THE QUANTITATIVE INTERPRETATION OF MAXIMUM IN SCATCHARD PLOTS

F. W. DAHLQUIST
Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, Oregon 97403, USA

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1. Introduction

A number of diagnostic tests of co-operative behavior in ligand binding to proteins has been developed [1]. These include the slope of the Hill plot [2]; curvature of the double reciprocal plot; curvature of the Scatchard plot [3]; the ratio of ligand concentration necessary to produce 10% and 90% saturation [4]; etc. In each case, the qualitative conclusion that co-operativity does or does not exist is rather easily drawn. However, a more quantitative conclusion is not easily reached. Since all methods of data treatment consider the fraction of sites bound, $\bar{y}$, versus a function of free ligand concentration, x, it seems reasonable that the various parameters of co-operative behavior should be related in a relatively simple fashion.

2. Discussion

The presence of a maximum in a Scatchard plot, $\bar{y}/x$ versus $\bar{y}$, has been recognized by several workers [5] as an indicator of positive co-operativity in ligand binding. The position of the maximum has been qualitatively discussed in terms of the fraction of the total number of sites participating in the co-operative phenomenon [6,7]. The exact nature of the maximum can be determined relatively simply by considering the derivative $d(\bar{y}/x)/d\bar{y}$.

In order for a maximum in the Scatchard plot to occur, this derivative must be zero.

$$d(\bar{y}/x) \ / \ d\bar{y} = \frac{1}{x}\left(1 - \frac{\bar{y}}{x}\frac{dx}{d\bar{y}}\right) = 0 \tag{1}$$

The meaningful condition for a maximum is therefore

$$\frac{x_m}{\bar{y}_m}\left(\frac{d\bar{y}}{dx}\right)_m = 1 \tag{2}$$

where the subscripts refer the values of these parameters at the maximum of the Scatchard plot. Eqn. 2 does not provide any immediate insights into the nature of the maximum. However, comparison of this equation with the expression for the Hill coefficient, n, helps to provide such insights. The Hill coefficient is defined as the slope of the Hill plot, $\log \frac{\bar{y}}{1-\bar{y}}$ versus $\log x$.

$$n = \frac{d \ln\left(\frac{\bar{y}}{1-\bar{y}}\right)}{d \ln x} = \left(\frac{1}{\bar{y}}\right)\left(\frac{1}{1-\bar{y}}\right)\frac{d\bar{y}}{d \ln x} \tag{3}$$

Combining Eqns. 2 and 3 gives a relationship between the Hill coefficient and the value of y at the maximum of the Scatchard plot

$$n = \frac{1}{1-\bar{y}_m} \tag{4}$$

Formally the value of n in Eqn. 4 is evaluated at the $\bar{y}_m$ and x_m. In practice, the majority of Hill plots is remarkably linear in the range between 10% and 90% saturation. Thus n is essentially constant over this range. For most co-operative interactions, the value of $\bar{y}_m$ is also in the range where n is essentially constant. For example, a Hill coefficient of 1.5 would generate a maximum in the Scatchard plot of the

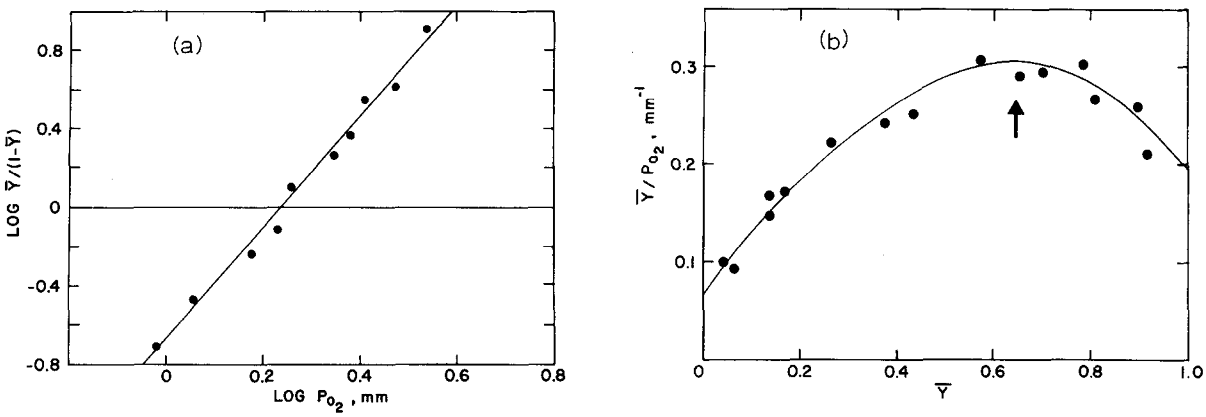

Fig. 1. a) A Hill plot of the data of Ogata and McConnell (1971) for the binding of oxygen to hemoglobin. b) A Scatchard plot of the same data. The arrow represents the position of the maximum predicted by Eqn. 4.

same data at $\bar{y} = 0.33$. A value of n equal to 4.0 would result in a Scatchard plot maximum at $\bar{y} = 0.75$.

Fig. 1a shows a Hill plot generated from the data of Ogata and McConnell [8] for the binding of oxygen to hemoglobin which has been stripped of diphosphoglycerate. The line drawn through the data points corresponds to a Hill coefficient of 2.8. Fig. 1b shows the Scatchard plot generated from the same data. There is somewhat more scatter in the data when presented in this fashion; however, a maximum is clearly present in the region of $\bar{y}$ values between 0.6 and 0.8. The arrow shown in the figure corresponds to the theoretical position of the maximum using Eqn. 4. The theoretical value is certainly consistent with the observed value of the maximum. Since the maximum is defined by only one or two data points, while the Hill coefficient is obtained using essentially all the data points, one would expect somewhat less precision in determination of the position of the Scatchard plot maximum than of the Hill coefficient. Since both plots contain similar information, the Hill plot is probably to be preferred, in most cases.

3. Conclusion

It should be noted that Eqn. 4 makes *no* assumptions as to models of co-operative binding. Since $\bar{y}$ can only vary from zero to unity, it follows that extrema in the Scatchard plot can only occur when n is greater than one. Thus a maximum implies positive co-operativity in the binding.

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