INTERACCIONES Y HETEROGENEIDAD EN LA FIJACION DE LIGANDOS A MACROMOLECULAS

TESIS

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CONTENIDO

I

INTRODUCCION GENERAL

Diversas áreas de la investigación bioquímica y biofísica están íntimamente relacionadas al fenómeno de fijación de ligandos. Ejemplos de estos fenómenos son: la fijación, ya sea de sustratos o inhibidores, a enzimas; la regulación de la expresión del genoma por inductores o represores; o bien la interacción de receptores con neurotransmisores, hormonas, drogas y otros efectores. Del mismo modo, varios eventos inmunológicos involucran o consisten de la fijación de ligandos; por ejemplo, el reconocimiento del antígeno por el anticuerpo, la proliferación de células inmunocompetentes que conducen a la producción de anticuerpos y a su eventual secreción, la citólisis inducida por el complemento, el disparo de reacciones de hipersensibilidad y la antigenicidad controlada por antígenos de histocompatibilidad.

Métodos para la caracterización de tales interacciones en el equilibrio cuando el ligando y el receptor son monovalentes y homogéneos en sus propiedades de fijación, existen desde hace varios años. Un procedimiento ampliamente usado e introducido por Scatchard (Scatchard, 1949), consiste en graficar la concentración del ligando unido a la macromolécula entre la concentración del ligando libre en contra de la concentración del ligando pegado. La pendiente de la línea recta obtenida es proporcional a la constante de equilibrio de la reacción, y el intercepto en las ordenadas a la concentración total de sitios activos.

Sin embargo numerosas macromoléculas son bivalentes o polivalentes, dando origen a la posibilidad de interacciones intramoleculares (efectos cooperativos) durante el proceso de fijación, y a la necesidad de

la determinación de parámetros adicionales para una adecuada caracterización de la reacción (Rodbard y Bertino, 1973). Las gráficas de Scatchard en estos casos ya no resultan en líneas rectas, sino que presentan segundas derivadas positivas o negativas, que reflejan interacciones negativas o positivas, respectivamente (Schwarz, 1976). Además, las gráficas de -- Scatchard obtenidas para poblaciones de macromoléculas que son heterogé-- neas con respecto a su energía libre de fijación por el ligando, pero no presentan interacciones, son indistinguibles de las gráficas obtenidas -- para sistemas con interacciones negativas (Fletcher, et al., 1970), complicando de este modo el análisis.

Aún cuando se obtenga información de experimentos independientes de que las macromoléculas son heterogénas y no presentan interacciones, extraer información de los detalles de la heterogeneidad es un problema difícil (Bowman y Aladjem, 1963). En esencia, el objetivo es obtener la distribución de energías libres, esto es, la fracción de receptores cuyo cambio en la energía libre en la fijación del ligando está entre ΔG y $\Delta G + d G$ (Delisi, 1978, Delisi y Thakur, 1978).

Existen numerosos intentos para extraer funciones de distribución de afinidades a partir de los datos de fijación; uno de los primeros intentos es el análisis de Sips (Sips, 1950) de la fijación de monómeros a una superficie catalítica heterogénea. Suponiendo una forma analítica particular para la curva de fijación (una isoterma modificada de --- Freundlich), Sips pudo expresar la función de distribución como una solución a una ecuación integral singular de primera clase. Este método fue

popularizado principalmente en investigaciones inmunoquímicas (Nisonoff y Pressman, 1958), pero adolece de la limitación de que la distribución es simétrica, y por lo tanto es sólo una aproximación adecuada cuando se sabe que la distribución es simétrica. Sin embargo, es raro que se tenga tal conocimiento a priori. Además, en algunas situaciones dinámicas, por ejemplo durante el curso de la respuesta inmune, la forma de la distribución de afinidades de los anticuerpos séricos varia (Congy y Mihaesco, 1978; Eisen y Siskind, 1964; Werblin y Siskind, 1972), y es precisamente esta variación la que debe caracterizarse para inferir los mecanismos selectivos que regulan la afinidad de los anticuerpos.

Cualquier distribución cuya forma se suponga a priori es inadecuada y por lo tanto se han desarrollado numerosas técnicas numéricas para calcular las funciones de distribución sin suponer la forma de las curvas de fijación (Bowman y Aladjem, 1964; Werblin y Siskind, 1972; Erwin y Aladjem, 1976). Sin embargo, aparte de lo complejo de estos métodos, lo cual, hace que su aplicación rutinaria sea difícil, las distribuciones de afinidad son muy inestables a mínimas perturbaciones de los datos de fijación (Delisi, 1976), y no consideran el caso en el que las macromoléculas heterogéneas sean cooperativas.

En este trabajo se presenta un análisis cualitativo en el que se permite que las poblaciones heterogéneas sean cooperativas. Se examina la sensibilidad de la gráfica de Scatchard a diversos parámetros de fijación, particularmente a las interacciones en ausencia o en presencia de heterogeneidad.

Este trabajo provee de reglas generales para la interpreta---
ción de curvas experimentales.

ALTERNATIVE INTERPRETATION OF UNUSUAL SCATCHARD PLOTS: CONTRIBUTION OF INTERACTIONS AND HETEROGENEITY

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ABSTRACT

Simulated binding reactions based on the stoichiometric binding model of Klotz [11] indicate that positive and negative site-site interactions in homogeneous and heterogeneous macromolecules result in varied binding isotherms, some with quite unusual shapes. Our findings provide the basis for some alternative interpretations of binding isotherms, distinct from those commonly found in the current literature.

1. INTRODUCTION

Many fields of contemporary interest in biochemical research are intimately related to ligand binding phenomena. The straightforward analysis of binding data is frequently complicated by the intrinsic heterogeneity of the binding sites and by positive and/or negative site-site interactions [8]. Both result in heterogeneous binding; however, the former situation, which is due to structural differences in the binding sites, is present from the start, while the latter is generated in the course of the reaction.

The need for assessing the individual contributions of initial heterogeneity and interactions in multiple ligand binding has previously been emphasized [12]. Without such a distinction, an analysis may lead to an erroneous interpretation of ligand binding data. For example, in the majority of the classical immunological studies, it is assumed that no interactions take place between the two binding sites of immunoglobulin G (IgG) when it reacts with monovalent hapten [10, 18, 27]; however, data have been obtained that are consistent with the hypothesis of cooperative binding of bivalent or monovalent hapten by IgG molecules [1, 2, 22, 23, 31]. In addition, problems have arisen in deciding whether the binding isotherms of several homogeneous and heterogeneous populations of multivalent macromolecules such as immunoglobulin M (IgM) [3, 9, 19, 20], nucleic acids [4, 17, 30], enzymes [13, 15], and others [14, 25] are evidence of site-site interactions and/or heterogeneity, due to the difficulty in detecting the occurrence of interactions when intrinsic heterogeneity is present.

