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Kinetic analysis of multi enzyme systems: A case study of the closed system of creatine kinase, hexokinase and glucose 6-phosphate dehydrogenase

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This paper describes a general methodology to handle closed multi enzyme systems using mixture of symbolic (which depends on the Gröbner Basis technique) and numerical computation methods. The applicability of the proposed method has been examined for the closed three-enzyme system of rabbit heart creatine kinase (EC 2.7.3.2), yeast hexokinase (EC 2.7.1.1) and human erythrocyte glucose 6-phosphate dehydrogenase (EC 1.1.1.49) using experimental data.

KEY WORDS: symbolic and numerical computation, kinetics of multi enzyme system, steady state assumption, parameter estimation

1. Introduction

When a sequence of enzyme reactions form a pathway (a multi enzyme system), its kinetic analysis can be difficult. A multi enzyme system is one whose biologically realistic mathematical description consists of a system of non-linear differential and algebraic equations including a number of unknown parameters that are difficult to estimate, although it is very important from the biochemical point of view to characterise the system. A mixture of symbolic and numerical computation techniques provides a tool for the analysis of such systems.

Modern techniques of computer algebra, which perform symbolic computations, allow previously insoluble problems to be tackled, and thus, offer a route to analyse the system.

Recently, novel techniques have been proposed for the kinetic analysis of multi enzyme systems including a mixture of symbolic and numerical computation methods [1]. Using these techniques, Bayram [2] has investigated the kinetics of two-enzyme systems. The method has been applied to more complex systems [3].

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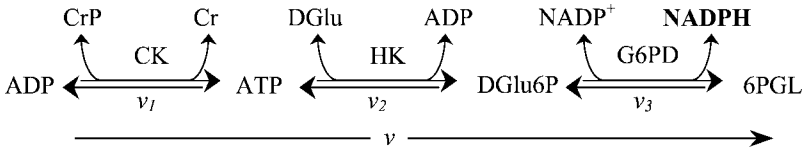

Figure 1. Shematic representation of the closed system of CK-HX-G6PD.

2. Construction of mathematical models for the kinetics of CK-HX-G6PD

The CK-HX-G6PD system can be represented as shown in figure 1. In this system, while kinetics of both CK and HX follow a Random Bi Bi mechanism, the kinetic mechanism of G6PD is Ordered Bi Bi with respect to Cleland's classification [4–7].

According to the mechanisms described in appendix A and B, rate equations v_1, v_2 and v_3 for each enzyme mechanism can be stated easily.

We make the assumption that

$$v_1 = v_2 = v_3 = v \tag{1}$$

at steady state. v is referred to as the overall rate law. Equation (1) can be written as $v - v_1 = 0$, $v - v_2 = 0$, $v - v_3 = 0$. Because the system is closed, the concentrations of the various metabolites are related. According to Reder's algorithms, Yildirim [9] has derived six linearly independent relationships related with its kinetics. The kinetics of the system must satisfy these relationships [10]. These are given by

$$\begin{aligned}
f_1 &= [\mathrm{ATP}] + [\mathrm{ADP}] - [\mathrm{ATP}]_0 - [\mathrm{ADP}]_0 = 0,\\
f_2 &= [\mathrm{DGlu}] - [\mathrm{CrP}] + [\mathrm{ADP}] - [\mathrm{DGlu}]_0 + [\mathrm{CrP}]_0 - [\mathrm{ADP}]_0 = 0,\\
f_3 &= [\mathrm{Cr}] + [\mathrm{CrP}] - [\mathrm{Cr}]_0 - [\mathrm{CrP}]_0 = 0,\\
f_4 &= \left[\mathrm{NADP}^+\right] - [\mathrm{CrP}] + [\mathrm{ADP}] - [\mathrm{DGlu6P}] - \left[\mathrm{NADP}^+\right]_0\\
&\quad + [\mathrm{CrP}]_0 - [\mathrm{ADP}]_0 + [\mathrm{DGlu6P}]_0 = 0,\\
f_5 &= [\mathrm{6PGL}] + [\mathrm{CrP}] - [\mathrm{ADP}] + [\mathrm{DGlu6P}] - [\mathrm{6PGL}]_0\\
&\quad - [\mathrm{CrP}]_0 + [\mathrm{ADP}]_0 - [\mathrm{DGlu6P}]_0 = 0,\\
f_6 &= [\mathrm{NADPH}] + [\mathrm{CrP}] - [\mathrm{ADP}] + [\mathrm{DGlu6P}] - [\mathrm{NADPH}]_0\\
&\quad - [\mathrm{CrP}]_0 + [\mathrm{ADP}]_0 - [\mathrm{DGlu6P}]_0 = 0,
\end{aligned} \tag{2}$$

