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References

Barnes, E. M., Jr., and Kaback, H. R. (1971), *J. Biol. Chem. 246,* 5518.
Cox, G. B., Newton, N. A., Gibson, F., Snoswell, A. M., and Hamilton, J. A. (1970), *Biochem. J. 117,* 551.
Davis, B. D., and Mingioli, E. S. (1950), *J. Bacteriol. 60,* 17.
Electr, S., Zakim, D., and Vessey, D. A. (1973), *J. Mol. Biol. 78,* 351.
Hong, K., and Hubbell, W. L. (1972), *Proc. Nat. Acad. Sci. U. S. 69,* 2617.
Hubbell, W. L., and McConnell, H. M. (1971), *J. Amer. Chem. Soc. 93,* 314.
Jost, P. C., Capeldil, R. A., Vanderkooi, G., and Griffith, O. H. (1973), *J. Supramol. Struct. 1,* 269.
Kaback, H. R. (1971), *Methods Enzymol. 22,* 99.
Kaback, H. R. (1972), *Biochim. Biophys. Acta 265,* 367.
Kaback, H. R. (1974), *Science* (in press).
Kaback, H. R., and Barnes, E. M., Jr. (1971), *J. Biol. Chem. 246,* 5523.
Kaback, H. R., and Hong, J. (1973), CRC Critical Reviews in Microbiology, Vol. 2, Laskin, A. I., and Lechevalier, H., Ed., Cleveland, Ohio, Chemical Rubber Co.
Keith, A., Sharnoff, M., and Cohn, G. E. (1973), *Biochim. Biophys. Acta 300,* 379.
Kohn, L. D., and Kaback, H. R. (1973), *J. Biol. Chem. 248,* 7012.
Lombardi, F. J., and Kaback, H. R. (1972), *J. Biol. Chem. 247,* 7844.
McConnell, H. M., and McFarland, B. G. (1970), *Quart. Rev. Biophys. 3,* 91.
Morrisett, J. D., and Drott, H. R. (1969), *J. Biol. Chem. 244,* 5083.
Rottem, S., and Samuni, A. (1973), *Biochim. Biophys. Acta 298,* 32.
Schuldiner, S., Kerwar, G. K., Weil, R., and Kaback, H. R. (1974), *J. Biol. Chem.* (in press).
Seelig, J. (1971), *J. Amer. Chem. Soc. 93,* 5017.
Yu, K., Baldassare, J. J., and Ho, C. (1974), *Biochemistry 13,* 4375.

Cooperativity in Associating Proteins. Monomer–Dimer Equilibrium Coupled to Ligand Binding†

Alexander Levitzki* and Joseph Schlessinger

ABSTRACT: The cooperativity due to ligand binding to a protein monomer and a protein dimer in equilibrium was studied. The system is: E + E ⇌ E_2 (K_1); E + S ⇌ ES (K_2); ES + E ⇌ E_2S (K_3); and ES + ES ⇌ E_2S_2 (K_4). Using a computer and a plotter, Hill plots for differenct combinations of the parameters K_1, K_2, K_3, and K_4 were constructed. It was found that high ratios of K_4/K_3 with moderate K_1 and K_2 ensure high positive cooperativity. In such cases the species E_2, ES, and E_2S never accumulate to significant concentrations throughout the titration. High K_4/K_3 ratios or high K_4 alone does not ensure binding of ligand. The monomer must have a finite affinity toward the ligand in order for the whole binding process to occur. Ratios of K_4/K_3 lower than 10^3 generate negative cooperativity and the transient accumulation of E_2S in the protein–ligand mixture. The possible physiological significance of positive cooperativity due to protein aggregation is discussed.

Cooperativity in multisubunit enzymes is due to changes in the strength of subunit interactions coupled to ligand binding (Monod *et al.*, 1965; Koshland *et al.*, 1966). The modulation of subunit interactions by ligand binding brings about positive cooperativity, negative cooperativity, and mixed-type cooperativity (Levitzki and Koshland, 1969).

