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KINETICS OF ENZYMIC HYDROLYSIS OF MALTO-OLIGOSACCHARIDES: A COMPARISON WITH ACID HYDROLYSIS

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ABSTRACT

The hydrolysis of malto-oligosaccharides G_3–G_6 catalysed by porcine pancreatic alpha-amylase was investigated kinetically at 25°. Kinetic parameters corresponding to different positions of enzymic attack were determined and product inhibition was evaluated. The enzymic hydrolysis was compared in terms of reaction rate and pattern of action with hydrolysis in 0.1M H_2SO_4 at 70°. Mathematical models for the mechanism of hydrolysis were developed and a good rationalisation of the experimental results was achieved.

INTRODUCTION

Malto-oligosaccharides contain only (1→4)-α-D-glucosidic linkages, which are hydrolysed by alpha-amylase [(1→4)-α-D-glucan 4-glucanohydrolase (EC 3.2.1.1)] several times faster than by mineral acids[1]. Alpha-amylases from various sources have been characterised and each has an inherent action pattern, differences in which have been explained on the basis of multiple attack[2] and preferred attack[3], and have been extensively discussed[4–6].

Human-pancreatic (HPA), human-salivary (HSA), and porcine-pancreatic (PPA) alpha-amylases have been investigated in detail. The kinetics of the action of these enzymes on carboxymethylamyloses[7] and the kinetic parameters for the action of purified human alpha-amylases on malto-oligosaccharides G_4–G_7 have been reported[8], and the action of these enzymes on C-1-modified malto-oligosaccharides has been examined[9,10].

Hydrolysis of the malto-oligosaccharides G_3–G_8, specifically labelled in the reducing unit with ^{14}C, by PPA has been studied in detail[11]. However, only a few kinetic parameters have been reported[12] for this reaction and, because of our interest in the hydrolysis of polysaccharides[13,14], we have performed a detailed

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kinetic study and now report on the enzymic and acid hydrolysis of G_3–G_6. Some preliminary results of this study have been described[15].

Recently, the initial rates of hydrolysis of several malto-oligosaccharides catalysed by PPA have been measured, and K_m and k_0 values have been obtained and correlated with chain length[16].

EXPERIMENTAL

Malto-oligosaccharides G_3–G_6 were purchased from Boehringer and their purity was checked by h.p.l.c. on a μ-Bondapack column (Waters). Porcine-pancreatic alpha-amylase (320 U/mg) was purchased from Merck.

Enzymic hydrolysis. — Solutions of substrate (5–25mM) and enzyme (0.5–15 μg/mL) in 0.02M phosphate buffer (pH 7.0) were mixed and stirred (300 r.p.m.) in a thermostatted reactor at 25.0 ±0.05°. Aliquots were withdrawn at intervals and analysed for malto-oligosaccharides. Product inhibition was determined by performing kinetic runs in the presence of an excess of the appropriate malto-oligosaccharide. Inhibition by the substrate was checked for maltopentaose by using various initial concentrations.

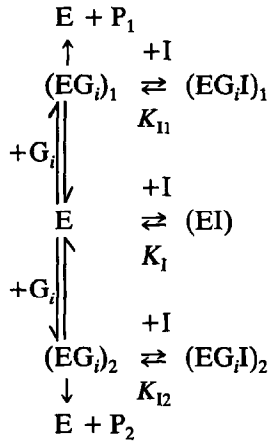
The samples (10–25 μL) were analysed by h.p.l.c. for glucose (G_1) and G_2–G_6 at 25°, using a μ-Bondapack column, elution with water, and detection with a Waters R401 refractometer. A pre-column containing LiChroprep S160 (Merck) was fitted between the pump and the injector in order to retain the enzyme. The column was conditioned immediately before use with 300 mL of water.

Specific response factors were determined for each malto-oligosaccharide at each concentration, and computational programs were used in which, by internal normalisation, the total number of glucose residues was kept constant within the same run.

