A STEADY--STATE ENZYME KINETICS MODELING SYSTEM

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Abstract

A technique is presented to generate rate equations for random enzymatic mechanisms in a systematic manner. The generation of constraint equations arrived from the principle of detailed balance is also discussed. The rate constants of the reaction mechanisms are then estimated by a stepwise least squares algorithm.

I. Generation of Initial Rate Equation

In 1956, King and Altman devised a systematic method, applicable to random as well as sequential binding order mechanisms, for deriving initial velocity rate equations (4). This manual technique uses rate constants and reactant concentrations obtained from all valid pathways of a mechanism in the formulation of an initial velocity rate equation.

As the complexity of the mechanism increases so does the difficulty in deriving the rate equation even with the technique of King and Altman. Utilizing linear graph theory concepts, the method developed by Lam and Priest (5) generates all valid patterns for an enzyme mechanism. An enzymatic mechanism is described as a connected graph with numbered branches and numbered nodes, corresponding to reaction steps and enzyme forms, respectively. Patterns can be generated from the graph by applying Wang algebra principles in a stepwise procedure. These patterns are then used to generate the initial velocity equations.

II. Generation of the Constraint Equations.

The value of the rate constants belonging to cycles within a random binding mechanism are restricted as a result of the law of microreversibility. The law of microreversibility, also known as the principle of detailed balance, requires forward and reverse fluxes in a time independent state to be equal. Consider an entire reaction for a mechanism at steady-state conditions. The concentrations of its intermediate enzyme complexes remain constant. If a cycle within the mechanism is at equilibrium, with no net change occurring for its reactant concentrations, then all enzyme complex concentrations for that cycle remain constant. For each of these enzyme complexes in the cycle, the change of its concentration over time, i.e., the forward flux minus the reverse flux of that reaction step, is equal to zero. It then follows that the forward flux is equal to the reverse flux in a cycle.

Due to the cyclic nature, the equality between forward flux and reverse flux in a cycle can be reduced by substitution and elimination of the enzyme complex and reactant concentrations. The resulting relationship equates the product of the ratios of rate constants for each reaction in the cycle to unity (2, 3). Specifically, the product of the rate constants of a cycle in a clockwise direction is equal to the product of the remaining rate constants in the counter clockwise direction. This type of relationship is hereby called a constraint equation. There are as many constraint equations as there are reversible cycles in the mechanism.

However, there could be more than one cycle in a mechanism. Conceivably, a particular rate constant could appear in many cycles. Thus, it further restricts the value of that rate constant. As the mechanism increases in complexity, it becomes very difficult, to determine all the cycles and their corresponding constraint equations by inspection. A systematic process for generating the constraint equations will be demonstrated with slides.

III. Parameters Estimation

Once the initial velocity rate equation and constraint equations are generated, the usual objective is to determine the values of the unknown rate constants. The parameters of the rate equation, namely, rate constants, are estimated in a least squares sense by means of a stepwise regression algorithm (1). Within each iteration, parameters are selected so that the greatest reduction in the error sum of squares would result. If necessary, only a subset of the parameters are modified in a given iteration to avoid the singularity problem of inverting a matrix. The partial derivatives needed in linearization are

computed by the difference method since they are difficult to obtain analytically. In order to retain enough accuracy in the value of the partial derivatives, double precision arithmetic is used throughout the program.

Testing the model depends a great deal on the derived equation (1). Since the expected mean square error may be a biased estimator of variance, the usual procedure for deciding on the "best" model by selecting the smallest variance is not really valid in the nonlinear case. An approximate F test (1) is provided to determine the lack of fit. A graphic comparison of observed values and predicted values from different models may pinpoint the "best" fitting model.

List of References

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