In this paper we present a qualitative graphical analysis of the sensitivity of the Scatchard plot [24] to site-site interactions of homogeneous multivalent and heterogeneous bivalent macromolecules, based on a computerized simulation of the sequential binding model of Klotz [11]. We have found that Scatchard plots may acquire very unusual shapes with several inflection points when there is a mixture of positive and negative intramolecular interactions in a

multivalent macromolecule containing homogeneous sites. Furthermore, some of our results show how the distribution of affinities in heterogeneous populations of bivalent macromolecules may influence the graphical expression of site-site interactions. Although most of the present discussion is in terms of antibody-hapten systems, the results are equally applicable to ligand-macromolecule systems in general.

2. MATHEMATICAL MODEL

The stoichiometric model of Klotz [11] has been shown to be a general formulation, applicable to binding studies [8, 16], which relates the parameters obtained from graphs of binding data with binding constants for all kinds of ligand-macromolecule interactions. In this model, the macromolecule is treated as a binding agent which is assigned a definite number of association constants, equal to the number of binding sites. This thermodynamic treatment defines a set of stoichiometric constants, K_1, K_2, ..., K_n, which appear in the equilibria between macromolecule A (a protein) and ligand L, and which describe the sequential stoichiometric macromolecule-ligand species AL_1, AL_2, etc.:

$$AL_{i-1} + L \rightleftharpoons AL_i \quad ; \quad K_i = \frac{(AL_i)}{(AL_{i-1})\ (L)} \tag{1}$$

For the purposes of simulation, the stoichiometric binding constants (or apparent affinity constants) can be assigned without making any assumptions regarding the physical nature of the interactions among the binding sites [12]. This model is expressed by the rational function

$$r = \frac{K_1\ (L) + 2K_1K_2\ (L)^2 + \ldots + i\,(K_1K_2 \ldots K_i)\ (L)^i + \ldots + n\,(K_1K_2 \ldots K_n)\ (L)^n}{1 + K_1\ (L) + K_1K_2\ (L)^2 + \ldots + (K_1K_2 \ldots K_i)\ (L)^i + \ldots + (K_1K_2 \ldots K_n)\ (L)^n} \tag{2}$$

where r is the number of moles of bound ligand ($1L_1 + 2L_2 + \ldots + nL_n$) per mole of total protein ($L_0 = L + L_1 + \ldots + L_n$). Equation (2) cannot distinguish the position of the occupied binding site which is involved in the interaction. However, if all binding sites are available to bind ligand and all free sites are identical and independent after each liganding reaction, the stoichiometric constants are related to the intrinsic site constant k by the linear factor [11]

$$K_i = \frac{n - i + 1}{i} k \tag{3}$$

If there are homotropic interactions between the active sites, they can be calculated in terms of an interaction factor θ_i defined as

$$\theta_i = \log \left[\frac{K_i \text{ simulated}}{K_i \text{ predicted}} \right] \tag{4}$$

where

$\theta_i > 0$ implies positive interaction

$\theta_i = 0$ implies no interaction

$\theta_i < 0$ implies negative interaction

Thus, the stoichiometric constants in a homogeneous system describe the sequential addition of ligand L to the macromolecular acceptor, while θ_i indicates the occurrence and nature of these interactions according to the prediction of Klotz for nonindependent and identical sites.

There is another interaction factor γ_i [7], suitable for describing interactions in bivalent macromolecules, defined as

$$\gamma_i = \log \left[\frac{K_{i+1}}{K_i} \cdot \frac{n}{n-i} \cdot \frac{i+1}{i} \cdot \frac{i+1}{i} \right] \tag{5}$$

where

$\gamma_i > 0$ implies positive interaction

$\gamma_i = 0$ implies no interaction

$\gamma_i < 0$ implies negative interaction

However, if γ_i is used for multivalent macromolecules, then a positive interaction between sites $i-1$ and i implies a negative interaction between sites i and $i+1$. This situation is excessively restrictive since the events related to the binding of site $i+1$ could depend on all those preceding it, and not necessarily on those only in the immediate past. Therefore, θ_i will be used to describe the simulated binding reactions throughout this paper.

The equation we have used to describe site-site interactions in heterogeneous populations of bivalent macromolecules is

$$r = \sum_{j=1}^{m} \frac{K_{1j}\,(L) + 2K_{1j}K_{2j}\,(L)^2}{1 + K_{1j}\,(L) + K_{1j}K_{2j}\,(L)^2} \tag{6}$$

where r is the ratio of the concentration of bound ligand to the total concentration of the macromolecule, (L) is the molar concentration of unbound ligand, m is the number of bivalent populations, and K_{1j} and K_{2j} are the stoichiometric constants of the jth population.

Equations (2) and (6) can be used for the purposes of simulation since θ_i values may be arbitrarily assigned. They both assume that positive or negative interactions result in deviations from the expected values of K_i, because of a change either in the statistical factor $(n-i+1)/i$ and/or in the intrinsic association constant k. In our simulations only the statistical factor was changed. The computer programs for simulation are available upon request.

3. SIMULATIONS AND RESULTS

A. *GRAPHICAL BEHAVIOR OF THE STOICHIOMETRIC BINDING CONSTANTS OF MULTIVALENT MOLECULES*

Figure 1 describes the reaction of a homogeneous decavalent molecule with ligand, illustrating the appearance of a Scatchard plot if the stoichiometric constant K_i in the ith binding step were ten times greater than its expected theoretical value given by Eq. (3). In each curve of Fig. 1 (except curve 0), only one K_i of the ten stoichiometric constants is ten times greater than its expected value. Curve 0 represents the situation in which the ten binding sites are identical and independent. Note that curves 2–10 each show an ascending limb whose height decreases in each succeeding binding step. In other words, the deviation from the ideal curve 0 is less pronounced with each subsequent interaction. We conclude that if some interactions take place during the last binding steps in a multivalent molecule such as IgM, it would be experimentally difficult to recognize them. If a steep ascending limb is to be evident at high concentrations of bound ligand, one or more of the last stoichiometric binding constants must have a high value.

