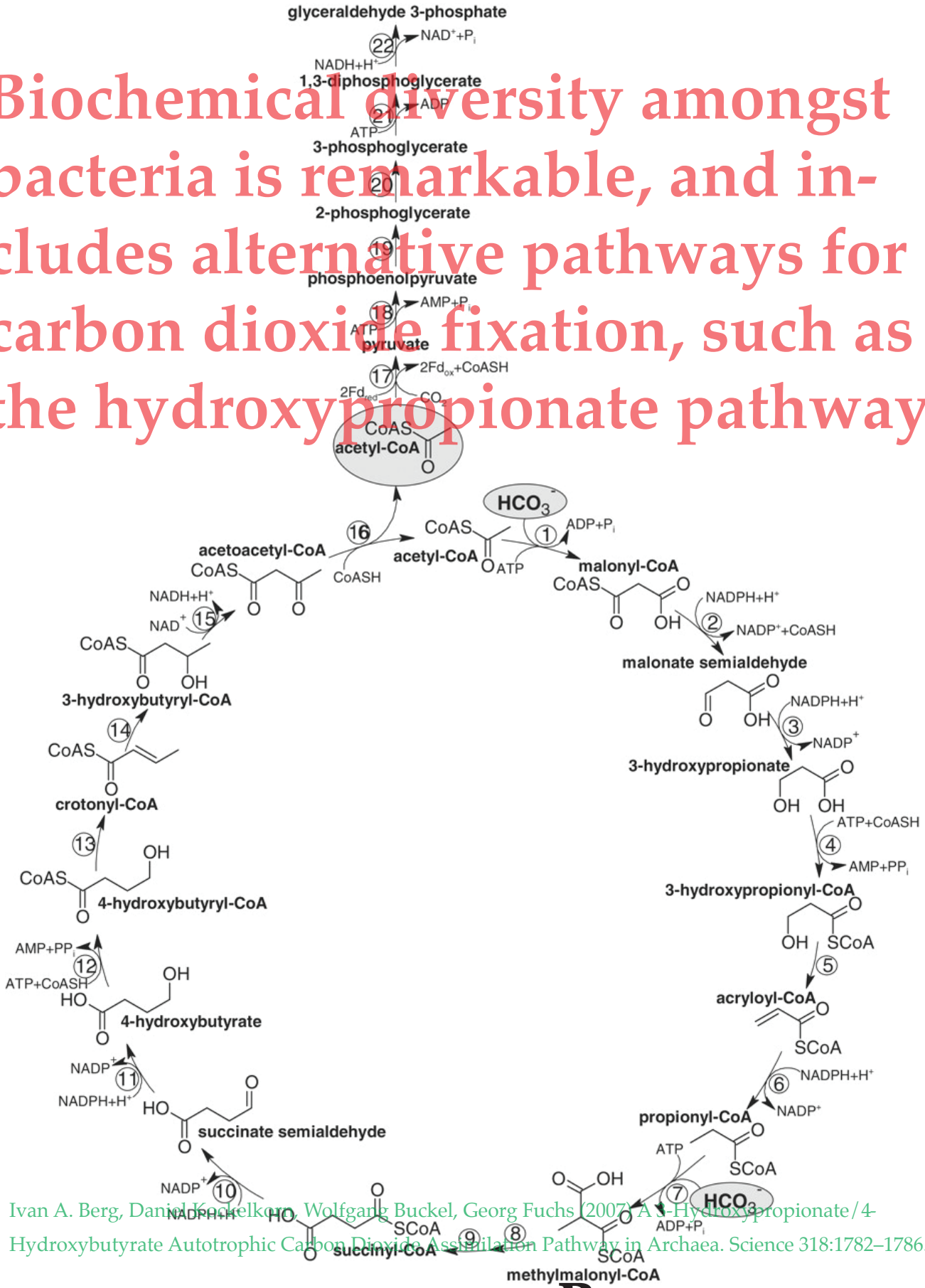
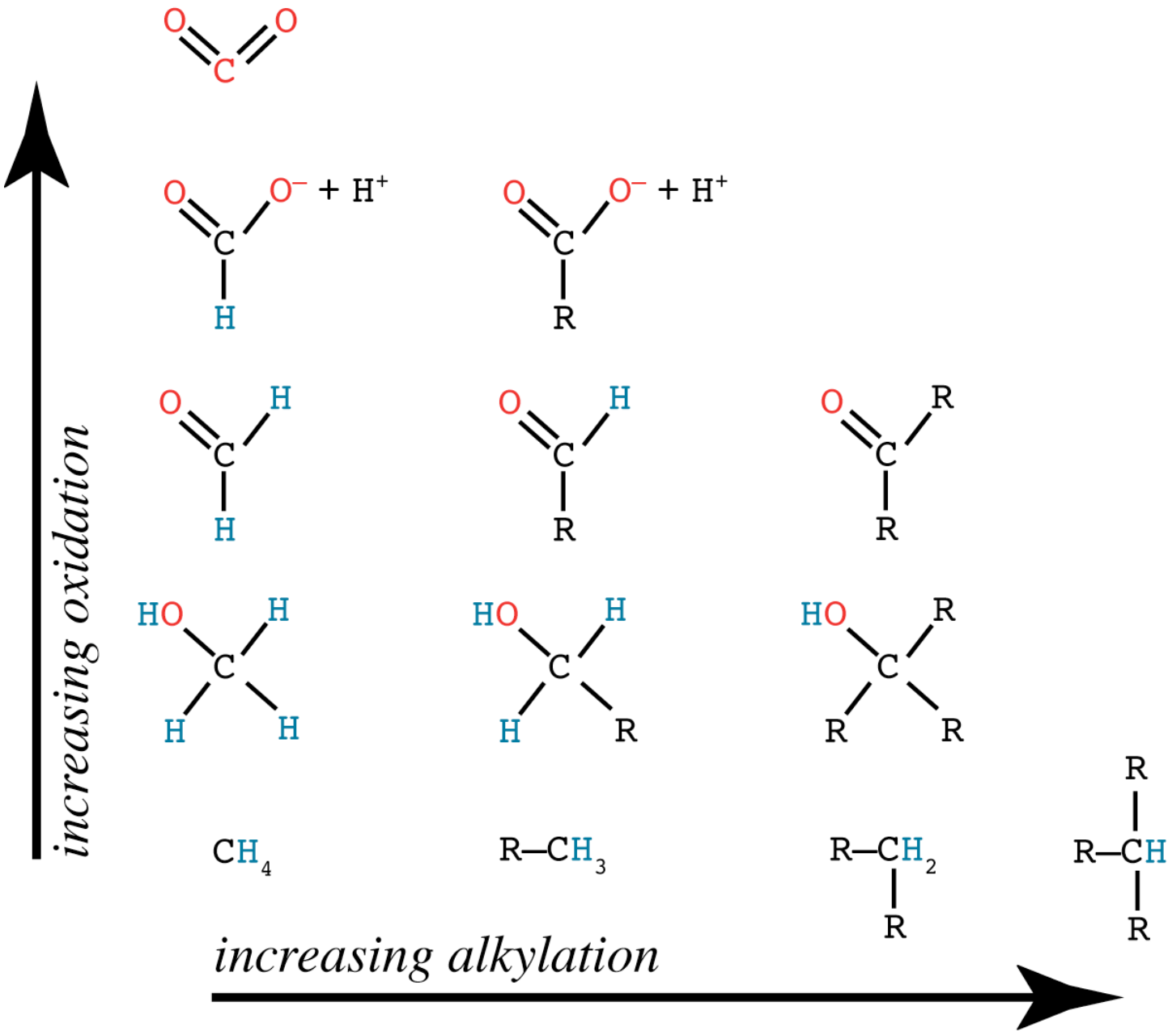
Biochemical diversity amongst bacteria is remarkable, and includes alternative pathways for carbon dioxide fixation, such as the hydroxypropionate pathway.



Ivan A. Berg, Daniel Kockelkorn, Wolfgang Buckel, Georg Fuchs (2007) A 3-Hydroxypropionate/4-Hydroxybutyrate Autotrophic Carbon Dioxide Assimilation Pathway in Archaea. Science 318:1782–1786.

Photosynthesis
SC/BIOL 4160

BIOENGINEERING CO_2 FIXATION


increasing oxidation
increasing alkylation
CH4
R—CH3
R—CH2
R
R—CH
R

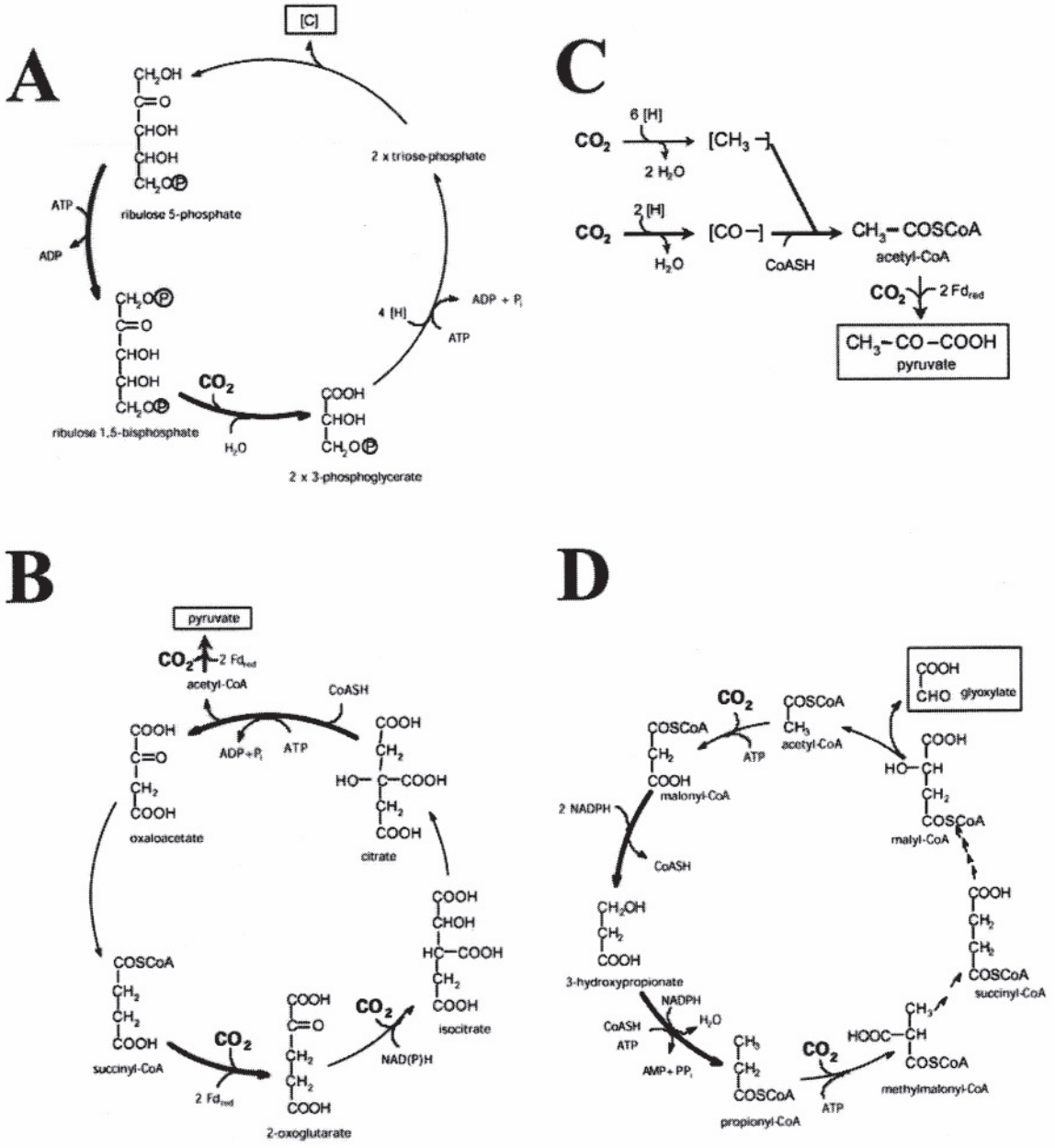
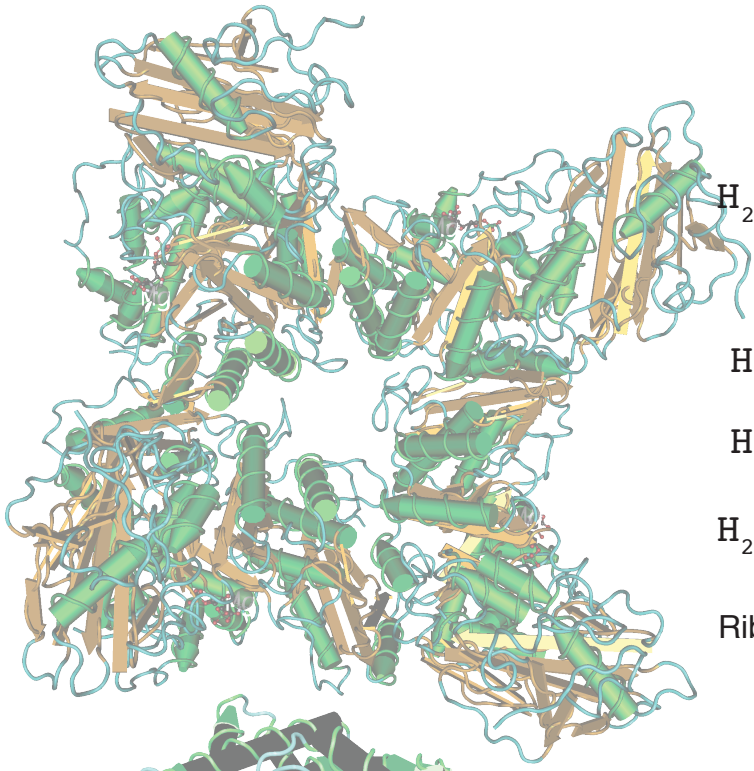
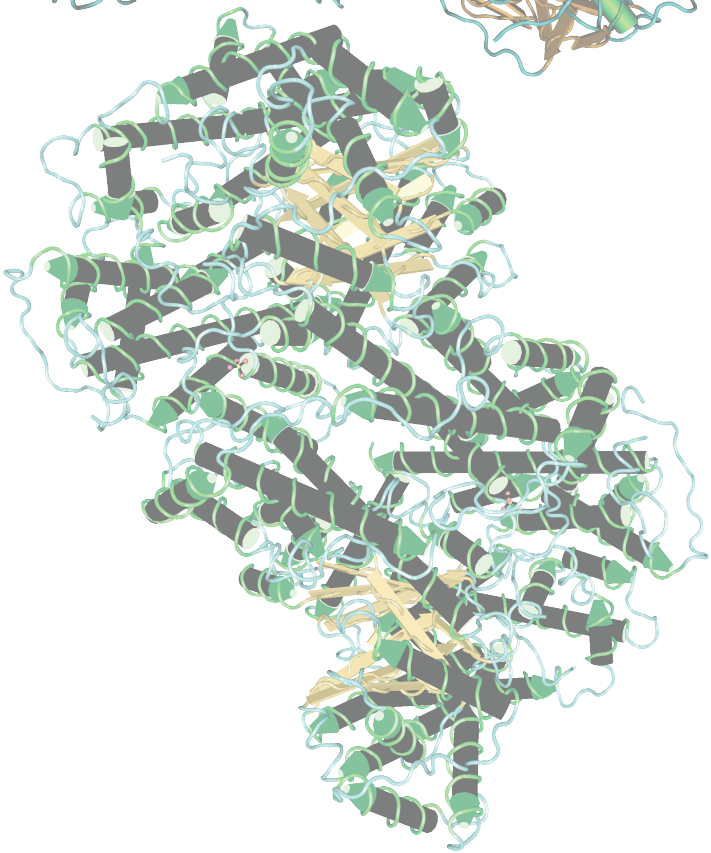
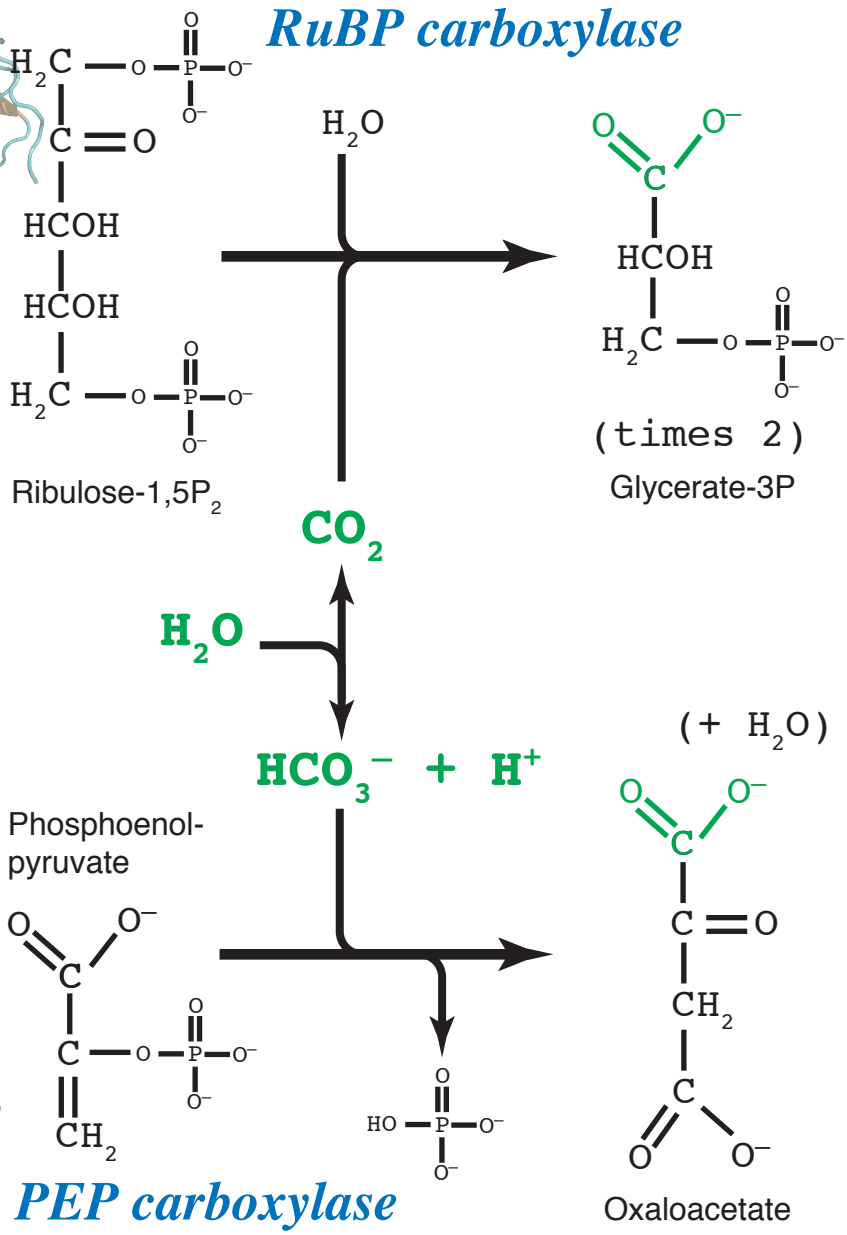


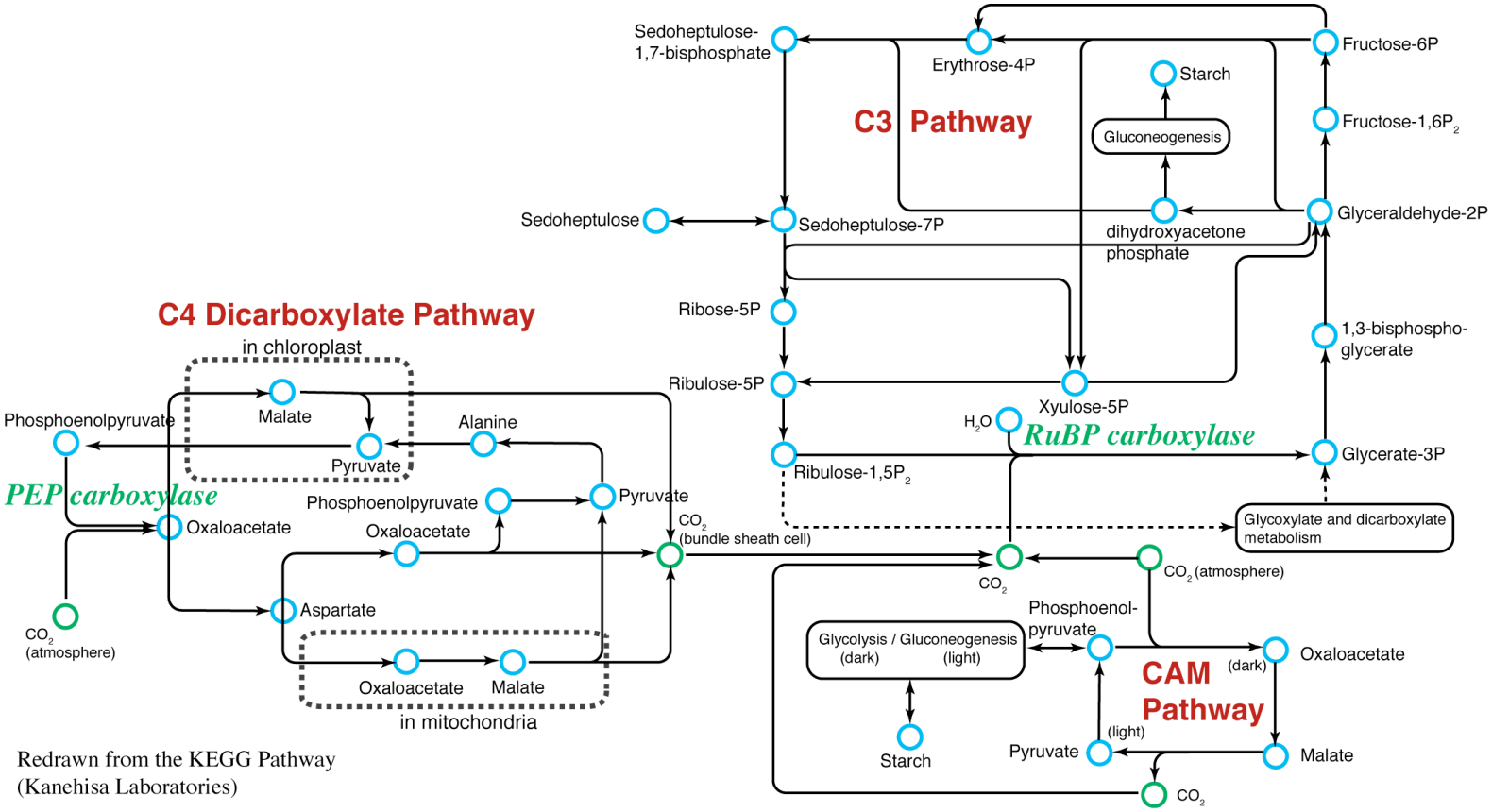
Fig. 1A–D. Outlines of the four known pathways for autotrophic CO_2 fixation[1]. The reactions catalyzed by key enzymes of these pathways are indicated by bold arrows. **A** Calvin-Bassham-Benson cycle; **B** reductive citric acid cycle; **C** reductive acetyl-CoA pathway; **D** 3-hydroxypropionate cycle. *[C]* Assimilated cell carbon, *[H]* reduction equivalent, Fd_{red} reduced ferredoxin, *[CH_3-]* enzyme-bound methyl group, *[CO-]* enzyme-bound carbon monoxide group.

