USE OF THE DIRECT LINEAR PLOT TO ESTIMATE BINDING CONSTANTS FOR PROTEIN-LIGAND INTERACTIONS

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SUMMARY: The direct linear plot (Eisenthal and Cornish-Bowden[1974] Biochem. J. 139, 715-720) for the determination of enzyme kinetic constants has been assessed as a means of describing specific steroid-protein interactions. In the rat uterine cytoplasmic estrogen receptor system, determination of the equilibrium dissociation constant (K_D) and of the total number of ligand-binding sites (B_{max}) has been made, and the results are in good agreement with those obtained by Scatchard and Lineweaver-Burk plot analyses. The usefulness of the direct linear plot lies in the speed and simplicity with which it can be constructed and interpreted.

INTRODUCTION

Characterization of hormone receptor interactions with respect to the nature and amount of specific binding within a given tissue system has been the subject of intensive investigation within recent years, as an indicator of the responsiveness of the tissue to the hormone. The most striking example of the direct physiological relevance of the determination of these parameters has been the correlation between human breast tumor regression and the level of estrogen receptors within the tumor cells (1). The parameters under consideration are primarily the equilibrium constants of association (K_A) or dissociation (K_D), and the concentration of receptor binding sites (B_{max}); the equilibrium constants define the affinity of the interaction, allowing distinction between receptor-like binding and nonspecific binding, and the concentration of binding sites dictates the amount of hormone which will be able to interact with specific nuclear chromatin binding regions and trigger the events which culminate in the expression of hormonal activity.

Measurement of the physicochemical parameters of steroid hormone-receptor association with a high degree of precision is generally performed by graphical analysis of saturation binding data using either the method of Scatchard

(2) or the double reciprocal plot of Lineweaver and Burk (3). Either of these analyses requires a fair amount of calculation and some degree of expertise for interpretation. The purpose of this paper is to present the application of the direct linear plot method of Eisenthal and Cornish-Bowden (4) to analysis of estrogen-receptor interaction, and to suggest that this method may be a useful and simple alternative to the more complex procedures mentioned.

MATERIALS AND METHODS

ANALYTICAL

The hyperbolic relationship between protein-bound ligand and free ligand can be described by a form of the Michaelis-Menten equation (5), as:

$$B = [B_{max} \cdot F] / [K_D + F] \quad (1)$$

where K_D and B_{max} are the equilibrium dissociation constant for the protein-ligand complex and the total number of ligand-binding sites, respectively. A plot of bound (B) versus free (F) ligand yields a rectangular hyperbola through the origin, with asymptotes, $B = B_{max}$ and $F = -K_D$.

It is not possible to obtain accurate estimates of K_D and B_{max} from this plot; however, linear transformations of this equation have been frequently used to evaluate these constants. The Lineweaver-Burk and Scatchard transformations have been most commonly used in the study of protein-hormone interactions. These and other methods of fitting enzyme kinetic data to the Michaelis-Menten equation have been examined by Atkins and Nimmo (6) with respect to their ability to handle data containing various types of error. These investigators have concluded that, unless the error is definitely known and of constant magnitude, the direct linear plot of Eisenthal and Cornish-Bowden (4) is the best method for fitting data to this equation. Equation (1) can be rearranged to the form:

$$[B_{max}/B] - [K_D/F] = 1 \quad (2)$$

The axes B_{max} and K_D are constructed on rectangular coordinates (Figure 1). For each data point (F,B), lines are drawn between $K_D = -F$ on the K_D axis and $B_{max} = B$ on the B_{max} axis, and extended into the first quadrant. The lines

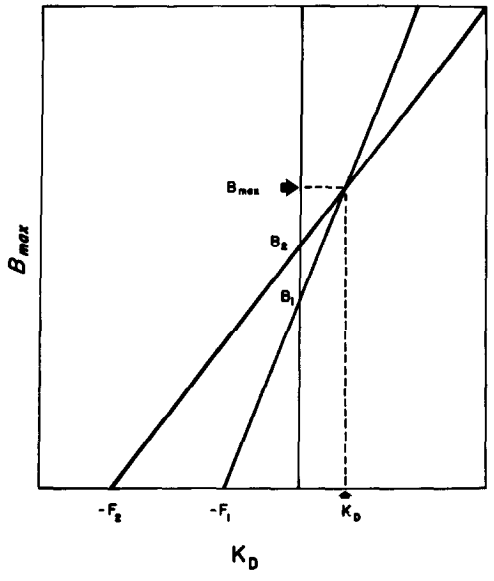

FIGURE 1. Coordinate system for construction of the direct linear plot.

intersect at a point which provides an estimate of the values of K_D and B_{max}. Equation (2) defines a straight line in $B_{max}K_D$ space. When data points are subject to error, there is no unique intersection point for all the lines. In this case, the median value in the range of each constant provides the best estimate of that constant (4,7).

The direct linear plot was applied to binding data obtained from the interaction of ^{3}H-17β-estradiol with the rat uterine cytoplasmic estrogen receptor.

EXPERIMENTAL

17β-Estradiol-2,4,6,7-^{3}H (91.3 Ci/mmol) was obtained from Amersham-Searle and brought to greater than 98% radiochemical purity by descending paper chromatography. Unlabeled 17β-estradiol was obtained from Mann Laboratories and used without additional purification.