Some proteins dissociate upon ligand binding, and others associate in the presence of ligands (Levitzki and Koshland, 1972, and references therein; Duncan *et al.*, 1972). In such cases the ligand binding is always positively cooperative, as was already pointed out (Klotz *et al.*, 1970).

The analytical treatment of ligand binding coupled to association or dissociation is rather complex. We therefore decided to conduct a quantitative treatment, using the computer, of a case in which a monomer ⇌ dimer equilibrium is coupled to ligand binding.

Theory

Let us consider an enzyme E which binds the ligand S according to the following scheme

$$E + E \overset{K_1}{\rightleftharpoons} E_2 \quad (1)$$

$$E + S \overset{K_2}{\rightleftharpoons} ES \quad (2)$$

$$ES + E \overset{K_3}{\rightleftharpoons} E_2S \quad (3)$$

$$ES + ES \overset{K_4}{\rightleftharpoons} E_2S_2 \quad (4)$$

where K_1, K_2, K_3, and K_4 are the intrinsic association constants. One can write

$$[E_2] = K_1[E]^2 \quad (5)$$

† From the Departments of Biophysics and Chemical Physics, The Weizmann Institute of Science, Rehovot, Israel. *Received March 11, 1974.* The work was partially supported by a grant from the Israel Academy of Sciences.

$$[ES] = K_2[E][S] \quad (6)$$

$$[E_2S] = K_3[E][ES] = K_2K_3[E]^2[S] \quad (7)$$

$$[E_2S_2] = K_4[ES]^2 = K_4K_2^2[E]^2[S]^2 \quad (8)$$

It is apparent that the total enzyme concentration $[E]_T$ is

$$[E]_T = [E] + 2[E_2] + [ES] + 2[E_2S] + 2[E_2S_2] \quad (9)$$

By inserting eq 5, 6, 7, and 8 into eq 9 one obtains

$$[E]_T = [E] + 2K_1[E]^2 + K_2[E][S] + 2K_2K_3[E]^2[S] + 2K_4K_2^2[E]^2[S]^2 \quad (10)$$

Rewriting eq 10 one obtains

$$2(K_1 + K_2K_3[S] + K_4K_2^2[S]^2)[E]^2 + (1 + K_2[S])[E] - [E]_T = 0 \quad (11)$$

Solving the quadratic equation one obtains an expression for the free enzyme concentration [E]

$$[E] = \frac{-(1 + K_2[S]) + \{(1 + K_2[S])^2 + 8(K_1 + K_2K_3[S] + K_4K_2^2[S]^2)[E]_T\}^{1/2}}{4(K_1 + K_2K_3[S] + K_4K_2^2[S]^2)} \quad (12)$$

The saturation function, $\bar{Y}$, for the system described in eq 1-4 is

$$\bar{Y} = \frac{[ES] + [E_2S] + 2[E_2S_2]}{[E] + 2[E_2] + [ES] + 2[E_2S] + 2[E_2S_2]} \quad (13)$$

Therefore

$$\frac{\bar{Y}}{1 - \bar{Y}} = \frac{[ES] + [E_2S] + 2[E_2S_2]}{[E] + 2[E_2] + [E_2S]} \quad (14)$$

Inserting eq 5-8 into eq 14 one obtains

$$\frac{\bar{Y}}{1 - \bar{Y}} = \frac{K_2[S] + K_2K_3[E][S] + 2K_4K_2^2[E]^2[S]^2}{1 + 2K_1[E] + K_2K_3[E][S]} \quad (15)$$

Using eq 12 one can calculate [E] for every [S] for each set of K_1, K_2, K_3, and K_4, insert it into eq 15, and obtain the value for $\bar{Y}/(1 - \bar{Y})$. Plotting log $[\bar{Y}/(1 - \bar{Y})]$ *vs.* log [S] yields a Hill plot which is a useful plot to investigate cooperativity. This procedure is extremely laborious and therefore was performed using the computer (IBM 370/165) and a plotter.[1]

The program was executed using different values for K_1, K_2, K_3, and K_4 which were varied in a systematic fashion. For each set the computer calculated the curve log $[\bar{Y}/(1 - \bar{Y})]$ *vs.* log [S] for 15 substrate concentrations ranging from 2×10^{-6} to 0.3 M.

