Analysis of Hill Interaction Coefficients and the Invalidity of the Kwon and Brown Equation*

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SUMMARY

The equation \( \log (1/v - 1/V') = \log \frac{K'_m/V'}{(n - 1) \log (S)} \) was derived by Kwon and Brown (J. Biol. Chem., 241, 1509 (1966)) as a criterion for estimation of \( n \), the number of substrate-binding sites per enzyme molecule. This equation is algebraically equivalent to the Hill equation, except that \((n - 1)\) replaces \( n \) of the Hill equation. The derivation of the Kwon-Brown equation is erroneous and the equation is consequently invalid.

Kwon and Brown (1) have derived the following general rate equation for the determination of the number of substrate molecules, \( n \), bound per mole of enzyme (\( V' \) and \( K' \) refer to high substrate concentrations):

\[
v = \frac{V[1 + \beta(S)^{-1}/K'_m]S}{(S) + K_m + S'K'_m} \tag{1}
\]

which can be written as

\[
v = \frac{VK'_m + V'(S)^{-1}}{K_mK'_m/(S) + K'_m + (S)^{-1}} \tag{2}
\]

where \( V' = \beta V \).

In the region of high substrate concentration, this equation was simplified to

\[
v = \frac{V'(S)^{n^{-1}}}{K'_m + (S)^{n^{-1}}} \tag{3}
\]

by the omission of \( V K'_m \) from the numerator and \( K_m K'_m/(S) \) from the denominator. Operationally, from the log form of the simplified equation

\[
\log (1/v - 1/V') = \log K'_m/V' - (n - 1) \log (S) \tag{4}
\]

a plot of \( \log (1/v - 1/V') \) against \( \log (S) \) was used to determine a value for \( n \). The unit slope they obtained was interpreted as evidence for the binding of 2 molecules of fructose 1,6-diphosphate per mole of aldolase. Murphey and Kaplan (2) also used this equation and have similarly concluded from the slope of 1 at high substrate concentrations that 2 molecules of oxalacetate reacted per mole of malate dehydrogenase.

It was recognized by Kwon and Brown that their data did not fit the unmodified Equation 2. When their data (1) or that of Murphey and Kaplan (2) are plotted according to the complete Equation 1, the data of both are found to differ significantly from expected rates calculated from the values given for \( V, V', K_m \), and \( K'_m \), with \( n \) set at 2. Although the data fit the logarithmic form of the modified equation, it is a necessary condition that it should also fit the unmodified equation. However, if the unmodified equation itself is erroneous, the relationship between the equation and the data is without significance.

The equation of Hill (3) also supplies a measure of \( n \). Based on the form

\[
\log \left[ \frac{v}{(V - v)} \right] = n \log (S) - \log K \tag{5}
\]

\( \log [v/(V - v)] \) is plotted against \( \log (S) \) to yield an estimate of \( n \). Hill plots for both enzymes, at both high and low substrate concentrations, have unit slope.

Atkinson, Hathaway, and Smith (4) have shown that the Hill equation follows from the usual Michaelis assumptions, with the added postulation of cooperative binding of substrate. The slope, \( n \), is a "function of the number of binding sites per enzyme molecule and of the strength of interaction." The interaction coefficient, \( n \), does not indicate the number of noninteracting sites on an enzyme. If the intrinsic interaction is weak, \( n \) becomes 1, regardless of the number of sites.

Kwon-Brown Equation 4 can be rearranged to show its analogy to Hill Equation 5:

\[
\log \left[ \frac{v}{(V - v)} \right] = (n - 1) \log (S) - \log K \tag{6}
\]

Equations 5 and 6 differ, therefore, only in their interpretation of the slope, which is \( n \) according to the Hill equation, and \((n - 1)\) according to Kwon and Brown (1). These equations cannot both be correct. The Kwon-Brown derivation is in fact erroneous, and the conclusion that the slope of the Hill plot is \( n - 1 \) is not valid. Since this treatment has been used to estimate a value of \( n \) in at least one other case (2), it seems necessary to point out the errors involved in the derivation.

