Biochimica et Biophysica Acta, 558 (1979) 179—186

BBA 78573

ON THE INTERPRETATION OF BINDING ISOTHERMS IN COMPLEX BIOLOGICAL SYSTEMS

APPARENT HOMOGENEITY OF SOME HETEROGENEOUS SYSTEMS

ALLEN P. MINTON *

Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, MD 20205 (U.S.A.)

(Received May 1st, 1979)

Key words: Site heterogeneity; Binding isotherm; Hormone binding; Drug binding; Lectin binding; Membrane

Summary

A simple treatment of the effect of site heterogeneity upon binding isotherms is presented, which is applicable to the analysis of data obtained from measurements of hormone, drug, or lectin binding to membranes and cell surfaces. Using this treatment, isotherms corresponding to various distributions of binding constants have been fitted to examples of experimental binding data ordinarily interpreted in the context of a homogeneous binding site model. It is found that these data do not permit one to exclude the alternate possibility of a broad distribution of the binding constant K. If a homogeneous binding site model can be satisfactorily fitted to the data, it is probable that the value of K obtained by this procedure is equal or nearly equal to the number average value of K in the actual (unknown) distribution.

Introduction

There are convincing reasons why preparations of membranes or whole cells might be expected to exhibit a broad distribution of affinities for the binding of any particular ligand (hormone, drug, lectin, etc.). These include:

1. The presence of a multiplicity of macromolecular components of the biological system which may bind the ligand with different intrinsic affinities; and

* From September 1978 through August 1979 as Guest Scientist in the Departments of Biophysics and Polymer Research, Weizmann Institute of Science, Rehovot, Israel.

2. The probable existence of local static or dynamic inhomogeneities in the geometry and/or composition of the membrane surface, leading to a distribution of local environments which may influence the affinity for ligand of a chemically homogeneous class of receptors.

Nonetheless, it is not infrequently reported (see for example Refs. 1 and 2) that preparations of cell membranes and whole cells exhibit binding isotherms for a particular ligand which are indistinguishable, to within experimental error, from that characteristic of a single homogeneous class of noninteracting binding sites. It is therefore pertinent to ask whether and to what extent experimental observations of this type are inconsistent with the expectation of significant heterogeneity in the distribution of binding site affinities for ligand.

The purpose of the present communication is 2-fold: (i) to introduce a simple representation of an arbitrary distribution of binding constants from which the corresponding binding isotherm may be easily calculated, and (ii) to demonstrate that experimental data of acceptable quality (by conventional standards) which might otherwise be taken as indicative of a single class of independent binding sites are not incompatible with the assumption of a broad distribution of binding constants.

Analysis

Expression of site heterogeneity in terms of a distribution of binding constants: General considerations and a trapezoidal approximation

The effect of site heterogeneity upon binding isotherms has been considered by several investigators [3—8]. In the development which follows we shall assume that the system is noncooperative, i.e., is composed of independent binding sites. The equilibrium average number of molecules of monovalent ligand X bound per binding site, $\bar{\nu}$, may be given as a function of the concentration of free X, c, by the relation [5]:

$$\bar{\nu}(c) = \int_0^\infty \frac{Kc}{1 + Kc} P(K)\, dK \tag{1}$$

where K is the equilibrium association constant for binding a molecule of X to a single binding site, and $P(K)$ is the probability that the equilibrium association constant for binding of X to a site selected at random will have a value between K and $K + dK$. $P(K)$ is therefore a distribution function which satisfies the normalization condition

$$1 = \int_0^\infty P(K)\, dK \tag{2}$$

In principle it is possible to invert Eqn. 1 and to obtain the function $P(K)$ if one has sufficiently complete knowledge of the form of $\bar{\nu}(c)$ [3,5]. However, it has been shown that data obtainable in the literature are almost never sufficiently complete or precise enough to permit this inversion to be reliably performed unless additional assumptions as to the functional form of $P(K)$ are

introduced [5]. Several investigators have assumed that $P(K)$ may be reasonably approximated by a normal [4] or quasi-normal [3] distribution of ln K (or the free energy of binding) about a mean value. While such distributions may indeed be realistic for certain systems, it is doubtful that such distributions would be appropriate for all systems in which heterogeneity of site affinity might be expected to occur. Let us consider a system comprised of numerous qualitatively similar chemical moieties capable of binding a particular ligand, such as the surface of a cell in which are embedded a variety of glycoproteins and glycolipids capable of binding lectin. In such a system there is no physical basis for the a priori postulation of random or quasi-random fluctuations in the free energy of binding about a mean value.

One possible alternative approach, which we pursue here, is to approximate an arbitrary distribution $P(K)$ by a set of m trapezoidal regions, as illustrated in Fig. 1. By increasing the number of trapezoidal regions, the accuracy of the approximation may be improved as desired. Quantitative expressions for the distribution function $P(K)$, the number fraction of sites in each trapezoidal region, and the binding isotherm calculated in the trapezoidal approximation are presented in the appendix.

Binding isotherm for distributions of K modeled by a single trapezoidal region

One may fully describe the ith trapezoidal region using three parameters defined in the appendix: K_i, the maximum value of K in the region, f_i, the ratio of the minimum to the maximum values of K in the region; and Δ_i, a measure of the slope of the distribution in the region. In Fig. 2 are presented four special cases of the general trapezoidal representation: the homogeneous distribution (a), two triangular distributions (b, d), and a uniform distribution (c). Using Eqn. A5, the isotherms corresponding to each of these distributions have been calculated and are presented in the form of titration and Scatchard plots in Figs. 3a and b, respectively. The most noteworthy feature of these isotherms is that although their positions are different, reflecting differences in average affinity, their shapes are very nearly (but not exactly) the same. This observation suggests that experimental data which are accommodated by the homogeneous site model (i.e., distribution a) might in many instances be accommodated nearly as well by a broad trapezoidal distribution model.

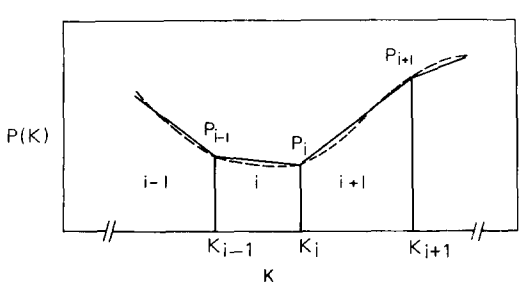

Fig. 1. Schematic representation of an arbitrary distribution of binding constants by a set of trapezoidal distributions. Dashed line represents 'true' distribution and upper edges of trapezoidal regions represent the approximation.

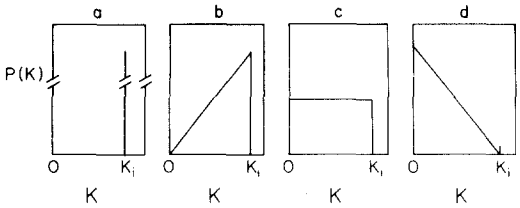

Fig. 2. Four special cases of the general trapezoidal distribution. (*a*) Homogeneous distribution: f_i = 0.99999 · · · · · ·, $-1 \leqslant \Delta_i \leqslant 1$. (*b*) Upper triangular distribution: f_i = 0.0, Δ_i = 1.0. (*c*) Uniform distribution: f_i = 0.0, Δ_i = 0.0. (*d*) Lower triangular distribution: f = 0.0, Δ_i = —1.0.

