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Generalized rate equation for single-substrate enzyme catalyzed reactions

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ABSTRACT

The most widely used rate expression for single-substrate enzyme catalyzed reactions, namely the Michaelis–Menten kinetics is based upon the assumption that enzyme concentration is in excess of the substrate in the medium and the rate is mainly limited by the substrate concentration according to saturation kinetics. However, this is only a special case and the actual rate expression varies depending on the initial enzyme/substrate ratio (E_0/S_0). When the substrate concentration exceeds the enzyme concentration the limitation is due to low enzyme concentration and the rate increases with the enzyme concentration according to saturation kinetics. The maximum rate is obtained when the initial concentrations of the enzyme and the substrate are equal. A generalized rate equation was developed in this study and special cases were discussed for enzyme catalyzed reactions.

Introduction

The rate expression for the single substrate–enzyme catalyzed reactions was developed by Henri in 1902 and later by Michaelis–Menten in 1913 for the known initial enzyme and substrate concentrations and the constant reaction volume and has the following form [1–4]

$$V = \frac{V_M[S]}{K_s + [S]} \quad (1)$$

where S is the unbound (free) substrate concentration, V_M is the maximum reaction rate and K_s is the saturation constant. According to the Michaelis–Menten kinetics the rate of enzymatic reactions vary with the enzyme concentration linearly and with the substrate concentration hyperbolically. Enzyme molecules have certain number of active sites on which the substrate molecules bind to form the enzyme–substrate complex. The rate of reaction is determined by the substrate bound enzyme concentration.

At low substrate concentrations ($S << K_s$), a small fraction of the enzyme active sites are occupied by the substrate molecules and the reaction rate increases with the increasing substrate concentration linearly yielding a first-order reaction kinetics [1]

$$V = (V_M/K_s)[S] = k_1[S] \quad (2)$$

At high substrate concentrations ($S >> K_s$), all active sites of the enzyme are occupied by the substrate (saturation) and increases in the substrate concentration do not increase the reaction rate yielding a zero-order reaction kinetics [1].

$$V = V_M \quad (3)$$

Michaelis–Menten rate expression (Eq. (1)) is based on the assumption of that the enzyme concentration (or the active sites)) is in excess of the substrate concentration which do not hold in all enzyme catalyzed reactions. The actual rate expression varies depending on the relative concentrations of the enzyme and substrate. Therefore, the major objective of this study is to provide a more systematic approach to single-substrate enzyme catalyzed reactions and to develop a generalized rate expression.

Generalized rate expression for the single-substrate enzyme catalyzed reactions

The single-substrate enzyme catalyzed reactions are described by the following equation

$$E + S \leftrightarrows ES \rightarrow E + P \quad (4)$$

where E is the enzyme, S is the substrate, ES is the enzyme–substrate complex and P is the product. The first reaction is assumed to reach a fast equilibrium forming the ES complex. The enzyme catalyzes some changes on the substrate and releases product to the reaction media. The second reaction is only in forward direction until a considerable amount of product is produced to cause a reverse reaction. For the initial stages of the enzyme catalyzed reactions the second reaction is assumed to be only in forward direction with no equilibrium. However, the reverse reaction may be important at later stages of the reaction and an equilibrium may be established for the second reaction.

The rate of substrate conversion or product formation can be written as follows,

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$$V = -\frac{dS}{dt} = \frac{dP}{dt} = k_2[\mathrm{ES}] \tag{5}$$

where V is the rate of substrate conversion or product formation (mg S L^{-1} h^{-1} or mg P L^{-1} h^{-1}), and k_2 is the rate constant for the second reaction. It is quite clear from the Eq. (5) that, the rate of enzymatic conversion depends upon the concentration of the enzyme–substrate complex (ES).

