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KINETIC COOPERATIVITY IN THE CONCERTED MODEL FOR ALLOSTERIC ENZYMES

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The cooperativity of enzyme–substrate interactions is investigated in the concerted allosteric model of Monod, Wyman and Changeux. The general case of K–V systems is considered, in which the two protomer conformational states R and T postulated in the theory differ in catalytic and binding properties. An expression for the Hill coefficient n_H defined with respect to the asymptotic velocity V_∞ is analyzed in conditions which exclude substrate inhibition. Kinetic cooperativity is always positive ($n_H > 1$) in the case of a dimer enzyme, and in the case of an inactive T state. Slight kinetic negative cooperativity ($n_H < 1$) occurs under restrictive conditions for larger numbers of protomers when the substrate binds significantly to the less active state of the enzyme, but the phenomenon remains negligible for trimers and tetramers.

These conclusions differ from those obtained [A. Goldbeter, J. Mol.Biol.90 (1974) 185] with the Hill coefficient based on the absolute maximum velocity, which may exceed the experimental value V_∞ in K–V systems. The results extend those of Paulus and DeRiel [J. Mol. Biol. 97 (1975) 667] and support the view that in most cases, negative cooperativity is not compatible with a mechanism based on a concerted and conservative allosteric transition. The Hill coefficients for binding and catalysis are compared in K–V systems.

1. Introduction

The cooperativity of allosteric interactions in multisubunit enzymes can be expressed by the slope of a Hill plot, known as the Hill coefficient n_H [1,2]. Depending on the value of the slope, cooperativity is either positive ($n_H > 1$) or negative ($n_H < 1$); absence of cooperative interactions, as in Michaelian enzymes, corresponds to a Hill number of 1 [3,4]. Several authors have emphasized the interest of considering profiles of the Hill coefficient as a function of ligand concentration, rather than linearized Hill plots [5–7]. This can be achieved explicitly in models for allosteric enzymes.

An analytical expression of the Hill coefficient related to the substrate is of special interest in the concerted model of Monod, Wyman and Changeux [8], since negative cooperativity is usually considered as the principal argument against this mechanism [9]. Watts-Tobin [10] has determined the Hill coefficient at equilibrium in the concerted model, showing that negative cooperativity of binding is excluded. This conclusion does not necessarily extend to nonequilibrium conditions [11]. The purpose of this study is to determine the modes of kinetic cooperativity in the concerted model, by analysis of the Hill slope for catalysis.

Paulus and DeRiel [12] have obtained an analytical expression for the kinetic Hill coefficient defined with respect to the asymptotic velocity V_∞, using the rate equation proposed by Dalziel [13] in his extension of the concerted model to nonequilibrium conditions. This expression of n_H differs from that obtained [14] for the Hill slope defined with respect to the maximum rate V_M. The discrepancy is due to the fact that the experimental value of the rate at saturating substrate concentration, V_∞, can be inferior to V_M in concerted K–V systems, i.e., when the two protomer conformational states R and T postulated in the theory differ by the affinity towards the substrate and by the catalytic activity.

We independently have obtained a similar expression for the kinetic Hill coefficient based on the asymptotic velocity. Reported here is a detailed analysis of this Hill coefficient in the case where the substrate binds with the

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largest affinity to the more active state of the enzyme. The analysis shows that cooperativity in the concerted model is always positive in the case of a dimer, and in the case of an inactive T state. There exists a narrow range of parameters for which slight kinetic negative cooperativity occurs for larger numbers of protomers when the activity of the T state is a fraction of that of the R conformation. This range and the magnitude of the corresponding Hill coefficients are precisely delineated here. The analysis extends the results of Paulus and DeRiel [12] who proved, under similar conditions, the absence of kinetic negative cooperativity for enzymes containing up to four protomers with inactive T state. These authors pointed to the possible occurrence of slight kinetic negative cooperativity while providing evidence, by numerical simulation, for the absence of the phenomenon over a wide range of parameter values. Paulus and DeRiel also showed that kinetic negative cooperativity may occur when the substrate binds with the largest affinity to the less active state of the enzyme. On the other hand, the present findings are in qualitative agreement with those of Kurganov et al. [6] who studied negative cooperativity in the concerted model by using an inconstant exponent graphically defined in preference to the classical Hill slope.

The results are discussed with regard to the possibility of discriminating between various models for oligomeric enzymes. Positive and negative cooperativity in binding and in catalysis are readily explained in the sequential model of Koshland et al. [15]. The present study favors the view that negative cooperativity is not compatible, in most cases, with a mechanism based on a fully concerted and conservative allosteric transition. Relaxation of either one of these constraints on the conformational change has been invoked as a source of negative cooperativity in extensions of the model of Monod et al. [16,17].

