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Cooperativity of ligand binding as a function of monomer-dimer equilibrium Parameters and Acceptor Concentration

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Abstract

COOPERATIVITY OF LIGAND BINDING AS A FUNCTION OF MONOMER-DIMER EQUILIBRIUM PARAMETERS AND ACCEPTOR CONCENTRATION

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A general monomer-dimer equilibrium system involving ligand interactions is presented. Cooperativity features of specific limited models are analyzed by selecting the appropriate family of equilibrium constants from this general scheme. Each system is then characterized in terms of Hill coefficient dependency on alterations in values of equilibrium constants and total acceptor concentration. This method permits comparison of predicted cooperativity trends between systems. Contrasting reports concerning cooperativity dependencies for certain defined equilibrium systems are compared and the discrepancies resolved. Characteristics of cooperativity binding patterns are shown to include symmetry about dimerization association constant values, both positive and negative cooperativity for a single set of parameters, and significant changes in cooperativity features with relatively small changes in equilibrium parameters.

LOMA LINDA

Graduate School

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by

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Each person whose signature appears below certifies that this manuscript in his opinion is adequate, in scope and quality, in lieu of a thesis for the degree Master of Science.

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INTRODUCTION

There are numerous examples of proteins which tend to self-associate under various conditions. At times, the degree of association can be influenced by small molecules (eg. substrates, receptor ligands or allosteric modifiers) which bind to the protein reversibly. Ligand-influenced polymerization of proteins is a subject of continuing interest, primarily due to the implications of these reactions in biological control mechanisms (for reviews see Hammes and Wu, 1974; Neet, 1980; Frieden and Nichol, 1981). Various theoretical aspects relating to this special type of monomer-oligomer equilibria have been discussed by a number of authors (Nichol, Jackson, and Winzor, 1967; Steiner, 1974; Levitzki and Schlessinger, 1974; Colosimo, Brunori and Wyman, 1976; Nichol and Winzor, 1976).

A characteristic behavior of these ligand-influenced aggregating systems is the dependence of ligand binding, and cooperativity, on the protein concentration. This dependence provides a means of distinguishing these systems from other cooperative mechanisms involving isomerization of subunits in a nondissociation oligomer. However, conflicting conclusions are reported regarding the trend of this cooperativity dependence.

Some authors (Levitzki and Schlessinger, 1974) indicate that the cooperativity, as measured by the Hill coefficient (Hill, 1910), decreases as a function of increasing protein concentration. Others (Nichol and Winzor, 1976) state that

positive cooperativity, as displayed by Scatchard plots (Scatchard, 1949), increases as the protein concentration increases for purely ligand-induced polymerizing systems. Yet another author (Frieden, 1967) reports that the directions of this trend might be related to the magnitude of the protein concentration. Added confusion is brought about by differing interpretations of equilibrium parameters as well as subtle constraints placed on some aggregation models.

Experimental work may be cited to support any of the above conclusions. Data obtained from the D-lactate dehydrogenase system has been used to support a decreasing degree of cooperativity as the enzyme concentration is increased for ligand-influenced monomer-dimer systems (Levitzki and Schlessinger, 1974; Sawula and Suzuki, 1970). On the other hand, data from the estrogen receptor system indicates that the Hill coefficient increases as the receptor concentration increases (Notides, Lerner, and Hamilton, 1981). Although binding equations can be derived, the analytical solutions are complex and are not readily obtained. Because of this, most workers have resorted to binding plots which were constructed using computer simulation. However, in many cases this numerical approach was conducted over a limited range of protein concentrations and the generality of the conclusions may be questioned.

The work presented here establishes the relationship between cooperativity and protein concentration for various

ligand-influenced monomer-dimer aggregation models using numerical analysis. The range of the parameters is extended beyond that reported previously, while applying greater refinement of the parameters within that range. This results in discovery of unique cooperativity dependencies that can, at times, be used to distinguish between alternate reaction schemes within a monomer-dimer framework while also allowing estimations of certain equilibrium parameters from data generated experimentally. In addition, it is also now possible to understand the apparent inconsistencies between the conclusions of previous workers.

THEORY

Consider the monomer-dimer association reaction with one ligand binding site per monomer and two sites on the dimer. A two step ligand-induced aggregation system can be illustrated by the equations

$$L + A \rightleftharpoons LA \qquad (1)$$

$$2LA \rightleftharpoons L_2A_2 \qquad (2)$$

where L and A represent ligand and acceptor (enzyme or receptor) respectively. The reaction scheme shown in fig 1, which includes reactions 1 and 2, illustrates the general system when dimerization can occur in the absence of ligand. Complexes involving various combinations of L and R are also

shown. The equilibrium association constants in fig 1 are defined as

$$K_1 = [A_2] / [A]^2 \quad (3)$$

$$K_2 = [LA] / [L][A] \quad (4)$$

$$K_3 = [LA_2] / [LA][A] \quad (5)$$

$$K_4 = [L_2A_2] / [LA]^2 \quad (6)$$

$$K_5 = [LA_2] / [A_2][L] \quad (7)$$

$$K_6 = [L_2A_2] / [LA_2][L] \quad (8)$$

The system shown in fig 1 can be defined by any four equilibrium constants. Association constants K_1-K_4 are chosen in this study because this allows clear comparison with previous analyses while also including the two step model illustrated by eq 1 and 2. The total acceptor concentration is given as

$$[A_t] = [A] + 2[A_2] + [LA] + 2[LA_2] + 2[L_2A_2] \quad (9)$$

where $[A_t]$ is the total concentration of the acceptor on a subunit basis (i.e., moles of monomer). By substitution of eq 3-8 into eq 9 and solving for the free monomer acceptor concentration, a quadratic in terms of K_1, K_2, K_3, K_4, [L] and $[A_t]$ is given as

$$[A] = \frac{-(1 + K_2[L]) + \{(1 + K_2[L])^2 + 8(K_1 + K_2K_3[L] + K_4K_2^2[L]^2)[A_t]\}^{1/2}}{4(K_1 + K_2K_3[L] + K_4K_2^2[L]^2)} \quad (10)$$

The fraction of ligand binding sites occupied (Y) is

$$Y = \frac{[LA] + [LA_2] + 2[L_2A_2]}{[A] + 2[A_2] + [LA] + 2[LA_2] + 2[L_2A_2]} \quad (11)$$

and

$$Y / 1-Y = \frac{K_2[L] + K_2K_3[L][A] + 2K_4K_2^2[L]^2[A]}{1 + 2K_1[A] + K_2K_3[L][A]} \quad (12)$$

On the basis of these equations one can, with the aid of a computer, readily construct Hill plots which can be used to show the dependence the maximum Hill coefficient has on the total acceptor concentration for various sets of parameters. The Hill coefficient (n), which can be defined as the slope of a Hill plot or

$$n = \frac{\partial (\log[Y/1-Y])}{\partial (\log[L])} \quad (13)$$

was calculated using eq 10 and 12 in the following way. A free ligand concentration was placed into quadratic eq 10 along with chosen values for $[A_t]$ and K_1-K_4. Next, eq 12 was solved using the free monomer acceptor concentration

from eq 10. When Hill plots (log(Y/1-Y) vs. log [L]) were desired, sufficient ordinate values were obtained by repeating the procedure at Δ log [L] = 0.01 intervals (for a representative example, see fig 5). If, instead, a Hill slope plot (n vs. log [L]) is required, two corresponding log(Y/1-Y) ordinate values for each individual free ligand interval were subtracted and the Hill slope (n) was obtained according to eq 13 above (Δ log [L] = 0.1). Representative Hill coefficient plots are shown in fig 2. If the dependence of n(max) on the total acceptor concentration ($[A_t]$) was desired, Hill slope values for all free ligand intervals at a given total acceptor concentration were compared and the maximum Hill slope identified. Additional coordinate values were obtained by incrementing the total acceptor concentration in eq 10 by log $[A_t]$ = 0.1 intervals and repeating the process.

