Department of Pharmaceutics
School of Pharmacy
State University of New York at Buffalo
Buffalo, NY 14214

Received October 4, 1976.
Accepted for publication November 9, 1976.
Supported in part by Grant GM 20852 from the National Institute of General Medical Sciences, National Institutes of Health.
[x] To whom inquiries should be directed.

Estimation of Free Energy Change Associated with Conformational Transition of Proteins Showing Cooperative Binding Properties

Keyphrases □ Protein binding—log Q plot used to estimate free energy change associated with conformational transition □ Conformational transition—unbound to complexed proteins, log Q plot used to estimate free energy change □ Cooperative protein binding—log Q plot used to estimate free energy change associated with conformational transition □ Binding, cooperative protein—log Q plot used to estimate free energy change associated with conformational transition

To the Editor:

Sokoloski and Hoener (1) recently pointed out some corrections necessary in the use, as suggested by Ma *et al.* (2), of a log Q plot to determine protein–ligand affinity constants in systems where interaction between binding sites is indicated. These plots are usually used to calculate the relevant binding parameters following assumption of a model. However, in this article, we shall show that such plots can be used to evaluate a parameter that plays a key role in cooperative binding processes.

A protein–ligand system exhibiting positive interactions is used to illustrate the calculations. It is generally accepted, and supported by experimental evidence, that the protein must be able to assume at least two different conformations, depending upon the state of ligation (3, 4), for such cooperativity to occur. Let the conformation in the absence of ligand be the T conformation and that of the fully saturated protein be the R conformation. During ligation, the molecule changes its conformation from the T to the R form. Whether this process occurs stepwise or not is irrelevant to the current problem; Scheme I (where L is the ligand, n is the number of binding sites, and b is the experimentally observable ligation reaction) can be assumed to hold.

$$\begin{array}{ccc} T + nL & \overset{b}{\rightleftharpoons} & RL_n \\ {\scriptstyle a}\searrow & & \nearrow{\scriptstyle c} \\ & R + nL & \end{array}$$

Scheme I

The standard free energy change of this step can be considered to be composed of at least two other steps, namely the T to R conformational change as indicated by a and the binding of ligands to the R conformation as indicated by c in Scheme I. The standard free energy change, ΔF_a, of step a can be viewed as the driving force for cooperativity, its magnitude and variation with experimental conditions being important for understanding the molecular mechanism of cooperative effects. It is possible to estimate ΔF_a from a log Q plot as shown below.

The observed mean number of sites occupied is ν, so the fractional saturation, Y, is equal to ν/n, where n is the total number of binding sites. The free ligand concentration is x, allowing Q to be defined by (5):

$$\log Q = \log(Y/1 - Y) - \log x \qquad \text{(Eq. 1)}$$

where Q can be considered to be the apparent affinity constant at a given value of Y. In the case of a single site or of independent and equivalent sites, Q is a constant. Figure 1, constructed from the data of Tyuma *et al.* (6), illustrates the variation of log Q with Y when cooperativity occurs. If a model is assumed, then such a plot can be used to estimate successive binding constants. The method described in this article permits the evaluation of ΔF_a when suitable values for Y and x are available.

Wyman (7) showed that ΔF_b, the total standard free energy of ligand binding, is equal to $nRT \int_0^1 \ln x \, dY$. This true thermodynamic expression can be related to log Q as follows:

$$\int_0^1 \ln x \, dY = -\int_0^1 [\ln(Y/1 - Y) - \ln x] dY \qquad \text{(Eq. 2a)}$$

$$\int_0^1 \ln(Y/1 - Y) dY = 0 \qquad \text{(Eq. 2b)}$$

Therefore:

$$\Delta F_b = -nRT \int_0^1 \ln Q_b \, dY \qquad \text{(Eq. 3)}$$

where b indicates that Q is concerned with step b. A similar argument can be used to obtain:

