PHRM 836 September 15, 2015

Enzyme Catalysis: regulation and inhibition

Devlin, section 10.10, 10.11, 10.9

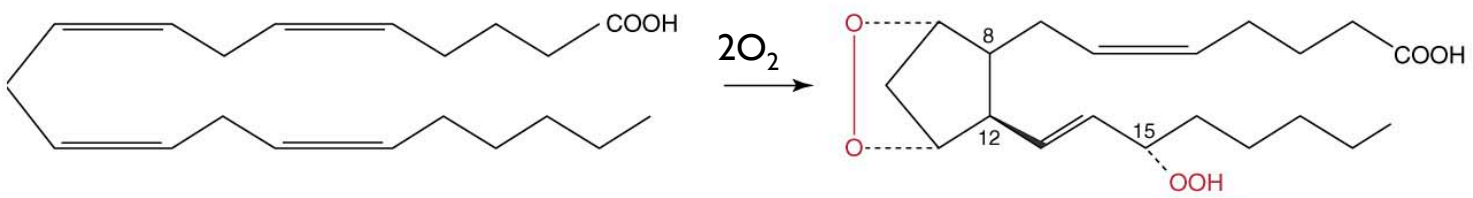
1. Discussion of statins: substrate-analogue inhibitors of HMG-CoA reductase
2. NSAIDs
   - Selectivity of COX inhibitors
3. Concept of cooperativity related to allosteric enzymes
4. Kinetics of enzyme inhibition
   - Mechanisms
   - Changes in K_M and V_{max}
5. Enzyme inhibitors
   - Irreversible
   - Mechanism-based

Statins: substrate-analogue inhibitors of HMG-CoA reductase

- Why are statins effective at lowering cholesterol?
- Why is it advantageous that statins have nanomolar affinity?
- Explain the observation that statins are competitive with HMG-CoA.
- Is it likely that statins are competitive with NADPH?
- If HMG-CoA reductase was a fully rigid molecule, would Pfizer be marketing Lipitor?