B. *MIXTURE OF INTERACTIONS IN A MULTIVALENT MACROMOLECULE AT HIGH AND LOW CONCENTRATIONS OF BOUND LIGAND*

The sensitivity of Scatchard plots to site-site interactions was shown to be inversely proportional to the concentration of bound ligand (see Fig. 1 and Eq. (1)). The effect of a mixture of interactions on the binding isotherm, not only at low but also at high concentrations of bound ligand, was then explored. Binding reactions in which positive and negative interactions alternate during the saturation process, thus increasing and decreasing, respectively, the probability of binding as a function of the occupancy of other binding sites, were simulated. Such

interactions are only possible in molecules such as IgM, IgA, DNA, RNA, etc., which have valences greater than two.

Figure 2 illustrates two Scatchard plots that represent binding reactions of a decavalent homogeneous population in which there are alternating positive and negative interactions. The most outstanding feature of both curves is that their shape is different from those that are usually fitted to experimental binding data. Both have several critical points, the number of which depends on the valence of the molecule and on the particular sequence of positive and negative interactions. Thus, experimental Scatchard plots with anomalous shapes may be indicative of very complicated binding processes involving alternating positive and negative interactions. This result is in contrast with Schwarz's proposal that points of inflection are present only when there are multiple classes of binding sites [26].

Figures 3 and 4 show the direct and Hill plots, respectively, that correspond to the Scatchard plots in Fig. 2. In order to test the sensitivity of each plot to mixed interactions, the area between the curves relative to the sum of the total areas in each figure was compared. We found that the alternation of positive and negative interactions is more obvious in the Scatchard plots, as is also the case for heterogeneity [5, 6, 28]. The direct plot has the advantage that one can simply read off the concentration of macromolecule-ligand species present at any given concentration of free ligand. The inflections of the direct plot are due to the presence of positive and negative interactions. When the data is represented in Hill plots (Fig. 4), there is an overlap at the extremes; the slope is the same at high and low concentrations of free ligand, a typical feature of this type of plot. A novel finding is the variation of the Hill coefficient as a function of the mixed interactions. Plots with the general form of those presented in Figs. 2 and 3 have been encountered experimentally [4, 30], and macromolecules exhibiting both positive and negative interactions have been described [15].

C. *Influence of Interactions, Heterogeneity, and Affinity Constant of Bivalent Molecules on the Shape of Scatchard Plots*

Panels *a* – *o* in Fig. 5 show Scatchard plots of simulated reactions of five populations of bivalent antibodies with monovalent hapten, all present in the same reaction system. Each simulated reaction involves the same concentration of total binding sites ($S_0 = 2 \times 10^{-7}$ moles/liter). Four of the five populations have dissimilar intrinsic affinity constants that remain constant in all plots. Only one population varies in its degree of interaction, intrinsic affinity constant, and relative concentration with respect to the other four. In every case, the x- and y-intercepts are independent of the degree of interaction, while the latter varies with the proportion of interacting antibodies. Although all the curves should reach the same S_0, which is the maximum value on the x axis, this is not graphically apparent. The total concentration of binding sites is always underestimated, particularly in the case of negative interactions and when the cooperative population is not very large.

The height and location of the ascending and descending limbs depend on the proportion of interacting antibodies and on the degree of interaction. Therefore, although an ascending limb is indicative of positive interactions in heterogeneous populations of antibodies, estimates of θ_i are not reliable if the concentration of the antibodies showing cooperativity relative to that of the total population is unknown. Experimentally, an ascending limb has been obtained in the reaction of antibodies to the monovalent hapten DNP (2,4-dinitrophenol) at very low concentrations of bound ligand [22, 23]. From the graphs presented here, we can conclude that the degree of cooperativity has been underestimated in many situations. It should be noted that the lack of a positive slope in a Scatchard plot does not rule out positive interactions since their detection in heterogeneous populations can be rather difficult if the cooperative population is not among the largest. Because the effects of positive interactions on

the curve compete with those of negative interactions and heterogeneity, an apparently straight plot may be obtained as a result of complicated binding processes, providing that the parameters have certain values. Moreover, it has previously been shown that a linear Scatchard plot can result from the creation of new binding sites [21].

The general conclusions that may be drawn from this set of curves are: 1) The Scatchard plot is sensitive to those binding constants that affect the most abundant populations, especially when the affinity of these populations is high with respect to that of the others; 2) The effects of positive interactions on a Scatchard plot compete with those of negative interactions and intrinsic heterogeneity; 3) A seemingly straight Scatchard plot derived from experimental data is no guarantee of homogeneity or of noninteractive binding.

4. DISCUSSION

The most important conclusions to be drawn from this qualitative graphical analysis of the sensitivity of the Scatchard plot to site-site interactions of homogeneous multivalent and heterogeneous bivalent macromolecules are:

a) Unusually shaped plots with several inflections are an indication of the presence of a mixture of positive and negative interactions in a multivalent macromolecule, and

b) The graphical representation of heterogeneity and negative interactions is antagonistic to that of positive interactions in a Scatchard plot; i.e., they tend to cancel each other.

Other noteworthy findings are:

c) Positive interactions are more easily detected during the initial binding steps in a multivalent macromolecule, and

d) The Scatchard plot is highly sensitive to complex binding reactions when the cooperative population(s) is among the largest.

The accidents in unusually shaped Scatchard plots may either be discarded as artifacts or interpreted as indicative of the presence of multiple classes of interacting binding sites. Although there have been no documented cases of the former situation, heterogeneity of binding has been invoked as an explanation of some binding isotherms of DNA and RNA [4, 26, 30]. Distinguishing between the graphical effects of a mixture of intramolecular interactions and those produced by multiple classes of interacting binding sites is, to our knowledge, still not possible.

Our results may be pertinent to the study of the binding of IgM since this decavalent molecule might bind hapten through a complicated mechanism involving site-site interactions. Although no unusual Scatchard plots for IgM have been published, it could be argued either that the available plots were obtained at high concentrations of bound ligand and thus were not sensitive to interactions, or that the intrinsic heterogeneity obscured the graphical characteristics of such interactions. The binding of IgM is certainly peculiar since there appears to be two classes of binding sites even in chemically homogeneous systems [19, 20].