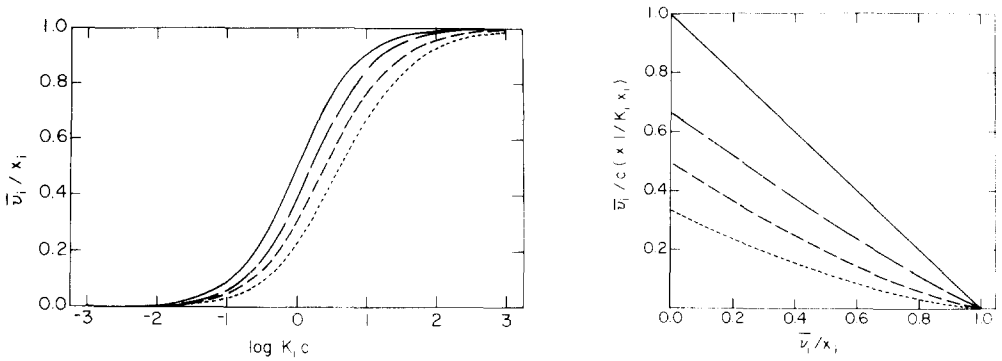

Fig. 3. (a) Titration plot of isotherms calculated according to Eqn. A5 for the four distributions shown in Fig. 2. ———, distribution *a*; — — —, distribution *b*; - - - - - -, distribution *c*; and · · · · · ·, distribution *d*. (b) Scatchard plot of same isotherms.

TABLE I

RESULTS OF FITTING EQN. A5 TO SELECTED EXPERIMENTAL DATA

Data set 1 was obtained from measurements of the binding of luteinizing hormone to plasma membranes of the corpus luteum [1]. Data set 2 was obtained from measurements of the binding of thyrotropin to cultured thyroid cells [2].

Data set	No. of points	f_i	Δ_i	Best-fit parameter values		log $\langle K \rangle_i$ **	Relative sum of squared residuals
				Relative No. of binding sites	log K_i *		
1	7	0.99999	0.0	(1.0)	8.55	(8.55)	(1.0)
		0.0	1.0	1.04	8.72	8.54	1.28
			0.0	1.10	8.86	8.56	1.46
			—1.0	1.17	9.00	8.52	1.81
2	10	0.99999	0.0	(1.0)	—1.77	(—1.77)	(1.0)
		0.0	1.0	1.02	—1.58	—1.76	1.00
			0.0	1.07	—1.45	—1.75	1.15
			—1.0	1.10	—1.26	—1.74	1.28

* K_i is in units of l/mol for data set 1 and ml/ng for data set 2.

** Obtained from the best-fit value of log K_i via Eqn. A6.

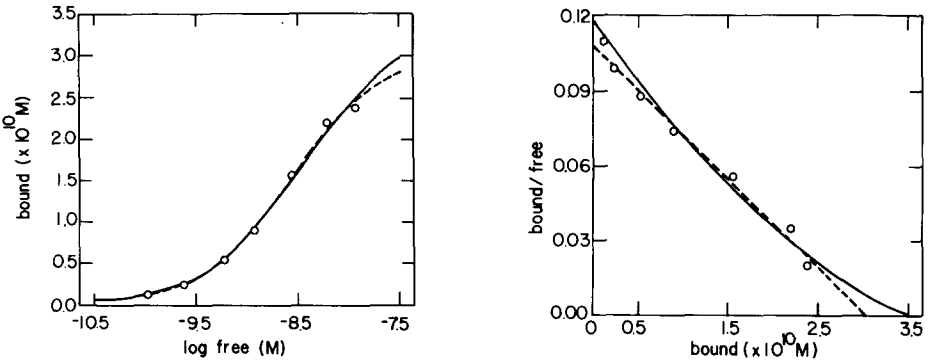

Fig. 4. (a) Titration plot of data set 1 [1] and best-fit isotherms calculated for distribution *a* (- - - - -) and distribution *d* (———). (b) Scatchard plot of same data and calculated isotherms.

