A NEW PLOT FOR ALLOSTERIC PHENOMENA

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SUMMARY: A new method to plot for allosteric phenomena is demonstrated. Hill-plot is used widely for energetic expressions of cooperativity of hemoglobin and allosteric enzymes. As the ordinate of this plot is ln [Y/(1-Y)], physical meaning of this ordinate is obscure. However if ln [Y/(1-Y)] - ln F is taken as ordinate, the ordinate is directly proportional to association energy of ligand. Using this new plot, Hill constant can be easily determined graphically with high accuracy.

Hill applied the multiple-ligand equation for the analysis of equilibrium between oxygen and hemoglobin (1). This equation has been known as the Hill-equation and the Hill constant $\underline{n}$ in this equation has been employed as one of the characteristic constants expressing cooperativity. It is easy to determine the Hill constant $\underline{n}$ from the inclination of the plot of ln [Y/(1-Y)] versus ln F, where Y is the fractional saturation of hemoglobin with oxygen, and F is ligand concentration. This plot has been called as Hill plot.

Wyman demonstrated that in the case of hemoglobin two of four Adair's constants, k_1 and k_4, could be calculated if Hill plot was used, and proposed that at low and high oxygen concentration the degree of saturation of hemoglobin must be measured in order to have accurate values of k_4 (2). One of the authors developed the improved method for automatic measurement of the oxygen equilibrium curve of hemoglobin (3). With this method, it became possible to measure accurately the degree of saturation of hemoglobin at low and high oxygen concentration, and then Adair's constants were calculated precisely (4).

Monod, Wyman and Changeux elaborated allosteric theory which explained well not only the conformational change of hemoglobin molecule by oxy- and deoxygenation, but also reaction of many allosteric enzymes (5). Even for the superiority of allosteric theory to Hill equation as the analyzing method, Hill plot is still used widely for energetic expressions of cooperativity of hemoglobin (6), because Hill plot can express some energetic aspect of the reaction.

However, one of disadvantages of Hill plot is that ln [Y/(1-Y)] does not correspond to the free energy change of the reaction, except only at ln F = 0, because Y/(1-Y) is a ratio of fraction of liganded molecules to unliganded one. From practical point of view, accuracy of the estimation of maximum inclination, which is Hill constant n, decreases as n increases.

The aim of this paper is to report a new graphical method to represent the association energy against ligand concentration. With this method, it becomes easy to calculate allosteric constants, and also Hill constant can be estimated more accurately.

According to the allosteric theory (2), the saturation function Y is expressed as follows:

$$Y = \alpha \frac{Lc(1 + c\alpha)^{n-1} + (1 + \alpha)^{n-1}}{L(1 + c\alpha)^{n} + (1 + \alpha)^{n}}, \qquad (1)$$

where $\alpha = F/K_R$, $L = T_0/R_0$ and $c = K_R/K_T$. K_R and K_T are microscopic dissociation constants of a ligand F bound to a stereospecific site, and n is the maximum number of sites of ligand. ln [Y/(1-Y)] is derived from equation (1), and is

$$\ln \frac{Y}{1-Y} = \ln F - \ln K_R + \ln \frac{Lc(1 + c\alpha)^{n-1} + (1 + \alpha)^{n-1}}{L(1 + c\alpha)^{n-1} + (1 + \alpha)^{n-1}}. \qquad (2)$$

It is clear that the inclination can never be less than one from equation (2). Even in allosteric theory, Hill plot is effective for representing energetic property of the reaction, but the disadvantages pointed earlier still remain. To eliminate these disadvantages, following transformations were performed. Apparent association constant of the reaction is equal to Y/[(1-Y)/F], because

