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Review

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

Elad Noor [a], Avi Flamholz [a], Wolfram Liebermeister [a,b], Arren Bar-Even [a], Ron Milo [a,*]

[a] Department of Plant Sciences, The Weizmann Institute of Science, Rehovot 76100, Israel
[b] Institut für Biochemie, Charité-Universitätsmedizin Berlin, 10117 Berlin, Germany

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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.

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:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EP \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E + P. \tag{1}$$

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

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

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]:

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

* Corresponding author.
E-mail addresses: elad.noor@weizmann.ac.il (E. Noor), ron.milo@weizmann.ac.il (R. Milo).
URL: http://www.weizmann.ac.il/plants/Milo/ (R. Milo).

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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]:

$$K_s = \frac{k_2k_4 + k_2k_5 + k_3k_5}{k_1(k_3 + k_4 + k_5)}$$
$$K_p = \frac{k_2k_4 + k_2k_5 + k_3k_5}{k_6(k_2 + k_3 + k_4)}$$
$$k^+_{cat} = \frac{k_3k_5}{k_3 + k_4 + k_5}$$
$$k^-_{cat} = \frac{k_2k_4}{k_2 + k_3 + k_4}. \quad (4)$$

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*:

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

1.2. Rohwer–Hofmeyr decomposition

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

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

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

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

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:

