Research Articles

Sigmoid shape of the oxygen equilibrium curve and the P_{50} of human hemoglobin

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Abstract. The fractional saturation of hemoglobin with oxygen was plotted against P/P_{50} and the slope of the abscissa at 1 was calculated for 38 OEC data sets of human Hb A. There was a linear correlation between the slope and the Hill coefficient (n_{max}), and the slope was about one-forth that of n_{max}. This implies that the slope of the abscissa at 1 of Y vs P/P_{50} plot can provide information about the magnitude of cooperativity in hemoglobin oxygen binding.
Key words. Hemoglobin; cooperativity; P_{50}; oxygen affinity; Hill coefficient; oxygen equilibrium curve.

The relationship between the fractional saturation of Hb with O_2 (Y) and the O_2 partial pressure (P) is expressed by the O_2 equilibrium curve (OEC)[1-3]. P_{50}, the O_2 partial pressure when Hb is half-saturated, is used as a parameter for the affinity of O_2. Generally, the OEC of Hb yields a sigmoid curve. This characteristic sigmoid shape indicates that there is cooperativity between the subunits of the Hb molecule and makes it possible to load or unload O_2 within a narrow range as the O_2 pressure changes. Hence, the sigmoid shape has great physiological importance. The magnitude of cooperativity is expressed in terms of the maximal slope of the Hill plot, $\log[Y/(1-Y)]$ vs log P plot, n_{max}[1,4]. However, the n_{max} value is not directly correlated with the O_2 transport ability of Hb. Physiologically, the significance of cooperativity for the practical O_2 transport efficiency of Hb can be better understood by the OEC slope. In this paper, the correlation between the OEC slope at P_{50} and n_{max} is investigated using precisely measured OECs of human Hb A.

Materials and methods

Thirty eight OEC data sets for human Hb A[5-8], determined precisely through a wide variety of experiments with an automatic recording apparatus, were used. In a tetrameric form, Y is described by the Adair's equation[9] as follows:

$$Y = (k_1P + 3k_1k_2P^2 + 3k_1k_2k_3P^3 + k_1k_2k_3k_4P^4)/(1 + 4k_1P + 6k_1k_2P^2 + 4k_1k_2k_3P^3 + k_1k_2k_3k_4P^4) \quad (1)$$

where Y is the fractional saturation of Hb with O_2, k_i ($i = 1, 2, 3, 4$) is the equilibrium constant (Adair constant) at the ith oxygenation step. All the parameter values were estimated by a least-squares curve-fitting procedure.

The slope of OEC (Y′) at P_{50} was calculated by computing the derivative of equation (1). All the calculations were carried out with an NEC personal computer PC-9801 model DA using MS-FORTRAN. Y′ at P_{50} was expressed as Y'_{50}.

Results and discussion

OEC and Y'_{50}. Figure 1 shows OEC *a* ($P_{50} = 5.32$ mmHg, $n_{max} = 3.02$) and OEC *b* ($P_{50} = 2.05$ mmHg, $n_{max} = 2.40$), and the Y'_{50} of *a* and *b* (dotted lines). The Y'_{50} of *b* (0.299) with lower cooperativity and high O_2 affinity is steeper than that of *a* (0.142) with higher cooperativity and low O_2 affinity. It shows the steepness of the OEC at P_{50} is greatly affected by O_2 affinity. Figure 2 shows the relationship between P_{50} and Y'_{50}, and it seems as though there is an inverse correlation between Y'_{50} and P_{50}. However, as shown in the figure, the $Y'_{50} * P_{50}$ value is not constant and ranges from 0.6 to 0.8.

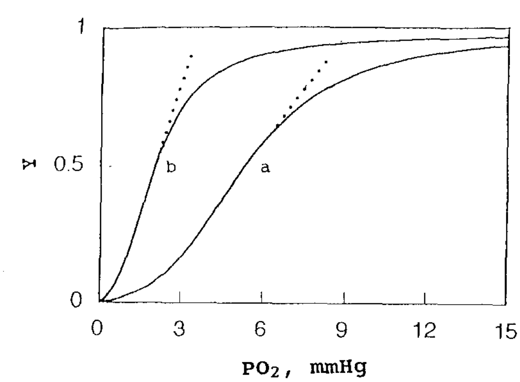

Figure 1. Oxygen equilibrium curve of human HbA. *a* ($P_{50} = 5.32$ mmHg, $n_{max} = 3.02$ and *b* ($P_{50} = 2.05$ mmHg, $n_{max} = 2.40$). OEC data from Imai and Yonetani[6].