Acid hydrolysis. — Solutions of each malto-oligosaccharide (20 mg) in 0.1M sulfuric acid (2 mL) were maintained at 70° (under reflux, glycerol bath). At intervals, portions (200 μL) were withdrawn, neutralised with Amberlite IR-45 (OH^-) resin, and analysed by h.p.l.c. as above.

Mathematical models and optimisation procedures

(a) Enzymic hydrolysis. — Each malto-oligosaccharide in the presence of PPA exhibits[11] a specific pattern of hydrolysis. In the series G_4–G_7, PPA preferentially splits the second glucosidic linkage from the reducing (first) residue. Since only maltopentaose was cleaved in a single position, giving maltose and maltotriose, a G_5-binding site was suggested with the catalytic groups located at the second residue. Thus, except for G_5, malto-oligosaccharides form several productive enzyme–substrate complexes, and the occurrence of competing attacks has been taken into account in the mechanistic model. For example, the hydrolysis of a malto-oligosaccharide G_i that involves two enzyme–substrate complexes and, for the sake of simplicity, a single inhibitor I involves the following profile ($i \neq 5$).



For maltotetraose, which gives G_1 and G_3 as well as G_2, the following kinetic equations were used (see Appendix).

$$-\frac{dc_{G4}}{dt} = \frac{dc_{G1}}{dt} + \frac{1}{2}\frac{dc_{G2}}{dt}, \qquad \left(\frac{dc_{G1}}{dt} \equiv \frac{dc_{G3}}{dt}\right)$$

$$\frac{dc_{G1}}{dt} = \frac{V_{max4,1} \cdot c_{G4}}{K_{m4,1}D_1 + c_{G4}D_2 + D_3(K_{m4,1}/K_{m4,2})},$$

$$\frac{dc_{G2}}{dt} = \frac{2V_{max4,2} \cdot c_{G4}}{K_{m4,2}D_1 + c_{G4}D_3 + D_2(K_{m4,2}/K_{m4,1})},$$

with

$$D_1 = 1 + \sum_j c_{Ij}/K_{Ij},$$

$$D_2 = 1 + \sum_j c_{Ij}/K_{Ij4,1}, \text{ and}$$

$$D_3 = 1 + \sum_j c_{Ij}/K_{Ij4,2},$$

where c_{Gi} is the concentration of the malto-oligosaccharide i, $V_{max4,1}$ and $K_{m4,1}$ refer to the reaction of G_4 to give G_1 ($+G_3$), $V_{max4,2}$ and $K_{m4,2}$ refer to the reaction of G_4 to give G_2, and $K_{Ij4,1}$ and $K_{Ij4,2}$ are the inhibition constants for the two enzyme–substrate complexes. When dealing with product inhibition in the hydrolysis of G_4, c_{Ij} assumes the values of c_{G3}, c_{G2}, and c_{G1}.

An analogous approach was applied to maltohexaose, whereas a single

enzyme–substrate complex was considered for maltopentaose. In the reaction of G_3, the distribution of products cannot reveal the pattern of attack and only indicative values of the reaction rate were measured. However, the linkage near to the reducing end is at least five-fold more susceptible to hydrolysis than the other linkage[11].

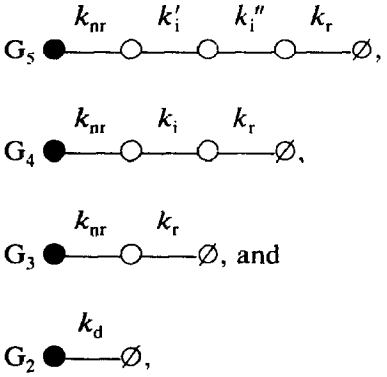
In the optimisation procedures, differential equations in line with those shown above were considered. The parameters were obtained by a computational program in which the optimisation routine VA04A[17] was associated with an integration routine based on the Runge–Kutta method[18]. Computations were carried out on an IBM 3083 computer. The minimised function was

$$\Phi = \sum_n \left(\sum_i [c_{Gi,\text{calc}} - c_{Gi,\text{exp}}]^2 \right),$$

where n is the number of the kinetic samples.

