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KINETIC STUDY OF THE INHIBITION OF THE HONEYBEE HAEMOLYMPH α-GLUCOSIDASE *IN VITRO* BY BAYe 4609, BAYg 5421 and BAYn 5595

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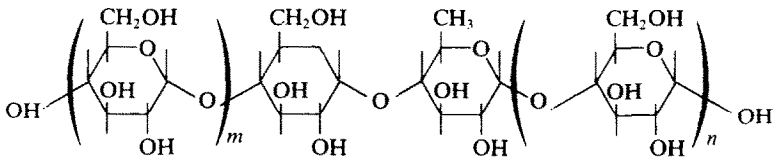
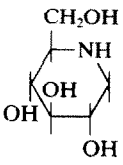
Abstract—Three therapeutic inhibitors of vertebrate α-glucosidases recently assayed in research on diabetes control, show high inhibitory potencies towards the *p*-NP-α-D-glucosidase activity of honeybee haemolymph. BAYe 4609 is an allosteric *V*-type (pure non-competitive) inhibitor with: $K_i \simeq K_i' \simeq I_{50} \simeq 180\ \mu M$; $n = 1.17$; $n_i = 1.15$. BAYg 5421, an hydrolysis derivative of the former, is a mixed allosteric inhibitor with: $K_i \simeq 0.17\ \mu M$; $K_i' \simeq 0.85\ \mu M$; $I_{50} \simeq 0.38\ \mu M$; $n = 1.19$; $n_i = 1.25$. BAYn 5595 is a pure competitive Michaelian inhibitor with: $K_i = 15\ \mu M$; $I_{50} \simeq 23\ \mu M$. All these properties reveal similarities to and differences from those of the natural inhibitors of the enzyme and analogies with their action on vertebrate enzymes. Accordingly, correlations have been emphasized between the structure and the activity of these inhibitors which finally lead to propositions of structures for new active molecules.

Dieting plays an important part in *diabetes mellitus*, so that intestinal inhibitors of amylases could be successfully assayed, almost 10 years ago, in the control of postprandial hyperglycaemia [1]. Recently, two novel generations of α-glucosidase inhibitors have been isolated from micro-organisms: the first type, including BAYe 4609 and BAYg 5421 (Acarbose) was extracted from *Actinomycetes* [2] and the second type, BAYn 5595 (Desoxynojirimycin), from several strains of *Bacillus* [3].



BAYe 4609: $2 \leq m \leq 3$; $5 \leq n \leq 28$
BAYg 5421: $m = 0$; $1 \leq n \leq 5$



BAYn 5595

All these compounds share positive effects in the control of both hyperglycaemia and hypertriglyceridaemia after oral administration in vertebrates, including man [4–6]. Acarbose, an hydrolysis product of BAYe 4609 is 100–500 times more effective in α-glucosidase inhibition than the latter, indicating a decrease in potency according to the length of the glucoside chains of the molecules [6]. *In vitro* studies have recently shown that BAYg 5421 acts as a competitive inhibitor towards the rat α-glucosidase [2].

In these conditions the activity of the inhibitors was compared, as determined in vertebrates, to their kinetic effects on honeybee α-glucosidases for the following reasons.

(a) In the case of similar potencies, the insect enzymes would provide an advisable cheap material for the screening of new inhibitors. Studies on the hormonal control of insect glycaemia [7, 8] (another side of the same problem) have already shown important similarities with vertebrate biochemistry.

(b) Thorough studies of the kinetic properties of honeybee haemolymph α-glucosidases have shown interesting particularities, including a feedback inhibition of the negative cooperativity type by glucose [9], and allosteric properties in some natural conditions, mainly at the foraging-adult stage [10]. The regulation properties, first shown by *in vitro* experiments, were confirmed *in vivo* [11], emphasizing their physiological significance. So, it can be expected that studying the molecular mechanisms of therapeutic inhibitors would provide important data for research into new active molecules.

The aim of this work was, therefore, to test the inhibition ability and the corresponding kinetics mechanisms of BAYe 4609, BAYg 5421 and BAYn 5595 towards honeybee α-glucosidases, and to compare their relative potencies with those already determined in vertebrates.