There are certain parameter values which give a Hill coefficient of less than one (i.e., negative cooperativity, see fig 3). In this case the same procedure is used to obtain the Hill and Hill slope plots, but it is the minimum Hill coefficient (n(min)), corresponding to the maximum negative cooperativity, that is plotted against the total acceptor concentration.

The derivatives shown in equation 13 may be obtained in terms of the equilibrium parameters for the two step reaction defined by eq 1 and 2 and the Hill coefficient n obtained as

$$n = \frac{1 + 4K_2K_4[A][L] + 2K_2K_4[L]^2\{\partial[A]/\partial[L]\}}{1 + 2K_2K_4[A][L]} \quad (14)$$

There are significant differences between key equations presented here and equations given by other authors (eg. equations 15, 16 and 21 of Levitzki and Schlessinger, 1974). In particular, these authors report the third term in the numerator of our eq 12 (their eq 15) as $2K_4K_2^2[A]^2[L]^2$. In addition, the second term in the numerator of their eq 21 (the equivalent to eq 14 in this work) has only one equilibrium constant (this applies to their eq 16 as well). It is difficult to determine which association constant is lacking since definitions of constants seemed to have changed during derivation of their equations. Yet dimensional analysis alone indicates a need for some alterations in their equations.

EXPRESSIONS FOR LIGAND BINDING CONSTANTS

Some confusion may arise since authors differ in their use of equilibrium constants. The reaction scheme in fig 1 is illustrated using six macroscopic association constants. It should, however, be noted that the system is fully described by any four of these constants and relationships can be developed between the remaining two constants and the four chosen. These macroscopic constants can, in turn, be defined in terms of microscopic association binding

constants by using appropriate statistical corrections for stoichiometry. This provides a way to compare the work of various authors and to draw general conclusions. Most authors (Nichol, Jackson and Winzor, 1967; Colosimo, Brunori and Wyman, 1976; Nichol, Winzor, 1976), but not all (Levitzki and Schlessinger, 1974), have placed constraints on the microscopic binding constants on the dimer. Namely, the ligand binding sites on the dimer are identical and independent. This in turn places constraints on the values of macroscopic constants K_1 through K_4.

Association constants K_5 and K_6 are related to macroscopic constants K_1-K_4 by

$$K_5 = K_3K_2/K_1 \qquad (15)$$

$$K_6 = K_4K_2/K_3 \qquad (16)$$

When the ligand binding sites on the dimer are identical and independent, the macroscopic association constant (K) is related to the microscopic constant (k) by (Klotz, 1946)

$$K = [q-(j-1)]k \; / \; j \qquad (17)$$

where q is the total moles of binding site per mole of dimer (two) and j is a number representing the j^{th} ligand being bound to the dimer which has j-1 ligands previously bound. Equations 15-17 can be used to show that if the microscopic binding sites on the dimer are identical and a finite amount of dimerization is allowed in the absence of ligand (i.e., $K_1 \neq 0$), then it can be shown that $K_5 = 4K_6$ and $K_4 = K_3^2/4K_1$.

ALTERNATE REACTION SCHEMES

There are several mechanistic schemes that can be derived from fig 1. A two ligand-acceptor complex species model results when both K_1 and K_3 are zero, and the two reactions are completely defined by K_2 and K_4. The only dimer complex present (L_2A_2) has a ligand to monomer subunit ratio of 1. Some authors (Levitzki and Schlessinger, 1974) suggest that this particular reaction scheme should always show a decrease in cooperativity as the total acceptor concentration is increased, while other (Nichol and Winzor, 1976) assert that the opposite may be possible. A different two species equilibrium model is obtained when K_1 and K_4 are zero, and the dimer (LA_2) has a ligand to monomer subunit ratio of 0.5. Previous authors (Nichol and Winzor, 1976) state that the cooperativity should increase in negativity as the total acceptor concentration increased for this two species model.

When all four association constants are assigned a finite value, and there are two identical and independent sites on the dimer, a more complex scheme is generated. It was noted (Nichol and Winzor, 1976) that under these conditions, and when the microscopic association constant on the dimer is greater than K_2, the degree of cooperativity decreased when the relative total acceptor concentration increased for several values tested. However, another author (Frieden, 1967) indicated that the direction of change in the cooperativity might be dependent on the

magnitude of the increasing acceptor concentration selected for the two identical and independent sites model.

A more general model still, one in which there are no assumptions made as to the affinities of the binding sites on the dimer (i.e., K_1-K_4 can vary independently) also has been analyzed (Levitzki and Schlessinger, 1974). These authors concluded that this system would predict the cooperativity to decrease as the total acceptor concentration increases.

HILL COEFFICIENT PLOTS

Two Species Equilibrium System (L2A2)

A system completely described by eq 1 and 2 (K_1 and K_3 = 0) appears to have a maximum Hill coefficient that continually increases as a function of increasing total acceptor concentration (fig 4, curve I) which contrasts with previous authors findings (Levitzki and Schlessinger, 1974). This is confirmed for a range of total acceptor concentrations far beyond that reported previously (Nichol and Winzor, 1976). The maximum Hill coefficient approaches 2 as $[A_t]$ increases to high values. The Hill plot slope curves for this particular two species model indicate that the free ligand concentration at which the maximum Hill coefficient occurs is dependent on the total acceptor concentration (fig. 2).

Alternate Two Species
Equilibrium System
(LA2)

A different two species system occurs when both K_1 and K_4 are zero. There are several differences between this two species mechanism (represented by K_2 and K_3) and the one presented above (defined by K_2 and K_4). This model gives a minimum Hill coefficient that is less than one (fig 3). A plot of n(min) vs. log $[A_t]$ continually decreases (fig 4. curve **A**). This is confirmed for a broad range of total acceptor concentrations.

The previous L_2A_2 two species equilibrium system (defined by K_2 and K_4) seems to show that the free ligand concentration ([L]) which corresponds to the point of maximal positive cooperativity (n(max)) is a function of the total protein concentration (fig 2). Yet this alternate LA_2 K_2-K_3 dimerization model gives the point of maximal cooperativity (negative) when [L] is equal to $1/K_2$ irrespective of the total protein concentration (fig 3). This finding compliments the results of previous studies. In particular, saturation curves of this LA_2 model intersect at $[L] = 1/K_2$ (Ingham, Saroff and Edelhoch, 1975) and Scatchard plots intersect at this same free ligand concentration (Nichol and Winzor, 1976). Another important characteristic of this alternate two species ligand-induced monomer-dimer model discussed here is that Hill plots are symmetrical about log [Y/1-Y)] = 0 (fig 5). The symmetry displayed by this alternate two species model is not

consistent with certain suggested tests for protein aggregating systems (Colosimo, Brunori, and Wyman, 1976).

