Analysis of Enzyme Kinetics in Invertebrates

- Derivation of the Michaelis-Menten Relation
- Experimental Use of the Michaelis-Menten Equation
- Enzyme Kinetics Experiment

Experimental Use of M-M Equation

I. Review

A. Chemical equation

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_3} E + P$$

where E=enzyme, S=substrate, C=complex, and P=product.

B. Mathematical Equations

$$\frac{dE}{dt} = -k_1SE + k_2C + k_3C$$

$$\frac{dS}{dt} = -k_1SE + k_2C$$

$$\frac{dC}{dt} = k_1SE - (k_2 + k_3)C$$

$$\frac{dP}{dt} = k_3C$$

C. Leads to the Michaelis-Menten equation

$$\frac{dP}{dt} = \frac{V_{\max}S}{K_m + S}$$

where $V_{\max}$ is the maximum rate of product formation and K_m is the 1/2-saturation coeffiicent.

II. Experimental Uses Effects of experimental treatments on enzyme kinetics (e.g., temperature, drugs, etc).

Compare K_m or $V_{\max}$ at different levels. Therefore, need estimates of the parameters under different conditions.

III. Two Kinds of Regression Problems

A. Models (equations) that are **Linear in the Parameters** (LITP). E.g.,

$$y = mx + b$$

All parameters (m = slope and b = intercept) appear as a constant (e.g., b) or as a simple multiplication of a constant (e.g., straight line above). Another example:

$$y = a_0 + a_1 x + a_2 x^2$$

The variable y is not linear over values of x, but it is linear if a_0, a_1, and a_2 are considered variables and x is considered constant.

$$y = a_0 + a_1 x_1 + a_2 x_2$$

Also LTIP, but has 2 independent variables (problem is to find slop and intercept of a plane, not a line.

B. Not LITP

Operations involving the parameters that cause it to not be LTIP: raise to power, use in denominator, use inside a function (e.g., $\sin(bx)$, $e^b x$). For example,

$$y = ax^b$$

a is okay, but the problem is b; it is not simple addition or multiplication.

$$y = \frac{ax}{b + x}$$

b is in the denominator.

IV. LTIP models can be solved using linear regression

A. Basic idea: (above) observed y equals straight line plus or minus some **error** (ϵ_i).

$$y_i = a_0 + a_1 x_i + \epsilon_i$$

where a_0 and a_1 must be estimated from data.

Another example:

$$y_i = a_0 + a_1 x_i + a_2 x_i^2 + \epsilon_i$$

B. Problem: What function to use for ϵ?

Let the error be the sum of squared deviations of the model from the data:

$$\epsilon = \sum_{i=1}^{N} [y_i - (a_0 + a_1 x_i)]^2$$

where N = number of data points.

C. Linear Regression is an Optimization (Minimization) Problem

Choose a_0^* and a_1^* such that

$$\epsilon = \min \left(\sum [y_i - (a_0^* + a_1^* x_i)]^2 \right)$$

D. Regression Terminology (next page)

V. What if the model is not in the form $y = mx + b$?

A. Transformation

How to transform this?

$$y = ax^b$$

Regression takes place in this space:

B. Lineweaver-Burke Transform of the M-M equation

Inverse transformation: invert both sides

$$\frac{1}{y} = \frac{K_m}{V_{\max}} \frac{1}{S} + \frac{1}{V_{\max}}$$

where S is the substrate concentration, y is the rate of product formation. (Algebra left for students.)

Regression takes place in this space:

C. SAS (Statistical Analysis System) Program and Output

(Next page)

D. Eadie-Hofstee Transformation

$$\frac{y}{S} = \frac{V_{\max}}{K_m} - \frac{y}{K_m}$$

(algebra left for students). SAS program and output on next pages.

Regression in this space:

E. Hanes

$$\frac{S}{y} = \frac{S}{V_{\text{max}}} + \frac{K_M}{V_{\text{max}}}$$

(algebra left for students). SAS program and output on next pages.

Regression in this space:

VI. Problems with the Inverse Transformation

Can inflate the R^2 of the model by creating groups of data. E.g., suppose we have these evenly spaced data:

x	1	2	3	4	5
y	1	2	3	4	5

Transformed:

$1/x$	1	0.5	0.333	0.25	0.2
$1/y$	1	0.5	0.333	0.25	0.2

Notice "clumping" of data; data are no longer uni-

formly distributed. This produces really just 2 "points" near 1.0 and small values near 0.25. Fitting a straight line to 2 points always gives a good R^2.
For this and other reasons, transformation is to be avoided if at all possible. Modern statistical packages make it easy to do regression without transforming data.

VII. Estimating parameters when the model in not LITP

$$y = ax^b$$

Error function is the same (usually)

$$\epsilon = \sum_{i=1}^{N} [y_i - (ax_i^b)]^2$$

where N = number of data points.

VIII. Can not solve for a and b directly.
Assume we don't want to do a log transform.

A. The problem:

B. Use computer iteration to move from point in parameter space that has high error to a point with low error.

C. Start at some point and choose new point to reduce ϵ.

D. Stop computer iteration until sufficiently close to minimum.

E. DANGER: Can stop at local minimum

F. How to choose new point? Levenberg-Marquardt method.

SAS progrm and output

IX. Advantages and Disadvantages

A. Linear Regression

Easy, quick, exact; but only straight lines and may require transformations that may distort the data.

B. Nonlinear Regression

Any function, any number of parameters, no transformations; but complex to program, may need multiple starting points