Results

Primary Condition for Substrate Binding. It can be demonstrated that as long as the affinity constant of the monomer form toward substrate (K_2) is very low, no binding of substrate will occur. Even when K_3 and K_4 acquire high values (10^6 each), the fact that K_2 remains low (for example, $K_2 = 10$) eliminates substrate binding altogether. In order for the substrate to bind significantly, although the monomeric form has a very low affinity toward the ligand, the dimerization constants K_3 and K_4 must acquire values much higher than 10^6. When the affinity of the monomeric form toward the ligand is allowed to increase, binding occurs at the required substrate concentration range.

[1] Program is available upon request.

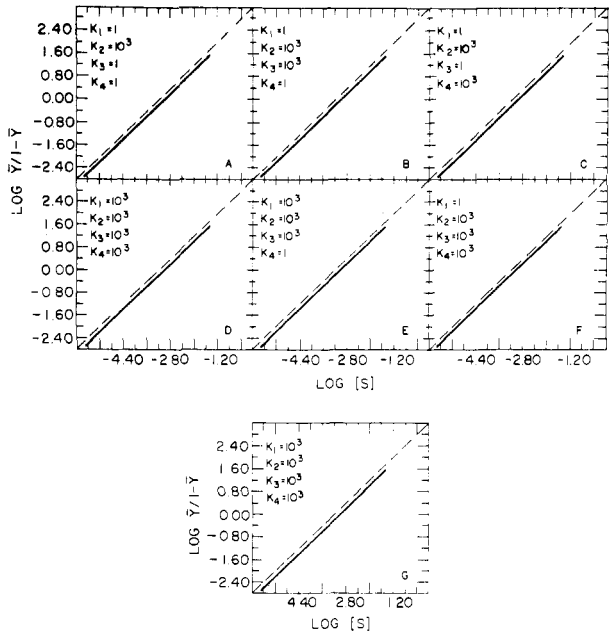

FIGURE 1: Cases with no dimer species formed. Total enzyme concentration = 10^{-5} M. Other details are given in the text.

When $K_1 = 10^6$, $K_2 = 10^3$, and $K_3, K_4 = 1$, saturation begins only at high ligand concentrations. From the above considerations one concludes that in order for a dimerizing monomer to bind substrate, one of the following two conditions must be fulfilled: (1) a moderate affinity of the monomer toward the ligand or (2) very high dimerization constants (K_3 and K_4) when the monomer affinity is low.

At high K_2 values ($K_2 > 10^4$) the main binding process occurring will be to the monomeric form and the main species throughout the titration is found to be ES. The cases considered in this paper deal mainly with situations in which the driving force for enzyme dimerization is ligand binding, namely cases in which [enzyme] $\ll 1/K_2$. Cases in which the monomer–dimer equilibrium is almost independent of total ligand binding are not dealt with. These latter cases correspond to situations in which the ligand affinities to E and E_2S are identical, namely when $K_2 = K_4K_2/K_3$. This condition implies that $K_3 = K_4$, namely that the intersubunit affinity is independent of whether the enzyme is liganded or not. Such cases were analyzed elsewhere (Cassman and King, 1972).

Cases in Which Dimerizations Are Absent. Figure 1 summarizes the cases where no dimerization occurs but monomer saturation occurs within the required substrate concentration range. Two requirements have to be fulfilled in order to obtain saturation without dimerization: (a) the intrinsic dissociation constant of the monomeric form toward the ligand is within the range of the relevant substrate concentrations tested (2×10^{-6}–0.33 M); (b) the dimerization constants K_3 and K_4 which describe the processes (see eq 3 and 4) are either smaller or equal to K_2 describing the monomer affinity toward the ligand. Whenever K_3 and/or K_4 becomes larger than K_2, dimer species (E_2S and/or E_2S_2) appear.