(b) Acid hydrolysis. Since the longest malto-oligosaccharide studied was maltopentaose, the mechanistic model employed for this compound covered all the lower homologues because the degradation of G_5 gives G_4, G_3, and G_2 in addition to G_1.

The following pseudo-first-order kinetic constants relative to the single bonds, all susceptible to acid hydrolysis, were considered first. Thus,



where ● is the non-reducing end, ∅ is the reducing end, k_{nr} and k_r refer to the non-reducing and reducing ends, respectively, k_i', k_i'', and k_i refer to the "internal" bonds, and k_d is the kinetic constant for maltose.

From a mathematical point of view, k_{nr} and k_r are indistinguishable, as well as k_i' and k_i''. Hence, in order to simplify the computational approach, $k_{nr} + k_r = 2\,k_e$ (for "external" bonds) and $k_i' + k_i'' = 2\,k_i$ (for "internal" bonds) were used. On the basis of these assumptions, the following differential equations were obtained for all first-order reactions.

$$\frac{dc_{G5}}{dt} = -2(k_i + k_e)c_{G5}$$

$$\frac{dc_{G4}}{dt} = 2k_e c_{G5} - (2k_e + k_i)c_{G4}$$

$$\frac{dc_{G3}}{dt} = 2k_i c_{G5} + 2k_e c_{G4} - 2k_e c_{G3}$$

$$\frac{dc_{G2}}{dt} = 2k_i c_{G5} + 2k_i c_{G4} + 2k_e c_{G3} - k_d c_{G2}$$

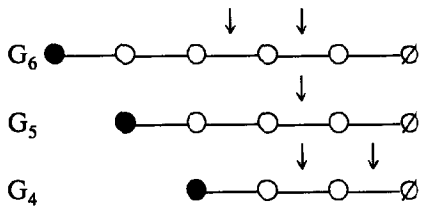
$$\frac{dc_{G1}}{dt} = 2k_e c_{G5} + 2k_e c_{G4} + 2k_e c_{G3} + 2k_d c_{G2}$$

The first-order behaviour could be verified by examining the kinetics of disappearance of the substrate starting from G_5, G_4, G_3, or G_2. The values of the parameters obtained served as initial values for the optimisation procedure applied for the treatment of the experimental results. An optimisation procedure and an objective function analogous to that described above for enzymic hydrolysis were used.

RESULTS AND DISCUSSION

The hydrolysis of maltopentaose at 25° with PPA (c_E^0 0.5 μg/mL) gave maltose and maltotriose. Maltohexaose afforded maltose, maltotriose, and maltotetraose, indicating the occurrence of competing attack as was found for maltotetraose, which gave D-glucose, maltose, and maltotriose. For G_4, however, a higher concentration of enzyme was used (c_E^0 15 μg/mL) since it appeared less susceptible to hydrolysis than G_5 and G_6. PPA hydrolysed maltotriose to give D-glucose and maltose only at concentrations as high as 1000 μg/mL.

In the series G_4–G_6, typical product distributions are shown in Fig. 1, which are consistent with the following pattern of attack observed by Robyt and French[11] using labelled substrates.



