# An analytic solution to the Monod-Wyman-Changeux model and all parameters in this model

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ABSTRACT Starting from the Monod-Wyman-Changeux (MWC) model (Monod, J., J. Wyman, and J. P. Changeux. 1965. *J. Mol. Biol.* 12:88-118), we obtain an analytical expression for the slope of the Hill plot at any ligand concentration. Furthermore, we derive an equation satisfied by the ligand concentration at the position of maximum slope. From these results, we derive a set of formulas which allow determination of the parameters of the MWC model ($k_R$, $c$, and $L$) from the value of the Hill coefficient, $n_H$, the ligand concentration at the position of maximum slope ($[A]_0$), and the value of $\bar{\nu}/(n-\bar{\nu})$ at this point.

We then outline procedures for utilizing these equations to provide a "best fit" of the MWC model to the experimental data, and to obtain a refined set of the parameters.

Finally, we demonstrate the applicability of the technique by analysis of oxygen binding data for *Octopus* hemocyanin.

## INTRODUCTION

A simple, yet versatile model for the cooperative binding of ligands by macromolecules has been provided by Monod et al. (1965). According to this Monod-Wyman-Changeux (MWC) model, the average number of ligands bound, at free ligand concentration [A], to an $n$-site molecule is given by

$$\bar{\nu} = nk_R[A]\,\frac{(1 + k_R[A])^{n-1} + Lc\,(1 + ck_R[A])^{n-1}}{(1 + k_R[A])^n + L\,(1 + ck_R[A])^n} \qquad (1a)$$

$$c = k_T/k_R \qquad (1b)$$

$$L = [T]/[R]. \qquad (1c)$$

Here $k_R$ and $k_T$ are respectively the binding constants in the "strong-binding" (R) state and "weak-binding" (T) states, and [R] and [T] are the concentrations of unliganded macromolecules in those two states. Thus, the MWC model involves three adjustable parameters, which may be taken to be either $k_R$, $c$, and $L$, or $k_R$, $k_T$, and $L$.

In attempting to fit experimental data to this model, two approaches are obvious. First, one may use a nonlinear least squares fitting routine, and attempt to find the best values of $k_R$, $c$, and $L$ to fit the data. This approach has been employed, for example, by Miller (1985). Satisfactory fits can be obtained, but the method is laborious, especially if preliminary estimates of the parameters are not available. An alternative precedure would be to obtain analytic expressions for measurable quantities in the Hill plot (or some other graphical representation) that would enable direct computation of the parameters. An approach of this kind has been utilized by Dahlquist (1978) for the much simpler variant of the MWC model in which binding occurs only to the R state, the "R-exclusive" binding model. This corresponds to the special case where $c = 0$ in Eq. 1. In Dahlquist's analysis, the slope of the Hill plot ($n_h$) was evaluated at the half-saturation point. This value, together with the concentration of ligand at half-saturation, allowed both $L$ and $k_R$ to be evaluated from simple expressions. However, as is shown by Colosimo et al. (1974), the more general model described by Eq. 1 can easily yield Hill plots in which the slope at the half-saturation point is unity, even though the maximum Hill coefficient ($n_H$) is much greater. Therefore it is apparent that the slope at half-saturation is not a useful parameter in the more general case.

The "R-exclusive" model was also examined by Kegeles (1974) who obtained an expression for the slope at any value of [A], and an equation for the [A] value at the position of maximum slope. However, most cooperative binding systems that have been examined exhibit significant binding to both R and T states, so that the utility of the "R-exclusive" model is limited.

Accordingly, we have carried out an analysis of the general MWC model (Eq. 1) to yield analytical expressions for the Hill slope, $n_h$, and the ligand concentration $[A]_0$ at the point of maximum slope ($n_H$). We show that these can be combined with the value of $[\bar{\nu}/(n-\bar{\nu})]$ at the point of maximum slope to yield equations which allow an analytical determination of $L$, $c$, and $k_R$.