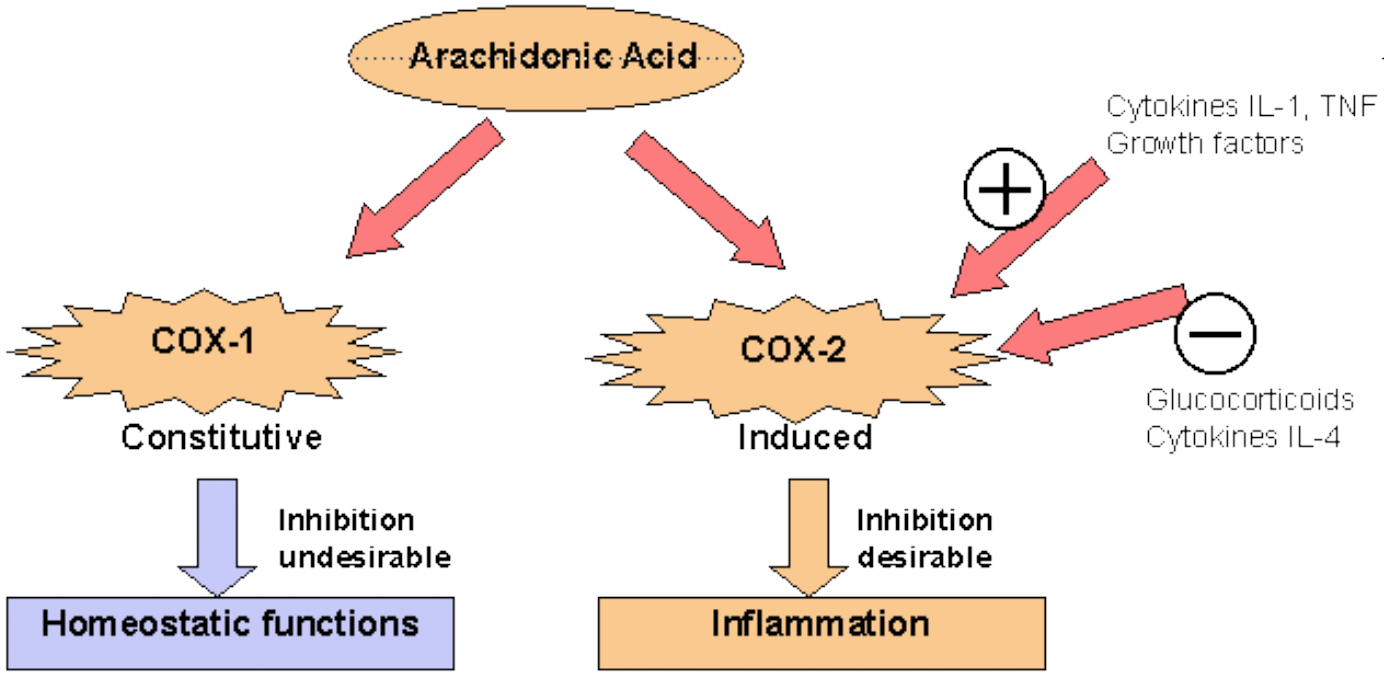
NSAIDs

- Non-steroidal anti-inflammatory drugs
- Block prostaglandin production.
- Irreversibly or reversibly inhibit cyclooxygenase (COX).
- Examples: aspirin, ibuprofen, naproxen
- Commonly inhibits COX-1 and COX-2 nonspecifically
  - COX-1: constitutive enzyme
  - COX-2: inducible and produced in response to inflammation
  - Nonspecific inhibition may result in side effects including gastrointestinal bleeding.



Arachidonic acid PGG_2

Comparison of two cyclooxygenases: need for selective inhibition

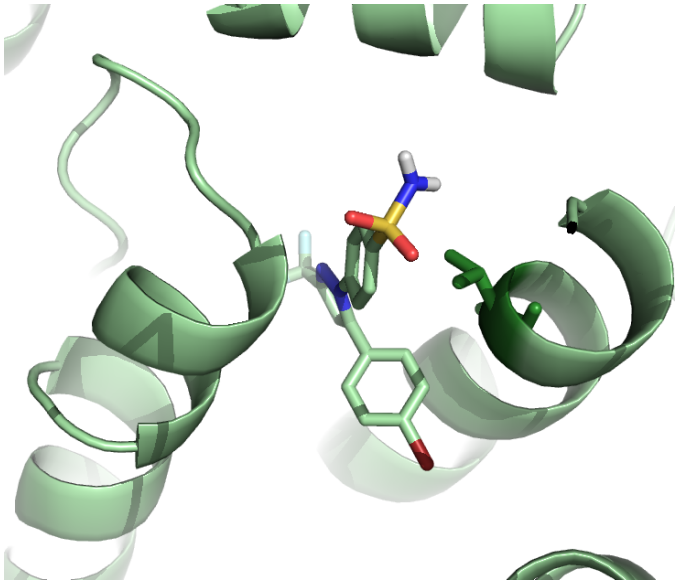


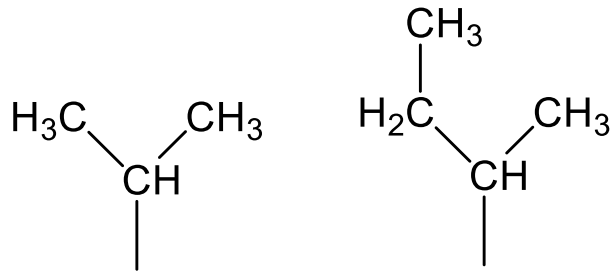
http://www.arthritis.co.za/cox.html

COX-2 selective inhibitors

- Examples:
  - Celecoxib (Celebrex®)
  - Rofecoxib (Vioxx®)
- COX-2 has a valine residue (V523) near the active site, which is smaller than isoleucine (I523) in COX-1.
- COX-2 selective inhibitors do not bind COX-1 due to the steric hindrance.