where subscripts indicate initial metabolite concentrations. These six relationships, together with the rate equations, give us a total of nine simultaneous (non-linear) equations under the assumption given in equation (1) describing the kinetics of the closed system.

3. Experimental procedure

Experimental data for NADPH concentration against time was obtained from the Biotechnology Application and Research Center in our university. For experimental procedure, we have followed the method given in Bergmeyer [11]. The temperature is kept

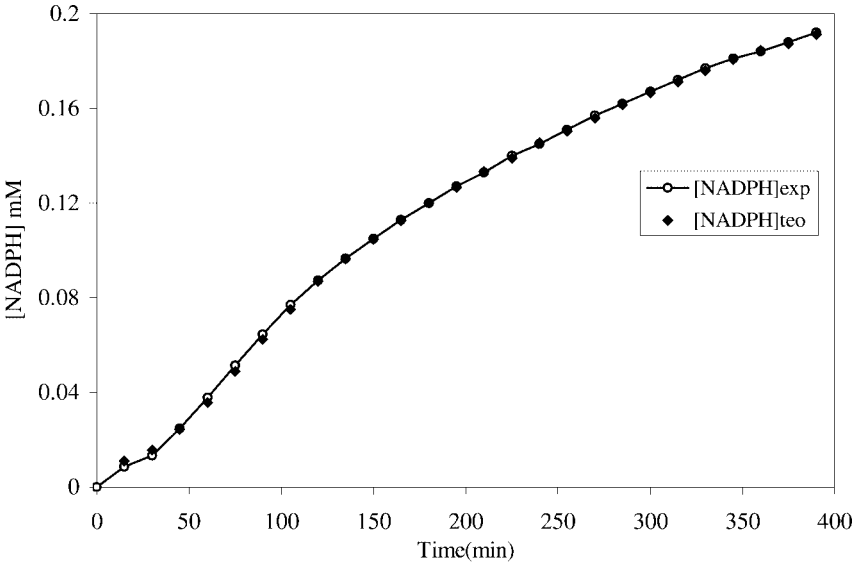

Figure 2. The experimental curve and the curve drawn substituting the estimated values of the selected parameters for NADPH concentration against the time.

constant at 25°C along the time course of the reaction, and we observed the concentration of NADPH over time using UV–VIS spectrophotometer. Data points were sampled every 15 min as depicted in figure 2. Initial metabolite and enzymes concentrations used in the experiment are given in by