MATERIALS AND METHODS

Emerging adults (*Apis mellifica* L.) were collected on the brood-combs from operculated alveoli, just before they broke out, and weighed. The average weight of the individuals used in the experiments was: 122.6 ± 5.8 mg ($N = 107$). Haemolymph was punctured out by use of microsyringes; a total of 400 μl was pooled from batches of 16 bees, then centrifuged for 15 min at 4000 *g* and kept at 0°C under a layer of decane. The α-glucosidase assays were performed by continuous recording of *p*-nitro-phenyl (NP)-α-D-glucosidase as previously described [9]. Progress curves were developed at a temperature of 30 ± 1°C for the incubating medium. In these conditions, the thermic activation coefficient was determined between 28 and 34°C as: A = 38.76 kJ/d ($N = 11$ points; $\rho = -0.925$). The kinetics parameters were determined by a new rigorous, non-iterative, algebraic method [12] applicable to the general case of the Hill equation: $v = V_M \cdot S^n/(K + S^n)$ (v = initial velocity, V_M = maximum velocity, S = substrate concentration, K = dissociation constant, n = Hill coefficient). The Augustinsson plot (v/S v v) was used for the illustration of saturation curves and also, together with the Hanes plot (S/v v S), for the regression calculation of the Michaelian parameters. The study of inhibitor action was completed with the Dixon plot: $v^{-1} = f(\mathrm{I})$ and the equation of Chou [13] $(v_o/v_i) - 1 = (\mathrm{I}/\mathrm{I}_{50})^{n_i}$ (v_o = control velocity; v_i = velocity in presence of the inhibitor concentration I; I_{50} = inhibitor concentration giving $v_i = v_o/2$; n_i = Hill coefficient of the inhibitor binding to enzyme). The inhibition constants and general mechanisms of action can be summarized:

(a) Mixed inhibition

$$\begin{array}{ccc} E + n\,S \overset{K_o}{\rightleftharpoons} (E\,Sn) & \text{-----}\rightarrow & E + \text{products} \\ {\scriptstyle +n_i\,\mathrm{I}\,(K_i)}\downarrow\uparrow \quad {\scriptstyle +n_i\,\mathrm{I}\,(K_i)}\downarrow\uparrow & & \\ (E\,\mathrm{I}n_i) \qquad (E\,Sn\,\mathrm{I}n_i) & & \text{inactive} \end{array}$$

(b) Non-competitive inhibition (a particular case of mixed inhibition)

$$\begin{array}{ccc} E + n\,S \overset{K_o}{\rightleftharpoons} (E\,Sn) & \text{-----}\rightarrow & E + \text{products} \\ {\scriptstyle +n_i\,\mathrm{I}\,(K_i)}\downarrow\uparrow \quad {\scriptstyle +n_i\,\mathrm{I}\,(K_i)}\downarrow\uparrow & & \\ {\scriptstyle +n\,S} & & \\ (E\,\mathrm{I}n_i) \rightleftharpoons (E\,\mathrm{I}n_i\,Sn) & \text{-----}\rightarrow & (E + n_i\mathrm{I}) + \text{products} \end{array}$$

(c) Inhibition parameters: the inhibition factors can affect V_M, K, or V_M/K in the following manner in Michaelian cases. (I) might be assumed to be replaced by $(\mathrm{I})^{n_i}$ in the other cases, but this needs further theoretical support.

V_M (inhibition) $= V_M/(1 + \mathrm{I}/K_i')$
$f_i = 1 + \mathrm{I}/K_i$
K_R (inhibition) $= K_o\,(1 + \mathrm{I}/K_i)/(1 + \mathrm{I}/K_i')$
f_i = (control/inhibition) parameters
$V_M/K_R = (V_M/K_o)/(1 + \mathrm{I}K_i)$
$f_i = 1 + \mathrm{I}/K_i$

The statistical analyses of data (variance, regressions, correlations, probabilities) were achieved with a TI-59 calculator fitted with the 'Applied Statistics Module'. The probabilities for significances were calculated according to the Student t test, with $A(t) = 2\,\mathrm{P} - 1$. All the kinetic points were determined at least in triplicate and the standard value of the coefficient of variation was established over all the experiments as S.E.M./$\bar{x} = \pm 0.068$.

The inhibitor concentrations in the reactive medium are expressed in mg/ml and in molar concentrations, according to the following conventionally estimated molecular weights: BAYe 4609, MW ≃ 1500–6000 [6], that is: MW ≃ 3750; BAYg 5421: MW ≃ 824 [2]; BAYn 5595: MW ≃ 163 [3].