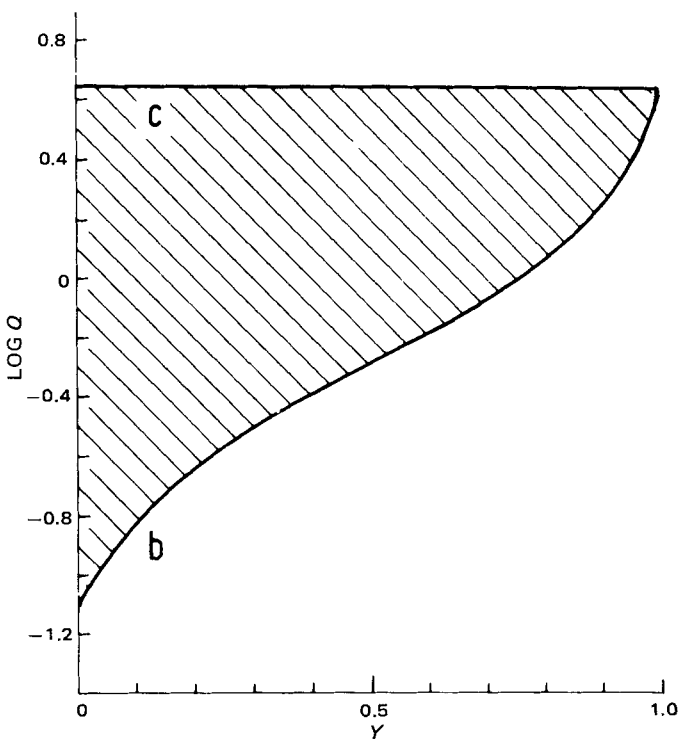

Figure 1—*Log Q plot of binding of oxygen to hemoglobin, calculated from the data in Ref. 6. In the calculation, partial oxygen pressure (in mm Hg) was used instead of molar oxygen concentration. Therefore, a conversion factor is involved in the calculation of* ΔF_b *or* ΔF_c *following the procedure outlined in the text. However, in the calculation of* ΔF_a, *this factor cancels out.*

$$\Delta F_c = -nRT \int_0^1 \ln Q_c \, dY \qquad \text{(Eq. 4)}$$

Therefore, as $\Delta F_a = \Delta F_b - \Delta F_c$:

$$\Delta F_a = 2.3nRT \int_0^1 (\log Q_c - \log Q_b) dY \qquad \text{(Eq. 5)}$$

In Fig. 1, curve b represents log Q_b, so the area under the curve multiplied by $-2.3nRT$ is equal to ΔF_b. However, Q_c cannot be obtained in a similar manner, because, in general, reaction c cannot be followed experimentally. A good approximation for log Q_c can be obtained, because all protein molecules are in the R state when Y approaches 1 so the limiting value of log Q_b and log Q_c must be the same. Therefore, an estimate of log Q_c can be obtained from Fig. 1, because line c should equal log Q_c when it is assumed that all sites are equal in the R state. This assumption immediately implies that, from Eq. 5, ΔF_a is equal to the shaded area of Fig. 1 multiplied by $2.3nRT$. In the figure, this area is equal to 5.0 kcal. From the figure, it can be seen that ΔF_a is always a positive quantity in a cooperative process, so the associated equilibrium constant is less than 1 or, in other words, the T conformation is highly favored in the absence of ligand.

This important result is obtained following the assumption that all sites in the R state are equal. A qualitative picture of the situation, when this assumption does not hold, can be obtained in the following way. All protein molecules are in the R state when Y approaches 1, so the limiting value of log Q_b when Y approaches 1 should form one point of the line representing log Q_c. However, when the binding sites are unequal, log Q_c is no longer equal to line c in Fig. 1. Log Q_c cannot lie below line c in Fig. 1, because this would indicate cooperativity in a molecule totally in the R state. Under these circumstances, log Q_c must lie somewhere above line c. This means that the true value of ΔF_a for nonidentical sites is higher than that obtained by applying the method for identical sites. Only when some specific model is assumed can the exact value of ΔF_a be calculated (8). It is obvious that dissimilarity of binding sites in the T state has no influence on the determination of ΔF_a.