Cases with Positive Cooperativity. Figure 2 and Tables I and II summarize the cases in which positive cooperativity is observed. From the difference between the cases with positive cooperativity and the cases with no cooperativity and those with no saturation, one observes that positive cooperativity occurs, if two conditions are fulfilled: (a) the

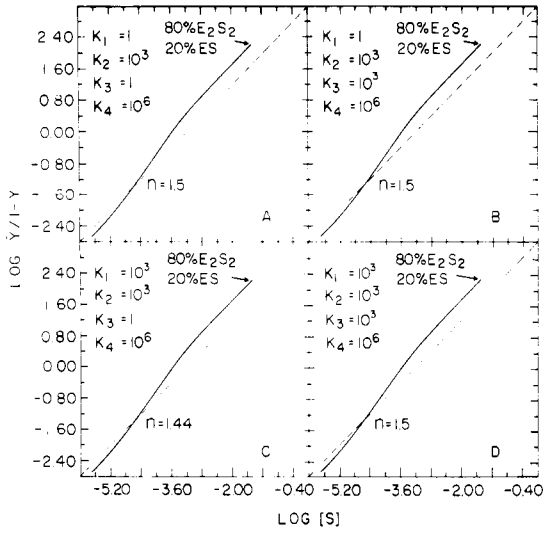

FIGURE 2: The conversion of E to E_2S_2 with no transient formation of E_2S. Total enzyme concentration = 10^{-5} M. The relative amounts of ES, E_2S, and E_2S_2 at the end of the titration are shown on the figure. Other details are given in the text.

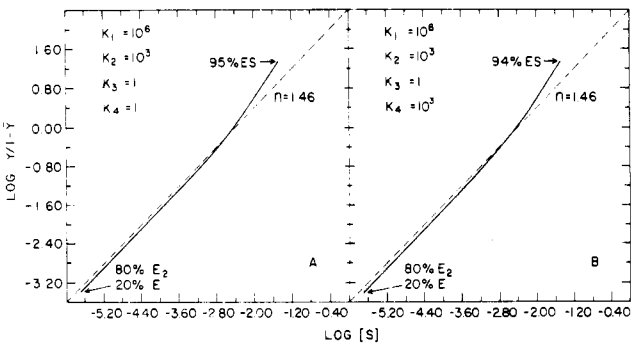

FIGURE 3: The conversion of E_2 to ES. Total enzyme concentration = 10^{-5} M. The relative amounts of ES, E_2S, and E_2S_2 at the end of the titration are shown on the figure. Other details are given in the text.

monomer species has a moderate affinity toward the ligand ($K_2 = 10^3$); (b) K_3 should be low compared to K_4 (Table I), namely, no transient accumulation of E_2S occurs. Thus, when the ratio K_4/K_3 is high, positive cooperativity will result (Tables I and II).

It is interesting to note that the degree of cooperativity or the value of $S_{0.5}$ is almost independent of K_3 and K_1 (Figure 2) within the range $K = 1$ to 10^3. When K_3 exceeds the value of 3×10^4, the cooperativity starts to decrease concomitantly with the transient accumulation of the species E_2S (see the following section).

When K_1 becomes large, a significant portion of the enzyme is in the dimer form in the absence of ligand. These cases actually involve the study of ligand-induced dissociation ($E_2 + 2S \rightarrow 2ES$) and can also lead to positive cooperativity (Figure 3). The treatment of such cases is equivalent to that applied for dimerization and therefore will not be discussed further.

In conclusion, in the positively cooperative cases, the species observed during the titration of E with S are E and E_2S_2. Although ES does not accumulate during the titration to any significant extent, *E must have a moderate affinity toward S* in order for the binding process to occur (see first section under Results), and thus for the cooperativity to be expressed.