RESULTS

The three studied inhibitors led to strong inhibition of honeybee α-glucosidases, but they differed in their molecular mechanisms of action, according to their structural differences. The enzyme properties can vary within some limits from one to another batch of bees, even coming from the same hive: this depends on the daily climatic and nutritive changes resulting in differences in the rearing of larvae, and consecutively in their biochemical characteristics; so each kinetic study has been run comparatively to corresponding controls performed on the same extracts.

BAYe 4609. The saturation curves plotted according to Augustinsson are illustrated in Fig. 1A. Controls show a linear (Michaelian) relation, whereas the experimental points obtained in presence of 0.5 mg/ml inhibitor (0.133 mM) show a curvature indicating a deviation from Michaelian kinetics. The linear part of this last curve gives a first estimate of the parameters: $\rho = -0.999$; $V_M = 12.45$ μM/min/μl and $K = 5.96$ mM. In controls, the linear regression calculated from the Augustinsson representation gives: $\rho = -0.998$; $V_M = 18.64$ μM/min and $K_m = 5.71$ mM; this agrees with the general equations of Bounias, giving ($N = 4$): $V_M = 20.7$ μM/min/μl (S.E.M. = 2.54); $K = 6.54$ mM (S.E.M. = 0.92); $n = 1.01$ (S.E.M. = 0.040). The average values leading to the determination of K_i and K_i' are indicated in Table 1.

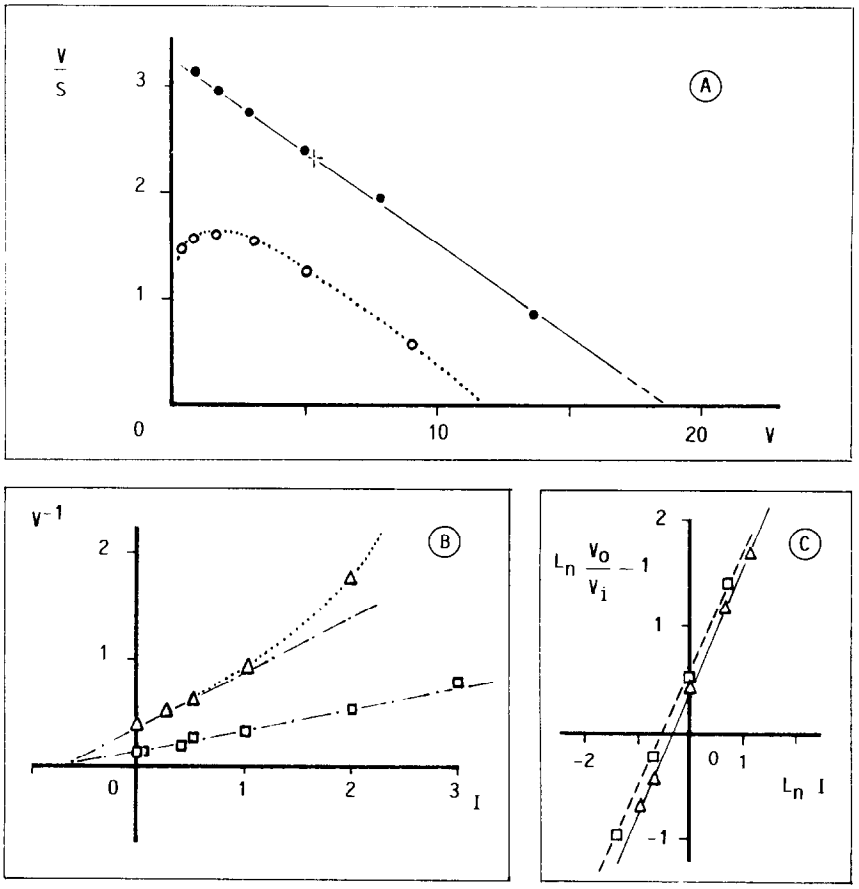


Fig. 1. Kinetic effects of BAYe 4609 on honeybee *p*-NP-α-D-glucosidase. (A) Augustinsson plots of the saturation curves: (●——●) controls, (○....○.....○) 0.5 mg/ml (0.133 mM) inhibitor. (B) Dixon plots with (△---△) S = 1 mM and (□---□) S = 4 mM. (C) Chou plots with (△---△) S = 1 mM and (□——□) S = 4 mM; v_o with I = 0, v_i with I. V is expressed in μM/min/μl, I in mg/ml.

