Enzymes: Regulation 1
Allosteric Regulation and Isozymes

Reading: Berg, Tymoczko & Stryer, 6th ed., Chapter 10, pp. 275-283

ATCase (aspartate transcarbamoylase): allosteric regulation

T state (inactive)  R state (active)

A nicely done Jmol routine on ATCase by students in Bioc 463A:
http://www.biochem.arizona.edu/classes/bioc463a/molecular_graphics_gallery/jmol/atcasea/ATCase.html
There is an R to T animation at the bottom of the page.

The Central Dogma:
DNA: 4 bases (A,T,C,G)

transcription
mRNA
translation
Proteins from 20 naturally occurring amino acids

Function!
Key Concepts

- **Amounts** of many key enzymes are regulated at the level of control of transcription, mRNA processing, and/or translation (mechanisms covered in BIOC 411 or BIOC 461), or destruction (proteolytic degradation) of old/unwanted enzymes.

- **Activities** of many key enzymes are regulated in cells, based on metabolic needs/conditions *in vivo*.

- **Regulation of enzyme activity can increase or decrease substrate binding affinity and/or** $k_{cat}$.

- **5 ways to regulate protein activity (including enzyme activity):**
  1. allosteric control
  2. multiple forms of enzymes (isozymes)
  3. interaction with regulatory proteins
  4. reversible covalent modification
  5. irreversible covalent modification, including proteolytic activation

Key Concepts, continued

1. **Allosteric control**
   - **conformational changes**
     - 2 conformations in equilibrium, "R" (more active) & "T" (less active)
     - allosteric activators (positive effectors/modulators)
     - allosteric inhibitors (negative effectors/modulators)
       - often *feedback* inhibitors (product of pathway inhibits *first committed step* in pathway)
   - Allosterically regulated enzymes always multi-subunit
   - Aspartate transcarbamoylase (*ATCase*) as an example
     - **homotropic effector = activator (substrate aspartate)**
     - **heterotropic effectors (activator = ATP; inhibitor = CTP)**

2. **Isozymes**
   - Multiple forms of an enzyme that catalyze the same reaction
   - Different kinetic parameters like $K_m$, and/or different allosteric regulation, with physiological consequences
   - Hexokinase -- different forms in liver vs. muscle reflect the different roles of those tissues in the body.
Regulatory enzymes

In general,
- Catalyze essentially irreversible metabolic reactions ($\Delta G'$ large, neg.)
- Catalyze the first committed step in a metabolic pathway
- Regulation of such steps permits efficient regulation of flux of metabolites through just that pathway

"Committed step": This step commits a metabolite (small molecule) down pathway to endproduct.
- No other branches lead to different endproducts that need to be regulated separately.
- FIRST committed step = most efficient step for regulation of the rate -- typically is the slowest step in pathway, controlling "flow" of matter to endproduct (whose concentration you want to regulate).

Feedback inhibition: endproduct acts as an allosteric inhibitor of enzyme catalyzing FIRST COMMITTED STEP in that pathway.

5 principal ways protein/enzyme activity is regulated

1. Allosteric control
   - Regulate binding affinity for ligands, and/or of catalytic activity, by conformational changes caused by binding of the same or other ligands at other sites on protein ("allostERIC effects")
   - Changes involve simple association/dissociation of small molecules, so enzyme can cycle rapidly between active and inactive (or more and less active) states.

2. Interaction with regulatory proteins
   - Binding of a different protein to the enzyme alters the enzyme activity (activates or inhibits the enzyme), usually by causing conformational change.

3. Multiple forms of enzymes (isozymes)
   - Isozymes (isoenzymes) = multiple forms of enzyme that catalyze same reaction but are products of different genes (so different amino acid sequences)
   - Isozymes differ slightly in structure, and kinetic and regulatory properties are different.
   - Can be expressed in different tissues or organelles, at different stages of development, etc.
5 principal ways protein/enzyme activity is regulated

4. Reversible covalent modification
   • Modification of catalytic or other properties of proteins by enzyme-catalyzed covalent attachment of a modifying group.
   • Modifications removed by catalytic activity of a different enzyme, so enzyme can cycle between active and inactive (or more and less active) states.

5. Proteolytic activation
   • Irreversible cleavage of peptide bonds to convert inactive protein/enzyme to active form.
   • Inactive precursor protein = a zymogen (a proenzyme).
   • Proteolytic activation irreversible, but eventually the activated protein is itself proteolyzed, or sometimes a tight-binding specific inhibitory protein inactivates it.

Allosteric Regulation

• Multisubunit enzymes (more than one active site per enzyme)
• Regulation of binding affinity for ligands (like substrates) and/or catalytic activity ($k_{cat}$)
• Conformational changes linked with ligand binding
  – homotropic effects: binding of "primary" ligand (substrate for an enzyme, $O_2$ for hemoglobin, etc.) can alter affinity of other binding sites on molecule for that same ligand
  – heterotropic effects: binding of other ligands (regulatory signaling molecules), to different sites from the primary ligand ("regulatory sites") can cause conformational changes that alter primary ligand binding affinity or catalytic activity
  – Sometimes regulatory sites are on different subunits ("regulatory subunits") from binding sites for primary ligand.
• Ligand binding-induced conformational changes:
  • Ligand concentration = signal (cell needs more or less of some metabolic product)
  • Signal detected by regulated enzyme
• Allosteric regulation permits very rapid cycling of enzyme between more active and less active conformations.