[1] Michael Hügler, Harald Huber, Karl Otto Stetter and Georg Fuchs 2003. Autotrophic CO_2 fixation pathways in archaea (Crenarchaeota). Archives of Microbiology 179:160–173.






RuBP carboxylase
Ribulose-1,5P2
H2O
(times 2)
Glycerate-3P
CO2
H2O
HCO3− + H+
Phosphoenol-
pyruvate
(+ H2O)
PEP carboxylase
Oxaloacetate

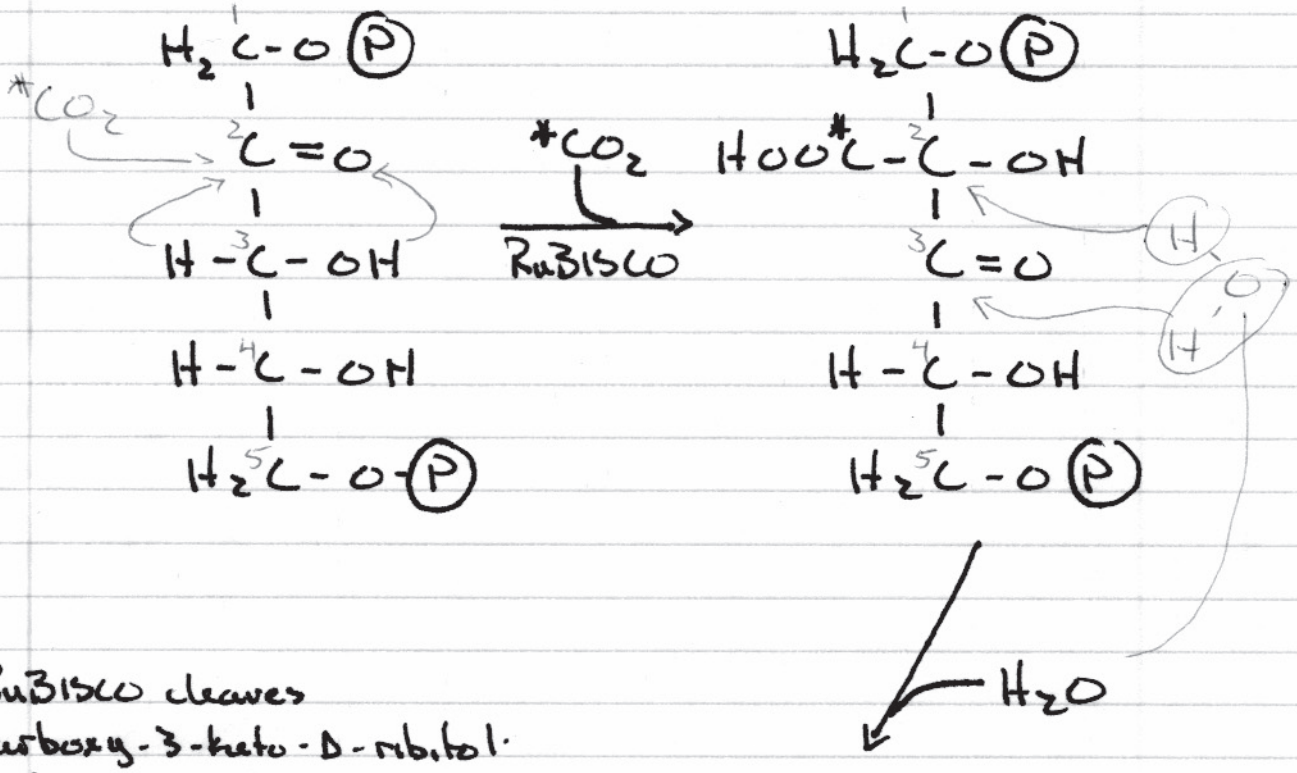


Redrawn from the KEGG Pathway
(Kanehisa Laboratories)

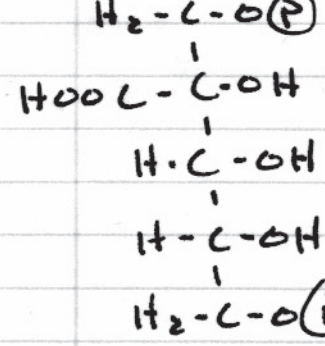
CO_2 FIXATION

(Ribulose 1,5-diphosphate)

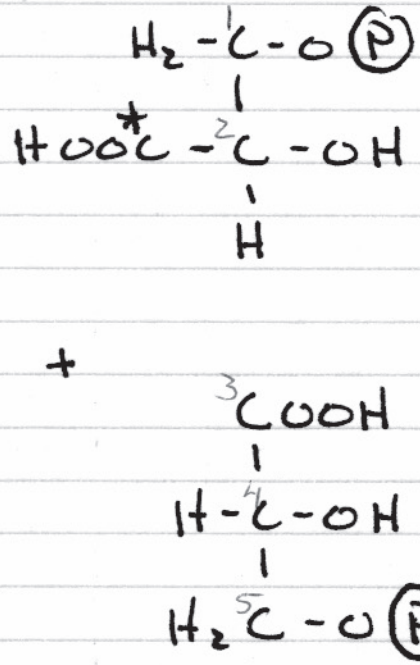
6-carbon intermediate* (2-carboxy-3-keto-D-ribitol 1,5-diphosphate)



*RuBISCO cleaves 2-carboxy-3-keto-D-ribitol 1,5-diphosphate to 3-PGA (2) and an analog



inhibits irreversibly (mimics an intermediate form during the enzymatic reaction)



3-phospho glycerate (3PGA)

ketoribitol (Hoober)

(x) also called ... ketoarabinitol (Bassham-Shop)

ξ ... ketopentitol (Goodwin & Mercer)

why? I don't know

Ribulose 1,5-bisphosphate carboxylase catalyzes the fixation of atmospheric carbon dioxide into carbohydrate. It is the most prevalent protein in the world. Without this enzyme, heterotrophic organisms (such as humans) could not survive. The carboxylation reaction is quite complex, as seen below.

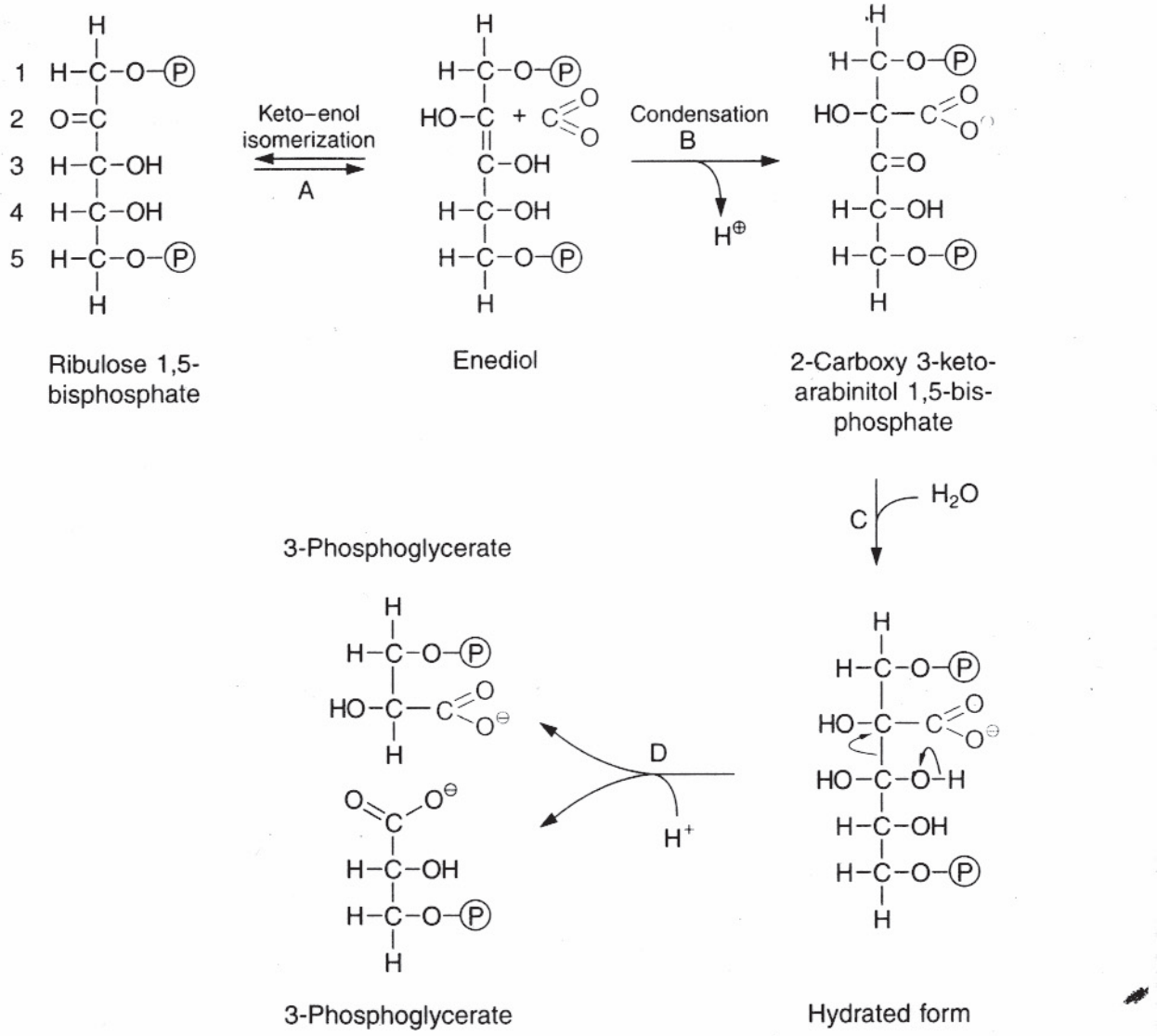


Figure 6.5 Reaction sequence in the carboxylation of RuBP by RubisCO. For the sake of simplicity $-PO_3^{2-}$ is symbolized as -P. An enediol, formed by keto–enol isomerization of the carbonyl group of the RuBP (A), allows the nucleophilic reaction of CO_2 with the C-2 atom of RuBP by which 2-carboxy-3-ketoarabinitol 1,5-bisphosphate (B) is formed. After hydration (C), the bond between C-2 and C-3 is cleaved and two molecules of 3-phosphoglycerate are formed (D).

Source: Heldt, Hans-Walter (1997) Plant Biochemistry and Molecuar Biology. Oxford University Press. page 152.

The substrate for RuBisCO is CO_2 rather than bicarbonate (HCO_3^-) based on

Substrate labeling	Rate of ^{14}C incorporation
$^{12}CO_2 + H^{14}CO_3^-$	slow
$^{14}CO_2 + H^{12}CO_3^-$	fast
$^{12}CO_2 + H^{14}CO_3^- + CA$	fast

where CA is carbonic anhydrase, which catalyzes the reaction:

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

The enzyme is multimeric, ~ 550 kDa

chlt genome: 8 large subunits (~54 kDa each)
nuclear genome: 8 small subunits (~12 kDa each)

assembled by chaperonins (ATP-required)

to yield L_8S_8 final form.

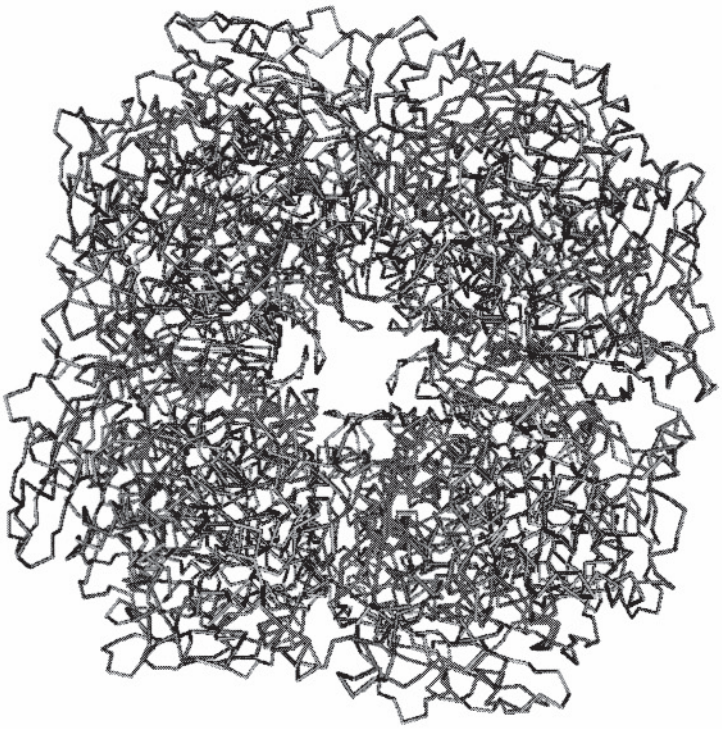
The catalytic active site is shared between two L subunits.

The L subunit is highly conserved, the S (small) subunit is not, and its role is not clear.

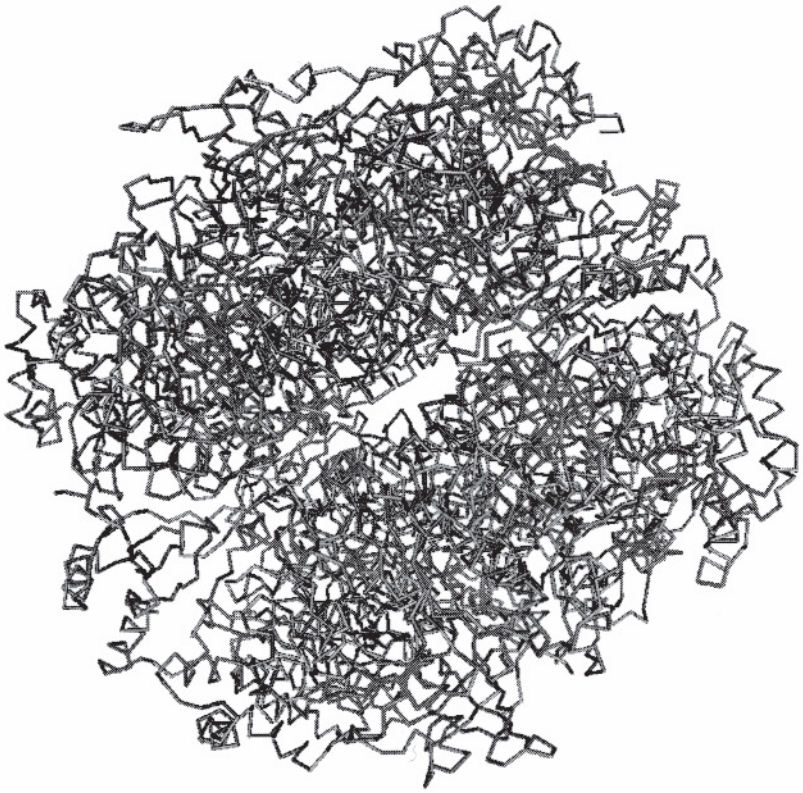
Dimers of L (L_2) have catalytic activity.

RuBISCO Structure[1]

Top_Down:



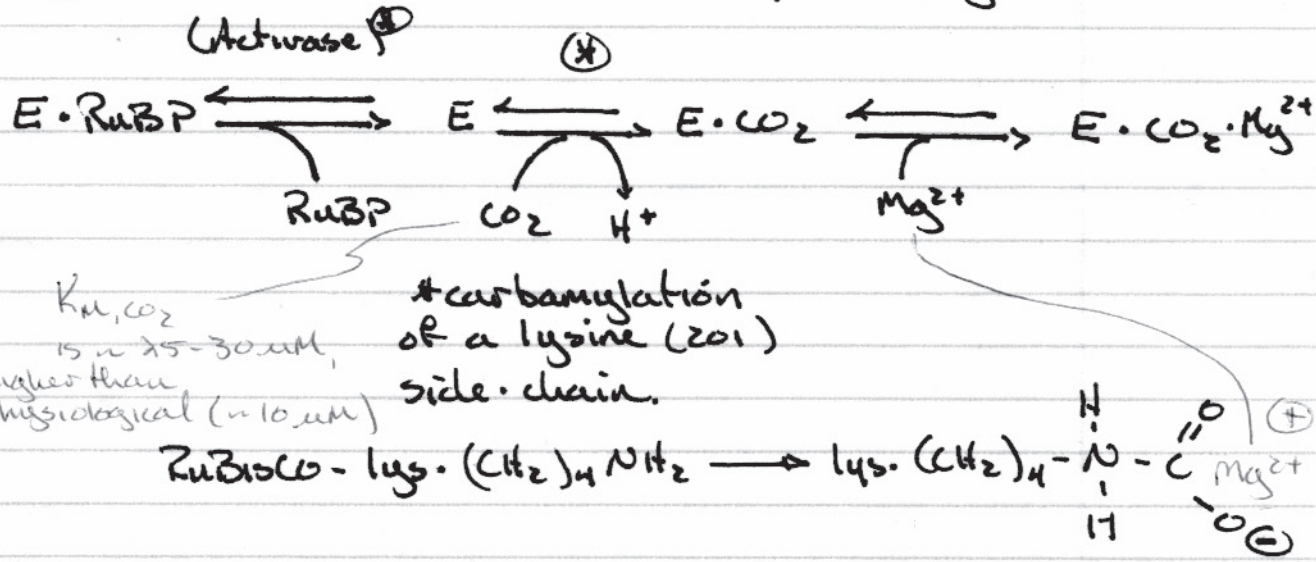
Sideways:



[1] Source: http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?form=6&db=t&Dopt=s&uid=6150

RuBISCO is located in the chloroplast stroma at a very high concentration for a protein (0.5 mM [≈ 250 mg/ml]) so, the active site concentration is about 4 mM. This is higher than the concentration of its substrate RuDP (Ribulose 1,5 - diphosphate) which is about 0.1 to 2 mM.

Regulation of RuBISCO activity is very complex:



$E \cdot CO_2 \cdot Mg^{2+}$ is the active form, binding RuBP at the active site.

(overhead)

⊛ The Mg^{2+} crosslinks to acidic residues: Glu 204 & Asp 203

⊛ The RuBISCO activase is itself light-activated and is very important in controlling the 'poise state' of carbon fixation as a function of light, and therefore ATP & NADPH production.

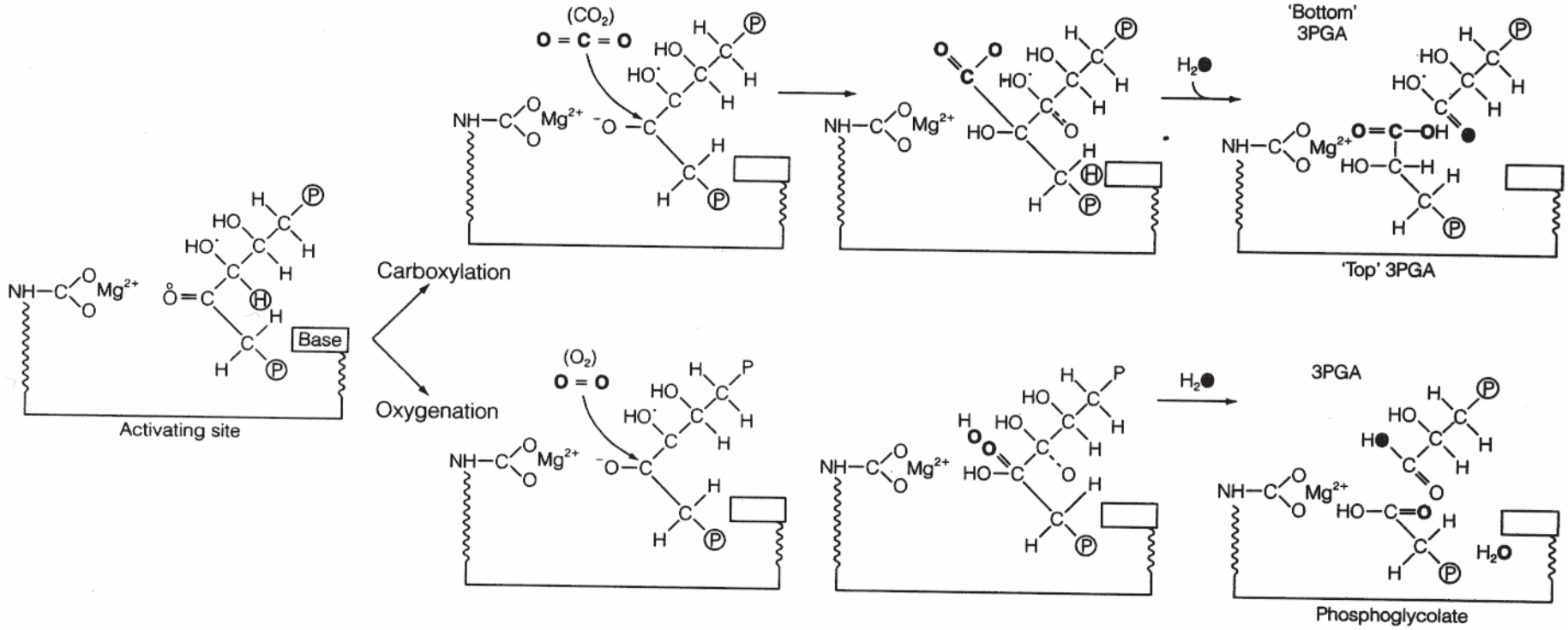
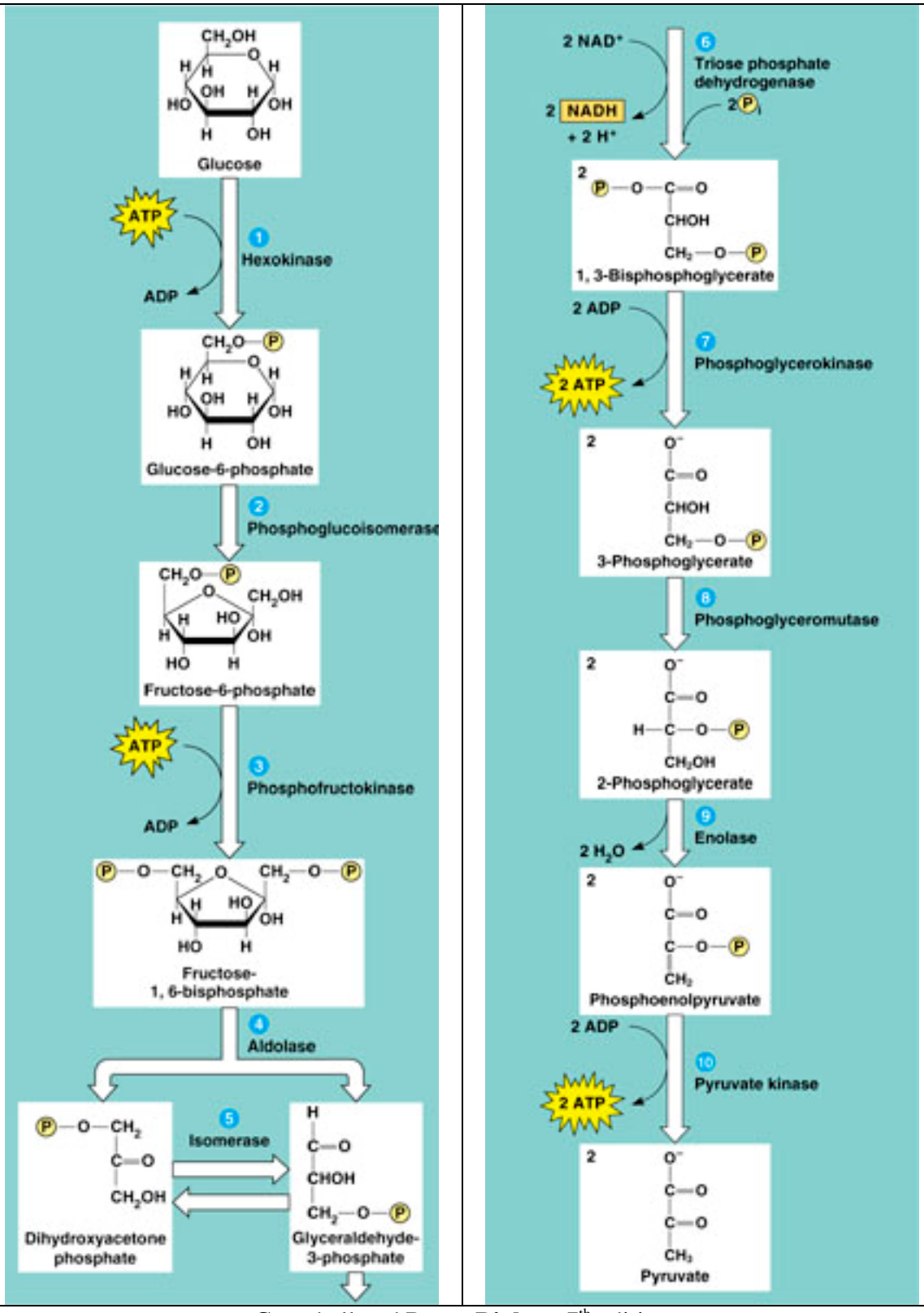


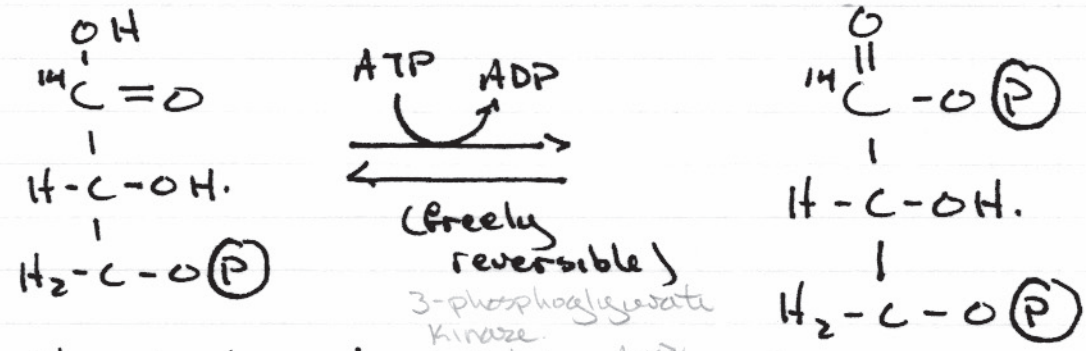
Figure 7.3. Schematic of the reaction site of ribulose bisphosphate carboxylase and the oxygenase showing molecular events in carboxylation and oxygenation of RuBP to give 3-phosphoglyceric acid (3PGA) or phosphoglycolate and 3PGA, respectively

Source:
Lawlor 2001 Photosynthesis



Campbell and Reece **Biology** 7th edition

Regeneration of Ribulose 1,5 - diphosphate.

