BI/CH 422/622

OUTLINE:

Introduction and review
Transport
Glycogenolysis
Glycolysis
Other sugars
Pasteur: Anaerobic vs Aerobic

Exam-1 material

Exam-2 material

Fermentations
Pyruvate
Krebs' Cycle
Oxidative Phosphorylation
- Electron transport
- Chemiosmotic theory: Phosphorylation

Exam-3 material

Fat Catabolism
Fatty acid Catabolism
- Mobilization from tissues (mostly adipose)
- Activation of fatty acids
- Transport; carnitine
- Oxidation: b-oxidation, 4 steps:
  - Saturated FA
  - Unsaturated FA
  - Odd-chain FA
  - Ketone Bodies

Protein Catabolism
- Digestion, lysosome, Ubiquitin-Proteosome

Amino-Acid Degradation
- Dealing with the nitrogen
  - Urea Cycle
- Dealing with the carbon
  - Seven Families
    - One-carbon (1-C) metabolism; THF, SAM
    - PLP uses
- Convergence with Fatty acid-odd chain

Nucleic Acid & Nucleotide Degradation
- Nucleic Acids
- Nucleotides
  - Salvage pathway
  - Degradation of purines
  - Degradation of pyrimidines

ANABOLISM I: Carbohydrates

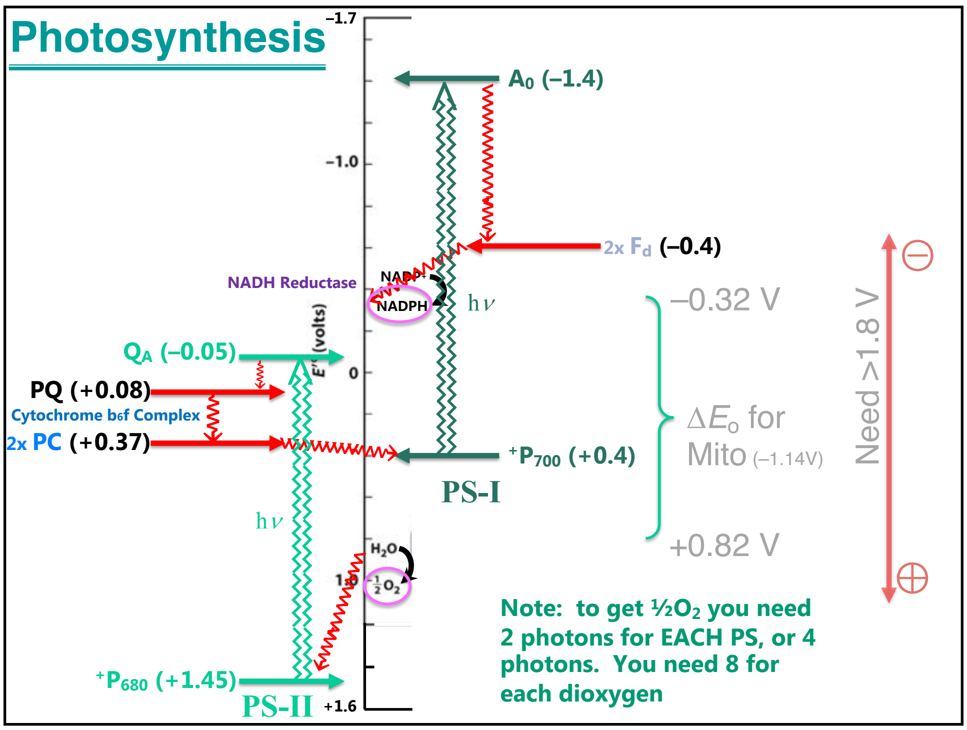
PHOTOSYNTHESIS:

Overview of Photosynthesis
Key experiments:
Light Reactions
- energy in a photon
- pigments
- HOW
- Light absorbing complexes-"red-drop expt"
  - Reaction center
  - Photosystems (PS)
    - PSII – oxygen from water splitting
    - PSI – NADPH

Proton Motive Force – ATP
Carbon Assimilation – Calvin Cycle
- Stage One – **Rubisco**
- Stage Two – making sugar
- Stage Three – remaking Ru $1,5P_2$