To test this hypothesis, we selected two sets of data from the experimental literature which appear to be consistent with the homogeneous binding site model. To these data sets we obtained the best least-squares fit of Eqn. A5 for all four distributions shown in Fig. 2. The results of the fitting procedures are shown in Table I, and the functions calculated using the best-fit parameter values for distribution *d* are presented together with the data in the form of titration and Scatchard plots in Figs. 4 and 5. Analysis of variance reveals that although the homogeneous site model (distribution *a*) fits both data sets as well or better than the other three distributions, the differences between the relative values of the sum of squared residuals are too small to characterize even the 'worst' of the fits — distribution *d* to data set 2 — as statistically significantly inferior to the 'best' fit with a 90% degree of confidence. Since distribution *d* is just that trapezoidal distribution which is most dissimilar to the homogeneous site model, we may conclude that given binding data of the quantity and precision characterizing the present examples, one cannot discriminate between the

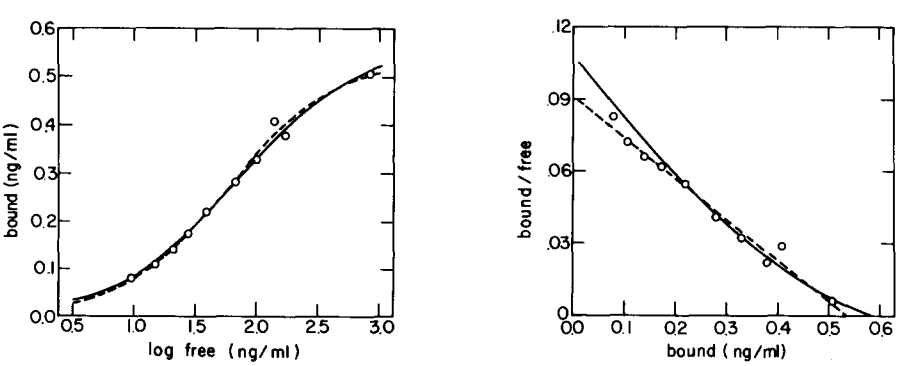

Fig. 5. (a) Titration plot of data set 2 [2] and best-fit isotherms calculated for distribution *a* (- - - - -) and distribution *d* (———). (b) Scatchard plot of same data and calculated isotherms.

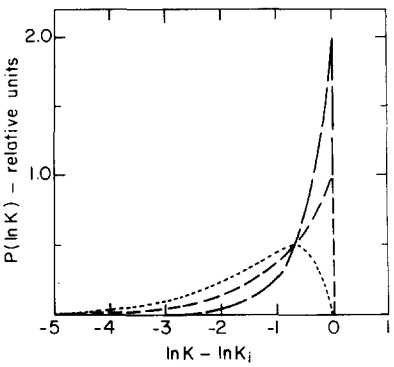

Fig. 6. Distributions of ln K corresponding to the distributions of K shown in Fig. 2. — — —, distribution b; - - - - - -, distribution c; and · · · · · ·, distribution d.

homogeneous binding site model and any trapezoidal distribution model. *

We may discern the underlying reason for the similarity of the isotherms generated by the four apparently dissimilar distributions of K shown in Fig. 2 by considering the corresponding distribution of ln K (or standard free energy of binding ΔG), which is related to the distribution of K by $P(\ln K) \propto K\,P(K)$. The distributions of ln K corresponding to the distributions b, c, and d in Fig. 2 are plotted in Fig. 6. It may be seen that the distributions of ln K or ΔG are reasonably narrow. In all cases the great bulk of sites (>75%) have values of ln K which differ from $\langle \ln K \rangle_i$ by less than one ln unit, i.e., have values of ΔG which differ from $\langle \Delta G \rangle_i$ by less than RT, where R is the gas constant and T the absolute temperature. Thus even the broadest trapezoidal distribution of K may be considered quasihomogeneous from the point of view of the distribution of ΔG.

Finally, we note in Table I that the best-fit value of $\langle K \rangle_i$ for a given data set is essentially independent of the type of distribution assumed and the best-fit value of the total number of binding sites is only mildly dependent (<±10%) upon the type of distribution assumed. It is therefore probably legitimate to conclude that *if* a set of data comparable to the examples we have chosen is capable of being fitted satisfactorily by a homogeneous binding site model, *then* the best-fit value of the binding constant in this model is equal to the number average value of the binding constant over whatever distribution of binding constants actually exists.