Our findings on the interference of heterogeneity in the graphical representation of interactions has at least two practical applications. The first has to do with the binding of hapten by naturally raised immunoglobulins in which heterogeneous binding sites are thought to be the rule. In this case, an ascending limb may be indicative of the presence of positive interactions, but the quantitative estimation of the interaction parameters from graphical data is not reliable [29]. The second is that a Scatchard plot with a "good straight line fit" is usually interpreted as evidence of independent and identical binding sites. This conclusion is no longer tenable due to experimental variation and the possible combined effect of heterogeneity and interactions on binding, and therefore on the shape of the curve.

Finally, although it is clear that some progress has been made in the interpretation of binding isotherms enabling the recognition in some cases of the presence of heterogeneity and

interactions, even when they occur simultaneously, we are still in need of a reaction model that would permit the precise estimation of all binding parameters.

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FIGURE LEGENDS

FIG. 1. Graphical [illegible] of stoichiometric binding constants. Simulated binding reactions of a decavalent macromolecule in the *i*th binding step, in which the corresponding K_i is ten times greater than the predicted value. All $\theta_i = 0$ for curves 0–10 except in curves 1–10 when $\theta_i = -\log 10$ where N = 1, 2, ..., 10, corresponding to the number of the curve.

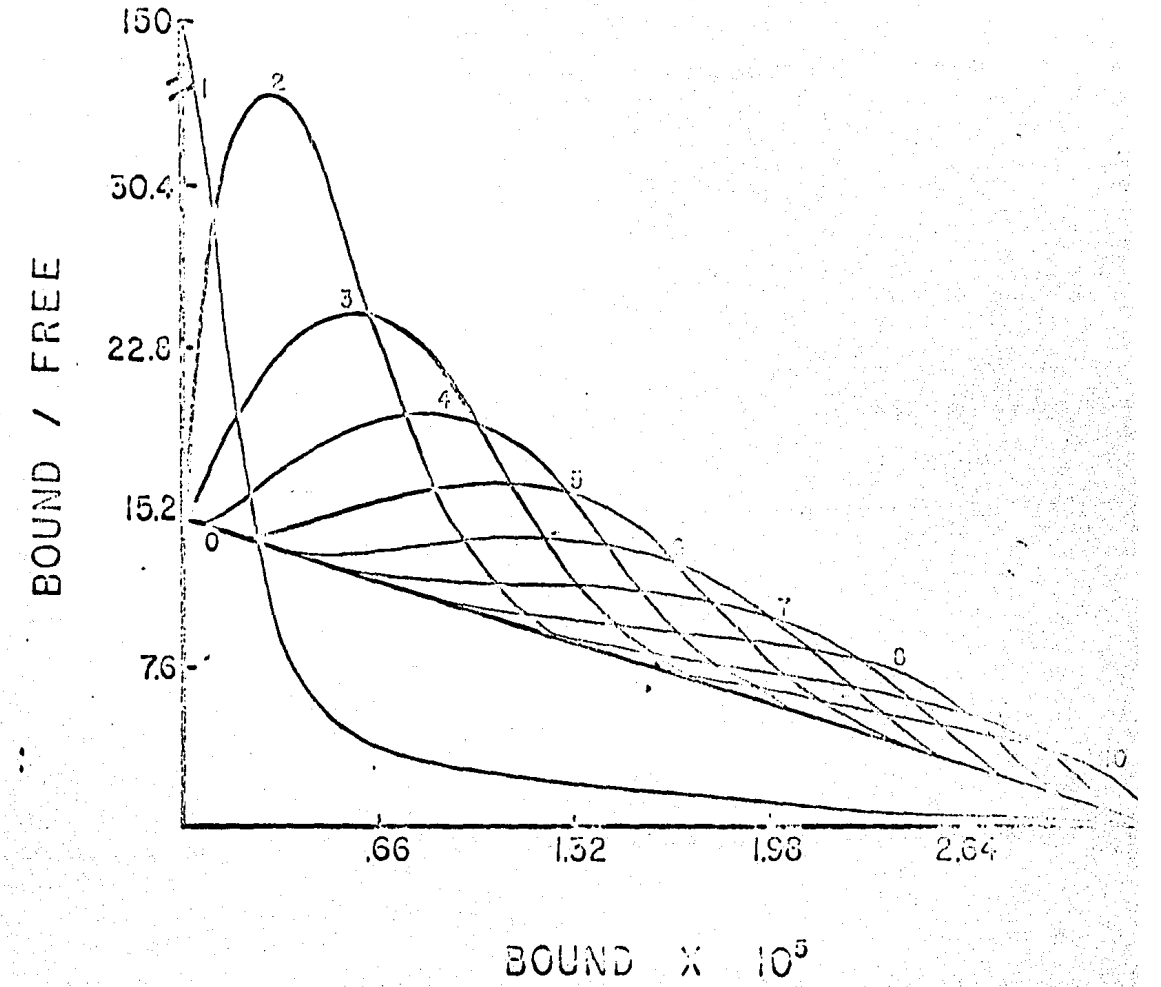
FIG. 2. Scatchard plots of simulated binding reactions in which positive and negative interactions alternate during the saturation process in a homogeneous decavalent molecule. The binding data of the solid curve are: total protein concentration $A_0 = 3.3 \times 10^{-7}$, $\theta_1 = \log 1$, $\theta_2 = \log 2.2$, $\theta_3 = \log .375$, $\theta_4 = \log 3.54 \times 10^9$, $\theta_5 = \log 8.3$, $\theta_6 = \log 12$, $\theta_7 = \log 1.75 \times 10^{-2}$, $\theta_8 = 2.26 \times 10^{-2}$, $\theta_9 = \log 4.5 \times 10^3$, $\theta_{10} = \log 10^4$. The binding data of the dotted curve are: $A_0 = 3.3 \times 10^{-7}$, $\theta_1 = \log 1$, $\theta_2 = \log .25$, $\theta_3 = \log .6$, $\theta_4 = \log 5.71$, $\theta_5 = \log 8.3$, $\theta_6 = \log 1.2 \times 10^{-2}$, $\theta_7 = \log 1.75 \times 10^{-2}$, $\theta_8 = \log 2.6 \times 10^{-2}$, $\theta_9 = \log 4.5 \times 10^3$, $\theta_{10} = \log 10^4$.

