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A generic rate law for surface-active enzymes

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ABSTRACT

Many biochemical reactions are confined to interfaces, such as membranes or cell walls. Despite their importance, no canonical rate laws describing the kinetics of surface-active enzymes exist. Combining the approach chosen by Michaelis and Menten 100 years ago with concepts from surface chemical physics, we here present an approach to derive generic rate laws of enzymatic processes at surfaces. We illustrate this by a simple reversible conversion on a surface to stress key differences to the classical case in solution. The available area function, a concept from surface physics which enters the rate law, covers different models of adsorption and presents a unifying perspective on saturation effects and competition between enzymes. A remarkable implication is the direct dependence of the rate of a given enzyme on all other enzymatic species able to bind at the surface. The generic approach highlights general principles of the kinetics of surface-active enzymes and allows to build consistent mathematical models of more complex pathways involving reactions at interfaces.

1. Introduction

Cell membranes are ubiquitous in living systems. Collagen, the most abundant protein in mammals, forms fibers in connective tissues. Carbohydrate polymers like cellulose, chitin and starch are by far the dominant sources of biomass on earth and fulfill important structural and energetic functions. These are common examples of aggregates, macromolecular entities made up by the interaction of similar elements and defined by an interface towards their, typically aqueous, environment. Evidently, spatially heterogeneous systems are the norm rather than an exception in biology.

Yet, surface-active (or interfacial) enzymes have not received the same attention as their classical counterparts, enzymes acting on dissolved compounds. This holds true especially for textbooks and undergraduate curricula. The awareness of surface-active enzymes, of their pervasiveness and their characteristic features seems to be rather low. However, by the very nature of their substrates, lipases, collagenases and amylases cannot be understood exclusively in those terms applying to classical enzymes. If catalytic activity is confined to an interface, constraints not present in aqueous solution become important [1]. This entails characteristic protein domains, mechanisms and kinetic properties of surface-active enzymes.

Probably, the chief reason for the lack of recognition of surface-active enzymes is the lack of consensus. Although Michaelis and Menten's approach [2] cannot be used in every circumstance, it captured the essence of enzymatic catalysis in solution and, by advocating initial-rate measurements, provided a blueprint for experimental design for years to come. A similar breakthrough for surface-active enzymes is missing. There is no canonical kinetic description although specific models [3–8] and conceptual treatments [9–11] have been put forward. It is only consequent that even recent authoritative treatments on enzyme kinetics [12] shy away from discussing interfacial catalysis or do so only with a focus on special systems like membrane surfaces [13, Section 7.12]. Marangoni's textbook [14, Ch. 10] and especially Berg and Jain's substantial contribution [1] are notable exceptions. The latter, unfortunately, had a limited impact on the mainstream, apparently due to the focus on membrane surfaces and lipases. While the fragmentation of disciplines is unavoidable and to a certain degree necessary, we believe that this was at the expense of developing a better conceptual understanding of surface-active enzymes.

As a contribution to this understanding, we propose to use generic rate laws for surface-active enzymes. Generic rate laws are

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useful to analyze characteristic properties for a given class of enzymes by deriving a mathematical form that is invariant towards certain mechanistic details. At the same time a generic rate law suggests the appropriate modification of a mathematical term in the rate law whenever a concrete situation applies. For example, Rohwer and Hofmeyr [15] discuss kinetic and thermodynamic contributions to control by means of a generic rate equation.

In addition to in vitro characterization, the development of generic rate laws is also driven by attempts to model metabolic systems consistently if detailed mechanistic information is missing or judged unnecessary [16–19]. Again, it is commonly acknowledged that Michaelis–Menten-like rate laws alone are insufficient to understand regulatory properties of enzymes and pathways. Still, in recognition of Occam's razor we are thankful having at least a simple foundation onto which we can build, layer by layer, more complexity as needed. Interfaces, in particular, force us to recognize this additional complexity.

Many metabolic models evade a mechanistically correct description of interfacial processes by treating aggregated substrates as external (i.e. source or sink) or applying classical rate laws to describe their turnover [20,21]. Both strategies become insufficient if interfacial reactions are crucial to understand regulatory features. An impressive example emerged recently in plant biology. Leaves of flowering plants store starch during the day to provide sink organs with carbon during the night. This turnover appears to be precisely controlled at different levels [22,23] but intriguingly the insoluble nature of starch and enzymes acting at its interface turn out to be crucial. Mutant plants lacking a native starch interface or functional surface-active enzymes show less capabilities to grow under stress and changing environments, conditions which a sessile organism hardly can avoid.