The relation of the theoretical results to equilibrium and kinetic measurements is discussed. A comparison of the Hill coefficients for binding and catalysis suggests a criterion for the discrimination between K and V systems.

2. Rate equation

The monosubstrate enzyme model consists of n protomers which exist under two conformations, R and T. The protomers undergo a concerted and conservative [8] transition between these states which may differ in their affinity towards the substrate (K effect) and in their catalytic properties (V effect). The enzyme exists in a single oligomeric form. Let a, a' be the kinetic constants for the binding of the substrate to the R and T states, respectively; the corresponding constants for dissociation are denoted d and d'. Let us denote $K_R = d/a$ and $K_T = d'/a'$ the dissociation constants of the enzyme–substrate complexes ($K_T \geq K_R$), and k, k' the catalytic constants related to the irreversible decomposition of these complexes in the R and T states, respectively ($k \geq k'$).

For a given substrate concentration, the steady-state reaction rate in such a system is given by the relation [13]:

$$\frac{v}{V_M} = \frac{\alpha(1+\alpha)^{n-1} + L\theta\alpha c(1+\alpha c)^{n-1}}{(1+\alpha)^n + L(1+\alpha c)^n}, \tag{1}$$

where α denotes the substrate concentration S normalized by division through the Michaelis constant of the R state: $\alpha = S/K_R(\epsilon+1)$, with $\epsilon = k/d$ and $\epsilon' = k'/d'$. The maximum reaction rate is given by $V_M = nkE$, where E represents the total enzyme concentration; L is the two-state equilibrium constant; $\theta = k'/k$ is the ratio of the turnover numbers of the T and R states, and $c = K_R(\epsilon+1)/K_T(\epsilon'+1)$ is an extended nonexclusive binding coefficient of the substrate. In regard to the preceding parameters, a perfect K system is defined by $\theta = 1, K_R < K_T$, whereas $\theta < 1$ and $K_R < K_T$ in a K–V system; a perfect V system is defined by $\theta < 1, K_R = K_T$ [8].

For simplicity, the notations α and c are used hereafter for binding and catalysis; in binding and kinetic expressions, they refer respectively to the equilibrium and nonequilibrium definitions of α and c. The latter coincide with the equilibrium definitions $\alpha = S/K_R$ and $c = K_R/K_T$ [8] when $\epsilon, \epsilon' \ll 1$ or, for c, when $\epsilon = \epsilon'$.

3. Hill coefficient for binding

In perfect K systems, the T and R states of the enzyme have the same catalytic activity. Then $\theta = 1$, and the ratio (v/V_M) becomes formally identical to the saturation function $\bar{Y}$ defined by Monod et al. [8] as the fraction of catalytic sites occupied by the substrate.

The Hill coefficient for binding is defined with respect to the saturation function by the relation [1]

$$n_H = \mathrm{d}\log[\bar{Y}/(1-\bar{Y})]/\mathrm{d}\log\alpha. \tag{2}$$

This relation, together with the expression of $\bar{Y}$ given by eq. (1) for $\theta = 1$, yields the expression [10]

$$n_H = 1 + [(n-1)L(1-c)^2\alpha(1+\alpha)^{n-2}(1+\alpha c)^{n-2}]/[(1+\alpha)^{n-1} + Lc(1+\alpha c)^{n-1}][(1+\alpha)^{n-1} + L(1+\alpha c)^{n-1}]. \tag{3}$$

In K systems, this relation gives the Hill coefficient for binding and for catalysis as a function of the substrate concentration. In K–V and V systems, the ratio (v/V_M) ceases to be identical to the saturation function $\bar{Y}$; eq. (3) then yields the Hill coefficient for binding only.

The Hill slope defined by eq. (3) is always larger than unity. Negative cooperativity of binding is thus excluded in the concerted model. In K systems, the same remark holds for catalysis also.

4. Asymptotic velocity in K–V systems

The maximum velocity V_M is reached in K–V systems when all the enzyme is present in the more active R state. At saturating substrate concentration, eq. (3) yields the following relation for the asymptotic velocity V_∞ [14]:

$$\lim_{\alpha\to\infty}(v/V_M) = V_\infty/V_M = (1 + L\theta c^n)/(1 + Lc^n). \tag{4}$$

The rate equation, expressed as a function of the asymptotic velocity, is thus given in the concerted model by the following relation free of V_M:

$$\frac{v}{V_\infty} = \left(\frac{1+Lc^n}{1+L\theta c^n}\right)\left(\frac{\alpha(1+\alpha)^{n-1} + L\theta\alpha c(1+\alpha c)^{n-1}}{(1+\alpha)^n + L(1+\alpha c)^n}\right). \tag{5}$$