Overview and regulation
Calvin cycle connections to biosynthesis
C4 versus C3 plants
Kornberg cycle – glyoxylate

Photosynthesis



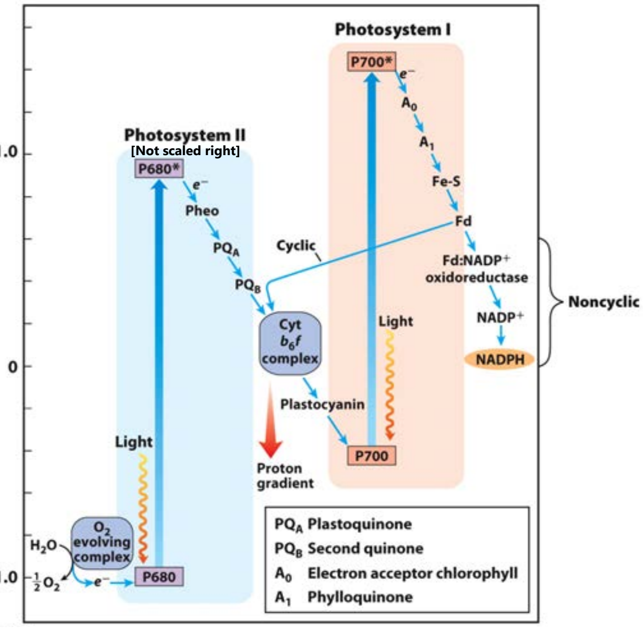
Note: to get ½O_2 you need 2 photons for EACH PS, or 4 photons. You need 8 for each dioxygen

Photosynthesis

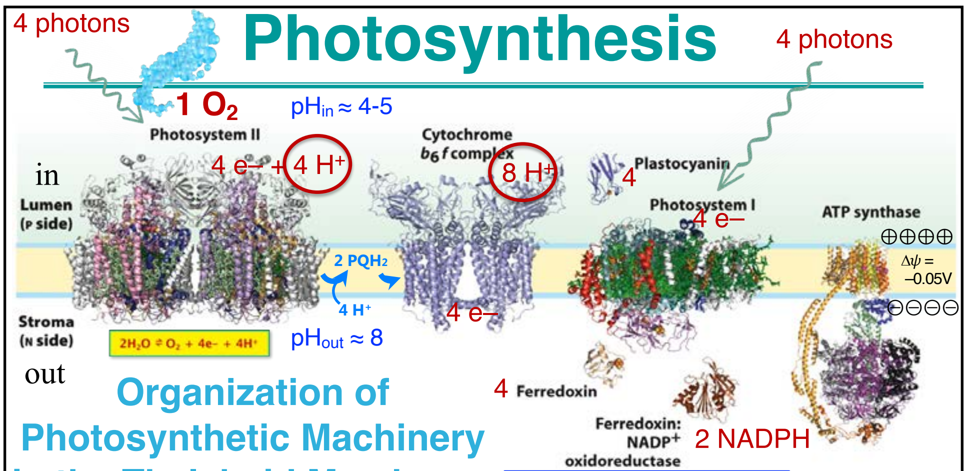
In Plants, the Two Reaction Centers Work Together

Cyclic Photosynthesis Can Be Utilized to Generate Excess ATP

This explains the energetics, but what about the stoichiometry?



