Two rules of enzyme kinetics for reversible Michaelis-Menten mechanisms

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In a Michaelis-Menten type reversible enzyme reaction (one substrate, one product) the rapid equilibrium kinetics in one direction excludes rapid equilibrium in the reverse direction. If rapid equilibrium functions in any direction, in the reverse reaction van Slyke type 'kinetic constant' appears in the rate equation independently of whether steady state is reached in finite time or the final equilibrium is attained at $t=\infty$. If the reaction proceeds in one direction with rapid equilibrium and in the reverse direction with steady-state kinetics, the thermodynamic equilibrium of the reaction determines that a higher equilibrium concentration of product (or substrate) can be reached only with steady-state kinetics.

Let us consider a reversible Michaelis-Menten type mechanism [1,2], where a single product is formed through a single enzyme-substrate complex from a single substrate (scheme 1).

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Scheme 1.

where E stands for enzyme, S for substrate and P for product. The k_1 and k_{-2} are second order, k_{-1} and k_2 first order rate constants. In this case, considering the initial velocity conditions in both directions:

$$K_{M,S} = (k_{-1} + k_2)/k_1 \text{ and}$$

$$K_{M,P} = (k_{-1} + k_2)/k_{-2} \quad (1)$$

where $K_{M,S}$ and $K_{M,P}$ are the Michaelis constants of the substrate and product, respectively.

If $k_2 \ll k_{-1}$ in the forward reaction, the rapid equilibrium kinetics holds. Consequently in this case

$$K_{M,S} \approx k_{-1}/k_1 = K_{S,S} \text{ and}$$

$$K_{M,P} \approx k_{-1}/k_{-2} = K_{k,P} \quad (2)$$

where K_S is the dissociation constant of ES and K_k is the van Slyke type 'kinetic constant' [3].

The condition for a rapid equilibrium in the reverse reaction would be $k_{-1} \ll k_2$, which is in contrast to the condition assumed for rapid equilibrium in the forward reaction. Since k_{-1} and k_2 are intrinsic rate constants of the enzyme action they are independent of the direction of the catalyzed reaction.

Therefore, if in the forward reaction the rapid equilibrium can be demonstrated, the same kinetics cannot hold in the reverse reaction. The enzyme in the reverse reaction would follow a mechanism which is described by a rate equation in which a van Slyke type constant is involved.

Walter [4] analyzed the conditions for steady state in the reversible Michaelis-Menten mechanism (scheme 1). The change of [ES] may be zero in finite time at the moment when the time course of [ES] passes through a local maximum, i.e. the second derivative of [ES] is negative at $d[ES]/dt = 0$.

$$d^2[ES]/dt^2 = (k_{-2} - k_1)(d[P]/dt)[E]$$

$$\text{at } d[ES]/dt = 0 \quad (3)$$

(cf. [4]) and the reaction follows steady-state

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kinetics in the forward reaction if $k_1 > k_{-2}$. Consequently, the reverse reaction cannot follow steady-state kinetics, since its condition would be $k_1 < k_{-2}$ [4].

It is to be noted that k_1 and k_{-2} are intrinsic, characteristic rate constants of the enzyme action only if $k_1, k_{-2} < k_D$, where k_D is the diffusion rate constant (it is assumed that $k_{D,S} \approx k_{D,P}$). If that is not the case the rates equal the diffusion rate and not the intrinsic association rate of the enzyme and S or P. In the special case $k_{-2} = k_1 = k_D$, i.e. in both directions the diffusion rate constant limits the rate of the reaction, no local maximum of [ES] would be found, since $d^2[ES]/dt^2 = 0$. During the course of the reaction [ES] starts from 0 and in a reversible reaction at $t = \infty$, [ES] = constant. This means that [ES] asymptotically reaches its equilibrium concentration from both directions, i.e. $d[ES]/dt = 0$ only at $t = \infty$ (final equilibrium).

If in the forward reaction rapid equilibrium kinetics holds, in the reverse reaction this is not possible and if the reaction in the forward direction reaches steady state in finite time in the reverse one this is impossible. Therefore, 'rapid equilibrium enzymes' or 'steady-state enzymes' do not exist. A reversible reaction in one direction may pass through a steady state in finite time or reach the final equilibrium at infinite time. In the reaction one elementary step may be rate limiting and in this case rapid equilibrium kinetics is to be considered. This latter is independent of whether the steady state is reached in the same direction in finite time or not at all.