Since these values are dependent upon a single point in the Hill plot, their determination does not, in itself, provide a test as to whether the model fits the data. Therefore, we utilize these values ($n_H$, $[A]_0$, and $[\bar{\nu}/(n-\bar{\nu})]_0$ as initial parameters in a nonlinear least squares

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fitting of the data, to arrive at a "best set" of $L$, $c$, and $k_R$. Such a directed fitting procedure can be much more efficient than one which initially chooses parameter values at random. To demonstrate the utility of this procedure, we have reanalyzed oxygen binding data for *Octopus* hemocyanin.

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## RESULTS

### An expression for the maximum Hill coefficient

Eq. 1, when rearranged in terms appropriate for the Hill equation, becomes

$$\frac{\bar{\nu}}{n-\bar{\nu}} = k_R[A]\left\{\frac{1 + Lc[(1+ck_R[A])/(1+k_R[A])]^{n-1}}{1 + L[(1+ck_R[A])/(1+k_R[A])]^{n-1}}\right\}. \tag{2}$$

In the Hill plot, log $[\bar{\nu}/(n-\bar{\nu})]$ is graphed vs. log [A]. The slope of the Hill plot (defined as $n_h$), at any value of [A], is found to be

$$\begin{aligned} n_h &= \mathrm{dlog}\,\{\bar{\nu}/(n-\bar{\nu})\}/\mathrm{dlog}[A] \\ &= 1 + \frac{Lc(n-1)(c-1)k_R[A](1+ck_R[A])^{n-2}}{\{1 + Lc[(1+ck_R[A])/(1+k_R[A])]^{n-1}\}(1+k_R[A])^n} \\ &\quad - \frac{L(n-1)(c-1)k_R[A](1+ck_R[A])^{n-2}}{\{1 + L[(1+ck_R[A])/(1+k_R[A])]^{n-1}\}(1+k_R[A])^n}. \end{aligned} \tag{3}$$

When $n = 1$, or $c = 1$, or in the limits $[A] \rightarrow 0$ or $[A] \rightarrow \infty$, we find that $n_h = 1$, as expected. Furthermore, when $c = 0$, the expression for $n_h$ reduces to that obtained by Kegeles (1974) for the case in which binding is only to the R state. (Note that the L used by Kegeles is the reciprocal of the value as defined here.)

To obtain the maximum value of the slope of the Hill plot, which has been defined as the Hill coefficient $n_H$, it is necessary to differentiate Eq. 3 and set $dn_h/\mathrm{dlog}[A]$ equal to zero. By doing so, we find the following result for the value of [A] (which we call $[A]_0$) at the maximum slope of the Hill plot.

$$\begin{aligned} &-\frac{Lc^2(n-1)(c-1)k_R[A]}{\{1 + Lc[(1+ck_R[A])/(1+k_R[A])]^{n-1}\}^2}\,\frac{(1+ck_R[A])^{n-1}}{(1+k_R[A])^{n-1}} \\ &+\frac{c[1+(n-1)ck_R[A]-(n-1)k_R[A]-ck_R^2[A]^2]}{1 + Lc[(1+ck_R[A])/(1+k_R[A])]^{n-1}} \\ =&-\frac{L(n-1)(c-1)k_R[A]}{\{1 + L[(1+ck_R[A])/(1+k_R[A])]^{n-1}\}^2}\,\frac{(1+ck_R[A])^{n-1}}{(1+k_R[A])^{n-1}} \\ &+\frac{\{1+(n-1)ck_R[A]-(n-1)k_R[A]-ck_R^2[A]^2\}}{1 + L[(1+ck_R[A])/(1+k_R[A])]^{n-1}}. \end{aligned} \tag{4}$$

Again, if we set $c = 0$, this reduces to the equivalent of Kegeles (1974) Eq. 3.