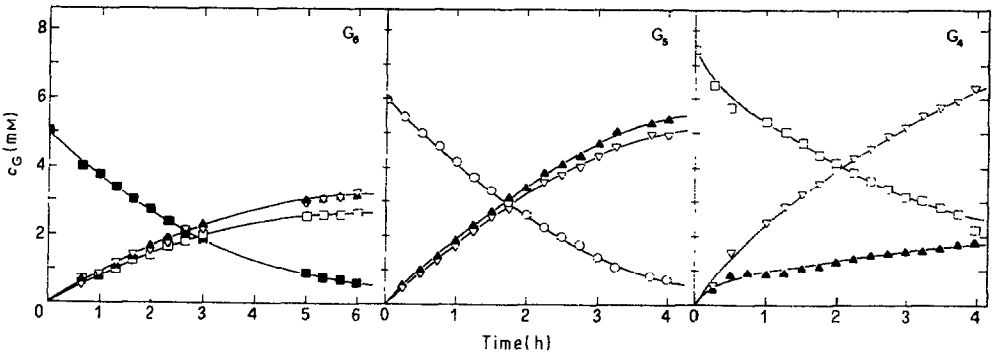


Fig. 1. Product distributions in the enzymic hydrolysis of malto-oligosaccharides: G_6, —■—; G_5, —○—; G_4, —□—; G_3, —▲—; G_2, —▽—.

The kinetics of the enzymic hydrolysis of G_5 was studied (seven runs) with the initial concentration c^0_{G5} close to 5mM. Treatment of the experimental data, according to the mathematical procedure outlined above, gave reliable K_m and V_{max} values (Table I). At this substrate concentration, there was negligible inhibition by the products. No substrate inhibition was observed up to an initial concentration of 12.1mM.

Three kinetic runs were carried out on G_6 for which, regardless of inhibition, the mechanistic model involves four parameters corresponding to the two positions of enzymic attack. In the first stage of computations, V_{max} values, determined by initial rate measurements, were used in the optimisation procedure to obtain the K_m values. Since these were all in the range 2.5–3.5mM, it seemed reasonable to assign the value 3.40mM to the K_m corresponding to position 2, by analogy with the value obtained for maltopentaose. By using this value, the K'_m values relative to position 3 were re-evaluated and gave the figures 2.93, 2.67, and 2.71mM (average 2.77). The similarity of these results supports the aforementioned assignment. By

TABLE I

KINETIC PARAMETERS AT 25° OF THE ENZYMIC HYDROLYSIS OF MALTO-OLIGOSACCHARIDES G_4–G_6 BY PORCINE PANCREATIC ALPHA-AMYLASE (c^0_E 0.5 μg/mL) AT pH 7.0 AND INITIAL RATES (V_0) WITH c^0_G 6mM

	$10^3 \times V_{max}$ (mM.min^{-1})	K_m (mM)	$10^4 \times V_{max}/K_m$ (min^{-1})	$10^4 \times V_0$ (mM.min^{-1})	Relative V_0	Pattern of attack[a] (%)
$G_6 \rightarrow G_2 + G_4$	31.7	3.4	93	114	29.2	64 (67)
$G_6 \rightarrow 2G_3$	14.6	2.8	52	63.8	16.3	36 (33)
$G_5 \rightarrow G_2 + G_3$	61.2	3.4	180	391	100	100 (100)
$G_4 \rightarrow 2G_2$	2.14	10.7	2.0	5.47	1.4	63 (70)
$G_4 \rightarrow G_1 + G_3$	1.12	9.5	1.2	3.23	0.8	37 (30)

[a]Values in brackets are from ref. 11.

using K_m values 3.40 and 2.77mM for positions 2 and 3, respectively, the optimised V_{max} values involved only minor modifications.

Three kinetic runs for maltotetraose, using the above approach, were carried out in order to obtain optimised V_{max} and K_m values.

All the results for G_4–G_6 are collected in Table I, together with the specificity constant (V_{max}/K_m), which gives a better indication of the effectiveness of a substrate[19].

By using the proper equations involved in the above models for single cleavages and with c^0_{Gi} 6mM, the following terms were evaluated: the initial rates (V_0), the relative initial rates (taking as reference the value for position 2 of G_5), and the corresponding percentages of attack within each malto-oligosaccharide which are compared with those obtained by Robyt and French[11] (Table I).