In the presence of inhibitor, the Hill coefficient increases to significantly above 1 ($A(t) = 0.028$) indicating an allosteric mechanism (positive cooperativity). The maximum velocity is depressed and the constant K not significantly affected (P = 0.99996) suggesting a non-competitive inhibition process.

On the Dixon plots, the point I = 2 mg/ml shows an important departure from linearity at the lower S concentration (1 mM). The regression equations plotted for the linear parts of the curves are, respectively:

◊ with S = 1 mM: $V^{-1} = 0.215\ \text{I} + 0.1035$ ($N = 7$; $\rho = 0.997$)

◊ with S = 4 mM: $V^{-1} = 0.578\ \text{I} + 0.347$ ($N = 4$; $\rho = 0.9996$)

Their intersection gives K_i = 0.67 mg/ml or $K_i \simeq 178.6$ μM.

Plotting the logarithmic form of the equation of

Table 1. Kinetics data for the inhibition of honeybee α-glucosidases by BAYe 4609. The S.E.M. are indicated between parentheses after N determinations

	V_M (μM/min/μl)	K or K_m (mM)	V_M/K or V_M/K_m	n
Controls (N = 5)	20.3 (2.4)	6.37 (0.89)	3.187	1.01 (0.004)
Inhibition (N = 5) I = 133.5 μM (0.5 mg/ml)	11.7 (1.5)	6.26 (0.96)	1.867	1.17 (0.04)
f_i	1.735	1.0	1.707	1.16
K_i' mg/ml	0.779	—	—	—
K_i' μM	181.6			
K_i μg/g	—	—	0.710	—
K_i μM			188.8	

$K_i'/K_i = 0.962 \simeq 1$.