### Determination of the parameters of the model

Eqs. 2, 3, and 4, evaluated at the point $[A]_0$, where $n_h = n_H$, and $\bar{\nu}/(n-\bar{\nu}) = [\bar{\nu}/(n-\bar{\nu})]_0$, provide three independent equations which can be solved to yield the three parameters $L$, $c$, and $k_R$. The solution of Eqs. 2–4 seems very complicated at first sight. For simplification, we first set

$$Y = \{(1 + ck_R[A]_0)/(1 + k_R[A]_0)\}^{n-1}. \tag{5}$$

Substituting this into Eq. 2 and rewriting, we get

$$1/(1 + LY) = W/[(1 + LcY)\,k_R], \tag{6}$$

in which

$$W = [\bar{\nu}/(n-\bar{\nu})]_0/[A]_0. \tag{7}$$

Eqs. 3 and 4 now become

$$\frac{\{L(n-1)(c-1)\,[A]_0(k_Rc - W)Y\}}{\{(1 + LcY)(1 + ck_R[A]_0)(1 + k_R[A]_0)\}} = Z, \tag{8}$$

in which $Z = n_H - 1$, and

$$\begin{aligned} &L(n-1)(c-1)k_R[A]_0Y\left\{\frac{-c^2}{(1+LcY)^2} + \frac{1}{(1+LY)^2}\right\} \\ &= \{1 + (n-1)ck_R[A]_0 - (n-1)k_R[A]_0 - ck_R^2\,[A]_0^2\} \\ &\qquad\left\{\frac{-c}{(1+LcY)} + \frac{1}{(1+LY)}\right\} \end{aligned} \tag{9}$$

This way, we get four equations with four unknowns, Eqs. 5, 6, 8, and 9. Using Eqs. 5 and 6, 6 and 8, 6 and 9, respectively, we can eliminate the variable $Y$ to obtain

$$\left\{\frac{1 + ck_R[A]_0}{1 + k_R[A]_0}\right\}^{n-1} = \frac{k_R - W}{L(W - k_Rc)} \tag{10}$$

$$\begin{aligned} &(n-1)(W - k_Rc)\,(k_R - W)[A]_0 \\ &\qquad = ZW(1 + ck_R[A]_0)(1 + k_R\,[A]_0) \end{aligned} \tag{11}$$

$$ck_R^2\{(n-1)[A]_0 - W[A]_0^2\} = (n-1)W^2[A]_0 - W. \tag{12}$$

We rewrite Eq. 12 as

$$c = X/k_R^2, \tag{13}$$

in which

$$X = \{(n-1)W^2[A]_0 - W\}/\{(n-1)[A]_0 - W[A]_0^2\}. \tag{14}$$

Substituting Eq. 13 into 11, we find

$$\begin{aligned} &(W - Q[A]_0)k_R^2 - (X + W^2 + QX[A]_0^2 + Q)k_R \\ &\qquad + X(W - Q[A]_0) = 0, \end{aligned} \tag{15}$$

in which

$$Q = ZW/\{(n-1)[\mathrm{A}]_0\}. \tag{16}$$

Eq. 15 is a simple quadratic equation. The solutions are

$$k_{\mathrm{R}1,2} = \frac{(X + W^2 + QX[\mathrm{A}]_0^2 + Q)}{2(W - Q[\mathrm{A}]_0)} \pm \frac{\{(X + W^2 + QX[\mathrm{A}]_0^2 + Q)^2 - 4X(W - Q[\mathrm{A}_0]^2\}^{1/2}}{2(W - Q[\mathrm{A}]_0)}. \tag{17}$$

All quantities in Eq. 17 are calculable from the experimental data, since, as defined above,

$$W = [\bar{\nu}/(n - \bar{\nu})]_0/[\mathrm{A}]_0$$

$$Z = n_\mathrm{H} - 1$$

$$X = \{(n-1)W^2[\mathrm{A}]_0 - W\}/\{(n-1)[\mathrm{A}]_0 - W[\mathrm{A}]_0^2\}$$

$$Q = ZW/(n-1)[\mathrm{A}]_0.$$

The values $k_{\mathrm{R}1}$ and $k_{\mathrm{R}2}$ correspond to the + and − solution of Eq. 17, respectively.