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

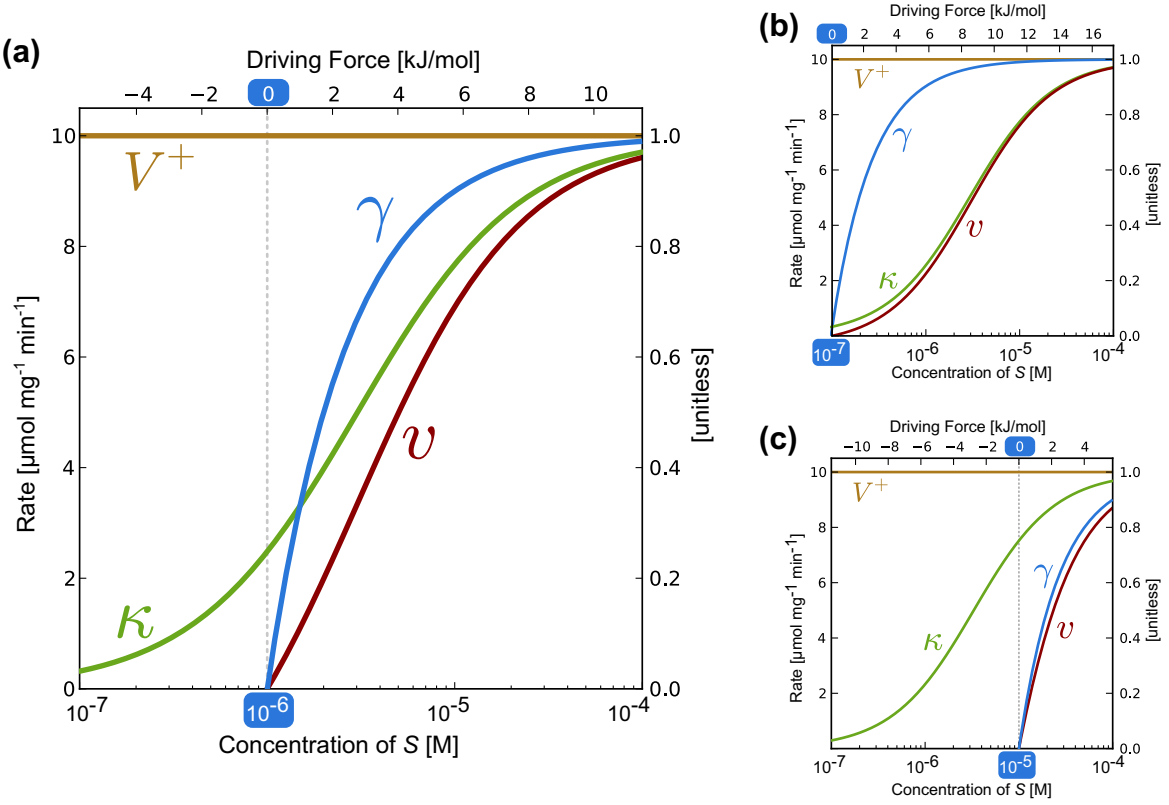

Fig. 1. The capacity, saturation and thermodynamic terms in the separable rate law as a function of the concentration of S and the driving force ($-\Delta_rG'$). The yellow and red lines show the value of the capacity term (V^+) and the net rate (v) in units of µmol mg^{-1} min^{-1}. The green and blue 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 K, V^+ = 10 µmol mg^{-1} min^{-1}, K_s = 3 µM, K_p = 100 µM, and $\Delta_rG'^\circ$ = 0. The concentration of product (p) is 1 µM in (a), 0.1 µM in (b), and 10 µM 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 $\Delta_rG'^\circ$ 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 $\Delta_rG'^\circ$, K_s, and K_p have similar effects on the relationships between the curves.

Notably, the resulting last term is the extent of thermodynamic disequilibrium, i.e. the driving force for the biochemical reaction $S \rightleftharpoons P$ (as appears in [13] – Eq. 4). To see this, we take the formula for the change in Gibbs energy of a reaction [14]:

$$\Delta_r G' = \Delta_r G'^{\circ} + RT \ln(p/s) \tag{9}$$

where $\Delta_r G'^{\circ} = -RT \ln K'_{eq}$, R is the gas constant, T is the temperature, and assuming the activity coefficients of S and P are both 1. It is therefore evident that $\frac{p/s}{K'_{eq}} = e^{\Delta_r G'/RT}$ and hence:

$$v = E\, k^+_{cat} \cdot \left(\frac{s/K_s}{1 + s/K_s + p/K_p}\right) \cdot (1 - e^{\Delta_r G'/RT}) \tag{10}$$

Using this separable rate law, we can identify three factors whose product determines the rate:

$$v = V^+ \cdot \kappa \cdot \gamma \tag{11}$$

where

$$\begin{aligned} V^+ &\equiv E\, k^+_{cat} \\ \kappa &\equiv \frac{s/K_s}{1 + s/K_s + p/K_p} \\ \gamma &\equiv 1 - e^{\Delta_r G'/RT} \end{aligned} \tag{12}$$

We denote (V^+) as the capacity term, (κ) as the fractional saturation term, and (γ) as the thermodynamic term. Examples for how the three terms vary as a function of the substrate concentration are given in Fig. 1. Note that k^-_{cat} does not explicitly appear in Eq. (12), since it is replaced by $\Delta_r G'$ through the use of the Haldane relationship.

It makes more sense to use Eq. (12) when the reaction proceeds in the $S \rightarrow P$ direction (i.e. $\Delta_r G' \leqslant 0$) as in such conditions v is positive and the thermodynamic term is bounded: $0 \leqslant \gamma < 1$. Nevertheless, this equation holds just as well when the reaction proceeds in the opposite direction. In such conditions, v will be negative and γ can be any negative number. Hence, we recommend switching the roles of S and P (and noting that $\Delta_r G'$ is negated) so that the rate law is written in the favorable (originally reverse) direction:

$$v_{reverse} = E\, k^-_{cat} \cdot \left(\frac{p/K_p}{1 + p/K_p + s/K_s}\right) \cdot \left(1 - e^{-\Delta_r G'/RT}\right) \tag{13}$$

2.2. Relation to the irreversible Michaelis–Menten equation

One outcome of our proposed formulation appears when considering very favorable reactions, i.e. when $\Delta_r G' \rightarrow -\infty$. Under this assumption, $\gamma = 1$ and the rate law becomes

$$\lim_{\Delta_r G' \rightarrow -\infty} v = E\, k^+_{cat} \cdot \left(\frac{s/K_s}{1 + s/K_s + p/K_p}\right) \tag{14}$$

Interestingly, the denominator in the fractional saturation term contains p/K_p, which is absent in the irreversible Michaelis–Menten rate law. This is an outcome of the reversible binding step $EP \rightleftharpoons E + P$, which decreases the amount of available free enzyme as a function of p. Michaelis and Menten derived their formula for initial rates, i.e. the rate of reaction at the initial state before the product has started to accumulate. In this setting, we can assume $p \ll K_p$ and arrive at the well-known irreversible Michaelis–Menten rate law:

$$v = E\, k^+_{cat} \cdot \frac{s}{s + K_s}. \tag{15}$$

Note that we can use the limit $\Delta_r G' \rightarrow -\infty$ here since it is trivially satisfied by $p \rightarrow 0$.