PDB entry 1CX2
Cox-2-celecoxib complx





Valine Isoleucine

Clinical correlation 10.8

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PDB entry 1CX2 & 1PTH
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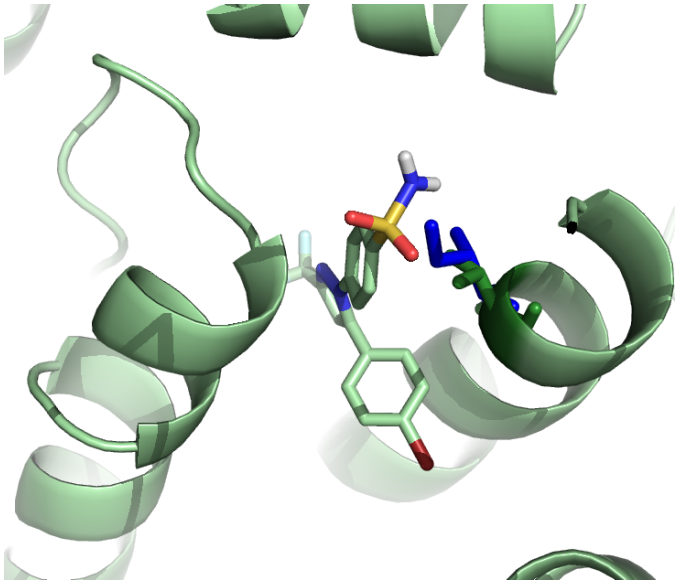

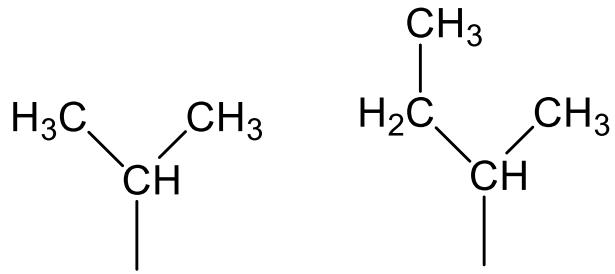

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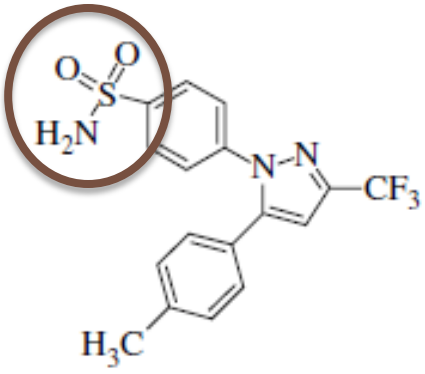
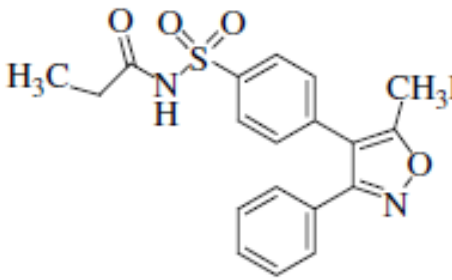
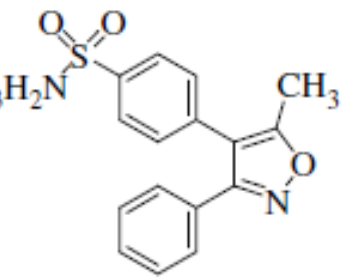


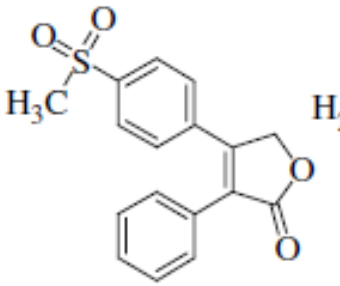
Celexoxib



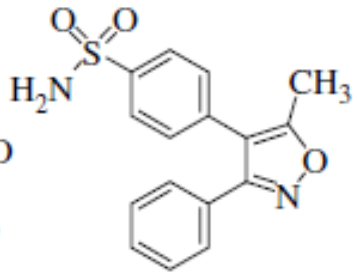
Etoricoxib



Parecoxib



Rofecoxib



Valdecoxib

Flower RJ, Nat Rev Drug Disco, 2:179, 2003

COX-2 selective inhibitors

Ratio indicates selectivity

IC_{50} values and COX2/COX-1 ratios of different NSAIDs in guinea pig peritoneal macrophage model.
(Engelhardt et al. Journal Inflammatory Research 1995, Volume 44, Pages 422 - 433.)

NSAIDs	COX-2 IC_{50} micromol/litre	COX-1-IC_{50} micromol/litre	Ratio COX-2 / COX-1
Meloxicam	0.0019	0.00577	0.33
Diclofenac	0.0019	0.000855	2.2
Piroxicam	0.175	0.00527	33
Tenoxicam	0.322	0.201	15
Indomethacin	0.00636	0.00021	30
Tenidap	47.8	0.393	122

Summary: examples of drugs as enzyme inhibitors

- The values determined for K_M and V_{max} in the presence of an inhibitor compound are the apparent values
- The apparent K_M and V_{max} differ by the amount $(1+[I]/K_I)$ relative to the actual K_M and V_{max}
- Whether K_M and/or V_{max} are affected by an inhibitor molecule depends on the type of inhibition
- Statins inhibit HMG-CoA reductase and are an example of two types of inhibition (competitive for HMG-CoA, but not for NADP)
- Binding of statins requires HMG-CoA reductase to be flexible in order to fit the large aromatic groups of statins into the binding site
- Some NSAIDS are examples of designing selective inhibitors; they preferentially inhibit COX-2 over COX-1.