Identical Sites on the Dimer

A system with all species present (K_1-K_4 are assigned finite values) and identical and independent ligand binding sites on the dimer ($K_4 = K_3^2/4K_1$) displays a marked difference in the trend in cooperativity as a function of increasing total acceptor concentration (fig. 6). The curves displayed only positive cooperativity for all values tested. These curves are concave down, symmetrical about $[A_t] \simeq 1/K_3$, and show one maximum. Thus, the trend in cooperativity can be either increasing or decreasing depending on which side of the maximum the chosen total acceptor concentration lies. The ratio of K_3/K_1 appears to affect only the relative size of the plot but not its shape (fig. 6, curves F-J). When the ratio of K_3/K_1 is kept constant, while the value of K_3 is varied, the n(max) vs. log $[A_t]$ plots are identical in shape and size but shifted to a maximum n(max) occurring at approximately $1/K_3$ (fig 6, curves A-E).

The condition of identical and independent ligand binding sites on the dimer seems to constrain the shape of a plot of n(max) vs. log$[A_t]$ to be symmetrical since removal of this constraint results in a modification of the curve and loss of symmetry (fig. 7). This appears to be true even for relatively slight changes in the condition that K_4 =

$K_3^2/4K_1$. When K_4 is increased slightly, both the shape and magnitude of the curves change markedly.

General Monomer-Dimer Equilibrium Systems

When all equilibrium constants are assigned finite values and no constraints are placed on K_4, a completely general ligand-induced monomer-dimer system is described. The cooperativity, as measured by the maximum Hill coefficient n(max), can be a complex function of the total acceptor concentration (fig 4, 8). Some selected values of K_1 through K_4 caused the cooperativity to exclusively decrease as $[A_t]$ increased (fig 4, curves A, B, and C). It also was possible to select a set of association constants that resulted in the cooperativity continually increasing as a function of increasing total acceptor concentration (fig 8, top curve). When K_3 was more than 2 decades greater that K_1 (i.e., $\log (K_3/K_1) > 2$), the n(max) vs. $\log [A_t]$ plot passes through a maximum at approximately $1/K_3$ (fig 8). Increasing the value of K_1 relative to K_3 results in a family of n(max) vs. $[A_t]$ curves that are similar for $[A_t]$ values below $1/K_3$, but in the $[A_t]$ region greater than $1/K_3$ they progressively become elevated. As K_1 comes within one decade of K_3, the curves continuously increase, approaching an n(max) value of 2.

Figure 4 shows the cooperativity trends for a general system in which the affects of changing K_4/K_3 ratios are emphasized, rather than the K_3/K_1 ratios discussed above.

Also included are the curves for both two species equilibrium systems discussed previously (i.e., the L_2A_2 (curve **I**) and LA_2 (curve **A**) dimer systems). When there is a maximum in a curve, it will occur when the total acceptor concentration approximates $1/K_3$. The trend in cooperativity thus can be either increasing or decreasing, depending on which side of the maximum a chosen total acceptor concentration lies. A striking feature of some curves is that they can show positive cooperativity (n(max) > 1) at one total acceptor concentration, but then display negative cooperativity (n(max) < 1) at other total acceptor concentrations. In addition, as K_3 approaches K_4 the degree of positive cooperativity progressively decreases until $K_3 = K_4$, when only negative cooperativity is displayed.

Comparison of maximum cooperativity curves for general equilibrium systems as a function of $[A_t]$, as shown in fig 4, further suggests that the two species L_2A_2 model would show only increasing positive cooperativity as the value for $[A_t]$ increases. All curves with positive cooperativity also show continually increasing cooperativity up to a maximum at $[A_t]$ 1 $1/K_3$. As K_3 becomes progressively smaller (ie. moving toward the value of $K_3 = 0$ which defines the specific L_2A_2 model) the position of the maximum in the n(max) vs. log $[A_t]$ curves occurs at ever increasing values of log $[A_t]$. In the limit as K_3 tends to zero the maximum cooperativity (n(max)) asymptotically approaches its maximum as $[A_t]$ tends to infinity (ie. $1/K_3$), thus showing that the

continuing increasing cooperativity of the special L_2A_2 model ($K_3 = 0$) is a natural extension of the more general case.

DISCUSSION

When characterizing an equilibrium system, the initial approach is generally to solve equilibrium equations analytically for key parameters. This has been undertaken for a monomer-dimer system with only partial success. Even with simplifying assumptions, the analytical solutions are not readily apparent. Because of this, numerical analysis was required in the past to characterize the system, but this was carried out in a somewhat limited fashion. When resorting to a numerical approach, the resolution of the system is only as good as the degree of refinement and extensiveness of variable values used. The work presented here increased the refinement and extended the independent variable values beyond that reported previously. This resulted in bringing cohesiveness to contradictory reports and discovery of several new patterns of cooperativity for a single, relatively simple equilibrium system. This illustrates the need for extensive analysis when using numerical approaches before generalizations can be made.

The relatively simple monomer-dimer model discussed here displays an amazing number of patterns in terms of cooperativity and the acceptor concentration. There are some subsets of the system that give only positive

cooperativity, others that give only negative cooperativity, and still others that will give both negative and positive cooperativity, depending on the total acceptor concentration. The trend in the maximal Hill coefficient as a function of an increasing total acceptor concentration can be increasing, decreasing or both increasing and decreasing. Cooperativity that is influenced by the protein concentration is known to distinguish an acceptor association mechanism from a nondissociating multisubunit allosteric model. However, the variability of patterns of cooperativity displayed by an aggregating system can also be exploited to help determine more precisely the particular reaction scheme within a monomer-dimer framework.

For instance, a system which gives a Hill coefficient that is greater than one cannot be completely described by a LA_2 two species monomer-dimer equilibrium system involving only one dimer species (ligand to monomer ratio of 0.5) and no dimerization in the absence of ligand. If a pattern with a decrease in the maximum Hill coefficient as a function of increasing acceptor concentration was obtained for a known monomer-dimer system, this would eliminate the L_2A_2 two species equilibrium model described by only one dimer species (ligand to monomer ratio of 1) with no dimerization in the absence of ligand. The D-lactate dehydrogenase system has shown a decreasing pattern of cooperativity as the enzyme concentration is increased (Sawula and Suzuki, 1970; Levitzki and Schlessinger, 1974). This enzyme system

could not involve a L_2A_2 two species equilibrium system with one dimer that has a ligand to subunit ratio of 1. If evidence pointed to a more general model, constraints would have to be placed on dimerization in the absence of ligand (K_3 must be substantially larger than K_1) to predict this decreasing trend in cooperativity.

The patterns of cooperativity vs. total acceptor concentration can also give insight into the relative magnitude of an important equilibrium constant. This constant (K_3) seems to be responsible for the inversion of an increasing total acceptor concentration to a decreasing one. If $1/K_3$ is within an obtainable acceptor concentration range, then the equilibrium constant might be estimated by locating the total acceptor concentration at which the maximum Hill coefficient occurs. Previous work has highlighted the importance of the degree of self association of the acceptor in the absence of ligand (represented by the value of K_1 in this work) in determining the cooperativity for systems with identical sites on the dimer. Our study extends this finding to general systems as well. However, the other acceptor association constant (K_3) appears to affect the cooperativity even when K_1 is zero. The importance of this particular parameter may not have been previously identified because in most cases the system has been described by constants other than K_3.

In addition to the estimation of one particular equilibrium constant, the equations that describe the

overall reaction scheme for the general monomer-dimer system can be used to refine data fitting to aggregating system models. Much of the data fitting procedures to date assume a dimer system with identical and independent ligand binding sites (Nichol and Winzor, 1972; Nichol, Smith and Winzor, 1969). The more general expressions given here might be used in the cases where data from known aggregating systems fail to conform to an identical and independent assumption. Initial elimination of some monomer-dimer models by Hill plot analysis might also help in estimating the parameter values by computer fitting of the data.