If we substitute Eq. 17 into 13 and 10, we find, respectively,

$$c_{1,2} = \{(n-1)W^2[\mathrm{A}]_0 - W\}/\{\{(n-1)[\mathrm{A}]_0 - W[\mathrm{A}]_0^2\}k_{\mathrm{R}1,2}^2\} \tag{18}$$

and

$$L_{1,2} = \{(k_{\mathrm{R}1,2} - W)/(W - k_{\mathrm{R}1,2}c_{1,2})\}\{(1 + k_{\mathrm{R}1,2}[\mathrm{A}]_0)/(1 + c_{1,2}\, k_{\mathrm{R}1,2}[\mathrm{A}]_0)\}^{n-1}. \tag{19}$$

Thus, we have obtained analytic solutions for the three parameters. We can show that

$$c_1 = k_{\mathrm{R}1}/k_{\mathrm{R}2};\ c_2 = 1/c_1;\ L_2 = 1/L_1.$$

**TABLE 1 The parameters obtained by using the equations derived in this paper and nonlinear least-square fitting, in which the meaning of $k_\mathrm{R}$, $k_\mathrm{T}$, $c$, and $L$ is the same as the definition in the MWC model and $\delta$ is the value of $\delta_{\min}$**

| | pH | $k_\mathrm{R}$ | $k_\mathrm{T}$ | $c$ | $L$ | $\delta$ |
|---|---|---|---|---|---|---|
| A | 8.00 | 0.93578 | 2.7369E−1 | 0.29247 | 5.6826E+2 | 7.77528E−3 |
| | 7.70 | 0.41150 | 7.7815E−2 | 0.18910 | 1.4360E+2 | 1.08586E−2 |
| | 7.66 | 0.37599 | 5.8628E−2 | 0.15593 | 2.6639E+3 | 9.98751E−3 |
| | 7.50 | 0.24616 | 2.1396E−2 | 0.08692 | 1.2685E+4 | 6.36919E−3 |
| | 7.20 | 0.24011 | 6.2211E−3 | 0.02591 | 8.9518E+6 | 1.59992E−2 |
| | 7.07 | 0.07436 | 3.5164E−3 | 0.04729 | 2.6522E+5 | 1.51871E−2 |
| | 7.00 | 0.14555 | 3.9373E−3 | 0.02705 | 1.6826E+8 | 5.22286E−3 |
| | 6.78 | 0.53928 | 3.7699E−3 | 0.00697 | 2.0726E14 | 5.93500E−3 |
| B | 8.00 | 0.96513 | 2.7649E−1 | 0.28648 | 7.0896E+2 | 7.74475E−3 |
| | 7.70 | 0.41086 | 7.7439E−2 | 0.18848 | 1.4230E+2 | 1.08569E−2 |
| | 7.66 | 0.37037 | 5.7847E−2 | 0.15619 | 2.4365E+3 | 9.96779E−3 |
| | 7.50 | 0.24607 | 2.1397E−2 | 0.08695 | 1.2643E+4 | 6.36906E−3 |
| | 7.20 | 0.24098 | 6.2317E−3 | 0.02586 | 9.1670E+6 | 1.59986E−2 |
| | 7.07 | 0.07563 | 3.5278E−3 | 0.04665 | 2.9546E+5 | 1.51826E−2 |
| | 7.00 | 0.15535 | 3.9517E−3 | 0.02544 | 2.6279E+8 | 5.21028E−3 |
| C | 8.00 | 0.97062 | 0.27702 | 0.28541 | 7.3761E+2 | 7.74391E−3 |
| | 7.70 | 0.41075 | 0.07739 | 0.18840 | 1.4209E+2 | 1.08569E−2 |
| | 7.66 | 0.37028 | 0.05783 | 0.15617 | 2.4338E+3 | 9.96778E−3 |
| | 7.50 | 0.24607 | 0.02140 | 0.08695 | 1.2643E+4 | 6.36906E−3 |
| | 7.20 | 0.24098 | 0.00623 | 0.02586 | 9.1669E+6 | 1.59986E−2 |
| | 7.07 | 0.07563 | 0.00353 | 0.04665 | 2.9556E+5 | 1.51826E−2 |
| | 7.00 | 0.15535 | 0.00395 | 0.02544 | 2.6268E+8 | 5.21028E−3 |
| | 6.78 | 0.54250 | 0.00376 | 0.00693 | 2.1615E14 | 5.93439E−3 |
| D | 8.00 | 0.9727 | 0.2767 | 0.2845 | 7.580E+2 | 7.74745E−3 |
| | 7.70 | 0.4102 | 0.0774 | 0.1887 | 1.410E+2 | 1.08579E−2 |
| | 7.66 | 0.3690 | 0.0577 | 0.1563 | 2.398E+3 | 9.97073E−3 |
| | 7.50 | 0.2588 | 0.0216 | 0.0836 | 1.698E+4 | 7.52374E−3 |
| | 7.20 | 0.2443 | 0.0063 | 0.0257 | 1.000E+7 | 1.60072E−2 |
| | 7.07 | 0.0942 | 0.0036 | 0.0386 | 1.230E+6 | 1.57487E−2 |
| | 7.00 | 0.1574 | 0.0040 | 0.0251 | 2.884E+8 | 1.82506E−1 |
| | 6.78 | 15.3800 | 0.0031 | 0.0002 | 3.160E24 | 2.88337E−2 |