Photosynthesis



Organization of Photosynthetic Machinery in the Thylakoid Membrane

$$H^+_{in} \rightleftharpoons H^+_{out}$$

$$\Delta G' = RT\,ln\frac{[H^+]_{out}}{[H^+]_{in}} + z\mathcal{F}\Delta\psi$$

$= RT2.3(pH_{in} - pH_{out}) + (+1)(96480)(-0.05)$

$= 5.9(-3) - 4.8$ kJ/mol

$= -17.7 - 4.8$ kJ/mol

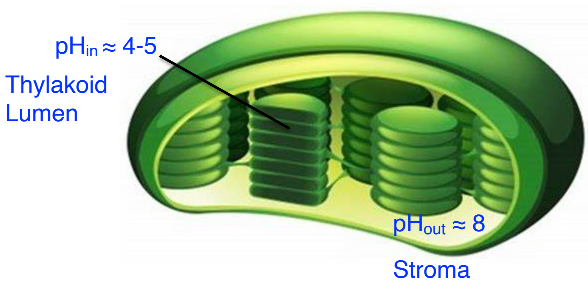
$= -22.5$ kJ/mol per proton

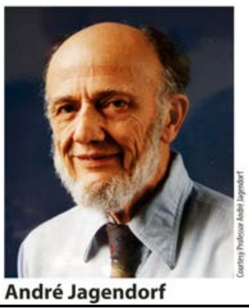
per 12 H⁺ $= 12 \times -22.5$ kJ/mol

$= -270$ kJ/mol

Enough for ~8 ATP

Photosynthesis

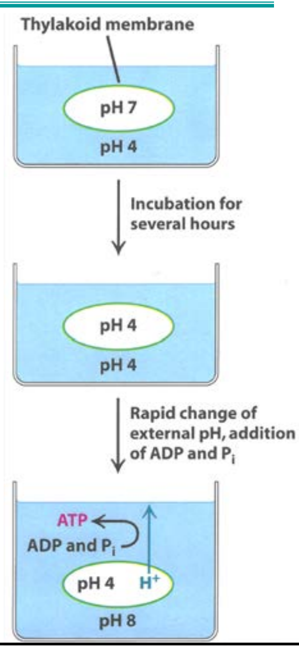




$$\Delta G' = RT\ln\frac{[H^+]_{out}}{[H^+]_{in}} + z\mathcal{F}\Delta\psi$$

$= -17.7$ kJ/mol $- 4.8$ kJ/mol

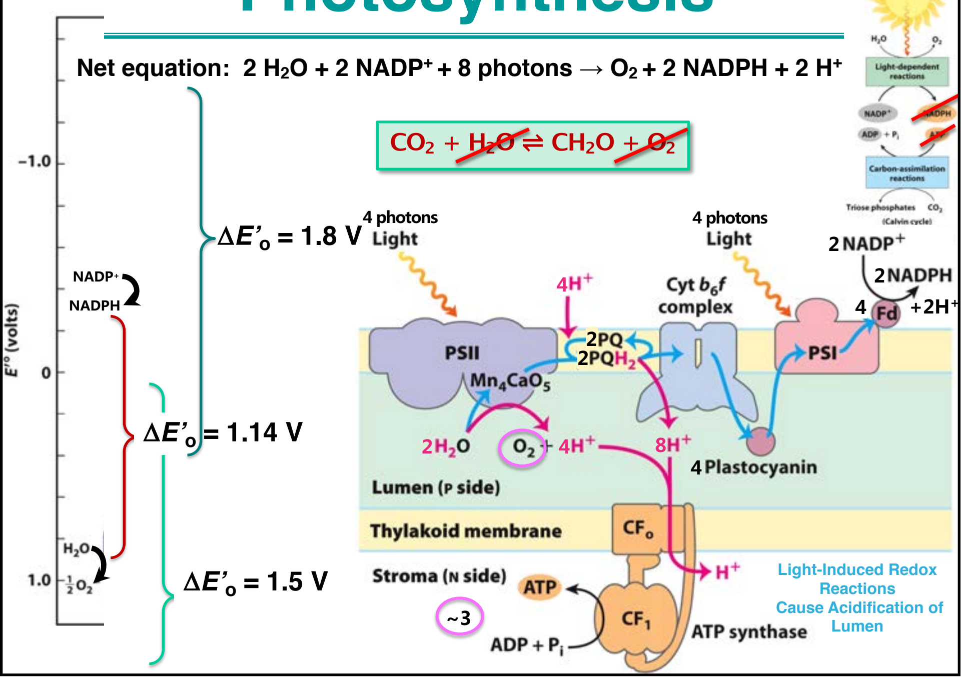
pH gradient — Membrane potential



Photosynthesis

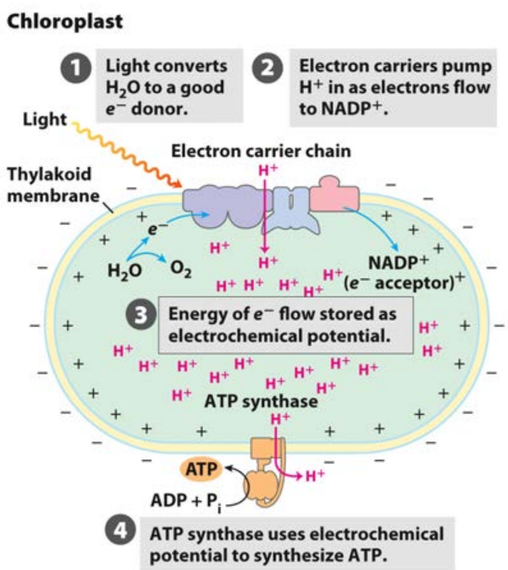
Net equation: $2\ H_2O + 2\ NADP^+ + 8$ photons $\rightarrow O_2 + 2\ NADPH + 2\ H^+$

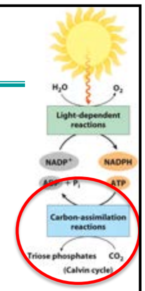
$CO_2 + \sout{H_2O} \rightleftharpoons CH_2O + \sout{O_2}$



Photosynthesis

Light Energy is Converted to ATP





- In contrast to oxidative phosphorylation, protons are pumped into the lumen, building up a large concentration gradient quickly in the small space.
- The proton-motive force across the thylakoid membrane drives the synthesis of ATP.
- The number of c subunits in F_0-rings is larger in chloroplasts (~12-14), so synthesis of ATP costs more H^+ per ATP (~4-5) than in oxidative phosphorylation.
- For the same light, the ratio of ATP-to-NADPH synthesized is **3:2**

Photosynthesis

Summary of light reactions:

- Photosynthetic organisms capture energy from light at a variety of wavelengths using accessory pigments and funnel it via excitons to reaction centers: P680 & P700.
- In plants, electrons are freed from H_2O, which generates O_2 and H^+. The electrons popping out of the reaction centers reduce PQ, which moves them through the photosynthetic electron-transport chain (cytb$_6$f).
- Per oxygen (O_2) produced from 4 photons in PS-II, 12 H^+ are mobilized into the lumen from the $2H_2O$ and cytb$_6$f (Q-cycle). This produces a proton-motive force sufficient for 3 ATPs
- The final electron acceptor for linear photosynthesis is $NADP^+$, which requires two electrons for conversion to NADPH. Per 8 photons (4 each to PS-I & PS-II), 2 NADPH are produced.
- When ATP stores are low, the electrons can be transported to cytochrome b_6f and cycled through the second half of photosynthesis. This cyclic photosynthesis resulting in an increased proton gradient and more ATP.

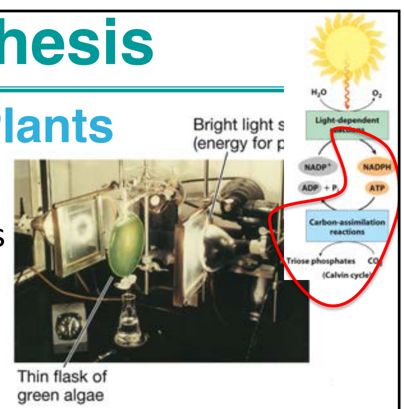
ANABOLISM I Carbohydrates

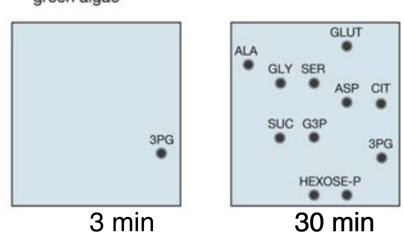
Photosynthesis and Carbohydrate Synthesis in Plants

Photosynthesis

Assimilation of CO_2 by Plants

- Taking CO_2 into biological, more reduced, carbon intermediates is ***CO_2 assimilation***.
- Question was: What is the first compound made by plants when they assimilated ***CO_2***?