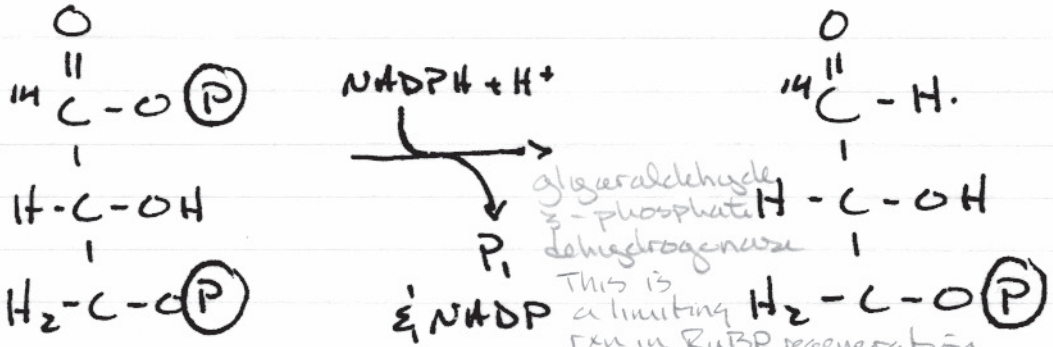


3-phosphoglycerate kinase. ADP & low ATP/ADP slow the rxn as does glyceraldehyde 3 phosphate.

3-phosphoglycerate

1,3 diphosphoglycerate

(phosphorylation of 3-PG to give 1,3-dPG)



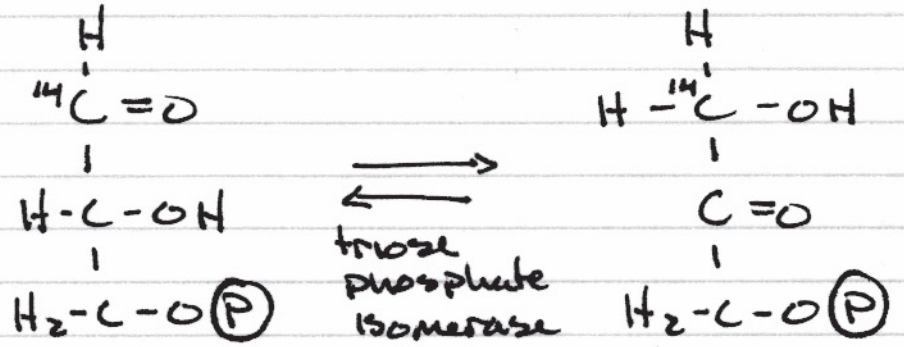
glyceraldehyde 3-phosphate dehydrogenase. This is a limiting rxn in RuBP regeneration.

(reduction of 1,3-dPG to yield glyceraldehyde 3 phosphate

Both of the reactions above should be familiar from glycolysis (though in the reverse direction)

The 3-phosphoglyceraldehyde can enter a number of alternate reactions to eventually regenerate the starting compound RuDP.

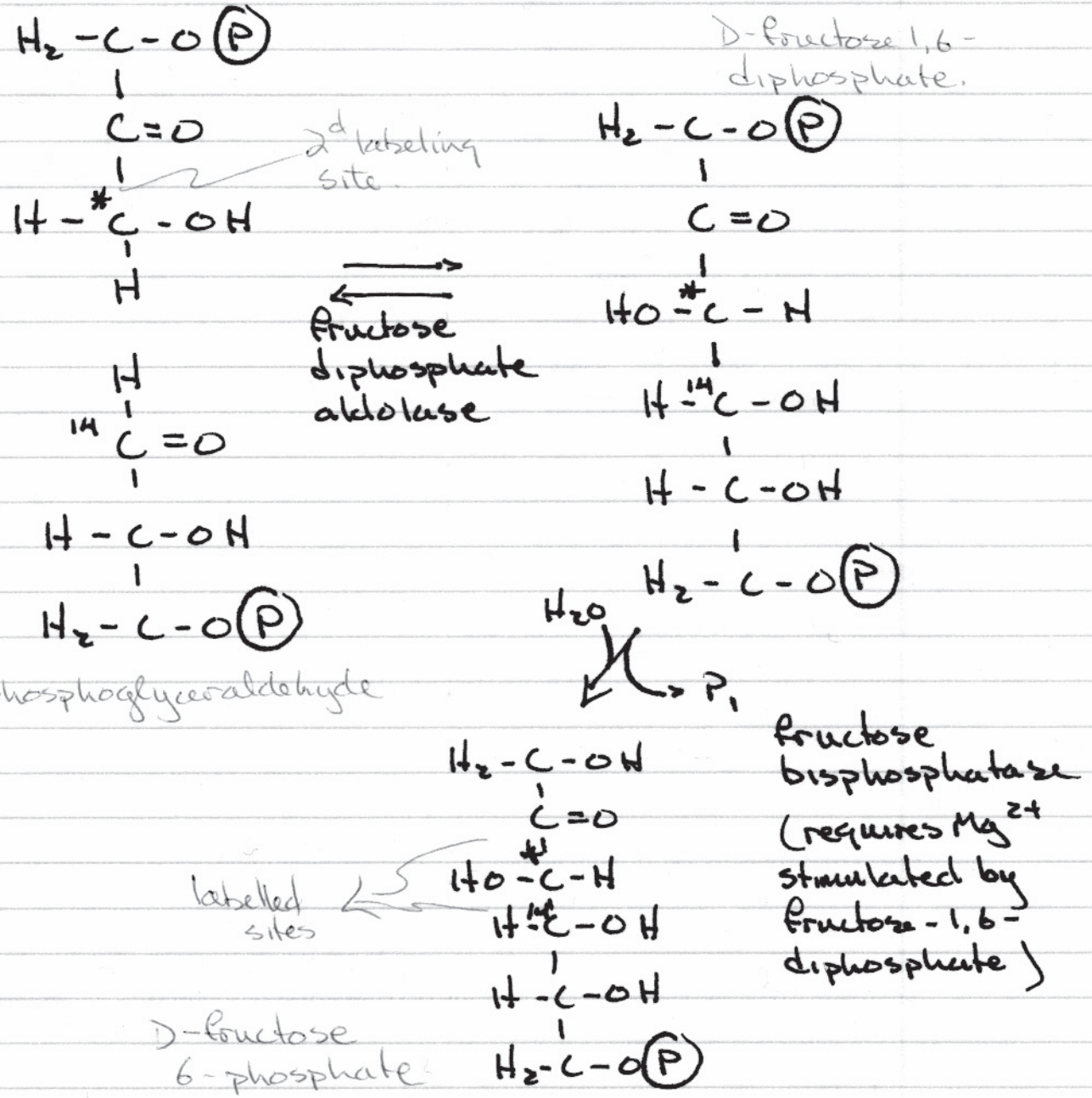
(1)



3-phosphoglyceraldehyde dihydroxyacetone phosphate

dihydroxyacetone phosphate

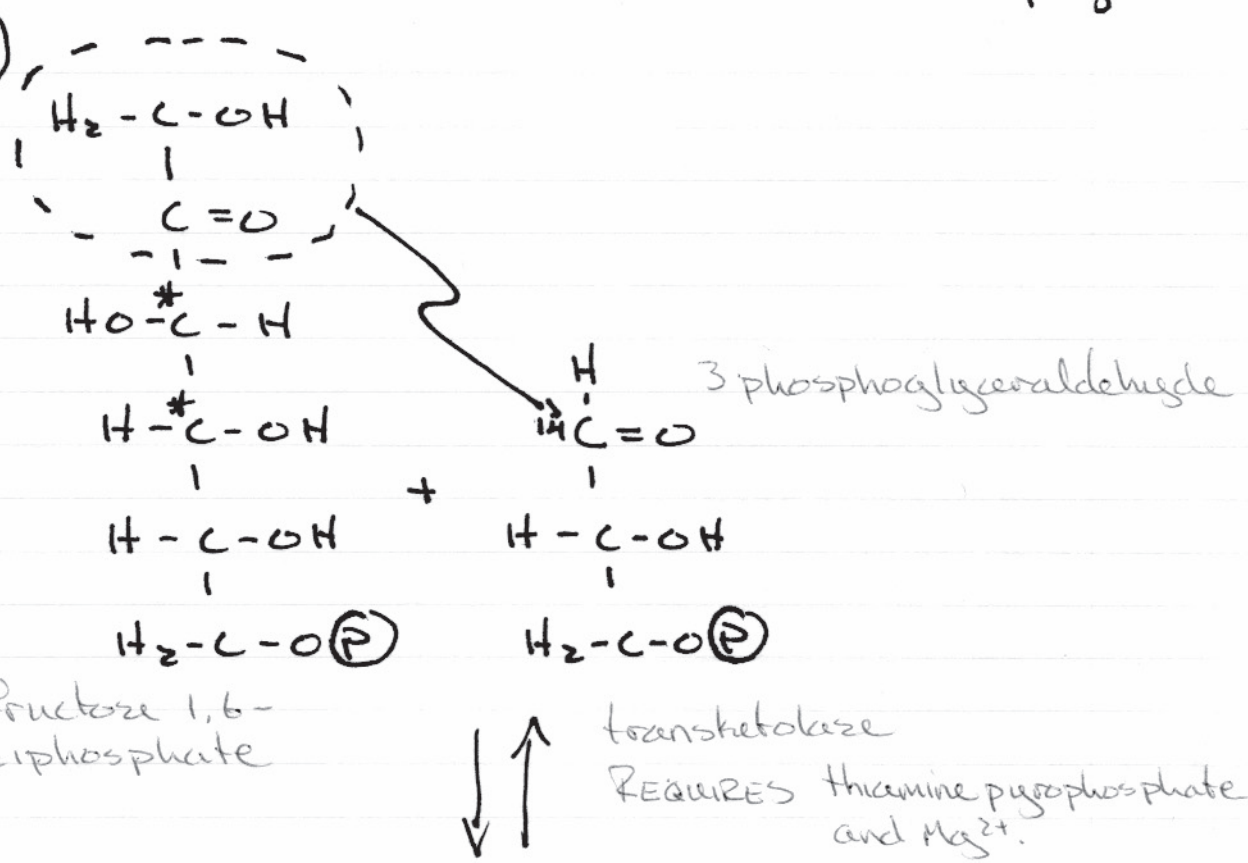
(2)



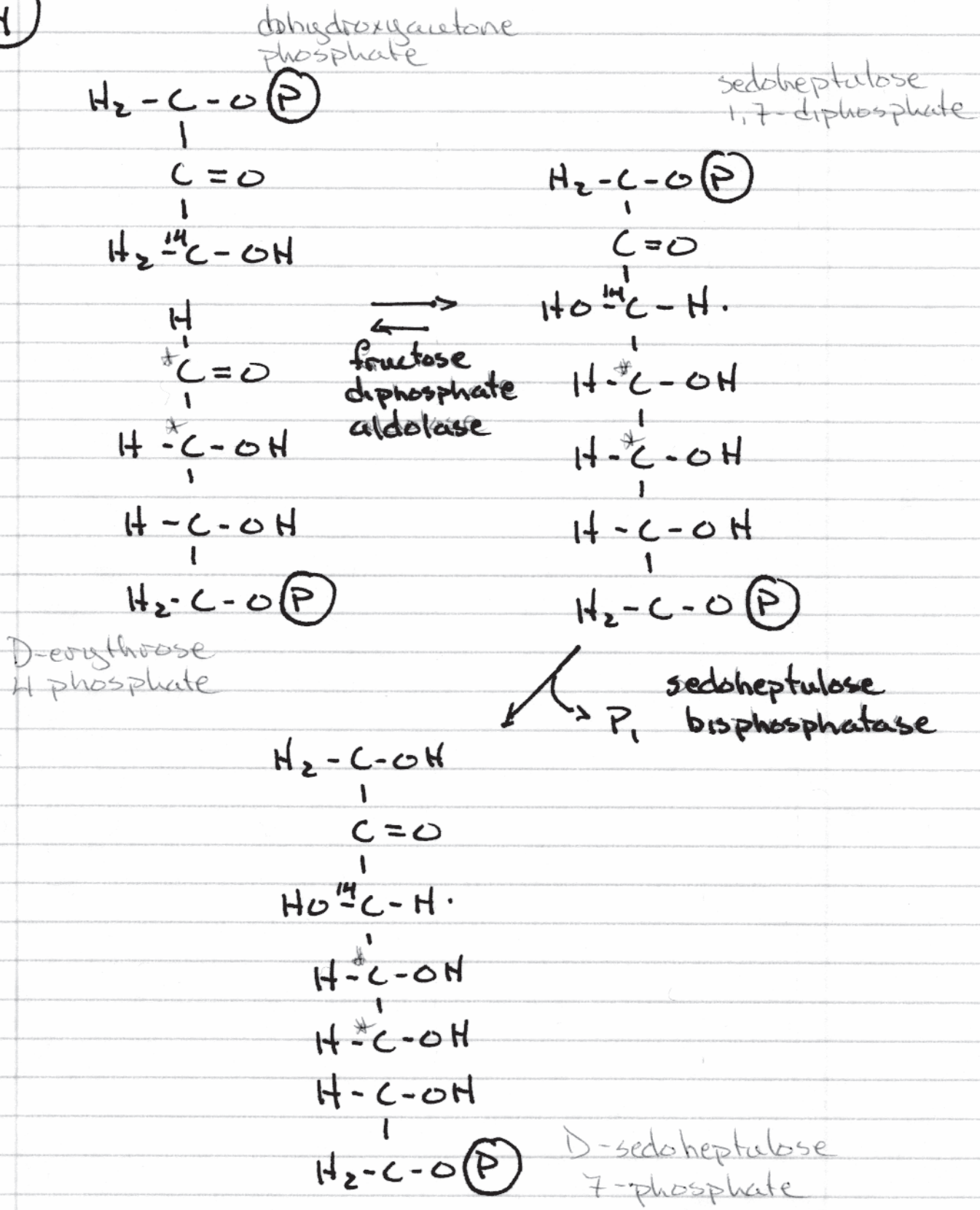
3 phosphoglyceraldehyde

D-fructose 6-phosphate

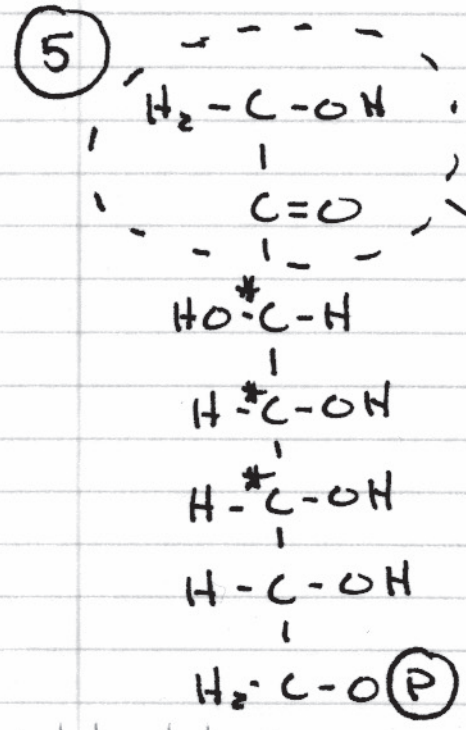
(3)


3 phosphoglyceraldehyde
fructose 1,6-diphosphate
transketolase
REQUIRES thiamine pyrophosphate and Mg^{2+}.
D-erythrose-4-phosphate
D-xylulose-5-phosphate

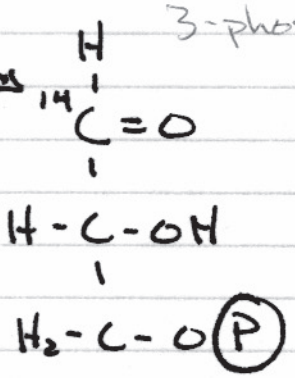
(4)


dihydroxyacetone phosphate
sedoheptulose 1,7-diphosphate
fructose diphosphate aldolase
D-erythrose 4 phosphate
sedoheptulose bisphosphatase
P_i
D-sedoheptulose 7-phosphate

5



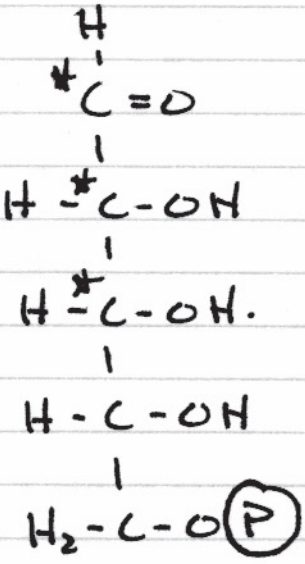
sedoheptulose 7 phosphate



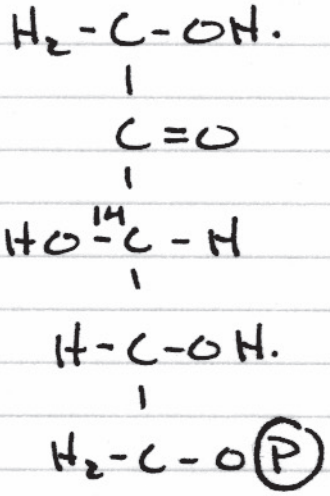
3-phosphoglyceraldehyde

transketolase

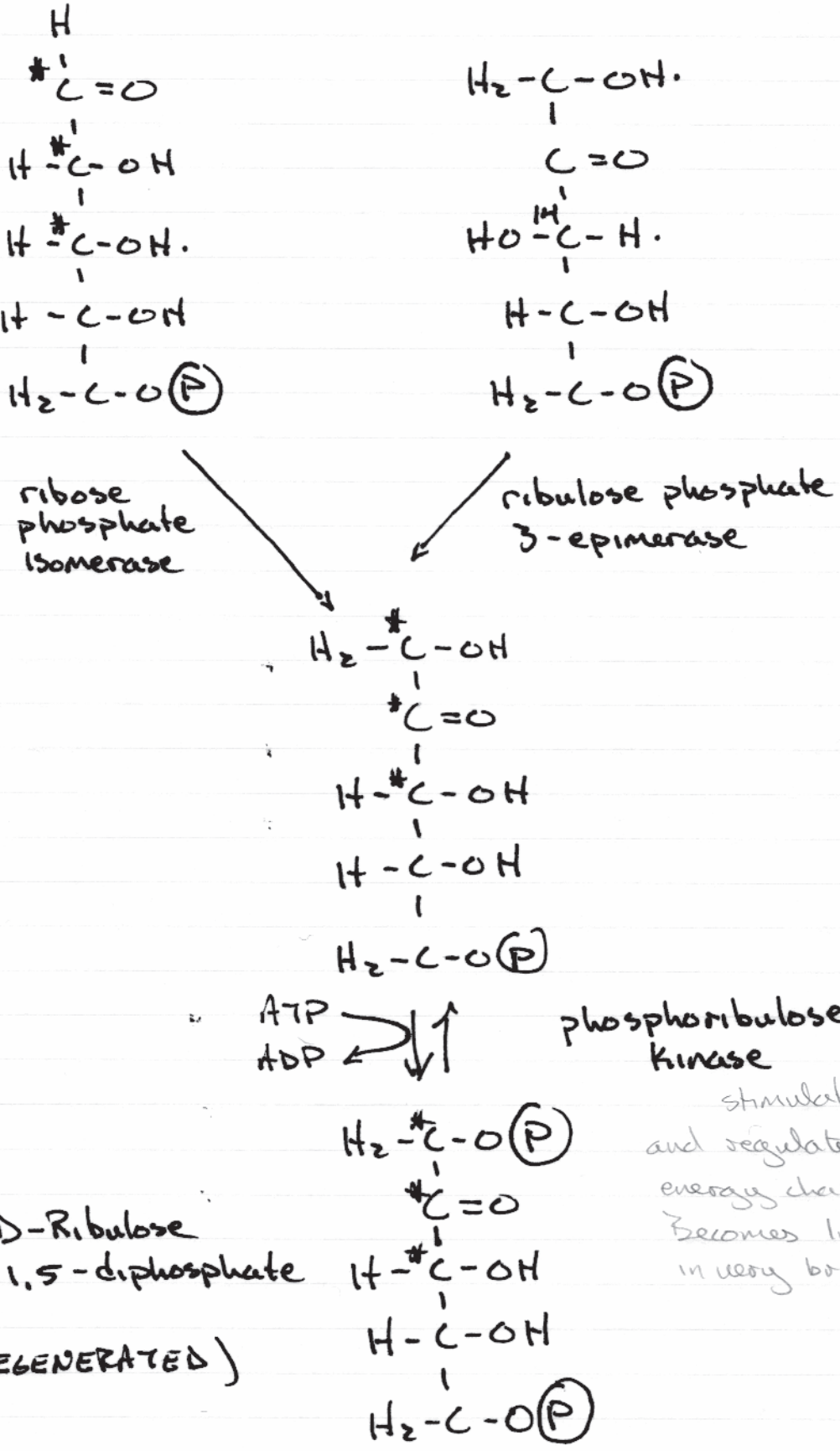
REQUIRES thiaminepyrophosphate and Mg^{2+}



D-ribose-5 phosphate



D-xylulose 5-phosphate



stimulated by ATP and regulated by energy charge. Becomes limiting in very bright light

$^{14}CO_2$ LABELING: Evidence for the Calvin Cycle

Distribution of ^{14}C after 5.4 sec incubation.