Eq. (4) shows that the asymptotic velocity V_∞ is equal to the absolute maximum velocity V_M when $\theta = 1$ (perfect K system), or when $Lc^n \ll 1$. The product Lc^n is directly linked to the extent of the allosteric transition at saturation, as

$$Lc^n = \lim_{\alpha\to\infty}(\Sigma T/\Sigma R), \tag{6}$$

where ΣT and ΣR denote the concentrations of enzyme species in the T and R states. When the T state is less active than the R state ($\theta < 1$), the asymptotic velocity thus approaches V_M as the equilibrium between the two protomer conformations shifts completely towards the R state. For large values of L and c, however, the condition $Lc^n \ll 1$ is not always ensured. Some enzyme then remains in the less active T state at saturation, so that $V_\infty \leqslant V_M$.

This point is essential for the kinetic definition of the Hill coefficient [12]. In a previous study [14], n_H was defined with respect to V_M. As V_∞ is the true experimental value of the reaction rate at saturating substrate concentration, it is necessary to determine the Hill coefficient with respect to the asymptotic velocity in order to assess kinetic cooperativity in K–V systems.

5. Kinetic cooperativity in concerted K–V systems

5.1. Analytical expression of the Hill coefficient

The kinetic Hill coefficient is defined with respect to the asymptotic velocity V_∞ by the relation [2]:

$$n_H = \mathrm{d}\log[v/(V_\infty - v)]/\mathrm{d}\log\alpha. \tag{7}$$

This definition, together with relation (5), yields an expression for the kinetic Hill coefficient in the concerted model, as in Paulus and DeRiel [12]. Some algebraic transformations yield the following form which is particularly suitable for later analysis:

$$n_H = 1 + L\alpha(P/Q), \tag{8}$$

with

$$P = [(1+\alpha)^{2n-2}c^n - (1+\alpha c)^{2n-2}L\theta c^2](1-\theta) + (1+\alpha)^{n-2}(1+\alpha c)^{n-2}$$
$$\times \{(1+L\theta c^n)(1-c)[(n-1)(1-\theta c) + (1-\theta)n\alpha c] + (1-\theta)c[(1+\alpha^2 c)(L\theta c^n - 1) + 2\alpha(L\theta c^{n+1} - 1)]\},$$
$$Q = [(1+\alpha)^{n-1} + L\theta c(1+\alpha c)^{n-1}]\{(1+L\theta c^n)[(1+\alpha)^{n-1} + L(1+\alpha c)^{n-1}] - (1-\theta)\alpha L[c^n(1+\alpha)^{n-1} - c(1+\alpha c)^{n-1}]\}. \tag{9}$$

When the T and R states have the same catalytic activity ($\theta = 1$), eq. (8) reduces to the expression of the Hill coefficient of a perfect K system [eq. (3)], as the ratios (v/V_∞) and (v/V_M) then coincide with the saturation function $\bar{Y}$.

However complicated the expression of the kinetic Hill coefficient in K–V systems, a discussion of relations (8) and (9) enables us to obtain analytical results, in several specific cases, as to the possible occurrence of kinetic negative cooperativity ($n_H < 1$).

Let us first note that the quantity Q defined in eqs. (8), (9) is always positive for $c < 1$, as in such a case it is clear that

$$c^n(1+\alpha)^{n-1} - c(1+\alpha c)^{n-1} < 0. \tag{10}$$

The inequality $c < 1$ generally holds when the substrate has the largest affinity for the more active R state ($K_R < K_T$). We limit ourselves hereafter to this case, which implies absence of inhibition by the substrate [8]. In these conditions, negative cooperativity will be ensured whenever P is a negative quantity.

5.2. Case of a dimer enzyme

The case of dimers is important, as they constitute the majority of multisubunit enzymes [18]. For $n = 2$, the expression of P reduces to the simple relation

$$P = (1-c)^2(1 + L\theta^2 c^2). \tag{11}$$

This shows that the sign of P is always positive, regardless of θ. Negative cooperativity in catalysis, as well as in binding, is thus excluded in the dimeric concerted model. The kinetic Hill coefficient for a dimer enzyme takes a simple form given in appendix 1.