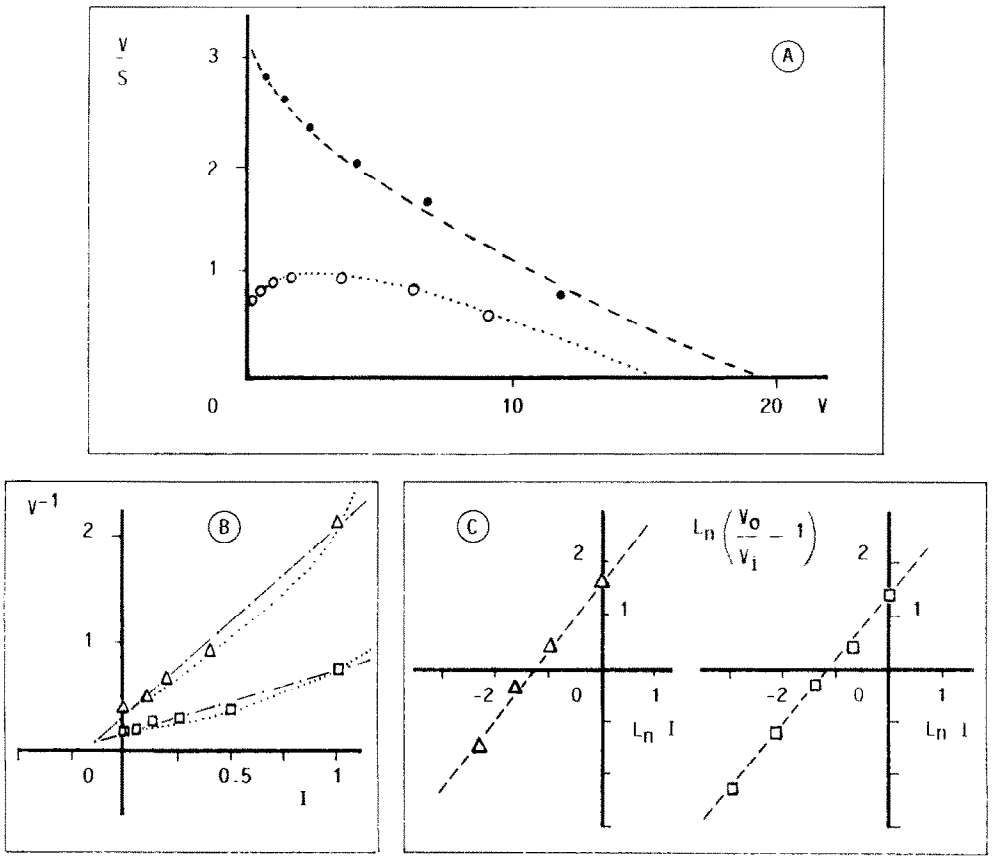


Fig. 2. Kinetic effects of BAYg 5421 on honeybee *p*-NP-α-D-glucosidase. (A) Augustinsson plots of the saturation curves: (●——●) controls; (○....○....○) 0.25 μg/ml (0.3 μM) inhibitor. (B) Dixon plots with (△---△) $S = 1$ mM and (□---□) $S = 4$ mM. (C) Chou plots with (△---△) $S = 1$ mM and (□——□) $S = 4$ mM; v_o with $I = 0$, v_i with I. v_i is expressed in μM/min/μl, I in μg/ml.

Chou: $L_N\ (v_o/v - 1) = n_i L_N I - n_i L_N I_{50}$ gives two linear curves (Fig. 1C) from which I_{50} and n_i can be determined:

◊ with $S = 1$ mM: $n_i = 1.12$; $I_{50} = 0.62$ mg/ml ($N = 4$) ≃ 164.9 μM ($\rho = +0.999$)
◊ with $S = 4$ mM: $n_i = 1.17$; $I_{50} = 0.725$ mg/ml ($N = 5$) ≃ 192.8 μM ($\rho = +0.999$)

The average values are $n_i = 1.145$ and $I_{50} = 0.707$ mg/ml or $I_{50} \simeq 178.9$ μM.

The two determinations of K_i give almost identical values, close to K_i' and to I_{50} which confirms the identification of a pure non-competitive inhibition of the allosteric '*V*-type'.

BAYg 5421 (Acarbose). The saturation curves of controls and inhibition series ($I = 0.25$ μg/ml, that is $I \simeq 0.3$ μM) are illustrated on Fig. 2A. In this case, the control curve shows a slight curvature corresponding to a weak expression of negative cooperativity, as often encountered at this stage [14]. In the presence of Acarbose, the curvature is the opposite way, as already seen with BAYe 4609. The kinetic parameters, determined from the general

Table 2. Kinetic parameters of the honeybee α-glucosidase inhibition by BAYg 5421. The S.E.M. are given between parentheses after *N* determinations

	V_M (μM/min/μl)	K or K_m (mM)	V_M/K or V_M/K_m	n
Controls ($N = 5$)	20.7 (3.0)	7.74 (1.28)	2.67	0.95 (0.05)
Inhibition ($N = 6$)				
$I = 0.3$ μM (0.25 μg/ml)	15.2 (1.9)	16.7 (2.3)	0.91	1.19 (0.03)
f_i	1.362	2.158^{-1}	2.93	1.25
K_i' μg/ml	0.69	—	—	—
K_i' μM	0.85			
K_i μg/ml	—	—	0.130	—
K_i μM			0.16	

$K_i'/K_i = 5.38$.

equations available in non-Michaelian cases, are given in Table 2.

Both V_M and K are affected by the inhibitor, predominantly for K, which indicates an important apparently-competitive part in the mechanism. The allosteric effect is even more pronounced than in the case of BAYe 4609.

The Dixon plots (Fig. 2B) show less pronounced deviations from linearity. The corresponding regression equations are

◊ with $S = 1$: $v^{-1} = 1.777\ \text{I} + 0.30$ ($N = 5$; $\rho = 0.9996$)
◊ with $S = 4$: $v^{-1} = 0.585\ \text{I} + 0.12$ ($N = 6$; $\rho = 0.990$)

Their intersection gives $K_i = 0.15\ \mu$g/ml or $K_i = 0.184\ \mu$M. This value is in good agreement with the previous estimation from f_i; the average is $K_i = 0.172\ \mu$M.