2.3. Saturation effects

It is useful to investigate the decomposition's behavior in cases where the concentrations of the substrate and/or the product are saturated ($\gg K_M$) or much below saturation ($\ll K_M$). We consider here four interesting cases.

2.3.1. Enzyme is both substrate and product sub-saturated: $s \ll K_s$ and $p \ll K_p$

In this case, the denominator of the fractional saturation term (κ) is approximately 1 and therefore $\kappa \approx s/K_s$. The kinetics in such a condition will be:

$$v \approx E\, k^+_{cat} \cdot s/K_s \cdot (1 - e^{\Delta_r G'/RT}). \tag{16}$$

This kinetics is identical to that assumed for the linear regime of an irreversible enzyme [15], modulated by the driving force of the reaction.

2.3.2. Enzyme is substrate saturated but product sub-saturated: $s \gg K_s$ and $p \ll K_p$

If the net flux through an enzyme is always in the forward direction ($S \rightarrow P$), we expect its K_s and K_p to be selected by evolution to achieve this condition. In this case, $s/K_s \gg 1 + p/K_p$, and therefore the fractional saturation term κ will approach 1 and thus:

$$v \approx V^+ \cdot \gamma = E\, k^+_{cat} \cdot (1 - e^{\Delta_r G'/RT}). \tag{17}$$

Note that, in this case, the net reaction rate is not affected by the concentrations of the substrate or the product, except through the Gibbs energy of the reaction. In addition, k^+_{cat} is the only kinetic parameter left in the formula. This approximation is especially useful for thermodynamic metabolic models which typically ignore saturation effects.

2.3.3. Enzyme is product saturated but substrate sub-saturated: $s \ll K_s$ and $p \gg K_p$

This mirrors the previous condition, reversing the roles of S and P. Therefore, we can use Eq. (13) for the reverse reaction and again approximate the fractional saturation term by 1, i.e. $v_{reverse} \approx E\, k^-_{cat} \cdot (1 - e^{-\Delta_r G'/RT})$. Note that the value of $v_{reverse}$ is negative when $\Delta_r G' < 0$, as expected. The rate of the original reaction is:

$$v = -v_{reverse} \approx E\, k^-_{cat} \cdot (e^{-\Delta_r G'/RT} - 1). \tag{18}$$

2.3.4. A generalization in which the enzyme is substrate or product saturated (or both): $s \gg K_s$ and/or $p \gg K_p$

In this last condition, we show how a simplified formula can be derived for the reaction rate whenever at least one of the reactants (the substrate and/or the product) is saturated. Note that Sections 2.3.2 and 2.3.3 are special cases of this more general condition.

Here, the saturation term is $\kappa \approx \frac{s/K_s}{s/K_s + p/K_p}$ and, therefore, we can rewrite the entire rate law as:

$$\begin{aligned} v &\approx E \cdot \left(\frac{k^+_{cat} s/K_s}{p/K_p + s/K_s}\right) \cdot (1 - e^{\Delta_r G'/RT}) \\ &= E \cdot \left(p/s \cdot 1/k^+_{cat} \cdot K_s/K_p + 1/k^+_{cat}\right)^{-1} \cdot (1 - e^{\Delta_r G'/RT}) \end{aligned} \tag{19}$$

From the Haldane relationship, in Eq. (5), we can replace $1/k^+_{cat} \cdot K_s/K_p$ with $1/\left(K'_{eq} \cdot k^-_{cat}\right)$ so we now get

$$v \approx E \cdot \left(\frac{p/s}{K'_{eq}} \cdot \frac{1}{k^-_{cat}} + \frac{1}{k^+_{cat}}\right)^{-1} \cdot (1 - e^{\Delta_r G'/RT}) = E \frac{1 - e^{\Delta_r G'/RT}}{1/k^+_{cat} + e^{\Delta_r G'/RT}/k^-_{cat}}. \tag{20}$$

Since this result generalizes Eqs. (17) and (18), both k_{cat}^{+} and k_{cat}^{-} play a part in it. It is interesting to note that all three cases lead to a rate law where concentrations of S and P only appear implicitly through their effect on $\Delta_r G'$.