(A) The parameters obtained from the first iterative cycle, in which the differences among three minimum $\delta$ are $<10^{-8}$. (B) The parameters obtained from the second iterative cycle, in which the differences among three minimum $\delta$ are $<10^{-10}$. (C) The parameters obtained from the final cycle in which the parameters are changed directly. (D) The parameters obtained from the previous paper by Miller (1985).

This simply means that mathematically the T and R states in the MWC model are indistinguishable. By convention, it is usually assumed that $c = k_T/k_R < 1$. Thus, after obtaining parameters, we choose only the set corresponding to $c < 1$. If, for example, $c_1 > 1$, then $k_R = k_{R1}$, $L = L_1$, and $k_T$ is defined as $k_T = k_{R2}$.

## Analysis of data

To deduce the MWC parameters from an experimental ligand binding curve, using the above equations, two approaches can be taken. The first is to evaluate the numerical derivative of the Hill plot as a function of log [A], and find its maximum value and the value of [A] and $\bar{\nu}/(n - \bar{\nu})$ at which this occurs. Eqs. 17–19 can then be used directly.

However, a better procedure, which makes full use of the experimental data, and also provides a test of the appropriateness of the MWC model, is the following.

First, use numerical drift to find approximate values for $n_H$, $[A]_0$, and $(\bar{\nu}/(n - \bar{\nu}))_0$. Then, varying these parameters independently over a narrow range, calculate the corresponding values for the MWC parameters from Eqs. 17–19, use these to compute binding curves, and seek a best fit.

We have applied this method to published data on oxygen binding by *Octopus dofleini* hemocyanin at 20°C (Miller, 1985). In that study, oxygen binding curves were determined at a number of pH values, under conditions in which the hemocyanin retained its native structure.[1] The measure of "goodness of fit" employed was

$$\delta = (\Sigma\{\log[\bar{\nu}/(n - \bar{\nu})]_{\text{exp}} - \log[\bar{\nu}/(n - \bar{\nu})]_{\text{theo}}\}^2)^{1/2}/N, \qquad (20)$$

where $N$ is the number of experimental points.

Analysis proceeded as follows: First we used a 9-points linear least-square fitting to get the approximate slope of the Hill plot so that we could determine the appropriate range of values of $[A]_0$, $n_H$, and $[\bar{\nu}/(n - \bar{\nu})]_0$. We then fixed $[A]_0$ and $n_H$, and allowed $[\bar{\nu}/(n - \bar{\nu})]_0$ to change, calculating $L$, $c$, and $k_R$ at each value. From these we calculated $\log[\bar{\nu}/(n - \bar{\nu})]$ corresponding to each point, and by comparison with the observed values, $\delta$ was obtained. This was continued until $\delta$ reached a minimum. Then, using this value of $[\bar{\nu}/(n - \bar{\nu})]_0$, we fixed it and $n_H$, and changed $[A]_0$ until $\delta$ was again minimized. Finally, $n_H$ was varied. This cycle was repeated until the difference among the three $\delta_{\min}$ values were $<10^{-8}$. The cycle was then repeated until the difference among the three $\delta_{\min}$ values was $<10^{-10}$. At this point, we expected the parameters to have five significant figures, so $k_R$, $c$, and $L$ were changed independently at the fifth figure until a minimum value of $\delta$ was found. The parameters obtained at each step are shown in Table 1A–C. The parameters obtained by a previous nonlinear least squares fitting (Miller, 1985) are shown in Table 1D. The parameters obtained by the new method yield curves in excellent agreement with the experimental data (Fig. 1), and give a somewhat better fit (according to the $\delta$ values) than the previous analysis.