Accumulation of E_2S, Negative Cooperativity. Figure 4 demonstrates that when the two dimerization processes (see eq 3 and 4) possess the same dimerization constant ($K_3 = K_4 = 10^6$, Figure 4C), the transient formation of E_2S occurs. This transient accumulation of E_2S brings about negative cooperativity ($n < 1$). the main species at high substrate concentration are ES and E_2S_2. When $K_4 < K_3$ (Figures 4A,B,D,E,G) strong negative cooperativity appears and incomplete saturation is observed where E_2S accumulates. When K_4 is increased so that $1 < K_4/K_3 < 10^3$ and K_2 is not changed the transient formation of E_2S can still be observed but its relative amount decreases as the K_4/K_3 ratio increases.

TABLE I: Cooperative Binding: The Transformation of E to E_2S_2 with No Transient Formation of E_2S.[a]

Intrinsic Assoc. Const.	Set	n^b	Set	n^b	Set	n^b	Set	n^b
K_1	1		1		10^3		10^3	
K_2	10^3	1.50	10^3	1.50	10^3	1.44	10^3	1.50
K_3	1		10^3		1		10^3	
K_4	10^6		10^3		10^6		10^6	

[a] Total enzyme concentration is 10^{-5} M. [b] Maximal Hill slope.

TABLE II: High Cooperativity Cases.[a]

Intrinsic Assoc. Const.	Set	n	Set	n	Set	n	Set	n
K_1	1		1		1		1	
K_2	10^3	1.50	10^3	1.65	10^3	1.77	10^3	1.94
K_3	10^3		10^3		10^3		10^3	
K_4	10^6		10^7		10^8		10^{10}	

[a] Enzyme concentration = 10^{-5} M.

TABLE III: Effect of Protein Concentration.

[Enzyme] (M)	n^a	n^b
10^{-5}	1.55	1.52
10^{-5}	1.50	1.50
10^{-4}	1.28	1.45
10^{-3}	1.17	1.43
10^{-2}	1.00	1.35

[a] $K_1 = 10^3$, $K_2 = 10^3$, $K_3 = 10^3$, $K_4 = 10^6$. [b] $K_1 = 1$, $K_2 = 10^3$, $K_3 = 10^3$, $K_4 = 10^3$. The Hill coefficients given are the maximal ones observed on the Hill plot.

It is easily seen that negatively cooperative behavior can be obtained when $K_1 > K_2$ and $K_3 > K_4$ as in Figure 4G. However, under these conditions the enzyme is largely a dimer in the absence of ligand. It can be seen that this case is an example of negative cooperativity in the transformation: $E_2 + 2S \leftrightarrow 2ES$.

Effect of Protein Concentration. The calculations described were performed, assuming an enzyme concentration of 10^{-5} M. The concentration is within the range of values chosen for $1/K_1$, $1/K_2$, $1/K_3$, and $1/K_4$. Under most conditions chosen no dimer is found in the absence of ligand since the aim of this study is to explore the transformation of monomer to dimer as a function of ligands bound.

Upon increasing enzyme concentrations enzyme dimer may form in the absence of ligand. If the enzyme concentration tested is within the range of $1/K_1$ significant dimer concentrations may exist in the absence of added ligand. The change in cooperativity of ligand binding as a function of protein concentration will depend on the relative values of K_1/K_2, K_3, and K_4. In the extreme case where $[\text{enzyme}]_{\text{total}} > 1/K_1$, the enzyme will be exclusively in the dimeric form prior to ligand binding. Under these conditions the cooperativity in binding will depend on the ratios K_4K_2/K_3, namely on the ratio of ligand affinity toward E_2S to that toward E_2. This case, therefore, represents the study of ligand binding to a dimer where the dimeric form is preserved as a function of ligand concentration. It is clear that the value of this ratio determines the type of cooperativity observed (Levitzki, 1974) and this cooperativity is exclusively due to the subunit interactions within the dimer. Some examples as to the dependence of the Hill coefficient

