University of Washington
Department of Chemistry
Chemistry 453
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Lecture 13 2/09/15

A Partial Cooperativity; Summary of Sequential Models

- Non-linear Hill plots of the type shown in Figure 13.1 are frequently observed for binding equilibria that display partial cooperativity. Such plots display slope one behavior in the low binding limit $\langle \nu \rangle << 1$ and in the high binding limit: $\langle \nu \rangle \gg 1$

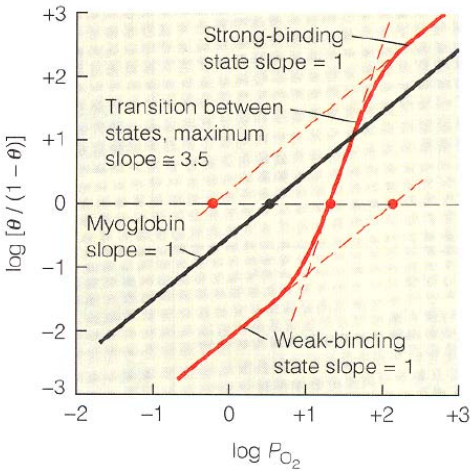

Figure 13.1: Hill Plot for a binding equilibrium that is partially cooperative.

- Sequential Binding models assume the site binding affinities change as a function of the number of sites bound . Starting with the Adair equation for binding of a ligand (i.e. oxygen) to four sites on a protein (i.e. Hb) we have

$$f_B = \frac{\langle \nu \rangle}{4} = \frac{1}{4}\frac{[O_2]}{Q}\frac{\partial Q}{\partial [O_2]} = \frac{\left(k_1[O_2] + 3k_1k_2[O_2]^2 + 3k_1k_2k_3[O_2]^3 + k_1k_2k_3k_4[O_2]^4\right)}{\left(1 + 4k_1[O_2] + 6k_1k_2[O_2]^2 + 4k_1k_2k_3[O_2]^3 + k_1k_2k_3k_4[O_2]^4\right)} \quad (13.1)$$

- .If the binding affinity increases with the number of sites bound then:

$$k_1 < k_2 < k_3 < k_4 \quad (13.2)$$

- Increase of binding affinity with number of ligands bound as shown in equation 13.2 is called positive cooperativity.

B. Pauling's Sequential Model is Positive Cooperativity

- In Pauling's model binding is enhanced by a factor of f for each additional site-site interaction. Therefore k1=k for $Hb + O_2 \overset{k}{\rightleftharpoons} Hb \cdot O_2$
  - $k_2 = e^{-\varepsilon_0/k_BT}k = fk$ where $f = e^{-\varepsilon_0/k_BT}$ is the enhancement factor from a single pair-wise interaction with energy ε_0: $Hb \cdot O_2 + O_2 \overset{fk}{\rightleftharpoons} Hb \cdot 2O_2$

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

- Using Paulings hypothesis the four adjustable parameters in Adair's equation are reduced to two adjustable parameters: k and f. The binding polynomial is

$$\begin{aligned} Q &= [Hb]\left(1 + 4k_1[O_2] + 6k_1k_2[O_2]^2 + 4k_1k_2k_3[O_2]^3 + k_1k_2k_3k_4[O_2]^4\right) \\ &= [Hb]\left(1 + 4k[O_2] + 6f k^2[O_2]^2 + 4f^3k^3[O_2]^3 + f^6k^4[O_2]^4\right) \end{aligned} \tag{13.3}$$

- With equation 13.3 the Binding equation is:

$$f_B = \frac{\langle \nu \rangle}{4} = \frac{[O_2]}{4Q}\frac{\partial Q}{\partial [O_2]} = \frac{\left(k[O_2] + 3fk^2[O_2]^2 + 3f^3k^3[O_2]^3 + f^6k^4[O_2]^4\right)}{\left(1 + 4k[O_2] + 6fk^2[O_2]^2 + 4f^3k^3[O_2]^3 + f^6k^4[O_2]^4\right)} \tag{13.4}$$

- Based in equation 13.4 the Hill equation is:

$$\frac{f_B}{1 - f_B} = k[O_2]\frac{1 + 3fk[O_2] + 3f^3k^2[O_2]^2 + f^6k^3[O_2]^3}{1 + 3k[O_2] + 3fk^2[O_2]^2 + f^3k^3[O_2]^3} \tag{13.5}$$

  - Assume $[O_2] \ll 1$. We obtain the simplified equation which results in a Hill plot with slope=1 and intercept lnk:

$$\frac{f_B}{1 - f_B} \approx k[O_2] \Rightarrow \ln\left(\frac{f_B}{1 - f_B}\right) \approx \ln k + \ln[O_2] \tag{13.6}$$

  - Next assume $[O_2] \gg 1$.