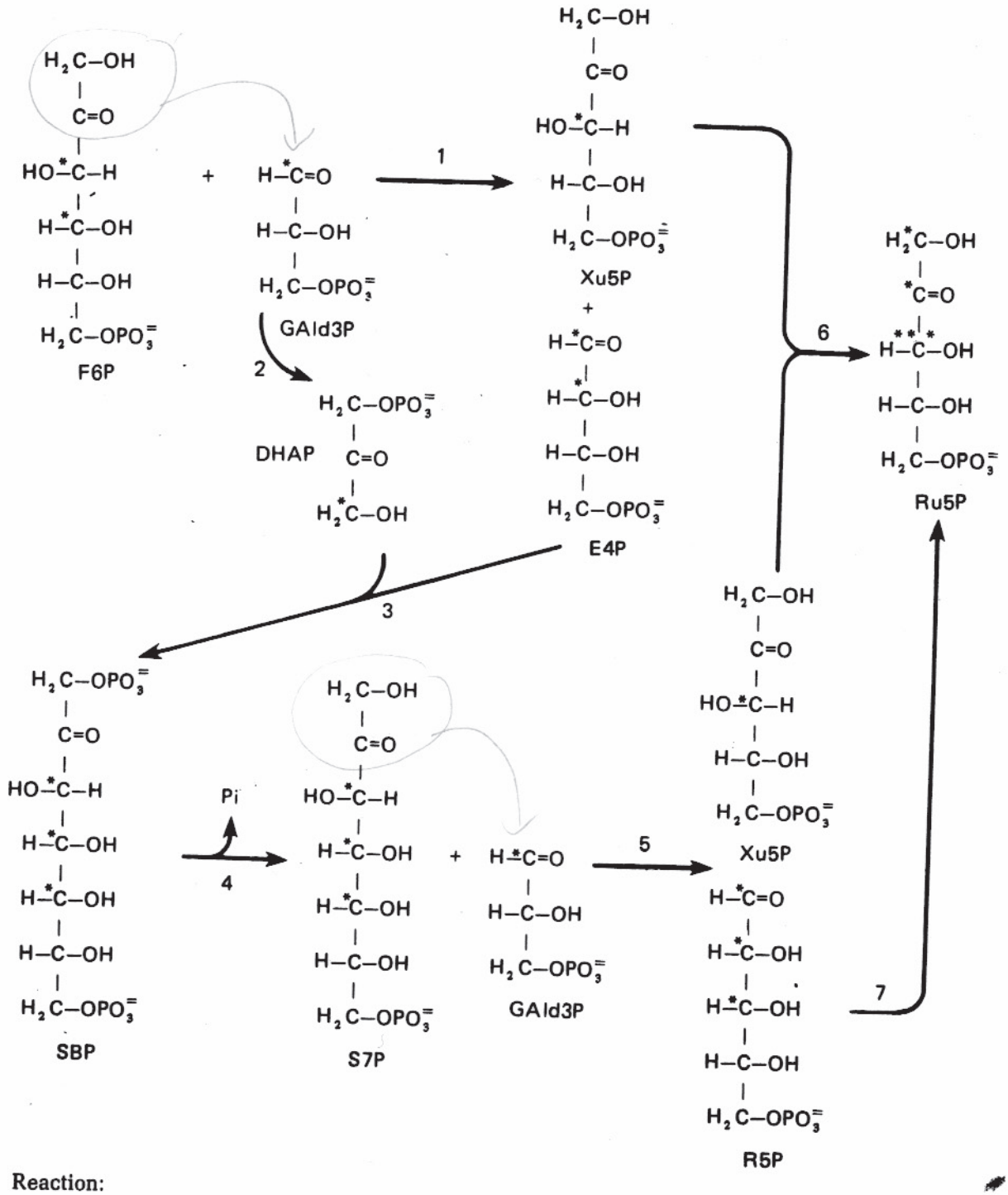
Carbon Atom	PGA	fructose	sedoheptulose	ribulose
1	82%	3%	2%	11%
2	9	3	2	10
3	9	43	28	69
4		42	24	5
5		3	27	3
6		3	2	
7			2	

Initial experiments were done with algae (Chlorella and Scenedesmus). After $^{14}CO_2$ addition, the cells were killed by dropping them into boiling alcohol.

30 sec labeling: ^{14}C in 3-PGA, triose phosphates, and hexose phosphates

5 sec labeling: ^{14}C in the carboxyl group of 3-PGA. Eventually, it was realized CO_2 was condensing with a pentose phosphate, then cleaving to (2) 3-PGA

In the hexose phosphates, C-3 & C-4 were most heavily labeled, indicating the hexose was formed by condensation of two triose phosphates.

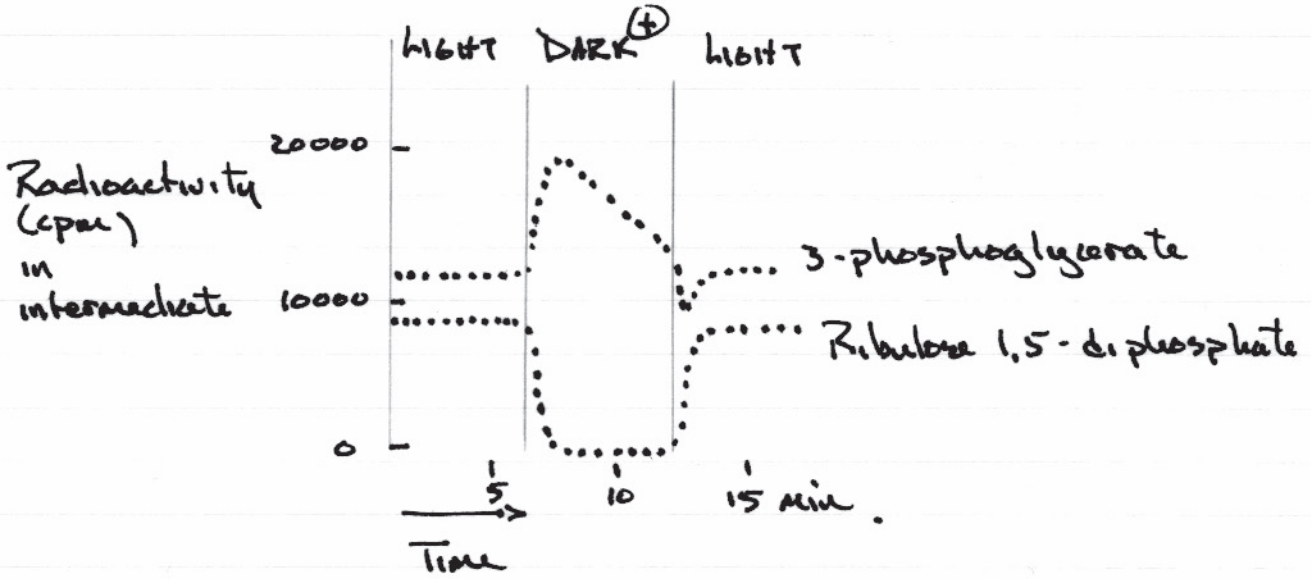


Reaction:

1. Fructose 6-phosphate + glyceraldehyde 3-phosphate $\xrightarrow{\text{transketolase}}$ xylulose 5-phosphate + erythrose 4-phosphate
2. Glyceraldehyde 3-phosphate $\xrightarrow{\text{triose phosphate isomerase}}$ dihydroxyacetone phosphate
3. Erythrose 4-phosphate + dihydroxyacetone phosphate $\xrightarrow{\text{aldolase}}$ sedoheptulose 1,7-bisphosphate
4. Sedoheptulose 1,7-bisphosphate $\xrightarrow{\text{sedoheptulose bisphosphatase}}$ sedoheptulose 7-phosphate + inorganic phosphate
5. Sedoheptulose 7-phosphate + glyceraldehyde 3-phosphate $\xrightarrow{\text{transketolase}}$ xylulose 5-phosphate + ribose 5-phosphate
6. Xylulose 5-phosphate $\xrightarrow{\text{ribulose phosphate-3-epimerase}}$ ribulose 5-phosphate
7. Ribose 5-phosphate $\xrightarrow{\text{ribose phosphate isomerase}}$ ribulose 5-phosphate

SOURCE:
Hoober, JK (1984)
Chloroplasts

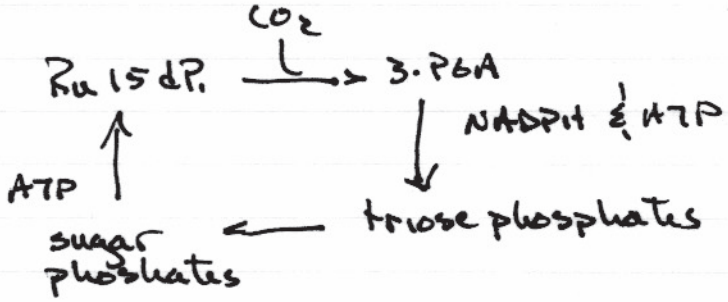
Light/dark transitions indicate there is a "connectivity" between 3-phosphoglycerate and ribulose 1,5 diphosphate ⊕



⊕ NADPH & ATP will decline.

Final evidence for the Calvin Cycle came from isolation & characterization of the enzymes acting at each step.

⊕ In simplified form



So Ru 1,5-di P & 3-PGA sample two different 'states'.

Source: Goodwin & Mercer Intro Biochem pages 138-139

LIGHT ACTIVATION OF CALVIN CYCLE.
(thioredoxin).

enzymes activated by light include

fructose bisphosphatase
sedoheptulose bisphosphatase

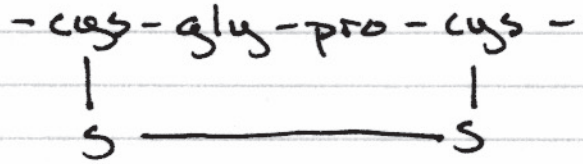
NADP+ - glyceraldehyde phosphate dehydrogenase

& phosphoribulokinase.

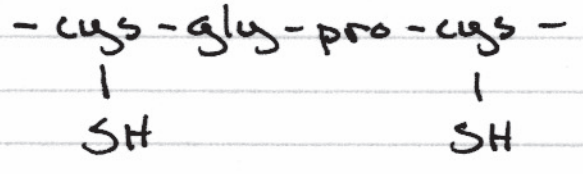
All are intrinsic to energy utilization.

Their activation is mediated by the redox poise* of the electron transport chain; specifically ferredoxin (in the ferredoxin·NADP dehydrogenase of PS I).

Reduced ferredoxin, produced in the light, in turn reduces thioredoxin:



(oxidized)



(reduced)

* not on/off, but modulated by light intensity. The higher the light intensity, the higher the $\frac{(\text{thioredox})\ \text{red}}{(\text{thioredox})\ \text{ox}}$ ratio.

The thioredoxin in turn reduces disulfide linkages (-S-S-) to sulfhydryls (-SH HS-) on the enzymes, activating them.

In addition to carboxylation, Ribulose 1,5-bisphosphate carboxylase catalyzes the fixation of atmospheric oxygen, without net carbohydrate production. Hence, the enzyme is commonly called Ribulose 1,5-bisphosphate carboxylase/oxygenase, or RuBisCO. Oxygen and carbon dioxide are very similar: small, linear molecules with analogous intrinsic dipoles. Thus the RuBisCO active site for carbon dioxide is 'fooled' into binding oygen instead.

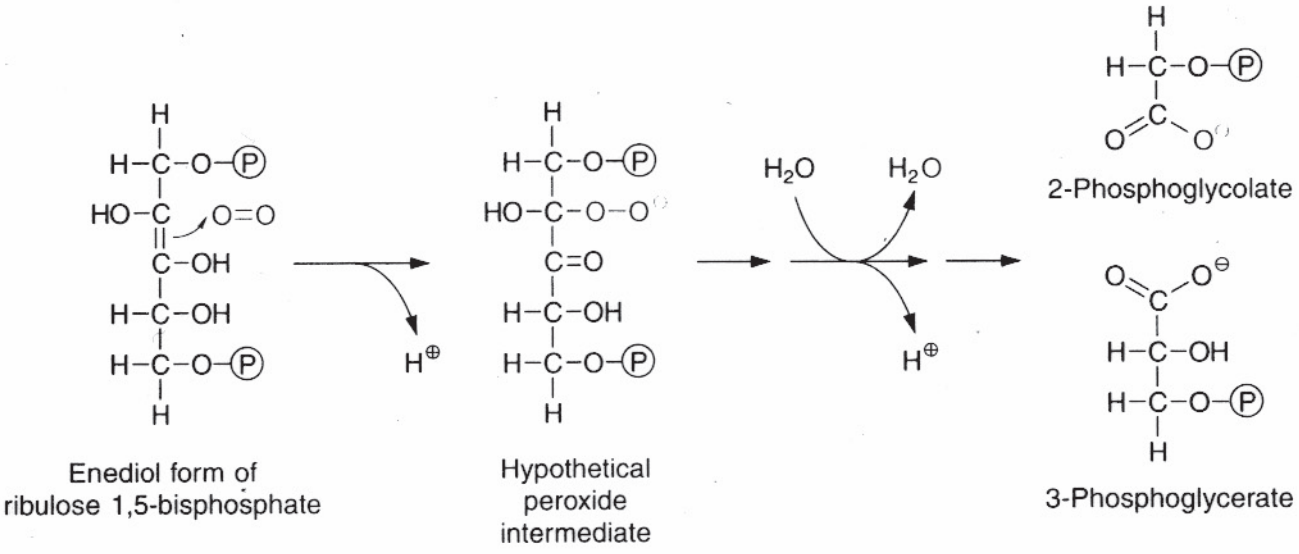


Figure 6.6 Part of the reaction sequence in the oxygenation of RuBP as catalysed by RubisCO. O_2 probably reacts in a similar way to CO_2 with the enediol of RuBP and thus forms a peroxide. In the subsequent cleavage of the O_2 adduct, one atom of the O_2 molecule is found in the water and the other in the carboxyl group of the 2-phosphoglycolate.

Kinetic Properties:

$K_M[CO_2]$: 9 μmol l^{-1}

$K_M[O_2]$: 535 μmol l^{-1}

K_M[RuBP]: 28 μmol l^{-1}

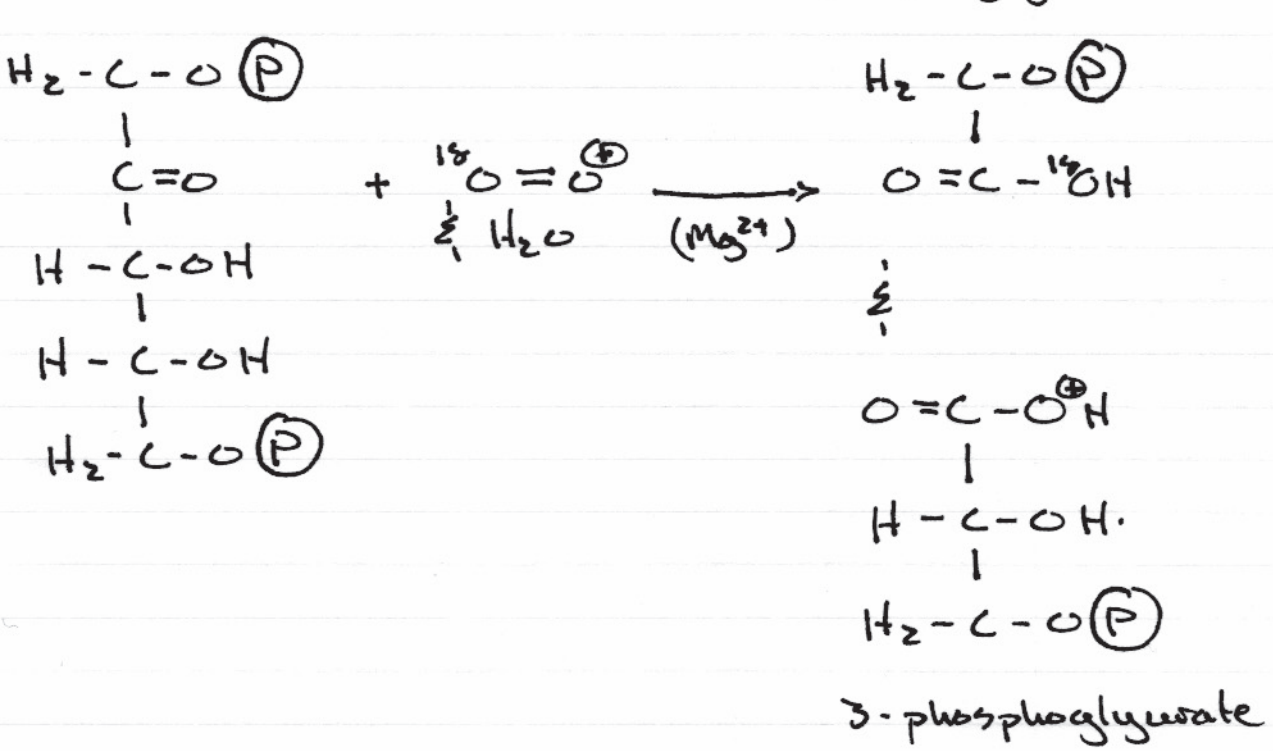
At normal atmospheric conditions (0.035%=350 ppm CO_2, 21% O_2), the concentrations in water at 25° C are: [CO_2], 11 μmol l^{-1}; [O_2], 253 μmol l^{-1}.

Source: Heldt, Hans-Walter (1997) Plant Biochemistry and Molecuar Biology. Oxford University Press. page 153-4.

PHOTORESPIRATION

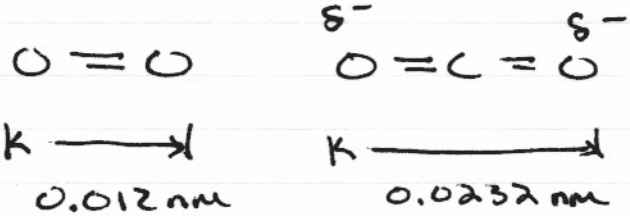
In addition to the carboxylation reaction of RuBISCO, it also has a biochemically significant oxygenase reaction.

The reaction:



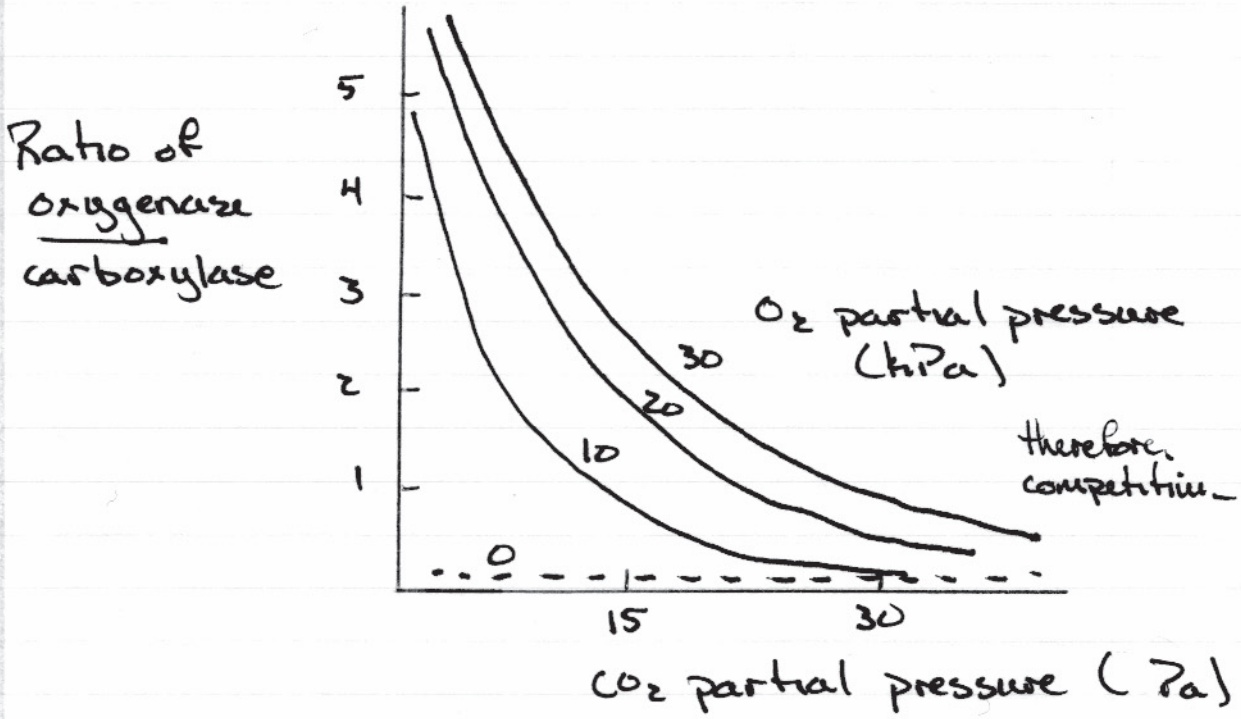
while the 3-phosphoglycerate can enter the Calvin Cycle, the 2-phosphoglycolate must be "regenerated" in a series of reactions in the chloroplast, peroxisome and mitochondria.

Oxygen and carbon dioxide are very different



Furthermore, oxygen reactions normally involve a transition metal (for example Fe) or an e^- donating redox group.

So the oxygenase reaction of RuBisCO remains unclear, but probably involves coordination to the Mg^{2+} ion in the active site.



(atmospheric O_2 partial pressure is ≈ 21 kPa
" CO_2 " " " is ≈ 38 Pa)

Note that during photosynthesis the partial pressure of O_2 will be higher. (respiration will increase CO_2) overall, a ratio of about 0.4 $\frac{\text{oxygenase}}{\text{carboxylase}}$ may occur. It is significant.

Because of the significance of the oxygenase reaction, much effort has been exerted on modifying RuBisCO specificity.

The specificity

$$\frac{v_{CO_2}}{v_{O_2}} = \frac{V_{max}^{CO_2} \cdot K_M^{O_2}}{V_{max}^{O_2} \cdot K_M^{CO_2}} \cdot \frac{pCO_2}{pO_2}$$

- $V_{max}^{CO_2}$: maximal carboxylation
- $K_M^{O_2}$: K_M for oxygen
- pCO_2: partial pressure CO_2
- $V_{max}^{O_2}$: maximal oxygenase
- $K_M^{CO_2}$: K_M for CO_2
- pO_2: partial pressure O_2.

