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The *Michaelis-Menten* Equation in the Case of Enzyme-Catalyzed Hydrolysis of Linear Homopolymer Substrates with Different Degrees of Polymerization

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A novel theory has been proposed allowing the derivation of the equation of *Michaelis-Menten* valid in the case of enzyme-catalyzed hydrolysis of linear homopolymer substrates with varying degrees of polymerization. This equation permits the definition of the maximal rate (V) of the enzyme reaction increasing with the molecular mass of the substrate and the *Michaelis-Menten* constant (Km) decreasing with the increase of the number of bonds in the substrate molecule. Two new methods have been developed permitting: 1. Determination of the *Michaelis-Menten* constant (Km') for a single substrate bond; and 2. Calculation of the free energy ($\Delta G^{\neq}$) required for the formation and degradation of a single enzyme-substrate complex. The theory explains a number of experimental results published by other authors.

(Keywords: Michaelis-Menten equation; Enzym hydrolyse; Linear homopolymer substrates; Maximal reaction rate; Michaelis-Menten constant; Free energy)

Über die Gültigkeit der Michaelis-Menten-Gleichung bei der enzymkatalysierten Hydrolyse von linearen homopolymeren Verbindungen unterschiedlichen Polymerisationsgrades

Aufgrund neuer Überlegungen ist es möglich, die Gleichung nach *Michaelis-Menten* auf die enzymkatalysierte Hydrolyse von homopolymeren Körpern linearer Struktur mit verschiedenem Polymerisationgrad anzuwenden. Die Gleichung erlaubt die Erfassung der maximalen Enzym-Reaktionsgeschwindigkeit (V), die sich mit dem Molekulargewicht des Substrates erhöht, sowie der *Michaelis-Menten*-Konstante (Km), die sich mit zunehmender Anzahl von Verknüpfungen vermindert. Es wurden zwei neue Verfahren entwickelt: Erstens die Bestimmung der *Michaelis-Menten*-Konstante (Km') für eine Substratbindung, zweitens die Berechnung der freien Energie ($\Delta G^{\neq}$) für die Bildung bzw. für die Zerlegung eines Enzym-Substrat-Komplexes. Die Theorie erklärt eine Reihe von Versuchsergebnissen, die von anderer Seite bereits veröffentlicht worden sind.

Introduction

During the past years several experimental studies[1-12] have been reported on the enzyme-catalyzed hydrolysis of linear homopolymer substrates with different degrees of polymerization (n, DP) or with different molecular masses (M_S). It was shown that by applying the *Michaelis-Menten* equation[13] for the initial reaction rate (v_0)

$$v_0 = \frac{V[S]}{Km + [S]} \tag{1}$$

in the form proposed by *Lineweaver* and *Burk*[14]

$$\frac{1}{v_0} = \frac{Km}{V}\frac{1}{[S]} + \frac{1}{V}, \tag{2}$$

for an enzyme reaction of the type

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} (ES) \overset{k_{+2}}{\rightarrow} E + P, \tag{3}$$

the determined value of the maximal reaction rate (V) increases, whereas the *Michaelis-Menten* constant (Km) decreases with the increase of the degree of polymerization, respectively the molecular mass of the substrate used. In this equation E, S, P, (ES), k_{+1}, k_{-1} and k_{+2} are the enzyme, the substrate, the product, the intermediate enzyme-substrate complex and the reaction rate constants, respectively. In the maximal reaction rate $V = k_{+2}[E]$ and in the *Michaelis-Menten* constant $Km = (k_{-1} + k_{+2})/k_{+1}$ (appearing in the equation of *Michaelis-Menten*) the influence of the degree of polymerization, respectively the molecular mass of the substrate used, is not accounted for.

In the present paper an attempt is made to supplement the *Michaelis-Menten* equation, with the aim to show the influence of the molecular mass on the values of the magnitudes determined above.

Theory

From the initial weight concentrations $[E]$ and $[S]$ of the enzyme and the substrate, having molecular masses M_E and M_S, it is possible to determine[15,16] the number of the active centres Z_E of the enzyme and that of the reactive bonds Z_S of the substrate in a unit of volume of the enzyme-substrate solution:

$$Z_E = a n_E = \frac{a[E]N_A}{M_E}, \tag{4}$$

$$Z_S = b n_S = \frac{b[S]N_A}{M_S}, \tag{5}$$

where a is the number of active centres in a single enzyme molecule, b is the number of reactive bonds in a single substrate molecule ($b = n - 1$), n_E and n_S are respectively the number of molecules in one unit of volume and N_A is the *Avogadro* number.