$$\frac{f_B}{1 - f_B} = kf^3[O_2] \tag{13.7}$$

  - This equation obtains a Hill plot with slope=1 and intercept $\ln f^3 k$

C. Protein Allostery and Concerted Models

a. Sequential Models assume oxygen binding sites are driven from weak to strong form by sequential addition of O_2 to Hb.
b. An alternative to sequential models are concerted models. Concerted models assume Hb exists in a form R where ALL binding sites are strong and form T where ALL binding sites are weak. R and T exist in equilibrium. All four O_2 binding sites change together (i.e. in a concerted

fashion) when R changes to T. Addition of O_2 shifts the equilibrium from favoring T forms at low O_2 levels to favoring R forms at high O_2 levels.

c. Monod-Wyman-Changeaux (MWC) Theory is a concerted model that was proposed as an explanation of cooperative oxygen binding in hemoglobin. X-ray studies have identified some intermediates ppredicted by MWC which indicate this model has validity.

d. According to MWC theory, in the absence of oxygen , Hb exists in two forms T and R that are in dynamic equilibrium $R \underset{\leftarrow}{\overset{K}{\rightleftharpoons}} T; K = \frac{[T]}{[R]}$,

- In the T state, all sites bind O_2 weakly
- In the R state, all binding sites bind O_2 tightly
  - In the absence of oxygen , the T form is favored, i.e. K>>1
  - As oxygen is added, the RL and RL_2 forms are favored over TL and TL_2.
  - MWC theory was demonstrated for 4 binding sites in Hb. For simplicity we only show results for two binding sites.
  - In the two binding site model there are six protein forms: T, TL, TL_2, R, RL, RL_2. The MWC model proposes a dynamic exchange between R and T forms as shown below for two oxygen binding sites:

$$\begin{array}{ccc} R & \underset{\leftarrow}{\overset{K}{\rightleftharpoons}} & T \\ \updownarrow k_R & & \updownarrow k_T \\ RL & \underset{\leftarrow}{\overset{cK}{\rightleftharpoons}} & TL \\ \updownarrow k_R & & \updownarrow k_T \\ RL_2 & \underset{\leftarrow}{\overset{c^2K}{\rightleftharpoons}} & TL_2 \end{array}$$

- The equilibria between the various forms of bound and unbound T are characterized by the equilibrium constant k_T. The equilibria between the various forms of bound and unbound R are characterized by the equilibrium constant k_R. The ratio $C = \frac{k_T}{k_R} << 1$ because R binds O_2 more strongly than T.
- Note that as more and more oxygen is added more RL, RL_2, TL, and TL_2 are formed. But because C<1 then $C^2K<CK<K$, and the equilibria between T and R forms shifts from favoring T over R to favoring RL_2 over TL_2.
- Assume two binding sites on T and R we start with the fraction of sites bound:

$$\langle \nu \rangle = \frac{[RL] + 2[RL_2] + [TL] + 2[TL_2]}{[R] + [T] + [RL] + [RL_2] + [TL] + [TL_2]} \tag{13.8}$$

- Substitute $K = \frac{[T]}{[R]} \; and \; C = \frac{k_T}{k_R}$ to obtain after some algebra the Hill equation for the MWC model:

$$\frac{f_B}{1 - f_B} = k_R [O_2] \frac{1 + KC\left(\frac{1 + Ck_R[O_2]}{1 + k_R[O_2]}\right)}{1 + K\left(\frac{1 + Ck_R[O_2]}{1 + k_R[O_2]}\right)} \tag{13.9}$$

- Equation 13.9 seems complicated but it also explains the Hill Plot for Hb.
    - Assume the weak binding limit where $[O_2] << 1$. Then:

$$\frac{f_B}{1-f_B} \approx k_R [O_2] \frac{1+KC}{1+K} \tag{13.10}$$

    - Note K>>1 because the T form is favored. Then

$$\frac{f_B}{1-f_B} \approx k_R C [O_2] \tag{13.11}$$

or

$$\ln\left(\frac{f_B}{1-f_B}\right) = \ln(k_R C) + \ln[O_2] = \ln k_T + \ln[O_2] \tag{13.12}$$

    - According to equation 13.12 the Hill Plot is linear at low $[O_2]$ with slope = 1 and intercept $\ln k_T$

    - In the high oxygen concentration limit $[O_2] >> 1$

$$\frac{f_B}{1-f_B} \approx k_R [O_2] \frac{1+KC^2}{1+KC} \tag{13.13}$$

    - R has a higher binding affinity so C<<1. Therefore the Hill equation becomes in the limit $[O_2] >> 1$:

$\ln\left(\frac{f_B}{1-f_B}\right) = \ln k_R + \ln[O_2]$, i.e. slope = 1 and intercept $\ln k_R$

    - These limiting equations, together the general equation that is effective at intermediate ligand concentrations, yields the Hill plot below.
- Although the sequential and concerted models both explain the Hill Plot data for Hb-O_2 binding , the simple form of the MWC concerted theory and the fact that many of its proposed intermediates have been identified by crystallography have caused this theory to be favored over sequential theories.
- By the late 1990's some of the intermediates proposed on sequential models had been detected with the result that the real model for binding between O_2 and Hb is likely a hybrid theory of the sequential and concerted models.