University of Washington
Department of Chemistry
Chemistry 453
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Lecture 12 2/02/15

A Partial Cooperativity; Adair Equation

- For non-cooperative binding a Hill plot will have slope = N=1. For fully cooperative binding the slope will be N>1. For Hemoglobin which binds 4 oxygen moleculesif this binding were fully cooperative, we would expect a linear Hill plot with slope N=4. But the appearance of hemoglobin's Hill plot is shown in Figure 12.1:
- Myoglobin is an oxygen storage protein for which N=1. Myoglobin has a linear Hill plot with slope=1 as expected.
- Hemoglobin (Hb) has a nonlinear Hill plot, shown in red in Figure 10.2.
- The Hill plot of Hb has three distinct reagions, a situation that results because Hb binds oxygen with partial cooperativity.

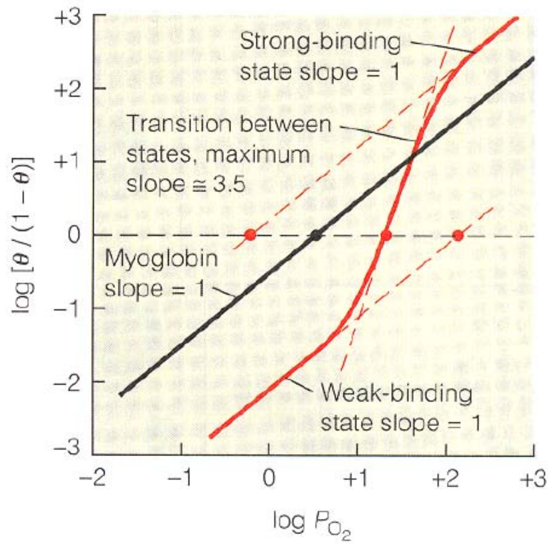


Figure 12.1: The oxygen storage protein myoglobin has N=1 and a linear Hill plot (black). Hemoglobin is an oxygen transport protein which has N=4 and a non-linear Hill plot (red).

- Partial cooperativity means a solution of Hb is a mixture of unbound Hb, singly bound hemoglobin Hb-O_2, doubly bound hemoglobin Hb-2O_2, triply bound hemoglobin Hb-3O_2 and filled hemobglobin Hb-4O_2, but the binding constants for these Hb-O_2 complexes are different. The binding affinity between the various forms of Hb and O_2 increases as Hb fills its binding sites with oxygen, i.e. $k_1 < k_2 < k_3 < k_4$. This means that Hb's binding affinity is regulated by the amount of O_2 bound, an effect called allosterism.
- The Adair equation was the first equation to quantify Hb-O_2 binding.The Adair equation is derived by writing out the binding polynomial for four binding sites with affinity constants $k_1 < k_2 < k_3 < k_4$

$$Q = q_B = [Hb]\left(1 + 4k_1[O_2] + 6k_1k_2[O_2]^2 + 4k_1k_2k_3[O_2]^3 + k_1k_2k_3k_4[O_2]^4\right) \qquad (12.1)$$

- In equation 12.1 the first term in the parenthesis is the amount of free hemoglobin [Hb]. The second term $4k_1$[L][Hb] is the amount of hemoglobin with one oxygen site bound etc.
- From equation 10.7 we obtain the fraction of oxygen sites bound in Hb for a certain concentration of oxygen:

$$f_B = \frac{\langle \nu \rangle}{4} = \frac{1}{4}\frac{[O_2]}{Q}\frac{\partial Q}{\partial [O_2]} = \frac{\left(k_1[O_2] + 3k_1k_2[O_2]^2 + 3k_1k_2k_3[O_2]^3 + k_1k_2k_3k_4[O_2]^4\right)}{\left(1 + 4k_1[O_2] + 6k_1k_2[O_2]^2 + 4k_1k_2k_3[O_2]^3 + k_1k_2k_3k_4[O_2]^4\right)} \quad (12.2)$$

- Equation 10.8 is the Adair equation and the constants $k_1 < k_2 < k_3 < k_4$ can be adjusted to fit Hb's non-linear Hill plot. This I smost easily seen by looking at the binding limits.
- In the weak binding limit where $[O_2]<<1$ equation 10.8 is

$$[O_2] << 1 : \frac{f_B}{1-f_B} \approx k_1[O_2] \; or \; \ln\left(\frac{f_B}{1-f_B}\right) = \ln k_1 + \ln[O_2] \qquad (12.3)$$

- So in the WEAK binding limit the Hill plot is linear , slope=1, and the y-intercept is $\ln k_1$.
- In the strong binding limit

$$[O_2] >> 1 : \frac{f_B}{1-f_B} \approx k_4[O_2] \; or \; \ln\left(\frac{f_B}{1-f_B}\right) = \ln k_4 + \ln[O_2] \qquad (12.4)$$

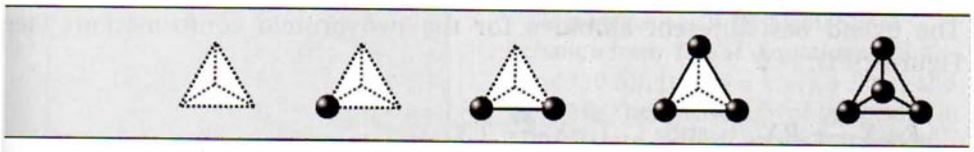
- So in the STRONG binding limit the Hill plot is linear , slope=1, and the y-intercept is $\ln k_4$.
- In the intermediate region the Hill plot must be fit using the entire equation 12.2 . The resulting slope is 2.9-3.5.