The K_m value of maltopentaose can be compared with those in the literature since a single position is involved, whereas for G_4 and G_6, it is necessary to compute the overall values from the specific values relative to the two positions of attack. The following equation was derived and applied (see Appendix).

$$K_m = \frac{K_{m1} \cdot K_{m2}}{K_{m1} + K_{m2}}$$

Computation gave overall K_m values of 1.54 (G_6), 3.40 (G_5), and 5.04mM (G_4), which indicate an increase in affinity with increase of the chain length. These values, obtained by measurements extended over a time course, differ from those observed by Laszlo *et al.*[12] (1.17mM for G_5 at 25°) and Prodanov *et al.*[16] (0.62 for G_6, 1.08 for G_5, and 0.72mM for G_4), and obtained on the basis of measurements of the initial increase of reducing power. The K_m value found for G_5 is identical to that obtained by Saito *et al.*[8] with HSA.

The sequence observed in the overall K'_m values does not appear for the overall V_{max} values which were computed by the equation (see Appendix)

$$V_{max} = \frac{V_{max1} \cdot K_{m2} + V_{max2} \cdot K_{m1}}{K_{m1} + K_{m2}},$$

and found to be 0.022 (G_6), 0.061 (G_5), and 0.002 (G_4), *i.e.*, $G_5 > G_6 \gg G_4$, and the corresponding relative overall rates were 100, 45.5, and 2.2 (with c^0_{Gi} 6mM). The large difference between the values for G_5 or G_6, and G_4 as well as between G_4 and G_3 (V_0, rel 0.07), is in line with the qualitative observations of Robyt and French[11].

As far as the pattern of attack is concerned, the findings of Robyt and French[11] were confirmed for G_6, whereas there was only a slight difference for G_4 (Table I). However, our data for G_4, as well as those of Robyt and French[11], indicate the formation of D-glucose which differs from the action pattern observed by Prodanov *et al.*[16].

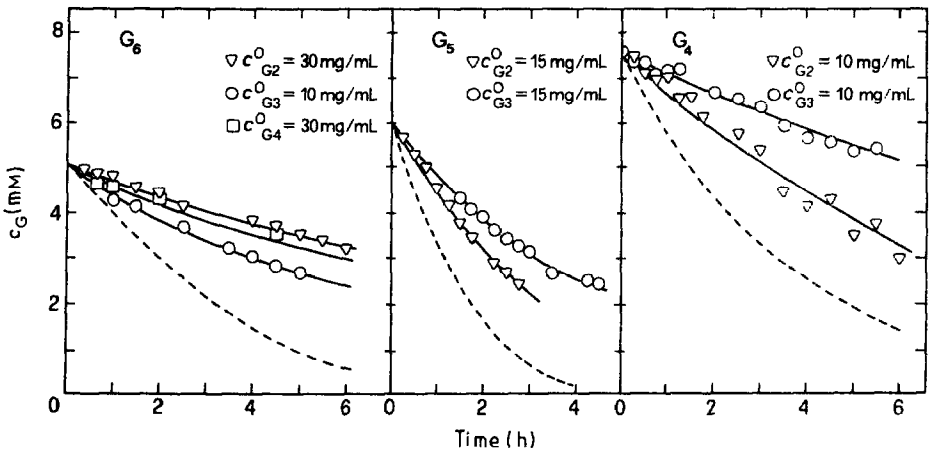


Fig. 2. Inhibition in the enzymic hydrolysis of malto-oligosaccharides (----, hydrolysis in the absence of inhibitor).

The inhibition by G_1, G_2, and G_3 in the hydrolysis of G_4–G_6 (c^0_{Gi} 5 mg/mL) was studied by using inhibitor excesses in the range 10–30 mg/mL. For malto-hexaose, inhibition by G_4 was examined since it is a product of hydrolysis. The overall inhibition study required fourteen kinetic runs.

The presence of an excess of D-glucose did not affect the rate of hydrolysis of G_5 and G_4, but enhanced the disappearance of G_6. Examples of inhibition by the malto-oligosaccharides are given in Fig. 2.