There is one other check which should be made when one is analyzing data from a multi-site binding protein like hemocyanin. Since the subunit of *Octopus* hemocyanin contains seven oxygen binding sites, we expected the allosteric domain to be of this size, and hence used $n = 7$ in our analyses. Once approximate values for $k_R$ and $c$

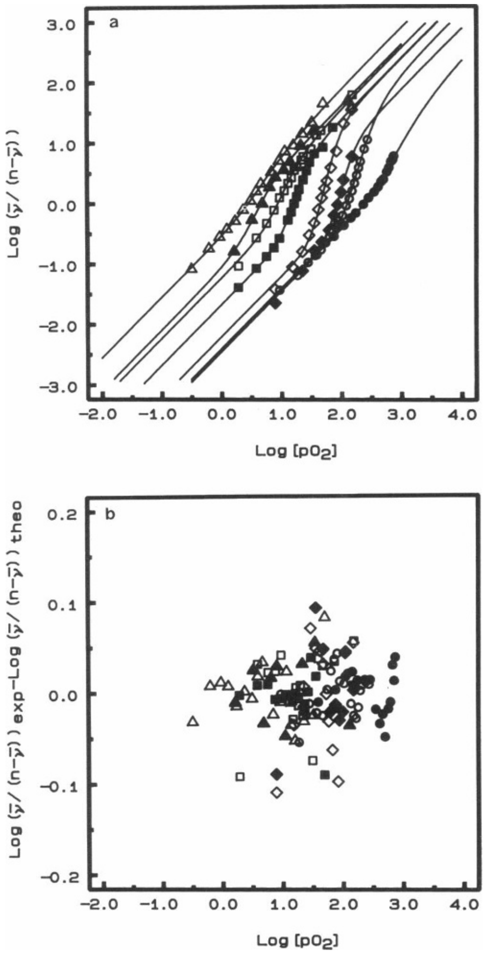

FIGURE 1 (*a*) Hill plots of oxygenation of hemocyanin at several pH values. The points are experimental, the curves are from the best fit (see Table 1C). All data obtained at 20°C, in 0.1 M ionic strength Tris buffer containing 370 mM NaCl, 45 mM $MgCl_2$, 10 mM $K_2SO_4$, and 8 mM $CaCl_2$. pH values are (from left to right) 8.00, 7.70, 7.66, 7.50, 7.20, 7.07, 7.00, 6.78. (*b*) A plot of the difference between experimental value of $\log[\bar{\nu}/(n - \bar{\nu})]$ and the theoretical value at different log [A] values for various pH conditions. Δ, pH 8.00; ▲, 7.00; □, 7.66; ■, 7.50; ◇, 7.20; ◆, 7.07; ○, 7.00; ●, 6.78.

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[1] It should be noted that in the Miller (1985) paper, the Hill plots of the binding data were accidentally printed upside down.

have been obtained, the method of Decker et al. (1983) can be employed to test the value of $n$. According to this method, a linear graph of slope $(n - 1)$ should be obtained when log $(1 - s/\alpha)/(S/\alpha - c)$ is plotted versus log $[(1 + c\alpha)/(1 + \alpha)]$, where $S = \theta/(1 - \theta)$ and $\alpha = k_R[A]$. Here $\theta$ is the fraction saturation, and for $O_2$ binding $k_R[A] = P_{O_2}/P_{50,R}$. Such graphs are shown in Fig. 2. Their linearity reaffirms the applicability of the MWC model, and the slopes range from 5.3 to 5.9, corresponding to $n = 6.3$–$6.9$, in good accord with expectation.

