Determination and Statistical Comparisons of Michaelis-Menten Kinetic Parameters[1]

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Summary. A method is described for determining and statistically comparing Michaelis-Menten kinetic parameters.

Many methods have been published for determination of Michaelis-Menten kinetic parameters. This paper describes a method for obtaining K_m and V when multiple measurements of v at each C must be made. It further presents a procedure in which statistical comparisons can be made between the constants found under various conditions. This procedure is valid when $v(C)$, the variance of C, is negligible compared to $v(v)$.

The Michaelis-Menten relationship can be expressed as: $C_i/v_i = (K_m/V) + ((1/V)C_i)$, where the regression coefficient (slope of the line), $1/V$, can be estimated by a weighted linear regression. In the following procedure each weight will equal the inverse of the variance of the dependent variable, C_i/v_i, since confidence in measurement of C_i/v_i increases as $v(C_i/v_i)$ decreases. $v(C_i/v_i)$ can be expressed as[2]: $(v(v_i)C_i^2)/\mu_i^4$, where μ_i is the true velocity at C_i. ($v(v_i)$ can be calculated from the following: $v(v_i) = \left(\sum_{j=1}^{r_i}(v_{ij}^2) - \left[\left(\sum_{j=1}^{r_i}(v_{ij})\right)^2/r_i\right]\right)\Big/ r_i(r_i-1)$ where r_i is the number of measurements taken at C_i. This value differs for each C_i depending on the scatter of the individual measured velocities, v_{ij}, and the number of replications, r_i.) Also the true velocities are unknown, but can be estimated as the means of the measured velocities. Our first approximation of w_i, the weight at C_i, is: $w_i = (v_i)^4/(v(v_i)C_i^2)$.

The regression coefficient, b, which is actually $1/V$, can be estimated as[3]

$$\hat{b} = \frac{\left[\sum_{i=1}^{n}(w_iC_i(C_i/v_i))\right] - \left[\left(\sum_{i=1}^{n}w_iC_i\right)\left(\sum_{i=1}^{n}w_i(C_i/v_i)\right)\Big/\sum_{i=1}^{n}w_i\right]}{\left[\sum_{i=1}^{n}(w_iC_i^2)\right] - \left[\left(\sum_{i=1}^{n}w_iC_i\right)^2\Big/\sum_{i=1}^{n}w_i\right]}. \quad (1)$$

The intercept, a, of the regression line can be estimated as:

$$\hat{a} = \left[\left(\sum_{i=1}^{n}w_i(C_i/v_i)\right)\Big/\left(\sum_{i=1}^{n}w_i\right)\right] - \hat{b}\left[\left(\sum_{i=1}^{n}w_iC_i\right)\Big/\left(\sum_{i=1}^{n}w_i\right)\right]. \quad (II)$$

The linear relationship between C and C/v can be approximated as $C/v = \hat{a} + \hat{b}C$.

Iteration leads to improved estimates of a and b. The true velocities can be better approximated by: $\hat{\mu}_i = ((\hat{a}/C_i) + \hat{b})^{-1}$. The refined weights now become: $w_i = [(\hat{a}/C_i) + \hat{b}]^{-4}/[v(v_i)\,C_i^2]$ and equations I and II give a refined estimate of the regression coefficient and intercept.

$v(b)$ can now be calculated using the residual mean square, s^2, to estimate the variance factor, σ^2. The following equations should be used:

$$s^2 = \left[\sum_{i=1}^{n}w_i((C_i/v_i) - (\hat{a} + \hat{b}C_i))^2\right]\Big/(n-2) \text{ and } v(b) = s^2\Big/\left[\sum_{i=1}^{n}w_i(Ci - (\sum_{i=1}^{n}w_iC_i\Big/\sum_{i=1}^{n}w_i))^2\right].$$

We now have a method for determining $1/V$ and $v(1/V)$ for various experimental conditions. If an estimate of V is desired, the reciprocal of $\hat{b}$ may be used.

Recently a method was described for determining K_m independently of V, and this procedure has been applied to determine the variance of K_m[4]. The new method employs the linear relationship $\bar{R} = C/K_m$ where

$$\bar{R}_i = \left[\sum_{\substack{j=1\\ i\neq j}}^{n}\frac{v_j(C_i - C_j)}{C_j(v_i - v_j)} - 1\right]\Big/(n-1)$$

and n is the number of substrate concentrations studied; v_j is the velocity of the reaction at C_j. If $\bar{R}_i$ is plotted versus C_i, the regression coefficient is $1/K_m$. The weight to be used is again the reciprocal of the variance of the dependent variable, $[v(\bar{R}_i)]^{-1}$. These weights can be obtained directly when calculating $\bar{R}_i$. The problem of determining $1/K_m$ is now one of performing a weighted linear regression through the origin. The applicable equations are:

$$\hat{b} = \left(\sum_{i=1}^{n}w_iC_i\bar{R}_i\right)\Big/\left(\sum_{i=1}^{n}w_iC_i^2\right) \text{ and } v(b) = s^2\Big/\left(\sum_{i=1}^{n}w_iC_i^2\right)$$

where $s^2 = \left(\sum_{i=1}^{n}w_i(\bar{R}_i - C_i\hat{b})^2\right)/(n-1)$. Since b is $1/K_m$, $\hat{b}$ is an estimate of $1/K_m$. An estimate of $v(1/K_m)$ has also been given. If an estimate of K_m is desired, $1/\hat{b}$ may be used.