5.3. Case of an inactive T state

A limit situation in K–V systems obtains when the T state of the enzyme is completely inactive ($\theta = 0$). The condition for kinetic negative cooperativity, $P < 0$, reduces then to the inequality

$$(1+\alpha)^n c^n + (1+\alpha c)^{n-1}[n(1-c) - (1+\alpha c)] < 0. \tag{12}$$

A necessary condition for P to be negative is clearly

$$\alpha > [(n-1)/c] - n. \tag{13}$$

This condition is precisely the one obtained when defining the Hill slope with respect to V_M [14]. In the latter case, condition (13) was both necessary and sufficient for ensuring kinetic negative cooperativity beyond a critical substrate concentration for $\theta = 0$. This is no more the case for calculations of n_H based on the asymptotic velocity V_∞; indeed inequality (12) is never satisfied, even when relation (13) holds, so that the sign of P is always positive. The demonstration by recurrence (see appendix 2) is made by showing that the inequality $P > 0$ holds for n if it holds for $(n-1)$ protomers. As this was already established for a dimer (see section 5.2), P is positive for all n. Kinetic negative cooperativity is thus excluded in concerted K–V systems with inactive T state.

5.4. Perfect V systems and exclusive binding

In a perfect V system, the T and R states differ by catalytic properties, but not by their affinity towards the substrate. At equilibrium, the requirement $K_R = K_T$ implies $c = 1$. When extended to nonequilibrium conditions (see section 2), the nonexclusive binding coefficient c is equal to unity in a perfect V system if $\epsilon = \epsilon'$, or if $\epsilon, \epsilon' \ll 1$. In any case, the value $c = 1$ corresponds to a peculiar situation in which the rate equation and the Hill coefficient take simple forms.

Regardless of θ, relation (5), for $c = 1$, reduces indeed to the Michaelian expression

$$(v/V_\infty) = \alpha/(1+\alpha). \tag{14}$$

In agreement with the fact that the Hill coefficient of Michaelian enzymes is equal to unity, eq. (8) yields $n_H = 1$ for $c = 1$. A similar value is found in those conditions for the Hill number defined for binding [eq. (3)], as the saturation function $\bar{Y}$ also reduces to the right-hand side of eq. (14).

On the other extreme, the distinction between K and V systems vanishes when c goes to zero. Both definitions (2) and (7) yield then the following expression for the Hill coefficient:

$$n_H = 1 + (n-1)\alpha L/(1+\alpha)\,[(1+\alpha)^{n-1} + L], \tag{15}$$

which predicts only positive cooperativity when the substrate binds exclusively to the R state of the enzyme.

It follows from the present and preceding sections that the kinetic Hill coefficient, as in the case of a dimer, is never less than unity when $\theta = 0$ (inactive T state), $\theta = 1$ (perfect K system), $c = 0$ (exclusive binding of substrate to the R conformation), or $c = 1$ (perfect V system). As shown below, this situation does not always prevail when parameters θ and c are comprised between 0 and 1.

5.5. General case ($n > 2$; $0 < \theta, c < 1$)

In contrast with the preceding cases, eqs. (8) and (9) do not yield easily tractable relations for the analysis of the Hill slope when the enzyme consists of more than two protomers, and when the affinity for the substrate and the catalytic activity of the T state are non-negligible fractions of those of the R conformation. Numerical analysis of eqs. (8) and (9) for various values of n, L, θ and c allows, in these conditions, to determine the patterns of kinetic cooperativity in the concerted model.

A general result of the analysis is that the Hill coefficient kinetically defined by eq. (8) goes to unity when the substrate concentration goes to zero or to infinity, behaving in this respect as the Hill coefficient defined for binding [1]. At intermediate substrate concentrations, positive cooperativity or the absence of cooperative interactions are the most common phenomena.

For $n = 3$ and 4, negative cooperativity occurs for large values of the allosteric constant L, of the order of 10^5, and for values of θ and c in the vicinity of 0.1. But the phenomenon is negligible, as the kinetic Hill coefficient remains close to unity (see table 1). The corresponding rate curves, accordingly, look hyperbolic.

Table 1
Kinetic negative cooperativity in the concerted model

n	3		4		6		8			
L	10^4	10^5	10^5	10^6	10^6	10^7	10^5	10^8	10^9	10^{12}
n_{Hm}	0.99	0.99	0.93	0.97	0.87	0.83	0.98	0.77	0.74	0.999
v in % V_∞	5	39	16	49	4	25	0.4	6	31	77
$n_{H1/2}$	1.04	0.99	1.02	0.97	1.38	0.94	3.41	1.40	0.87	1
n_{HM}	1.04	1	1.03	1	1.48	1.01	3.75	1.48	1	1
v in % V_∞	52		67		28	81	22	31		

The minimum value of the Hill slope (n_{Hm}) is given, with the corresponding rate of reaction, as a function of the allosteric constant L and of the number n of protomers constituting the enzyme. Also indicated are the Hill coefficient at half asymptotic velocity ($n_{H1/2}$) and the maximum value of the Hill slope (n_{HM}), with the corresponding reaction rate. The data are obtained by simulation of the model on an IBM 370/165 computer, for $c = \theta = 0.1$. The Hill coefficient is determined according to eqs. (8) and (9). Lower values of parameters c and θ favor positive cooperativity.

