FEBS Letters 587 (2013) 2818–2824

journal homepage: www.FEBSLetters.org

Effect of substrate competition in kinetic models of metabolic networks

Sascha Schäuble [a,1], Anne Kristin Stavrum [b,1], Pål Puntervoll [c], Stefan Schuster [d], Ines Heiland [e,*]

[a] Theoretical Systems Biology Group, Friedrich-Schiller-University Jena, Germany
[b] Department of Informatics, University of Bergen, Bergen, Norway
[c] Computational Biology Unit, Uni Computing, Uni Research, Bergen, Norway
[d] Department of Bioinformatics, Friedrich-Schiller-University Jena, Germany
[e] Department of Arctic and Marine Biology, University of Tromsø, Norway

ARTICLE INFO

Article history:
Received 8 May 2013
Revised 18 June 2013
Accepted 18 June 2013
Available online 26 June 2013

Edited by Athel Cornish-Bowden

Keywords:
Substrate competition
Kinetic rate laws
Modelling
Metabolic pathway

ABSTRACT

Substrate competition can be found in many types of biological processes, ranging from gene expression to signal transduction and metabolic pathways. Although several experimental and *in silico* studies have shown the impact of substrate competition on these processes, it is still often neglected, especially in modelling approaches. Using toy models that exemplify different metabolic pathway scenarios, we show that substrate competition can influence the dynamics and the steady state concentrations of a metabolic pathway. We have additionally derived rate laws for substrate competition in reversible reactions and summarise existing rate laws for substrate competition in irreversible reactions.

1. Introduction

Substrate competition has been reported to have implications in different biochemical processes, including degradation of polymeric carbohydrates [1], plant secondary metabolism [2], metabolic transport [3–5], signal transduction pathways [6] and gene regulation [7,8]. All these have in common that different substrates compete for the active site of the same enzyme. Substrate competition is also used to describe biochemical mechanisms where two different enzymes compete for the same substrate. L-Arginine, for example, is a substrate for both nitric oxide synthase and arginase, and competition between the enzymes plays a role in asthma development [9]. This type of competition has also been described as a possible mechanism behind changes in methylation patterns in cancer cells [10]. While the reaction rates of the latter type of competition can be described by standard Michaelis–Menten kinetics (MMK), descriptions of reaction rates of the first type of substrate competition require some modifications. Surprisingly, applicable rate laws describing competition between different substrates for the same enzyme are not available for reversible reactions. Although the rate laws described here can be used for substrate competition in different cellular processes, our examples will focus on metabolic processes.

For irreversible reactions substrate competition is comparable to enzyme inhibition. The different substrates can be viewed as inhibitors of each others reactions, and hence, the mechanism of substrate competition can be described by adapting the kinetic rate laws from competitive inhibition. The mechanisms of enzyme inhibition have been thoroughly investigated for decades, and the kinetics are mostly based on the original reaction rate equation by Henri, Michaelis and Menten [11–13]. In 1977, Chou and Talaly published a generalised equation for the analysis of multiple inhibitors for various mechanisms of irreversible reactions [14]. Furthermore, they provided general rules for the different inhibition mechanisms that can be applied to various kinetic rate laws. Dingerkus et al. [15] used a rate law that is similar to irreversible MMK with competitive inhibition to describe the competition between tryptophan and other amino acids to get across the blood–brain barrier. Although the reverse transport rate might be low under physiological conditions, the amino acid transporters are indeed reversible. Thus, to describe the dynamics of these transport processes more accurately, reversible rate laws are required. To our knowledge, explicit kinetic rate laws that describe steady states of reversible reactions, which include substrate competition, are not available in the literature. In contrast to irreversible reactions, the competitive binding of the product must also be considered for reversible reactions. So far, only kinetic rate laws for the initial

* Corresponding author. Tel.: +47 77646889
E-mail address: ines.heiland@uit.no (I. Heiland).
[1] These authors have contributed equally to this work.

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.06.025

velocity in the absence of products have been described [16,17]. However, these rate equations are not suitable for steady state calculations. To close this gap we applied the rules provided by Chou and Talaly [14] to derive rate laws for reversible reactions, based on the quasi steady state assumption.

To study the impact of substrate competition, we constructed three toy models that resemble real pathway scenarios. We used the models to simulate the impact of substrate competition on: (i) substrate accumulation over time, (ii) steady state concentrations of intermediates for increasing substrate concentrations, and (iii) the metabolic capacity of the system. Although it may be valid to neglect competition in some cases, we show that it is very difficult to safely judge whether this is appropriate in complex pathways.