Regulation of enzyme activity

Misregulation causes disease: ***Gout*** Clinical Correlation 10.13:

- Inflammatory disease caused by overproduction of uric acid, a highly insoluble compound.
- Uric acid is end product of purine degradation
- Hyperuricemia can be due to overproduction of purine nucleotides resulting from abnormal enzyme activity at various metabolic steps.
  - One candidate enzyme is PRPP synthetase.
  - The product PRPP (5-phospho-ribosyl pyrophosphate) is an intermediate in purine biosynthesis;
  - excessive PRPP synthetase activity leads to uric acid overproduction
- Patient study of gout:
  - Increased PRPP levels in red blood cells
  - But, PRPP synthetase had normal K_M and V_{max} and normal cellular levels

So WHAT's THE PROBLEM?

Regulation of enzyme activity

- Substrate cooperativity in enzyme catalysis
- Activators and inhibitors
  - Allosteric effectors
  - Feedback inhibition by the final product of a pathway
- Cellular enzyme concentration
  - Regulation at the gene expression level
- Covalent modification
  - Phosphorylation
  - Proteolysis
- Localization in the cell
  - Spatial separation between enzyme and substrate

Regulation of enzyme activity: cooperativity

- Cooperativity: multimeric (i.e. multiple subunits) enzyme in which the activity of one subunit affects the activity of the other subunits; subunits are not independent.
  - Usually K_M is changed, but for some enzymes k_{cat} changes, or both change
  - Positive cooperativity is common; negative cooperativity does occur
  - Apparent in velocity curves and in Lineweaver-Burk double reciprocal plots

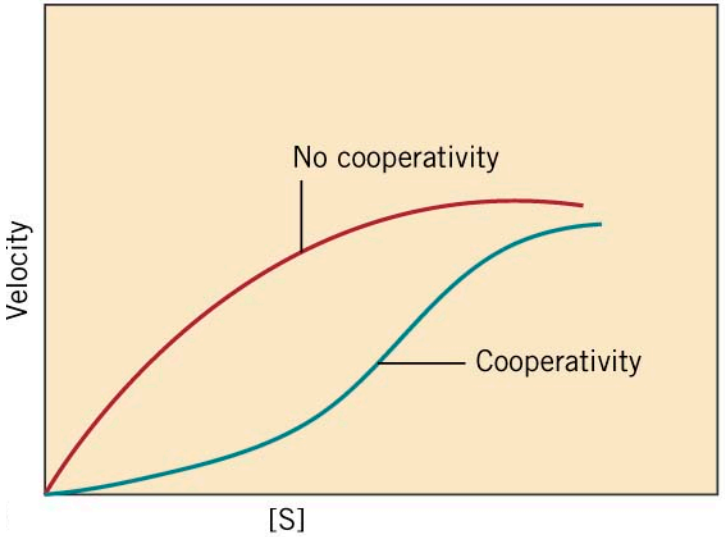
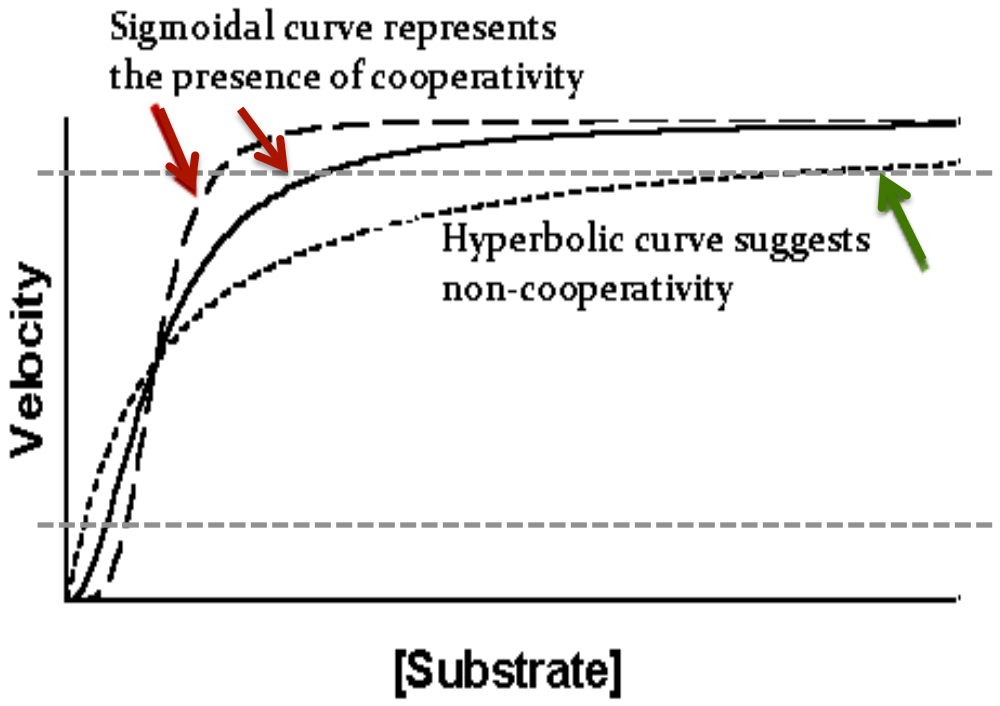


