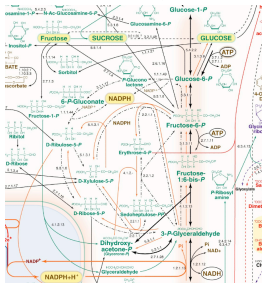
Chem 452 - Lecture 6
Regulatory Strategies
Part 1

Living cells contain thousands of metabolites linked to one another by a dizzying array of chemical reactions. These reactions link one metabolite to another and collectively are arranged into metabolic pathways, which crisscross and intersect to form a large interconnected network. Each reaction is catalyzed by one or more enzymes and many of these enzymes play a large role in controlling the flow of material through the network. In this lecture we will focus on some of the strategies used to regulate enzyme activity, and consequently, metabolic processes.

Introduction

- Metabolism comprises a vast network of interconnecting metabolic pathways.

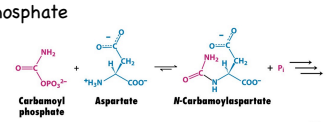


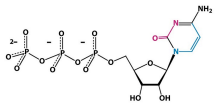
Introduction

- One of the primary strategies for regulating metabolism is to regulate the activity of some of the key enzymes in this network.
- There are several mechanisms used to do this:
  - **Allosteric Control**
  - **Multiple Forms of Enzymes (Isozymes)**
  - **Reversible Covalent Modifications**
  - **Proteolytic Activation**
  - **Controlling the level of Enzyme Present**

ATCase is Allosterically Regulated

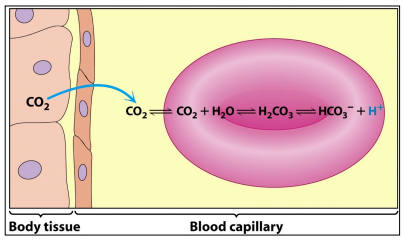
- Aspartate transcarbamoylase (ATCase) provides an good example of allosteric control.
  - ATCase catalyzes the first reaction in the pathway leading to the synthesis of the nucleotide cytidine triphosphate





Allosteric Regulation

- CO_2 lowers Hb's affinity for O_2 by two different mechanisms.
  - It lowers the pH



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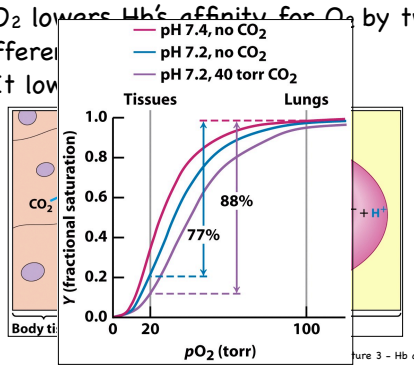

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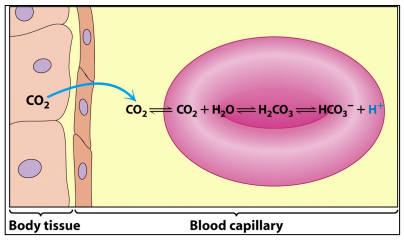
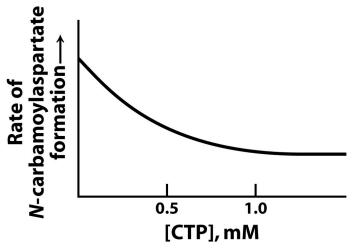


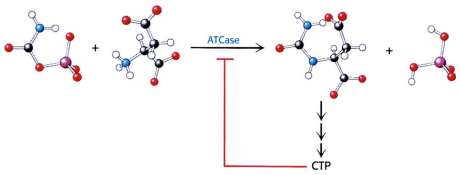
ATCase is Allosterically Regulated

- ATCase is inhibited by the end-product of this pathway (CTP).



ATCase is Allosterically Regulated

- ATCase is inhibited by the end-product of this pathway (CTP).
  - This is an example of **feedback inhibition**.
  - This is a common strategy used to regulate biosynthetic pathways.



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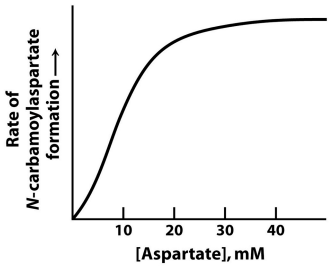
- Most allosterically regulated enzymes do not obey Michaelis-Menten kinetics.