$$\begin{aligned}
&[\mathrm{ADP}]_0 = 5.9 \times 10^{-1}\ \mathrm{mM}, && [\mathrm{DGlu}]_0 = 1.13 \times 10^{1}\ \mathrm{mM},\\
&[\mathrm{CrP}]_0 = 2.55 \times 10^{1}\ \mathrm{mM}, && \left[\mathrm{NADP}^{+}\right]_0 = 3.3 \times 10^{-1}\ \mathrm{mM},\\
&[\mathrm{ATP}]_0 = 0.0 \times 10^{0}\ \mathrm{mM}, && [\mathrm{Cr}]_0 = 0.0 \times 10^{0}\ \mathrm{mM},\\
&[\mathrm{DGlu6P}]_0 = 0.0 \times 10^{0}\ \mathrm{mM}, && [\mathrm{6PGL}]_0 = 0.0 \times 10^{0}\ \mathrm{mM},\\
&[\mathrm{NADPH}]_0 = 0.0 \times 10^{0}\ \mathrm{mM}, && [\mathrm{CK}]_0 = 8.0 \times 10^{-6}\ \mathrm{mM},\\
&[\mathrm{HX}]_0 = 3.4 \times 10^{-5}\ \mathrm{mM}, && [\mathrm{G6PD}]_0 = 1.4 \times 10^{-5}\ \mathrm{mM}.
\end{aligned} \tag{3}$$

4. Computation methods and Gröbner Basis technique

The kinetics of the system depicted in figure 2 was analysed using both symbolic and numerical computation methods. We have used the Gröbner Basis method as a symbolic technique. As numerical methods, the solution of initial value problem, finding roots of a univariate polynomial and optimisation of a multivariable function have been used.

For numerical computations, we used NAG FORTRAN library [12] and PRAXIS [13]. C02AEF was implemented for finding all roots of a univariate polynomial with constant coefficients using a search algorithm. D02AEF that integrates a

stiff system of first-order ordinary differential equations over a range with suitable initial conditions, using a variable-order, variable-step method implementing the backward differentiation formulae is used as an integration routine. Finally, for the optimization of multivariable function, PRAXIS has been implemented which uses Brent's algorithm. All floating-point computations are performed in double precision.

We have used MAPLE 4.0 as a computer algebra system for symbolic computations [14]. Both symbolic and numerical computations were performed on an IBM compatible PC with 64 MB RAM and Pentium III processor running under the Windows 98 operating system.

4.1. Gröbner Basis as a symbolic method and its computation via MAPLE

In this section, the basic concepts of a Gröbner Basis will be presented. We have restricted the discussion to the parts related to our work. Details can be found in [15–17].

Let R be the ring of all polynomials in $x_1, x_2, \ldots, x_n$ with real coefficients. A product $x_1^{m_1} x_2^{m_2} \cdots x_n^{m_n}$, with nonnegative exponents, is called monomial. A set of power products is denoted by $\boldsymbol{T}^n = \{x_1^{\beta_1} \cdots x_n^{\beta_n} \mid \beta_i \in \mathbb{N}, i = 1, \ldots, n\}$. Sometimes $x_1^{\beta_1} \cdots x_n^{\beta_n}$ is represented by $\boldsymbol{x}^{\boldsymbol{\beta}}$ where $\boldsymbol{\beta} = (\beta_1, \ldots, \beta_n) \in \mathbb{N}$. To give the definition of Gröbner Basis, we first have to fix a term order.

By a term order on $\boldsymbol{T}^n$ we mean a total order $<$ on $\boldsymbol{T}^n$ satisfying the following two conditions:

(a) $1 < \boldsymbol{x}^{\boldsymbol{\beta}}$ for all $\boldsymbol{x}^{\boldsymbol{\beta}} \in \boldsymbol{T}^n$, $\boldsymbol{x}^{\boldsymbol{\beta}} \neq 1$.

(b) If $\boldsymbol{x}^{\boldsymbol{\alpha}} < \boldsymbol{x}^{\boldsymbol{\beta}}$, then $\boldsymbol{x}^{\boldsymbol{\alpha}} \boldsymbol{x}^{\boldsymbol{\gamma}} < \boldsymbol{x}^{\boldsymbol{\beta}} \boldsymbol{x}^{\boldsymbol{\gamma}}$, for all $\boldsymbol{x}^{\boldsymbol{\gamma}} \in \boldsymbol{T}^n$.

The lexicographical ordering has been used in our computations that is the most suitable to eliminate variables from a set of equations.