Previous authors (Levitzki and Schlessinger, 1974) used an incorrect form of eq. 14 (discussed under theory section) to conclude that the maximum Hill coefficient should decrease as a function of the total acceptor (enzyme) concentration for monomer-dimer systems, including the L_2A_2 two species equilibrium model defined entirely by K_2 and K_4. This is in contrast to what is predicted here. That analysis using equation 14 required determining the Hill coefficient as a function of the total acceptor concentration at a constant free ligand concentration. However, eq. 14 only gives the Hill coefficient (not the maximum Hill coefficient) at a given free ligand concentration. In addition, as fig. 4 shows, the free ligand concentration at which the maximum Hill coefficient occurs varies as the total acceptor concentration changes. Thus it is difficult to see how eq. 14 can be used to

support any argument that requires the determination of the maximum Hill coefficient at different and unknown free ligand values. Moreover, since this equation was derived only for this particular two step mechanism, its applicability to more general systems cannot be generalized.

A changing degree of cooperativity in response to a changing acceptor concentration has long been known to be an indicator of acceptor association. Previous analysis of certain protein association models seemed to suggest yet another association indicator. Hill plots of studied association systems were found to be asymmetrical about the ordinate axis corresponding to a half saturated system (Colosimo, Brunori, and Wyman, 1976). However, as shown in fig. 5, at least one particular acceptor association system (LA_2) does show symmetry. Thus Hill plot asymmetry cannot be an absolute test of an aggregating system.

Finally, there is much evidence indicating that a ligand induced monomer to dimer transition is involved in the estrogen receptor system. The hormone binding data presented in support of this shows an increase in the Hill coefficient as the receptor concentration is raised (Notides, Lerner and Hamilton, 1981). This trend is said to support a two-step model (Notides, Sasson and Callison, 1985; Notides and Sasson, 1983). Ironically, these authors used the theoretical analysis of Levitzki and Schlessinger to support their claim. As stated before, that analysis predicted the opposite trend for ligand-influenced

polymerization reactions (i.e., a decreasing Hill coefficient as the acceptor concentration increases). Our analysis shows that the hormone binding data is consistent with the proposed dimerization mechanism for the estrogen receptor, placing that data in harmony with many other findings that support a monomer-dimer estrogen receptor system.

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Fig. 1. A ligand-influenced monomer-dimer equilibrium system with corresponding species and association constants. Symbols A and L represent the monomer acceptor and free ligand respectively.

THE GENERAL REACTION SCHEME

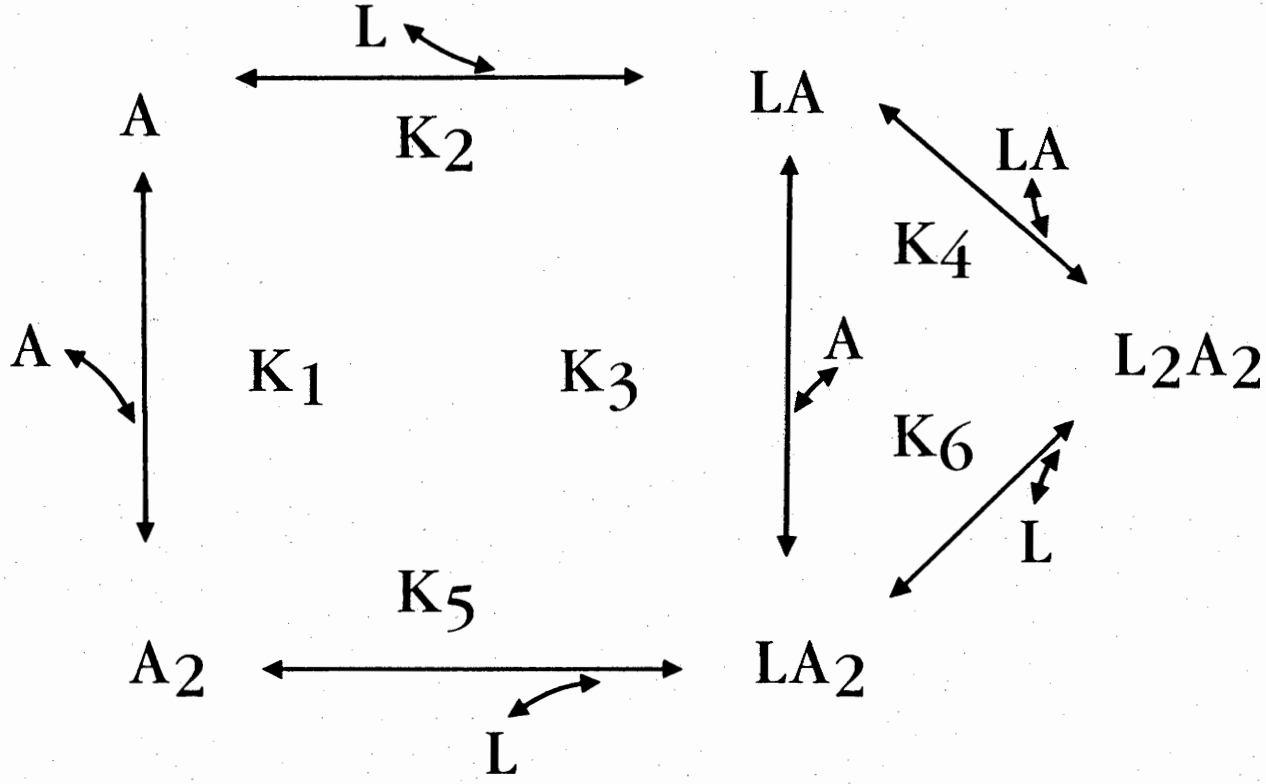


Fig. 2. Hill slope plots for a L_2A_2 two species equilibrium system at various total acceptor concentrations. K_1, K_2, K_3 and K_4 are 0, 10^3, 0 and 10^6, respectively. The total acceptor concentration for curves A-E are 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}, respectively.

