Slide 1

Photosynthesis

Slide 2

Magnitude of the Process

- 1.5×10^{22} kJ energy per day from the sun
- 1% transduced by photosynthesis into chemical energy
- 10^{11} tons of CO_2 fixed globally per year
- In green plants, the process occurs in the chloroplast (See Fig. 22.2)

Notice membrane organization of the chloroplast. An internal thylakoid membrane is folded into structures called lamellae that stack to form grana. The interior of the thylakoid vesicles is the "lumen", while the space outside the thylakoid vesicles but inside the chloroplast membrane is called the stroma. Chloroplasts, like mitochondria, contain some DNA that codes for some of its proteins.

Slide 3

Overall Reaction

Opposite of respiration:

$$6\,CO_2 + 6\,H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6\,O_2$$

$$\Delta G^{o\prime} = +2860 \frac{\text{kJ}}{\text{mol of glucose}}, \text{ or } +477 \frac{\text{kJ}}{\text{mol of C reduced}}$$

van Niel's generalized equation:

$$CO_2 + 2\,H_2A \xrightarrow{\text{light}} (CH_2O) + 2\,A + H_2O$$

Hydrogen acceptor; Hydrogen donor; Reduced acceptor; Oxidized donor

In photosynthetic bacteria, H_2A can be H_2S, isopropanol, or other oxidizable substrate.

Slide 4

Overall Reaction, con't.

Better representation:

$$CO_2 + 2H_2O \xrightarrow{\text{light}} (CH_2O) + O_2 + H_2O$$

- Water is split, not CO_2.
  - Shown by experiments with $H_2{}^{18}O$
- Can be separated into two reactions:
  - O_2 generation (the **light** reaction)
  - CO_2 reduction (the **dark** reaction)
  - See Figure 22.4.

Slide 5

The Light Reaction

$$2\,H_2O + 2\,NADP^+ + x\,ADP + x\,P_i \xrightarrow{nh\nu} O_2 + 2\,NADPH + 2\,H^+ + x\,ATP + x\,H_2O$$

Reducing NADP to NADPH requires

$$\Delta G^{o'} = -nF\Delta E_o{}' = -(2)(96.5\frac{kJ}{mol-volt})(-1.136\ volts) = +219\ \frac{kJ}{mol\ NADPH}$$

or $438\ \frac{kJ}{mol\ of\ O_2 produced}$

Making ATP requires $30.5\frac{kJ}{mol\ of\ ATP}$

or an additional $30.5x\ \frac{kJ}{mol\ of\ O_2 produced}$

We will see that at least 3 ATP's will be required in the Dark Reaction, so that more than 530 kJ of light energy are needed per mole of oxygen produced.

Slide 6

The Dark Reaction

$$6\,CO_2 + 12\,NADPH + 12\,H^+ + 18\,ATP + 12\,H_2O \longrightarrow C_6H_{12}O_6 + 12\,NADP^+ + 18\,ADP + 18\,P_i$$

$12 \times -219\ \frac{kJ}{mol}$ for NADPH and $18 \times -30.5\ \frac{kJ}{mol}$ for ATP

or a $\Delta G^{o'}$ of -2628 kJ - 549 kJ = -3177 kJ energy input

More than enough for the reduction of $6\,CO_2$

which required + 2860 $\frac{kJ}{mol}$

Remember, these numbers are **standard free energies**. The actual energy need is greater in order to push the reaction towards completion.

Slide 7

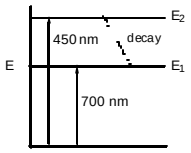
Harvesting Light Energy

- Chlorophyll is the main pigment for trapping the energy of light quanta.
  - See structure, Fig. 22.5
  - See Absorption spectrum, 22.6
- Two major energy levels corresponding to major absorption peaks
  - ~ 450 nm and ~ 700 nm
  - (slightly different for chlorophyll a and b)

Slide 8

Harvesting Light Energy, con't.