We define the *lexicographical order on* $\boldsymbol{T}^n$ with $x_1 > x_2 > \cdots > x_n$ as follows. For $\boldsymbol{\alpha} = (\alpha_1, \ldots, \alpha_n)$, $\boldsymbol{\beta} = (\beta_1, \ldots, \beta_n) \in \mathbb{N}$ we define: $\boldsymbol{x}^{\boldsymbol{\alpha}} < \boldsymbol{x}^{\boldsymbol{\beta}} \Leftrightarrow$ the first coordinates α_i and β_i in $\boldsymbol{\alpha}$ and $\boldsymbol{\beta}$ from the left, which are different, satisfy $\alpha_i < \beta_i$.

Let R be a field, $R[x_1, x_2, \ldots, x_n]$ is a ring of n-variable polynomials over R. If $F = \{f_1, f_2, \ldots, f_n\}$ is a finite subset of $R[x_1, x_2, \ldots, x_n]$, then the *ideal generated by* F denoted by I is defined as

$$I = \left\{ \sum_{i=1}^{n} u_i f_i \;\middle|\; u_i \in R[x_1, x_2, \ldots, x_n],\ f_i \in R,\ i = 1, \ldots, n \right\}.$$

Let $f = a_1 p_1 + a_2 p_2 + \cdots + a_m p_m$ with $a_i \neq 0$ constant, and p_i are monomials satisfying $p_m < p_{m-1} < \cdots < p_1$. The leading term of f, written $\mathrm{lt}(f)$, is $a_1 p_1$. If $f_1, f_2, \ldots, f_s$ are polynomials then the ideal generated by these polynomials is denoted by $\langle f_1, f_2, \ldots, f_s \rangle$. For an ideal $I \subseteq R$ denote by $\mathrm{lt}(I)$ the set of leading terms of elements of I, and by $\langle \mathrm{lt}(I) \rangle$ the ideal generated by the elements of $\mathrm{lt}(I)$.

A finite subset $G = \{g_1, g_2, \ldots, g_s\}$ of an ideal I is said to be *Gröbner Basis* if $\langle \mathrm{lt}(g_1), \mathrm{lt}(g_2), \ldots, \mathrm{lt}(g_s) \rangle = \langle \mathrm{lt}(I) \rangle$. A Gröbner Basis is called a reduced Gröbner Basis

for an ideal I for any g_i, if the coefficient of $\mathrm{lt}(g_i)$ is 1 and no monomial of g_i lies in $\langle \mathrm{lt}(G - \{g_i\})\rangle$.

Proposition 1. Let I be a polynomial ideal. For a given monomial order, I has a unique reduced Gröbner Basis.

Proposition 2. Any Gröbner Basis for an ideal I is a basis for I.

Proposition 3. Let G be a Gröbner Basis of an ideal and f a polynomial. The remainder on division of f by G does not depend on the ordering of the elements of G. Moreover, f is an element of I if and only if the remainder is zero.

Proposition 4. Let $f_1, f_2, \ldots, f_m$ be polynomials. If the reduced Gröbner Basis of $\langle f_1, f_2, \ldots, f_m\rangle$ is $\langle 1\rangle$ then the equations $f_1 = \cdots = f_m = 0$ have no solutions; if the basis is not $\langle 1\rangle$ then they must have a solution which may be complex [16].

In 1965, Buchberger [15] presented an algorithm in order to compute the Gröbner Basis of any given ideal. Many computer algebra systems implement a version of Buchberger's algorithm. These systems usually compute a reduced Gröbner Basis. MAPLE's Gröbner Basis package includes a sub-packages of *gbasis* that computes reduced Gröbner Basis. Syntax of *gbasis* is "*gbasis*$(F, X,$ *termorder*$)$". Here, F is a list of polynomials, X is a list of intermediates and *termorder* is either *tdeg* or *plex* that are the names of term ordering which will be used. *plex* represents lexicographical ordering while *tdeg* means total degree ordering which is out of this study's scope. It computes the reduced Gröbner Basis of the ideal $\langle G\rangle$ with respect to the intermediates X and given term ordering.

