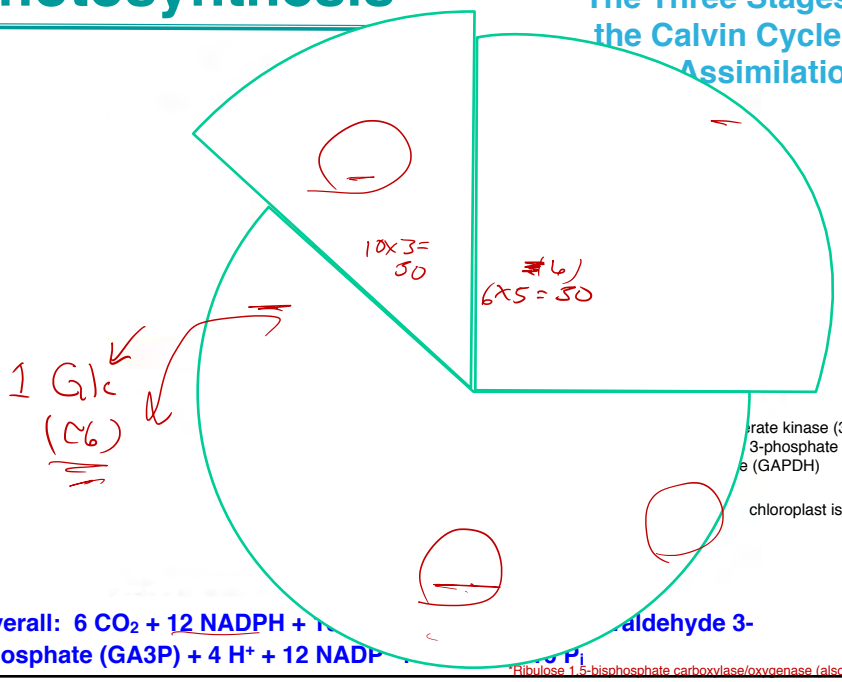
Photosynthesis

The Three Stages of the Calvin Cycle of Assimilation



rate kinase (3PGK)
3-phosphate
e (GAPDH)