3. The thermodynamic term γ and the flux–force relationship

The flux–force relationship [16,17] states that the thermodynamic driving force determines the ratio of forward and backward reaction rates in the following way:

$$\frac{v^+}{v^-} = e^{-\Delta_r G'/RT} \tag{21}$$

where the forward rate (v^+) is defined as the rate in which a free substrate molecule (S) binds to an enzyme (ES), undergoes some arbitrary path as a bound enzyme–substrate complex (i.e. within the shaded cyan box in Fig. 2) and is eventually released as a free product molecule (P). The backward rate (v^-) is similarly defined as the rate in which P binds to the enzyme to form EP, undergoes an arbitrary path as a complex and eventually is released as S. The net reaction rate v is the difference between these two rates:

$$v = v^+ - v^- \tag{22}$$

If we follow Haldane's derivation of the reversible rate law, as appears in Eq. (3), we find that the expressions for v^+ and v^- are:

$$v^+ = E\frac{k_{cat}^{+} \cdot s/K_s}{1 + s/K_s + p/K_p} \tag{23}$$

$$v^- = E\frac{k_{cat}^{-} \cdot p/K_p}{1 + s/K_s + p/K_p} \tag{24}$$

The forward rate is equal to the product of the first two terms in the separable rate law, $v^+ = V^+ \cdot \kappa$. The last term, γ is equivalent to the ratio between the net rate and the forward rate [18]:

$$\frac{v}{v^+} = \frac{v^+ - v^-}{v^+} = 1 - \frac{v^-}{v^+} = 1 - e^{\Delta_r G'/RT} = \gamma \tag{25}$$

This all comes together in the simple formula for the separable rate law:

$$v = v^+ \cdot \frac{v}{v^+} = V^+ \cdot \kappa \cdot \gamma \tag{26}$$

Thus, the last term in the separable rate law, γ, can be interpreted in two ways: (a) as the multiplicative term in the separable rate law which depends only on $\Delta_r G'$ or (b) as the ratio between the net rate and the forward rate using the flux–force relationship.

A similar approach focuses on the net rate as a fraction of the total, i.e. the sum of the forward and backward rates [19]:

$$\frac{v}{v^+ + v^-} = \frac{1 - \frac{v^-}{v^+}}{1 + \frac{v^-}{v^+}} = \frac{1 - e^{-\Delta_r G'/RT}}{1 + e^{-\Delta_r G'/RT}} = \frac{\gamma}{2 - \gamma} \tag{27}$$

Since the flux–force relationship described in Eq. (21) holds for any biochemical reaction at steady state, this approach is useful even when considering non-Michaelis–Menten reaction mechanisms. By assuming the total rate is proportional to the amount of enzyme ($v^+ + v^- \propto E$), the rate law becomes a simple function of the thermodynamic term:

$$v \propto E \cdot \frac{\gamma}{2 - \gamma} \tag{28}$$

3.1. Sensitivity analysis through the elasticity coefficients

Elasticity coefficients quantify the effect of changing the concentration of a substrate (or other effectors, such as products or allosteric regulators) on the rate of a reaction, while keeping all other factors constant. The definition of the scaled elasticity coefficient with respect to the substrate concentration is:

$$\varepsilon_s^v \equiv \frac{\partial \ln v}{\partial \ln s} \tag{29}$$

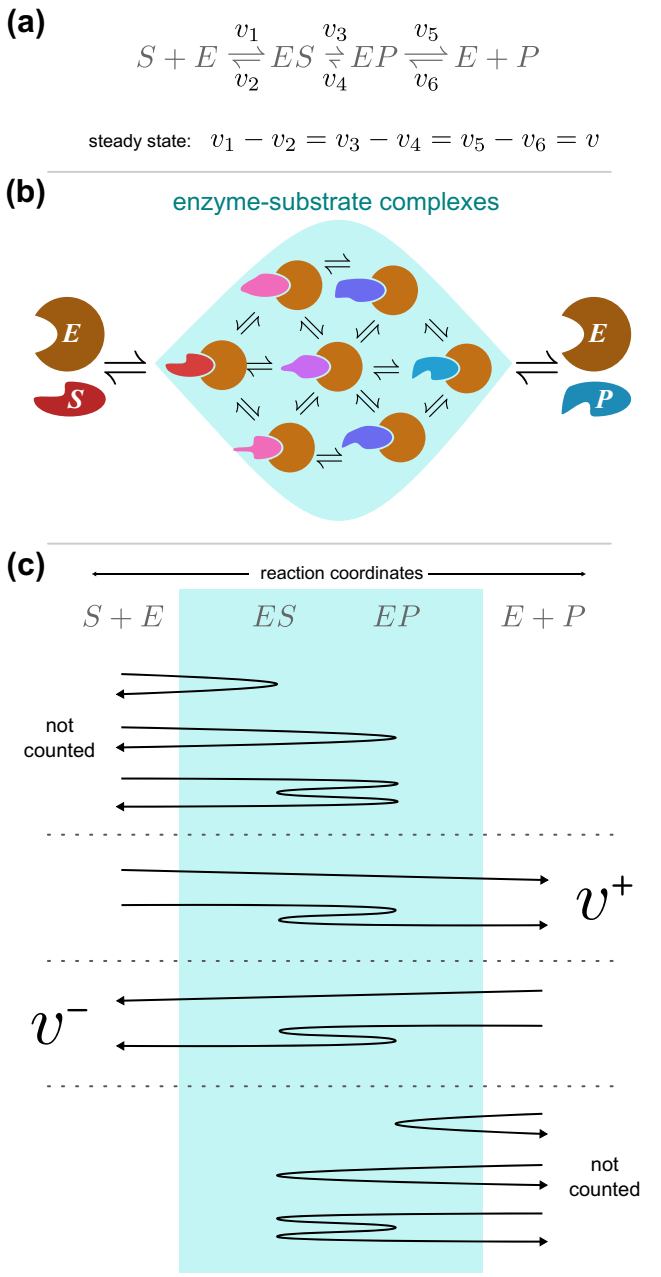

Fig. 2. Definition of the forward and backward rates in a reversible reaction. In general, a reaction mechanism consists of a series of reversible steps (a). In steady state, the net flux is constant thus determining the difference between forward and backward fluxes, but their absolute values in each step can vary. It is then essential to make a rigorous definition of the forward and backward flux for the whole reaction (for example, to be used in the flux–force relationship). The naïve definition of the forward rate as $ES \rightarrow EP$ (as is typically done for irreversible reactions) is not suitable, both because ES and EP can refer to various different complexes, as shown in panel (b), and because these are bound states whose energetics is different from that of the overall reaction. (c) This can be solved by defining v^+ as the rate at which E binds to S and remains as an enzyme–substrate complex until a product molecule is released. Thus, for instance, a binding of E to S giving ES that subsequently decomposes back into $E + S$ is not counted for the forward (or backward) flux. Similarly, binding of S to E to give ES that is transformed to EP but then reverts back to ES and then $S + E$ without product release, is also not counted. This definition is robust to the choice of how to represent the internal reaction mechanism in terms of the ES and EP micro-states.

Elasticity coefficients are used extensively in Metabolic Control Analysis [20–23], a mathematical framework that describes, for instance, how the activity of a single enzyme controls the pathway flux. Since control coefficients [20,21,24] are essentially derived from the elasticity coefficients (and the network topology), understanding the different factors that determine the elasticity in different regimes may help to get a more intuitive understanding of the control of flux in multi-enzyme systems. We provide here the formulas for the reaction elasticity coefficient using the terms defined in the previous sections (namely γ and κ), but leave it to the motivated reader to make use of these results in the broader context of Metabolic Control Analysis.

As pointed out by Rohwer and Hofmeyr [10], the multiplicative nature of the decomposition enables us to express ε_s^v as a sum of the elasticities of the three terms:

$$\varepsilon_s^{V^+} = 0 \tag{30}$$

$$\varepsilon_s^{\kappa} = \frac{\partial \ln(s/K_s) - \ln(1 + s/K_s + p/K_p)}{\partial \ln s} = 1 - \frac{s/K_s}{1 + s/K_s + p/K_p} = 1 - \kappa \tag{31}$$

$$\varepsilon_s^{\gamma} = \frac{\partial \ln(1 - p/s \cdot K_{eq}'^{-1})}{\partial \ln s} = \gamma^{-1} - 1 \tag{32}$$

$$\varepsilon_s^{v} = \varepsilon_s^{V^+} + \varepsilon_s^{\kappa} + \varepsilon_s^{\gamma} = \gamma^{-1} - \kappa \tag{33}$$

Fig. 3 illustrates how these elasticity coefficients change with the substrate concentration. At low driving forces, the total elasticity (ε_s^v) is dominated by thermodynamics ($\varepsilon_s^{\kappa} \ll \varepsilon_s^{\gamma}$) and can therefore be determined without knowing any of the kinetic constants.