5. Parameter estimation procedure for the system of CK-HX-G6PD

To show applicability of the proposed methods to analyse kinetics of a multi enzyme system, three parameters of $K_{\mathrm{M}}^{\mathrm{ADP}}$, $K_{\mathrm{M}}^{\mathrm{CrP}}$ and $K_{\mathrm{I}}^{\mathrm{ADP}}$ for the individual system of CK were selected to be estimated looking at the sensitivity of the overall rate law to variations in the kinetic parameters. For each parameter, k_i, we determine the scaled derivative

$$\frac{k_i}{v}\cdot\frac{\partial v}{\partial k_i} \tag{4}$$

taking all the other parameters at their published values, and concentrations at their experimental levels. A large value of the scaled derivative indicates that fitting k_i by minimising residuals in v will lead to good estimates of k_i. Any small error in k_i will greatly affect v. Correspondingly, where the scaled derivative is small, variation in the estimate for k_i will have little effect on v, and so the estimate is unlikely to be reliable. Values for the scaled derivatives in fact vary through the time course of the experiment, since they are a function of NADPH concentration [2].

We have determined the scaled derivatives at starting concentrations and seen that $K_{\mathrm{M}}^{\mathrm{ADP}}$, $K_{\mathrm{M}}^{\mathrm{CrP}}$ and $K_{\mathrm{I}}^{\mathrm{ADP}}$ greatly affect v.

To estimate the parameters, a Gröbner Basis calculation for the system involving six equations given in equation (2), along with rate equations for individual reactions under the assumption given in equation (1), must be performed. This assumption reduces the mathematical model for kinetics of the system to system of polynomial equations that allow us to make the Gröbner Basis calculation. The mathematical model of the system has 24 distinct kinetic parameters as seen in appendices A and B. Values obtained, under the conditions similar to our experiment conditions in the sense of sources, pH, temperature, enzyme resources etc., for each of these parameters for individual reactions in our system can be found in the literature [4–6]. Substitution of these literature values of all of these parameters leaving the selected parameters in symbolic form, the Gröbner Basis calculation yields a system of polynomials in triangular form. In Gröbner Basis calculation via MAPLE, parameters involved in *gbasis* syntax for our system of polynomials are as follows:

$$\begin{aligned} X &= \{\mathrm{CrP}, \mathrm{ADP}, \mathrm{Cr}, \mathrm{ATP}, \mathrm{DGlu}, \mathrm{DGlu6P}, \mathrm{NADP}^{+}, \mathrm{6PGL}, v\}, \\ F &= \{f_1, f_2, f_3, f_4, f_5, f_6, \mathit{numer}(v - v_1), \mathit{numer}(v - v_2), \mathit{numer}(v - v_3)\}. \end{aligned} \tag{5}$$

$GB = \mathit{gbasis}(F, X, \mathit{plex})$ computes the reduced Gröbner Basis of the ideal $\langle F \rangle$ with respect to the intermediates X and given term ordering, where f_j $(j = 1, 2, \ldots, 6)$ are polynomials given in equation (2) and v_j $(j = 1, 2, 3)$ are rates for individual reactions. In this case, MAPLE's $\mathit{numer}(v - v_i)$ $(i = 1, 2, 3)$ command simply picks off the numerators of $(v - v_i)$ that are clearly polynomials. $\mathit{gbasis}(F, X, \mathit{plex})$ transforms the system F into a Gröbner Basis which can be written in triangular form.