For larger numbers of protomers, negative cooperativity is slightly more pronounced. Depending on the allosteric constant, it can either precede positive cooperativity or extend over a large range of the velocity curve (see table 1 for $n = 6$ and 8). In the latter case, the Hill coefficient at half asymptotic velocity can be less than unity, but remains generally greater than 0.9; the minimum value found for $n_{H1/2}$ by numerical simulation is 0.87, for $L = 10^9$, in the case of an octamer. When mixed cooperativity occurs, the Hill slope is usually less than 1 in the range 1–10% V_∞, and passes through a maximum before 50% V_∞. This situation may result in small undulation in the velocity curve at low substrate concentration, as for $n = 6$, 8 and $c = \theta = 0.1$ (figs. 1,2), but the phenomenon is never so significant as to induce an intermediary plateau.

The dependence of the velocity and of the Hill coefficient upon θ and c is given in figs. 1 and 2, respectively. Kinetic negative cooperativity occurs in the restricted range 0.1–0.01 of these parameters. These relatively large values of θ and c required for anticooperative behavior are responsible for the fact that the maximum Hill coeffi-

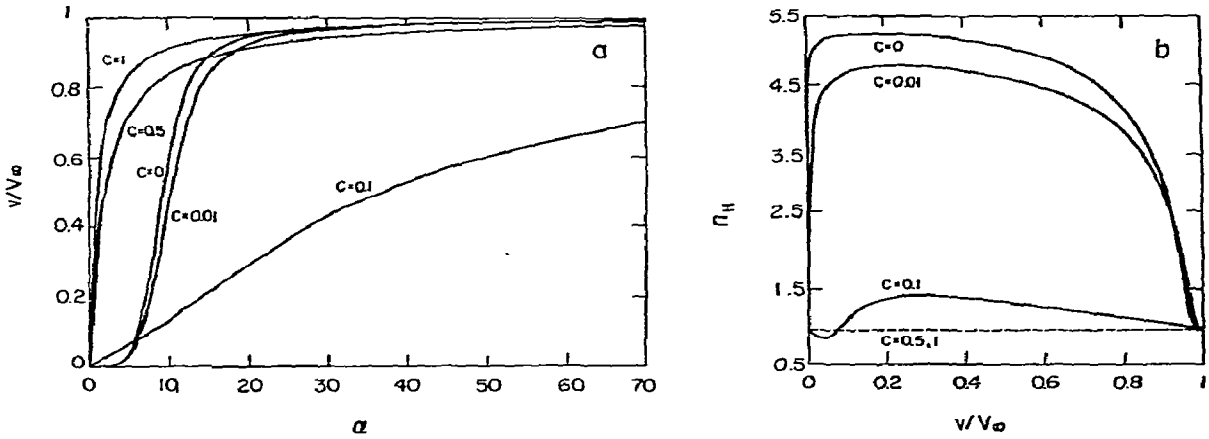

Fig. 1. Dependence of the velocity and of the kinetic Hill coefficient on the nonexclusive binding coefficient of the substrate. The curves are established for $n = 6$, $\theta = 0.1$ and $L = 10^6$. The velocity (a) and the Hill coefficient (b) are determined according to eqs. (5) and (8) on an IBM 370/165 computer. Highest cooperativity occurs for $c = 0$, whereas a small undulation in the velocity curve, for $c = 0.1$, is associated with mixed-type cooperativity. When $c = 1$, the rate curve is hyperbolic and the Hill coefficient (dashed line) remains equal to 1.