3.2. Generalization for a simplified form of multi-substrate/multi-product reactions

The derivations performed in this work have all been made for enzymatic reactions with one substrate and one product. However, most enzymes catalyze reactions with multiple substrates and products. Therefore, we show how our decomposition can be extended to describe the kinetics of such enzymes, but focus on a simple case where the enzyme can only exist in one of three distinct states: free, all substrates bound, or all products bound. Under this assumption, the reaction rate v is given by the following rate law [25]:

$$v = E\, \frac{k_{cat}^{+} \prod_i (s_i/K_{s,i})^{m_i^+} + k_{cat}^{-} \prod_j (p_j/K_{p,j})^{m_j^-}}{1 + \prod_i (s_i/K_{s,i})^{m_i^+} + \prod_j (p_j/K_{p,j})^{m_j^-}} \tag{34}$$

where for each substrate s_i is its concentration, m_i^+ is its stoichiometric coefficients, and $K_{s,i}$ – its Michaelis–Menten constant (and similarly for products p_j).

By applying the same methodology as in Section 2.1, we introduce $\Delta_r G'$, eliminate k_{cat}^- using the Haldane relationship and rewrite Eq. (34) to arrive at a separable form:

$$v = E\, k_{cat}^{+} \cdot \left(\frac{\prod_i (s_i/K_{s,i})^{m_i}}{1 + \prod_i (s_i/K_{s,i})^{m_i} + \prod_j (p_j/K_{p,j})^{m_j^-}} \right) \cdot \left(1 - e^{\Delta_r G'/RT}\right) \tag{35}$$

3.3. Summary

The formulation presented here – a rate law as a product of the capacity, saturation and thermodynamic terms – provides an easy conceptual framework to understand how different factors affect the net reaction rate. We highlight the didactic value by showing how the simple and well-studied irreversible rate law is derived easily by taking the limit $p \to 0$.

In addition, the separable rate law helps clarify the often ignored connection between thermodynamics and rate. It is a common misconception to assume that the Gibbs energy change only determines whether a reaction is feasible, but does not affect the kinetics. This misconception might arise from to the fact that enzymes cannot change the equilibrium constant of areaction. However, reaction thermodynamics does limit the net flux by imposing a counter-productive backward flux. In a recent report [26], we compared the Embden–Meyerhoff–Parnass (EMP) pathway with the Entner–Doudoroff (ED) pathway and demonstrated how the relationship between thermodynamics and flux affects the efficiency of whole pathways. By utilizing the separable rate law described in Eq. (35) to formulate a protein cost function, we were able to show that the ED pathway is expected to require several-fold less enzymatic protein to achieve the same glucose conversion rate as the EMP pathway.

We hope that the decomposition presented here will be useful for teaching about reversible Michaelis–Menten kinetics as well as for research purposes – by clarifying the interrelationships between enzyme kinetics, capacity, fractional binding saturation andthe reaction thermodynamics.

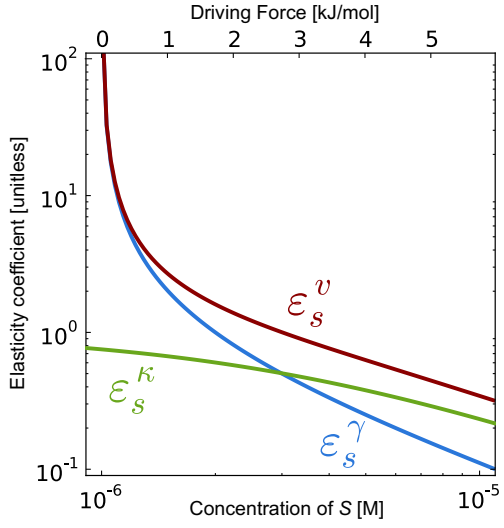

Fig. 3. The elasticity coefficients in the separable rate law as a function of the concentration of S and the driving force ($-\Delta_r G'$). The parameters used for the plot are the same as in Fig. 1a, i.e. $T = 300$ K, $K_s = 3$ μM, $K_p = 100$ μM, $p = 1$ μM and $\Delta_r G'^{\circ} = 0$). One can identify two regimes: $[S] < 3$ μM – where the green line is below the blue line (i.e. $\varepsilon_s^{\kappa} < \varepsilon_s^{\gamma}$) and the response of v to changes in $[S]$ is thermodynamically dominated; and $[S] > 3$ μM – where the response of v to changes in $[S]$ is dominated by substrate binding.

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