



Review

A note on the kinetics of enzyme action: A decomposition that highlights thermodynamic effects

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ABSTRACT

Michaelis and Menten's mechanism for enzymatic catalysis is remarkable both in its simplicity and its wide applicability. The extension for reversible processes, as done by Haldane, makes it even more relevant as most enzymes catalyze reactions that are reversible in nature and carry in vivo flux in both directions. Here, we decompose the reversible Michaelis–Menten equation into three terms, each with a clear physical meaning: catalytic capacity, substrate saturation and thermodynamic driving force. This decomposition facilitates a better understanding of enzyme kinetics and highlights the relationship between thermodynamics and kinetics, a relationship which is often neglected. We further demonstrate how our separable rate law can be understood from different points of view, shedding light on factors shaping enzyme catalysis.

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1. Introduction

The kinetic rate law of simple irreversible enzymatic reactions – introduced by Victor Henri [1] and later rationalized by Michaelis and Menten [2], Briggs and Haldane [3,4] – is a hallmark of quantitative biochemistry [5]. Haldane extended this rate law to reversible reactions to reach a mathematical description, often referred to as *reversible Michaelis–Menten kinetics* (the history of the field is clearly summarized in [6]).

Here, we present a new decomposition of the reversible Michaelis–Menten rate law. By rewriting Haldane's formula as a product of three factors – the maximal rate, the enzyme saturation level and the thermodynamic driving-force – we analyze the relative importance of different factors affecting enzyme kinetics. The original irreversible rate law (i.e. Michaelis–Menten kinetics) emerges naturally when assuming a thermodynamically highly favorable reaction and low product concentration.

1.1. Reversible uni-molecular reactions

Reversible Michaelis–Menten kinetics is given by the following mechanism:



The steady-state assumption is formulated by equating the time derivatives of the concentrations of the enzyme complexes to zero, i.e.:

$$\begin{aligned} E &= [E_{\text{free}}] + [ES] + [EP] \\ 0 &= \frac{d[ES]}{dt} = k_1 \cdot s \cdot [E_{\text{free}}] + k_4 \cdot [EP] - (k_2 + k_3) \cdot [ES] \\ 0 &= \frac{d[EP]}{dt} = k_6 \cdot p \cdot [E_{\text{free}}] + k_3 \cdot [ES] - (k_4 + k_5) \cdot [EP]. \end{aligned} \quad (2)$$

E being the total enzyme concentration; $[E_{\text{free}}]$, $[ES]$ and $[EP]$ corresponding to the concentrations of the free enzyme, the enzyme bound to the substrate and the enzyme bound to the product, respectively; s and p represent the concentrations of the substrate (S) and the product (P). Solving these equations for s and p yields the following rate law [7]:

$$v = E \frac{k_{\text{cat}}^+ \cdot s / K_s - k_{\text{cat}}^- \cdot p / K_p}{1 + s / K_s + p / K_p}. \quad (3)$$

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The apparent enzymatic parameters, i.e. k_{cat}^+ , k_{cat}^- , K_s and K_p , are directly derived from the mass-action kinetic parameters by [7]:

$$\begin{aligned} K_s &= \frac{k_2 k_4 + k_2 k_5 + k_3 k_5}{k_1 (k_3 + k_4 + k_5)} \\ K_p &= \frac{k_2 k_4 + k_2 k_5 + k_3 k_5}{k_6 (k_2 + k_3 + k_4)} \\ k_{\text{cat}}^+ &= \frac{k_3 k_5}{k_3 + k_4 + k_5} \\ k_{\text{cat}}^- &= \frac{k_2 k_4}{k_2 + k_3 + k_4}. \end{aligned} \quad (4)$$

The k_{cat} values are the maximal forward and backward rates per unit of enzyme (E), and K_s and K_p are the *Michaelis constants*, denoted more generally by K_M .

In his original paper, Haldane noticed an inherent dependency between the kinetic parameters and reaction thermodynamics [7]. When assuming a reaction has reached equilibrium, and equating Eq. (3) to zero, the ratio between enzyme efficiencies, i.e. k_{cat}/K_M , in both directions equals K'_{eq} – a thermodynamic constant representing the ratio between the concentrations of the product and the substrate at equilibrium [8]. This was later denoted the *Haldane relationship*:

$$\frac{k_{\text{cat}}^+/K_s}{k_{\text{cat}}^-/K_p} = K'_{\text{eq}}. \quad (5)$$

1.2. Rohwer–Hofmeyr decomposition

Rohwer and Hofmeyr [9,10] highlighted the fact that the reversible Michaelis–Menten equation can be rewritten as

$$v = \frac{E \cdot k_{\text{cat}}^+}{K_s} \cdot \frac{1}{1 + s/K_s + p/K_p} \cdot \left(s - p \cdot \frac{k_{\text{cat}}^-/K_p}{k_{\text{cat}}^+/K_s} \right). \quad (6)$$

To simplify this equation, they defined the rate capacity V^+/K_s (where $V^+ \equiv E \cdot k_{\text{cat}}^+$) and the binding term $\Theta \equiv 1/(1 + s/K_s + p/K_p)$. Using the Haldane relationship, the last term was reduced to $(s - p/K'_{\text{eq}})$. Therefore, the reaction rate is:

$$v = \frac{V^+}{K_s} \cdot \Theta \cdot \left(s - \frac{p}{K'_{\text{eq}}} \right). \quad (7)$$

The initial rate of reactions in the linear regime, i.e. when $s \ll K_s$ and $p = 0$, is approximated by $v \approx (V^+/K_s) \cdot s$. Therefore, the rate capacity can be directly measured as the slope of v as a function of s in such conditions.