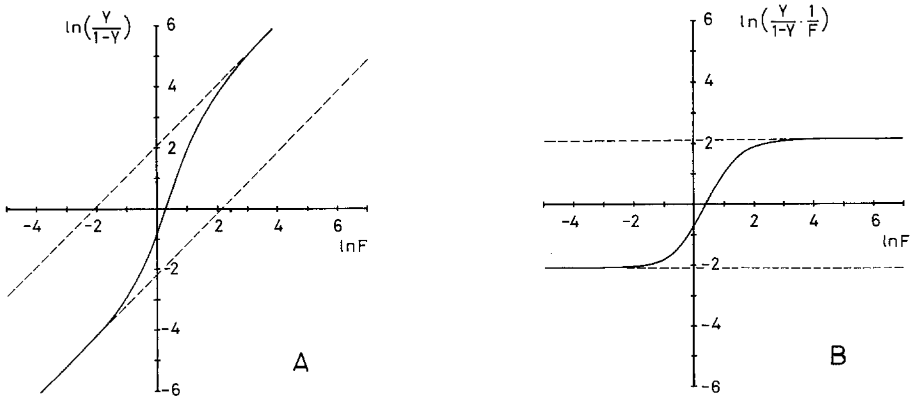

Fig. 1 A is Hill plot and B is the new plot (ln Y/[(1-Y)F] versus ln F). These curves are plotted by YHP personal computer model 20 with graphic display using constants as follows: K_R = 0.1124 mmHg, K_T = 8.065 mmHg, L = 15000 and N = 4.

Y and 1-Y are propotional to the concentration of liganded and unliganded protein, respectively. In order to correlate ln (Y/[(1-Y)F]) with ln F, equation (2) must be transformed to the following equation:

$$\ln\left(\frac{Y}{1-Y}\cdot\frac{1}{F}\right) = -\ln K_R K_T + \ln\frac{LK_R(1+c\alpha)^{n-1} + K_T(1+\alpha)^{n-1}}{L(1+c\alpha)^{n-1} + (1+\alpha)^{n-1}} . \qquad (3)$$

When ln F decrease to minus infinity, equation (3) will be written as follows:

$$\lim_{\ln F\to -\infty} \ln\left(\frac{Y}{1-Y}\cdot\frac{1}{F}\right) = -\ln K_R K_T + \ln\frac{LK_R + K_T}{L+1} \qquad (4)$$

Also in the case of ln F→ ∞, equation (3) will be

$$\lim_{\ln F\to \infty} \ln\left(\frac{Y}{1-Y}\cdot\frac{1}{F}\right) = -\ln K_R K_T + \ln\frac{LK_R c^{n-1} + K_T}{Lc^{n-1} + 1} . \qquad (5)$$

Equation (4) and (5) are two asymptotes of equation (3), and are independent of ln F, that is, pararell to abscissa. It can be seen that the value of ordinate is directly proportional to free energy change of ligand binding reaction, and the maximum inclination is less by one compared to that of Hill plot.

Hill plot and new plot are shown in Fig. 1 A and B for comparison, and the allosteric constants are taken from reference of Imai et al. (7). In Fig. 1 B, apparent association free energy is shown clearly with function of ln F. By using two asymptotes, L and K_R can be easily estimated if n is known and c is zero. The use of least square method with a computer enables one to estimate the allosteric constants accurately.

In this new plot, inclination of Hill plot, $\Delta \ln [Y/(1-Y)] / \Delta \ln F$, is transformed to $\Delta \ln \{Y/[(1-Y)F]\} / \Delta \ln F$. Accordingly, the inclination is reduced by one but Hill constant can be estimated easily with high accuracy.

REFERENCES

1. Hill, A. V. (1910) J. Physiol. (London) 40, iv-vii.
2. Wyman, J. (1964) Adv. Protein Chem. 19, 223-286.
3. Imai, K., Morimoto, H., Kotani, M., Watari, H., Hirata, W., and Kuroda, M., (1970) Biochim. Biophys. Acta 200, 189-196.
4. Tyuma, I., and Imai, K. (1971) Biochem. Biophys. Res. Commun. 43, 423-428.
5. Monod, J., Wyman, J., and Changeux, J. P. (1965) 12, 88-118.
6. Imai, K. (1973) Biochemistry 12, 798-808.
7. Imai, K., and Yonetani, T. (1975) J. Biol. Chem. 250, 2227-2231.