A competitive inhibition mechanism was suggested for G_3 and G_4 by the lowest values of the objective functions obtained in the optimisation procedure carried out according to this inhibition model. Support for this type of inhibition was obtained for maltotriose by examing the hydrolysis of G_6, G_5, and G_4 in the presence of an excess of G_3 as inhibitor, which gave competitive inhibition constants of 4.3–6.5, 4.3, and 4.4–5.0mM respectively. The similarity of these values indicates interaction of the inhibitor with the free enzyme. Support for the competitive inhibition mechanism of G_4 rests on the overlap of the value of its inhibition constant (3.2–5.1mM) with the overall K_m value (5.04mM) obtained for

TABLE II

COMPETITIVE INHIBITION CONSTANTS K_I OF G_I FOR THE ENZYMIC HYDROLYSIS OF G_4–G_6 BY PPA AT 25° AND pH 7.0

Inhibitor	K_I (mM)
G_4	4.1 ±1.0
G_3	4.8 ±0.6
G_2	16.9 ±7.4

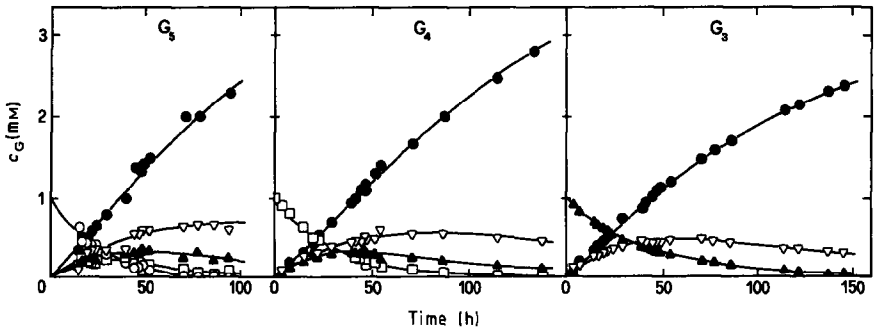


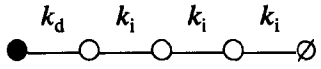
Fig. 3. Product distributions in the acid hydrolysis of malto-oligosaccharides: G_5, —○—; G_4, —□—; G_3, —▲—; G_2, —▽—; G_1, —●—.

its hydrolysis. Both constants refer to the same enzymic complex.

An unequivocal indication of the inhibition mechanism was not achieved for G_2, but a competitive mechanism was compatible with the experimental results even if the corresponding inhibition constants in the series of substrates G_4–G_6 varied by a factor of two. Hence, in order to allow useful comparisons to be made, the apparent competitive inhibition constants are reported also for maltose (Table II).

The product distribution on hydrolysis of G_3–G_5 with 0.1M H_2SO_4 was studied at 70°. The disappearance of each substrate followed pseudo-first-order kinetics and examples are shown in Fig. 3.

When the experimental results were treated according to the above mathematical model, the best optimisation was obtained by assuming the same rate coefficient for the cleavage of all the linkages except that at the non-reducing end, and putting $2k_e = k_d + k_i$. For example, for G_5,



k_d coincides with the pseudo-first-order hydrolysis rate constant of maltose (2.40×10^{-4} min^{-1})[14]. The optimisation procedure gave k_i values of 1.34×10^{-4} (G_5), 1.21×10^{-4} (G_4), and 1.31×10^{-4} min^{-1} (G_3). These values are similar and underline the validity of the assumption. Thus, on the basis of the average value of 1.29×10^{-4} min^{-1}, the pseudo-first-order rate constant (k') for the acid hydrolysis of G_1 is given by

$$10^4 k' = 2.40 + 1.29\,(i - 2).$$

Since, for 0.05–0.5M H_2SO_4, the equation $k' = k\,[H_3O^+]$ applies, k being the

second-order rate constant, the following expression analogous to that for k' can be derived for k ($min^{-1}.N^{-1}$).

$$10^3k = 1.20 + 0.65\ (i - 2)$$

Inspection of Table III reveals that the values obtained using the above equation accord with the experimental data.