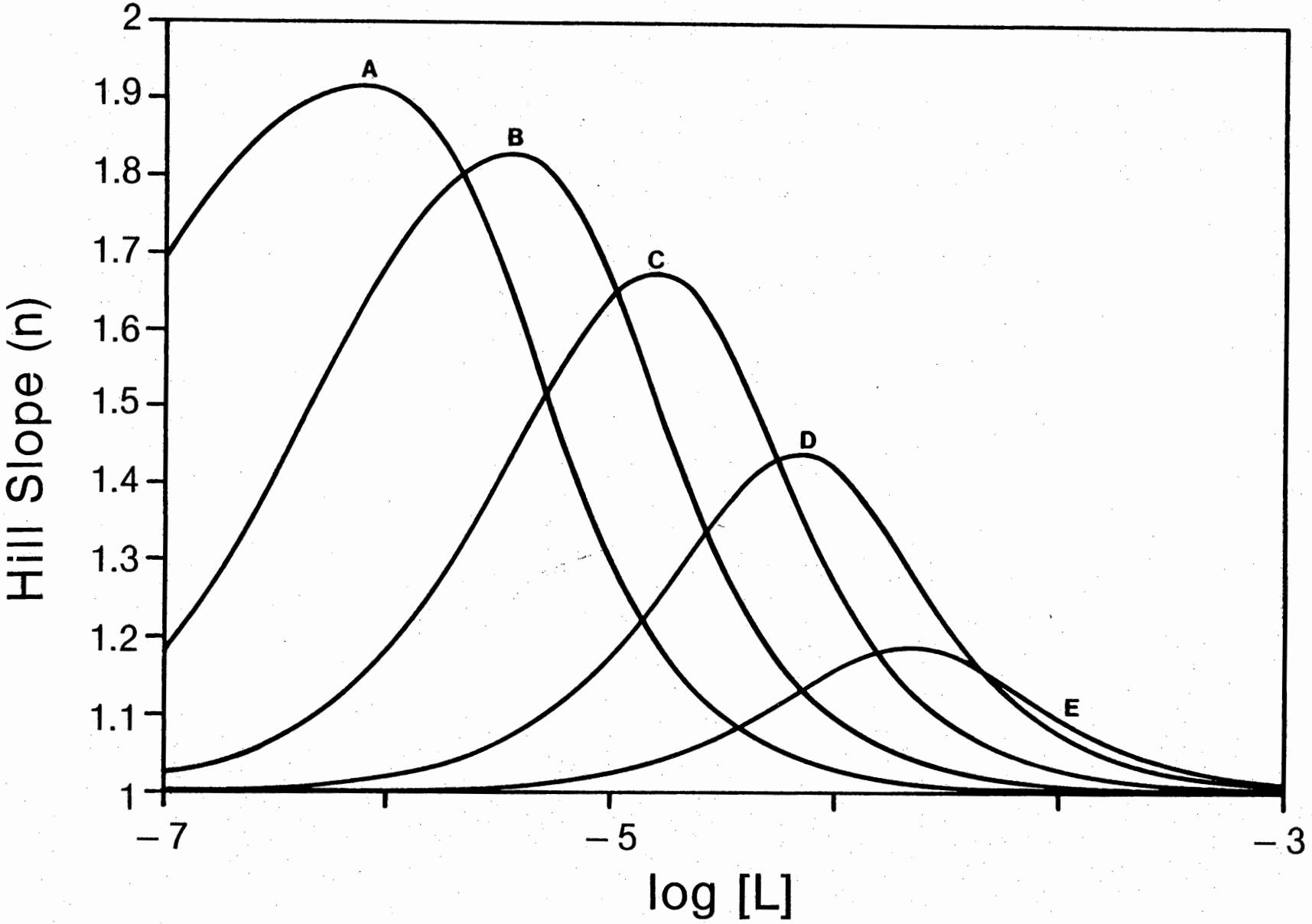

Hill Slope (n)
2
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
A
B
C
D
E
−7
−5
−3
log [L]

Fig. 3. Hill slope plots for an alternate LA_2 two species equilibrium system at various total acceptor concentrations. K_1, K_2, K_3 and K_4 are 0, 10^4, 10^6 and 0, respectively. The total acceptor concentrations for curves A-D are 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4}, respectively. The minimum Hill slope occurs when the free ligand concentration ([L]) equals $1/K_2$.

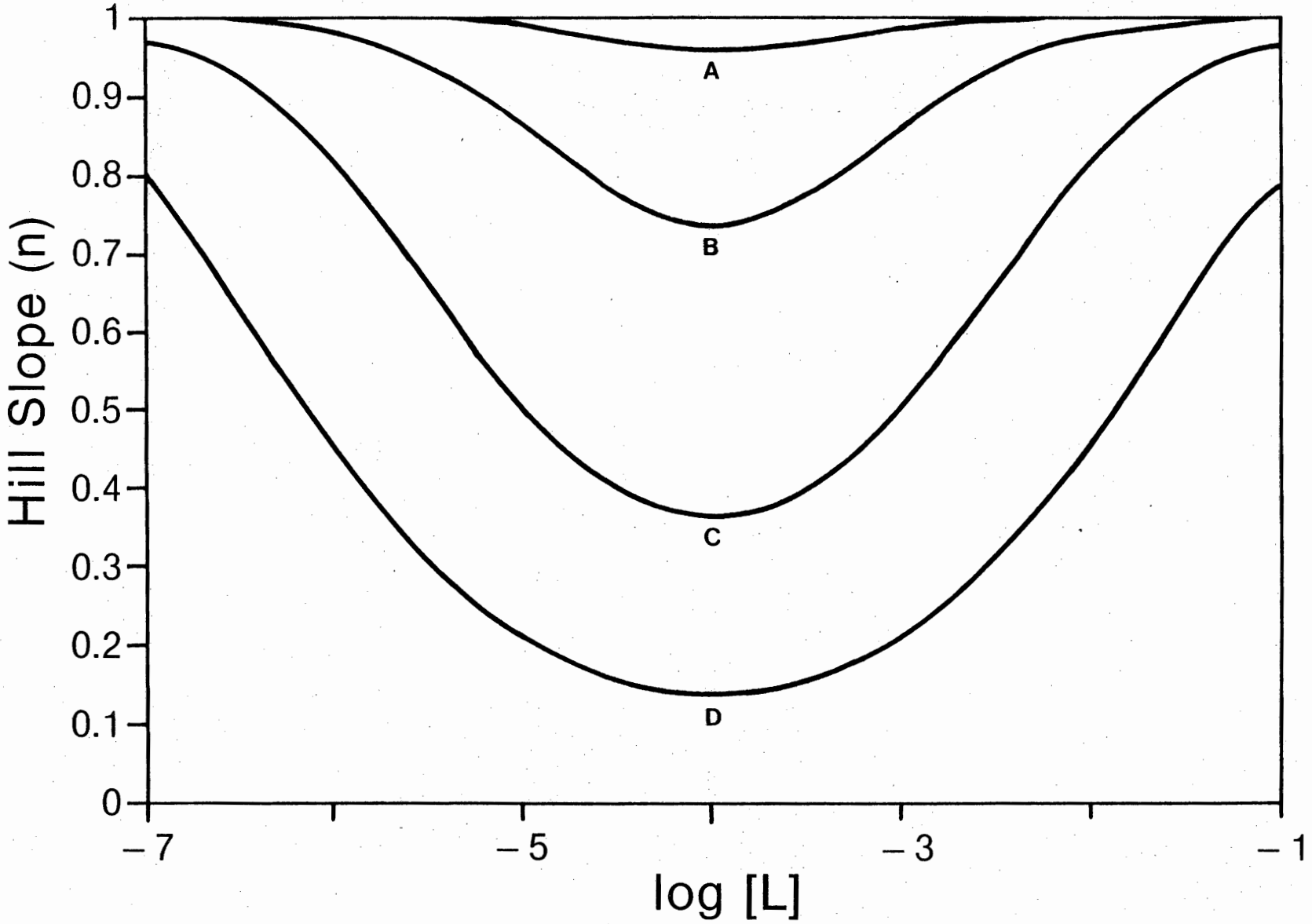

Hill Slope (n)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
A
B
C
D
−7
−5
−3
−1
log [L]

Fig. 4. Representative n(max) vs. log[A_t] plots for a generalized monomer-dimer system without dimerization in the absence of ligand. Also included are cooperativity curves for a L_2A_2 two species equilibrium system (curve I), and an alternate LA_2 two species equilibrium model (curve A). K_1 and K_2 values are 0 and 10^3, respectively. Values of K_3 and K_4 are: Curve A (10^6, 0); curve B (10^6, 10^4); curve C (10^6, 10^6); curve D (10^5, 10^6); curve E (10^4, 10^6); curve F (10^3, 10^6); curve G (10^2, 10^6); curve H (10^1, 10^6); curve I (0, 10^6).