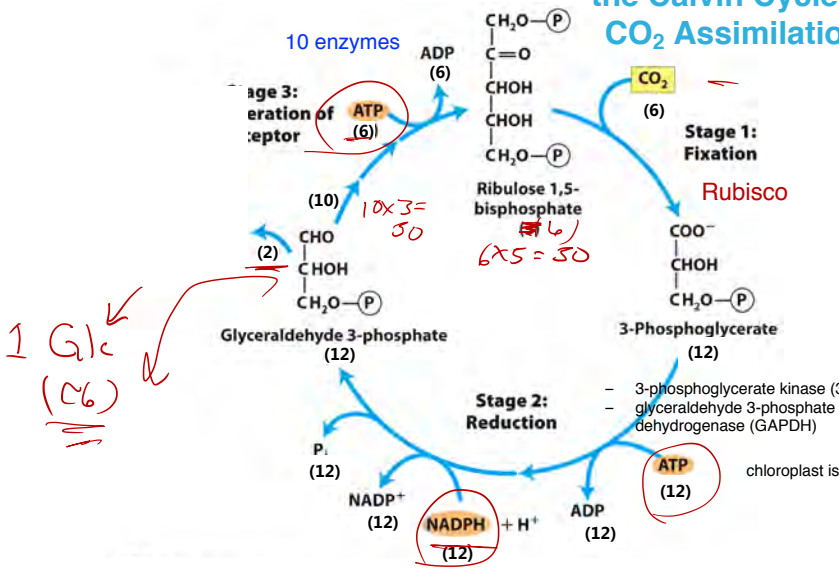
chloroplast isoforms

Overall: 6 CO_2 + 12 NADPH + 1 aldehyde 3-phosphate (GA3P) + 4 H^+ + 12 NADP P_i

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

Photosynthesis

The Three Stages of the Calvin Cycle of CO_2 Assimilation



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

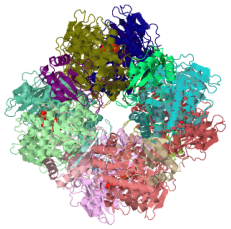
chloroplast isoforms

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

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

Photosynthesis

First Stage of Calvin Cycle

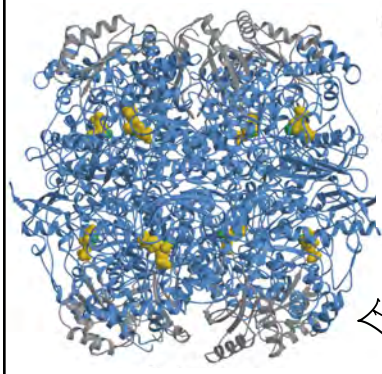


CO_2 Fixation Is Catalyzed by Rubisco

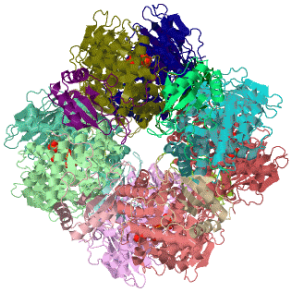
- Most plentiful, most important, enzyme on Earth (4 tons/yr)
  - **Low turnover # of k_{cat} = 3 s^{-1} at 25 C°**
  - **means a LOT of the enzyme needed!**
  - **50% of plant enzymes are rubisco.**
- Large (560 KDa; $\alpha_8\beta_8$)(L=56 kDa, S=14 kDa) Mg^{2+}–dependent enzyme; activated by CO_2 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)

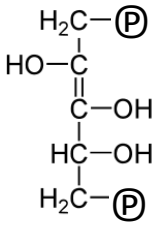


Structure of Rubisco (Form I)

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.

Photosynthesis

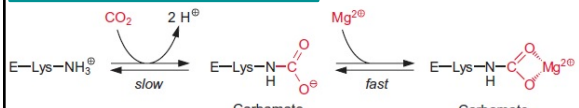
First Stage of Calvin Cycle



Ribulose
1,5-bisphosphate

endiol intermediate

CO_2 carbamoylation



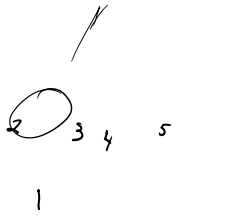
Carbamate (inactive)

Carbamate–$Mg^{2⊕}$ complex (active)

- 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}.

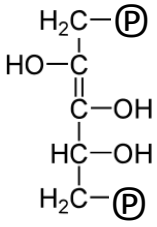
Role of Mg^{2+}

- 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 of enediolate to CO_2.



Photosynthesis

First Stage of Calvin Cycle



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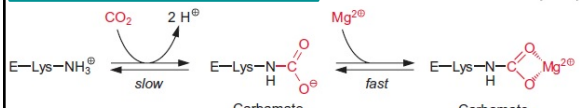

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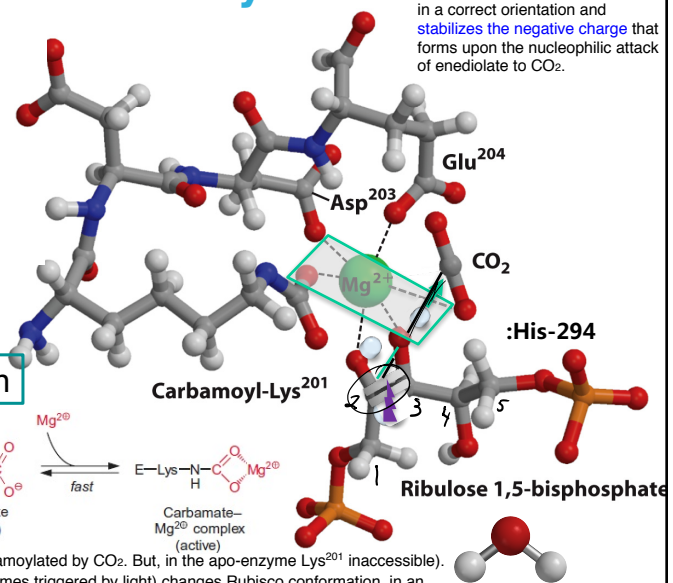
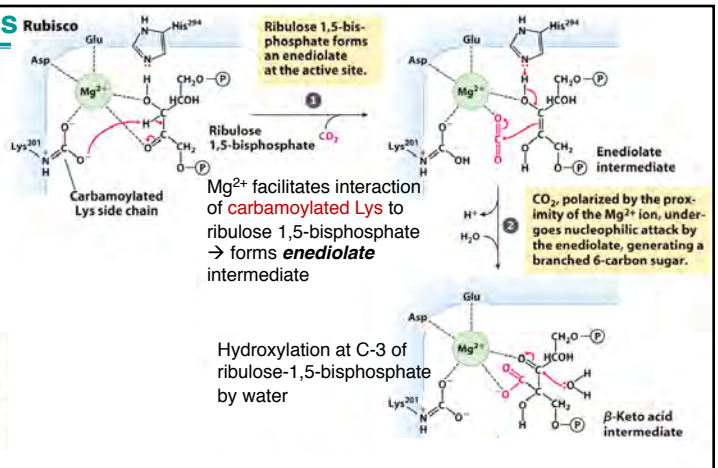