Melvin Calvin, 1911–1997

Calvin and Benson used the ^{14}C radioisotope to determine the sequence of reactions in CO_2 fixation.

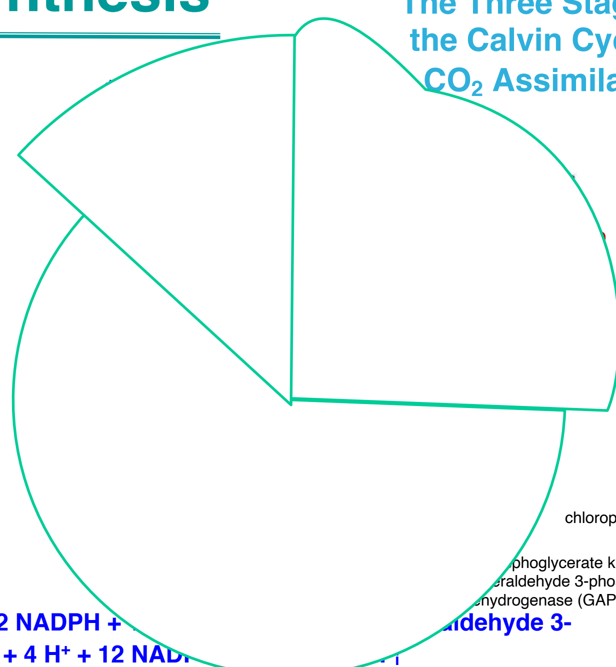
They exposed *Chlorella* to $^{14}CO_2$, then extracted the organic compounds and separated them by paper chromatography.

Photosynthesis

- Plant cells: can also *make* 3-C intermediates for further synthesis
  - made from CO_2, H_2O, plus ATP and NADPH from photosynthesis
- It occurs in the stroma of chloroplasts via a cyclic process known as the Calvin cycle.
- Key intermediate **ribulose 1,5-bisphosphate** is constantly regenerated using energy of ATP.
- It produces **3-phosphoglycerate,** which is rapidly converted to **glyceraldehyde 3-phosphate** (GA3P) & **dihydroxyacetone phosphate** (DHAP), which are important intermediates for all other compounds.
- The net result is the reduction of CO_2 with the NADPH that was generated in the light reactions of photosynthesis.

Photosynthesis

The Three Stages of the Calvin Cycle of CO_2 Assimilation



chloroplast isoforms

...phoglycerate kinase (3PGK)
...eraldehyde 3-phosphate
...ehydrogenase (GAPDH)

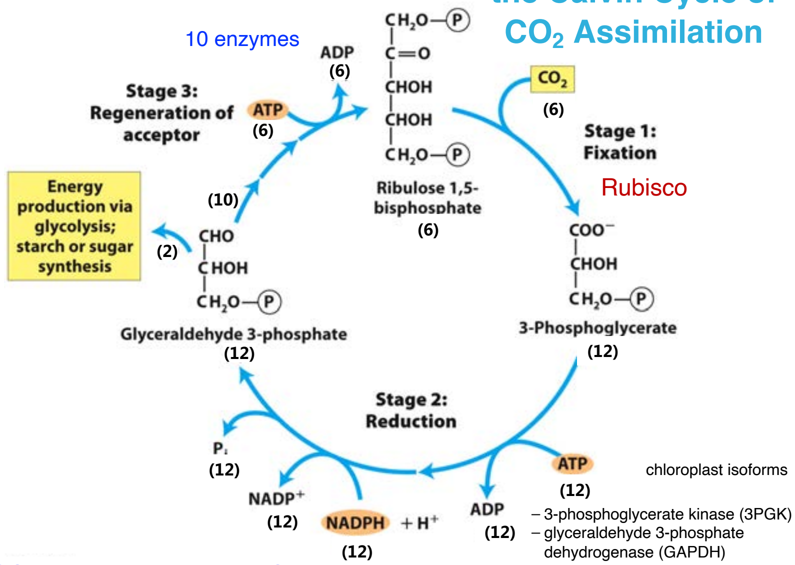
Overall: 6 CO_2 + 12 NADPH + ... aldehyde 3-phosphate (GA3P) + 4 H^+ + 12 NAD...