Figure 10.65

Regulation of enzyme activity: cooperativity

- Cooperativity: what is its purpose?

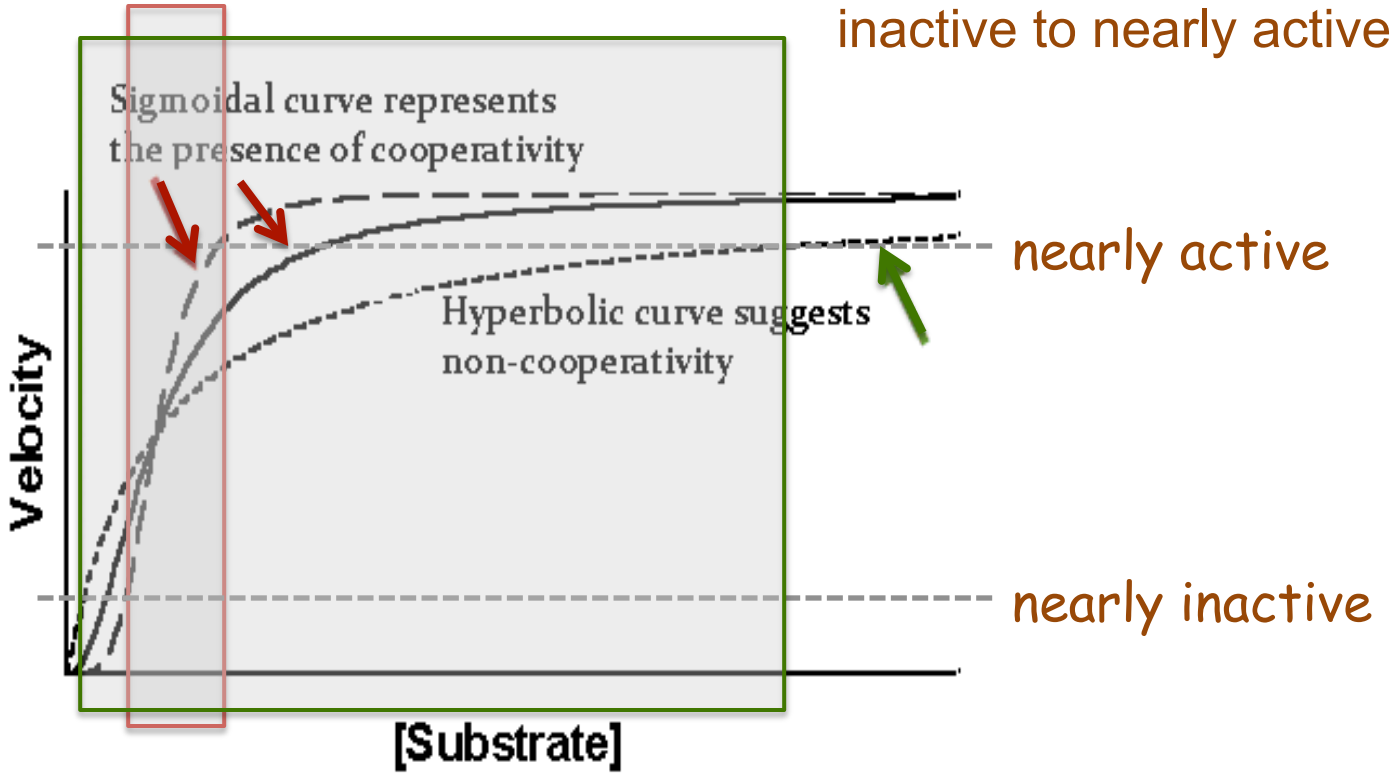


http://chemwiki.ucdavis.edu/

Regulation of enzyme activity: cooperativity

- Cooperativity: what is its purpose?

Consider the range of substrate concentrations needed to vary from nearly inactive to nearly active.

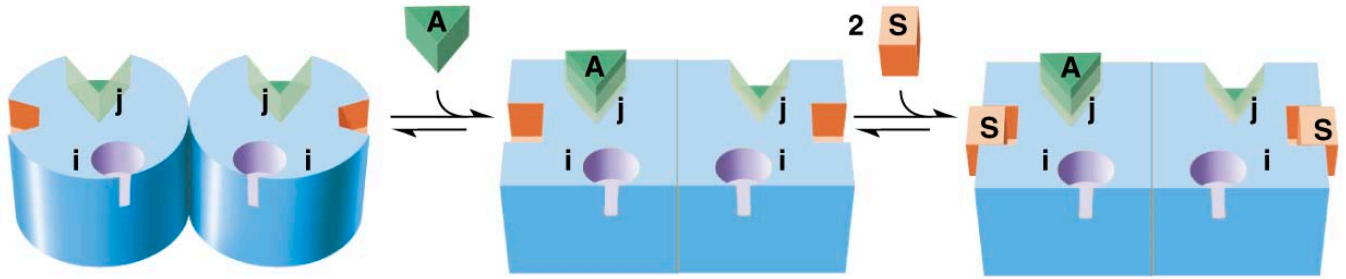


http://chemwiki.ucdavis.edu/

Regulation of enzyme activity: allosteric activation and inhibition

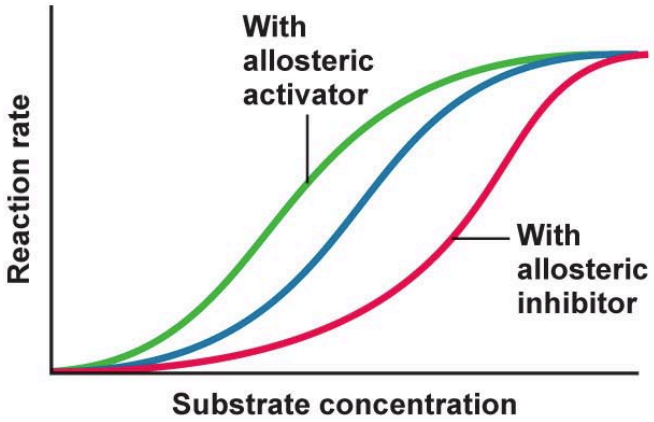
- Effectors are metabolic inhibitors or activators (small molecules) affect activity of allosteric enzymes
  - Alter the affinity of substrates (K_M) and/or the reactivity (V_{max})
  - Can be either positive (activator) or negative (inhibitor) effect
- Bind noncovalently to a site distinct from the active site
  - not modified chemically during reaction
- Most often multiple subunits, either identical or nonidentical
  - Effector binding leads to a conformational change that propogates to other subunits via contacts at the subunit interface
  - Heterotropic: ligand in effector site not the same as the substrate ligand
  - Homotropic: same ligand as substrate (not common)