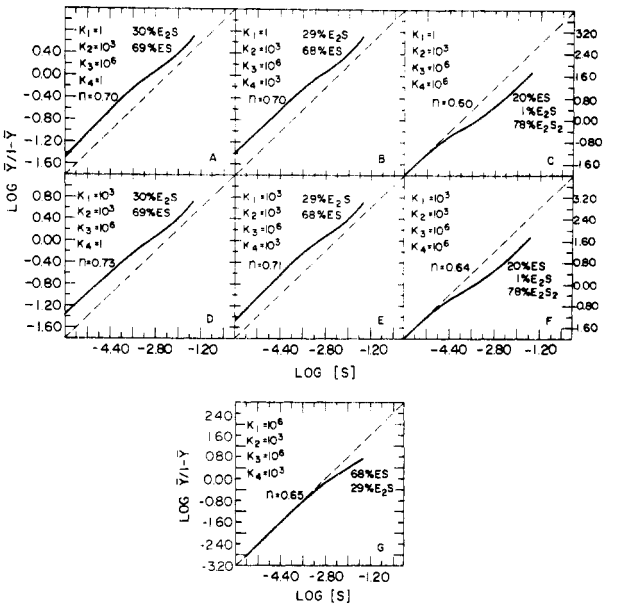

FIGURE 4: The formation of E_2S. Total enzyme concentration = 10^{-5} M. The relative amounts of ES, E_2S, and E_2S_2 at the end of the titration are shown on the figure. Other details are given in the text.

on protein concentration for different sets of K values are summarized in Table III. In general, for the same set of K values the cooperativity will either remain unchanged, or decrease as a function of protein concentration. One can in fact prove (see Appendix) that the effect of increasing protein concentration is to diminish the cooperativity.

Discussion

In a system where a monomer to dimer transformation coupled to ligand binding the two types of cooperativity, positive and negative, can be generated. The basic rules which govern the system can be summarized as follows (see Table IV). (a) At any protein concentration used, in order for ligand binding to occur at all, the monomer form must have *finite affinity* toward the ligand. If the monomer–ligand dissociation constant ($1/K_2$) is one or two orders of magnitude higher than total enzyme concentration, positive cooperativity can be generated at fairly low ligand concentrations. If the monomer–ligand dissociation constant is within the range of enzyme concentration ES will form and the cooperativity in ligand binding will decrease. (b) The

TABLE IV: Behavior of the Dimerizing System as a Function of the Characteristic Parameters.

Parameter	Range of Other Parameters	Predominant Species during Titration	Type of Cooperativity (Max. Hill Coeff.)
$K_2 = 1$	K_1, K_3, K_4 = 1–10^6, all combinations	No binding	No binding
$K_2 = 10^3$	K_1, K_3, K_4 = 1–10^3, all combinations	E, ES	No cooperativity ($n_H = 1$)
$K_4/K_3 > 10^3$	$K_2 = 10^3$, K_1 = 1–10^3, all combinations	E, E_2S_2	Positive ($n_H = 1.5$)
$K_4/K_3 > 10^6$	$K_2 = 10^3$, K_1 = 1–10^3, all combinations	E, E_2S_2	($n_H = 2.0$)
$K_3 = 10^6$ $K_4/K_3 = 1$	$K_2 = 10^3$, K_1 = 1–10^3	E, E_2S, E_2S, E_2S_2	Negative ($n_H < 1.0$)
$K_3 = 10^6$ $K_4/K_3 < 1$	$K_2 = 10^3$, K_1 = 1–10^3	E, ES, E_2S	Negative ($n_H < 1.0$)