*Ribulose 1,5-bisphosphate carboxylase/oxygenase (also RuBisCo)

Photosynthesis

The Three Stages of the Calvin Cycle of CO_2 Assimilation

10 enzymes



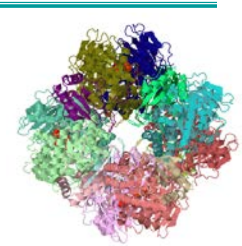
chloroplast isoforms

- 3-phosphoglycerate kinase (3PGK)
- glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

Overall: $6\ CO_2 + 12\ NADPH + 10\ H_2O + 18\ ATP \rightarrow 2$ glyceraldehyde 3-phosphate (GA3P) $+ 4\ H^+ + 12\ NADP^+ + 18\ ADP + 16\ P_i$

*Ribulose 1,5-bisphosphate carboxylase/oxygenase (also RuBisCo)

Photosynthesis



RUBISCO

Photosynthesis

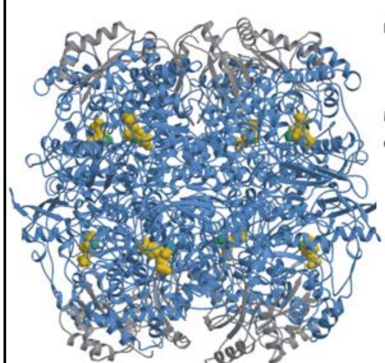
First Stage of Calvin Cycle

CO_2 Fixation Is Catalyzed by Rubisco

- Most plentiful, most important, enzyme on Earth (4 tons/yr)
  - **Low turnover # of k_{cat} = 3 s^{-1} at 25 C°**
  - **means a LOT of the enzyme needed!**
  - **50% of plant enzymes are rubisco.**
- Large (560 KDa; $\alpha_8\beta_8$)(L=56 kDa, S=14 kDa); Mg^{2+}–dependent enzyme; activated by CO_2 carbamylation
- pH dependence is sharp, optimal activity at pH=8
- Catalyzes the reaction:
  - ribulose 1,5-bisphosphate + CO_2 → **2** 3-phosphoglycerate
  - $\Delta G°'$ = –35.1 kcal/mol

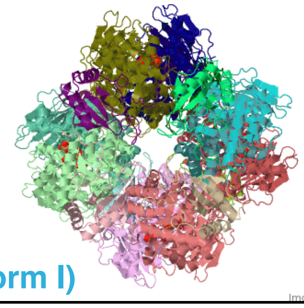
Photosynthesis

First Stage of Calvin Cycle



Structure of Rubisco (Form II)

There are two distinct forms of rubisco. Form I is found in vascular plants, algae, and cyanobacteria; Form II is confined to certain photosynthetic bacteria.



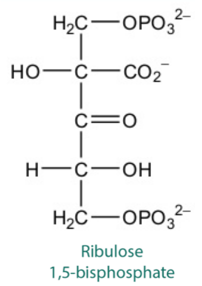
Structure of Rubisco (Form I)

