LECTURE 3

In lecture 2 we saw that enzymes can work as catalysts for biochemical reactions within the cell - However their role as catalytic agent is not guaranteed all the time

One of the most common scenarios is:

- Competitive Inhibition:

Molecules other than the substrate are able to bind with the enzyme and - if this happens - the enzyme is no longer able to catalyze the reaction ⇒ To model this, we need to pair the reaction

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \xrightarrow{k_2} E + P$$

with a competing reaction

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} C_2$$

$I \triangleq$ molecule inhibiting E by forming C_2

Let us introduce ODEs:

$s \triangleq [S]$

$c_1 \triangleq [C_1]$

$c_2 \triangleq [C_2]$

$p \triangleq [P]$

$i \triangleq [I]$

$e \triangleq [E]$

$e + c_1 + c_2 = e_0$

$$\begin{cases} \frac{ds}{dt} = -k_1 s(e_0 - c_1 - c_2) + k_{-1} c_1 \\ \frac{dc_1}{dt} = k_1 s(e_0 - c_1 - c_2) - (k_{-1} + k_2) c_1 \\ \frac{dc_2}{dt} = k_3 (e_0 - c_1 - c_2) i - k_{-3} c_2 \\ \frac{di}{dt} = -k_3 (e_0 - c_1 - c_2) + k_{-3} c_2 \\ V = \frac{dp}{dt} = k_2 c_1 \end{cases}$$

By using the QSS approximation we have:

$$\frac{dc_1}{dt} = 0 \iff k_1 s\,(e_0 - c_1 - c_2) = (k_{-1} + k_2)\,c_1$$

$$\frac{dc_2}{dt} = 0 \iff k_{-3}\,c_2 = k_3\,(e_0 - c_1 - c_2)\,i$$

$$\left.\begin{aligned} k_m &\triangleq \frac{k_2 + k_{-1}}{k_1} \\ k_{eq_3} &\triangleq \frac{k_{-3}}{k_3} \end{aligned}\right\} \text{ from previous lectures}$$

Hence we have:

$$\begin{cases} k_m c_1 = s\,(e_0 - c_1 - c_2) \\ k_{eq_3}\,c_2 = i\,(e_0 - c_1 - c_2) \end{cases} \iff \begin{cases} c_1 = \dfrac{s}{i}\,\dfrac{k_{eq_3}}{k_m}\,c_2 \\ k_{eq_3}\,c_2 = e_0 i - s\,\dfrac{k_{eq_3}}{k_m}\,c_2 - i\,c_2 \end{cases}$$

$$\iff \begin{cases} c_2 = \dfrac{e_0 i}{k_{eq_3} + s\,\dfrac{k_{eq_3}}{k_m} + i} \\ c_1 = \dfrac{s e_0}{k_{eq_3} + s\,\dfrac{k_{eq_3}}{k_m} + i}\,\dfrac{k_{eq_3}}{k_m} \end{cases} \iff \begin{cases} c_1 = \dfrac{k_{eq_3}\,s\,e_0}{k_m k_{eq_3} + s\,k_{eq_3} + k_m i} \\ c_2 = \dfrac{k_m e_0 i}{k_m k_{eq_3} + s\,k_{eq_3} + k_m i} \end{cases}$$

And the velocity of the reaction results:

$$V = k_2 c_1 = \frac{k_2\,k_{eq_3}\,e_0\,s}{k_m k_{eq_3} + k_{eq_3} s + k_m i} = \frac{V_{max}\,s}{k_m + s + k_m\,(i/k_{eq_3})}$$

$$V_{max} \triangleq k_2 e_0$$

Now let us compare to what we had at the end of lecture 2:

lect. 2: $V = \dfrac{V_{max}\,s}{k_m + s}$ Now: $V = \dfrac{V_{max}\,s}{k_m + s + k_m\,(i/k_{eq_3})}$