C. Protein Allostery &: Pauling's Sequential Model

- The Adair equation can fit the Hill plot for Hb but it has four adjustable parameters and there is no physical insight as to why $k_1 < k_2 < k_3 < k_4$.
- Linus Pauling first proposed a sequential model for Hb allosterism where in Hb, O_2 binding was enhanced as a result of pair-wise interactions between bound sites which are gradually increased in number by sequential binding of oxygen.
- Pauling assumed that the oxygen binding sites occupied the vertices of a tetrahedron in Hb and thus are all equidistant. This allowed him to increased the O_2 binding affinity of Hb as a simple function of the number of pair-wise interactions between occupied binding sites.
- Assuming an equilibrium between Hb and Hb-O_2, only a single site is bound in the product so no pair-wise interactions are present. Therefore k_1=k: $Hb + O_2 \overset{k}{\rightleftharpoons} Hb \cdot O_2$
- For the equilibrium between HbO_2 and Hb-$2O_2$, the product has one pair-wise interaction so that the affinity constant is enhanced by

$k_2 = e^{-\varepsilon_0/k_BT}k = fk$ where $f = e^{-\varepsilon_0/k_BT}$ is the enhancement factor from a single pair-wise interaction with energy ε_0: $Hb\cdot O_2 + O_2 \xrightleftharpoons{fk} Hb\cdot 2O_2$

- For the equilibrium between Hb-$2O_2$ and Hb-$3O_2$
  $k_3 = e^{-2\varepsilon_0/k_BT}k = f^2k$ reflecting the two additional pair-wise interactions in Hb$3O_2$ versus Hb$2O_2$: $Hb\cdot 2O_2 + O_2 \xrightleftharpoons{f^2k} Hb\cdot 3O_2$
- For the equilibrium between Hb-$3O_2$ and Hb-$4O_2$
  $k_4 = e^{-3\varepsilon_0/k_BT}k = f^3k$ reflecting the three more pairwise interactions in Hb$4O_2$ versus Hb$3O_2$: $Hb\cdot 3O_2 + O_2 \xrightleftharpoons{f^3k} Hb\cdot 4O_2$



Number of Ligands Bound	0	1	2	3	4
Number of Pairwise Interactions	0	0	1	3	6

Figure 12.2: The pairwise interactions between oxygen bound sites that enhance oxygen binding according to pauling's Sequential Model.

- It is assumed that the binding is enhanced as sites are progressively bound so *f*>1.
- Using Paulings hypothesis the four adjustable parameters in Adair's equation are reduced to two adjustable parameters: k and f. The binding polynomial is

$$Q = [Hb]\left(1 + 4k_1[O_2] + 6k_1k_2[O_2]^2 + 4k_1k_2k_3[O_2]^3 + k_1k_2k_3k_4[O_2]^4\right)$$
$$[Hb]\left(1 + 4k[O_2] + 6fk^2[O_2]^2 + 4f^3k^3[O_2]^3 + f^6k^4[O_2]^4\right) \quad (12.5)$$

  - Note each term in the binding polynomial has f raised to the power of the number of pair-wise interactions in the Hb-O_2 complex. For example, Figure 12.2 shows that in Hb where all four sites are filled with O_2, there are 6 pairwise interactions so the fifth term in Q has contains f^6.
- With equation 12.5 the Adair equation becomes

$$f_B = \frac{\langle \nu \rangle}{4} = \frac{\left(k[O_2] + 3fk^2[O_2]^2 + 3f^3k^3[O_2]^3 + f^6k^4[O_2]^4\right)}{\left(1 + 4k[O_2] + 6fk^2[O_2]^2 + 4f^3k^3[O_2]^3 + f^6k^4[O_2]^4\right)} \quad (12.6)$$

- Equation 12.6 can be fitted to the Hill Plot in Figure 12.1 . To see thi s we calculate

$$\frac{f_B}{1-f_B} = k[O_2]\frac{1+3fk[O_2]+3f^3k^2[O_2]^2+f^6k^3[O_2]^3}{1+3k[O_2]+3fk^2[O_2]^2+f^3k^3[O_2]^3} \quad (12.7)$$

- To test whether equation 12.7 can fit Hill plots like Figure 12.1 let us see if in the limits of low and high $[O_2]$ we obtain linear Hill plots with slope=1.
  - Assume $[O_2] \ll 1$. Then neglect all terms in the numerator and denominator of equation 12.7 except for the 1's. We obtain the very simplified equation which results in a Hill plot with slope=1 and intercept lnk:

$$\frac{f_B}{1-f_B} \approx k[O_2] \Rightarrow \ln\left(\frac{f_B}{1-f_B}\right) \approx \ln k + \ln[O_2] \quad (12.8)$$

  - Next assume $[O_2] \gg 1$. In this case we retain the largest terms in the numerator and denominator of equation 12.7: