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A generalised enzyme kinetic model for predicting the behaviour of complex biochemical systems

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ABSTRACT

Quasi steady-state enzyme kinetic models are increasingly used in systems modelling. The Michaelis Menten model is popular due to its reduced parameter dimensionality, but its low-enzyme and irreversibility assumption may not always be valid in the *in vivo* context. Whilst the total quasi-steady state assumption (tQSSA) model eliminates the reactant stationary assumptions, its mathematical complexity is increased. Here, we propose the differential quasi-steady state approximation (dQSSA) kinetic model, which expresses the differential equations as a linear algebraic equation. It eliminates the reactant stationary assumptions of the Michaelis Menten model without increasing model dimensionality. The dQSSA was found to be easily adaptable for reversible enzyme kinetic systems with complex topologies and to predict behaviour consistent with mass action kinetics *in silico*. Additionally, the dQSSA was able to predict coenzyme inhibition in the reversible lactate dehydrogenase enzyme, which the Michaelis Menten model failed to do. Whilst the dQSSA does not account for the physical and thermodynamic interactions of all intermediate enzyme-substrate complex states, it is proposed to be suitable for modelling complex enzyme mediated biochemical systems. This is due to its simpler application, reduced parameter dimensionality and improved accuracy.

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1. Introduction

Systems modelling of intracellular biochemical processes can provide quantitative insight into a cell's response to stimuli and perturbations [1]. If the model is mechanistic, it has the power to both infer molecular mechanisms and predict biological responses [2]. This requires the simulation of biochemical reaction kinetics typically described using ordinary differential equations (ODEs). Modelling enzymatic cascade networks, however, requires the simulation of multiple reactions. This inevitably increases the complexity of the ODE model, which increases the number of free kinetic parameters. It then becomes more difficult to constrain all parameters simultaneously using a limited amount of available

experimental data [3]. This can result in the derivation of multiple well fitting models with limited predictive power because of their non-uniqueness. Thus, an optimum parameter dimensionality should be selected to reduce non-uniqueness without reducing the topological complexity required to capture key kinetic features in the system [4].

Of the biochemical processes that need to be modelled, many are enzyme reactions [5]. Enzymatic cascades are based on enzyme kinetics within which additional interactions such as inhibition and allosteric effects can be included using mass action kinetics [6]. Basic enzyme kinetics is modelled using the following series of reactions:

$$S + E \underset{k_f^d}{\overset{k_f^a}{\rightleftharpoons}} ES \underset{k_f^c}{\overset{k_r^c}{\rightleftharpoons}} EP \underset{k_r^a}{\overset{k_r^d}{\rightleftharpoons}} P + E \qquad (1)$$

where S, E, ES, EP and P denote the substrate, enzyme, enzyme-substrate complex, enzyme-product complex and product, respectively.

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List of abbreviations

Model names

ODE   ordinary differential equation
tQSSA   total quasi-steady state assumption
dQSSA   differential quasi-steady state assumption
SBML   Systems Biology Markup Language

Chemical species

ATP   adenosine triphosphate
NAD^+ nicotinamide adenine dinucleotide
NADH   reduced nicotinamide adenine dinucleotide
LDH   lactate dehydrogenase

Modelling states and parameters

S_F free substrate
E_F free enzyme
S_T total substrate (sum of bound and free)
E_T total enzyme (sum of bound and free)
ES enzyme-substrate complex
EP enzyme-product complex
P_F free product
P_T free product (sum of bound and free)
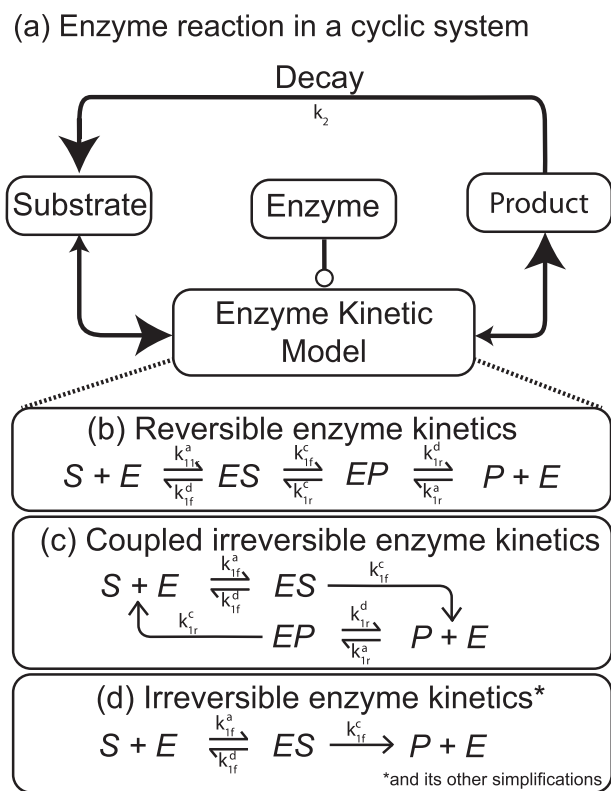k rate parameter
K^m Michaelis constant