By analogy with equation (3), the enzyme-substrate reaction between the enzyme active centres and the reactive bonds of the substrate in one unit of volume will be of the type

$$Z_E + Z_S \underset{k'_{-1}}{\overset{k'_{+1}}{\rightleftharpoons}} (Z_E Z_S) \overset{k'_{+2}}{\rightarrow} Z_E + Z_{S\infty}, \tag{6}$$

where $(Z_E Z_S)$ is the concentration of the intermediate complexes of reactive bonds bound to the active centres, whereas $Z_{S\infty}$ is the final number of unbroken bonds of the substrate after an infinite time of development of the enzyme reaction. In this equation k'_{+1}, k'_{+2} are reaction rate constants with dimensions in SI:

$$k'_{+1}(\mathrm{dm^3\,s^{-1}}),\ k'_{-1}(\mathrm{s^{-1}}) \text{ and } k'_{+2}(\mathrm{s^{-1}}).$$

The reaction rates of formation and degradation of the intermediate complexes of substrate bonds connected with enzyme active centres are:

$$v'_{+1} = k'_{+1} Z_E Z_S = k'_{+1} (C_b^a)^\alpha n_E n_S = k'_{+1} (C_b^a)^\alpha [n_E^\circ - (n_E n_S)] n_S, \tag{7}$$
$$v'_{-1} = k'_{-1} (Z_E Z_S) = -k'_{-1} a (n_E n_S), \tag{8}$$
$$v'_{+2} = -k'_{+2} (Z_E Z_S) = -k'_{+2} a (n_E n_S), \tag{9}$$

where n_E° and $(n_E n_S)$ are the total number of enzyme molecules and the number of the intermediate complexes in one unit of volume.

$$\text{The magnitude } (C_b^a)^\alpha = \left[\frac{b(b-1).....(b-a+1)}{1.2.3.....a}\right]^{\frac{\sigma_S}{\sigma_E}} \tag{10}$$

is the effective number of possible combinations between the reacting active centres of the enzyme with the reactive bonds of the substrate, respectively, C_b^a is the combination free of repetition.

$$\text{The exponent } \alpha = \frac{\sigma_S}{\sigma_E} \gtrless 1 \tag{11}$$

is the relation of the effective crossections of the reactive substrate bond σ_S and that of the active enzyme centre σ_E. In enzymology $a = 1$ and $C_b^1 = b$ is the case most frequently found.

From the equation for the stationary state of the enzyme-substrate reaction

$$v'_{+1} + v'_{-1} + v'_{+2} = 0 \tag{12}$$

and equations (7), (8) and (9), the concentration of the enzyme-substrate complex $(n_E n_S)$ is determined

$$(n_E n_S) = \frac{n_E^{\circ} n_S}{\frac{k'_{-1} + k'_{+2}}{k'_{+1}} \frac{a}{(C_b^a)^{\alpha}} + n_S}. \tag{13}$$

Taking into consideration the relationship between volume $(n_E n_S)$ and weight $[ES]$ concentrations of the enzyme-substrate complex

$$(n_E n_S) = \frac{[ES] N_A}{M_S + M_E}, \tag{14}$$

the initial reaction rate (v_0) of the said reaction in a weight concentration can be determined

$$v_0 = k_{+2}[ES] = \frac{V[S]}{Km + [S]} = \frac{k'_{+2}[E](1 + M_S/M_E)[S](b - b\,\infty)}{\frac{k'_{-1} + k'_{+2}}{k'_{+1}} \frac{a}{(C_b^a)^{\alpha}} \frac{M_S}{N_A} + [S]} \tag{15}$$

In this novel equation the maximal reaction rate of the reaction is determined by the expression

$$V = k'_{+2}[E]\left(1 + \frac{M_S}{M_E}\right)(b - b\,\infty) \tag{16}$$

where by $M_S \ll M_E$, the reaction rate constants k_{+2} and k'_{+2} are related by the equation

$$k_{+2} = k'_{+2}(b - b_{\infty}) = k'_{+2} \frac{M_S - M_{S\infty}}{N_A m_1}, \tag{17}$$

where b_{∞} is the number of unbroken bonds of the molecule and m_1 is the molecular mass of the monomer.