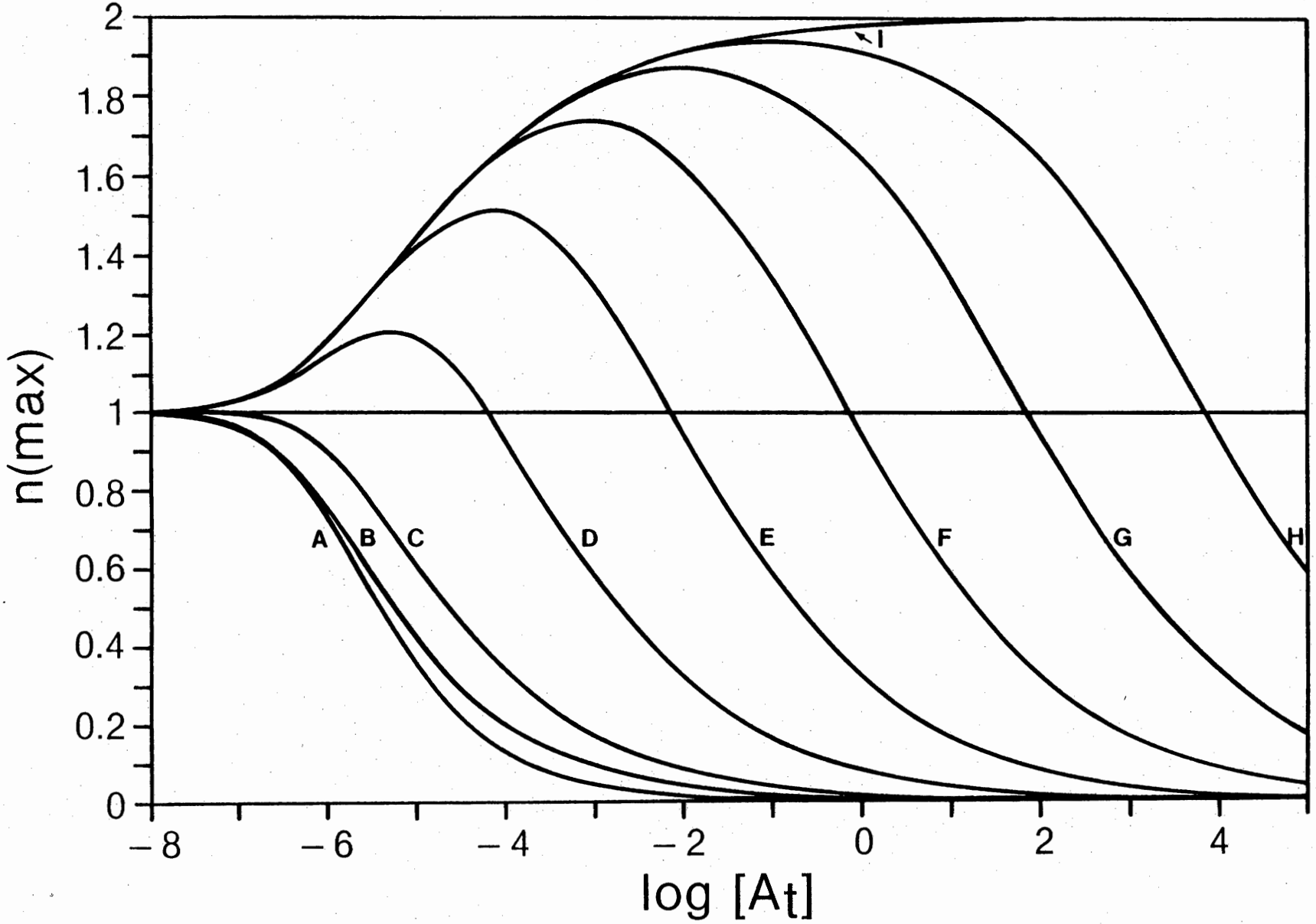

n(max)
log [At]
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
−8
−6
−4
−2
0
2
4
A
B
C
D
E
F
G
H
I

Fig. 5. Hill plots for an alternate LA_2 two species system, defined by K_2 and K_3, with various total acceptor concentrations. The total acceptor concentrations are: Curve A (10^{-6}); Curve B (10^{-5}); Curve C (10^{-4}); and Curve D (10^{-3}). K_1-K_4 values are 0, 10^3, 10^6 and 0. All plots intersect when the free ligand concentration equals $1/K_2$.

