Enzyme Regulation

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“Enzyme” Regulation: Hemoglobin

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   b. CO₂ binding
   c. Blood buffer: Bohr effect
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Enzyme Regulation

Control by Allostery:
Allosteric Effectors can change binding/kinetic behavior

EXAMPLE: Aspartate Transcarbamoylase (ATCase)

\[
\begin{align*}
\text{Carbamoyl phosphate} + \text{Aspartate} & \rightleftharpoons \text{Aspartate transcarbamoylase} \\
& \rightarrow \text{N-Carbamoylaspartate}
\end{align*}
\]
Pyrimidine Biosynthesis: ATCase Feedback Inhibition

Control by Allostery:

Enzyme Regulation

CTP is a negative heterotropic allosteric effector molecule

ATP is a positive heterotropic allosteric effector molecule

Enzyme Regulation

Control by Allostery:
Allosteric Effectors can change binding/kinetic behavior

Other examples:

**Why has sigmoidal kinetics evolved?**

A→S→P→Q

If [S] decreases, the rate of its use goes down. Eventually, the [S] is replenished.

If [S] increases, the rate of enzyme goes up, and it gets used up. Eventually, the [S] is lowered.

This acts to "buffer" the activity at around \( \frac{1}{2} V_{\text{max}} \), often right at the homeostatic [S].

Figure 10.4. The abolition of positive cooperativity on the binding of allosteric effectors to some enzymes. Note the dramatic increases in activity at low substrate concentrations on the addition of adenine monophosphate to isocitrate dehydrogenase, of deoxyxysteine diphosphate to deoxyxysteine kinase, and of fructose 1,6-diphosphate to pyruvate kinase; this shows how the activity may be "switched on" by an allosteric effector (PEP = phospho-ethanolamine). (From J. A. Hartman and D. E. Ackerman, J. Biol. Chem. 138, 2175 (1960); R. Ghanem and A. Kornberg, J. Biol. Chem. 239, 975 (1964); R. Haeckel, B. Hess, W. Lütter, and K.-H. Wiesen, Hoppe-Seyler's Z. Physiol. Chem. 249, 699 (1968).)
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Control by Allostery:

What is the degree of cooperativity?

Michaelis-Menten Equation

\[ v_0 = \frac{V_{\text{max}}[S]}{K_m + [S]} \]

Like binding, we can also express as fraction of maximum rate

\[ \frac{v_0}{V_{\text{max}}} = \frac{[S]}{K_m + [S]} \]

Recall for cooperative binding, there is an exponent term on the [S].

\[ \frac{v_0}{V_{\text{max}}} = \frac{[S]^{n'}}{K'_m + [S]^{n'}} \]

What is \( K'_m \) term? The \( K'_m \) term is a mixture of values for both poor-(at low [S]) and high-affinity (at higher [S]) binding sites.

What is the value of this \( n' \) term? The \( n' \) term is related to the degree of cooperativity.

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Control by Allostery:

In 1913, Archibald Hill derived an equation to measure both the \( K'_m \) term, and the \( n' \) term: \textbf{The Hill Equation}

\[
\frac{\text{Fraction of [E] as [E] (Bound)}}{\text{Fraction of [E] as [E] (Free)}} = \frac{Y}{1-Y} = \frac{[S]^{n'}}{K'_m + [S]^{n'}} \\
\text{Related to } \frac{v_0}{V_{\text{max}}} = \frac{[S]^{n'}}{K'_m + [S]^{n'}}
\]

\[
\frac{[S]^{n'}}{K'_m} = \left( \frac{K'_m + [S]^{n'}}{K'_m + [S]^{n'}} \right)
\]

Sometimes you will see: \( n' = h \), the Hill coefficient.

\[ \log \left( \frac{Y}{1-Y} \right) = n' \log [S] - \log K'_m \]

(Eqn for line \( y = ax + b \))

This has the form of a line with \( n' \) as the slope and \( -\log K'_m \) as the x-intercept in a plot of \( \log Y/(1-Y) \) \textit{versus} \( \log [S] \). This is a "Hill Plot."
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Control by Allostery:

The Hill Plot

\[ \log \left( \frac{Y}{1-Y} \right) = n \log [S] - \log K_m \]

Positive cooperativity: \( n > 1 \)
Non-cooperative: \( n = 1 \)
Negative cooperativity: \( n < 1 \)

Theoretical maximum cooperativity = # of binding sites

at low and high [S]
Physically, how does this cooperativity work?

In other words, how is this cooperativity accomplished at the molecular level?

Recall that sigmoidal behavior requires more than one subunit. Therefore, subunits must “communicate,” or binding of one subunit changes the subunit-subunit interface, and this changes how the non-bound subunits will bind.

EXAMPLE:

- Dimer
- Two conformations
  - unbound enzyme = T (tense) state
  - bound enzyme = R (relaxed) state
- Conformation at interface differs
- Binding site differs with binding easier (tighter) in the R-state

In other words, how is this cooperativity accomplished at the molecular level?

Two ways have been proposed to explain the transition for T-state to R-state binding.

This type of cooperativity would be homotropic (caused by the substrate itself).

All at once = CONCERTED
[Proposed by Monod, Wyman, & Changeux (MWC)]

One at a time = SEQUENTIAL
[Proposed by Koshland, Nemethy, & Filmore (KNF)]
Heterotropic cooperativity can be achieved by binding at a different allosteric site.

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Control by Allosteric:

If it binds to the T-state, it would be an inhibitor.
(negative heterotropic allosteric effector)

If it binds to the R-state, it would be an activator.
(positive heterotropic allosteric effector)

How about for a tetramer?

Sequential Model of cooperative regulation

- S does not bind to the T-state.
- Binding of ligand to one subunit changes its conformation.
- That conformational change influences neighboring subunits through changes at the subunit-subunit interface.
- The binding to the neighboring subunits is easier ($K_d$ decreases).
- Model has many different binding and dissociation constants (very complicated rate equations).
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Control by Allostery:
Concerted Model of cooperative regulation

• Looks more complicated, but its simpler in concept and mechanism

• An equilibrium exists between the T-state and the R-state

• Binding of substrate is easier to the R-state

• Binding of substrate to one subunit shifts the equilibrium.

• That shift brings neighboring subunits, which are also high affinity; more "good" sites.

Two Models of Cooperativity:
Concerted vs. Sequential

All at once = CONCERTED [MWC]
One at a time = SEQUENTIAL [KNF]
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Control by Allostery:

We can write the rate equation for the Concerted Model of cooperative regulation:

The equilibrium between the T-state and the R-state = \([T_0]/[R_0] = L\)

Binding of substrate is easier to the R-state, which is reflected in a ratio of dissociation constants = \(c = K_R / K_T\) (less than 1.0).

A rate equation can be derived using these values: For a dimer......

\[
\frac{V_o}{V_{\text{max}}} = \frac{k_{\text{cat}} [\text{ES}]}{k_{\text{cat}} [\text{E}]_T} = \frac{[\text{ES}]}{[\text{E}]_T} = \frac{\text{Bound S}}{\text{Total sites}} = Y = \frac{[S]}{K_R} \left( 1 + \frac{[S]}{K_R} \right)^{n-1} / L \left( 1 + \frac{[S]}{K_R} \right)^{n}.
\]

For dimer, exponent in denominator
If L=0 (no T-state) .......

Recall eqn for coop:

\[
\frac{v}{v_{\text{max}}} = \frac{[S]}{K_R} \left( 1 + \frac{[S]}{K_R} \right)^{n-1} = \frac{[S]}{K_R} \left( 1 + \frac{[S]}{K_R} \right)^n.
\]


Is there structural evidence for these T- and R-states?
Enzyme Regulation
Aspartate Transcarboxylase (ATCase)

Control by Allostery:

ATCase from *E. coli*
PDBids 5AT1 and 8ATC

Enzyme Regulation
Control by Allostery:
ATCase: Conformational Changes: T-State vs. R-State

(a) Inactive T state
(b) Active R state
Enzyme Regulation

Control by Allostery:

Aspartate Transcarboxylase (ATCase)