Photosynthesis

Rubisco Carboxylase Mechanism—Overview

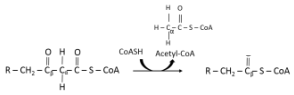


Mg^{2+} facilitates interaction of carbamoylated Lys to ribulose 1,5-bisphosphate → forms ***enediolate*** intermediate

Hydroxylation at C-3 of ribulose-1,5-bisphosphate by water

C3-tetrahedral intermediate

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



Photosynthesis

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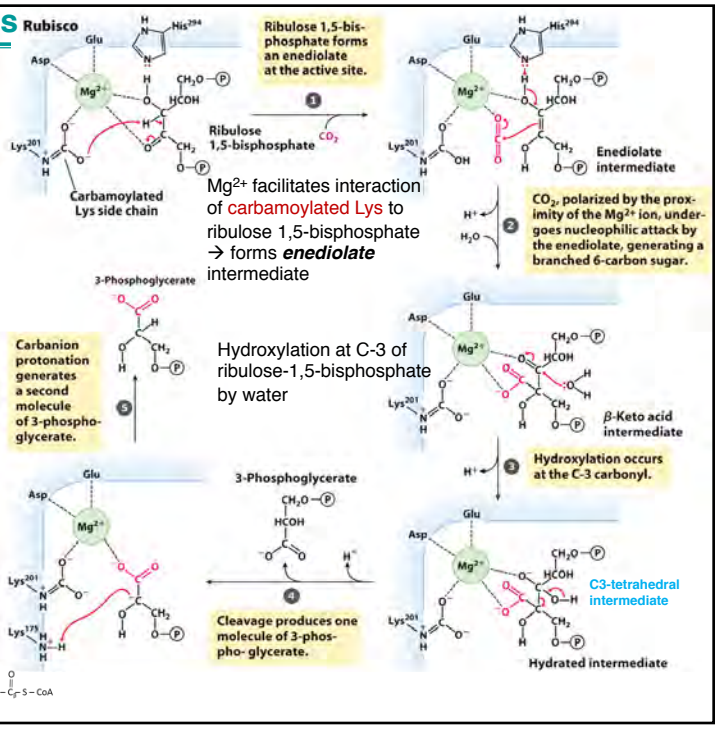

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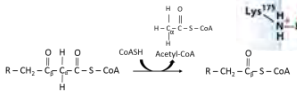