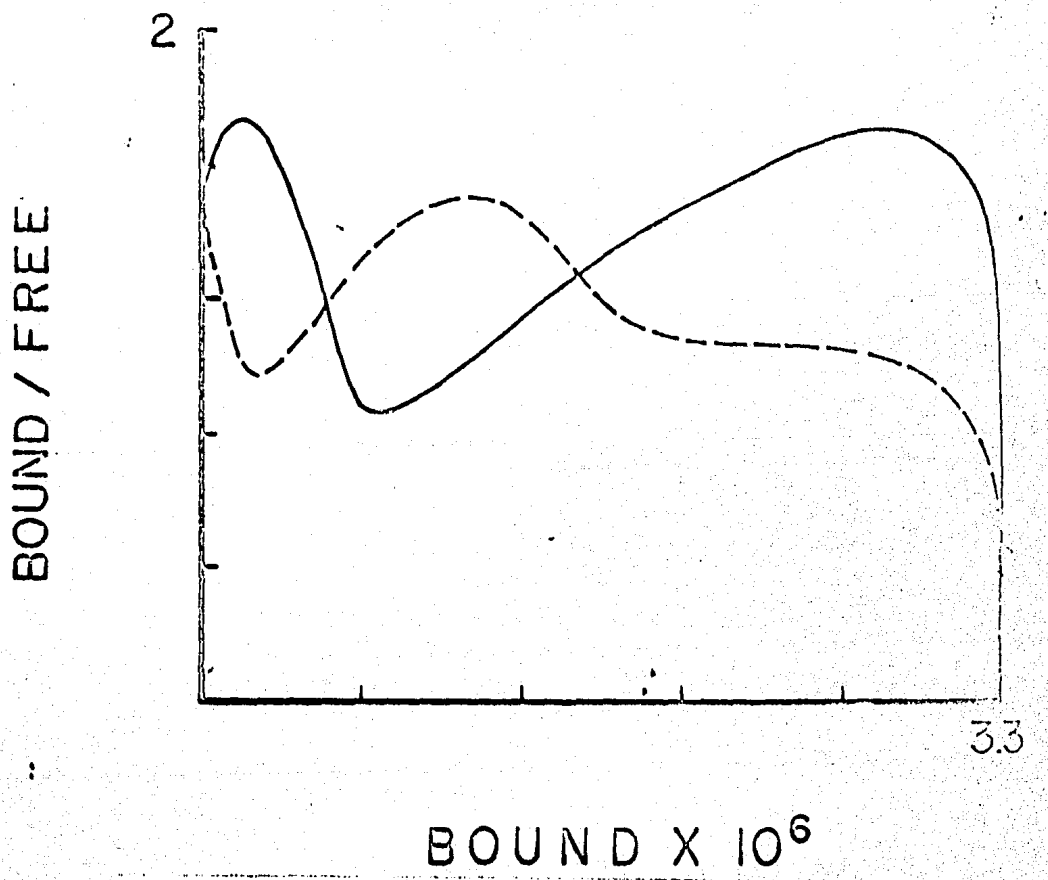
FIG. 3. Direct plots corresponding to the binding data used in Fig. 2.

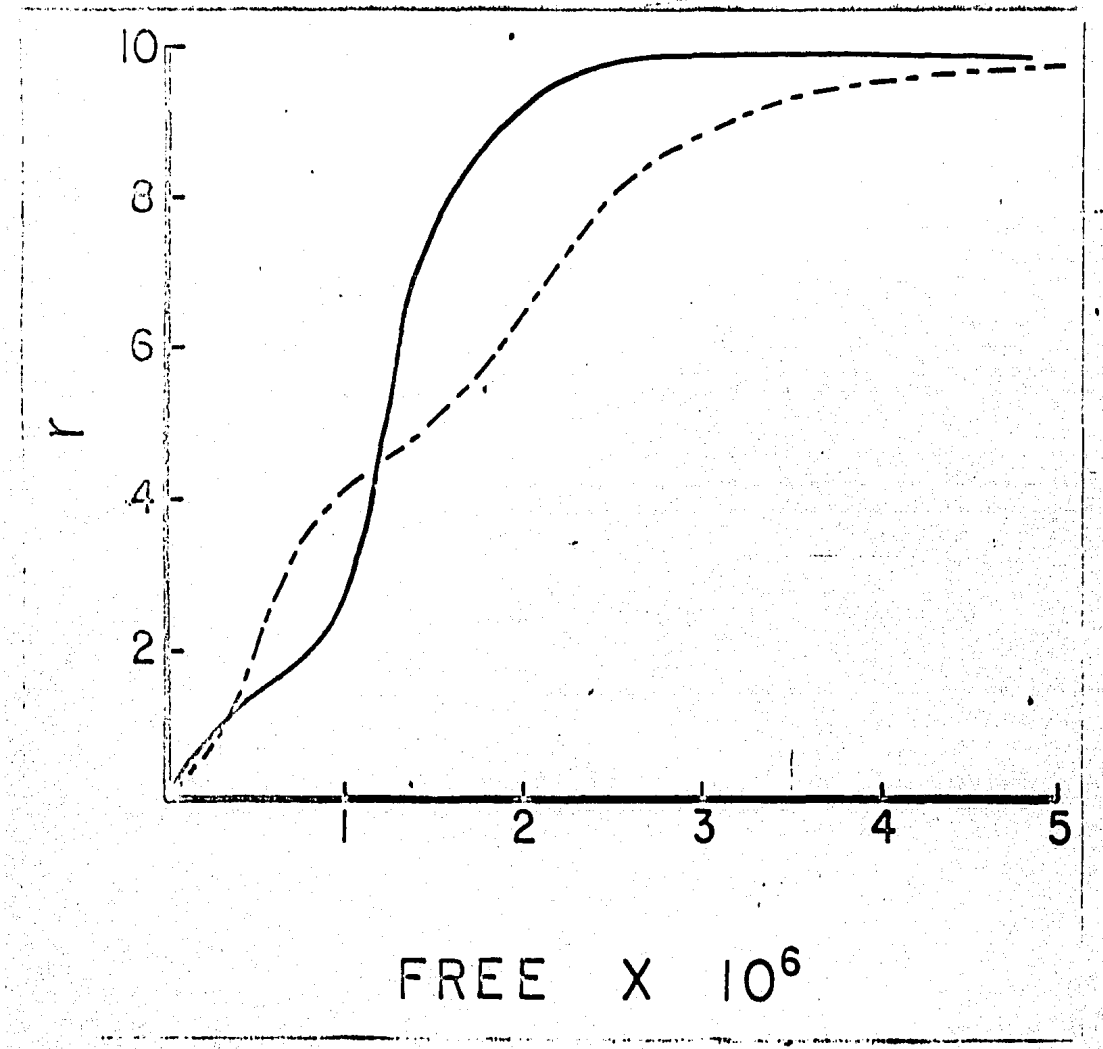
FIG. 4. Hill plots corresponding to the binding data used in Fig. 2.