Figure 10.64



Green: positive effector binding changes both subunits to a higher affinity form

Regulation of enzyme activity: allosteric activation and inhibition



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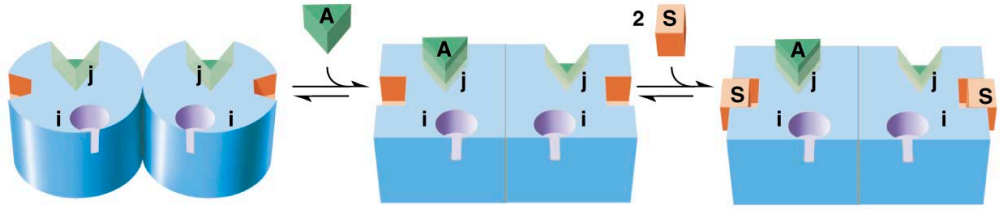
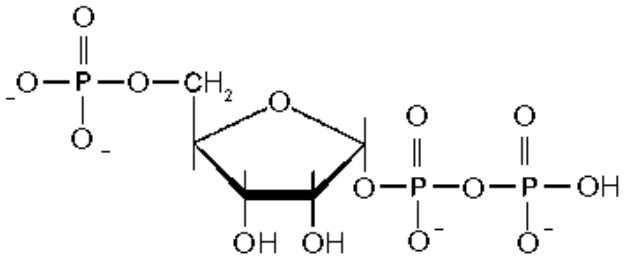


Purple: negative effector (not shown) binding changes both subunits to a low affinity form → inhibits activity

Regulation of enzyme activity

Clinical Correlation 10.13, Gout:

- So what about PRPP synthetase?
  - Increased PRPP levels in red blood cells of patients
  - PRPP synthetase had normal K_M and V_{max} and normal cellular levels



PRPP

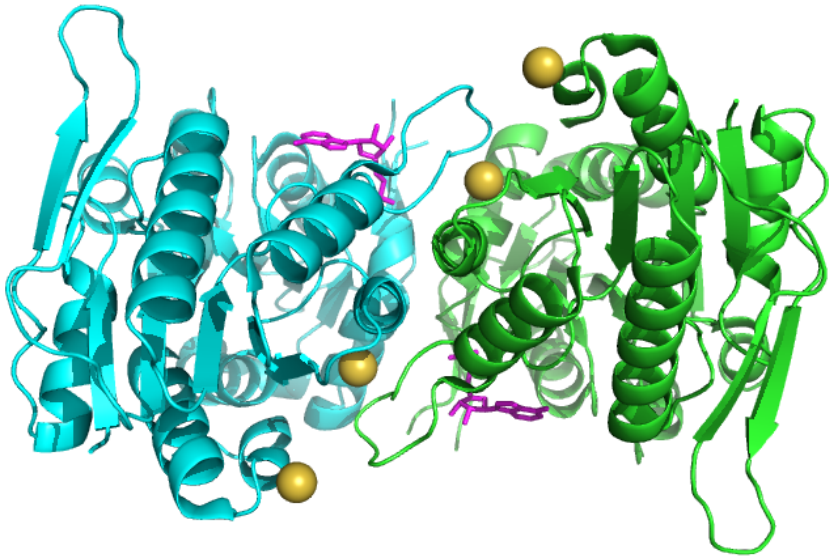
PRPP is a metabolite precursor in purine nucleotide biosynthesis.
PRPP synthetase:

- catalyzes synthesis from ribose 5-phosphate and ATP
- regulated by phosphate ion (activator) and ADP (inhibitor)
  - absolute requirement for P_i
  - …but sensitivity to P_i is normal in these patients

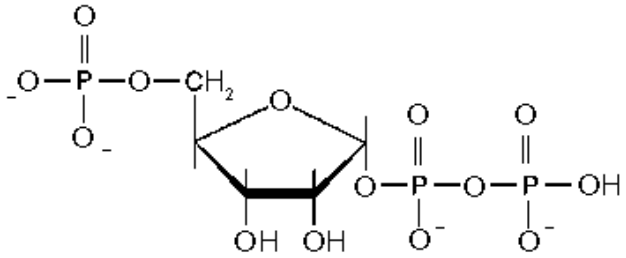
Regulated by effectors

Regulation of enzyme activity

Clinical Correlation 10.13, Gout:



Allosteric sites



PRPP

PRPP is also inhibited by ADP; the increase in PRPP arose b/c ADP did not inhibit the synthase … thus, a remaining possibility is that a mutation in this allosteric site led to failure of feedback control.

Summary of Enzyme Regulation and Inhibition

- Multimeric enzymes are often allosteric and show cooperative behavior in an initial velocity vs [S] curve. The sigmoidal behavior enables a strong dependence on small changes in [S].
- Allosteric enzymes are often regulated by effector molecules that are either activators or inhibitors. Effectors often bind at the oligomeric interfaces to change the allosteric response.