The concentration of the ES complex vary according to the following equation,

$$\frac{d[\mathrm{ES}]}{dt} = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{ES}] - k_2[\mathrm{ES}] \tag{6}$$

where [E] and [S] are the unbound (free) enzyme and substrate concentrations in the reaction media; k_1 and k_{-1} are the rate constants for the forward and reverse reactions. The concentration of the ES complex increases at the initial stages of the reaction with further decreases due to product formation by the second reaction at the later stages. The concentration of the ES complex does not change considerably during the later stages and remains almost constant to assure the quasi steady-state. Therefore, with the assumption of quasi steady-state and $d[\mathrm{ES}]/dt = 0$ (approximately), Eq. (6) may be written as follows,

$$[\mathrm{ES}] = \frac{k_1[\mathrm{E}][\mathrm{S}]}{k_{-1} + k_2} = \frac{[\mathrm{E}][\mathrm{S}]}{(k_{-1} + k_2)/k_1} = \frac{[\mathrm{E}][\mathrm{S}]}{K_s} \tag{7}$$

where K_s is the ES complex dissociation constant which is a measure of stability of the ES complex.

Concentrations of free (unbound) enzyme (E) and substrate (S) concentrations depend on the relative initial concentrations of the enzyme and the substrate. Three different cases can be identified on the basis of initial $[\mathrm{E}]_0/[\mathrm{S}]_0$ ratio.

(a) The initial enzyme concentration is in excess of the initial substrate concentration. That is $[\mathrm{E}]_0/[\mathrm{S}]_0 >> 1$:

In this case, only part of the enzyme is bound to the substrate or a fraction of the enzyme is free. The rate is limited by the availability of the substrate and increases with increasing substrate concentration or with the addition of extra substrate to the reaction medium.

The free enzyme and substrate concentrations in this case are

$$[\mathrm{E}] = [\mathrm{E}]_0 - [\mathrm{ES}] \tag{8}$$

$$[\mathrm{S}] = [\mathrm{S}]_0 - [\mathrm{ES}] \tag{9}$$

Since almost all substrate molecules are bound to the enzyme, [ES] is approximately equal to initial substrate concentration ($[\mathrm{ES}] = [\mathrm{S}]_0$) and therefore, the free substrate concentration is very low, limiting the rate of enzymatic reaction.

Therefore, Eq. (7) takes the following form in this case,

$$[\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{K_s} = \frac{([\mathrm{E}]_0 - [\mathrm{ES}])[\mathrm{S}]}{K_s} \tag{10}$$

$$\text{or } [\mathrm{ES}] = \frac{([\mathrm{E}]_0[\mathrm{S}])}{K_s + [\mathrm{S}]} \tag{10a}$$

Substitution of Eq. (10a) into Eq. (5) yields the well known Michaelis–Menten or Henry equation for enzymatic reaction rate.

$$V = \frac{k_2[\mathrm{E}]_0[\mathrm{S}]}{K_s + [\mathrm{S}]} = \frac{V_M[\mathrm{S}]}{K_s + [\mathrm{S}]} \tag{1}$$

where $V_M = k_2[\mathrm{E}]_0$ is the maximum reaction rate when the substrate concentration is the rate limiting factor.

In this case, the reaction rate varies with the substrate concentration hyperbolically since free substrate concentration is very low and is the rate limiting. K_s is the substrate concentration at which the reaction rate is equal to the one-half of the maximum rate known as the half-velocity constant.

(b) The initial substrate concentration is in excess of the initial enzyme concentration. That is $[\mathrm{E}]_0/[\mathrm{S}]_o << 1$:

In this case, only part of the substrate is bound to the enzyme and a fraction of the substrate is free. However, all enzyme molecules are bound to the substrate and concentration of the free enzyme is very low or negligible. The rate is limited by the availability of the enzyme and increases with increasing enzyme concentration or with the addition of extra enzyme to the reaction medium.

The free enzyme and substrate concentrations in this case are

$$[\mathrm{E}] = [\mathrm{E}]_0 - [\mathrm{ES}] \tag{8}$$

$$[\mathrm{S}] = [\mathrm{S}]_0 - [\mathrm{ES}] \tag{9}$$

Since the initial enzyme concentration is low, almost all enzyme molecules are occupied by the substrate and the free enzyme concentration is low. That is, $[\mathrm{E}]_0$ is approximately equal to [ES] and the free enzyme concentration limits the rate of reaction.