RuBisCO specificity is significantly higher in higher plants compared to algae and cyanobacteria.

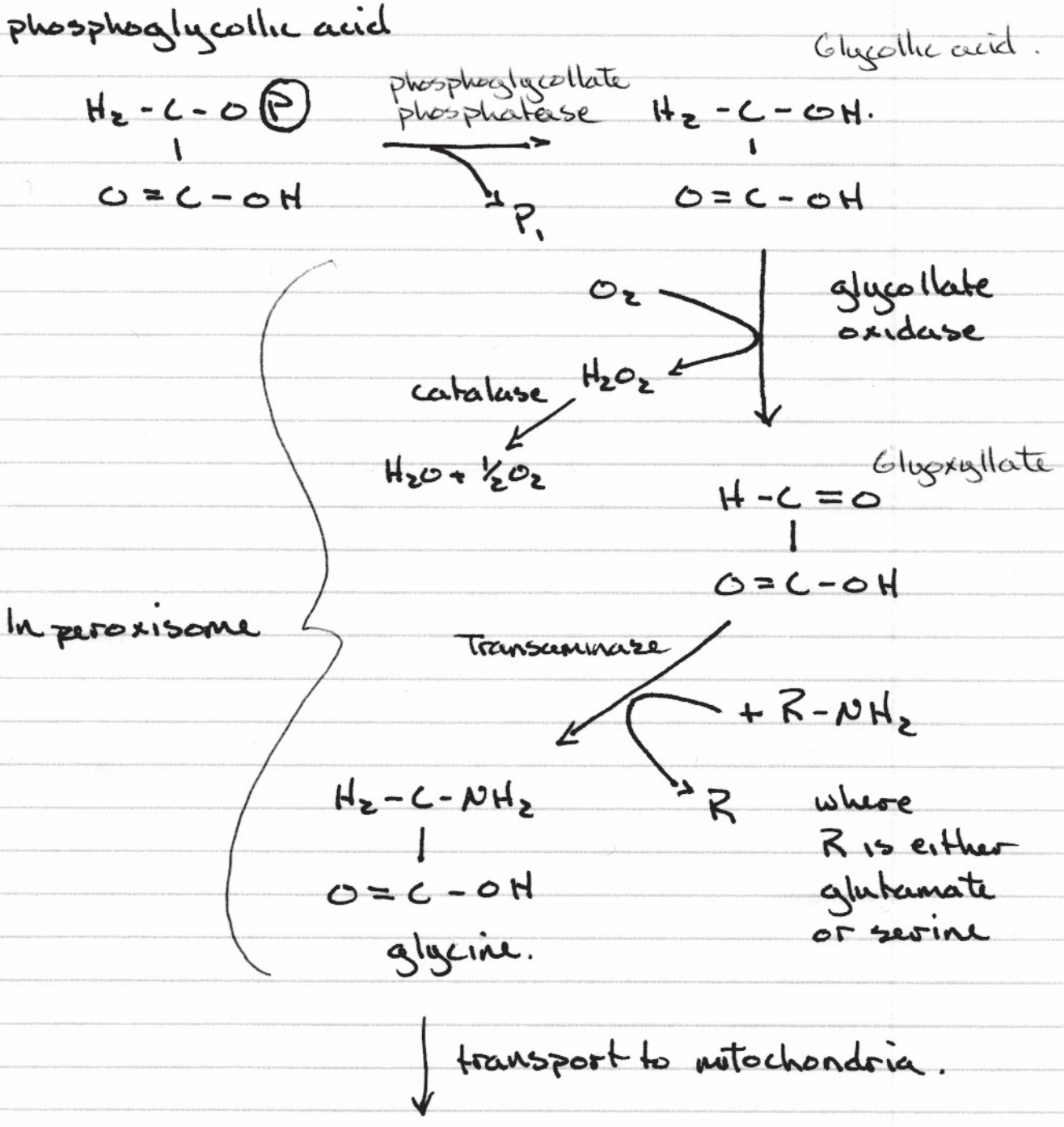
	Specificity	
C_3 plants	77 - 94	mol CO_2 / mol O_2
C_4 plants	58 - 82	
green algae	50 - 60	
cyanobacteria	35 - 48	
Rhodospirillum	10	(purple bact. range 10 - 60)

SOURCE: Lawlor 2001 Photosynthesis pages 152-153

* That is, $v_{CO_2} = \frac{V_{max}^{CO_2}}{K_M^{CO_2}} \cdot pCO_2$

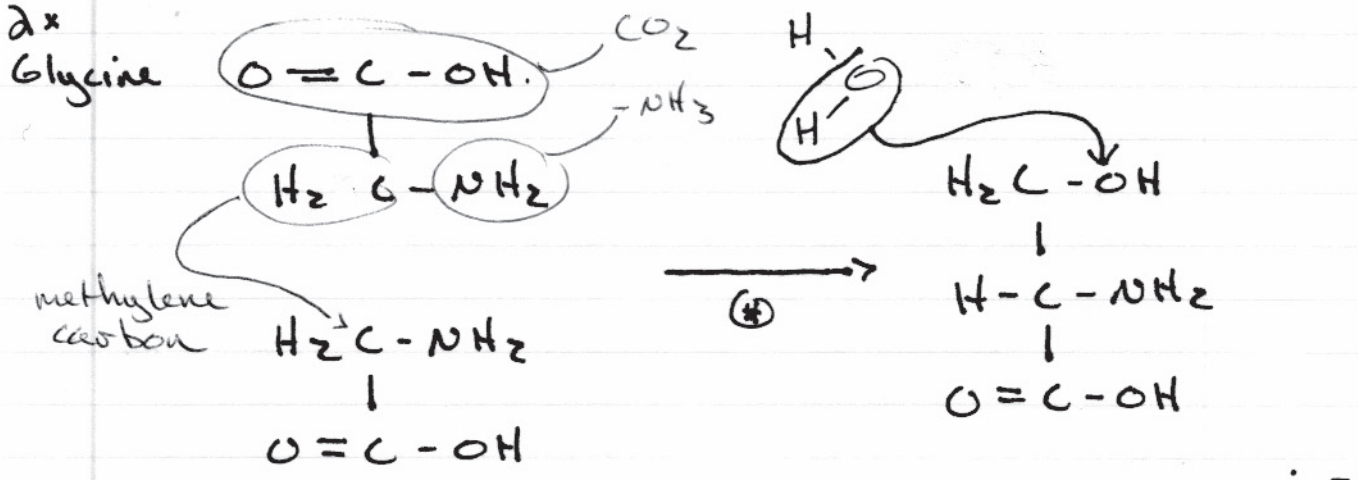
It is not related directly to Michaelis Menten $v = \frac{V_{max}[S]}{K_M + [S]}$ because competitive inhibition will exist.

Regeneration of 3-phosphoglycerate



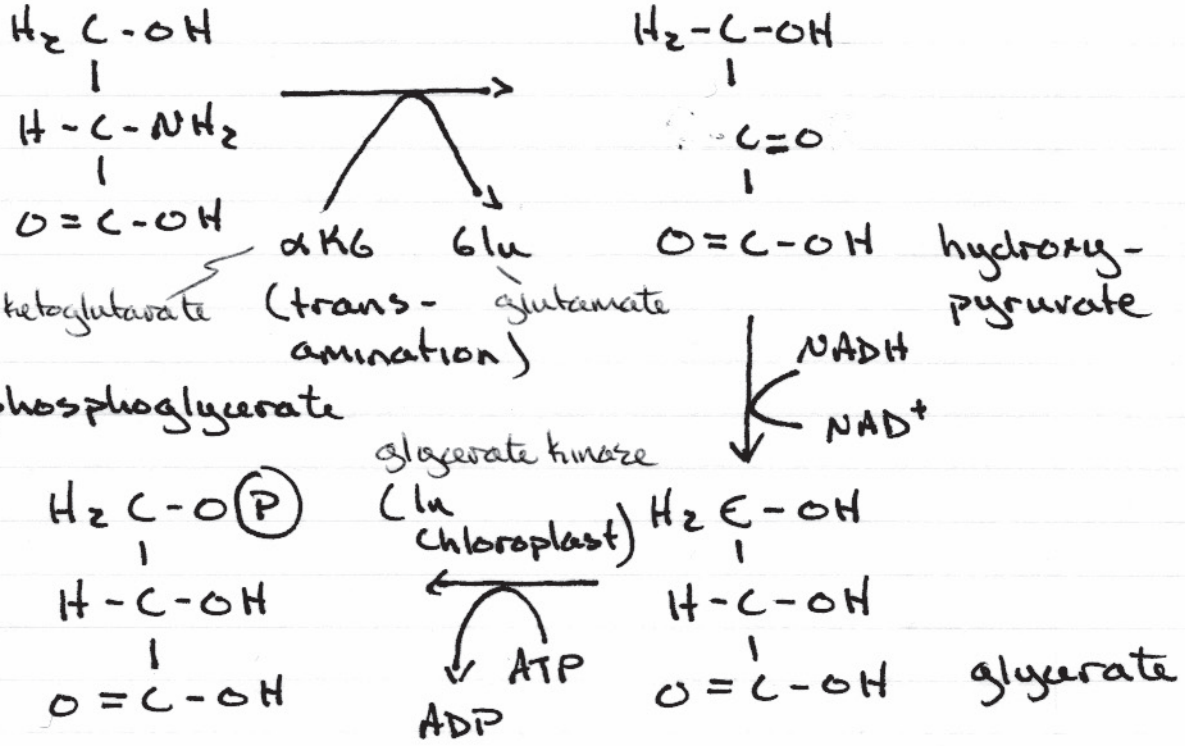
3-phosphoglycerate regeneration (continued)

(In Mitochondria)



(4) the conversion of 2 glycines to a serine, plus CO_2 & NH_3 (ammonia) involves 2 enzymatic reactions and the intermediate co-factor tetrahydrofolate (to which the methylene carbon is bound)

(In Peroxisome)



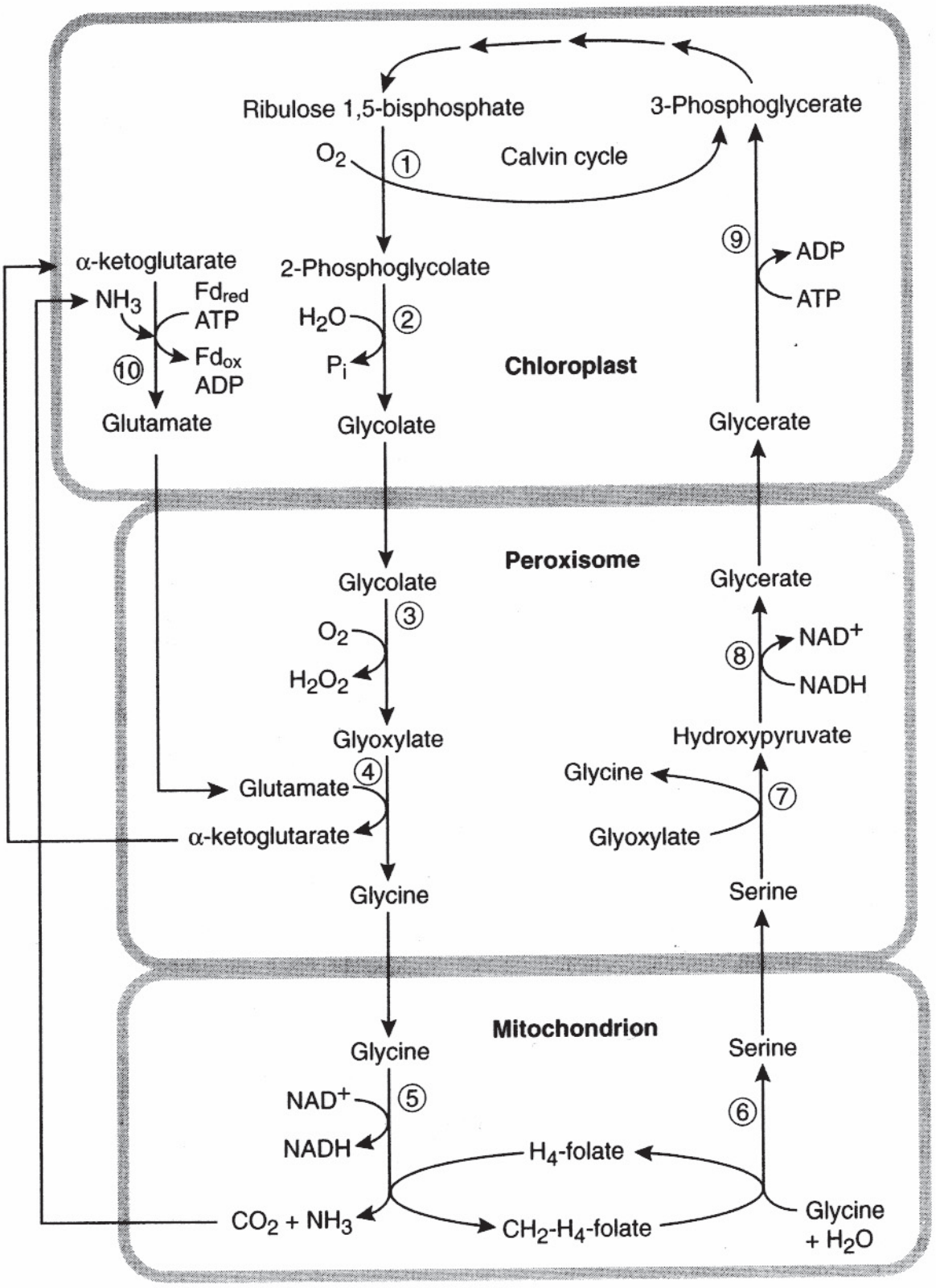


Figure 9.11 The photorespiratory cycle. The 2-phosphoglycolate formed in the oxygenation reaction is converted to glycolate, exported from the chloroplast, and is imported into the peroxisome, where it is metabolized into glycine. The glycine is exported from the peroxisome and taken up by the mitochondrion, where two molecules are combined and decarboxylated to form one molecule of serine. The serine is then transported to the peroxisome, where it is converted to glycerate and then reimported into the chloroplast and phosphorylated to form PGA. The individual reactions are given in Table 9.3.

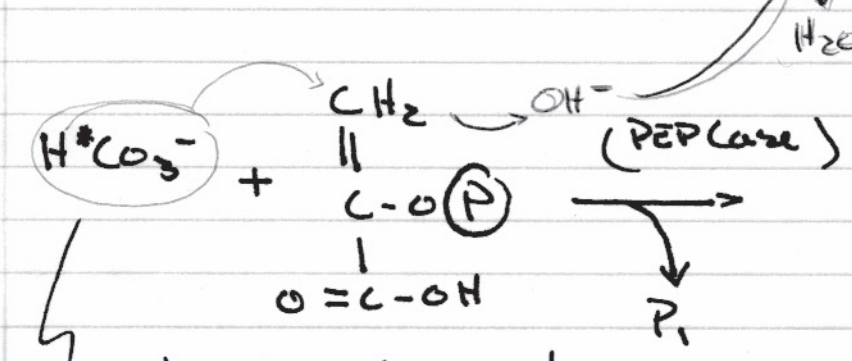
C_4 PATHWAY OF CARBON FIXATION

To overcome the intrinsic problem of oxygenase activity of RuBisCO, photosynthetic organisms have evolved
CARBON DIOXIDE CONCENTRATING MECHANISMS.

The "simplest" of these is to actively take up CO_2 into the cell/chloroplast to increase $[CO_2]$ for RuBisCO. This is done in aquatic organisms, both eukaryotic algae and prokaryotic cyanobacteria.

In terrestrial plants "concentrating mechanisms" involve biochemical fixation of CO_2 via a different enzyme: phosphoenolpyruvate carboxylase (PEP case).

$$^*CO_2 + H_2O \rightleftharpoons H_2{}^*CO_3 \rightleftharpoons H^+ + H^*CO_3^-$$

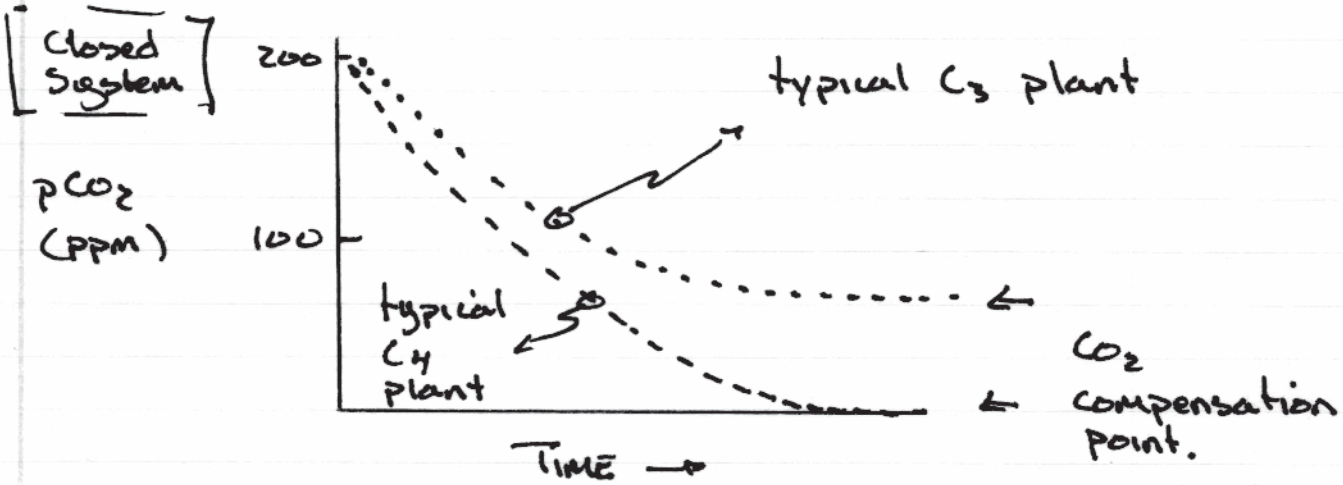


an anion, no competition with O_2

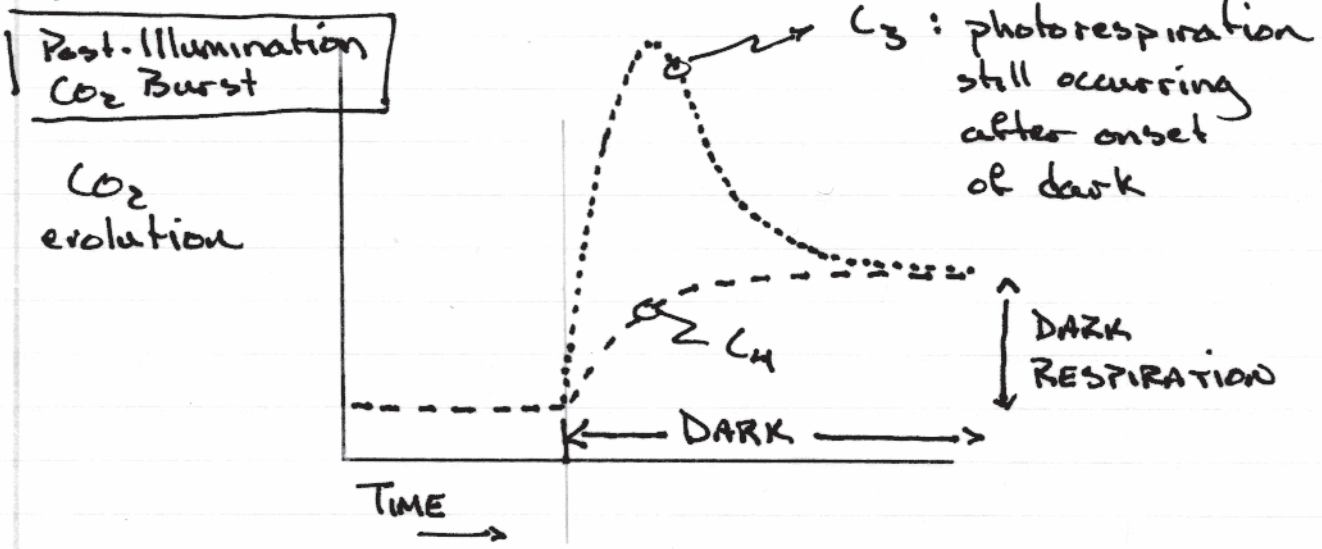
oxaloacetic acid
(4-carbons, hence C_4)
(3-phosphoglycerate is C_3)

C_4 PATHWAYS of CARBON FIXATION.

The consequences of C_4 - carbon fixation:



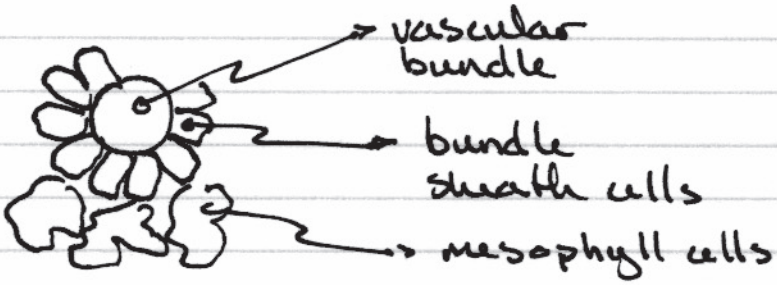
C_4 plants (for example, maize and sugar cane) have a compensation point of approximately '0'
C_3 plants (for example, soybean) have a compensation point of ~ 80 ppm.



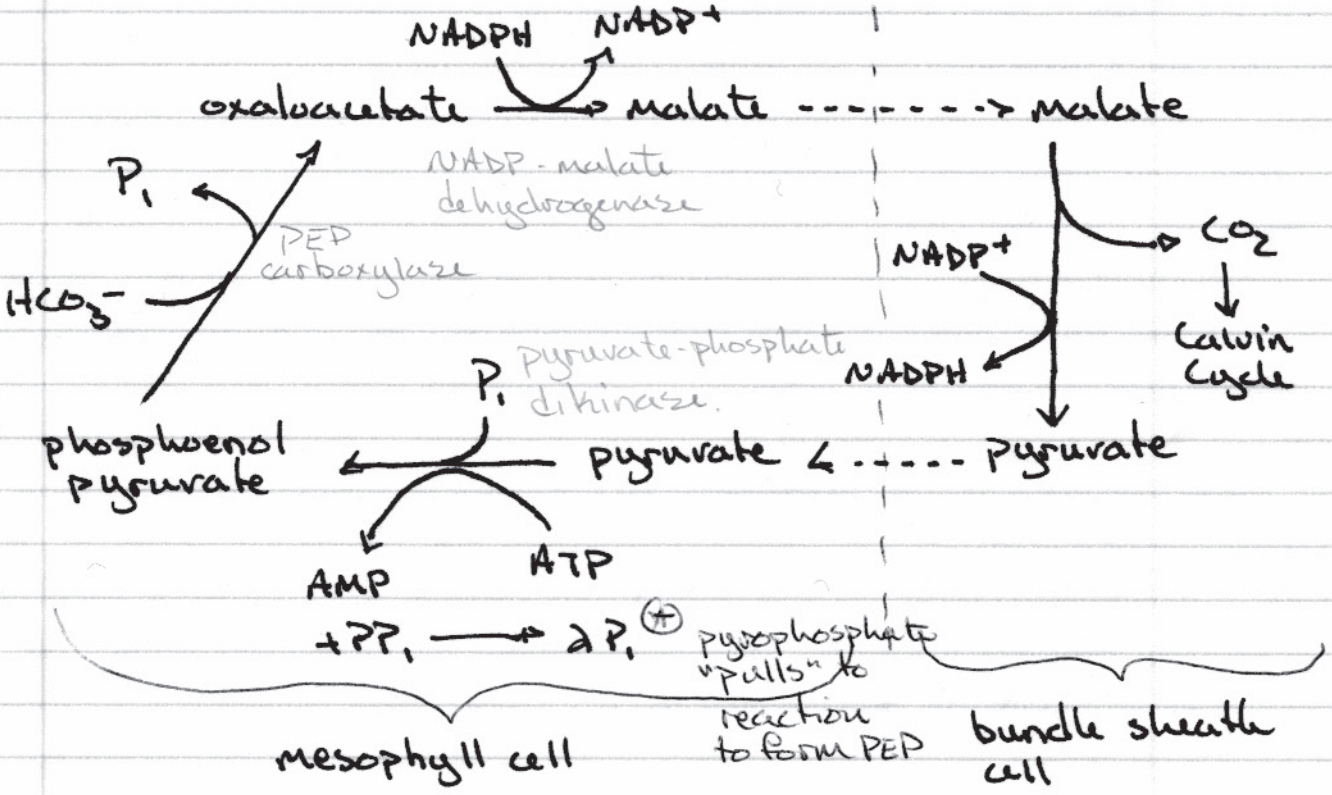
As would be expected, C_4 plants' photosynthesis is insensitive to O_2 levels up to 21% atmospheric.