2. Methods

Three generalised toy models were made to study the effect of substrate competition as a result of enzymes catalysing multiple reactions in (A) different pathways, (B) different branches within one pathway or (C) multistep reactions (schemes see Figs. 1–3). Four versions of each model were set up: the first two models describe an irreversible mechanism, of which one includes competition and the other neglecting it. The other two models contain reversible reactions, again one containing competition and the other neglecting it. To ensure that the observed effect was due to competition alone, we standardised the setup of the models. The input reactions were modelled using mass action kinetics (Eq. (1)), whereas transitions between intermediate species and output reactions were modelled using standard Michaelis–Menten kinetics for irreversible (Eq. (2)) and reversible (Eq. (3)) reactions. The Michaelis–Menten constant (K_m) was arbitrarily set to 0.02 mM and maximal velocity (V) was set to 1 mM/h, for the respective parameters in all reactions.

$$v = k_1 S \tag{1}$$

$$v = \frac{VS}{K_m + S} \tag{2}$$

$$v = \frac{V_f \frac{S}{K_m^S} - V_r \frac{P}{K_m^P}}{\frac{S}{K_m^S} + \frac{P}{K_m^P} + 1} \tag{3}$$

The full description of all used models can be found in the Supplementary material.

2.1. Substrate competition

Based on the rules described by Chou and Talaly [14] the rate law for monomolecular irreversible reactions with any number of competing substrates can be described as follows:

$$v_1 = \frac{VS_1}{K_{m_1}\left(1 + \sum_{i=2}^{n} \frac{S_i}{K_{m_i}}\right) + S_1} \tag{4}$$

where S_1 competes with n-1 substrates $S_2,\ldots,n$ for the binding site of the catalysing enzyme. The variables K_{m_i} describe the Michaelis constants for the respective substrates S_i. An equally simple relationship could not be found in the literature for reversible reactions. A general formulation for reversible reactions including competition between multiple substrates can be deduced by recognising that not only the substrates but also the products compete for the binding site of the free enzyme. Thus the K_m-values have to be modified as follows:

$$K_{m_1} = K_{m_1}\left(1 + \sum_{i=2}^{n}\left(\frac{S_i}{K_m^{S_i}} + \frac{P_i}{K_m^{P_i}}\right)\right) \tag{5}$$

The resulting kinetic rate law for mono-molecular mechanism then becomes:

$$v_1 = \frac{V_f \frac{S_1}{K_m^{S_1}} + V_r \frac{P_1}{K_m^{P_1}}}{\sum_{i=1}^{n}\left(\frac{S_i}{K_m^{S_i}} + \frac{P_i}{K_m^{P_i}}\right) + 1} \tag{6}$$

with S_1 and P_1 competing with n-1 other substrates $S_2,\ldots,n$ and n-1 other products $P_2,\ldots,n$ for the binding site of the catalysing enzyme.

Kinetic rate laws for other reaction mechanisms as well as the derivation for the mono-molecular reaction (Eq. (6)) can be found in the Supplementary materials.

2.2. Steady state analysis and time course simulations

Steady state analyses are commonly performed to predict species concentrations and reaction rates. To see the effect of competition on predicted species concentrations, we used COPASI [18] to analyse the influence of increasing input-species concentrations on the steady state of the respective toy model. External concentrations were varied from 0.001 to 1 mM with a step size of 0.001 mM. The results for the concentrations at which a steady state was found were used to calculate the difference between the corresponding models including or neglecting competition. The last concentration in the scan that yielded a steady state was considered to be the metabolic capacity of the model corresponding to the saturating concentration of the system.

Additionally, time course calculations were performed to study the differences over time. The uptake rate was set to 1 mM/h and the concentration of the external metabolites A_{ex} and B_{ex} was set to 0.05 mM.

3. Results

In irreversible reactions substrate competition can be described by competitive inhibition kinetics by substituting inhibitory Michaelis–Menten constants K_I with the respective K_m values of the competing substrates (see Section 2 for details). This is possible as reactions with substrate competition and reactions with competitive inhibition both have the same number of competing ligands. In reversible reactions, however, both substrates and the respective products compete for the active binding site of an enzyme. The number of compounds that influence enzyme kinetics is 2n, which is the sum of n competing substrates and the n competing products. In contrast, an irreversible competitive inhibition describes n ligands influencing the kinetics of an enzyme, as it is accessible to n-1 inhibitors and one substrate. Hence, substrate competition in reversible reactions cannot be simulated by rate laws describing competitive inhibition.

Surprisingly, explicit kinetic rate laws for substrate competition of reversible reactions were not available in the literature. To derive these rate laws we modified the rule provided for irreversible competitive inhibition by Chou and Talaly [14]. The modification was based on the consideration that in reversible reactions both substrate and product compete for the binding to the active site of the enzyme (details see Section 2). This modified rule (Eq. (5)) was subsequently applied to derive kinetic rate laws for mono- and bimolecular reactions of different types (see Eq. (6) and Supplementary material). Our modified rule was proven to be correct for a monomolecular reaction, by deriving the kinetic rate law with an independent method (Supplementary material).