The *Michaelis-Menten* constant in a molar concentration is determined by the expression

$$Km(M) = \frac{Km}{M_S} = \frac{k'_{-1} + k'_{+2}}{N_A k'_{+1}} \frac{1}{b^{\alpha}} = Km'(M) \frac{1}{b^{\alpha}}, \tag{18}$$

with $a = 1$.

The reaction rate constant k'_{+2} and the *Michaelis-Menten* constant $Km'(M)$, corresponding to a single broken substrate bond, can be determined graphically by plotting the reaction rate constant k_{+2} against the number of reactive bonds (b), according to equation (17) and the *Michaelis-Menten* constant $Km(M)$ against b, according to equation (18).

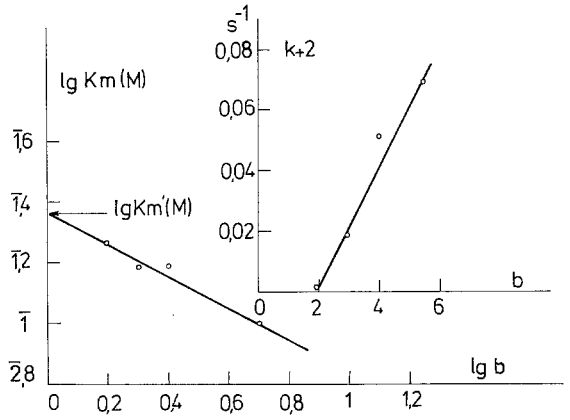

Fig. 1. Plots of *Km* and k_{+2} against the number of bonds (*b*) in the reaction cellulase – oligosaccharides[9,10]

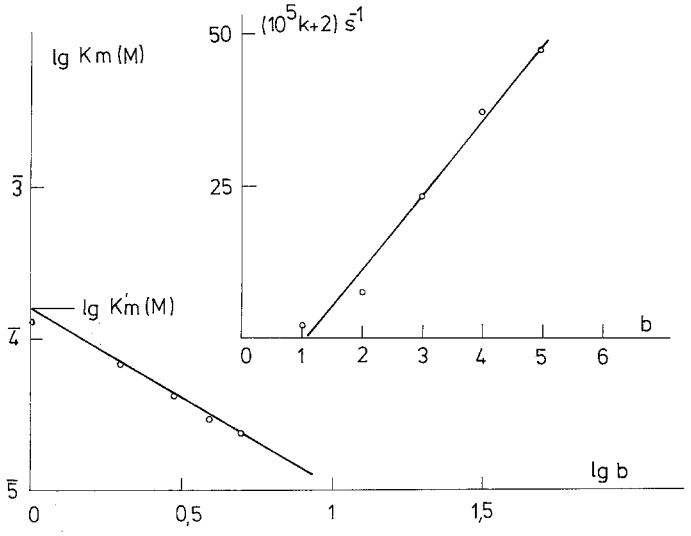

Fig. 2. Plots of *Km* and k_{+2} against the number of bonds (*b*) in the reaction exonuclease – oligonucleotides[2]

Table 1. *Km′ (M), k'_{+2}, ΔG^{+}, ΔE^{+} and α for some hydrolases, catalyzing the*

Nr.	Enzyme	M_E	Substrate	*n, DP*
1	Fraction from cellulase (*Trichoderma viride*)	62 400	Unreduced oligo-saccharides	2–6
2	Exonuclease—Snake venom phosphodiesterase (*Crotalus adamanteus*)	20 000	Oligonuc-leotides	1–6
3	Glucoamylase (*Rhizopus delemar*)	70 000	Oligo-saccharides	1–15