According to the representation of Chou (Fig. 2C), the following values can be determined

◊ with $S = 1$ mM: $n_i = 1.27$; $\text{I}_{50} = 0.295\ \mu$g/ml or $\text{I}_{50} = 0.36\ \mu$M ($N = 4$; $\rho = 0.996$)
◊ with $S = 4$ mM: $n_i = 1.21$; $\text{I}_{50} = 0.328\ \mu$g/ml or $\text{I}_{50} = 0.40\ \mu$M ($N = 5$; $\rho = 0.998$)

the average values are $n_i = 1.24$ and $\text{I}_{50} = 0.38\ \mu$M, which is situated between K_i and K_i'.

All these data agree with a mixed ($V + K + n$) allosteric inhibition, in which the high value of the ratio K_i'/K_i indicates a larger affinity of the inhibitor for pure enzyme than for the (ES) complex. The fact that the inhibition does not level off at high values of [I] rules out the possibility of a kinetically competent (ESI) complex.

BAYn 5595 (Desoxynojirimycin). The kinetics of enzyme saturation have been studied in controls and in presence of $\text{I} = 15\ \mu$M (2.5 μg/ml). The Augustinsson plots, illustrated in Fig. 3A, show linear curves as confirmed by the determination of Hill coefficients from the general equation: in controls ($N = 6$) $n = 1.002$ (S.E.M. = 0.038) and with inhibitor ($N = 4$) $n = 1.017$ (S.E.M. = 0.033).

In these conditions, the determinations of V_M and K_m give similar results from the general equations as well as from the Augustinsson and the Hanes plots. The average results are shown in Table 3.

The maximum velocity is not significantly altered by the presence of inhibitor (P = 0.99993) whereas the constant K is almost doubled. This indicates a pure competitive Michaelian inhibition. No value of K_i' can be determined.

The Dixon representations are linear (Fig. 3B) and their respective equations of regression are

◊ with $S = 1$ mM: $v^{-1} = 0.157\ \text{I} + 0.469$ ($N = 5$; $\rho = 0.9995$)
◊ with $S = 4$ mM: $v^{-1} = 0.056\ \text{I} + 0.187$ ($N = 6$; $\rho = 0.9975$).

Their intersection gives $K_i = 2.49\ \mu$g/ml or $K_i = 15.2\ \mu$M which is in close agreement with the previously determined value. The average is $K_i = 15.06\ \mu$M. The intersection point also gives $V_M = 12.62\ \mu$M/min/μl in agreement with the results of Table 3.