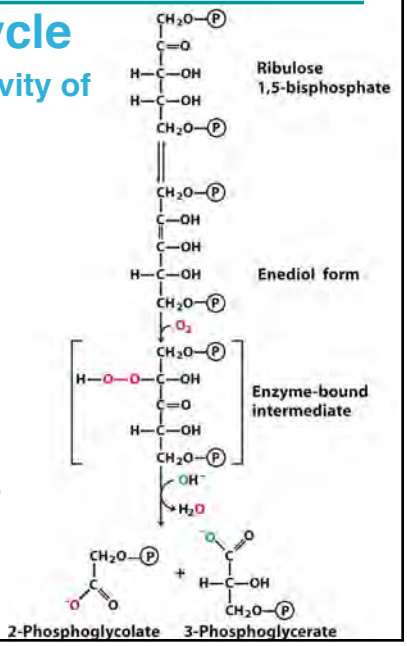
Photosynthesis

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



Photosynthesis

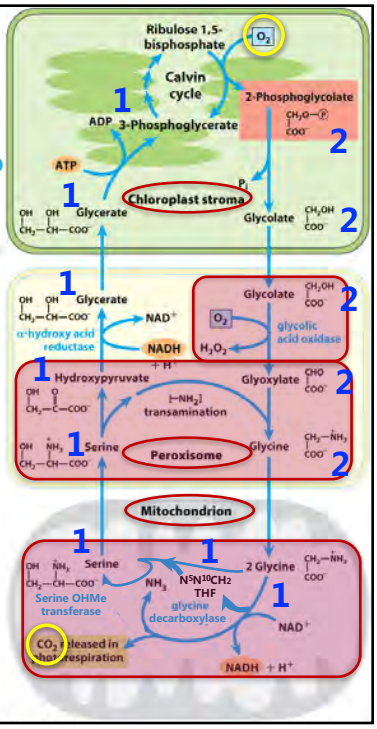
First Stage of Calvin Cycle

Dealing with the oxygenase activity of rubisco

The Glycolate Pathway*

- Complex ATP-consuming process for the recovery of C_2 fragments from photorespiration. 1st Glycolate is made.
- Uses three organelles
- Loss of C as CO_2 by mitochondrial decarboxylation of glycine (see Pyr family)
- **Two 2PGAs are converted to Ser + CO_2.**
- The Ser is cycled back to the chloroplast to generate one 3PGA.
- Whole cycle costs an ATP per **1** 3PGA converted back

*Don't confuse with the glyOXYlate Cycle



Photosynthesis

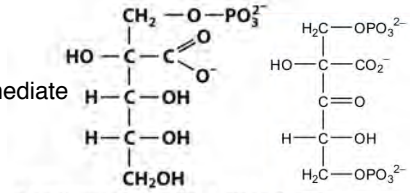
First Stage of Calvin Cycle

Regulation of rubisco activity

Rubisco is Inhibited by Several Criteria:

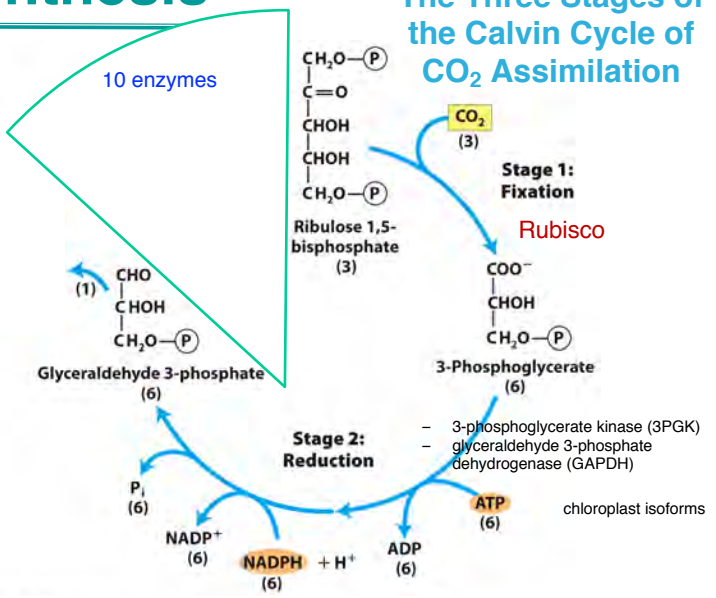
1. **pH**
   - In the stroma the pH is ~8 when light is on
   - pH decreases in the dark; rubisco inactive at low pH
2. **CO_2** (for the carbamoylation of Lys-210; also better at higher pH)
3. **NADPH** (indirectly through rubisco activase)
4. **a "Noctural" Inhibitor**