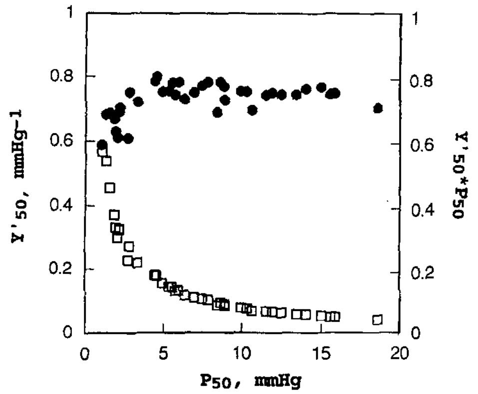

Figure 2. Dependence of the slope at P_{50} upon P_{50} calculated from the 38 sets of OEC of human HbA. OEC data taken from Imai[5], Imai and Yonetani[6], Imaizumi et al.[7], and Tyuma et al.[8].

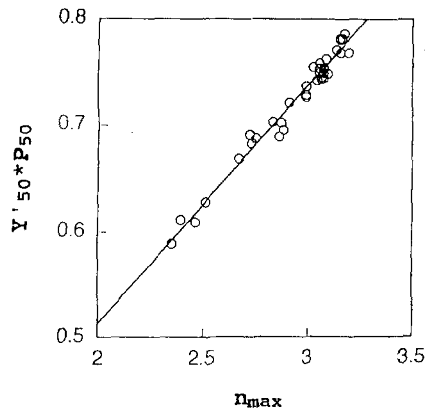

Figure 4. Correlation between n_{max} and slope at 1 of the abscissa of Y vs P/P_{50} plot of human HbA. The slopes were calculated from the 38 sets of OEC data. The OEC are the same as in figure 2.

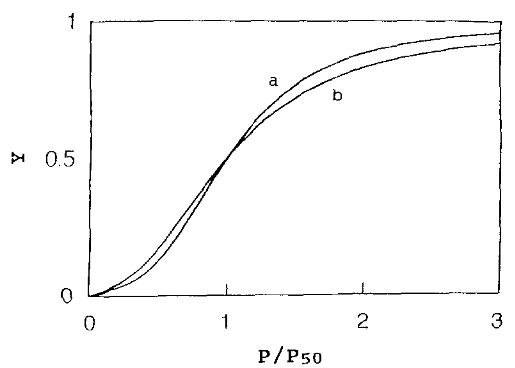

Figure 3. Y vs P/P_{50} plot of the OEC on human HbA. The OEC (*a* and *b*) are the same as in figure 1.

Y vs P/P_{50} plot and its slope of the abscissa at 1. Figure 3 illustrates the Y vs P/P_{50} plot of Hb. In this plot, the abscissa is normalized by P_{50}, and expressed as a dimensionless parameter to eliminate the effects of O_2 affinity on the OEC slope. The normalized OECs coincide at 0.5 of the ordinate and 1 of the abscissa. The slope of the abscissa at 1 can be given by $Y'_{50} * P_{50}$.

In the OEC slope, which is not shown in the figure, *a* (0.75) with high cooperativity is steeper than that of *b* (0.61) with low cooperativity. It seems to show that $Y'_{50} * P_{50}$ values are correlated with the magnitude of cooperativity.

Significance of the slope of the abscissa at 1 of Y vs P/P_{50} plot. Figure 4 shows the relationship between the slope of the abscissa at 1 of the normalized plot and n_{max} values for 38 OEC data sets. These relationships are linear and expressed as:

$$\mathrm{slope}(Y'_{50} * P_{50}) = 0.23 * n_{max} + 0.06 \quad (R^2 = 0.97) \qquad (2)$$

This implies that the slope of Y vs P/P_{50} of the abscissa at 1 can provide information about the intrinsic magnitude of the cooperativity of Hb.

The slope of the abscissa at 1 of the normalized plot and the slope of the Hill plot at P_{50}, which is known as $n_{1/2}$ and has also been used as the index of the magnitude of cooperativity[3], were compared.

$$\mathrm{slope}(Y'_{50} * P_{50}) = 0.25 * n_{1/2} + 0.01 \quad (R^2 = 0.99) \qquad (3)$$

The slope of the OEC of the abscissa at 1 is proportional to $n_{1/2}$.

