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LETTERS TO THE EDITOR

The Physiological Significance of Negative Co-operativity

Negative co-operativity was first predicted as a consequence of the sequential model of subunit interactions in proteins (Koshland, Némethy & Filmer, 1966). Since then it has been observed in rabbit muscle glyceraldehyde 3-phosphate dehydrogenase (Conway & Koshland, 1968) and numerous other proteins (Levitzki & Koshland, 1969). Although it is clearly a widespread phenomenon, its physiological role has remained obscure. It may be advantageous for some enzymes to operate almost independently of substrate concentration, but it is not easy to see why negative co-operativity should be a better way of achieving this than the evolution of enzymes that bind their substrates very tightly. For example, mammalian hexokinases achieve such insensitivity to glucose concentration at physiological levels without the need for negative co-operativity (Walker, 1966). It is of interest, therefore, that Levitzki (1974) has recently suggested that the function of negative co-operativity may be to permit binding systems to be highly sensitive to ligand at low ligand concentrations, at the expense of less sensitivity at high but physiologically irrelevant concentrations. Although this proposal was made in the context of membrane receptors, it applies equally well to negative co-operativity in enzymes and other proteins. The purpose of this letter is to point out that an alternative, and possibly more reasonable, analysis of negative co-operativity leads to a conclusion contrary to that reached by Levitzki.

For simplicity we may assume that the binding system obeys the Hill equation with sufficient accuracy for this discussion, i.e.

$$\bar{Y} = \frac{L^n}{K + L^n} \tag{1}$$

where $\bar{Y}$ is the fractional saturation, L is the free ligand concentration, and K and n are constants. Levitzki (1974) has shown that this equation leads to the following relationship:

$$L_{0\cdot5} = 9^{1/n} L_{0\cdot1} \tag{2}$$

where $L_{0\cdot1}$ and $L_{0\cdot5}$ are the values of L that give $\bar{Y} = 0\cdot1$ and $\bar{Y} = 0\cdot5$ respectively. He concludes that the value of $L_{0\cdot1}$ decreases with n, and that in consequence small values of n provide the greatest sensitivity of $\bar{Y}$ to ligand concentration at low levels of saturation.

The difficulty with this argument is that it tacitly assumes that $L_{0.5}$ is unchanged when n is changed. But since two proteins with different values of n must be fundamentally different from one another, it seems arbitrary to assume that they share a common value of $L_{0.5}$. This arbitrary feature can be removed by considering the sensitivity of $\bar{Y}$ to *relative* changes in L, which can be estimated as $\mathrm{d}\bar{Y}/\mathrm{d}\ln L$. Differentiation of equation (1) gives:

$$\frac{\mathrm{d}\bar{Y}}{\mathrm{d}\ln L} = \frac{nKL^n}{(K+L^n)^2}. \tag{3}$$

Eliminating L between equations (1) and (3), we have:

$$\frac{\mathrm{d}\bar{Y}}{\mathrm{d}\ln L} = n\bar{Y}(1-\bar{Y}). \tag{4}$$

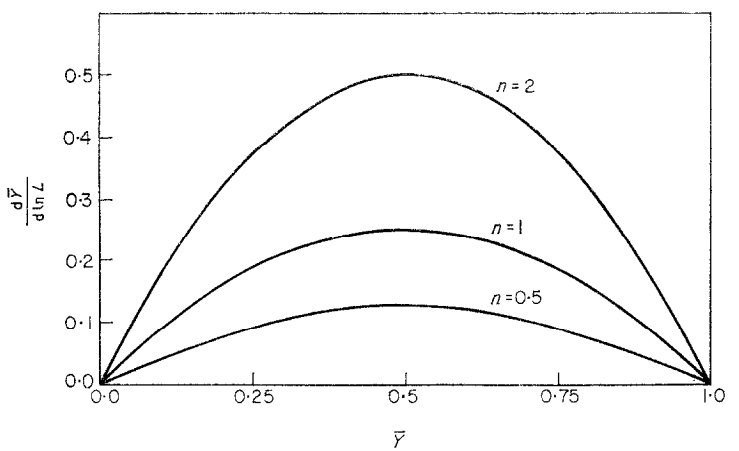

FIG. 1. Sensitivity of fractional saturation to changes in the ligand concentration, shown for values of $n = 0{\cdot}5$ (negative co-operativity), $n = 1$ (no co-operativity) and $n = 2$ (positive co-operativity).

This transformation also eliminates K, and so no arbitrary assumptions need be made about the constancy of K between unlike proteins. Since $\bar{Y}$ is restricted to values between 0 and 1, it follows from equation (4) that $\mathrm{d}\bar{Y}/\mathrm{d}\ln L$ increases with n at all levels of saturation, as illustrated in Fig. 1. In other words, the more negatively co-operative a system is the *less* sensitive it is to relative changes in ligand concentration at *all* ligand concentrations.

It follows from this result that the rationalization of negative co-operativity suggested by Levitzki (1974) is reasonable only if arguments can be

advanced to justify treating $L_{0.5}$ as an absolute quantity. Only then can one justify measuring ligand concentrations on an absolute rather than a relative scale.

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