- ***2-carboxyarabinitol 1-phosphate*** inhibits carbamoylated Rubisco.
  - transition state analog of β-keto acid intermediate
  - synthesized in the dark in some plants



Photosynthesis

The Three Stages of the Calvin Cycle of CO_2 Assimilation



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

chloroplast isoforms

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

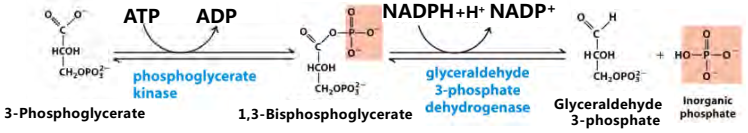
Photosynthesis

Second Stage of Calvin Cycle: making Glucose

3-PGA Reduced to GA3P

Twelve 3-phosphoglycerate + 12 ATP + 12 NADPH + 12 H^+ → 12 glyceraldehyde 3-phosphate + 12 ADP + 12 $NADP^+$ + 12 P_i

- Requires most of the ATP and NADPH from photosynthesis at ratio of 3:2
- Reverse of the key reactions in glycolysis (except NADPH used rather than NADH)
  - uses enzymes 3-phosphoglycerate kinase (3-PGK) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH)
- Driven forward by high concentration of NADPH and ATP in the chloroplast stroma; and the pulling of GA3P and P_i by the cycle and ATP synthase



$\Delta G^{\circ\prime}$ = +5.5 kcal/mol −1.8 kcal/mol +3.7 kcal/mol

Photosynthesis

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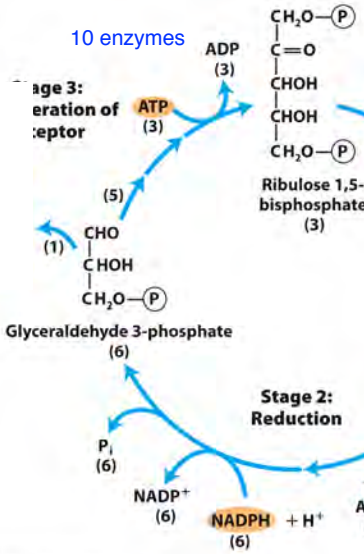


- 3-phosphoglycerate kinase (3PGK)
- glyceraldehyde 3-phosphate dehydrogenase (GAPDH)

chloroplast isoforms

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

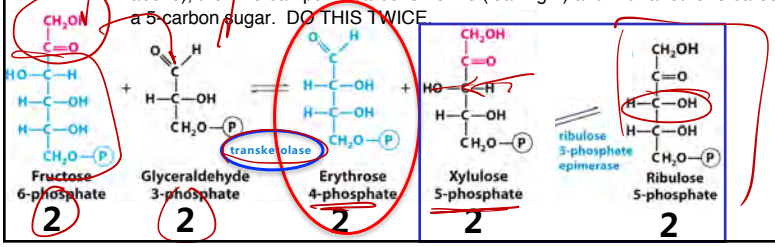
Photosynthesis 10→use 6 GA3P

Third Stage of Calvin Cycle

Stage 3: Regeneration of Ru 1,5-P_2

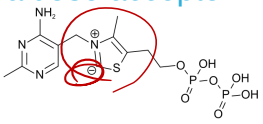
- 12 glyceraldehyde-3-phosphate are produced during the Calvin cycle in the assimilation of 6 carbons into Glc.
- 2/12 glyceraldehyde-3-phosphate products are pulled out of the cycle and used for glucose and other carbos.
  - Reverse of glycolysis: GA3P converted to DHAP by *triose phosphate isomerase;* GA3P and DHAP converted to fructose 1,6-bisphosphate (Fru 1,6-P_2) via *aldolase*, the to Fru 6-P via *fructose 1,6-bisphosphatase*, then Glc 6-P