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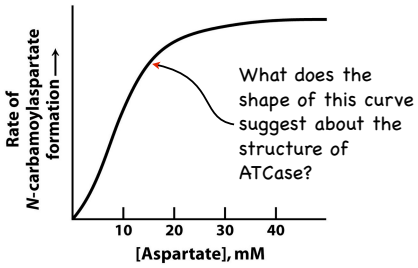

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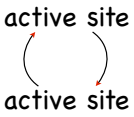

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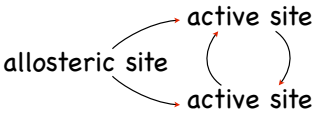
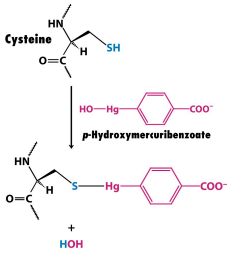


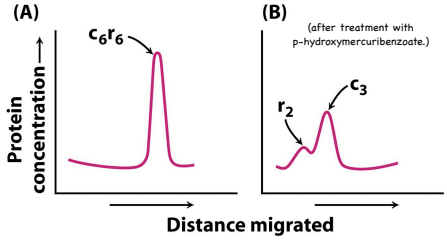
ATCase is Allosterically Regulated

- ATCase can be separated into individual subunits by reacting it with p-hydroxymercuribenzoate.



ATCase is Allosterically Regulated

- The subunits can then be separated by ultracentrifugation.



ATCase is Allosterically Regulated

- The subunit arrange was determined by the ultracentrifugation studies:
  - There are 2 types of subunits (c and r)
  - There are 6 of each type (c_6r_6)
  - The c subunits form trimers (c_3)
  - The r subunits form dimers (r_2)

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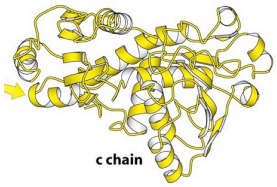
$$2\ c_3 + 3\ r_2 \longrightarrow c_6r_6$$

ATCase is Allosterically Regulated

- The larger, **c** subunits (34 kDa each) are the **catalytic** subunits and contain the active sites.



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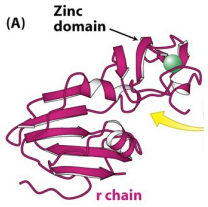
ATCase is Allosterically Regulated

- The smaller **r** subunits (17 kDa each) are the **regulatory** subunits and contain the allosteric sites



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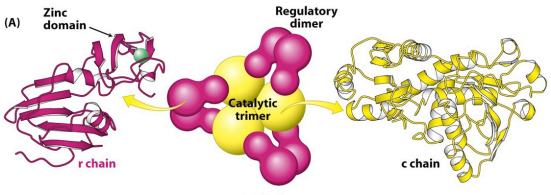
ATCase is Allosterically Regulated

- The three dimers of **r** subunits bridge the two trimers of **c** subunits.



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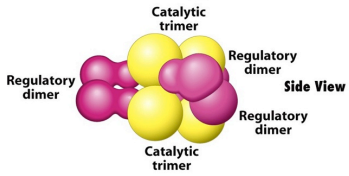

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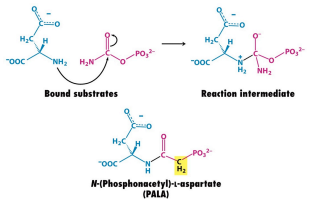
ATCase is Allosterically Regulated

- A substrate analogue, N-phosphoacetyl-L-aspartate (PALA), was used to locate the active sites.



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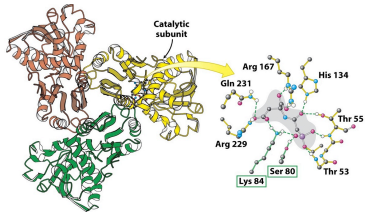

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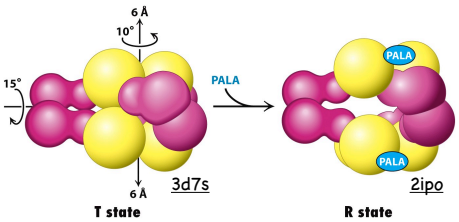
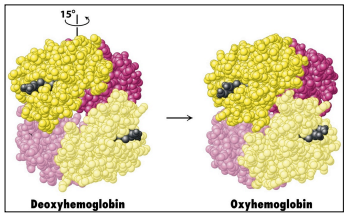
ATCase is Allosterically Regulated

- The binding of PALA produces a large conformational change in the enzyme.