Let us consider the case when rapid equilibrium holds in the forward reaction, i.e. eqn 2 is valid. In the reverse reaction steady state is reached in finite time if $k_1 < k_{-2}$, i.e. $K_{S,S} > K_{k,P}$. If $k_1 > k_{-2}$ steady state can be reached only in the forward direction where rapid equilibrium holds, but not in finite time in the reverse reaction (the last inequality means a local minimum of [ES] in the reverse reaction, which is physically impossible). Therefore, if in the forward reaction rapid equilibrium kinetics exists, in the reverse direction either the steady state is reached at finite or the final equilibrium at infinite time.

In the following we shall deal with the assumption that in the forward reaction steady-state kinetics holds (i.e. $k_1 > k_{-2}$) but rapid equilibrium is not established (i.e. $k_2 \ll k_{-1}$ is not fulfilled). In this case eqn 1 is valid. In the reverse reaction steady state in finite time cannot be reached, since it is not true that $k_1 < k_{-2}$. We can assume that either k_{-1} is smaller or greater than, or equal to, k_2 or $k_{-1} \ll k_2$. In the first case in the reverse reaction neither steady state is reached in finite time, nor rapid equilibrium, only the final equilibrium at $t = \infty$. If we assume that $k_{-1} \ll k_2$ we will have rapid equilibrium in the reverse reaction ($K_{M,P} \approx k_2/k_{-2} = K_{S,P}$, the equilibrium constant of ES $\rightleftharpoons$ E + P reaction). However, in this case $K_{M,S} \approx k_2/k_1 = K_{k,S}$, i.e. equals the van Slyke type kinetic constant in the forward reaction. Briefly, if in one direction steady state can be reached in finite time and in the opposite direction rapid equilibrium kinetics holds, it follows that in the steady state the van Slyke constant should occur. Steady state with Michaelis constant as defined by Briggs and Haldane [5] may exist only in an irreversible enzyme reaction or in a reversible one where in the forward reaction steady-state kinetics holds and in the reverse one no rapid equilibrium exists, but [ES] reaches its constant value only in the final equilibrium.

Let us consider the equilibrium of the reversible reaction. The kinetic Haldane relation [6,7] is:

$$K_{eq} = k_1k_2/k_{-1}k_{-2} = [P]_{eq}/[S]_{eq} \quad (4)$$

If we assume rapid equilibrium in one of the directions and steady state in the other, the equilibrium concentrations of the substrate and the product correspond to the direction in which the reaction will follow rapid equilibrium or steady-state kinetics. The latter always favours the higher equilibrium concentration (condition for rapid equilibrium in the forward reaction: $k_{-1} \gg k_2$, for steady state in finite time in the reverse direction: $k_{-2} > k_1$, i.e. in the Haldane relation: $k_{-}k_{-2} \gg k_1k_2$, consequently $[S]_{eq} \gg [P]_{eq}$).

Let us consider the more realistic scheme 2 in which both enzyme-substrate and enzyme-product complexes are kinetically relevant:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-e}}{\overset{k_e}{\rightleftharpoons}} EP \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Scheme 2

In this case we have as V_{max} and K_M in both directions the following expressions, if the initial

velocity conditions are considered [4,7]:

$$K_{M,S} = (k_{-1}k_e + k_{-1}k_2 + k_ek_2)/k_1(k_{-e} + k_2 + k_e) \quad (5)$$

$$V_{max,f} = k_ek_2[E]_T/(k_{-e} + k_2 + k_e) \quad (6)$$

$$K_{M,P} = (k_{-1}k_{-e} + k_{-1}k_2 + k_ek_2)/k_{-2}(k_{-e} + k_{-1} + k_e) \quad (7)$$

$$V_{max,r} = k_{-1}k_{-e}[E]_T/(k_{-e} + k_{-1} + k_e) \quad (8)$$

We may have rapid equilibrium in the forward direction only for ES or for both ES and EP. In the following analysis only the latter case will be considered.

Rapid equilibrium in the forward direction for both ES and EP holds, if $k_2 \ll k_{-e} \ll k_e$ and $k_{-1} \gg k_ek_2/k_{-e}$. In this case

$$K_{M,S} \approx k_{-1}k_{-e}/k_1k_e = K_{S,S}K_{EP} \text{ and}$$

$$V_{max,f} \approx k_2[E]_T \quad (9)$$

where K_{EP} is the equilibrium constant of ES $\rightleftharpoons$ EP transformation.