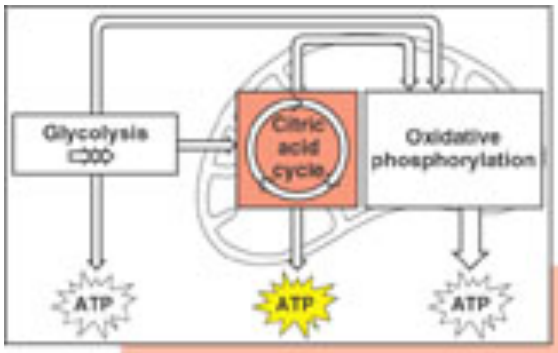
C_4 MECHANISMS.

Some of the C_4 mechanisms are associated with a well defined leaf anatomy (Kranz)



The mesophyll cells fix CO_2 with the phosphoenolpyruvate carboxylase. The product, oxaloacetate is reduced to malate (or transaminated to aspartate), which is transported to the bundle sheath chloroplasts, then decarboxylated so that the CO_2 can be re-fixed with RuBisCO.





Acetyl CoA

Oxaloacetate

Citrate

Isocitrate

α-Ketoglutarate

Succinyl CoA

Succinate

Fumarate

Malate

Citric acid cycle

NADH + H⁺

NAD⁺

CO₂

H₂O

CoA—SH

FADH₂

FAD

GTP GDP

ADP

ATP

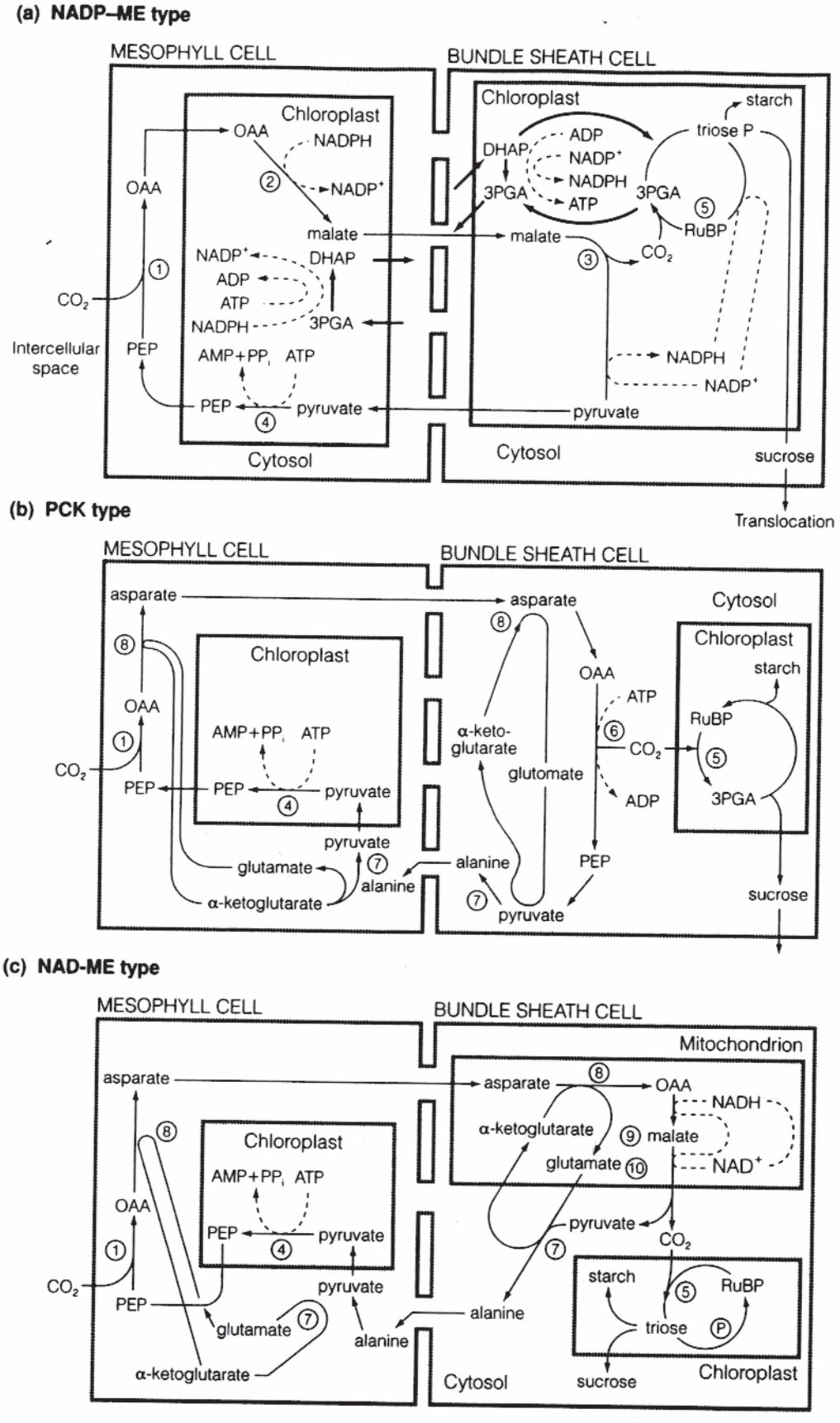
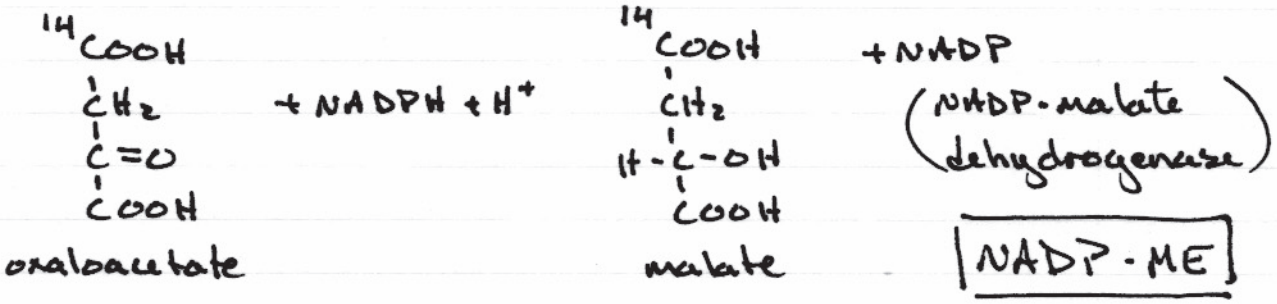


Figure 9.2. Photosynthetic metabolism of C4 plants, compounds transferred between mesophyll and bundle sheath decarboxylation with: (a) NADP requiring malic enzyme or 'NADP-ME' type; (b) aspartate-forming and PEP type of C4 metabolism; (c) aspartate-forming and NAD requiring malic enzyme 'NADME'-type of C4 metabolic enzymes listed below: (1) PEP carboxylase; (2) NADP malate dehydrogenase; (3) NADP malic enzyme; (4) pyruvate, P; dikinase; (5) RuBP carboxylase/oxygenase; (6) PEP carboxykinase; (7) alanine aminotransferase; (8) aspartate aminotransferase; (9) NAD malate dehydrogenase; (10) NAD malic enzyme

SOURCE: DAU LAUER. PHOTOSYNTHESIS 3rd edition

C_4 Biochemical Details

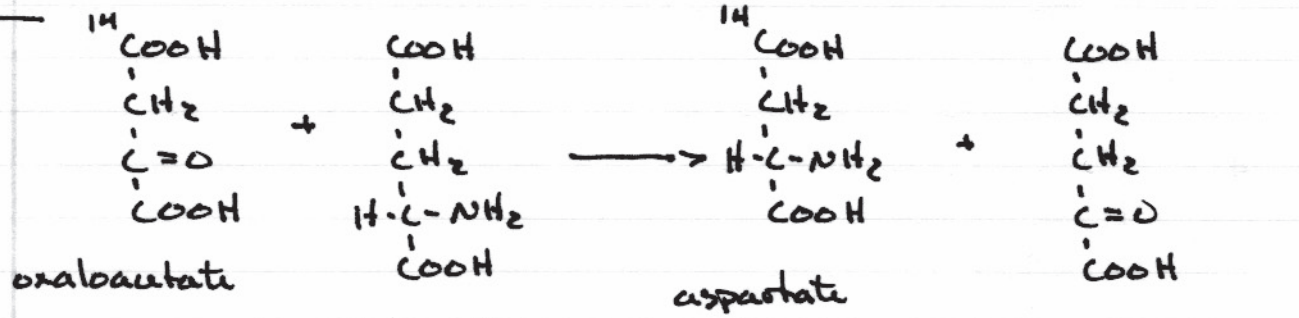
To malate....



oxaloacetate — malate — **NADP-ME**

PCK
NAD-ME

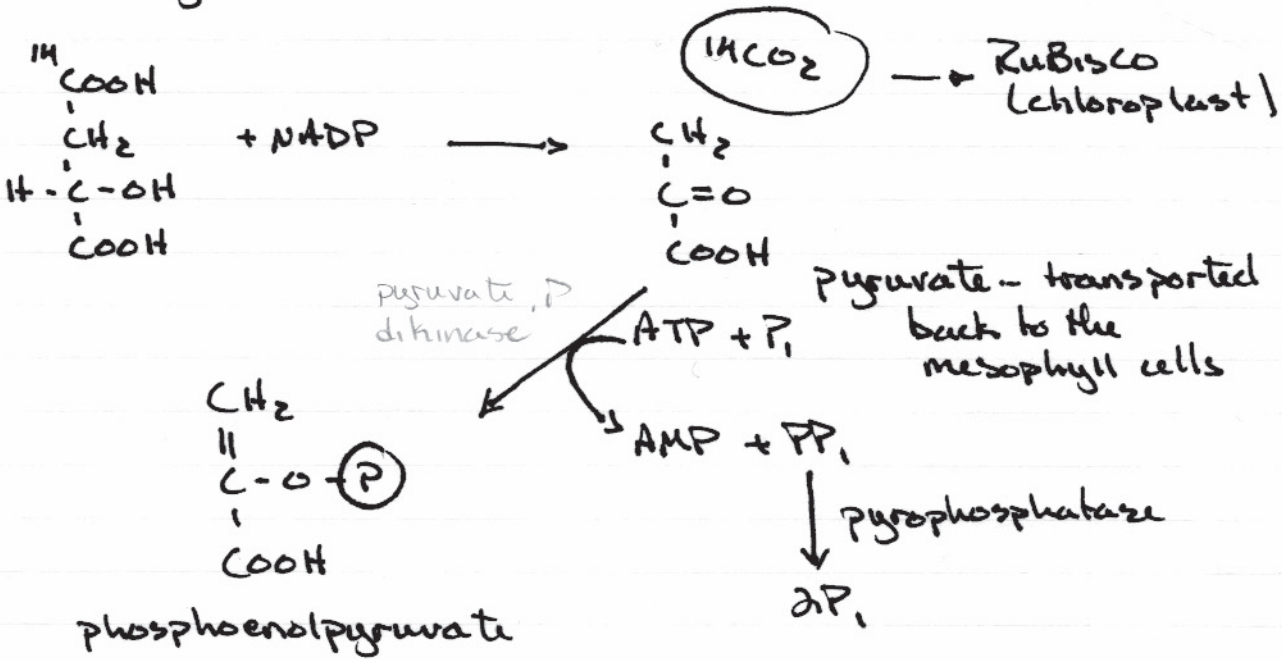
or to aspartate (transaminase)



oxaloacetate + glutamate → aspartate + α-ketoglutarate

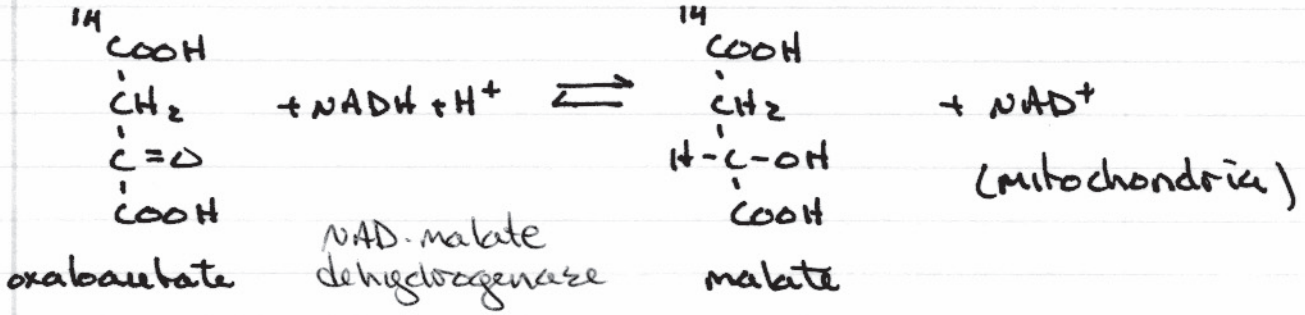
NADP ME

once transported to the bundle sheath, the malate is decarboxylated

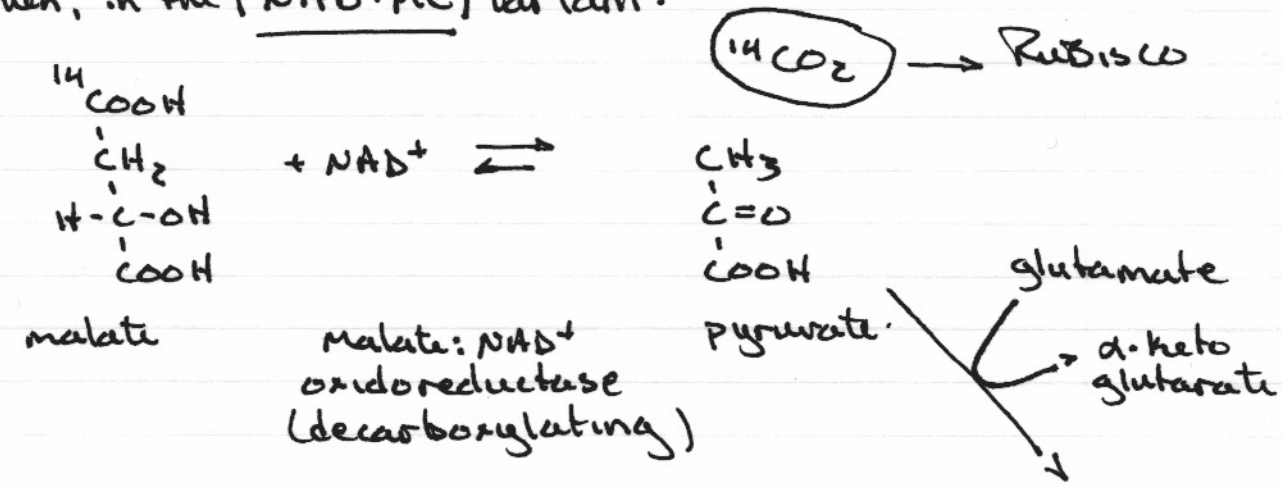


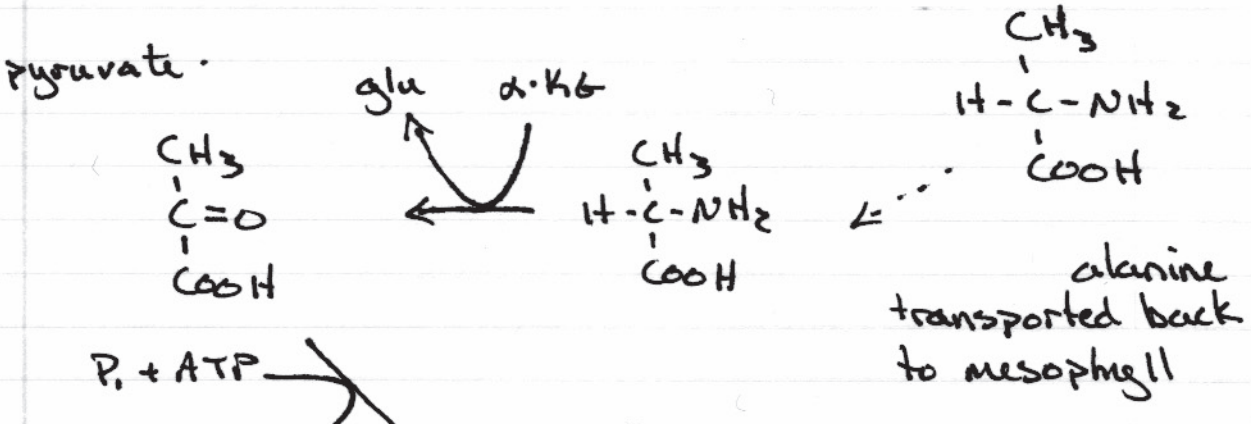
phosphoenolpyruvate

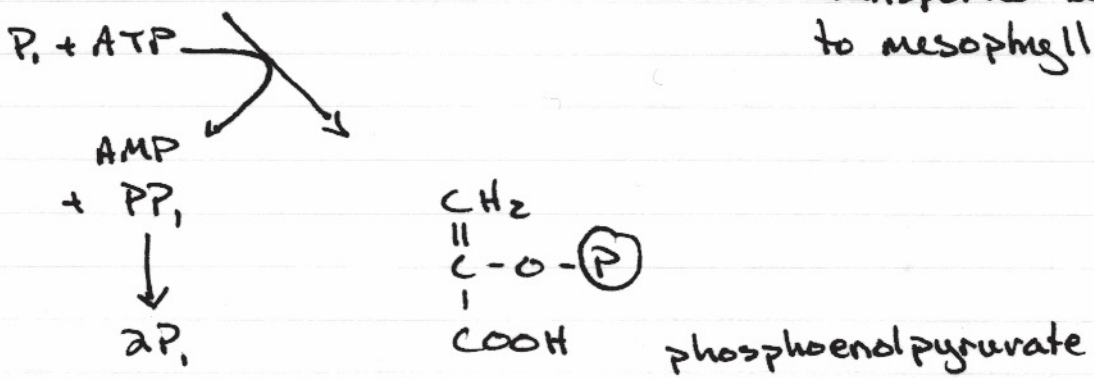
for NAD·ME & PCK variants, the aspartate is transported to the bundle sheath cell, and oxaloacetate re-formed by transamination



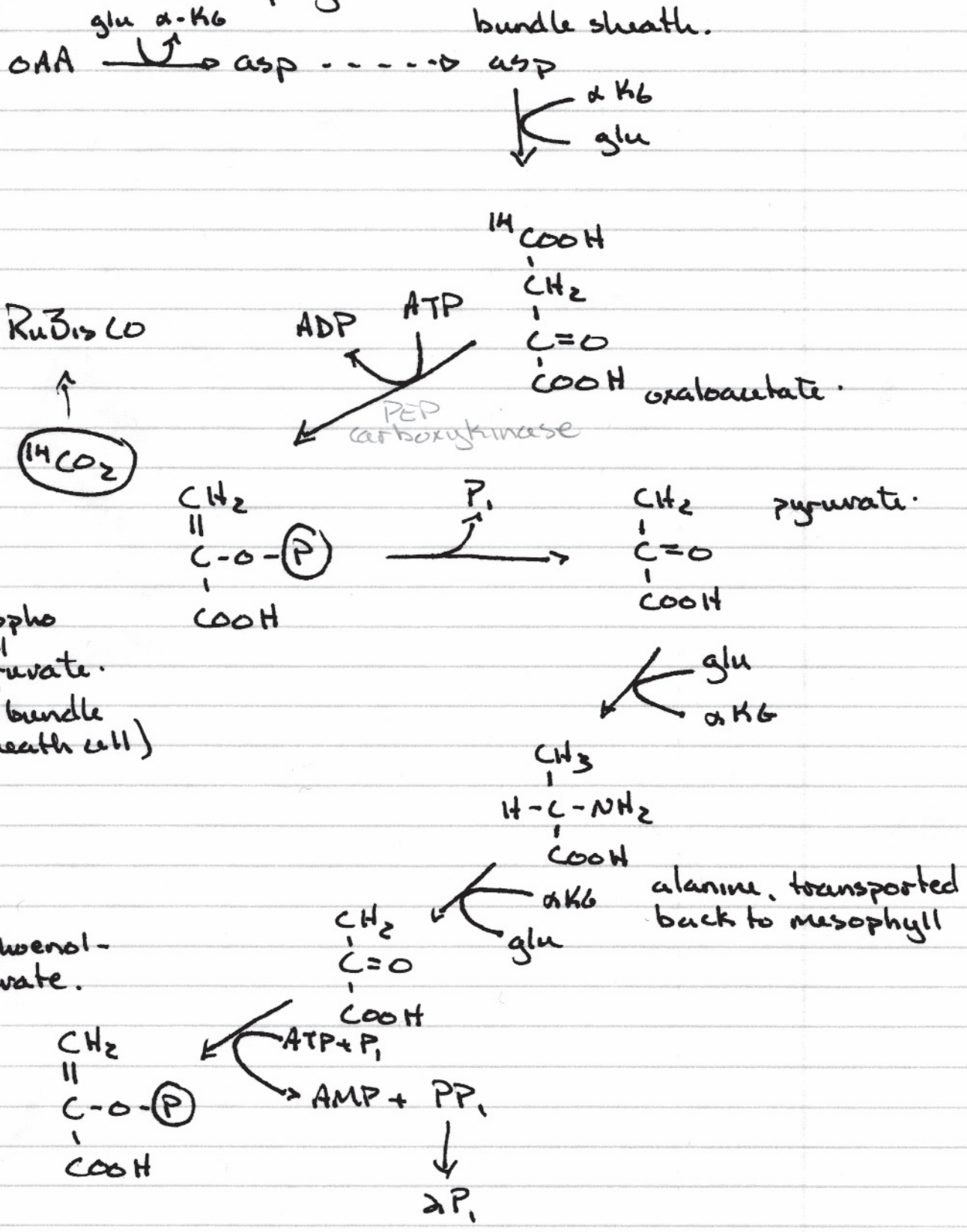
Then, in the NAD·ME variant.







In the PCK pathway, the oxaloacetate produced by transamination of the aspartate transported from the mesophyll cell ...