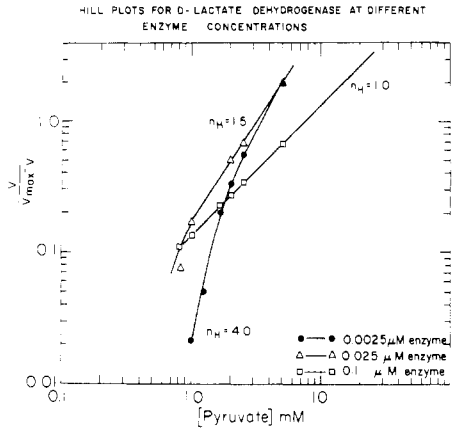

FIGURE 5: Hill plots for D-lactate dehydrogenase from *Aerobacter aerogenes*. The Hill plots were constructed from the data published by Sawula and Suzuki (1970). The plots demonstrate the characteristic features of cooperativity in aggregating systems. The system is highly cooperative at low enzyme concentrations. The Hill coefficient of 1.0 at high enzyme concentration indicates the existence of aggregated species exclusively, throughout the titration range. In this study it is assumed that $\bar{Y} = v/V_{max}$.

degree of positive and negative cooperativity strongly depends on the ratio K_4/K_3. (c) Negative cooperativity becomes more pronounced as the ratio K_4/K_3 decreases. When the ratios K_4/K_3 become very low, no E_2S_2 is formed and E_2S is converted to ES during the titration (Figure 4G). (d) Increasing protein concentration generally decreases cooperativity (Figure 5).

Effect of Total Enzyme Concentration. When $[E]_T$ is increased the cooperativity in the system decreases for each set of K_1, K_2, K_3, and K_4 (Table III). This effect is seen in the expression (eq 16) which describes the dependence of the Hill coefficient (see also the Appendix). If one selects certain [S] values for different $[E]_T$ values and investigates the value of n as a function of increasing $[E]_T$, the following picture emerges. As $[E]_T$ goes up, [E] also will go up, and the rate of change of $4K_2[E][S]$ and $2K_2K_1[E][S]$ is identical. However, upon increasing $[E]_T$, the value $\partial[E]/\partial[S]$ also increases. This is true since more protein will bind ligand for every increment of substrate at high total

$$n = \frac{\partial \log \frac{\bar{Y}}{1-\bar{Y}}}{\partial \log [S]} = \frac{1 + 4K_2[E][S] + 2K_2K_1[S]^2\frac{\partial[E]}{\partial[S]}}{1 + 2K_2K_1[E][S]} = \frac{1 + 4K_2[E][S]}{1 + 2K_2K_1[E][S]} + \frac{2K_2K_1[S]^2\frac{\partial[E]}{\partial[S]}}{1 + 2K_2K_1[E][S]} \quad (16)$$

enzyme concentration than at low enzyme concentration for each set of association constants. Since $\partial[E]/\partial[S]$ is a negative number, the term $2K_2K_1[S]^2(\partial[E]/\partial[S])$, in the nominator in eq 16, will become more negative as $[E]_T$ increases. The net result is, therefore, that n decreases with increasing $[E]_T$ (Table III).

That this is indeed the case in real systems is seen in Figure 5, where the effect of protein concentration on the cooperative response of D-lactate dehydrogenase toward pyruvate was studied. Although the data presented in Figure 5 are based on saturation kinetics rather than on binding studies, in every regulatory enzyme thus far studied the assumption that $\bar{Y} = v/V_{max}$ was found to be valid.

General Significance. Equations 12-16 can be used to fit ligand binding data and obtain the values for K_1 through K_4. If experiments are conducted at different total protein concentrations, $[E]_T$, the quality of the fitting can be analyzed.

Biological Significance. The response of a protein to a ligand in a situation where the protein associates or dissociates upon ligand binding offers a means of controlling the response, by the change in the total concentration of the protein. This device can be extremely efficient especially when a large difference in ligand affinity exists between the dissociated and the aggregated species of the protein. It is interesting that in very cooperative proteins where the Hill coefficient measured approaches the total number of binding sites, it is found that the protein represents an aggregating system in which the aggregation process is tightly coupled to ligand bindings (Table V). It may be suggested that whenever high degrees of cooperativity had to be attained

TABLE V: High Hill Coefficients in Aggregating Enzymes.