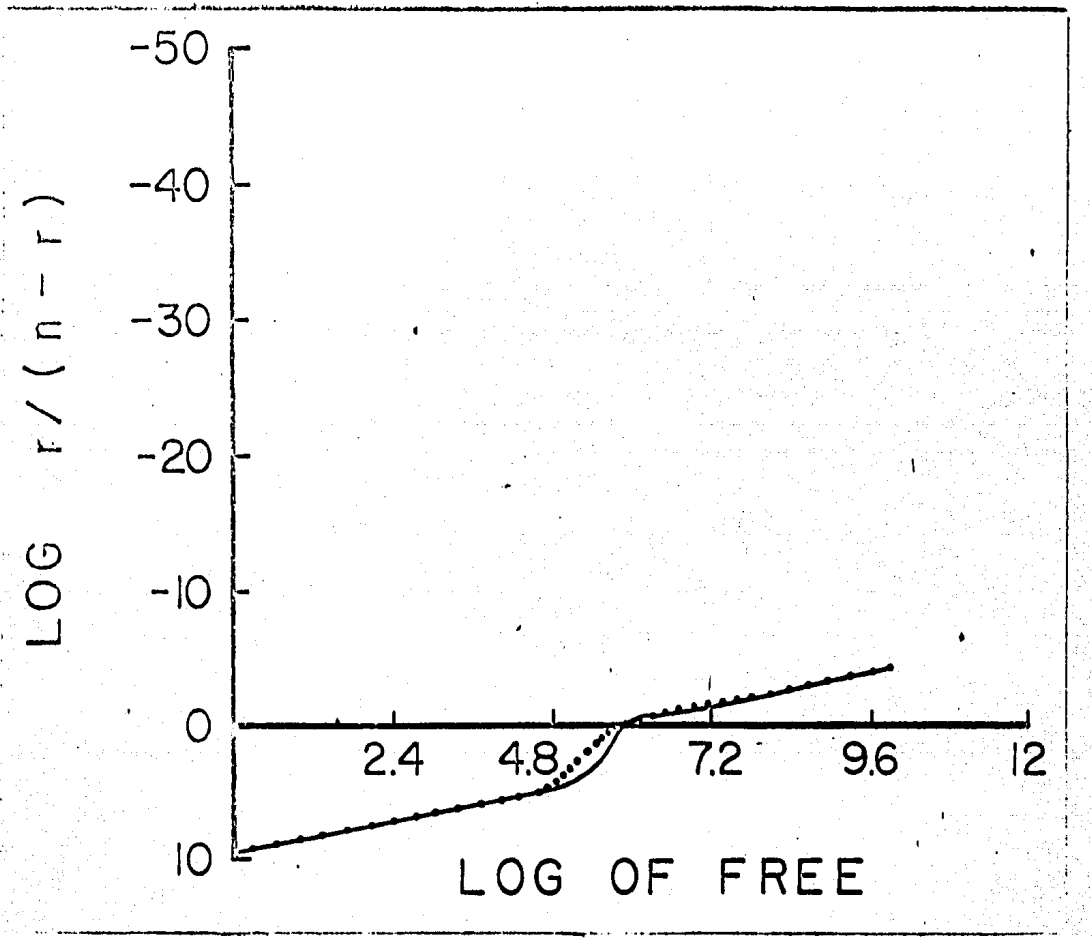
FIG. 5. Influence of interactions, heterogeneity, and affinity constant on the shape of Scatchard plots. All simulated reactions have the following data in common: total protein concentration ($A_0 = 10^{-7}$ moles/liter); total concentration of binding sites ($S_0 = 2 \times 10^{-7}$ moles/liter); five populations, of which four are noninteracting ($K_1 = 2 \times 10^4$, $K_2 = 10^4/2$;