Appendix

Properties of the trapezoidal distribution and calculation of the associated binding isotherm

In the trapezoidal approximation,

$$P(K) = P(K_{i-1}) + \frac{K - K_{i-1}}{K_i - K_{i-1}} [P(K_i) - P(K_{i-1})] \quad \text{for } K_{i-1} \leqslant K \leqslant K_i \qquad \text{(A1)}$$

* Although rarely attempted, it is possible to accumulate enough data of sufficient precision to permit statistically valid discrimination between homogeneous and broadly heterogeneous trapezoidal distributions. One example of a data set permitting such discrimination may be found in Fig. 4 of Ref. 9.

and the number fraction of sites present in the ith trapezoidal region is

$$x_i = \frac{(K_i - K_{i-1})[P(K_i) + P(K_{i-1})]}{\sum_j (K_j - K_{j-1})[P(K_j) - P(K_{j-1})]} \tag{A2}$$

To simplify notation, we introduce the variables f_i and Δ_i, defined as follows:

$$K_{i-1} \equiv f_i K_i$$

$$P(K_i) \equiv x_i(1 + \Delta_i)/(K_i - K_{i-1})$$

$$P(K_{i-1}) \equiv x_i(1 - \Delta_i)/(K_i - K_{i-1}) \tag{A3}$$

where $0 \leqslant f_i < 1$ and $-1 \leqslant \Delta_i \leqslant 1$. The parameter f_i is thus a measure of the breadth of the ith trapezoidal region and the parameter Δ_i is a measure of the average slope of $P(K)$ in the interval $K_{i-1} \leqslant K \leqslant K_i$. By combining Eqn. 1 with Eqns. A1 and A3 and evaluating the resulting integral expression, we obtain

$$\bar{\nu}(c) = \sum_{i=1}^{m} \bar{\nu}_i(c) \tag{A4}$$

where

$$\bar{\nu}_i(c)/x_i = \left[\frac{1 - \Delta_i}{1 - f_i} - \frac{2\Delta_i f_i}{(1 - f_i)^2}\right] \times \left[1 - f_i - \frac{1}{K_i c} \ln \frac{1 + K_i c}{1 + f_i K_i c}\right]$$

$$+ \left[\frac{2\Delta_i}{(1 - f_i)^2}\right] \times \left[\frac{1 - f_i^2}{2} - \frac{1 - f_i}{K_i c} + \frac{1}{K_i^2 c^2} \ln \frac{1 + K_i c}{1 + f_i K_i c}\right] \tag{A5}$$

The number average value of K within a given trapezoidal region may be calculated to be

$$\langle K \rangle_i = \frac{1}{x_i} \int_{K_{i-1}}^{K_i} KP(K)\, dK$$

$$= K_i \left\{(1 - f_i^2)\left[\frac{1 - \Delta_i}{2(1 - f_i)} - \frac{\Delta_i f_i}{(1 - f_i)^2}\right] + \tfrac{2}{3}\Delta_i \frac{(1 - f_i^3)}{(1 - f_i)^2}\right\} \tag{A6}$$

Acknowledgements

I thank N. Sharon and J. Gilboa for critically reading and commenting upon early drafts of this report. I also thank the Departments of Biophysics and Polymer Research, Weizmann Institute of Science, Rehovot, Israel, for their hospitality.

References

1 Gospodarowicz, D. (1973) J. Biol. Chem. 248, 5042—5049
2 Verrier, B., Fayet, G. and Lissitzky, S. (1974) Eur. J. Biochem. 42, 355—365

3 Sips, R. (1948) J. Chem. Phys. 10, 490—495
4 Karush, F. and Sonnenberg, M. (1949) J. Am. Chem. Soc. 71, 1369—1376
5 Bowman, J.D. and Aladjem, F. (1963) J. Theor. Biol. 4, 242—253
6 Werblin, T.P. and Siskind, G.W. (1972) Immunochemistry 9, 987—1011
7 Goldstein, B. (1975) Biophys. Chem. 3, 363—367
8 Thakur, A. and Delisi, C. (1978) Biopolymers 17, 1075—1089
9 Kloog, J. and Sokolovsky, M. (1978) Brain Res. 144, 31—48