Energetic Comparison:

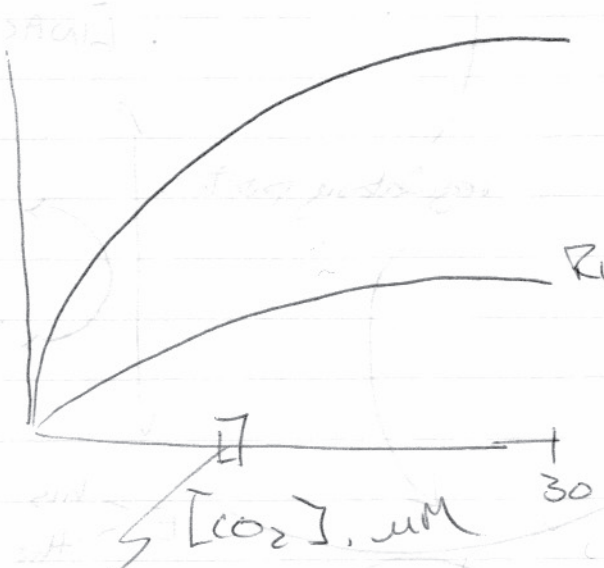
C_3 3ATP & 2 NADPH per CO_2

C_4 5ATP & 2 NADPH per CO_2.

But, one must also consider the lack of photorespiration in C_4 plants.

The primary function is presumably concentration of CO_2.

Typically, in a C_4 plant. (no Oxygenase)



PEPCase
20-40 μmol/mg Chl/min
K_m = 7 μM

RuBPCase V_{max} ~ 4-10 μmol/mg Chl/min
K_m ~ 15 μM

With concentration. RUBISCO operates without Oase, thus efficiently.

Regulation of C_4

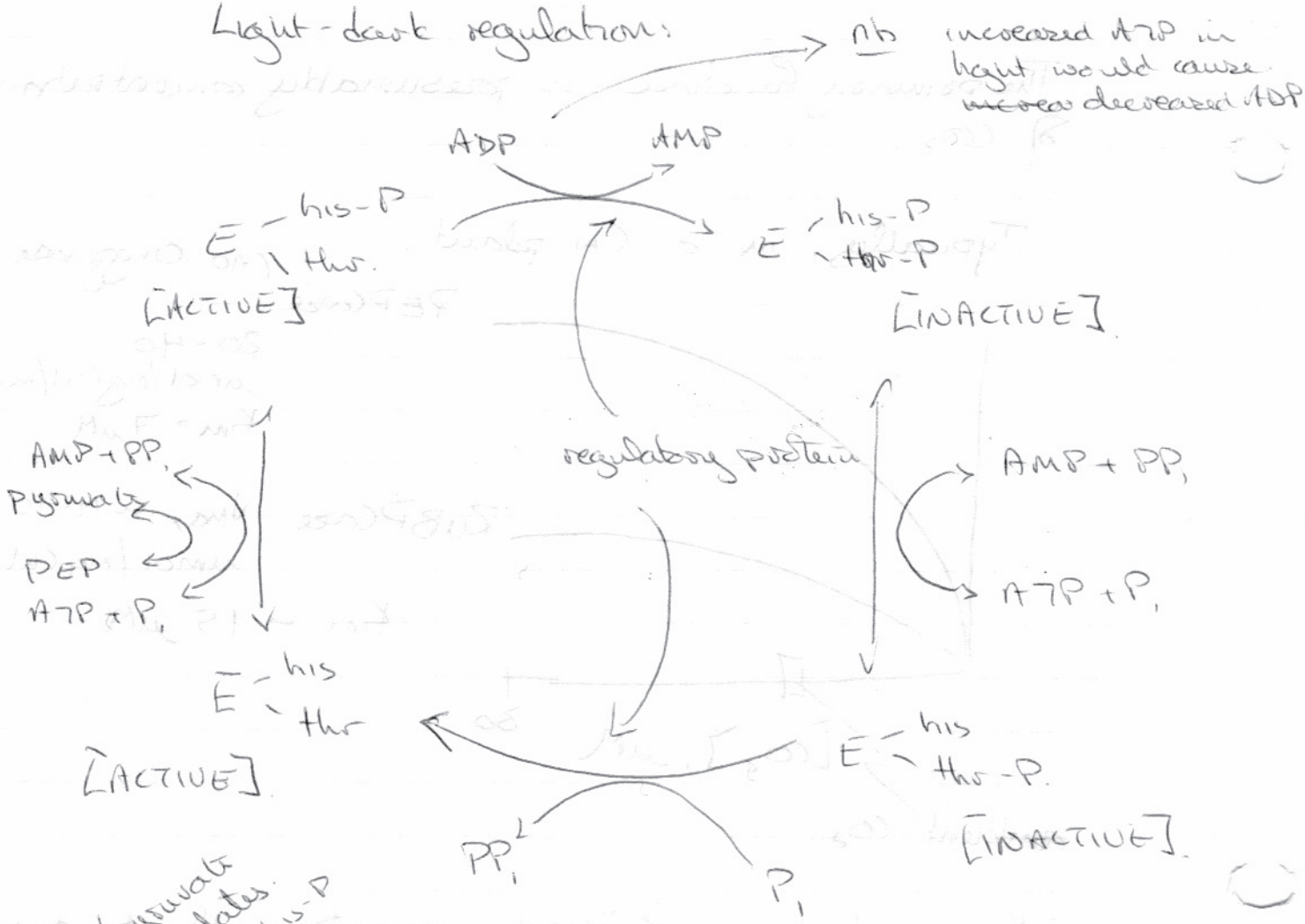
Pyruvate, P_i dikinase is light regulated.

The reactions:

$$E\text{-his} + AMP\text{-}\overset{\beta}{P}\text{-}\overset{\gamma}{P}\text{-}P + P \rightleftharpoons E\text{-his-}\overset{\beta}{P} + AMP + \overset{\gamma}{PP_i}$$

$$E\text{-his-}\overset{\beta}{P} + \text{Pyruvate} \rightleftharpoons \overset{\beta}{\overset{\beta}{PEP}} + E\text{-his}$$

Light-dark regulation:



PEP/Pyruvate ratio regulates E^{his}_{-thr} / $E^{his\text{-}P}_{-thr}$

ref Burnell & Hatch 1985.

Light-dark modulation of leaf pyruvate, P_i - dikinase TIBS 10 288. 1985.

C_4 Photosynthesis: Ecophysiology

C_4 photosynthesis is less efficient energetically, but allows efficient CO_2 assimilation under conditions of limiting CO_2.

These conditions, in general, occur under conditions of water stress when stomates are closed, limiting gas exchange between the leaf and external atmosphere.

Thus, C_4 is common in semi-arid climates. Some C_4 plants (*Spartina* sp.) are found in salt marshes, where water stress is an issue.

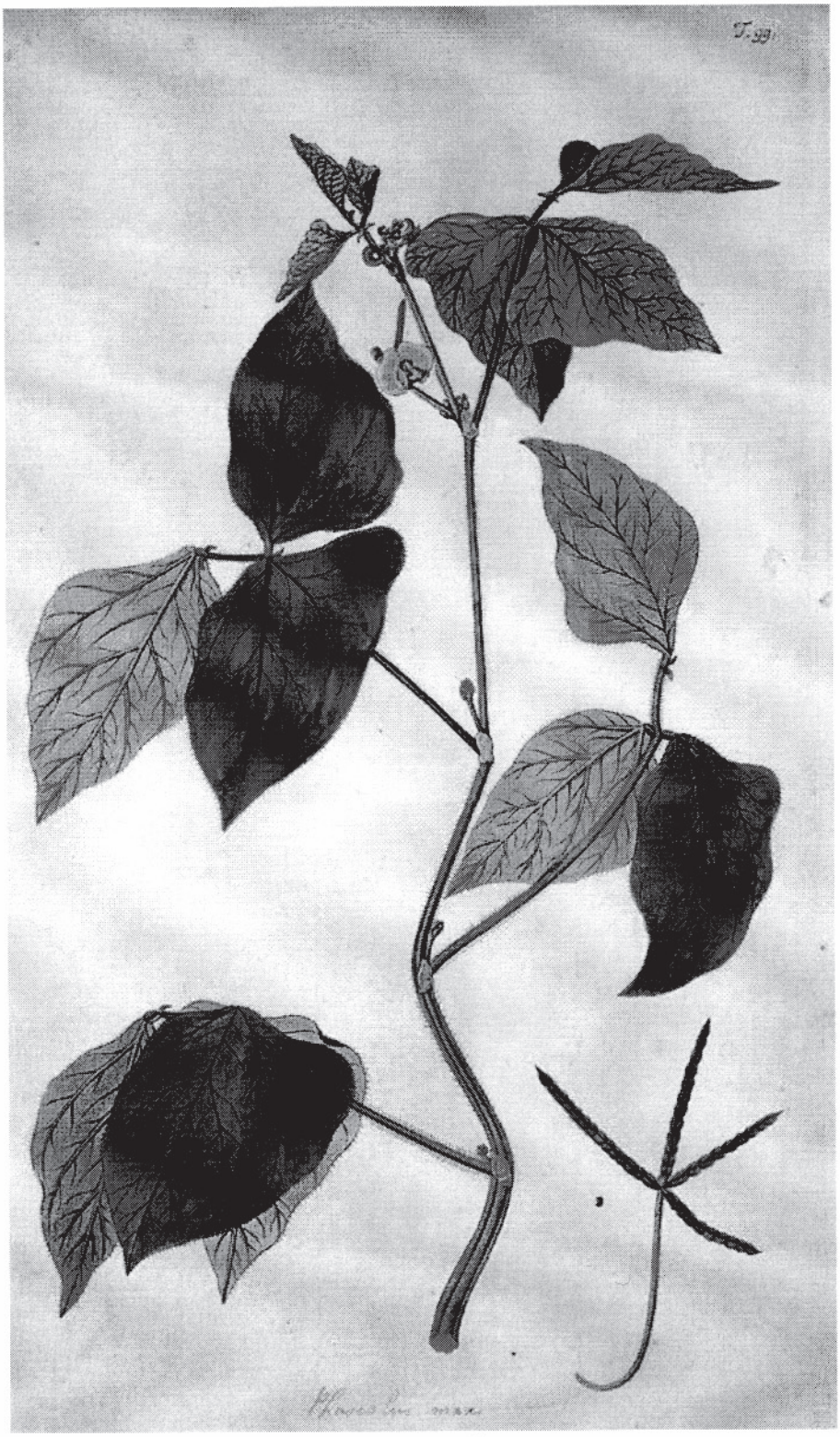
Evidence for C_4 photosynthesis, based on carbon isotope ratios, suggest it appeared recently (perhaps 12.5 million years ago) and $\delta^{13}C$ ratios of -10 to -14 ‰ (*) appear 7 million years ago with fossils of known C_4 grasses.

Monocotyledons are more commonly C_4, but it is clear from the distribution of C_4 among diverse families that C_4 evolved (and is evolving) many times in different groups.

(*) ^{13}C is discriminate against less in C_4 because HCO_3^- is the substrate in C_4 photosynthesis. C_3 δ^{13} ratios are -22 to -34 ‰ because CO_2 is the substrate.

Lawlor, 2001 page 295.

Glycine max[1] uses the C_3 Pathway



[1] Source: Hortus botanicus vindobonensis (ca 1773)
http://www.illustratedgarden.org/mobot/rarebooks/page.asp?relation=QK98J3151770V1&identifier=0250

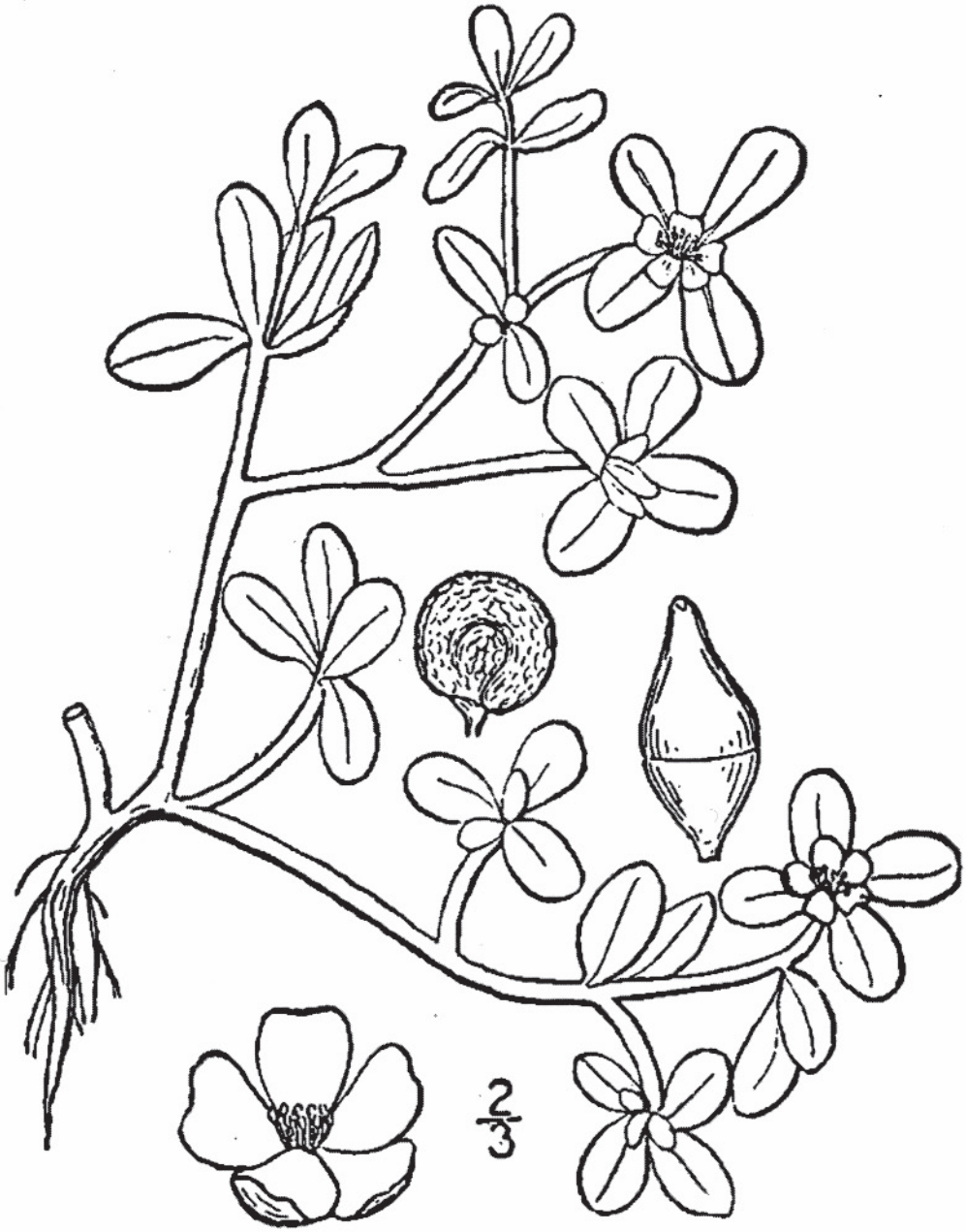
Panicum maximum[1] uses the PEP-CK variant of the C_4 Pathway

[1] Source: http://www.hear.org/pier/species/panicum_maximum.htm

Zea mays[1] uses the NADP-ME variant of the C_4 Pathway

[1] Source: Hitchcock, A.S. (rev. A. Chase). 1950. Manual of the grasses of the United States. USDA Misc. Publ. No. 200. Washington, DC. 1950. Usage Guidelines.
http://plants.usda.gov/java/profile?symbol=ZEMA&photoID=zema_001_avd.tif

Portulaca oleracea[1] uses the NAD-ME variant of the C_4 Pathway



[1] Source: Britton, N.L., and A. Brown. 1913. Illustrated flora of the northern states and Canada. Vol. 2: 40. http://plants.usda.gov/java/profile?symbol=POOL&photoID=pool_001_avd.tif

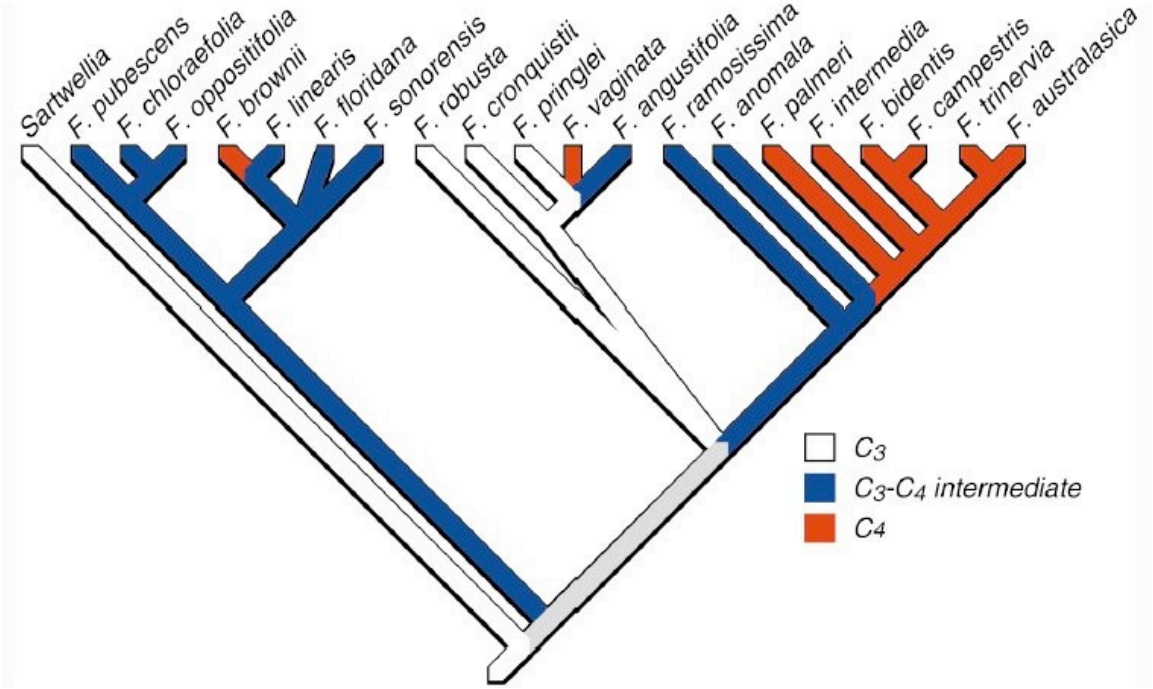


Figure 7. Evolution of C4 photosynthesis in Flaveria (Asteraceae; Monson 1996). The colors along each branch of the phylogeny represent a hypothesized reconstruction of the evolution of photosynthetic pathways based on phylogenetic parsimony methods (i.e., the reconstruction that requires the fewest evolutionary transitions leading to the observed present-day distribution of photosynthetic types). The hatched bar indicates an uncertain reconstruction. If this branch is inferred to be C3, there are three independent origins of C3–C4 intermediate pathways (including F. angustifolia); alternatively, if this branch is reconstructed as C3–C4 intermediate, there is one origin of C3–C4 and one subsequent reversal to C3. The C4 pathway is inferred to have evolved independently three times, and in at least two of these cases, the C3–C4 type represented an intermediate evolutionary stage. A more recent molecular phylogeny of Flaveria, for 12 of the 20 species shown here, suggests at least two independent origins of C4 photosynthesis (Kopriva et al. 1996). Redrawn with permission from Monson (1996). Bioscience 50(11):979–995.

CARBON-CONCENTRATING MECHANISMS

The C_4 pathway can be considered a mechanism for concentrating CO_2, and appears to be specific to land plants.

There are other mechanisms for concentrating CO_2 in aquatic organisms: both prokaryotes (cyanobacteria) and eukaryotes (algae).

These mechanisms are closely allied with the aqueous chemistry of CO_2:

$CO_2 + H_2O \rightleftharpoons CO_{2(aq)}$ → CO_2 solubility 1.45 kg/m³ → 33 μM

$CO_{2(aq)} + H_2O \rightleftharpoons H_2CO_3$ carbonic acid

pK 6.1 $H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$ bicarbonate.

pK 10.3 $HCO_3^- \rightleftharpoons H^+ + CO_3^{2-}$ carbonate

DIC (dissolved inorganic carbon) accounts for all the inorganic carbon species, and increases markedly with pH. That is, at pH more alkaline than 6.1, HCO_3^- and CO_3^{2+} are the majority.

In "dirty" aqueous chemistry Mg^{2+} and Ca^{2+} can complex with the anions to form fairly insoluble salts.

The reaction:

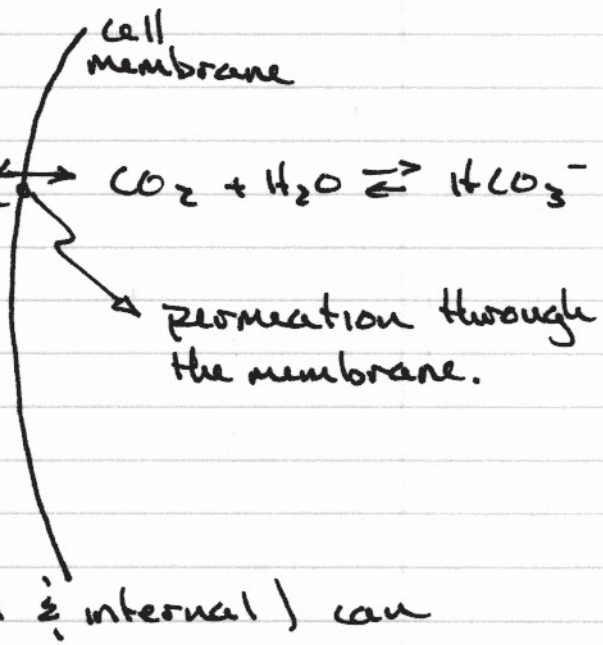
$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$$

is fairly slow. The enzyme carbonic anhydrase catalyzes the reaction.

Now, in terms of concentrating mechanisms, CO_2 is very permeable and passes across cell membranes very quickly.

In contrast, the anions (HCO_3^- at physiologically relevant pH) are fairly impermeant.

