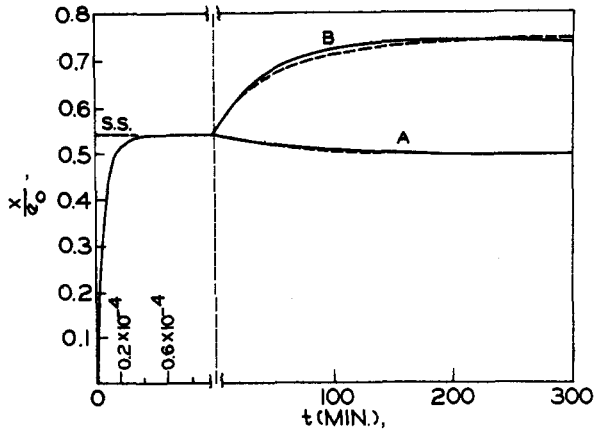

Fig. 4.—Plots of x/e_0 *vs.* t: Curve A was calculated using $k_1 = 7.9 \times 10^8\ M^{-1}$ min.$^{-1}$, $k_2 = 6.58 \times 10^4$ min.$^{-1}$, $k_3 = 1.49 \times 10^3$ min.$^{-1}$, $k_4 = 7.9 \times 10^7\ M^{-1}$ min.$^{-1}$, $s_0 = 10^{-4}\ M$ and $e_0 = 3 \times 10^{-10}\ M$. Curve B was calculated using the same values for k_1 and s_0 but with $k_2 = 2.07 \times 10^4$ min.$^{-1}$, $k_3 = 4.67 \times 10^4$ min.$^{-1}$, $k_4 = 7.9 \times 10^9\ M^{-1}$ min.$^{-1}$ and $e_0 = 10^{-11}\ M$. The first two perturbation terms were used in calculating the solid curve. The dashed curves were calculated using the steady-state assumption.

MADISON, WISCONSIN

[CONTRIBUTION NO. 2335 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY]

Statistical Factors in the Correlation of Rate Constants and Equilibrium Constants

BY SIDNEY W. BENSON[1,2]

RECEIVED APRIL 26, 1958

It is shown that in comparisons of equilibrium constants with each other or of rate constants among a set of rate constants, it is the intrinsic or "chemical" constants which should be compared and not the observed constants. These chemical constants, K_{chem} (or k_{chem}), are related to the observed constants K (or k) by the relation: $K_{chem} = K/K_\sigma$; $k_{chem} = k/K^{\ddagger}_\sigma$ where K_σ and $K^{\ddagger}_\sigma$ are the ratios of symmetry numbers for reactant and product species in equilibrium and chemical reaction, respectively. This leads to some interesting changes in the "relative" base strengths of amines. The symmetry corrections are derivable from statistical mechanics and are equivalent to some of the more intuitive methods in current use. In the case of the Brönsted relation, correlating general acid-base catalytic behavior with acid strength, it leads to a consistent method of assigning symmetry corrections to both k and K_{ion}. The need for such corrections in other "linear free-energy relationships" is pointed out.

Introduction

Since the early success of Brönsted and Pedersen[3] in correlating the catalytic rate constants of acids and bases with their ionization constants, it has become popular to extend this procedure to other reactions. Thus the Hammett[4] treatment of the acidities of substituted benzene derivatives and their correlation with rate constants is a further example of a correlation which is now come to be known more generally as the "linear free energy" relationship. It was early realized[3,5] that such correlations offer ambiguities when species of

(1) Visiting Research Fellow in Chemistry in the Division of Chemistry and Chemical Engineering. On sabbatical leave from the Chemistry Department, University of Southern California.

(2) The author wishes to acknowledge his appreciation to the National Science Foundation for a Senior Post-Doctoral Fellowship which made possible the present work.

(3) J. N. Brönsted and K. Pedersen, *Z. physik. Chem.*, **108**, 185 (1924).

(4) L. P. Hammett, *Chem. Revs.*, **17**, 125 (1935); *Trans. Faraday Soc.*, **34**, 156 (1938).

(5) J. N. Brönsted, *Chem. Revs.*, **5**, 322 (1928).