The derivation by Kwon and Brown begins with Equation 7 at low values of \( (S) \) and Equation 8 at high values.

\[
E + S \xrightleftharpoons[k_{-1}]{k_{+1}} ES \xrightarrow{k_{+2}} E + P \tag{7}
\]
Reaction of the $ES_0$ complex is assumed to be more rapid than that of $ES$; hence, $\beta > 1$. The Michaelis constants for $ES$ and $ES_n$ are denoted by $K_n$ and $K'_n$, respectively. At low substrate concentrations, $ES$ is assumed to be more likely to react than to bind further molecules of substrate; thus, Equation 7 applies. At high substrate concentrations, on the other hand, binding of additional substrate is more likely, and in this case Equation 8 is assumed to apply. These considerations led to Equations 9, 1, and 2,

$$v = k_{+1} (ES) + \beta k_{+2} (ES_n)$$

where $V$ is the maximal velocity corresponding to Equation 7 (that is, $k_{+4} (E)$).

Kwon and Brown point out that as substrate concentrations become small, terms containing $(S)^n$ vanish, and the equation reduces to the classical Michaelis equation.

$$v = V (S)/[K_n + (S)]$$

It is obvious that, as $(S)$ becomes large, the terms in $(S)^n$ become dominant, and the equation reduces to

$$v = \frac{\partial V}{\partial n} = V$$

This is in accord with the necessary kinetic conclusion that the reaction velocity approaches a maximum value as the substrate concentration increases. Kwon and Brown, as noted above, suggest that there is a region of high substrate concentration in which Equation 2 may be simplified to

$$v = V' (S)^{n-1}/[K'_n + (S)^{n-1}]$$

It is clear that Equations 7 and 8 do not adequately describe any situation other than that where $n$ equals 2. For higher values of $n$, the situation at high substrate concentrations must approach

$$ES_{n-1} + S \rightarrow ES_n \rightarrow ES_{n-1} + P$$

That is, when a substrate-binding site is uncovered by dissociation of product, it will very rapidly adsorb another molecule of substrate; thus, enzyme molecules bearing fewer than $n - 1$ molecules of substrate will be virtually nonexistent. Since the two forms differ by only 1 molecule of substrate, the slope of the Hill plot in this region must have a value of 1. At very low substrate concentrations, the enzyme exists almost entirely as free enzyme, $E$, with a small amount of $ES$. No other species is kinetically significant; thus, the value of the Hill slope in this region is again 1. Thus, although the simple Hill equation, based as it is on the assumption of infinitely cooperative binding sites, corresponds to a straight line of slope $n$, any real chemical situation to which it might be applied must in principle yield plots with slopes of unity at both high and low extremes of concentration. The situation has been discussed and curves presented by Wyman (5). The slope may approach $n$ only at intermediate values of substrate concentration. Evidently, as the substrate concentration is increased above this intermediate range, the slope of the line decreases from $n$ to 1. At some intermediate point, the slope necessarily has the value of $n - 1$. In this region, however, the slope will be changing relatively rapidly, and no linear segment with slope $n - 1$ will be observed. As Kwon and Brown point out, when $n$ equals 2, Equation 1 is identical with an equation derived by Webb (6). However, as shown above, 2 is the only value of $n$ for which Equation 1 is valid, since it depends on the kinetic assumptions of Equations 7 and 8 and, for any value of $n$ other than 2, Equation 8 is based on an implausible assumption. If, for the sake of illustration, $n$ is taken as 4, Equation 8 implies that only $ES$ and $ES_4$ are kinetically significant at high substrate concentrations. The distribution of enzyme between the species $E$, $ES$, $ES_n$, and $ES_4$ has been briefly discussed for the situation of noncooperative and of highly cooperative binding (4). No plausible pattern of binding energies could result in near-total partitioning of enzyme between $ES$ and $ES_4$. The virtual impossibility of such a situation is the basis for the invalidity of the Kwon-Brown equations and of the criterion of $n$ drawn from them.

The relation of the Kwon-Brown treatment to experimental results may be summarized as follows. For any likely situation, the maximal slope of the Hill plot supplies a minimal estimate of $n$, the number of interacting ligand-binding sites on the enzyme molecule. At both high and low values of substrate concentration, the limiting slope of the experimental curve is 1. Thus, the slope will vary between 1 and a maximal value that may approach $n$. In no case corresponding to a realistic enzymic reaction will it be valid to attribute a value of $(n - 1)$ to the slope in any operationally specifiable region.

Kwon and Brown proposed their equation, which implies a value of $n - 1$ for the slope of the Hill plot, for use in the evaluation of experimental results. The use of this equation as proposed will inevitably lead to incorrect conclusions and should therefore be discontinued.

REFERENCES

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