2. Methods

Interfacial catalysis spans reaction spaces where different concentration measures apply. We propose some notational conventions to reduce the burden of bookkeeping. Interfacial species are typically denoted by an asterisk [1], a convention we will abide by. Furthermore, it is convenient to distinguish between cis- ($*$X) and trans-elements (U$*$) akin to the terminology used in gene regulation. A cis-element denotes a species that is either a genuine component of the substrate surface or originates from it. On the contrary, trans-elements are diffusible species usually residing in the aqueous phase. The notation for interfacial complexes is straightforward: cis–cis complexes ($*$X+$*$Y → $*$XY), trans–trans complexes (U$*$ + V$*$ → UV$*$) and cis–trans complexes (U$*$ + $*$X → U$*$X). Square brackets ($[\cdot]$) and angle brackets ($\langle\cdot\rangle$) denote concentrations per unit volume and per unit surface area, respectively.

A biochemical rate law is a single equation describing the rate of an enzymatically catalyzed reaction. Since these reactions are made up of several chemical steps (association, dissociation, catalysis) representing the mechanism of the enzyme, a rate law implies certain approximations. These approximations allow to reduce the set of ordinary differential equations (ODEs), describing the dynamics of the complete system, to a single ODE for product formation which is usually assumed to be the rate-limiting step. Two well-known assumptions are the rapid-equilibrium (RE) approximation, that was used by Michaelis and Menten [2], and the (standard) quasi-steady state (sQSS) approximation due to Briggs and Haldane [24]. The validity of these and other approximations from a mathematical viewpoint has been studied extensively elsewhere [25].

Here, we will apply Cha's method [26] assuming a partial equilibrium mechanism. We apply the RE approximation for enzyme adsorption, assuming this process to be close to equilibrium at the time scale of catalytic turnover. The sQSS approximation is then applied to the resulting reaction scheme to derive the steady state rate equation. This hybrid approach allows to formulate the rate law for surface-active enzymes in terms of the so-called *adsorption isotherm*. This well-known concept from surface science is used to quantify the partitioning of an adsorbate (here enzyme) between two phases (here aqueous solution and substrate surface) at equilibrium for a given temperature, hence isotherm. Adsorption isotherms, unlike equilibrium constants, define the mass action ratio at equilibrium not by a single number but by some, in general implicit, equation $[E*]_{eq}/[E]_{eq} = f([E*]_{eq})$ (eq denotes equilibrium concentrations). The confinement to equilibria may appear to be a severe restriction. Still, the observed diversity of adsorption isotherms [27–29] allows to cover a wide range of adsorption mechanisms and to study their effects on the rate of catalysis. Also, the RE approximation usually leads to simpler rate laws and is often advocated as a first approach [12,30]. It should be noted that the equilibrium assumption only relates to binding and dissociation processes and does not preclude lateral diffusion of the enzyme. Similarly, the RE approximation in the original Michaelis–Menten approach does not preclude the enzyme, the substrate or the complex to move in solution.

3. Results and discussion

To illustrate our generic approach, we assume a simple reversible uni–uni mechanism at the interface (Fig. 1). Upon adsorption of the enzyme E at the interface of the aggregated substrate (area A), the trans-species E$*$ binds the cis-reactant $*$S in a bimolecular reaction to form the cis–trans complex E$*$S. The catalytic step immediately follows and releases the cis-product $*$P and E$*$. The enzyme can either desorb or engage in another catalytic cycle. The two-step process of adsorption followed by finding the reaction partner at the interface assumes that either the enzyme, or the reactant or both can perform lateral movements.

Before we embark on the derivation of the rate law for surface-active enzymes, we will consider adsorption as an isolated process which is essentially completed and, thus, in equilibrium during the phase of catalytic turnover. There is some experimental evidence [31,32] justifying the assumption of such a temporal hierarchy and several studies of interfacial enzyme kinetics used adsorption isotherms [5,33,34], albeit without applying Cha's method.

Following this, we will derive the rate law and study some of its qualitative properties, most notably the effects of substrate amount, surface properties and enzyme amount.

3.1. Adsorption equilibrium

Following Langmuir's seminal work [35], we may think of adsorption as a bimolecular reaction between the enzyme E and an empty "elementary space" $*$ at the surface. We define this space such that its surface area equals the parking area of the adsorbate. In this case a simple stoichiometric relation applies, E+$*$ = E$*$, and we can write the on-rate as

$$v_{on} = k'_{on}[*][E], \tag{1}$$

where k'_{on} is a second-order rate constant.

Langmuir's model assumes that the adsorption sites are independent and that adsorbed molecules do not interact, essentially behaving like a two-dimensional ideal gas. We seek a phenomenological description that allows a departure from these restrictive assumptions. To this end, we introduce the so-called *available area function*, Φ, into enzyme kinetics. This concept from surface physical chemistry [28,36] allows to describe many different adsorption scenarios. The value of Φ lies between 0 and 1, and quantifies the