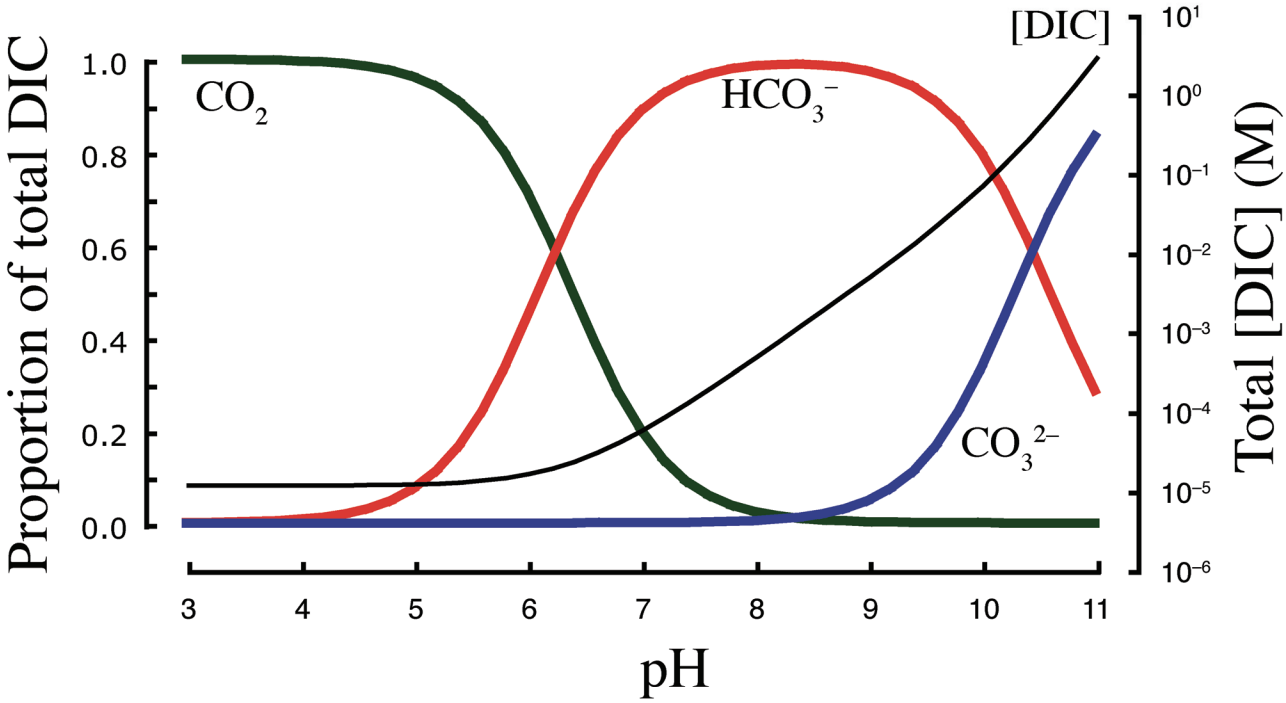
So:



can "trap" DIC inside the cell. Most effective if the external pH is acidic compared to the cell pH (typically 6.8 - 7.2).

Carbonic anhydrase (external & internal) can speed the 'passive' uptake.

Carbon Species Availability (as a function of aqueous pH)

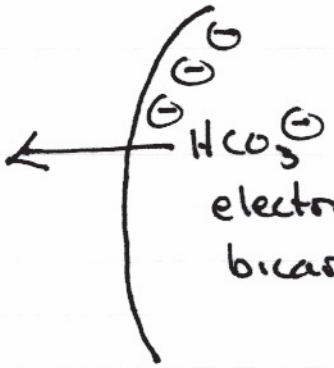


The relative proportions of the various DIC (dissolved inorganic carbon) species are shown as a function of pH, based upon the equilibria shown in the chemical equation below:

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \xleftrightarrow{pK_a = 6.4} H^+ + HCO_3^- \xleftrightarrow{pK_b = 10.4} H^+ + CO_3^{2-}$$

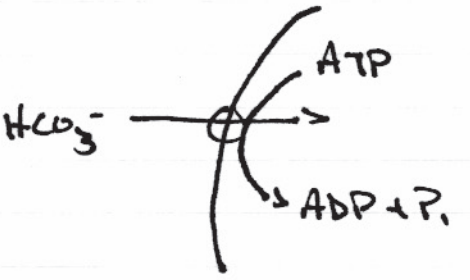
Total [DIC] increases dramatically at alkaline pH, but the predicted concentrations shown do not account for solubility.

A further complication is that the cell will have an inside -ve potential:

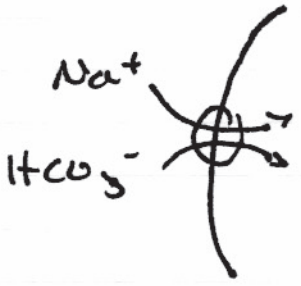


electrophoresis of the anion bicarbonate <u>out</u> of the cell

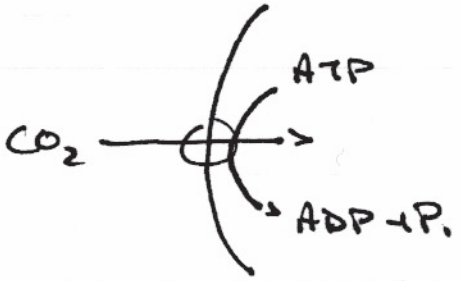
So, active uptake requires other mechanisms.



An ATP-dependent pump
This could be an ABC family transporter (reported in cyanobacteria)



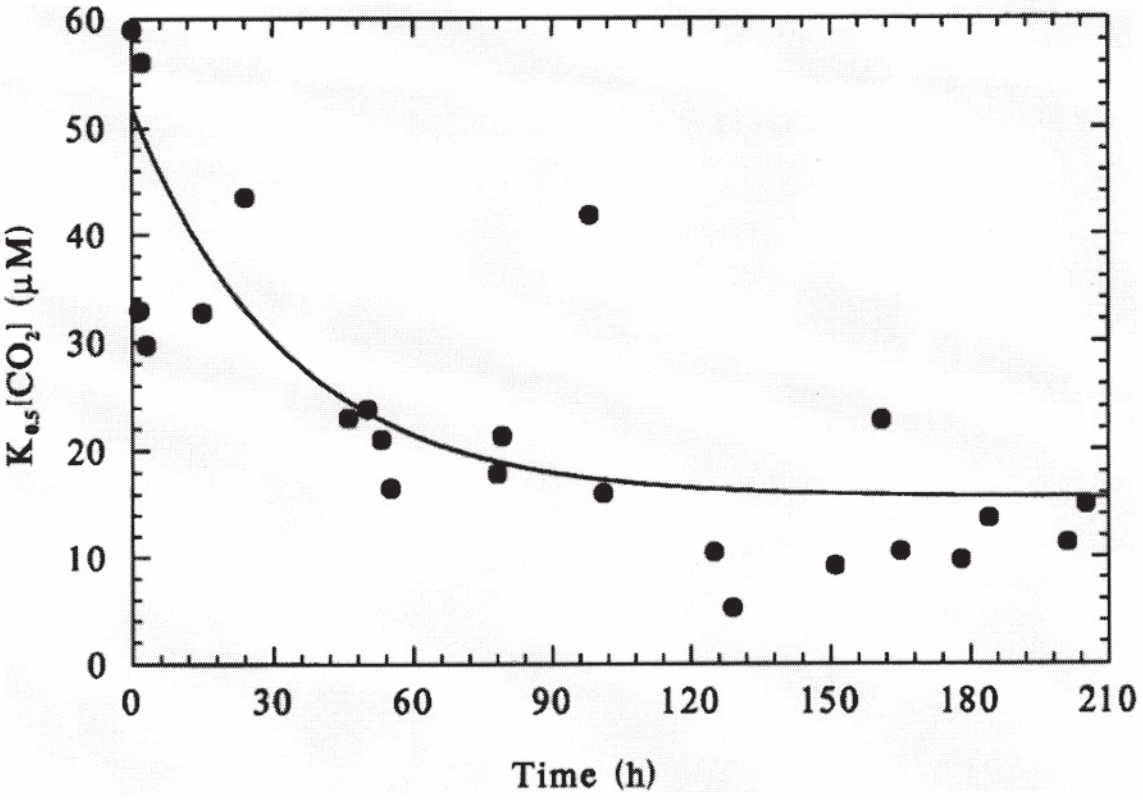
A co-transport system
An Na^+ : HCO_3^- symport in this example. (also reported in cyanobacterium).



A CO_2-ATPase has also been proposed.....

Induction of active DIC (CO_2).[1]

Time course of change in $K_{0.5}[CO_2]$ of *Eremosphaera viridis* at pH 5 when switched to 0.03% CO_2 after growth on 5% CO_2. Cultures of *E. viridis* were switched from 5% CO_2 to 0.03% CO_2 at pH 5.0 and sampled over a 200-h period.



Active CO_2 uptake is induced under conditions of low CO_2. The uptake can be differentiated from HCO_3^- uptake because of the acid pH (5.0), at which CO_2 is the major DIC species.

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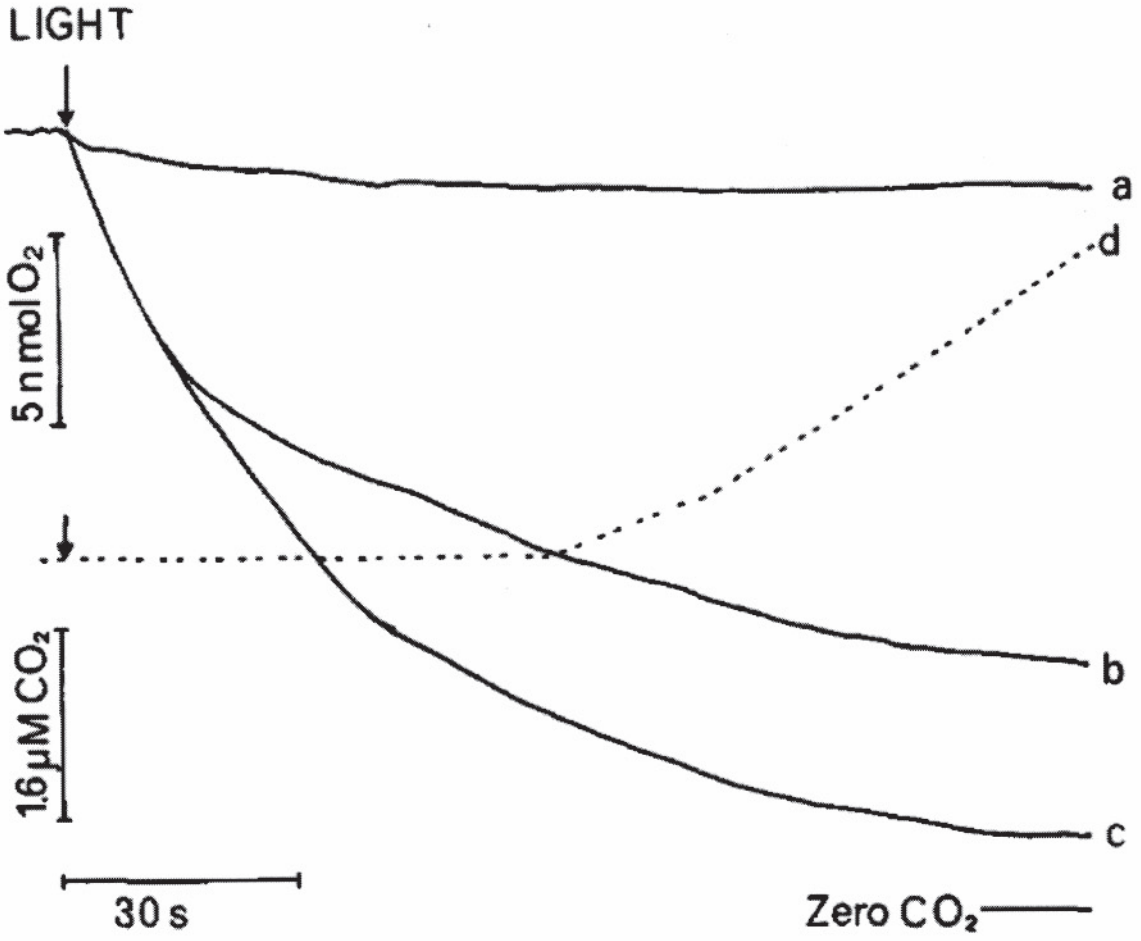
[1] Source: Jason S.T. Deveau, Roger R. Lew, and Brian Colman 1998 Evidence for active CO_2 uptake by a CO_2–ATPase in the acidophilic green alga *Eremosphaera viridis*. Canadian Journal of Botany 79:1274–1281.

CO_2 uptake and O_2 evolution measured by mass spectrometry.[1]

Measurement of CO_2 uptake (——) and O_2 evolution (-----) by mass spectrometry during illumination of *Eremosphaera viridis* cell suspensions (containing 55-60 μg Chl) in BTP-HC1 buffer (pH 7.5) in the presence of 100 μM DIC. ***Curve a***, cells treated with Carbonic Anhydrase (50 WA units ml^{-1}). ***Curve b***, cells pretreated with glycolaldehyde (100 mM) for 5 minutes to inhibit CO_2 fixation ***Curve c***, untreated cells. ***Curve d***, 0_2 evolution of untreated cells.



The results indicate that CO_2 uptake precedes O_2 evolution and is not a consequence of CO_2 uptake caused bu CO_2 utilization during carbon fixation.

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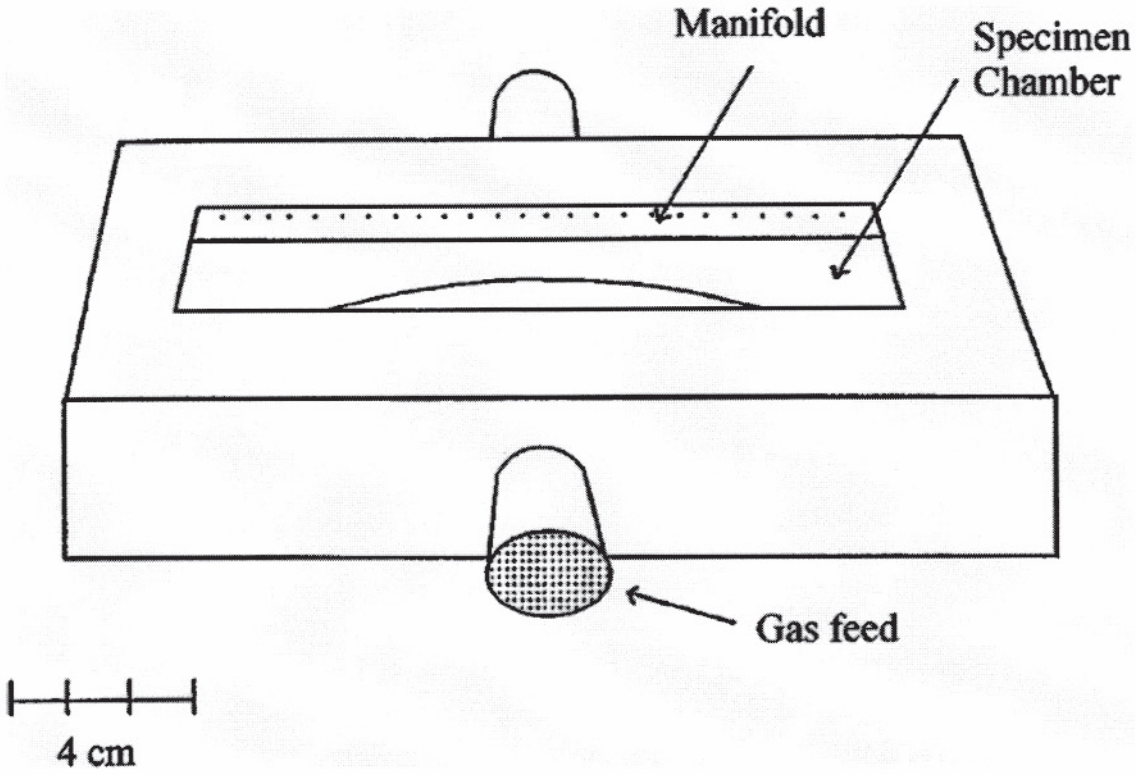
[1] Source: Caterina Rotatore, Roger R. Lew, and Brian Colman 1992 Active uptake of CO_2 during photosynthesis in the green alga *Eremosphaera viridis* is mediated by a CO_2-ATPase. Planta 188:539–545.

Apparatus for controlling DIC levels during microimpalement.[1]

To control DIC levels during electrical measurements, a brass chamber with an inlaid glass bottom was designed to perfuse cells with a media containing variable concentrations of DIC, while creating a laminar-flow N_2 shield to prevent any DIC contamination from the atmosphere. The chamber rested on the stage of a light transmission microscope so that, while in the chamber, cells could still be observed and impaled for electrical analysis. Preliminary oxygen-electrode studies indicate that for the conditions used in the electrophysiology, the affinity for CO2 uptake has a $K_{0.5}$ of approximately 15 μM.



The apparatus was used to determine if active CO_2 (or HCO_3^-) uptake involved electrogenic transport at the plasma membrane.

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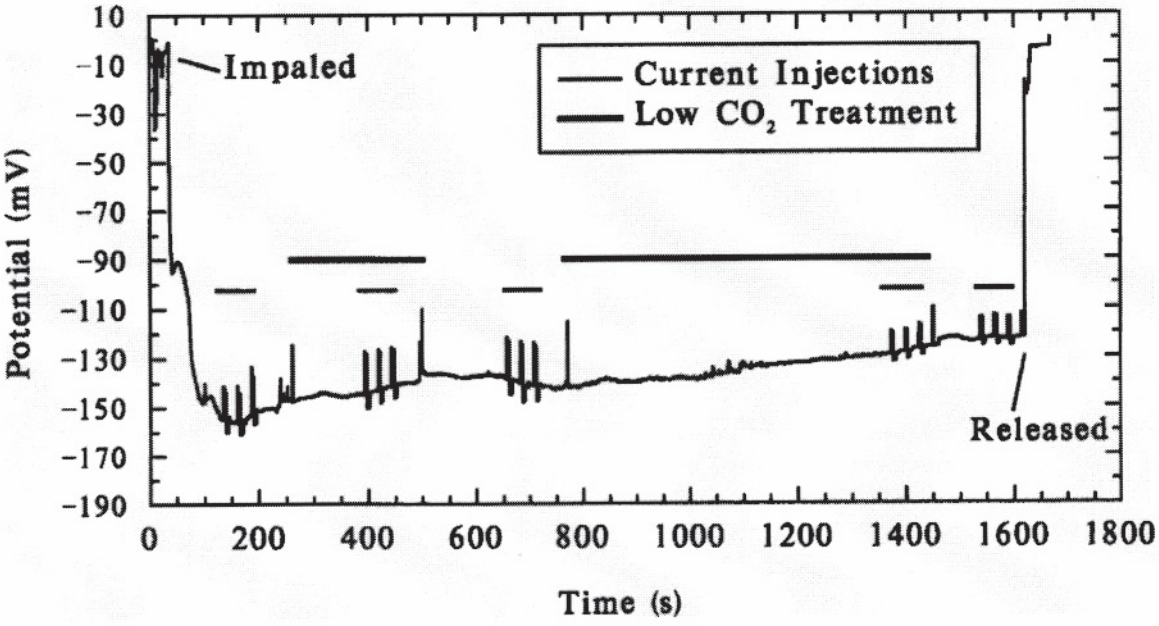
[1] Source: Jason S.T. Deveau, Houman Khosravani, Roger R. Lew, and Brian Colman 1998 CO_2 uptake mechanism in *Eremosphaera viridis*. Canadian Journal of Botany 76:1161–1164.

Example of a typical electrophysiological trial in APW (pH 5.0) in the light.[1]

Following impalement, when the potential had reached a stable negative value, input resistance was measured by current injection. The DIC concentration was then changed from high to low, and the input resistance was measured again. Cells remained in each concentration for a minimum of
300 s.

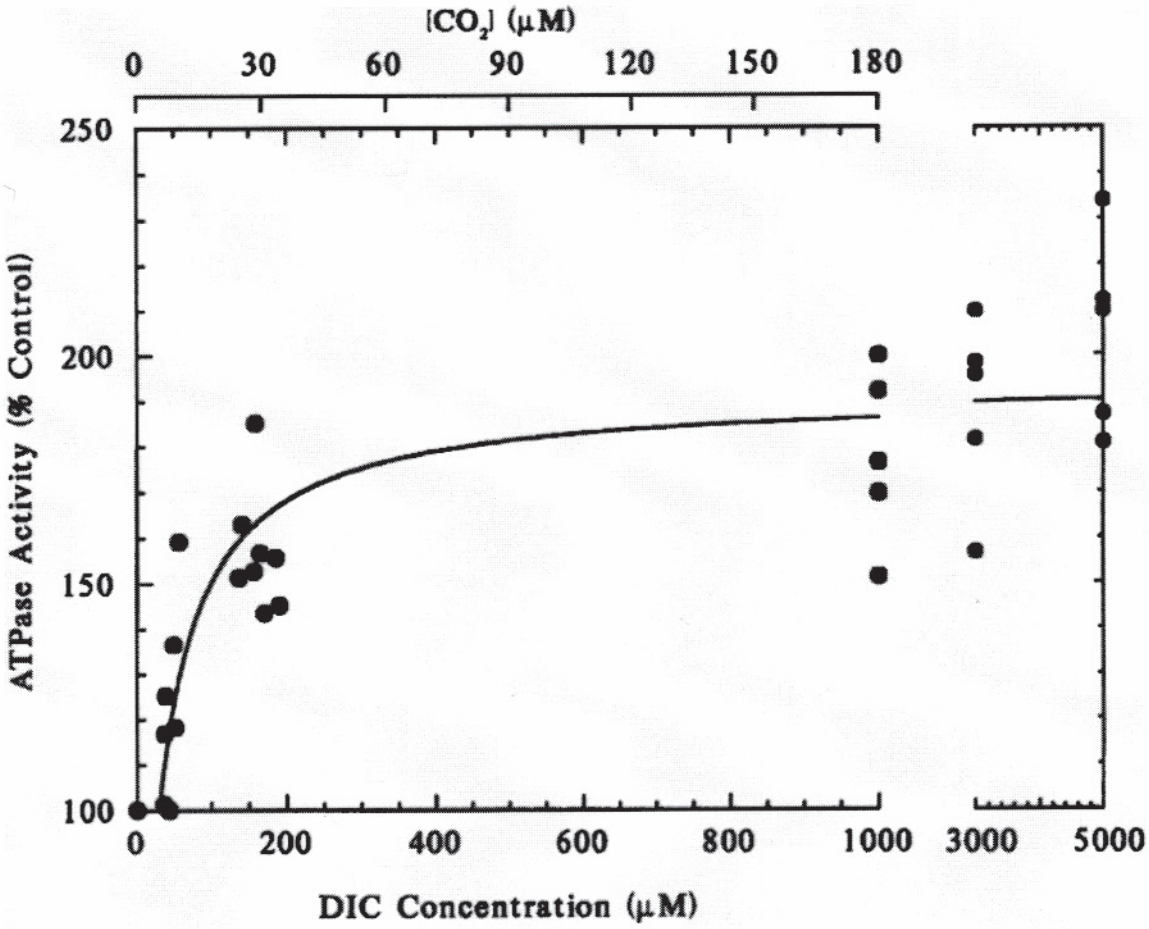


There was no electrical signal during CO_2 uptake. Therefore, the uptake mechanism is not electrogenic.

[1] Source: Jason S.T. Deveau, Roger R. Lew, and Brian Colman 1998 Evidence for active CO_2 uptake by a CO_2–ATPase in the acidophilic green alga *Eremosphaera viridis*. Canadian Journal of Botany 79:1274–1281.

A CO_2–ATPase may be responsible for active CO_2 uptake.[1]

ATPase activity (percent control) as a function of DIC concentration ($n = 5$). The control activity was 2.00 ± 0.58 μmol·mg protein^{-1}·h^{-1}. The curve is a best fit to the Michaelis–Menten equation. The $K_{0.5}$[DIC] was 124.6 μM; equivalent to a $K_{[CO]}$ of 22.5 μM.



Activation of ATPase by CO_2 can be explained as activation by a substrate of the ATPase reaction. The similarity of the half-maximal activating concentration, and affinity for CO_2 uptake in whole cells supports this explanation. Biochemical isolation and characterization is crucial for confirmation.

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[1] Source: Jason S.T. Deveau, Roger R. Lew, and Brian Colman 1998 Evidence for active CO_2 uptake by a CO_2–ATPase in the acidophilic green alga *Eremosphaera viridis*. Canadian Journal of Botany 79:1274–1281.