The full mass action description of this reaction requires six kinetic parameters: k_f^a, k_f^d and k_f^c are the forward association, dissociation and catalytic rate parameters, respectively, and k_r^a, k_r^d and k_r^c are the corresponding reaction rate parameters in the reverse direction.

Many models of biochemical systems use the simplified irreversible form of the reaction (Fig. 1d), which only requires three kinetic parameters [5,7–15]. Whilst this is an approximation of real enzyme action, *in vitro* spectroscopic studies of single molecule enzyme kinetics have shown that this approximation is sufficient in experiments where there is no product inhibition [16,17]. Further simplifications have led to other enzyme kinetic models such as the Michaelis–Menten model and the Tzafriri total quasi-steady state assumption (tQSSA) model [7,9,18–21]. Whilst the Michaelis–Menten model is more widely used, it is strictly accurate at low enzyme concentrations. Since this may not be true under *in vivo* conditions, unrealistic conclusions may be drawn from models using the Michaelis–Menten equation [18,22–24]. The tQSSA is not subject to the same limitation, but it has a more complex mathematical form that requires reanalysis for each distinct network to which it is applied [24]. Currently, systems modellers must choose between complex enzyme models with high parameter dimensionality, or simpler models at the cost of accuracy.

A further compounding factor is that *in vitro* investigations of enzyme action are generally performed in closed thermodynamic systems which achieve thermodynamic equilibrium, as reflected in the model described by Eq. (1). Cellular systems, however, are not thermodynamically closed, and so achieve only homeostatic equilibrium. This is achieved by constant energy inflow through coenzymes such as ATP which allows the network to form cyclic reactions made of counteracting enzymatic reaction pairs which maintain and regulate this equilibrium. Examples of cyclic reactions are the cyclic interconversion of nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$), mediated by NAD kinase and $NADP^+$ phosphatase in metabolism, and the cyclic interconversion of phosphatidylinositol (4,5)-bisphosphate to (3,4,5)-triphosphate, mediated by PI3K kinase and PTEN phosphatase in insulin and cancer signalling [25–27]. Thus, models of cellular systems need to account for the continual energy consumption in these cyclic reactions. Conventionally, the global coenzyme concentration is not the focus of study, hence systems models implicitly account for the effects of coenzyme concentration [CoE] by asserting that $k_c' \approx k_c[\text{CoE}]$ and then directly varying the catalytic rate k_c' to vary energy input rate. This allows the thermodynamically closed enzyme kinetic model to be used in a thermodynamically open context [4].

To address these issues, we have developed a generalised enzyme kinetic model that retains its mathematical form for systems with multiple enzymes, whilst minimising the number of simplifying assumptions and parameters needed to characterise the system. This enables more accurate simulation of the biochemical mechanisms involved.

2. Theoretical background

As enzymes form the basis of many biochemical processes, models of enzyme kinetics are fundamental components of mathematical models of biochemical networks. The difficulty in implementing

Fig. 1. Various models of enzyme kinetics in a cyclic reaction system. (a) Shows the simple reaction cycle which interconverts a substrate and product involving an enzyme reaction and a backward decay reaction. (b) Shows the mechanism of the reversible enzyme kinetic model, (c) shows the coupled irreversible enzyme kinetic model, (d) shows the irreversible enzyme kinetic model, which includes the Michaelis Menten model and tQSSA model.