Enzyme	No. of Subunits in Dissociated Species	Aggregated Species	Ligand Promoting Assn	Highest Hill Coefficient Obsd	Ref
D-Lactate dehydrogenase	1	4	Pyruvate	4	Sawula and Suzuki (1970)
N^{10}-Formyltetrahydrofolate synthetase	1	4	NH_4^+	4	McKenzie and Rabinowitz (1971)
Phosphofructokinase	2	2*n*	Fructose	3.4	Froede *et al.* (1968); Mansour and Ahlfors (1968)
CTP synthetase	2	4	UTP	4[a]	Long and Pardee (1967)
			ATP	4[b]	Levitzki and Koshland (1972)

[a] In the presence of subsaturating ATP. [b] In the presence of subsaturating UTP.

strong subunit interactions had to evolve in the protein. The largest change in subunit interactions expected upon ligand binding would indeed be when the subunits are physically separated when unliganded and tightly associated when liganded. Similarly, high cooperativities can be generated when the nonliganded subunits are strongly aggregated in an oligomeric structure and dissociate upon ligand binding.

Appendix

A relatively simple analytical expression for the Hill coefficient in a dimerizing system can be arrived at for a case in which the species E_2S and E_2 never accumulate to a significant extent. The cases which qualify to this restriction actually are the cases where the highest cooperativity is seen. With these restrictions eq 14 simplifies to

$$\frac{\bar{Y}}{1 - \bar{Y}} = \frac{[ES] + 2[E_2S_2]}{[E]} \tag{17}$$

namely

$$\frac{\bar{Y}}{1 - \bar{Y}} = \frac{K_1[E][S] + 2K_4K_2^2[E]^2[S]^2}{[E]} \tag{18}$$

or

$$\frac{\bar{Y}}{1 - \bar{Y}} = K_1[S] + 2K_4K_2^2[E][S]^2 \tag{18}$$

Therefore

$$\log \frac{\bar{Y}}{1 - \bar{Y}} = \log (K_1[S] + 2K_4K_2^2[E][S]^2) \tag{19}$$

but

$$n = \frac{\partial \log \frac{\bar{Y}}{1 - \bar{Y}}}{\partial \log [S]} = [S] \frac{\partial \log \frac{\bar{Y}}{1 - \bar{Y}}}{\partial [S]} \tag{20}$$

so one obtains

$$n = \frac{1 + 4K_2[E][S] + 2K_2K_1[S]^2 \frac{\partial [E]}{\partial [S]}}{1 + 2K_2K_1[E][S]}$$

References

Cassman, M., and King, R. C. (1972), *Biochemistry 11,* 4937.

Duncan, B. K., Diamond, G. R., and Bessman, M. J. (1972), *J. Biol. Chem. 247,* 8136.

Froede, H. C., Geraci, G., and Mansour, T. E. (1968), *J. Biol. Chem. 243,* 6021.

Klotz, I. M., Langerman, N. R., and Darnall, O. W. (1970), *Annu. Rev. Biochem. 39,* 25.

Koshland, D. E., Nemethy, G., and Filmer, D. (1966), *Biochemistry 5,* 365.

Levitzki, A. (1974), *in* Subunit Structure and Interactions of Proteins, Ebner, K. E., Ed., New York, N.Y., Marcel Dekker, in press.

Levitzki, A., and Koshland, D. E. (1969), *Proc. Nat. Acad. Sci. U. S. 62,* 1121.

Levitzki, A., and Koshland, D. E. (1972), *Biochemistry 11,* 247.

Long, C. W., and Pardee, A. B. (1967), *J. Biol. Chem. 242,* 4715.

Mansour, T. E., and Ahlfors, C. E. (1968), *J. Biol. Chem. 243,* 2523.

McKenzie, R. E., and Rabinowitz, J. C. (1971), *J. Biol. Chem. 246,* 3731.

Monod, J., Wyman, J., and Changeux, J. P. (1965), *J. Mol. Biol. 12,* 88.

Sawula, R. V., and Suzuki, I. (1970), *Biochem. Biophys. Res. Commun. 40,* 1096.