Apparently, the value of $Y'_{50} * P_{50}$ is identical to that of one-fourth $n_{1/2}$. This implies that the slope at 1 can be an index of the magnitude of cooperativity. There are linear correlations between n_{max} and $n_{1/2}$.

$$n_{1/2} = 0.92 * n_{max} + 0.17 \quad (R^2 = 0.98) \qquad (4)$$

It is worthwhile to mathematically consider the slope ratio of the normalized plot to that of the Hill plot at about Y = 1/2. The ratio is calculated as follows:

$$\{dY/d(P/P_{50})\}/\{d \log[Y/(1-Y)]/d \log P\} =$$
$$\{P_{50} * dY/dP\}/\{[P/[Y(1-Y)]] * dY/dP\} =$$
$$(P_{50}/P) * [Y(1-Y)] = (P_{50}/P) * \{1/4 - (Y - 1/2)^2\} \qquad (5)$$

Equation (5) states that when the Y and P values are equal to 1/2 and P_{50}, respectively, the quotient is 1/4. Theoretically, the slope ratio of the normalized plot to that of the Hill plot at Y = 1/2 is 1/4.

In the case of n_{max}, the fractional saturation (Y_{nmax}) at P_{nmax}, the O_2 partial pressure when n_{max} occurs, for 38 OEC data sets was 0.678 ± 0.05. Hence, the square of $(Y - 1/2)$ becomes very small and the value of $\{1/4 - (Y - 1/2)^2\}$ is almost 1/4. This supports the fact that the slope ratio of the abscissa at 1 of the normalized plot to the n_{max} (eq. 2) is roughly 1/4. These results imply that the ordinate of Hill plot lengthens by four times that of Y vs P/P_{50} plot at about P_{50}.

Until now, the O_2 binding characteristics of Hb have generally been described in terms of two parameters, P_{50} of the OEC and the n_{max} of the Hill plot. However, P_{nmax} does not allow a maximum of O_2 loading or unloading with a minimum change in O_2 pressure. These phenomena are attributed to the fact that both plots have different abscissa and ordinate axes. Therefore, the OEC and Hill plot have been discussed independently.

In the case of the Hill plot, the magnitude of the slope is not dependent on O_2 affinity because the ordinates have been normalized at $Y = 1/2$. Therefore, if the Hb has a different O_2 affinity, the curve shifts along the abscissa either to the right or left without changing the intrinsic magnitude of the slope. In this paper, the O_2 partial pressure of OEC was normalized, and it was found that a simple linear relationship exists between the slope of both plots. It is considered that the Y vs P/P_{50} plot is not only useful in comparing the shape of the OEC, but also gives information concerning the magnitude of cooperativity through its slope of the abscissa at 1.

Cooperative ligand binding behavior is not restricted to O_2 carrying proteins (hemoglobin, hemocyanin, chlorocruorin etc.). It is well known that there are regulatory mechanisms in enzyme activity. These enzymes do not obey Michaelis-Menten Kinetics and are called allosteric enzymes (for example, Aspartate transcarbamoylase[10], Threonine deaminase[11], Pyruvate kinase[12] etc.). The reaction velocity (V) vs substrate concentration (S) plot (V vs S plot) for an allosteric enzyme often shows a S-shape. The S-shape also indicates the presence of the cooperativity between the subunits of the enzyme molecule. The intensity of the cooperativity of allosteric enzymes have been estimated from the maximum slope of the Hill plot (n_{max}). The results of this paper are applicable to allosteric enzymes and the intensity of the S-shape can be estimated from the slope of V vs S/Km plot when S is equal to Km, the substrate concentration at which the reaction velocity is half maximal ($1/2\ V_{max}$).

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1 Wyman, J., Adv. Protein Chem. *19* (1964) 223.
2 Imai, K., Meth. Enzym. *76* (1981) 438.
3 Wyman, J., in: Hemoglobin, Molecular Genetic and Clinical Aspects, p. 37. Eds H. F. Bunn and B. G. Forget, W. B. Sanders Company, Philadelphia, 1986.
4 Hull, A. V., J. Physiol. *40* (1910) 4.
5 Imai, K., Allosteric effects in hemoglobin. Cambridge University Press, Cambridge, 1982.
6 Imai, K., and Yonetani, T., J. biol. Chem. *250* (1975) 2227.
7 Imaizumi, K., Imai, K., and Tyuma, I., J. molec. Biol. *159* (1982) 703.
8 Tyuma, I., Imai, K., and Shimizu, K., Biochemistry *12* (1973) 1491.
9 Adair, G. S., J. biol. Chem. *63* (1925) 529.
10 Gerhart, J. C., in: Current Topics in Cellular Regulation, vol. 2, p. 275. Eds B. L. Horecker and E. R. Standtman. Academic Press, New York, 1970.
11 Monod, J.-P., Changeux, F., and Jacob, J., J. molec. Biol. *6* (1963) 306.
12 Haeckel, R., Hess, B., Lauterborn, W., and Wuster, K.-H., Hoppe-Seyler's Z. physiol. Chem. *349* (1963) 699.