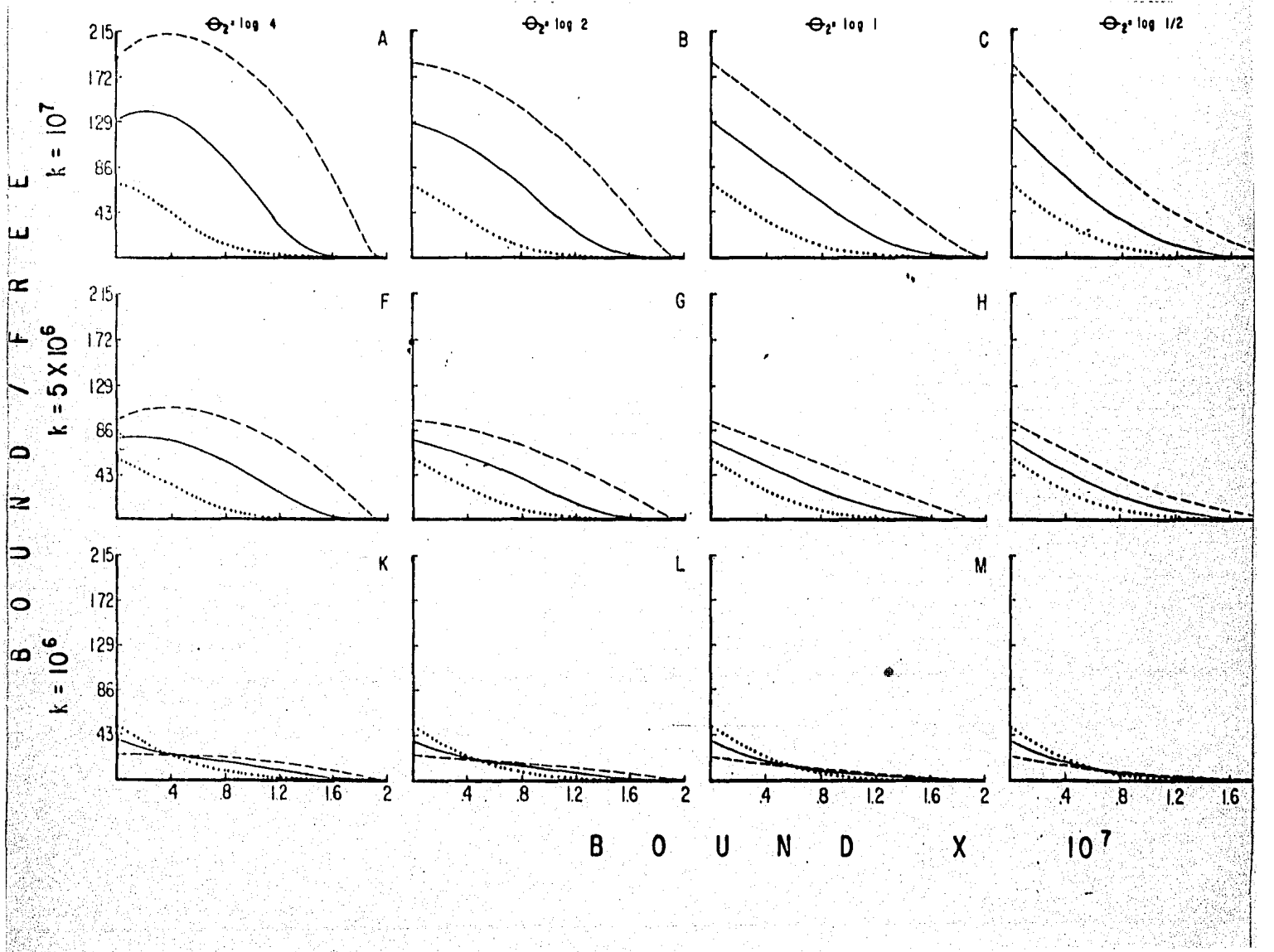
$K_1 = 2 \times 10^5$, $K_2 = 10^5/2$; $K_1 = 2 \times 10^6$, $K_2 = 10^6/2$; $K_1 = 2 \times 10^7$, $K_2 = 10^7/2$, respectively). The concentration of these four populations represents 10 o/o (dashed line), 50 o/o (solid line), and 90 o/o (dotted line) of the total protein concentration; the fifth population represents the rest of the protein in the system. In the panels, the fifth population varies in its θ_2 value, as follows: column 1 (*a, f, k*), $\theta_2 = \log 4$; column 2 (*b, g, l*), $\theta_2 = \log 2$; column 3 (*c, h, m*), $\theta_2 = \log 1$; column 4 (*d, i, n*), $\theta_2 = \log 1/2$; column 5 (*e, j, o*), $\theta_2 = \log 1/4$. The population that varies in θ_2 also varies in its intrinsic affinity constant, as follows: row 1 (*a–e*), $k = 10^7$; row 2 (*f–j*), $k = 5 \times 10^6$; row 3 (*k–o*), $k = 10^6$.