In the reverse reaction, applying the same inequalities, we will obtain

$$K_{M,P} \approx k_{-1}k_{-e}/k_{-2}(k_{-1} + k_e) \text{ and}$$

$$V_{max,r} \approx k_{-1}k_{-e}[E]_T/(k_{-1} + k_e) \quad (10)$$

where $K_{M,P}$ is a 'degenerated' Michaelis constant. If $k_{-1} \gg k_e$

$$K_{k,P} \approx k_{-e}/k_{-2} \text{ and } V_{max,r} \approx k_{-e}[E]_T \quad (11)$$

where $K_{k,P}$ is a van Slyke constant. It is an interesting feature of this mechanism that in the V_{max} of the reverse reaction the rate constant of intramolecular transformation of EP appears instead of k_{-1}.

Walter and Morales [8] have shown that the steady-state assumption for reversible mechanisms containing more than one enzyme-substrate (product) complex can never be exactly correct. The concentration change in time of all the complexes cannot be simultaneously zero. Therefore, we define $d[EP]/dt = 0$ a steady-state condition in the forward reaction and $d[ES]/dt = 0$ in the reverse one for the mechanism in scheme 2.

However, one should be aware of two problems arising:

(i) Eqns 5–8 are derived by assuming $d[ES]/dt = 0$ and simultaneously $d[EP]/dt = 0$. This is not correct, but it is possible that the time derivatives of both complexes in scheme 2 could be relatively small simultaneously (but not exactly zero) so that a steady-state approximation could be made for an appropriate time interval. If the time interval during which the two time derivatives are small occurs during the initial stage of the overall reaction (initial velocity) we may use eqns 5–8 (cf. [4]).

(ii) The conditions of the exact steady state for a mechanism with more than one enzyme complex have not yet been elaborated. It seems that the necessary and sufficient condition for an exact steady state is very complicated and contains a set of relations between the rate constants involved in the mechanism.

As a first approximation we may use an analogous reasoning as above. Preliminary investigations indicate that in this case the same rules may be obtained as for the simple Michaelis-Menten mechanism with a single enzyme-substrate complex.

The Haldane relation for the reaction in scheme 2 is:

$$K_{eq} = k_1k_ek_2/k_{-1}k_{-e}k_{-2} \quad (12)$$

Since the condition for the rapid equilibrium mechanism in the forward reaction necessitates $k_{-1} \gg k_ek_2/k_{-e}$ and $k_{-e} \gg k_2$ if k_1/k_{-2} is not much greater than $k_{-1}k_{-e}/k_ek_2$ we will have $k_{-1}k_{-e}k_{-2} \gg k_1k_ek_2$ and consequently $[S]_{eq} \gg [P]_{eq}$.

As an example one may consider the interconversion of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate catalyzed by triosephosphate isomerase. In the thermodynamic equilibrium the ratio of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate is 96:4. Consequently, if the reaction is not diffusion limited in either direction, the enzyme will exhibit rapid equilibrium kinetics in the direction of glyceraldehyde 3-phosphate formation while it shall work in steady state in the direction of dihydroxyacetone phosphate production. In agreement with the above theoretical prediction, experimental results indicate rapid equilibrium kinetics in the direction of glyceraldehyde 3-phosphate formation [9].

Accordingly, analyzing the initial velocity of a reversible reaction with one substrate and one product the two rules of enzyme kinetics are as follows:

(i) It is impossible that the same kinetics would

be valid for both directions (except if the diffusion is rate limiting for both substrate and product binding). If the enzyme reaction follows rapid equilibrium kinetics in the forward direction, in the reverse direction either reaches the steady state in finite time or the final equilibrium at infinite time, in both cases with a van Slyke constant. If the reaction reaches the steady state in finite time in the forward direction, either follows the rapid equilibrium mechanism in the reverse reaction (the Michaelis constant of the steady state being degenerated or of van Slyke type), or only reaches the final equilibrium at $t = \infty$.

(ii) If the reaction works in one direction with rapid equilibrium kinetics and in the opposite direction with steady state, from the thermodynamic equilibrium of the reaction one may determine in which direction the enzyme catalysis follows rapid equilibrium and in which steady-state kinetics. The higher equilibrium concentration is produced by the reaction in steady state.

Further analysis is needed to determine the rules of enzyme kinetics in the case of more than one substrate and/or product, as well as the exact conditions for steady state in mechanisms with more than one enzyme complex.

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