(1) T. D. Sokoloski and B. Hoener, *J. Pharm. Sci.*, **64**, 1892 (1975).
(2) J. K. H. Ma, H. W. Jun, and L. A. Luzzi, *ibid.*, **62**, 2038 (1973).
(3) J. Monod, J. Wyman, and J. P. Changeux, *J. Mol. Biol.*, **12**, 88 (1965).
(4) D. E. Koshland, G. Nemethy, and P. Filmer, *Biochemistry*, **5**, 365 (1966).
(5) J. T. Edsall, C. Felsenfeld, D. S. Goodman, and F. R. N. Curd, *J. Am. Chem. Soc.*, **76**, 3054 (1954).
(6) I. Tyuma, K. Shimizu, and K. Imai, *Biochem. Biophys. Res. Commun.*, **43**, 423 (1971).
(7) J. Wyman, *Adv. Protein Chem.*, **19**, 223 (1964).
(8) L. H. M. Janssen and S. H. de Bruin, *Biophys. Chem.*, **1**, 130 (1973).

Lambert H. M. Janssen [x]
John H. Perrin *
School of Pharmacy
State University of Utrecht
Catharijnesingel 60
Utrecht, The Netherlands

Received July 26, 1976.
Accepted for publication October 8, 1976.

* Present address: College of Pharmacy, J. H. Miller Health Center, University of Florida, Gainesville, FL 32610.
[x] To whom inquiries should be directed.

BOOKS

REVIEWS

Topics in Infectious Diseases. Vol. 1. Drug Receptor Interactions in Antimicrobial Chemotherapy. Edited by J. DREWS and F. E. HAHN. Springer-Verlag, 175 Fifth Ave., New York, NY 10010, 1975. 314 pp. 17 × 24.5 cm. Price $19.40.

This book contains the papers presented at the Sandoz Symposium held in Vienna on September 4–6, 1974. It is divided into five general areas, *i.e.*, receptor hypothesis, DNA as a drug receptor, ribosomes as drug receptors, mode of action of chloramphenicol, and microbial enzymes as drug receptors, and includes papers that were contributed by 20 participants. The text of the book has 300 pages, 130 figures and illustrations, and 60 tables.

Drug–receptor interaction is the key to the effect and fate of a drug in the biological system. The authors exemplified the underlying mechanism of antibiotic–receptor interactions by systematically quantifying the relationship between physicochemical parameters and biological responses elicited by interactions of the drug with bioreceptors. Recent advances in research on binding sites of drug molecules to DNA and ribosomal subunits are presented.

The book offers a further insight into the mechanism of development of resistance in microorganisms and the role of plasmids in transmitting resistant genetic elements into the new cell line. Evidence has been produced demonstrating that there can be a surge of R factors in nonpathogenic enterobacteria which may be transferred to the pathogens such as *Shigellae* and *Salmonellae* due to the worldwide indiscriminate use of antibiotics. This is part of the endless race between medical science and microorganisms. The authors discussed the elimination of the genetic determinant elements from plasmids by binding DNA with a number of antibiotic as well as nonantibiotic agents. Enzyme inhibitory actions demonstrated by antibiotics and nonantibiotics suggest potential development of a bacterial enzyme inhibitor as an antimicrobial agent.

The authors suggest that when theory and knowledge of drug–receptor interactions are put into practice, a more ideal drug molecule with precise effect and anticipated mode of action may be designed with less time and expense.

Reviewed by Chian L. Huang
College of Pharmacy and Allied Health Professions
Wayne State University
Detroit, MI 48202

Medication Law and Behavior. By J. TYRONE GIBSON. Wiley, 605 Third Ave., New York, NY 10016, 1976. 407 pp. 16 × 23.5 cm. Price $15.95.

"The book is designed to help the reader learn more about the influence of medication law on the behavior of health care personnel who assist in providing medication and medication services." The author establishes this objective in the preface and in a highly readable style achieves it in the text.

Nonlawyers and those not in the health professions, as well as health professionals, can learn from reading this book. The book