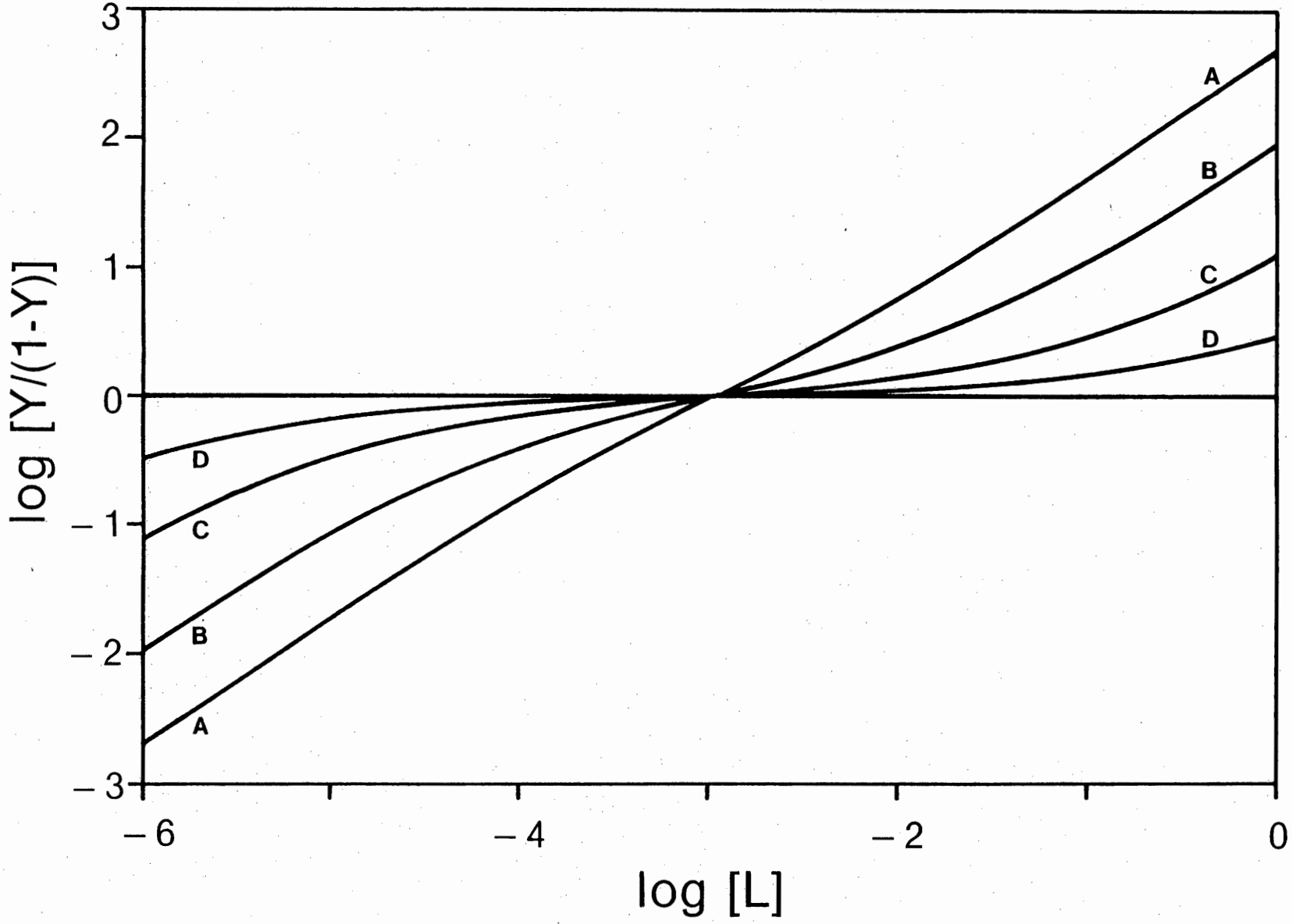

3
2
1
0
−1
−2
−3
log [Y/(1-Y)]
A
B
C
D
D
C
B
A
−6
−4
−2
0
log [L]

Fig. 6. Maximum Hill coefficient plots when the association constants conform to the condition of identical and independent binding sites. K_4 was calculated for each set of K_1-K_3 by the equation $K_4 = K_3/4K_1$. In curves A-E, the ratio of K_3/K_1 is constant while K_3 varies. Values of K_1-K_4 are: Curve A (10^4, 10^3, 10^6, 2.5×10^7); Curve B (10^3, 10^3, 10^5, 2.5×10^6); Curve C (10^2, 10^3, 10^4, 2.5×10^5); Curve E (1, 10^3, 10^2, 2.5×10^3). In curves F-J, K_3 remains constant while the ratio of K_3/K_1 changes. The values of K_2 and K_3 are 10^3 and 10^4, respectively. K_1 values for curves F-J are: 10^2, $10^{2.2}$, $10^{2.4}$, $10^{2.6}$, $10^{2.8}$ and 10^3.

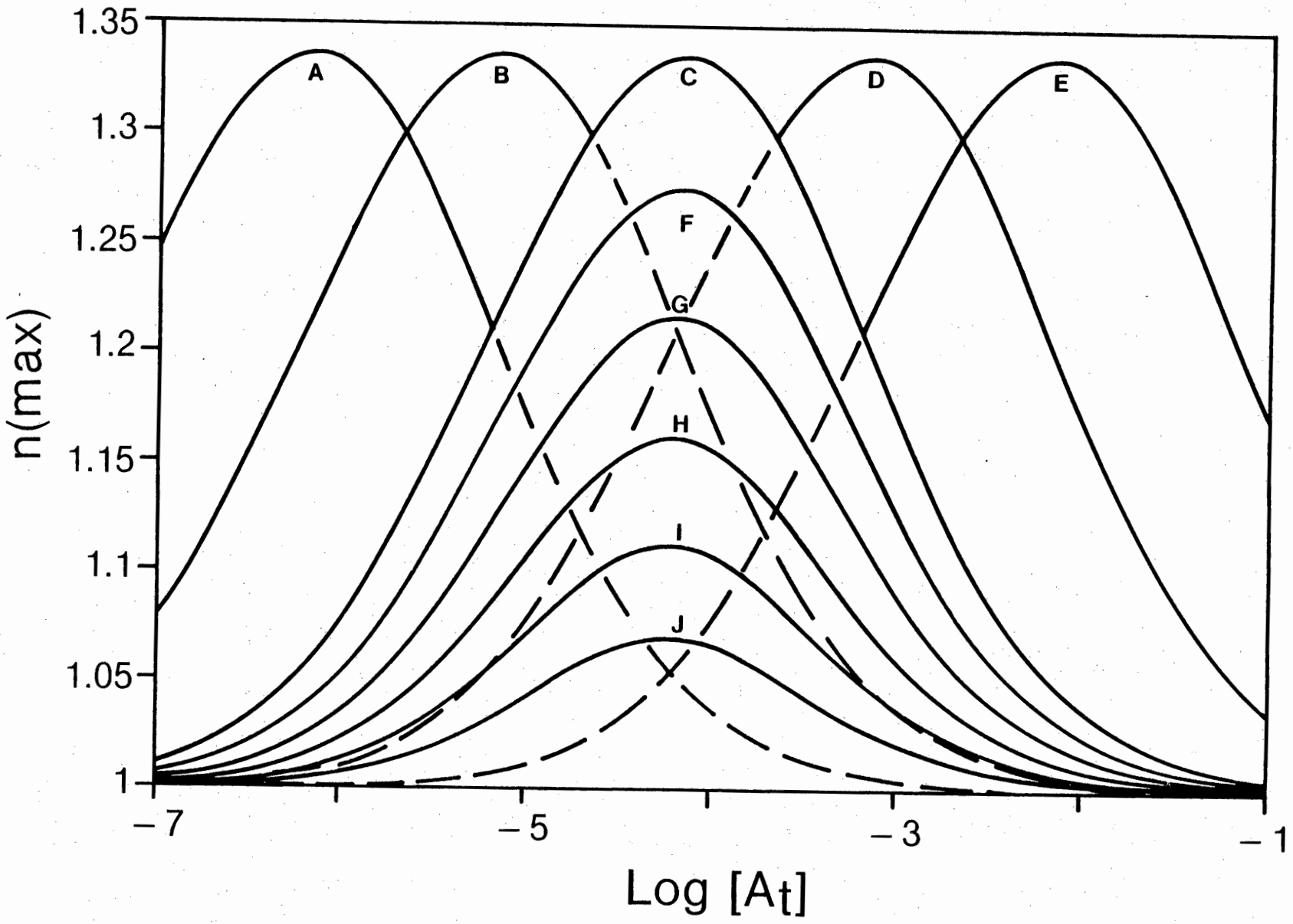

1.35
1.3
1.25
1.2
1.15
1.1
1.05
1
n(max)
A
B
C
D
E
F
G
H
I
J
−7
−5
−3
−1
Log [At]

Fig. 7. Maximum Hill coefficient plots when the constraints of identical and independent sites on the dimer are gradually removed. Curve A conforms to the condition of identical and independent sites ($K_4 = K_3^2/4K_1$). Values for K_1, K_2, and K_3 are 10^4, 10^3, and 10^5 for all curves. K_4 for curves A-F are 2.5×10^5, 2.8×10^5, 3.2×10^5, 3.8×10^5, 4.4×10^5 and 5.0×10^5, respectively.