• Allosteric activators ---> increase activity
  – Homotropic effector (substrate itself)
  – Heterotropic effectors, e.g.
    – Metabolite (product) from a reaction upstream (“feed-ahead activation”)
    – Other metabolites (ligands) from other pathways that act as indicator(s) of cellular metabolic need

• Allosteric inhibitors ---> decrease activity
  – Heterotropic effectors/modulators
  – Endproduct of whole pathway (“feedback inhibition”)
  – another ligand that acts as indicator that cell needs less of that pathway’s product

Homoallostery
• Allosteric enzymes have Sigmoidal $V_o$ vs. [S] plots. (look familiar?)
• Cooperative substrate binding/activation (cooperativity): S binding to one active site alters S binding affinity and/or catalytic activity at other active sites on same enzyme molecule.
• Homoallostery: effect is due to the Substrate alone.
Heteroallostery

Regulation by heterotrophic effectors (non-Substrate):
- positive (activation, favors R state), or
- negative (inhibition, favors T state)
- Heterotropic effectors bind to different site from active site.

Aspartate transcarbamoylase ("ATCase")

- first committed step in the pathway for biosynthesis of pyrimidine nucleotides
- Nucleotides:
  - compounds whose 3 covalently linked components are
    - heterocyclic "base" (A, G, C or T in DNA; usually A, G, C or U in RNA)
    - sugar (deoxyribose in DNA, ribose in RNA)
    - phosphate
  - building blocks of nucleic acids
  - other major roles
    - coenzymes
    - energy storage compounds
    - regulators of enzyme activity
ATCase reaction

- Condensation of Asp + carbamoyl phosphate

\[ \text{Asp} + \text{carbamoyl phosphate} \rightarrow \text{carbamoyl aspartate} + P_i \]

endproduct of pathway: CTP

Berg et al., Fig. 10-1

PALA: a bisubstrate analog for ATCase

- A molecule used to stabilize R state (active conformation) of ATCase to study its structure
- Can’t react to form products

Berg et al., Fig. 10-7
Homoallostery in ATCase

- In absence of any substrate or regulators, ATCase R-T equilibrium favors T state by a factor of about 200: $[T_o] / [R_o] = \sim 200$
- ATCase binds the substrate aspartate cooperatively (sigmoidal kinetics).
- T state has a very HIGH $K_m$ for Asp.
- R state (predominates at high [S]) has a much LOWER $K_m$ for Asp.

**ATCase -- Substrate Binding**

- **cooperative substrate binding**: mixture of R and T states
- Equilibrium at very low [S] lies far toward T conformation (T/R = 200/1).
- As Asp binds, T $\Rightarrow$ R equilibrium shifted to right.

As [Asp] increases enzyme shifts from T to R:
1. Activity increases steeply
2. apparent $K_m$ decreases giving the sigmoid plot of $V_o$ vs. [Asp]

Look familiar?

Berg et al., Fig. 10-10

Enzymes: Regulation 1
Structural basis for allosteric regulation in ATCase

- **Quaternary structure (subunit structure) of ATCase:**
  - 12 subunits:
    - 6 catalytic chains total, arranged in 2 $c_3$ catalytic trimers (blue)
    - 6 regulatory chains total, arranged in 3 $r_2$ regulatory dimers (red)

- Regulatory dimers NOT required for catalytic trimers ($c_3$) activity
- Asp binding to isolated catalytic trimers ($c_3$) NOT cooperative (no communication between catalytic subunits).
- CTP (feedback inhibitor) has no effect on activity of isolated catalytic trimers
- Regulatory subunits bind heterotrophic effectors, CTP (feedback inhibitor) and ATP (allosteric activator)

Another view of ATCase structure
ATCase active sites at interfaces between catalytic subunits

- 3 active sites per catalytic trimer
- PALA (bisubstrate analog) binds very tightly, interacting with residues on both sides of subunit interfaces.

Quaternary structural changes T → R in ATCase

T state, “tense” form, more compact, less active

R state, “relaxed” form, expanded, more active; favored by PALA binding (PALA a bisubstrate analog) and by ATP binding.

To see how large the actual changes are check out this Jmol routine:
http://www.biochem.arizona.edu/classes/bioc463a/molecular_graphics_gallery/jmol/atcasea/ATCase.html
There is an R to T animation at the bottom of the page.
T state, less active, stabilized by CTP binding

CTP is a feedback inhibitor, the endproduct of the pathway of pyrimidine nucleotide biosynthesis.

CTP bind to regulatory subunit and locks enzyme in T state.