Rearrangement of the polynomials in the basis gives us an 11th order polynomial as the last polynomial of the form given in equation (6) in *GB*:

$$\sum_{i=0}^{11} a_i v^i = 0, \tag{6}$$

where $a_i = h_i([\mathrm{NADPH}], K_{\mathrm{M}}^{\mathrm{ADP}}, K_{\mathrm{M}}^{\mathrm{CrP}}, K_{\mathrm{I}}^{\mathrm{ADP}})$, which is easy to solve numerically, if an experimental value for NADPH concentration and initial values for the parameters to be estimated are substituted. In our study, last eight equations in *GB* are linear in the metabolite concentrations. We can then find the values of all metabolites concentrations by back substituting them into these equations and solving them in turn.

Since equation (6) is an 11th order polynomial, there is more than one root, and of these a meaningful one (which makes all metabolites concentrations positive) has to be chosen. It should be noted that in the real world there must be only one value for v because the enzyme reaction cannot go at two different velocities. For substituting initial values for the parameters in equation (6), the authors are not sure that there is only one positive-real root of equation (6) making all concentrations positive. We noted that improvement by optimisation routine in the parameters values to be estimated carries out this restriction especially in the neighbourhood of the optimal values. This situation

Table 1
Estimated values for the parameters of the closed system.

Parameters		Best fit (mM)	Literature values (mM)	Estimated values (mM)
CK	K_M^{ADP}	0.054	0.050	0.055 ± 0.0041
	K_M^{CrP}	3.114	2.900	3.113 ± 0.0020
	K_I^{ADP}	0.019	0.140	0.018 ± 0.0027

is controlled for several initial values of the parameters and each of experimental values for NADPH concentrations within the program we have developed.

The experimental data consists of NADPH concentration against time. The rate laws we are fitting relate $v = \mathrm{d[NADPH]}/\mathrm{d}t$ to [NADPH], but we need to integrate the rate laws to fit to the data. Since analytical integration of the rate laws is not in general possible, we used numerical integration instead. When fitting to integrated equations, it is important not to perform a complete numerical integration over the range and minimise residuals.

This is essentially an overconstrained multi-point integration problem. We provide estimates for each of three kinetic parameters, integrate numerically from one experimental data point to the next, and record the difference between the numerical and experimental values as a residual error. We do this for all N data points, yielding $N - 1$ residual errors. We then use standard least-squares minimisation to obtain better estimates of parameters that will reduce these residual errors.

The process is iterated. It is terminated either after a predetermined number of iterations, or when F becomes approximately constant over several iterations.

6. Results and discussion

We have used the algorithm explained in the previous section to estimate a total of three parameters. Estimated values of these parameters are given in table 1. Confidence intervals for the parameters are determined by the Bootstrap method [18]. Same estimations were obtained for several starting points. Error tolerances chosen for C02AEF, D02EAF and PRAXIS are 10^{-8}, 10^{-2} and 10^{-3}, respectively. The sum of error squares obtained is 73.49×10^{-12} M^2.

The results of the least-squares fit (a best-fit curve superimposed on the experimental data) are illustrated in figure 2, showing that our parameters are very well.

Although the method presented here is mathematically accurate and can handle more complex situations, there is a problem with this approach, i.e., computer algebra systems may generate massive expressions in Gröbner Basis computation. On evaluation with specific values in floating point arithmetic by numerical packages, such expressions are prone to serious rounding, overflow and underflow errors. Some alternative procedures including interfacing computer algebra and numerical analysis systems have been developed to overcome these difficulties [19].

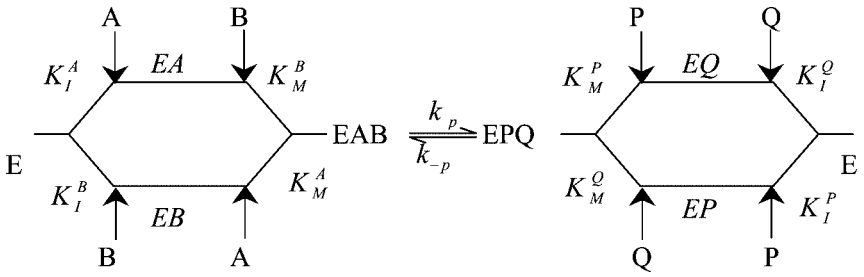

Figure 3.