2. Decomposing the reversible Michaelis–Menten rate law

2.1. A separable rate law

We choose to rewrite the reversible rate law to reflect the combined effect of the maximal rate, the enzyme saturation level and the thermodynamic driving-force. We recast Hofmeyr's Eq. (7) by moving K_s from the first term to the second term, like in Refs. [11,12], and moving s from the third term to the second:

$$v = E k_{\text{cat}}^+ \cdot \left(\frac{s/K_s}{1 + s/K_s + p/K_p} \right) \cdot \left(1 - \frac{p/s}{K'_{\text{eq}}} \right) \quad (8)$$

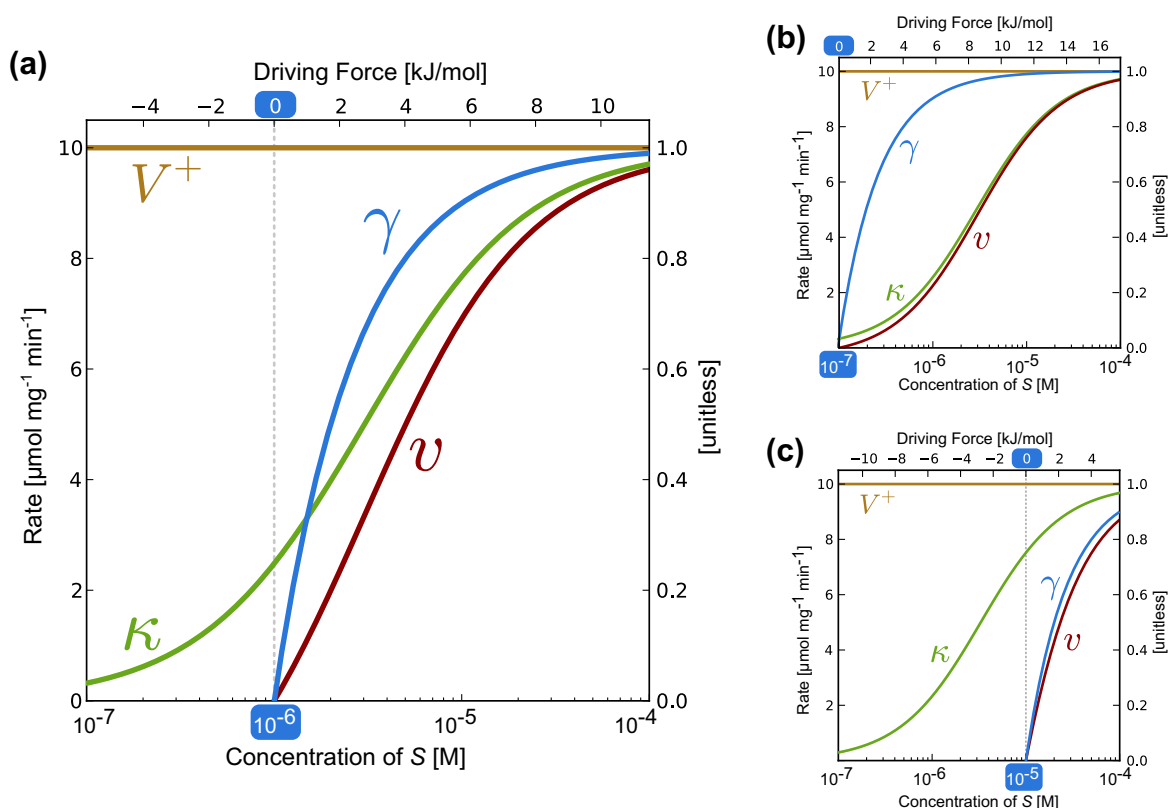


Fig. 1. The capacity, saturation and thermodynamic terms in the separable rate law as a function of the concentration of S and the driving force ($-\Delta_r G^\circ$). The yellow and red lines show the value of the capacity term (V^+) and the net rate (v) in units of $\mu\text{mol mg}^{-1} \text{min}^{-1}$. The green and blue lines show the values of the saturation (κ) and thermodynamic terms (γ) – which are without units. The parameters used for the plot are $T = 300 \text{ K}$, $V^+ = 10 \mu\text{mol mg}^{-1} \text{min}^{-1}$, $K_s = 3 \mu\text{M}$, $K_p = 100 \mu\text{M}$, and $\Delta_r G^\circ = 0$. The concentration of product (p) is $1 \mu\text{M}$ in (a), $0.1 \mu\text{M}$ in (b), and $10 \mu\text{M}$ in (c). The places on the x-axis where the reaction is at equilibrium are highlighted in blue, i.e. where the reaction driving force is 0. With the $\Delta_r G^\circ$ chosen in this example, this occurs when the substrate and product concentrations are equal. Any point with a lower concentration of S will have a negative net rate ($v < 0$) – not shown in this plot. These examples show that, depending on the concentration of the product, the response of the reaction net rate (v) to changes in the concentration of substrate can be dominated by thermodynamics (c), saturation (b), or both (a). Similarly, the values of $\Delta_r G^\circ$, K_s , and K_p have similar effects on the relationships between the curves.

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