- Photochemistry occurs generally from the lowest singlet state. (Higher energy states decay rapidly to lowest state).



$$E = \frac{hc}{l} = \frac{(6.625\times10^{-34}\ \text{Js})(3.00\times10^{8}\ \text{ms}^{-1})}{700\times10^{-9}\ \text{m}}$$

$$= 2.84\times10^{-19}\ \frac{\text{J}}{\text{quantum}} \times 6.02\times10^{23}\ \frac{\text{quanta}}{\text{mol}}$$

$$= 171\ \text{kJ per mol of quanta}$$

Slide 9

Harvesting Light Energy, con't.

- Need at least 533 kJ of energy per mol of O_2 produced
- Can obtain 171 kJ per mole of quanta
- 533/171 = 3.11; Expected that 4 quanta would be sufficient for each O_2
- It actually takes 8 quanta per O_2
  - "Inefficiency" because one needs large overall negative ΔG to make reaction irreversible

Slide 10

Harvesting Light Energy, con't.

- Chlorophyll is transparent to most of visible light.
- Absorption of light in 450-650 nm region accomplished by accessory pigments
  - Beta-Carotene and Phycocyanobilin (Fig. 22.7)
  - Responsible for colorful fall foliage
- Light is trapped by a "pigment system", and energy funneled to a reaction center (Fig 22.9)

Increasing light intensity leads to saturation of rate of photosynthesis. If this is done with flashing light, then dark reaction will not be limiting. Still get one CO_2 produced for about every 2500 chlorophyll molecules. This was evidence for a "photosynthetic unit" of about 400 chlorophylls for each electron promoted. Most cholorphyll and the accessory pigments serve as "antennae" which trap the photon. Energy is then transferred to a reaction center, which is a special type of cholorphyll.

Slide 11

Trapping Light Energy

- Possible fates of the quantum of absorbed light energy are diagrammed in Figure 22.8
  - Thermal dissipation
  - Fluorescence
  - Exciton transfer (which funnels energy to the reaction center)
  - Transfer of excited electron to another acceptor.
    - The excited state has a much lower reduction potential (is a stronger reducing agent) than the ground state.

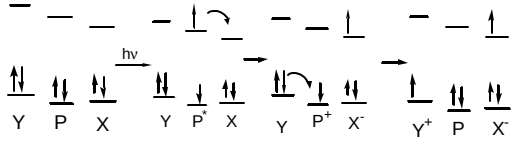
Slide 12

Trapping Light Energy, con't.

$$P \xrightarrow{h\nu} P^*$$

$$P^* + X \longrightarrow P^+ + X^-_{red}$$

$$P^+ + Y \longrightarrow P + Y^+_{ox}$$



The trick in trapping the energy in this way is to prevent the electron in X^- from falling back to reduce P^+. That is accomplished by proper orientation of the components so that Y preferentially reduces P^+, and the charge is rapidly separated.

Slide 13

Two Photosystems in Eukaryotes

- Evidence from "Red Drop"
  - (aka "Emerson effect")
- 700 nm light is inefficient unless supplemented by <680 nm light. (See Fig. 22.10)
- Photosystem I (PSI) absorption max. at 700 nm
  - Also called P_{700}
- Photosystem II (PSII) absorption max at 680 nm
  - Also called P_{680}

Chloroplasts given light at both 680 and 700 nm simultaneously yield more O_2 than the sum of O_2 produced when each wavelength is used alone.

Slide 14

Effect of Each Photosystem

- PS I (P_{700}) produces:
  - X^-, a strong reductant (E_o' ~ -0.6 volts)
  - Y^+, a weak oxidant (E_o' ~ 0.45 volts)
  - Strong reductant capable of reducing $NADP^+$
- PSII (P_{680}) produces:
  - X^-, a weak reductant (E_o' ~ 0.0 volts)
  - Y^+, a strong oxidant (E_o' ~ >+0.8 V volts)
  - Strong oxidant capable of oxidizing water
- The weak reductant of PSII reduces the weak oxidant of PS I (See Fig. 22.11)