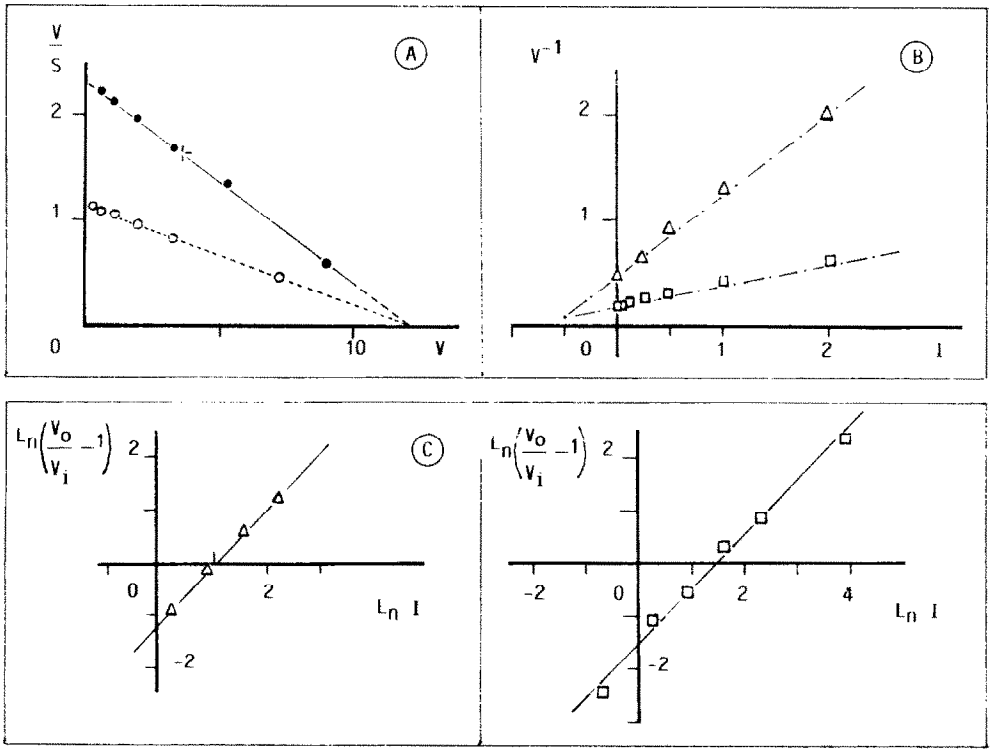


Fig. 3. Kinetic effects of BAYn 5595 on honeybee *p*-NP-α-D-glucosidase. (A) Augustinsson plots of the saturation curves: (●——●) controls; (○....○....○) 2.5 μg/ml (15 μM) inhibitor. (B) Dixon plots etc. and (C) Chou plots etc. as in Fig. 2.

Table 3. Kinetic parameters of honeybee α-glucosidase inhibition by BAYn 5595

	V_M (μM/min/μl)	K_m (mM)	V_M/K_m
Controls ($N = 7$)	12.54 (1.61)	5.40 (0.82)	2.32
Inhibition ($N = 6$) $I = 15\ \mu M$ ($2.5\ \mu g/ml$)	11.81 (1.23)	10.33 (1.19)	1.14
f_i	0.94	1.91^{-1}	2.03
K_i'	∞	—	—
K_i $\begin{cases}\mu g/ml \\ \mu M\end{cases}$	—	—	$\begin{cases}2.43 \\ 14.87\end{cases}$

The logarithmic form of the equation of Chou (Fig. 3C) then gives

◊ with $S = 1$ mM: $n_i = 1.03$; $I_{50} = 2.99\ \mu g/ml$ or $I_{50} = 18.36\ \mu M$ ($N = 4$; $\rho = 0.999$)
◊ with $S = 4$ mM: $n_i = 1.02$; $I_{50} = 4.50\ \mu g/ml$ or $I_{50} = 27.67\ \mu M$ ($N = 6$; $\rho = 0.994$)

The average values are $n_i = 1.025$; $I_{50} = 23.0\ \mu M$.

DISCUSSION AND CONCLUSION

The relative potencies of the 3 inhibitors on the honeybee *p*-NP-α-D-glucosidases can be classified as follows, according to the determined I_{50} values expressed either in molarities or in ponderal concentrations:

(with I_{50} in μM)
BAYe 4609 ..... level 1
BAYn 5595 ..... level 7
BAYg 5421 ..... level 447

(with I_{50} in μg/ml)
BAYe 4609 ..... level 1
BAYn 5595 ..... level 189
BAYg 5421 ..... level 2273

In vertebrates, the comparison of the doses giving 50% inhibition of various α-glucosidases by BAYe 4609 and BAYg 5421 shows the same classification order, but the potency of BAYg 5421 reaches a maximum of 810 times the potency of BAYe 4609 [6], that is 1/2.8 of the amplitude observed for the honeybee enzyme.

Then, Schmidt *et al.* [2] have estimated the K_i of BAYg 5421 for porcine sucrase to be: $K_i = 0.26\ \mu M$ *in vitro*: this is close to our result for honeybee enzyme ($0.17\ \mu M$). Our experiments allow us to conclude that close similarities exist between the 'kinetic behaviour' of these inhibitors towards insect and vertebrate enzymes. The results then show that BAYn 5595 is an efficient inhibitor, too.

The mechanisms of action are different for each of the three studied inhibitors, depending on their molecular structures. BAYe 4609 and BAYg 5421 belong to the same chemical class. Their common mode of action results in allosteric binding of both inhibitor and substrate to enzyme (average $n \simeq 1.23$ over all the experiments, that is $\simeq 10/8$), but BAYg 5421 shows a mixed inhibition with a higher f_i for the competitive aspect, whereas the action of BAYe 4609 is strictly non-competitive. The inhibition factor in the latter case ($f_i = 1.73$) is identical to the average f_i of the former ($f_i(V_M, K_m) = 1.76$), indicating a distribution of the V and K types instead of focusing on the V type. From a molecular viewpoint, this means that the (enzyme–substrate–inhibitor) complex might be inactive in the case of Acarbose, whereas it might be dissociated in the case of BAYe 4609. The corresponding structural difference is the free cyclitol terminal of Acarbose which could link to a receptor site of the enzyme and/or block the substrate binding by steric effect. On the other hand, the two terminal glucose radicals would reversibly link to one of the enzyme active sites and allow a certain rate of dissociation of the complex. Consequently, it would be interesting to check if the glucoside terminals of BAYe 4609 can play the role of a substrate.