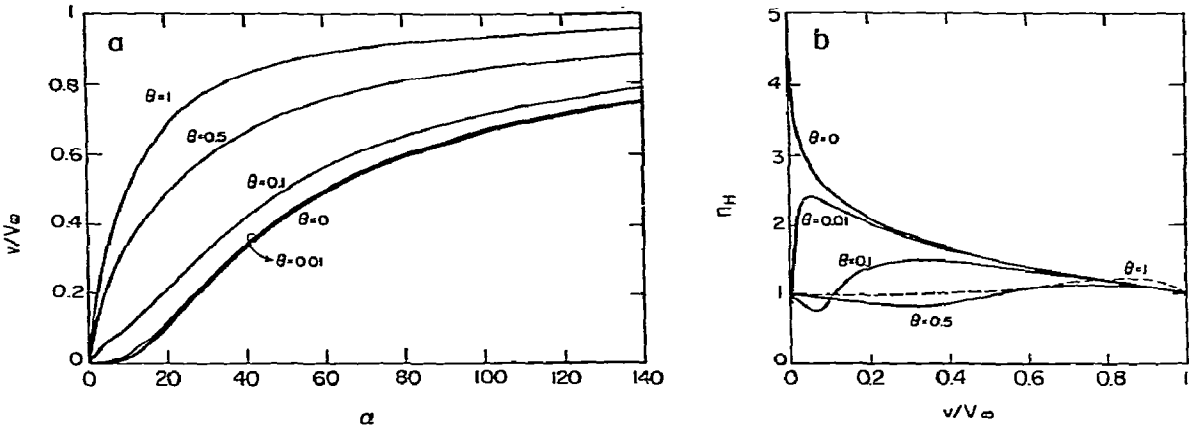

Fig. 2. Dependence of the velocity and of the kinetic Hill coefficient on the ratio of turnover numbers of the T and R states. The curves are established for $n = 8$, $c = 0.1$ and $L = 10^8$. The velocity (a) and the Hill coefficient (b) are determined according to eqs. (5) and (8). Maximum cooperativity occurs in the case of an inactive T state ($n_H = 4.40$ for $v = 1.2 \times 10^{-4} V_\infty$). For $\theta = 0.1$, a small undulation in the velocity curve results from the succession of negative and positive cooperativity. The quasi-hyperbolic rate curve obtained for $\theta = 1$ coincides with the saturation function $\bar{Y}$; the corresponding Hill coefficient (dashed line) coincides with the Hill coefficient for binding, given by eq. (3).

cients in the domain of positive cooperativity (table 1) are small in comparison with the number of protomers. As in the case of binding [19], maximum positive cooperativity is observed when c goes to zero (fig. 1); a similar result is obtained with respect to parameter θ (fig. 2).

The detailed dependence of kinetic cooperativity on the allosteric constant is indicated in table 1 for an octamer. Upon increasing L from 10^5 to 10^{12}, one successively observes positive cooperativity, mixed-type cooperativity, negative cooperativity and absence of cooperative interactions. It should be noted that positive effectors decrease the value of the two-state equilibrium constant, whereas negative effectors bring about an increase in L [8,13].

6. Discussion

The analysis of the kinetic Hill coefficient defined with respect to the asymptotic velocity V_∞ in the concerted model for allosteric enzymes yields results which differ from those obtained with a definition of n_H based on the maximum rate V_M. Indeed, the maximum velocity can exceed the experimental value V_∞ when the two conformational states of the enzyme differ in catalytic properties. One of the main differences, already pointed out by Paulus and DeRiel [12], is that the Hill coefficient determined with respect to the asymptotic velocity goes to 1 as the substrate concentration tends to zero or to infinity; this rules out kinetic negative cooperativity beyond a critical substrate concentration [14].

Negative cooperativity is usually considered as the most compelling argument against the concerted mechanism [9]. This view is supported in many respects by the present analysis. Negative cooperativity in the concerted model is indeed excluded for binding; it is also ruled out for catalysis in the case of a dimer enzyme, and in the case of an inactive T state. Kinetic negative cooperativity is allowed in concerted K–V systems for larger numbers of protomers when the substrate binds significantly to the T state of the enzyme, and when the catalytic activity of this state is a significant fraction of the activity of the R conformation. The phenomenon remains negligible for trimers and tetramers. For $n > 4$, a small undulation may result from the succession of negative and positive coop-