It should be noted that this procedure is, to a certain degree circular, since the value of $n$ was first assumed (on a structural basis) to be 7, and the parameters calculated from this then used in constructing the graphs in Fig. 2. However, the fact that data sets taken over a wide pH range give in each case a straight line with slope consistent with $n = 7$ we take to be strong evidence that the assumption is correct. Such problems will not be encountered with most smaller allosteric proteins, where $n$ is usually identifiable with the number of binding sites. It may become a serious problem in cases like those found in some arthropod hemocyanins, where the apparent value of $n$ is not only smaller than the number of subunits, but seemingly changes with conditions. In such cases we would expect that $n$ would have to be systematically varied in order to obtain good fits to the data, or a more general nonlinear fitting scheme, with $n$ a floating parameter, would have to be employed.

## DISCUSSION

This paper provides what is, to our knowledge, the first analytic solution for the parameters of the general MWC

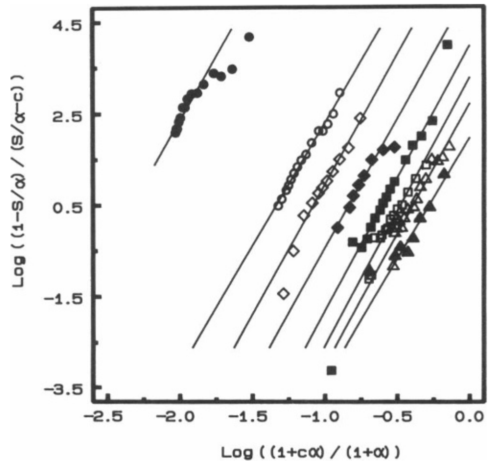

FIGURE 2 Data plotted according to the methods of Decker et al. (1983). Symbols are as in Fig. 1 *b*.

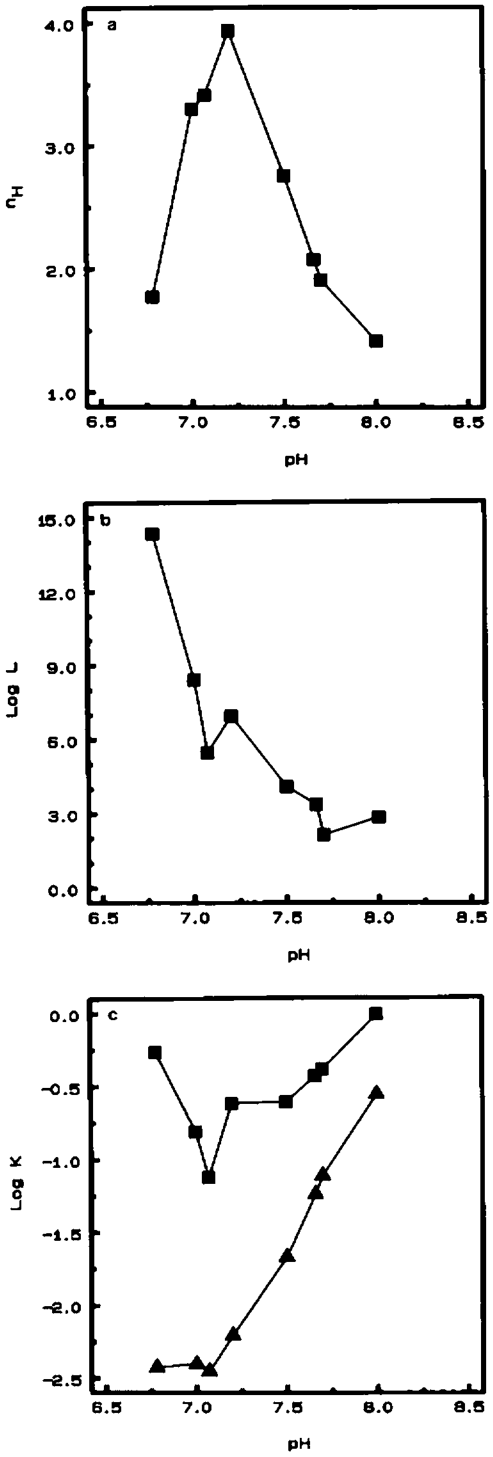

FIGURE 3 (*a*) The maximum Hill slope as a function of pH; (*b*) Logarithm of $L$ as a function of pH; (*c*) Logarithm of $k_R$ (■) and $k_T$ (▲) as a function of pH.