The velocity is reduced. More specifically, the effective equilibrium constant is increased from k_m to $k_m\left(1+{}^{i}/_{k_{eq_3}}\right)$

- Cooperativity:

There are situations where the model

$$S+E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \xrightarrow{k_2} E+P$$

is not enough to estimate V or the evolution of [S]. The problem is not the inhibition of the enzyme E but it rather depends on the dynamics of the reaction between E and S

Ex: E has two sites for binding (i.e., it can bind with two molecules of S) but the reactions do not need to occur at the same time

⇓

We cannot write: $2S+E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \xrightarrow{k_2} E+P$

Moreover, E can catalyze the production of P both if it is bounded to one molecule of S or two molecules of S but the reaction rate changes with the number of molecules of S

⇓

We need a new model to capture these facts

Ex: An analogy comes with Hb (hemoglobin), that is a protein-based compound that binds with up to 4 O_2 molecules to transport oxygen in the blood - The binding happens in cascade (one O_2 at the time) with each step facilitating

the following one

Let us focus on the case with E binding with up to two molecules of S:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \xrightarrow{k_2} E + P \qquad k_2 \neq k_4$$

$$C_1 + S \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} C_2 \xrightarrow{k_4} C_1 + P$$

$C_1 \triangleq$ type 1 complex (one S involved)
$C_2 \triangleq$ type 2 complex (two S involved)

Let us write the ODE model:

$s \triangleq [S]$
$p \triangleq [P]$
$c_1 \triangleq [C_1]$
$c_2 \triangleq [C_2]$
$e \triangleq [E]$

$$\begin{cases} \frac{ds}{dt} = -k_1 s e + k_{-1} c_1 - k_3 s c_1 + k_{-3} c_2 \\ \frac{dc_1}{dt} = k_1 s e - (k_{-1} + k_2) c_1 - k_3 s c_1 + (k_{-3} + k_4) c_2 \\ \frac{dc_2}{dt} = k_3 s c_1 - (k_{-3} + k_4) c_2 \\ \frac{dp}{dt} = k_2 c_1 + k_4 c_2 \end{cases}$$

Remember: $e + c_1 + c_2 = e_0$ - constant $\Rightarrow$ We can drop one ODE

QSS approximation: $\frac{dc_2}{dt} = 0 \iff k_3 s c_1 = (k_{-3} + k_4) c_2$

$$\frac{dc_1}{dt} = 0 \iff k_1 s e = (k_{-1} + k_2) c_1$$

From these conditions it results still: $\frac{ds}{dt} = -\frac{dp}{dt}$

In analogy with what we did last time, let us call:

$$k_m \triangleq \frac{k_{-1}+k_2}{k_1} \qquad k_r \triangleq \frac{k_{-3}+k_4}{k_3}$$

We have:

$$\begin{cases} s c_1 = k_r c_2 \\ s(e_0 - c_1 - c_2) = k_m c_1 \end{cases} \iff \begin{cases} c_2 = s c_1 / k_r \\ s e_0 - s c_1 - s^2 c_1 / k_r = k_m c_1 \end{cases}$$

$$\iff \begin{cases} c_1 = \dfrac{k_r e_0 s}{k_m k_r + k_r s + s^2} \\ c_2 = \dfrac{e_0 s^2}{k_m k_r + k_r s + s^2} \end{cases}$$

Hence the velocity of the reaction is:

$$v = \frac{1}{k_m k_r + k_r s + s^2}\left(k_2 k_r e_0 s + k_4 e_0 s^2\right)$$

Case 1: Binding sites act independently and identically with reaction rates k_+ (forward) and k_- (backward) - Then we have:

$$k_1 = 2k_+ \qquad k_{-1} = k_- \qquad k_4 = 2k_2$$

$$k_3 = k_+ \qquad k_{-3} = 2k_-$$

Hence: $k_m = \dfrac{k_{-1}+k_2}{k_1} = \dfrac{k_- + k_2}{2k_+}$ $\qquad k_r = \dfrac{k_{-3}+k_4}{k_3} = \dfrac{2k_- + 2k_2}{k_+}$