We are now able to determine whether or not the difference in the regression coefficient, be it $1/V$ or $1/K_m$, caused by altering the conditions is significant. Indeed, if we can show that $1/V$ (or $1/K_m$) changes significantly, then V (or K_m) also changes significantly. One of two procedures must now be followed depending upon whether or not the variance factors of the lines to be compared are homogeneous. The equality of s_1^2 and s_2^2 may be tested by the standard F-test[5]. For homogeneous variance factors, their joint estimate is given by[6]: $s_p^2 = [(n_1 - A)s_1^2 + (n_2 - A)s_2^2]/(n_1 + n_2 - 2A)$ with A = 2 when working with $1/V$ and A = 1 with $1/K_m$. A test of the hypothesis that V_1 is equal to V_2 is then given by: $t = (b_1 - b_2)\Big/\left(s_p\sqrt{\frac{v(b_1)}{s_1^2} + \frac{v(b_2)}{s_2^2}}\right)$ which will have a Student's distribution with $n_1 + n_2 - 4$ degrees of freedom, or for the test that K_{m1} is equal to K_{m2}, $n_1 + n_2 - 2$ degrees of freedom[6].

When the variance factors of the two regression coefficients are not homogeneous, both t and df, the degrees of freedom, should be modified[7]. To test the hypothesis that V_1 and V_2 (K_{m1} and K_{m2}) are not statistically different, the following equations are used: $t = (b_1 - b_2)/\sqrt{v(b_1) + v(b_2)}$ and $df = [(v(b_1)/s_1^2) + (v(b_2)/s_2^2)]^2/[(v(b_1)^2/s_1^4(n_1 - A)) + (v(b_2)^2/s_2^4(n_2 - A))]$ with A = 2 when testing $1/V$ and A = 1 when testing $1/K_m$.

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Kinetic parameters and statistical tests for L-leucine transport in chicken intestine

Experiment	Preincubation	Incubation	n	$1/V$	V	σ^2	$v\,(1/V)$	$1/K_m$	K_m	σ^2	$v\,(1/K_m)$
1	KHB	KHB	4	0.0107	94	0.00914	4.12×10^{-7}	0.337	2.97	0.855	5.88×10^{-4}
2	ChKHB	KHB	5	0.0249	40	0.0693	2.30×10^{-6}	0.229	4.37	0.621	1.26×10^{-6}
3	ChKHB	ChKHB	8	0.0721	14	0.0301	9.05×10^{-5}	0.227	4.41	0.613	1.05×10^{-3}

	Tests of V			Tests of K_m		
Experiments	t	df	P	t	df	P
1 versus 2	7.5467	5	< 0.001	4.8572	7	< 0.001
1 versus 3	7.0553	8	< 0.001	2.7118	10	< 0.05
2 versus 3	4.1204	9	< 0.01	0.0571	11	N.S.

For details on symbols, see text. The velocity of transport of L-leucine was studied in the chicken ileum and for each substrate concentration was the mean of 8–40 determinations on tissue from 4–20 animals. V is reported in nmol/cm² intestine/min and K_m in mM. Preincubation was for 30 min and incubation with tracer substrate was for 1 min. KHB, Krebs Henseleit buffer; ChKHB, Na^+ -free, choline cation-substituted Krebs Henseleit buffer. For other details, see BURRILL et al.[8]. The t-values were computed on the basis of homogeneous variance factors (see text).

To illustrate the method we evaluated the kinetic parameters for the initial velocity of L-leucine transport in the chicken ileum (Table). The results indicate that the replacement of Na^+ by choline cation in the preincubation solution significantly decreased V and increased K_m. Incubation in the absence of Na^+ led to a further significant decrease in V with no further change in K_m. An objective, properly weighted analysis is essential for all data if the constants are to be compared statistically. This comparison requires an estimate of the variance of the constants; these variances cannot be estimated by simple graphical methods.

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The Effects of Various Agents in vitro on Homocarnosine-Carnosine Synthetase from Rat Brain[1]

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Summary. The present paper reports the first evidence that homocarnosine-carnosine synthetase from rat brain requires free sulfhydryl groups for activity. The activity of the synthetase can be stabilized by dithioerythritol and inhibited strongly by Cu^{2+}, Cd^{2+}, Hg^{2+}, Zn^{2+}, and to a lesser extent by Ca^{2+}, Ni^{2+}, Li, chlorpromazine, α-methyl DOPA and norepinephrine at the concentrations tested.

The L-histidine-containing dipeptides carnosine (β-alanyl-L-histidine) and homocarnosine (γ-aminobutyryl-L-histidine) are found in excitable tissue. Carnosine was first discovered in muscle in 1900 by GULEWITSCH and AMIRADZIBI[3]. In 1962 ABRAHAM et al.[4] found homocarnosine and small amounts of carnosine in the brains of man and several other mammalian species. Although relatively little is known about the biological roles of these two dipeptides, recent studies[5–7] suggested that they may have unique functions in the central nervous system (CNS). Homocarnosine-carnosine synthetase catalyzes the formations of carnosine from β-alanine and histidine, and of homocarnosine from GABA and histidine. Its isolation and partial purification from rat brain have been reported by SKAPER et al.[8]. However, instability of the enzyme has hindered further purification. The purpose of the present study was to investigate the effects of various agents on the enyzme in order to stabilize its activity. Furthermore, in view of the findings[9,10] that certain drugs lowered levels of homocarnosine and carnosine in rat brain in vivo, several CNS agents were tested in the present study.

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