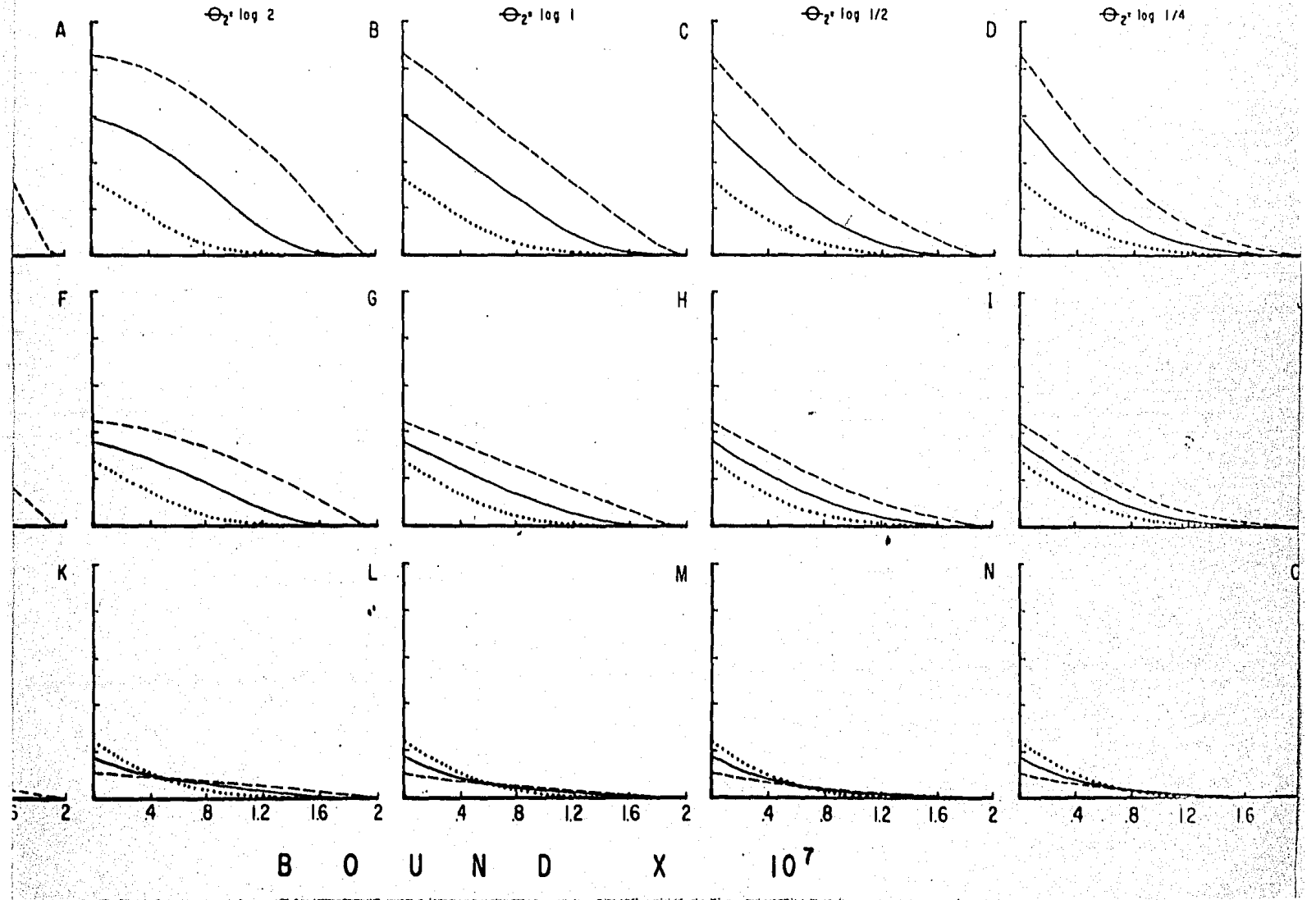

150
30.4
22.8
15.2
7.6
BOUND / FREE
1
2
3
4
5
6
7
8
9
0
.66
1.32
1.98
2.64
BOUND X 10⁵


2
BOUND / FREE
3.3
BOUND X 10⁶


10
8
6
4
2
r
1
2
3
4
5
FREE X 10⁶


-50
-40
-30
-20
-10
0
10
LOG r/(n-r)
2.4
4.8
7.2
9.6
12
LOG OF FREE


Θ_2 log 4
Θ_2 log 2
Θ_2 log 1
Θ_2 log 1/2
A
B
C
F
G
H
K
L
M
215
172
129
86
43
.4
.8
1.2
1.6
2
k = 10^7
k = 5×10^6
k = 10^6
BOUND / FREE
BOUND X 10^7


A
B
C
D
F
G
H
I
K
L
M
N
O
Θ_2 log 2
Θ_2 log 1
Θ_2 log 1/2
Θ_2 log 1/4
.4
.8
1.2
1.6
2
BOUND X 10^7

III

DISCUSION GENERAL

La sensibilidad de la gráfica de Scatchard a diversos parámetros de fijación así como algunas características cualitativas de la reacción de fijación han sido examinadas en este trabajo. Las gráficas de Scatchard proveen de información cualitativa valiosa referente a la naturaleza de las interacciones, la constante de afinidad promedio, y a la heterogeneidad del sistema. Sin embargo, los estimados cuantitativos y, en algunos casos, la interpretación cualitativa de estas gráficas no es confiable.

Las principales reglas a seguir cuando se interpreten las diversas formas que una gráfica de Scatchard puede adquirir son:

1. Una región con pendiente positiva indica interacciones positivas entre los sitios de fijación. Esto es cierto aún en presencia de interacciones negativas y/o heterogeneidad inicial. Estas últimas reducen los efectos gráficos de la interacción positiva.

2. Una pendiente negativa surge de heterogeneidad inicial y/o interacciones negativas, aun en la presencia de interacciones positivas.

3. Dado que los efectos de interacción positiva compiten con los de interacciones negativas y heterogeneidad, una aparente línea recta puede obtenerse como resultado de procesos complicados de fijación, siempre que los parámetros presenten ciertos valores. De este modo, una línea recta no necesariamente indica sitios idénticos e independientes.

4. Curvas bizarras de fijación que presenten un número de accidentes pueden ser indicativas de un proceso complejo de fijación, y por lo tanto no deben ser experimentalmente consideradas como resultado de artefactos.

5. Un estimado preciso de la concentración total de sitios activos - So es necesario para la confiable estimación de las constantes de afinidad, ya que las interacciones negativas y la heterogeneidad favorecen la subestimación de So. Atención especial debe darse - a la porción aparentemente horizontal de la gráfica antes de que se interprete el sistema en su totalidad.

Estas reglas enfatizan el concepto de que cualquier modelo de fijación puede ajustar un conjunto de datos experimentales, pero hasta -- que todas las alternativas sean descartadas, un ajuste no establece un modelo particular (Fletcher, et al, 1970; Koshland, et al, 1966; Magar y -- Steiner, 1971). Cualquier desviación discreta de la región de fijación - provocada por la unión de una molécula de ligando, debería producir un -- cambio en las propiedades de fijación de la macromolécula (De Lean, et al, 1979). Se requieren de técnicas químicas que distingan entre una posibble heterogeneidad de los sitios y de interacciones entre sitios idénticos --- (Boeynaems y Cantraine, 1980; Fletcher, et al., 1970). Las situaciones - presentadas en las gráficas incluídas en este trabajo no son atribuíbles - a ninguna reacción en particular. Sin embargo, no parece haber ninguna -- razón lógica para descartar algunas de las formas de las gráficas de --- Scatchard mostradas en este trabajo, en base a los datos de equilibrio --- existentes en la literatura. Otras formas de las curvas de adsorción pueden fácilmente ser generadas en base al modelo estequiométrico empleado. Es claro que las gráficas de Scatchard deben ser consideradas como un primer paso en el análisis de datos de fijación de ligandos.

APENDICE

En este apéndice se mencionan los temas principales de los diversos artículos provistos en la sección de referencias. Lo anterior tiene como objeto que el lector interesado en el fenómeno de fijación de ligandos a macromoléculas pueda ampliar y profundizar la información que hasta la fecha se tiene de este tipo de fenómenos.

TEMA	REFERENCIAS
1.- Análisis y Desarrollo matemático del modelo estequiométrico de fijación.	1, 21, 24, 28, 29, 30, 31, 32, 33.
2.- Análisis de las diferentes representaciones gráficas del fenómeno de fijación en base al modelo estequiométrico.	5, 8, 24, 31, 47, 49.
3.- Formulación estocástica del modelo estequiométrico.	56
4.- Análisis y desarrollo matemático del modelo de mecánica estadística de fijación.	12, 52, 54, 55.
5.- Análisis de las diferentes representaciones gráficas del fenómeno de fijación en base al modelo de mecánica estadística.	10, 12, 38, 53.

6.- Modelos conformacionales de la fijación.	14, 27, 37.
7.- Problemas teóricos de los modelos de fijación.	20, 21, 22, 23, 32, 33.
8.- Otros modelos de fijación.	10, 36, 42, 48.
9.- Problemas en la interpretación de las isotermas de adsorción.	8, 10, 13, 16, 19, 32, 42, 49.
10.- Propiedades generales de las isotermas de adsorción.	8, 13, 16, 31, 53, 54, 61.
11.- Problemas en la medición de los parámetros de fijación.	13, 16, 21, 27, 34, 35, 45, 53, 59, 62, 63.
12.- Desarrollo de técnicas tanto teóricas como experimentales para determinar distribuciones de afinidad.	4, 15, 18, 22, 24, 25, 43, 50, 52, 60.
13.- Sistemas gráficos para la representación de la heterogeneidad del sistema.	13, 24, 39, 40, 43, 50, 51, 53.
14.- Problema de distinción entre heterogeneidad e interacciones.	3, 20, 22, 23, 32, 53, 57.

IV

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