So if we call: $k \triangleq \dfrac{k_- + k_2}{k_+}$, we have: $k_m = \dfrac{k}{2}$; $k_r = 2k$

$$V = \frac{2k_2 e_0 (k+s)s}{\underbrace{k^2+2ks+s^2}_{=(k+s)^2}} = \frac{\overbrace{2k_2 e_0 s}^{V_{max}}}{k+s}$$

- We obtain the formula for V seen last time multiplied by 2 (because of two binding sites)

This defines cooperation ←

Case 2: The rate of reaction of the first binding is much slower than the rate of the second binding, e.g.:

$k_1 \cdot k_3 = \alpha$ - constant and $k_1 \to 0$; $k_3 \to \infty$

In this case we have:

$$k_m = \frac{k_{-1}+k_2}{k_1} \to \infty \qquad k_r = \frac{k_{-3}+k_4}{k_3} \to 0$$

$$k_m \cdot k_r = \frac{(k_{-1}+k_2)(k_{-3}+k_4)}{\underbrace{k_1 \cdot k_3}_{=\alpha}} \text{ - constant}$$

So we have:

$$V = \frac{1}{\underbrace{k_r k_m}_{=\alpha} + \underbrace{k_r s}_{\to 0} + s^2} \left(\overbrace{k_2 k_r e_0 s}^{\to 0} + k_4 e_0 s^2\right) \Rightarrow V \cong \frac{1}{\alpha + s^2} k_4 e_0 s^2$$

$\to 0$ because $k_r \to 0$ and s is bounded

If we call: $\hat{k}_m^2 \triangleq \alpha$ $\hat{V}_{max} \triangleq k_4 e_0$, we have:

$$V = \frac{V_{max} s^2}{\hat{k}_m^2 + s^2}$$

Formally, we obtained a formula that is similar to the formula we originally

had for the case with enzymes having only one binding site. The major differences are:

s^2 instead of s

$k_m k_r$ instead of k_m^2

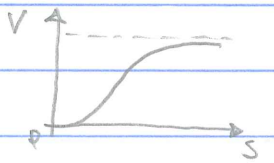
In general, it can be shown that if the enzyme can bind sequentially to n molecules and each molecule has an equilibrium constant K_i $i=1,2,\dots,n$ and $K_1 \to \infty$ while $K_n \to 0$ and $K_1 \cdot K_n = \text{constant}$, then:

$$V = \frac{V_{max}\, s^n}{\prod_{i=1}^{n} K_i + s^n} \qquad \text{(Hill Equation)}$$

$\prod_{i=1}^{n} K_i = K_1 \cdot K_2 \cdot K_3 \cdots K_n$

The relationship between V and s is sigmoidal

Note that:



$$V\left(s^n + \prod_i K_i\right) = V_{max}\, s^n$$

$$\Updownarrow$$

$$(V_{max} - V)\, s^n = V\left(\prod_i K_i\right)$$

Applying $\ln$ to both sides: $\Updownarrow$

$$\ln(V_{max} - V) + n \ln s = \ln V + \sum_{i=1}^{n} \ln K_i$$

$$\Updownarrow \quad K_i = K^* \quad i = 1,2,\dots,n$$

$$n \ln s = \ln\left(\frac{V}{V_{max} - V}\right) + n \ln K^*$$

$$\Updownarrow$$

$$\ln s = \frac{1}{n} \ln\left(\frac{V}{V_{max} - V}\right) + \ln K^*$$

The plot of $\ln\left(\frac{V}{V_{max}-V}\right)$ vs. $\ln(S)$ should be a line

Case Study: Binding of oxygen to Hemoglobin

The equivalent reaction between Hb (hemoglobin) and O_2 (oxygen) is

$$Hb + 4O_2 \rightleftharpoons HbO_8$$

Hb, though, is a protein-based molecule with 4 binding sites and a cooperative behavior:

$$Hb + O_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \qquad \text{(binding 1 site)}$$

$$C_1 + O_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C_2 \qquad \text{(binding 2 sites)}$$

$$C_2 + O_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_3 \qquad \text{(binding 3 sites)}$$

$$C_3 + O_2 \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} HbO_8$$

Full disclosure: Hb is not an enzyme (although it shares a protein-based nature as many enzymes). However, the cascade of reactions is a good example of cooperation