The above findings underline the different pattern of attack in acid and enzymic hydrolysis, whereas Table IV shows the different reaction rates involved in the two cases.

TABLE III

COMPARISON OF THE EXPERIMENTAL KINETIC COEFFICIENTS OF THE ACID HYDROLYSIS OF MALTO-OLIGOSACCHARIDES G_i WITH H_2SO_4 AT 70° AND THE CORRESPONDING VALUES CALCULATED ACCORDING TO THE EQUATION $10^3k = 1.20 + 0.65(i - 2)$.

G_i	$10^3k(min^{-1}.N^{-1})$	
	Exptl.	*Calc.*
G_3	1.9	1.85
G_4	2.5	2.50
G_5	3.2	3.15
G_6	3.9[a]	3.80

[a]Ref. 14b.

TABLE IV

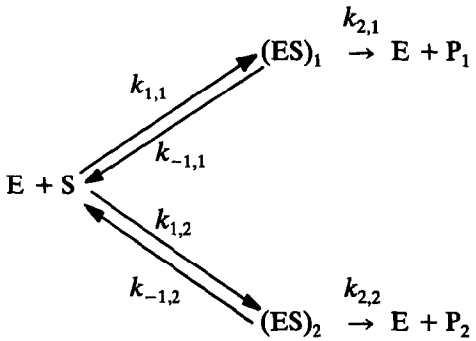
COMPARISON OF ENZYMIC AND ACID HYDROLYSIS IN TERMS OF THE INITIAL RATE OF DISAPPEARANCE OF MALTO-OLIGOSACCHARIDE G_i WITH c^0_{Gi} 6mM

Oligosaccharide	V_0 (mM.min^{-1})	
	Enzymic[a]	*Acid*[b]
G_6	1.8×10^{-2}	4.7×10^{-3}
G_5	3.9×10^{-2}	3.8×10^{-3}
G_4	8.7×10^{-4}	3.0×10^{-3}

[a]25°; c^0_E 0.5 μg/mL. [b]70°; 0.1M H_2SO_4.

APPENDIX

If a substrate S is susceptible to enzymic attack at two different positions so that its rate of disappearance (V) is the sum of the two corresponding specific rates (V_1 and V_2), the following mechanism must be considered.



Writing out differential equations for $c_{(ES)_1}$ and $c_{(ES)_2}$, and applying the pseudo-steady-state assumption, results in equations *1* and *2*.

$$\frac{dc_{(ES)_1}}{dt} = k_{1,1}\, c_E c_S - (k_{-1,1} + k_{2,1})\, c_{(ES)_1} = 0 \qquad (1)$$

$$\frac{dc_{(ES)_2}}{dt} = k_{1,2}\, c_E c_S - (k_{-1,2} + k_{2,2})\, c_{(ES)_2} = 0 \qquad (2)$$

By adopting definitions *3* and *4*,

$$K_{m1} = (k_{-1,1} + k_{2,1})/k_{1,1} \qquad (3)$$

$$K_{m2} = (k_{-1,2} + k_{2,2})/k_{1,2} \qquad (4)$$

expressions for $c_{(ES)_1}$ and $c_{(ES)_2}$ can be derived from equations *1* and *2*, as follows.

$$c_{(ES)_1} = c_E c_S / K_{m1} \qquad (5)$$

$$c_{(ES)_2} = c_E c_S / K_{m2} \qquad (6)$$

Equations *5* and *6*, combined with equation *7* representing the conservation of enzyme,

$$c_E^0 = c_E + c_{(ES)_1} + c_{(ES)_2} \qquad (7)$$

result in the following expression for c_E.

