Mb, Hb, Allostery, and Motors

QUATERNARY STRUCTURES AND COMPLEX ENZYMES
Myoglobin
Hemoglobin
Motor Proteins
Actin-Myosin
Conclusion

QUATERNARY STRUCTURES AND COMPLEX ENZYMES

Advantages of complex (quaternary) protein structures

• Stability: decreased surface-to-volume -> more hydrophobic interactions
• New sites: e.g., immunoglobulin binding sites
• Coupled reactions:
  - tryptophan synthetase:
    - Indoleglycerol-P --> G3P + indole
    - Indole + ser --> trp
  - purine (A, G) synthesis: 10 reactions, 6 enzymes, 1 complex (in purine depleted medium)
• Cooperativity: e.g., allostery

Example of Cooperativity: Mb/Hb (Myoglobin and Hemoglobin)

• shows advantage of quaternary structure
• shows examples of flexibility: low ΔG of shape change
Myoglobin

- MW ca 17,000 daltons
- 75% helix
- Heme prosthetic group: protoporphyrin ring plus Fe$^{2+}$
- Heme binds O$_2$ as porphyrin-Fe$^{2+}$-O$_2$, color change from brown → red
- Serves as an O$_2$ buffer
- Hyperbolic saturation curve shows that there is no coordinate activity

Why is this an O$_2$ buffer? High slope below the P$_{50}$ means that considerable Mb is charged (or uncharged) for a small change in pO$_2$ (as pO$_2$ drops, MbO$_2$ replenishes O$_2$)
Hemoglobin

- Tetramer of myoglobin-like subunits, each with...
- Heme prosthetic groups: protoporphyrin ring plus Fe2+
- MW ca 4 x 17,000 daltons
- 75% -helix
- Complexed with O2, porphyrin-Fe2+-O2, brown --> red

- Better O2 buffer (at a higher [O2])
- Sigmoid saturation curve shows that there is coordinate activity: “positive, homotropic, allosteric effector”
Bohr effect: $H^+$, $CO_2$ promote dissociation of $O_2$ from Hb-O$_2$: "negative, heterotropic, allosteric effector." The Bohr effect in hemoglobin can also be depicted as an oxygen-binding curve. There is a proportional relationship between the affinity of oxygen and pH level. As the pH level decreases, the affinity of oxygen in hemoglobin also decreases. As hemoglobin approaches low pH, more oxygen is released.

2,3-bisphosphoglycerate also promotes dissociation of $O_2$. Purified hemoglobin binds much more tightly to the oxygen, making it less useful in oxygen transport. The difference in characteristics is due to the presence of 2,3-Bisphosphoglycerate(2,3-BPG) in human blood, which acts as an allosteric effector. An allosteric effector binds in one site and affects binding in another. 2,3-BPG binds to a pocket in the T-state (taut) of hemoglobin and is released as it forms the R-state (relaxed). The presence of 2,3-BPG means that more oxygen must be bound to the hemoglobin before the transition to the R-form is possible.
Lung conditions (Low H+, CO2) promotes O2 saturation; tissue conditions (high H+, CO2) promote O2 release; 2,4-BPG magnifies the allosteric effects. Allosteric effects match the saturation curve to the conditions in lung and tissue.

Motor Proteins

Types (substrate-motor)

Linear:
- Microtubules (tubulin) – dynein (+ to -), kinesin (- to +, with exception—Science 1April 2011)
- DNA – helicases
- Microfilaments (actin) – myosin

Rotary:
- Bacterial flagella
- F0F1 ATP synthases

Motion depends on
- Flexible 3Å structure
- Reversible binding
- ATP hydrolysis affecting binding

Microtubule--kinesin

Microtubule: right handed hollow helix of tubulin α/β dimers
kinesin: left-handed helix with two globular heads

Each step depends on flexibility ("rotation")
Each step hydrolyzes one ATP (→ ADP + Pi)
Each step involves an exchange reaction
(There is another motor protein, dynein, which moves along microtubules. Its 4-A crystal structure was recently reported (Science 331:1159, 3/4/11), but its mechanism of action is still unknown.)

Actin-Myosin

Microfilaments: right-handed double helix of actin monomers

G-actin ionic strength

F-actin

myosin: left-handed coil- (alpha-helix) coil
Myosin is an ATPase. Would you expect the addition of actin to increase or decrease ATP hydrolysis activity? (Reaction rate: $0.05 \text{s}^{-1} \rightarrow 10 \text{s}^{-1}$)

**Actomyosin in Muscles**

Contraction: sliding in the A band from myosin-actin connections

Why rigor mortis? When there is a loss of ATP, the muscles cannot relax because it cannot be broken down into ADP

**Conclusion**

Flexibility in protein structures allows more complex functions

- Reversible O2 and CO2 binding
- Reversible protein-protein (kinesin-MT) binding

Shows the importance of low $\Delta G$ in protein shape changes