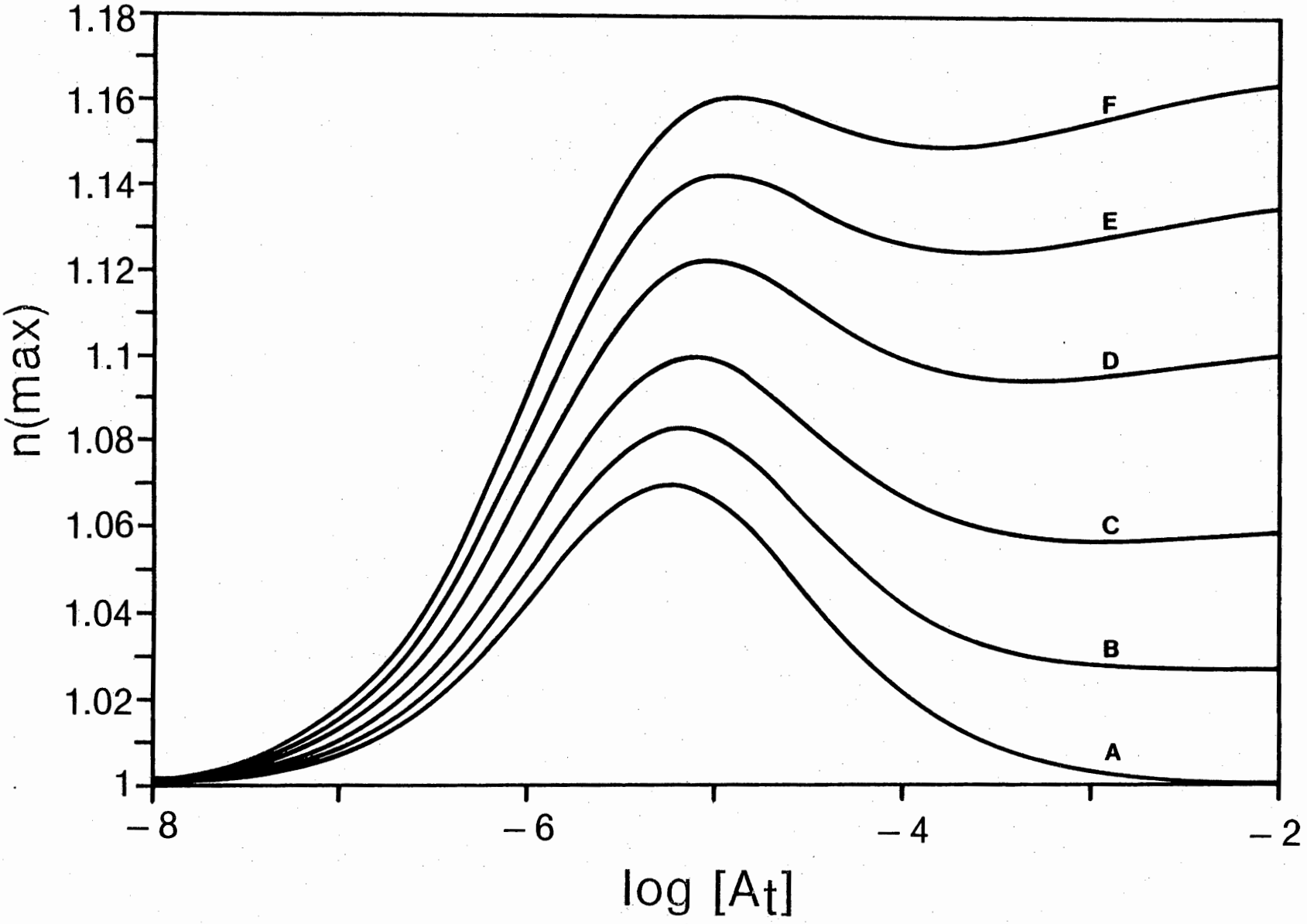
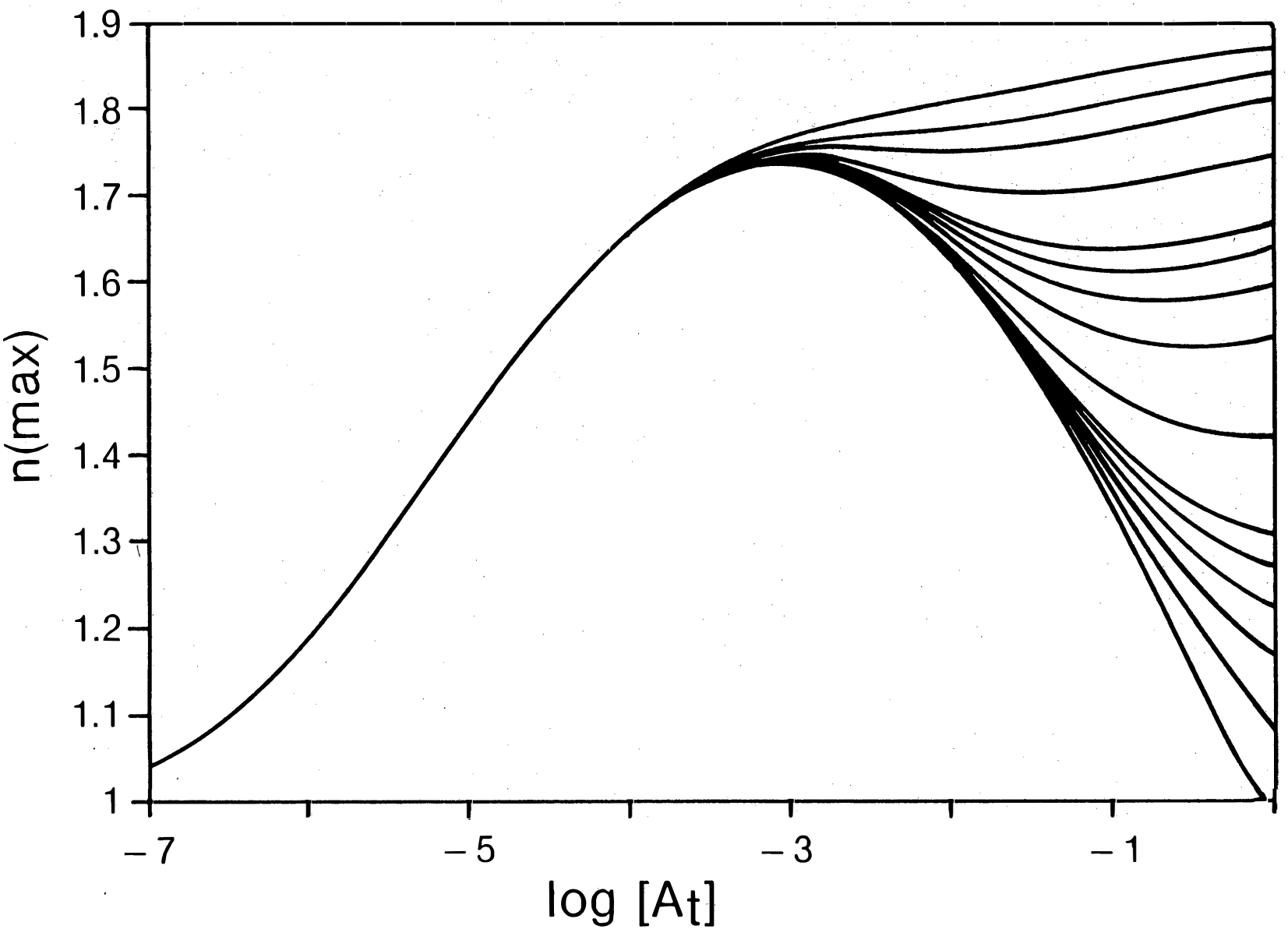

1.18
1.16
1.14
1.12
1.1
1.08
1.06
1.04
1.02
1
n(max)
F
E
D
C
B
A
−8
−6
−4
−2
log [At]

Fig. 8. Representative n(max) vs. log[A_t] plots for a completely generalized monomer-dimer system. The different curves are constructed for a changing K_3/K_1 ratio while K_3 remains constant. The values of K_2, K_3 and K_4 are 10^3, 10^3 and 10^6. The values of K_1 are (bottom to top): 0, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0, 10, 20, 40, 60, and 10^2.


1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
n(max)
– 7
– 5
– 3
– 1
log [At]