Hb Binds O_2 Cooperatively

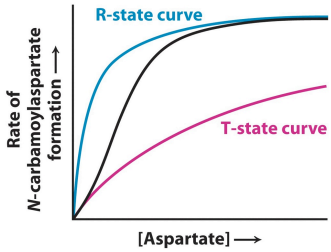
- Cooperativity is associated with changes in the quaternary structure of Hb



Tense (T) State Relaxed (R) State

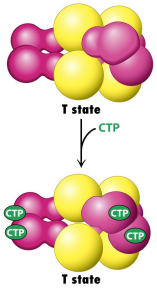
ATCase is Allosterically Regulated

- The kinetics can be interpreted in the same way used to interpret the O_2 binding to hemoglobin.



ATCase is Allosterically Regulated

- Cytidine triphosphate (CTP) binds to the allosteric sites and stabilizes the T-state of the enzyme.



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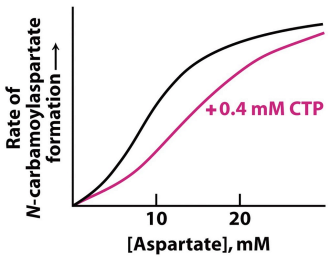
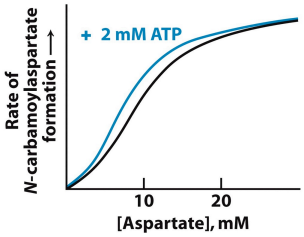
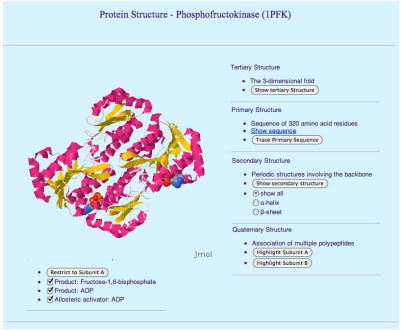


ATCase is Allosterically Regulated

- Adenosine triphosphate (ATP) also an **allosteric effector** for ATCase.
  - It enhances rather than inhibits the ATCase activity.



Phosphofructokinase is also Allosterically Regulated



LDH Has Multiple Isozymes

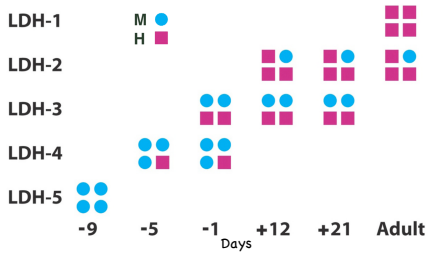
- Another strategy for regulating enzyme activity is to produce multiple forms of an enzyme.
- These multiple forms are called **isozymes**.
  - They are homologous proteins with different amino acid sequences
  - They usually display different K_M values with respect to the substrate.
- **Lactate Dehydrogenase** (LDH) provides a good example.

LDH Has Multiple Isozymes

- There are two isozymes of LDH
  - M (for skeletal muscle)
  - H (for heart muscle)
    - Has lower K_M than M isozyme
    - Is allosterically inhibited by pyruvate
- LDH is tetrameric
  - H and M subunits can be combined in different ratios:
    - H_4
    - H_3M
    - H_2M_2
    - HM_3
    - M_4

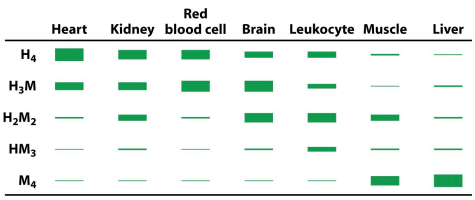
LDH Has Multiple Isozymes

- Altering the ratio of the two isozymes of LDH is used to modify LDH activity during the life of a rat.



LDH Has Multiple Isozymes

- It is also used to modify the activities of LDH in different tissues.



Next up

- Regulatory Strategies, con'd (Chapter 10)
  - Reversible covalent modifications
  - Proteolytic activation of proenzymes