Nomenclature

[A]	concentration of A
CrP	creatine phosphate
DGlu6P	D-glucose 6-phosphate
DGlu	D-glucose
Cr	creatine
ATP	adenosine triphosphate
ADP	adenosine diphosphate
$NADP^+$	nicotinamide adenin dinucleotide phophate (oxidized form)
NADPH	nicotinamide adenin dinucleotide phophate (reduced form)
6PGL	6-phosphoglucono-δ-lactone
CK	creatine kinase
HX	hexokinase
G6PD	glucose 6-phosphate dehydrogenase

Appendix A. Rate equation for rapid equlibrium Random Bi Bi mechanism with dead-end EAP and EBQ complexes

The reaction mechanism is of the random type and can be represented as shown in figure 3.

It will be assumed that the interconvention of the central complexes, EAB and EPQ, is the slowest step of the reaction, that rapid equilibrium is established at all other steps, and that the dead-end complexes EAP and EBQ are formed by the reactions:

$$\mathrm{EA} + \mathrm{P} \overset{K_{\mathrm{II}}^{\mathrm{P}}}{\rightleftharpoons} \mathrm{EAP} \overset{K_{\mathrm{II}}^{\mathrm{A}}}{\rightleftharpoons} \mathrm{A} + \mathrm{EP},$$
$$\mathrm{EB} + \mathrm{Q} \overset{K_{\mathrm{II}}^{\mathrm{Q}}}{\rightleftharpoons} \mathrm{EBQ} \overset{K_{\mathrm{II}}^{\mathrm{B}}}{\rightleftharpoons} \mathrm{B} + \mathrm{EQ}.$$

It should be noted that $K_{\mathrm{I}}^{\mathrm{A}}$, $K_{\mathrm{I}}^{\mathrm{B}}$, $K_{\mathrm{I}}^{\mathrm{P}}$ and $K_{\mathrm{I}}^{\mathrm{Q}}$ represent dissociation constants for the reaction of the free enzyme with A, B, P and Q, respectively, and the Michaelis–Menten constants K^{A}, K^{B}, K^{P} and K^{Q} are dissociation constants for the reaction of A, B, P and Q with EB, EA, EQ and EP, respectively. $K_{\mathrm{II}}^{\mathrm{A}}$, $K_{\mathrm{II}}^{\mathrm{B}}$, $K_{\mathrm{II}}^{\mathrm{P}}$ and $K_{\mathrm{II}}^{\mathrm{Q}}$ are also dissociation

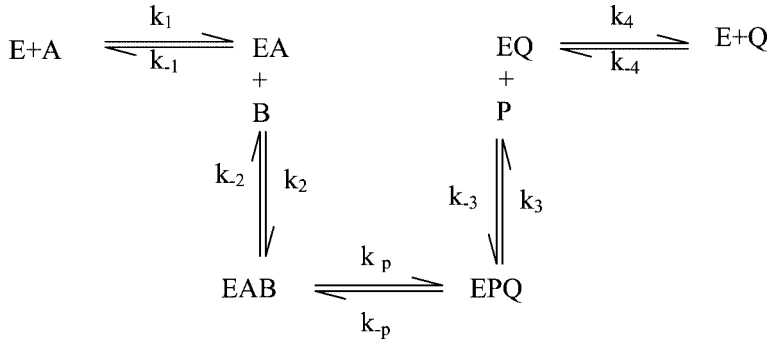

Figure 4.

constants, but for the reaction of A, B, P and Q with different forms of the enzyme, namely, EP, EQ, EA and EB, respectively.