Cytosol was prepared as the 105,000 x g supernatant fraction of homogenates of uterine tissue from adult Holtzman rats. The buffer employed was Tris-EDTA (0.01M Tris, 1.5mM Na_2EDTA, pH 8.0) and the cytosol concentration was ad-

justed to 1 mg protein per ml. Cytosol protein was determined by the method of Lowry et al. (8). To 100 µl aliquots of cytosol was added ^{3}H-17β-estradiol at concentrations varying from 5 x 10^{-11}M to 3.5 x 10^{-10}M, and these samples were incubated to equilibrium (18 hrs at 0°C). Duplicate samples contained a 100-fold molar excess of unlabeled 17β-estradiol to correct for nonspecific binding. Bound and free hormone were separated by adsorption onto dextran-coated charcoal (0.5% Norit "A", 0.05% dextran [60,000-90,000 MW]) (9) and bound hormone was quantified by liquid scintillation spectrometry (Beckman LS250, efficiency for tritium: 40%). Free hormone was determined as the difference between the bound hormone and the total hormone added. The representative data chosen for examination of the direct linear plot were obtained from experiments as described above and are shown in Table 1.

TABLE 1. Equilibrium concentration of unbound and receptor-bound 17β-estradiol in samples of rat uterine cytosol[1]

Unbound (x 10^{-10}M)	Bound (x 10^{-10}M)
3.421 ± 0.055	1.867 ± 0.054
5.382 ± 0.051	2.548 ± 0.051
7.712 ± 0.045	2.862 ± 0.045
12.522 ± 0.040	3.340 ± 0.040
15.105 ± 0.118	3.399 ± 0.118
17.631 ± 0.032	3.517 ± 0.032
22.788 ± 0.078	3.648 ± 0.078
25.414 ± 0.016	3.665 ± 0.016
27.980 ± 0.062	3.743 ± 0.063
33.122 ± 0.179	3.824 ± 0.076

[1]Each row of values corresponds to a different concentration of total ^{3}H-17β-estradiol added. The columns are arranged in order of increasing total ^{3}H-17β-estradiol. Figures represent the mean ± S.D. for triplicate determinations.

RESULTS AND DISCUSSION

The binding data in Table 1 were analyzed by the direct linear plot (Figure 2). Lines drawn between free and bound 17β-estradiol data points in-

tersect at a common point in the first quadrant. The values of 3.50×10^{-10}M for K_D and 4.20×10^{-10}M (4.20×10^{-13} moles/mg cytosol protein) for B_{max} were estimated. The same data were also evaluated by the method of Scatchard and that of Lineweaver and Burk (Figure 3). Identical values for K_D and B_{max} were obtained by these two methods. These values were in good agreement with those

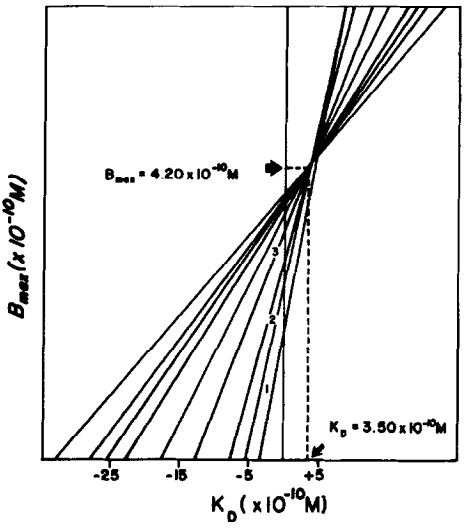

FIGURE 2. Direct linear plot of the data of Table 1. K_D and B_{max} are estimated from the common intersection point of the binding lines.

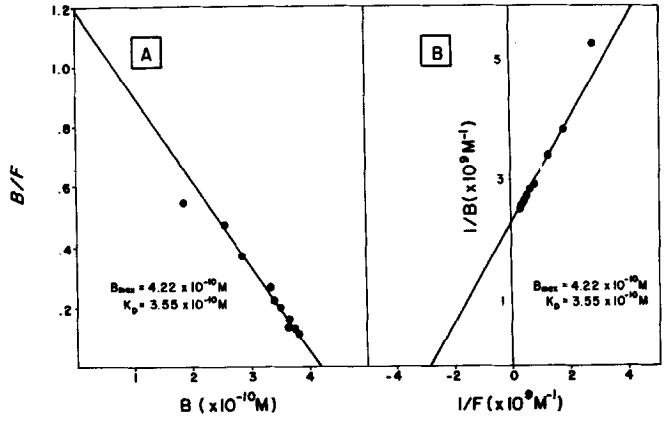

FIGURE 3. Scatchard (A) and Lineweaver-Burk (B) plots of the binding data of Table 1. K_D and B_{max} are calculated from the slopes and intercepts of the lines.

determined from the direct linear plot. A notable observation is that the same outlying data point is easily detected by all three methods of plotting the data.

Thus, the direct linear plot provides estimated of K_D and B_{max} for the interaction of 17β-estradiol with the rat uterine receptor similar to those provided by other methods (10), but offers several advantages over these other methods of analyzing binding data: the plots are simple to construct; they require no calculations; binding constants are read directly from the graph; data points which are in error are easily detected; and estimates of K_D and B_{max} can be obtained without making unwarranted assumptions concerning the magnitude and distribution of error in determining bound and free hormone (4,6).

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