For BAYg 5421, the ratio K_i'/K_i is high, indicating a greater affinity for the enzyme than for the complex (enzyme–substrate). In both cases there must be at least 2 acceptor-sites for glucose terminals on the 'elementary' enzyme molecule, which is in agreement with the conditions for an allosteric transition.

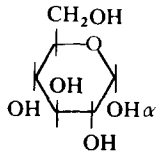
A comparison can be made with the inhibitory action of 2 other glucosides, sucrose and trehalose, on the *p*-NP-α-D-glucosidases of emerging honeybees. Sucrose acts as a mixed inhibitor of ($V + K + n$) type, as does BAYg 5421, but it is weaker: $I_{50} \simeq 154$ mM; $K_i \simeq 262$ mM; $K_i' = 477$ mM and the Hill coefficients are lower than 1: $n = 0.90$; $n_i = 0.656$ [15]. Trehalose may act in the same way ($I_{50} \simeq 98$ mM, $K_i \simeq 63$ mM; $K_i' \simeq 168$ mM; $n = 1$; $n_i = 0.91$) [16] but in non-Michaelian cases ($n_o < 1$), n decreases to 0.8 while n_i goes up to 1.4 and the inhibition has a slight tendency to the non-competitive type [14]. By comparison, it seems that the presence of 2 glucoside chains at the extreme parts of the inhibitor molecule (BAYe 4609 and trehalose) leads to non-competitive tendencies, whereas a single glucoside terminal associated with an inactive moiety (sucrose = fructo-glucoside, BAYg 5421 = cyclitol-deoxyglucose-glucoside) gives a more marked mixed inhibition.

The major differences coming from the presence of the cyclitol-deoxyglucose moiety are: the strongest potencies specially expressed on K, and the allosteric effects both on n and n_i; in this case, the cyclitol terminal of BAYg 5421 plays a part that the fructose moiety of sucrose does not.

At last, it can be pointed out that within some limits, the cooperative effects of BAYe 4609 could

be affected by the lengths of the chains. However, the observation of similar allosteric properties with BAYg 5421 (a more homogeneous molecule) enfeebles this objection. Then, these cooperative properties could not be mimicked by the superimposition of several kinetics, since this would give aberrant results when plotting the data for calculating Hill equations.

The case of BAYn 5595 is different. The mechanism of inhibition is of the pure competitive type and the molecular structure of this compound shows a great analogy with D-glucose

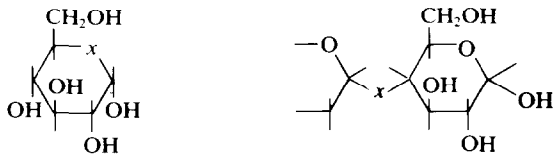


(α-D-glucopyranose).

This first indicates a competition for the same site of the enzyme molecule. But the comparison with glucose effects on the *p*-NP-α-D-glucosidase activity brings additional information: in emerging bees, glucose also acts as a competitive inhibitor *in vivo* as well as *in vitro* [17] with $K \simeq 25$ mM but the Hill coefficient shows a tendency to decrease below 1, which is particularly evident at other stages [9, 12]. So, it seems likely that the modifications made to the natural hexose structure play an essential part in the deviations of Hill coefficients, either from below 1 to 1 (glucose and BAYn 5595) or from 1 to above 1 (sucrose and BAYg 5421).

Different types of new inhibitors would probably result from the following modifications:

(1) from glucose analogs (2) from pseudo-glucoside bonds



Competitive inhibitors Mixed inhibitors

In conclusion, these experiments show that honeybee haemolymph-α-glucosidases might provide an adequate cheap material for screening the potency of new inhibitors and studying their structure/activity mechanisms of action. Additionally, the two inhibitors BAYg 5421 and BAYe 4609 gave us *for the first time* an allosteric inhibition at a stage during which the non-Michaelian properties observed in natural conditions of regulation are oriented to negative cooperativity: this confirms the universality of the kinetic processes of the regulation of honeybee haemolymph α-D-glucosidases.

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