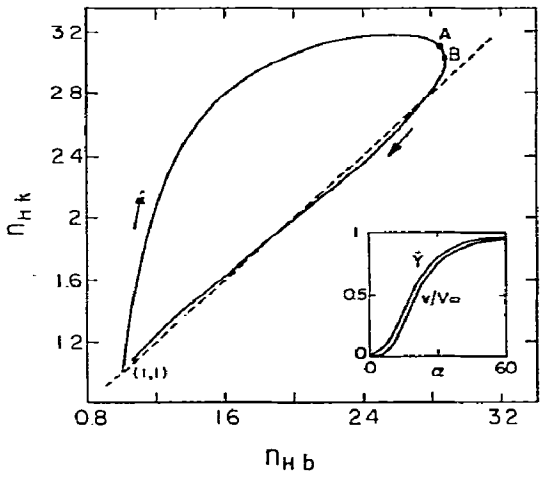

Fig. 3. Comparison of Hill coefficients for binding and catalysis. The Hill coefficient for binding, n_{Hb}[eq. (3)], is plotted as a function of the kinetic Hill coefficient, $n_{H\,k}$ [eq. (8)], as the substrate concentration α varies from 0 to 600 in the case $n = 4, c = 0.01, \theta = 0.1, L = 10^5$. The resulting curve is closed as the system departs from the initial point (1,1) in the presence of substrate and returns to it at saturation (arrows). Points A and B refer, respectively, to half saturation ($\alpha = 19$) and half asymptotic velocity ($\alpha = 21$). The dashed line represents the locus of equal Hill coefficients for binding and catalysis. The saturation function $\bar{Y}$ and fractional velocity v/V_∞ are plotted in the inset for α varying from 0 to 60. It is assumed that the catalytic steps are rate-limiting ($\epsilon, \epsilon' \ll 1$), so that the equilibrium and nonequilibrium definitions of α and c coincide.

erativity, but this could hardly account for intermediary plateaux which are sometimes observed in the kinetics of multisubunit enzymes [3,4,20]. Furthermore, the values of $n_{H1/2} < 1$ obtained for hexamers and octamers in the concerted model (table 1) cannot account for the range 0.2–0.75 found for the Hill coefficient at half asymptotic rate in most negatively cooperative enzymes [21].

The above results are obtained for values of c comprised between 0 and 1. When the nonexclusive binding coefficient is larger than unity in K–V systems, the velocity generally passes through a maximum as the substrate concentration increases [8]. Definition (7) for the kinetic Hill coefficient does not apply in such a case. Paulus and DeRiel have shown that significant kinetic negative cooperativity without intermediary maximum in velocity is allowed in the concerted model when the substrate has the largest affinity for the less active state of the enzyme, that is when $c > 1$. The phenomenon occurs in a restricted domain of parameter values, and corresponds to very low (V_∞/V_M) ratios [12].

In contrast to the predictions of the concerted mechanism, negative cooperativity in binding and in catalysis, as well as intermediary plateaux in activity curves, are readily explained by the sequential model of Koshland et al., provided that suitable inequalities hold among the successive dissociation or catalytic constants postulated in this model [4,15,20].

Association–dissociation phenomena involving several molecular enzyme species are another type of process which may give rise to anticooperative behavior [6,22]. It should be noted that several extensions of the concerted model, which depart from the maximum symmetry postulated by Monod et al., account for negative cooperativity at equilibrium [16,17]. In particular, the phenomenon can be explained when the allosteric transition between the T and R states is partially concerted [16], or partially conservative [17]. Viratelle and Seydoux have shown, in the latter case, that with an additional assumption of asymmetry in binding properties between pairs of protomers in the R state, the concerted mechanism can give rise to significant negative or mixed cooperativity. Furthermore, the existence of a concerted but rate-limiting allosteric transition has been implicated in the occurrence of an intermediary plateau region in velocity curves [23].

The difference between the Hill slopes for binding and for catalysis is illustrated in fig. 3. Both definitions coincide only for K systems; the less active the T state, the larger the difference between the coefficients. Fig. 3 indicates that the kinetic Hill coefficient is usually larger, and reaches its maximum value before the Hill coefficient for binding. The rate curve, in such a case, looks more sigmoidal than the saturation curve obtained by binding measurements (see also fig. 2). As expected, the difference subsides as the nonexclusive binding coefficient c goes to zero. Such a comparison between binding and kinetic Hill plots suggests a criterion for the discrimination between K and V or K–V systems. The two kinds of representation should yield identical results for K systems only.

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Appendix 1. Case of a dimer enzyme

For $n=2$, eq. (9) yields the following expression for P:

$$P=[(1+\alpha)^2-L\theta(1+\alpha c)^2]c^2(1-\theta)+(1+L\theta c^2)(1-c)[(1-\theta c)+2(1-\theta)\alpha c]$$
$$+(1-\theta)c[(1+\alpha^2 c)(L\theta c^2-1)+2\alpha(L\theta c^3-1)], \tag{A.1}$$

This relation takes the successive forms:

$$P=(1-\theta)[(c^2+2\alpha c^2+\alpha^2 c^2)-(c+\alpha^2 c^2+2\alpha c)]+(1-c)[(1-\theta c)+2\alpha c(1-\theta)]$$
$$+L\theta c^2\{(1-c)[(1-\theta c)+2\alpha c(1-\theta)]+(1-\theta)[(c+\alpha^2 c^2+2\alpha c^2)-(1+2\alpha c+\alpha^2 c^2)]\}$$
$$=(1-c)[(1-\theta c)-c(1-\theta)]+L\theta c^2(1-c)[(1-\theta c)-(1-\theta)].$$

Hence,

$$P=(1-c)^2(1+L\theta^2 c^2)\geqslant 0. \tag{A.2}$$

In a dimer system, the Hill coefficient kinetically defined by eq. (8) is thus given by

$$n_H=1+\alpha L(1-c)^2(1+L\theta^2 c^2)/[(1+\alpha)+L\theta c(1+\alpha c)]\{(1+L\theta c^2)[(1+\alpha)+L(1+\alpha c)]+\alpha Lc(1-\theta)(1-c)\} \tag{A.3}$$

The value of this coefficient at half asymptotic velocity is given in appendix 3, in the case of an inactive T state.