Compared to the enzyme reaction paradigm, we have no product released at the first 3 steps and no intermediate complex at step 4

Let us write the equations:

$h \triangleq [Hb]$

$o \triangleq [O_2]$

$c_i \triangleq [c_i] \quad i = 1,2,3$

$c_4 \triangleq [HbO_8]$

$$\begin{cases} \frac{do}{dt} = \sum_{i=1}^{4} k_{-i} c_i - (k_1 h + k_2 c_1 + k_3 c_2 + k_4 c_3)\, o \\ \frac{dc_1}{dt} = k_1 h o + k_{-2} c_2 - (k_{-1} + k_2 o) c_1 \\ \frac{dc_2}{dt} = k_2 c_1 o + k_{-3} c_3 - (k_{-2} + k_3 o) c_2 \\ \frac{dc_3}{dt} = k_3 c_2 o + k_{-4} c_4 - (k_{-3} + k_4 o) c_3 \\ \frac{dc_4}{dt} = k_4 c_3 o - k_{-4} c_4 \end{cases}$$

Remember: $h + c_1 + c_2 + c_3 + c_4 = h_0$ - constant $\Rightarrow$ We can drop one equation

$$\frac{dc_1}{dt} = 0 \Leftrightarrow (k_{-1} + k_2 o) c_1 = k_1 h o + k_{-2} c_2$$

$$\frac{dc_2}{dt} = 0 \Leftrightarrow (k_{-2} + k_3 o) c_2 = k_2 c_1 o + k_{-3} c_3$$

$$\frac{dc_3}{dt} = 0 \Leftrightarrow (k_{-3} + k_4 o) c_3 = k_3 c_2 o + k_{-4} c_4$$

$$\frac{dc_4}{dt} = 0 \Leftrightarrow k_4 c_3 o = k_{-4} c_4$$

Hence: $k_4 c_3 o = k_{-4} c_4 \Rightarrow k_{-3} c_3 + \cancel{k_{-4} c_4} = k_3 c_2 o + \cancel{k_{-4} c_4} \Rightarrow k_3 c_2 o = k_{-3} c_3$

$k_3 c_2 o = k_{-3} c_3 \Rightarrow k_{-2} c_2 + \cancel{k_{-3} c_3} = k_2 c_1 o + \cancel{k_{-3} c_3} \Rightarrow k_2 c_1 o = k_{-2} c_2$

$k_2 c_1 o = k_{-2} c_2 \Rightarrow k_{-1} c_1 + \cancel{k_{-2} c_2} = k_1 h o + \cancel{k_{-2} c_2} \Rightarrow k_1 h o = k_{-1} c_1$

And: $c_4 = \left(\frac{k_4}{k_{-4}}\right) o \cdot c_3 \qquad c_2 = \left(\frac{k_2}{k_{-2}}\right) o\, c_1$

$c_3 = \left(\frac{k_3}{k_{-3}}\right) o\, c_2 \qquad c_1 = \left(\frac{k_1}{k_{-1}}\right) o h$