Berg et al., Fig. 10-11

Quaternary structural changes T --> R in ATCase

In absence of any substrate or regulators, ATCase R-T equilibrium favors T state by a factor of about 200:

\[
\frac{[T_o]}{[R_o]} = \sim 200
\]

Berg et al., Fig. 10-12
Heterotropic Effects in ATCase

- Heterotropic ligands bind to the **regulatory subunits** of ATCase.
- **CTP** (endproduct of whole pathway): allosteric **inhibitor** of ATCase.
  - binds preferentially to **T state** of whole ATCase
  - thus decreasing binding affinity for Asp (substrate) at active sites on catalytic subunits.
  - Lower affinity for Asp means apparent $K_m$ increases, so at any given Asp concentration, $V_o$ is decreased.
  - This is the essence of **FEEDBACK INHIBITION**: the endproduct of pathway (CTP) signaling back and slowing down the first committed step.

![Graph showing the effects of CTP on the rate of N-carbamoylaseptide formation](image)

Heterotropic Effects in ATCase

- **ATP**, a purine, is an allosteric **activator** of ATCase.
  - preferentially binds to **R state**
  - shifts R-T equilibrium toward R state, which binds Asp more tightly, so $V_o$ vs. [S] curve shifts toward LEFT, as shown in blue.
  - Competes with CTP for binding the regulatory/nucleotide-binding site on regulatory subunits.

![Graph showing the effects of ATP on the rate of N-carbamoylaseptide formation](image)

Berg et al., Fig. 10-14
Heterotropic Effects in ATCase, continued

- ATP activates ATCase and thus leads to more pyrimidine biosynthesis
- ATP is a purine nucleotide, not related to the pyrimidine biosynthetic pathway.
- Why would ATP be an allosteric activator of ATCase?
  - ATP is used to "store" metabolic energy in the cell.
  - High concentration of ATP an indicator that the cell is energy-rich.
  - High [ATP] concentration thus "tells" the cell
    - there are lots of purine nucleotides available, so more pyrimidine nucleotides are needed to keep nucleotide pool balanced for nucleic acid biosynthesis, and
    - cell is in great shape metabolically and wants to replicate its DNA and divide; high concentration of nucleotides is needed for cell division.
  - High [ATP] thus can "override" inhibitory signal of high [CTP] and activate ATCase.
- ATP binds to the same nucleotide binding site on the regulatory subunits that CTP binds to -- if CTP binds, equilibrium shifts toward T state; if ATP binds, equilibrium shifts toward R state.

Isozymes (Isoenzymes)

- Multiple forms of enzyme that catalyze same reaction
- Different amino acid sequences (products of different genes)
- Expressed in different tissues or organelles, at different stages of development, to meet different metabolic/regulatory criteria.
- Different kinetic parameters like $K_m$, and/or different allosteric regulation, with physiological consequences
- Example: hexokinase (in muscle) vs. glucokinase (in liver)
  - Both enzymes phosphorylate glucose inside cells using ATP trapping it inside
  - Hexokinase (muscle): low $K_m$ for glucose, ~0.1 mM (so working at ~V$_{max}$, since cellular [glucose] ~2-5 mM); inhibited by product, glucose-6-phosphate -- if G-6-P is building up, muscle won’t take more in from blood.
  - muscle contraction requires a lot of energy, derived from blood glucose.
  - Glucokinase (liver): high $K_m$ for glucose, ~10 mM, so activity regulated by blood [glucose]; not inhibited by product G-6-P
  - 1 major liver function = maintenance of blood [glucose] at ~4-5 mM; liver takes up and stores excess glucose, or makes more glucose and exports it, as needed.
Isozymes of hexokinase: different metabolic roles

Blood glucose ~4-5 mM

- **Hexokinase (muscle)** $K_m \sim 0.1$ mM, already operating near $V_{max}$ when blood [glucose] increases above 5 mM, so little change
- **Glucokinase (liver)** $K_m \sim 10$ mM, regulated directly by changes in conc. of blood glucose -- $V_o$ vs. [glucose] changing steeply in [glucose] range below $K_m$

Dr. Tischler will talk about this in more detail, later.

Nelson & Cox, *Lehninger Principles of Biochemistry*, 4th ed., Fig. 15-16

Learning Objectives

- **Terminology** (some are review): quaternary structure, multimeric protein, homopolymeric protein, heteropolymeric protein, ligand, binding site, fractional saturation, feedback inhibition, cooperativity, cooperative binding, allosteric, homotropic effector/regulator, heterotropic effector/regulator, allosteric activator (positive heterotropic effector/regulator), allosteric inhibitor (negative heterotropic effector/regulator), protomer, prosthetic group, isozyme.
- Briefly explain the allosteric regulation of ATCase, including its quaternary structure, its role in metabolism, and how its activity is regulated by allosteric inhibition and activation. Include the physiological rationale for the inhibition and activation.
- Sketch plots of $V_o$ vs. [S] for an allosteric enzyme that illustrate positive homotropic regulation and positive and negative heterotropic regulation, with ATCase as an example. Specifically, sketch (all on the same axes) for ATCase: $V_o$ vs. [aspartate] curves with no heterotropic regulators present, with an allosteric inhibitor present, and with an allosteric activator present.
- Explain the biological usefulness of isozymes, and discuss the example of muscle hexokinase vs. liver glucokinase in terms of difference in function of the tissues.