$$c_E = \frac{c_E^0}{1 + c_S(1/K_{m1} + 1/K_{m2})} \tag{8}$$

By using equations *8*, *5*, and *6*, the following expressions for rates are derived.

$$V_1 = k_{2,1}c_{(ES)_1} = \frac{k_{2,1}c_E^0 c_S}{K_{m1} + c_S(1 + K_{m1}/K_{m2})} = \frac{V_{max1} c_S}{K_{m1} + c_S(1 + K_{m1}/K_{m2})} \tag{9}$$

$$V_2 = k_{2,2}c_{(ES)_2} = \frac{k_{2,2}c_E^0 c_S}{K_{m2} + c_S(1 + K_{m2}/K_{m1})} = \frac{V_{max2} c_S}{K_{m2} + c_S(1 + K_{m2}/K_{m1})} \tag{10}$$

If the rate of disappearance of S is considered in terms of overall K_m and V_{max} values, the following relation can be obtained.

$$\frac{V_{max}c_S}{K_m + c_S} = \frac{V_{max1}c_S}{K_{m1} + c_S(1 + K_{m1}/K_{m2})} + \frac{V_{max2}c_S}{K_{m2} + c_S(1 + K_{m2}/K_{m1})} =$$

$$= \frac{[(V_{max1}K_{m2} + V_{max2}K_{m1})/(K_{m1} + K_{m2})]c_S}{(K_{m1}K_{m2})/(K_{m1} + K_{m2}) + c_S}, \tag{11}$$

whence

$$V_{max} = \frac{V_{max1}K_{m2} + V_{max2}K_{m1}}{K_{m1} + K_{m2}} \tag{12}$$

and

$$K_m = \frac{K_{m1}K_{m2}}{K_{m1} + K_{m2}} \tag{13}$$

In the presence of a single inhibitor I interacting with enzyme E as well as with both enzyme–substrate complexes $(ES)_1$ and $(ES)_2$, the equation for enzyme conservation becomes

$$c_E^0 = c_E + c_{(ES)_1} + c_{(ES)_2} + c_{EI} + c_{(ESI)_1} + c_{(ESI)_2}. \tag{14}$$

Equations *5* and *6*, combined with equations *14* and *15–17*, give the inhibition constants for inhibitor I,

$$K_I = c_E c_I / c_{EI}, \tag{15}$$

$$K_{I1} = c_{(ES)_1} c_I / c_{(ESI)_1}, \text{ and} \tag{16}$$

$$K_{I2} = c_{(ES)_2} c_I / c_{(ESI)_2}, \tag{17}$$

and result in the following expression for c_E.

$$c_E = \frac{c_E^0}{(1 + c_I/K_I) + (c_S/K_{m1})(1 + c_I/K_{I1}) + (c_S/K_{m2})(1 + c_I/K_{I2})} \tag{18}$$

By using equations *18*, *5*, and *6*, the following expressions for rates are derived.

$$V_1 = k_{2,1} c_{(ES)_1} = \frac{V_{max1} c_S}{K_{m1}(1 + c_I/K_I) + c_S(1 + c_I/K_{I1}) + c_S(1 + c_I/K_{I2})(K_{m1}/K_{m2})} \tag{19}$$

$$V_2 = k_{2,2} c_{(ES)_2} = \frac{V_{max2} c_S}{K_{m2}(1 + c_I/K_I) + c_S(1 + c_I/K_{I2}) + c_S(1 + c_I/K_{I1})(K_{m2}/K_{m1})} \tag{20}$$

In the presence of a multiplicity of inhibitors, the terms $(1 + c_I/K_I)$, $(1 + c_I/K_{I1})$, and $(1 + c_I/K_{I2})$ assume the forms $(1 + \sum_j c_{Ij}/K_{Ij})$, $(1 + \sum_j c_{Ij}/K_{I1,j})$, and $(1 + \sum_j c_{Ij}/K_{I2,j})$.

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