Photosynthesis

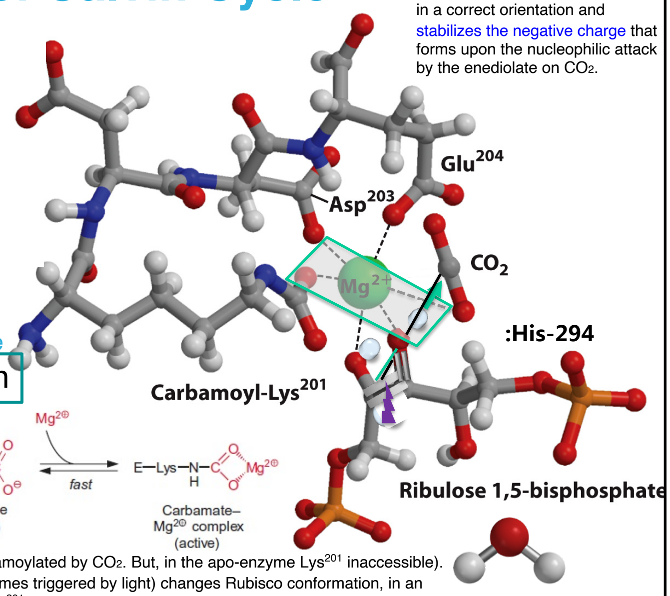
First Stage of Calvin Cycle

Role of Mg^{2+}

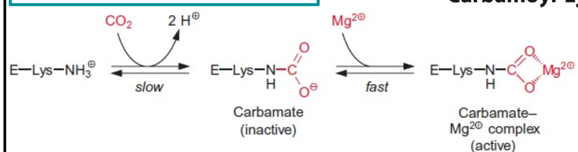
- Notice that Mg^{2+} is held by negatively charged side chains of:
- Glu, Asp, and carbamoylated Lys
- Mg^{2+} brings together the reactants in a correct orientation and stabilizes the negative charge that forms upon the nucleophilic attack by the enediolate on CO_2.



~~endiol intermediate~~
β-ketoacid intermediate



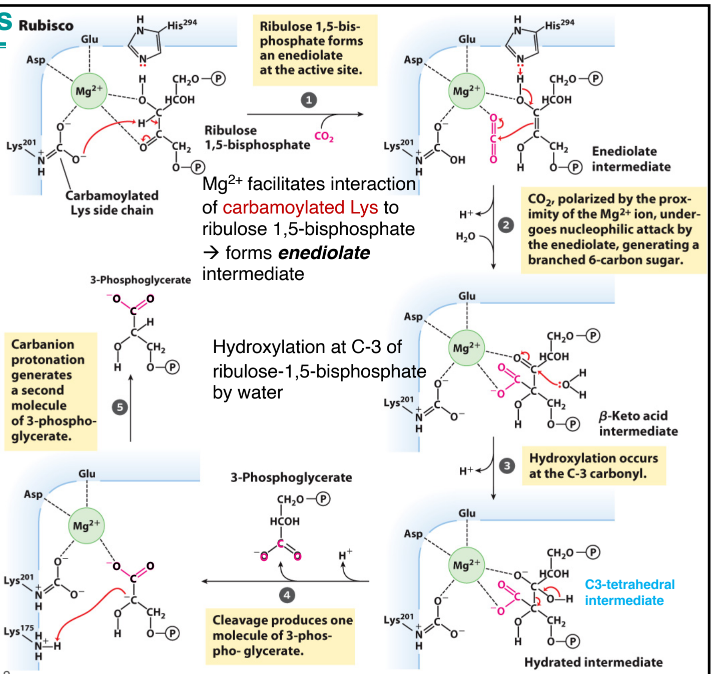
CO₂ carbamoylation



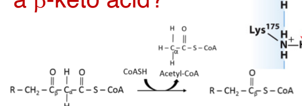
- Rubisco is inactive until Lys^{201} is carbamoylated by CO_2. But, in the apo-enzyme Lys^{201} inaccessible).
- ***Rubisco activase*** (an enzyme sometimes triggered by light) changes Rubisco conformation, in an ATP-dependent manner, to expose Lys^{201}.

Photosynthesis

Rubisco Carboxylase Mechanism— Overview



Recall another C-C cleavage of a β-keto acid?



Photosynthesis

First Stage of Calvin Cycle

Oxygenase Activity of Rubisco

- O_2 competes with CO_2 for the active site.
  - ~1 in every 3 or 4 turnovers, O_2 binds
- The reactive nucleophile in the rubisco reaction is the electron-rich enediol form of ribulose 1,5-bisphosphate.
- The nucleophile adds to O_2 to form 3-phosphoglycerate (same as in Calvin cycle) and ***2-phosphoglycolate (2-PG)***.
  - *–2-PG is metabolically difficult.*
  - *–Salvaging its Carbons requires energy*
  - *–As it is produced in appreciable amounts, an elaborate pathway has been cobbled together.*

This process is called PHOTO-RESPIRATION