Symbols used

E	enzyme
$[E]$	weight concentration of enzyme E
S	substrate
$[S]$	weight concentration of substrate S
ES	enzyme-substrate complex
P	product
n_E	number of enzyme molecules in one unit of volume
n_S	number of substrate molecules in one unit of volume
$(n_E n_S)$	number of intermediate complexes in one unit of volume
n°_E	total number of enzyme molecules in one unit of volume
Z_E	number of active centres of the enzyme
Z_S	number of reactive bonds of the substrate
$(Z_E Z_S)$	number of intermediate complexes of reactive bonds bound to the active centres
$Z_{S\infty}$	final number of unbroken bonds of the substrate after an infinite time of development of the enzyme reaction
a	number of active centres in a single enzyme molecule
b	number of reactive bonds in a single substrate molecule
$b\infty$	number of unbroken bonds of the substrate molecule
m_1	molecular mass of the monomer
M_S	molecular mass of substrate S
M_E	molecular mass of enzyme E
n, DP	degree of polymerization
k_{+1}, k_{+2}	forward rate constants
k_{-1}	reverse rate constant
k'	rate constant, corresponding to a single broken substrate bond
Km	*Michaelis-Menten* constant
Km'	*Michaelis-Menten* constant, corresponding to a single broken substrate bond
v	rate of reaction
v_0	initial rate of reaction
V	maximal reaction rate
C_b^a	combination free of repetition
σ_S	effective crossection of the reactive substrate bond

hydrolysis of linear homopolymer substrates with varying degrees of polymerization

pH	*T* (°C)	Km' (M/dm^3)	k'_{+2} (s^{-1})	$\Delta G^{\neq}$ (J mol^{-1})	$\Delta E^{\neq}$ (eV)	α	Ref.
5.0	30	$1.44 \cdot 10^{-1}$	0.0221	79 500	1.09	0.65	[9,10]
9.7	25	$1.58 \cdot 10^{-4}$	$1.65 \cdot 10^{-4}$	73 600	1.02	1.11	[2]
5.15	15	$3.16 \cdot 10^{-4}$	8.15	47 500	0.65	0.72	[6]

σ_E effective crossection of the active enzyme centre
$\Delta G^{\neq}$ free energy for the formation and degradation of an enzyme-substrate complex
$\Delta E^{\neq}$ free energy for a single substrate bond in electron-volt (eV) units
R gas constant
k *Boltzmann* constant
h *Planck* constant
N_A *Avogadro* number
T absolute temperature

From the values of k'_{+2} and $Km'(M)$ the free energy ($\Delta G^{\neq}$) for the formation and degradation of an enzyme-substrate complex, corresponding to a certain bond in an enzyme-substrate reaction of a polymer substrate, can be estimated from the thermodynamic and transition state theory equation

$$\Delta G^{\neq} = \Delta G_0^{\neq} + \Delta G = RT \ln \frac{kTKm'(M)}{hk'_{+2}} (\text{J mol}^{-1}), \qquad (19)$$

where R, k, h and T are the gas constant, the *Boltzmann* constant, the *Planck* constant and the absolute temperature at which the enzyme reaction takes place. This energy for a single substrate bond can be estimated in the electron-volt (eV) units by means of the equation

$$\Delta E^{\neq} = \frac{\Delta G^{\neq}}{1.2 \cdot 10^{-19} N_A} \qquad (20)$$

Application

The validity of relationships deduced above can be illustrated by kinetic data reported for enzyme-catalyzed hydrolysis of linear homopolymer substrates with varying degrees of polymerization. The experi-

mental values of $Km(M)$ and k_{+2} from the literature according to equations (17) and (18) are presented in Figs. 1, 2 and 3 for some enzym-substrate reactions. The values determined for $Km'(M)$, k'_{+2}, $\Delta G^{\neq}$, $\Delta E^{\neq}$ and α from these data are presented in Table 1.

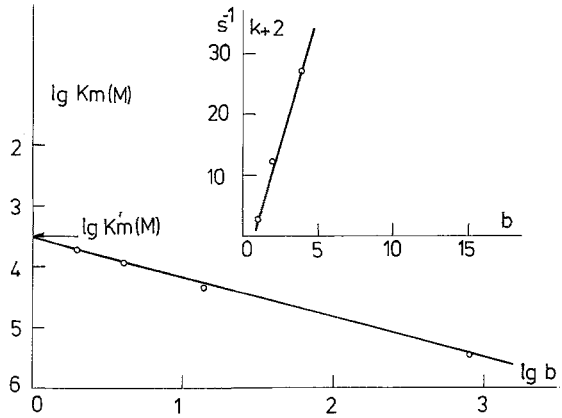

Fig. 3. Plots of Km and k_{+2} against the number of bonds (b) in the reaction glucoamylase – oligosaccharides[6]

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