Because: $h = h_o - c_1 - c_2 - c_3 - c_4$ we have:

$$keq_i \triangleq k_{-i}/k_i$$

$$c_4 = \frac{1}{keq_4} o\, c_3 = \frac{1}{keq_4} \cdot \frac{1}{keq_3} o^2 c_2 = \frac{1}{keq_4\, keq_3\, keq_2} o^3 c_1$$

$$c_3 = \frac{1}{keq_3\, keq_2} o^2 c_1 \qquad c_2 = \frac{1}{keq_2} o\, c_1$$

$$c_1 = \frac{1}{keq_1} o h = \frac{1}{keq_1} o \left(h_o - c_1 - \frac{o c_1}{keq_2} - \frac{o^2 c_1}{keq_2\, keq_3} - \frac{o^3 c_1}{keq_2\, keq_3\, keq_4} \right)$$

$$\left(1 + \frac{o}{keq_1} + \frac{o^2}{keq_1\, keq_2} + \frac{o^3}{keq_1\, keq_2\, keq_3} + \frac{o^4}{keq_1\, keq_2\, keq_3\, keq_4} \right) c_1 = \frac{o\, h_o}{keq_1}$$

Let us call: $K^4 \triangleq keq_1\, keq_2\, keq_3\, keq_4$ - We have:

$$c_1 = \frac{K^4}{keq_1} \frac{o\, h_o}{\left(K^4 + \frac{K^4}{keq_1} o + \frac{K^4}{keq_1\, keq_2} o^2 + keq_4\, o^3 + o^4 \right)}$$

$$c_4 = \frac{o^4 h_o}{K^4 + \frac{K^4}{keq_1} o + \frac{K^4 o^2}{keq_1\, keq_2} + keq_4\, o^3 + o^4}$$ ⇐ This is the final product

Now, let us remember the assumptions of the Hill Equation:

$k_1 \to \infty$

$k_n \to 0$ - So let us assume (this is the case in reality) that:

$\prod_{i=1}^{n} k_i = \text{constant}$

$keq_1 = \frac{k_{-1}}{k_1} \to \infty$ (i.e., $k_1 \to 0$) $\qquad keq_4 = \frac{k_{-4}}{k_4} \to 0$ (i.e., $k_4 \to \infty$)

So we have:

$$k_{eq_4} o^3 \cong 0$$

$$\frac{k^4 o^2}{k_{eq_1} k_{eq_2}} = \frac{\text{constant} \cdot o^2}{k_{eq_1} \cdot k_{eq_2}} \to 0 \quad (k_{eq_1} \cdot k_{eq_2} \to \infty)$$

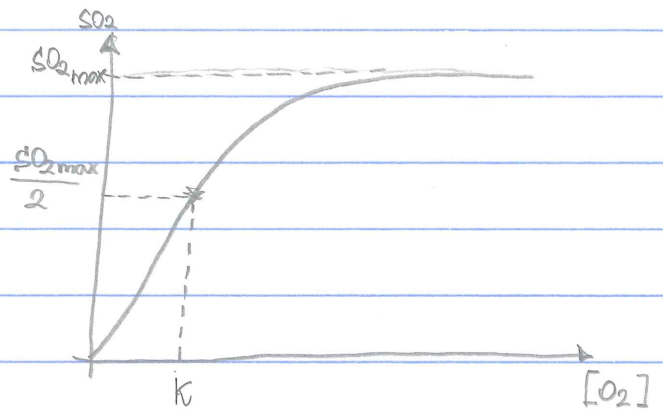
$$\frac{k^4}{k_{eq_1}} o = \frac{\text{constant} \cdot o}{k_{eq_1}} \to 0 \quad (k_{eq_1} \to \infty)$$

$$\Rightarrow c_4 \cong \frac{h_0 o^4}{k^4 + o^4}$$

Note this: the concentration c_4 of fully-bound hemoglobin HbO_8 is less than the original concentration $h_0 \Rightarrow$ There is a saturation: and the percentage of available hemoglobin sites that are bound to oxygen is:

$$SO_2 \triangleq 100 \cdot \frac{o^4}{k^4 + o^4}$$

where k^4 can be estimated sperimentally from the saturation curve:



REFERENCE:

Textbook (volume 1): chapter 1, sec 1.4.3 - 1.4.4. (till page 17)

Textbook (volume 2): chapter 13, sec 13.3 - 13.3.1 (in part)