Appendix 2. Case of an inactive T state

In the case $\theta=0$, the condition $P>0$ for the absence of kinetic negative cooperativity takes the form:

$$(1+\alpha)^n c^n+(1+\alpha c)^{n-1}[n(1-c)-(1+\alpha c)]>0. \tag{A.4}$$

This inequality can be rewritten as

$$[(1+\alpha)c/(1+\alpha c)]^n > [1-n+c(\alpha+n)]/(1+\alpha c). \tag{A.5}$$

The Hill coefficient defined by eq. (8) is always larger than unity in the case $n=2$ (see appendix 1). If we show that inequality (A.5) is satisfied for n if it holds for $n-1$, when $n\geqslant 3$, then condition (A.4) will be satisfied for all $n\geqslant 2$. Suppose that inequality (A.5) is verified for $n-1$, i.e.,

$$[(1+\alpha)c/(1+\alpha c)]^{n-1} > [1-(n-1)+c(\alpha+n-1)]/(1+\alpha c). \tag{A.6}$$

Inequality (A.6) can be rewritten as

$$[(1+\alpha)c/(1+\alpha c)]^n > [(1+\alpha)c/(1+\alpha c)][2-n+c(\alpha+n-1)]/(1+\alpha c). \tag{A.7}$$

By comparison of inequalities (A.5) and (A.7), it is clear that inequality (A.4) will be verified if the following inequality is proven:

$$\left(\frac{(1+\alpha)c}{(1+\alpha c)}\right)\left(\frac{2-n+c(\alpha+n-1)}{(1+\alpha c)}\right) > \frac{1-n+c(\alpha+n)}{(1+\alpha c)}. \tag{A.8}$$

This inequality takes successively the forms

$$(1+\alpha)c[2-n+c(\alpha+n-1)] > (1+\alpha c)[1-n+c(\alpha+n)], \tag{A.9}$$

or

$$c[2-n+c(n-1)] > nc-(n-1), \tag{A.10}$$

or

$$(n-1)(1-c)^2 > 0, \tag{A.11}$$

which is always true, when $c < 1$. For $c = 1$, the Hill coefficient equals unity (see section 5.4). Inequality (A.4) is thus verified for all relevant α, c and n.

Appendix 3. Hill coefficient at half asymptotic velocity

Hill plots are often linearized in the vicinity of half saturation or half asymptotic rate. The expression for the kinetic Hill coefficient at half asymptotic velocity is much more complex than that obtained at half saturation for the Hill coefficient for binding. In the simple case of an inactive T state ($\theta = 0$), the relation $v = V_\infty/2$ corresponding to the ratio $[v/(V_\infty - v)] = 1$ takes the form

$$[\alpha(1+2Lc^n)-1](1+\alpha)^{n-1} = L(1+\alpha c)^n. \tag{A.12}$$

The kinetic Hill coefficient corresponding to the half asymptotic velocity is then given, from eqs. (8), (9) and (A.12), by the relation

$$n_{H1/2} = 1 + L\{(1+\alpha_{1/2}c)^{n-1}[n(1-c)-(1+\alpha_{1/2}c)] + c^n(1+\alpha_{1/2})^n\}/(1+\alpha_{1/2})^n(1+Lc^n), \tag{A.13}$$

where the substrate concentration at half asymptotic velocity, $\alpha_{1/2}$, is solution of eq. (A.12). In the case of a dimer with inactive T state, relation (A.13) yields

$$n_{H1/2} = 1 + L(1-c)^2/(1+\alpha_{1/2})^2(1+Lc^2). \tag{A.14}$$

For binding, the Hill coefficient [eq. (3)] at half saturation is given by the relation [24]:

$$n_{H1/2} = 1 + (n-1)(\alpha_{1/2}-1)(1-c\alpha_{1/2})/(\alpha_{1/2}+1)(1+c\alpha_{1/2}), \tag{A.15}$$

where $\alpha_{1/2}$ is solution of the equation

$$(\alpha-1)(1+\alpha)^{n-1} = L(1+c\alpha)^{n-1}(1-c\alpha). \tag{A.16}$$

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