The complete rate equation for the above mechanism may be written as

$$v = \left(V^{\mathrm{F}}_{\max}[\mathrm{A}][\mathrm{B}] - \frac{V^{\mathrm{R}}_{\max}[\mathrm{P}][\mathrm{Q}]K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{B}}}{K^{\mathrm{P}}K^{\mathrm{Q}}_{\mathrm{I}}} \right) \times \left(K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{B}} + K^{\mathrm{B}}[\mathrm{A}] + K^{\mathrm{A}}[\mathrm{B}] + [\mathrm{A}][\mathrm{B}] + \frac{K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{B}}[\mathrm{A}][\mathrm{P}]}{K^{\mathrm{P}}_{\mathrm{I}}K^{\mathrm{A}}_{\mathrm{II}}} + \frac{K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{B}}[\mathrm{P}]}{K^{\mathrm{P}}_{\mathrm{I}}} + \frac{K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{B}}[\mathrm{Q}]}{K^{\mathrm{Q}}_{\mathrm{I}}} + \frac{K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{B}}[\mathrm{P}][\mathrm{Q}]}{K^{\mathrm{P}}K^{\mathrm{Q}}_{\mathrm{I}}} + \frac{K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{B}}[\mathrm{B}][\mathrm{Q}]}{K^{\mathrm{Q}}_{\mathrm{I}}K^{\mathrm{B}}_{\mathrm{II}}} \right)^{-1}.$$

Algebraic expressions for all parameters can found be in [8].

Appendix B. Steady state rate equation for Ordered Bi Bi mechanism

The reaction mechanism is of the Ordered Bi Bi type and can be represented as shown in figure 4.

Under steady state assumptions, the rate equation for this mechanism can be written as

$$v = \left(\frac{V^{\mathrm{F}}_{\max}[\mathrm{A}][\mathrm{B}]}{K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{B}}_{\mathrm{M}}} - \frac{V^{\mathrm{R}}_{\max}[\mathrm{P}][\mathrm{Q}]}{K^{\mathrm{P}}_{\mathrm{M}}K^{\mathrm{Q}}_{\mathrm{I}}} \right) \times \left(1 + \frac{[\mathrm{A}]}{K^{\mathrm{A}}_{\mathrm{I}}} + \frac{K^{\mathrm{A}}_{\mathrm{M}}[\mathrm{B}]}{K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{B}}_{\mathrm{M}}} + \frac{K^{\mathrm{Q}}_{\mathrm{M}}[\mathrm{P}]}{K^{\mathrm{P}}_{\mathrm{M}}K^{\mathrm{Q}}_{\mathrm{I}}} + \frac{[\mathrm{Q}]}{K^{\mathrm{Q}}_{\mathrm{I}}} + \frac{[\mathrm{A}][\mathrm{B}]}{K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{B}}_{\mathrm{M}}} + \frac{K^{\mathrm{Q}}_{\mathrm{M}}[\mathrm{A}][\mathrm{P}]}{K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{P}}_{\mathrm{M}}K^{\mathrm{Q}}_{\mathrm{I}}} + \frac{K^{\mathrm{A}}_{\mathrm{M}}[\mathrm{B}][\mathrm{Q}]}{K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{B}}_{\mathrm{M}}K^{\mathrm{Q}}_{\mathrm{I}}} + \frac{[\mathrm{P}][\mathrm{Q}]}{K^{\mathrm{P}}_{\mathrm{M}}K^{\mathrm{Q}}_{\mathrm{I}}} + \frac{[\mathrm{A}][\mathrm{B}][\mathrm{P}]}{K^{\mathrm{A}}_{\mathrm{I}}K^{\mathrm{B}}_{\mathrm{M}}K^{\mathrm{P}}_{\mathrm{I}}} + \frac{[\mathrm{B}][\mathrm{P}][\mathrm{Q}]}{K^{\mathrm{B}}_{\mathrm{I}}K^{\mathrm{P}}_{\mathrm{M}}K^{\mathrm{Q}}_{\mathrm{I}}} \right)^{-1}.$$

Parameters in terms of rate constants can be obtained algebraically as in [8].

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