Therefore, Eq. (7) takes the following form in this case,

$$[\mathrm{ES}] = \frac{[\mathrm{E}][\mathrm{S}]}{K_s} = \frac{[\mathrm{E}]([\mathrm{S}]_0 - [\mathrm{ES}])}{K_s} \tag{11}$$

$$\text{or } [\mathrm{ES}] = \frac{[\mathrm{S}]_0[\mathrm{E}]}{K_s = [\mathrm{E}]} \tag{11a}$$

Substitution of Eq. (11a) into Eq. (5) yields the following rate equation for the enzymatic reaction when the enzyme concentration is much lower than the substrate and is the rate limiting

$$V = \frac{k_2[\mathrm{S}]_0[\mathrm{E}]}{K_s + [\mathrm{E}]} = \frac{V_M[\mathrm{E}]}{K_s + [\mathrm{E}]} \tag{12}$$

where $V_M = k_2[\mathrm{S}]_0$ is the maximum reaction rate when enzyme concentration is the limiting factor.

(c) Initial enzyme and substrate concentrations are equal. That is, $[\mathrm{E}]_0/[\mathrm{S}]_0 = 1$

In this case neither the enzyme nor the substrate is the rate limiting parameter. All substrate molecules are bound to the enzyme and all enzyme molecules are occupied by the substrate.

The free (unbound) enzyme and substrate concentrations are

$$[\mathrm{E}] = [\mathrm{E}]_0 - [\mathrm{ES}] \tag{8}$$

$$[\mathrm{S}] = [\mathrm{S}]_0 - [\mathrm{ES}] \tag{9}$$

Since the free enzyme and substrate concentrations are almost zero, $[\mathrm{E}] = [\mathrm{S}] = 0$, then

$$[\mathrm{ES}] = [\mathrm{E}]_0 = [\mathrm{S}]_0 \tag{13}$$

The rate is maximum in this case as given below,

$$V = k_2[\mathrm{ES}] = k_2[\mathrm{E}]_0 = k_2[\mathrm{S}]_0 = V_M \tag{14}$$

Therefore, the rate expression for the single-substrate enzyme catalyzed reactions varies depending on the relative initial concentrations of the enzyme and the substrate. The generalized rate expression for the enzyme catalyzed reactions can be written as follows,

$$V = \frac{V_M[\mathrm{S}]}{K_s + [\mathrm{S}]}\,\frac{[\mathrm{E}]}{K_s + [\mathrm{E}]} \tag{15}$$

When the enzyme concentration is in excess, ($[\mathrm{E}] >> K_s$), K_s is negligible as compared to [E] and the second term can be neglected in Eq. (15). The rate equation takes the following form in this case,

$$V = \frac{V_M[\mathrm{S}]}{K_s + [\mathrm{S}]} \quad \text{where } V_M \text{ is } k_2[\mathrm{E}]_0 \tag{1}$$

This is the case known as the Michaelis–Menten kinetics.

On the other extreme, where the substrate concentration is in excess, ([S] >> K_s), K_s is negligible as compared to [S] and the first term in Eq. (15) can be neglected. The rate equation takes the following form in this case,

$$V = \frac{V_M[E]}{K_s + [E]} \quad \text{where } V_M \text{ is } k_2[S]_0 \tag{12}$$

This equation is different from the Michaelis–Menten expression since enzyme concentration is the rate limiting parameter instead of the substrate.

Conclusion

The rate expression for the single-substrate enzyme catalyzed reactions varies depending on the relative initial concentrations of the enzyme and the substrate. The well known Michaelis–Menten equation is a special case where the enzyme concentration is in excess of the substrate concentration. The rate varies with the limiting substrate concentration in form of a hyperbolic function in this case. However, the Michaelis–Menten equation is not valid when the substrate is in excess as compared to the enzyme concentration. In this case the rate varies with the enzyme concentration hyperbolically and increasing enzyme concentrations increase the reaction rate according to the saturation kinetics. The generalized rate expression for enzyme catalyzed reactions contain both substrate and enzyme limitations in hyperbolic forms. One or the other limitations may be neglected depending on the relative concentrations of the initial enzyme and substrate concentration. The generalized rate expression can be used for all cases no matter what the rate limiting parameter is.

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