model. Furthermore, it outlines methods for the practical use of these equations to deduce these parameters.

One way to obtain the parameters is to get accurate numerical derivatives of the experimental data, find the values of $n_H$, $[A]_0$ and $[\bar{\nu}/(n - \bar{\nu})]_0$ and then substitute these into Eqs. 17–19. An alternative approach is to use these equations followed by nonlinear least-square fitting to get the best fit to the curve. We think the latter method is better. One reason is that the exact numerical derivative is not easy to obtain from experimental data. Furthermore, the latter procedure utilizes all of the experimental data. A remaining problem with this latter approach is to decide which quantity is the best criterion for goodness-of-fit. In our study, we utilized a simple parameter, considering only the error in $[\bar{\nu}/(n - \bar{\nu})]$ and used the difference between the experimental data and corresponding theoretical value. Presumably, if we utilized the quantity described by Johnson (1985), who considered the errors both in $[A]$ and in $[\bar{\nu}/(n - \bar{\nu})]$ and used the average minimum distance between the experimental point and the theoretical curve as the fitting criterion, more reliable parameters might be obtained.

This study confirms that the MWC model describes oxygen binding to *Octopus* hemocyanin very well, over a range of pH values. We note that for this system, not only $P_{50}$ and $n_H$, but all three MWC parameters ($k_R$, $k_T$, and $L$) are functions of pH. From Fig. 3 *a* we see that the maximum $n_H$ exists at pH 7.2, which means that the maximum cooperativity is found at this pH. However, the decrease in cooperativity at pH values below and above this is accounted for in a complex way, as can be seen by comparing Fig. 3, *b* and *c*.

From Fig. 3 *b*, we see that when pH $< 7.2$, $L$ becomes very large. Almost all the deoxyhemocyanin is in the T state, so it is not easy to drive all the T state to R state as the oxygen concentration increases. On the other hand, at high pH, where $L$ is not so large, the ratio between $k_T$ and $k_R$ becomes closer to unity (Fig. 3 *c*). Thus, even though the R and T states are more equally populated than at low pH, their similarity in binding affinity decreases the maximum Hill slope.

It should be noted that the data obtained at pH 7.07 appear anomolous in Figs. 1 and 3, *b* and *c*. We believe that this is a consequence of the truncation of this binding curve, which has led to incorrectly low values of $k_R$ and $L$. This points out the necessity of obtaining data over as wide a range of saturations as possible.

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## REFERENCES

Colosimo, A., M. Brunori, and J. Wyman. 1974. Concerted changes in an allosteric macromolecule. *Biophys. Chem.* 2:338–344.

Dahlquist, F. W. 1978. The meaning of Scatchard and Hill plots. *Methods Enzymol.* 48:270–299.

Decker, H., A. Savel, B. Linzen, and K. E. van Holde. 1983. A new graphical test for the MWC-model and its application to some hemocyanins. *Life Chem. Rep. Suppl. Ser.* 1. E. J. Wood, ed. Harwood, New York. 251–256.

Johnson, M. L. 1985. The analysis of ligand-binding data with experimental uncertainties in the independent variables. *Anal. Biochem.* 148:471–478.

Kegeles, G. 1974. The Hill coefficient for a Monod-Wyman-Changeux allosteric system. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 103:5–6.

Miller, K. I. 1985. Oxygen equilibria of *Octopus dofleini:* hemocyanin. *Biochemistry.* 24:4582–4586.

Monod, J., J. Wyman, and J. P. Changeux. 1965. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* 12:88–118.