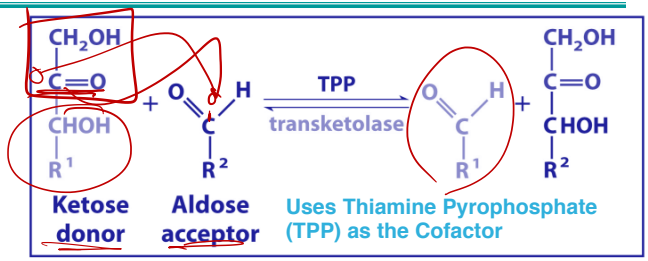
- 10/12 glyceraldehyde-3-phosphate products (30 carbons) MUST be used to regenerate 6 ribulose-1,5-bisphosphate (30 carbons).
  1. We have 3-carbon compounds and need 5-carbon compounds: put 2 together to make 6 (as above), then we can pull 2 carbons from 6 (leaving 4) and with another 3 carbon compound make a 5-carbon sugar. DO THIS TWICE.



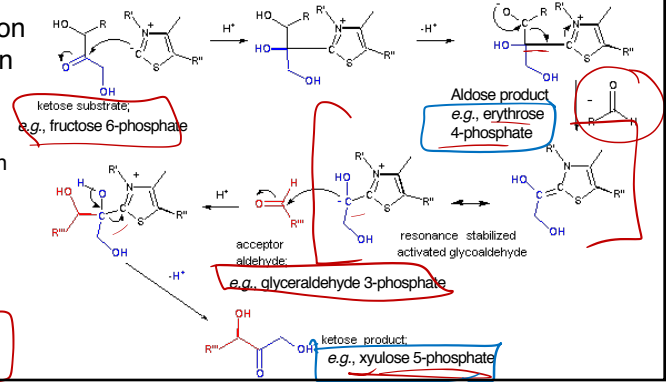
Photosynthesis

Transketolase: exchange of 2C from ketose to an aldose acceptor



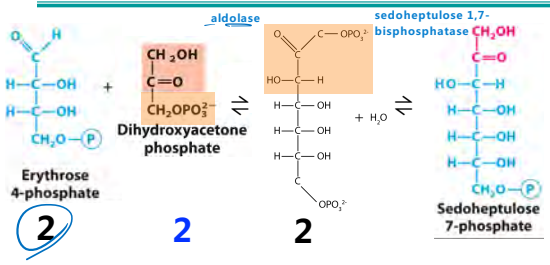


- Contains thiazolium anion for nucleophilic attack on carbonyls of ketose
- Also used by:
  - pyruvate dehydrogenase in acetyl CoA formation
  - pyruvate decarboxylase in ethanol metabolism
  - α-ketoglutarate dehydrogenase in CAC
  - transketolase in pentose phosphate pathway



Stage 3 Photosynthesis

4→ use 2 GA3P
2→0 GA3P left

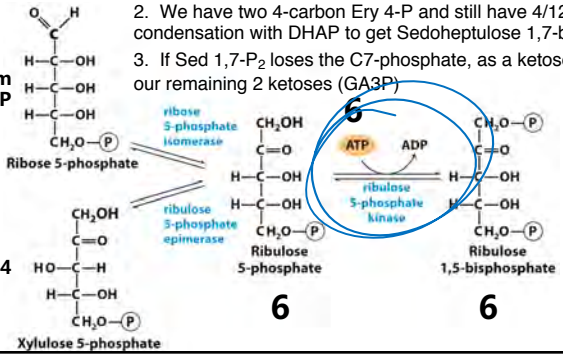


2 2 2 2 2 2 2

2. We have two 4-carbon Ery 4-P and still have 4/12 GA3Ps. Do it again; aldol condensation with DHAP to get Sedoheptulose 1,7-bisphosphate (Sed 1,7-P_2).

3. If Sed 1,7-P_2 loses the C7-phosphate, as a ketose, we can pull 2 carbons off and put on our remaining 2 ketoses (GA3P)

2 from Fru6-P



2+2=4

6 6 6

Sedoheptulose 1,7-bisphosphate dephosphorylated to sedoheptulose 7-phosphate (S7P) via *sedoheptulose 1,7-bisphosphatase*

- This enzyme is unique to plants.

Finally, RuP phosphorylated to ribulose 1,5-bisphosphate by *phosphoribulokinase*

- This enzyme is also unique to plants and regulated by NADPH