Slide 15

Organization of Photosystems

- PSI and PSII are macromolecular assemblies found in the thylakoid membrane of the chloroplast.
- The pigment systems are connected by an electron transport chain so that the weak reductant of PSII will oxidize the weak oxidant of PSI.
- The connecting electron transport chain resembles complex III of mitochondria.
- There is also a chlorophyll containing complex that is a **light harvesting complex** (LHC)

Slide 16

The Z Scheme

- Arranging these systems and the intermediate carriers according to reduction potentials creates a pattern resembling a Z laid on its side.
  - See Fig 22.12
- Two quanta of light are necessary to take an electron from a very high reduction potential (species D) to a very low reduction potential (species A_0)
- A cytochrome complex connects the reductant from PSII to the oxidant from PSI.

Slide 17

Oxygen Evolution

- Oxygen evolution involves a manganese complex on the lumen face of the thylakoid membrane. (Fig. 22.13)
- The complex cycles through 5 oxidation states
- 1 e^- is removed in each of 4 steps by D, a tyrosyl radical, which is the strong oxidant produced by P_{680}^+
  - S_0 to S_1 to S_2 to S_3 to S_4
- Fifth step involves O_2 release
  - S_4 to S_0
- Evidence from effect of light flashes on O_2 production (See Fig 22.14)

Slide 18

Electron Transport Through a Cytochrome Complex

- Pheophytin, a special chlorophyll missing the Mg^{2+} is the weak reductant produced by P_{680}^*
- Plastoquinone accepts electrons from pheophytin
  - (See Figure 22.15)
- Electrons passed from plastoquinone to plastocyanin, a Cu protein, by the cytochrome b/f complex, containing an Fe/S protein.
  - (See Figure 22.12)
- The complex pumps protons into the lumen via a Q cycle

Note the similarity of the cytochrome b/f complex with complex III of the mitochondrial electron transport chain. In both cases, electrons are passed from a quinone to a peripheral membrane protein, and protons are pumped across the membrane. In this case, the protons are pumped from the stroma to the lumen.

Slide 19

$NADP^+$ Reduction

- P_{700}^* reduces A_0, a special chlorophyll
- A_0 reduces A_1, a quinone called **phylloquinone**
  - (phylloquinone is vitamin K_1)
- Electrons then passed through several Fe/S membrane proteins to a soluble Ferredoxin (Fd)
- Fd reduces a flavoprotein which reduces $NADP^+$
- NADPH is made on the stromal face
- P_{700}^+ is reduced at the lumen face by plastocyanin

Slide 20

ATP Synthesis

- The proton gradient drives an ATP synthase located in the thylakoid membrane.
- It consists of CF_0, an integral membrane component, and CF_1, a peripheral complex, both similar to the F_0F_1 complex of mitochondria.
- CF_1 lies on the stromal (outside) face of the thylakoid membrane.
- ATP is made in the stroma.

The detailed structure, organization, and mechanism of the chloroplast ATP synthase is probably almost identical to that of the mitochondria enzyme except for orientation.

Slide 21

ATP Synthesis, con't.

- Chloroplasts provided early direct evidence for the chemiosmotic hypothesis.
  - See description of Jagendorf and Uribe exp., p 728
- The chloroplast gradient differs in two ways from the mitochondrial gradient
  - Protons are pumped **into** the lumen instead of **out of** the organelle
  - $\Delta\psi$ is discharged by movement of Mg^{2+} from the lumen into the stroma
    - The proton motive force comes primarily from ΔpH

Chloroplasts were incubated at low pH so the inside equilibrated at that pH. Upon transfer to a solution of high pH to artificially create a proton gradient, and ATP synthesis was observed as the gradient collapsed.

Slide 22

Cyclic Photophosphorylation

- The dark reaction needs 3 ATP's per two NADPH's reduced.
- The Z scheme may provide one, or less than one, depending on proton stoichiometry.
- Additional ATP's can be made by PSI recycling electrons to the cytochrome chain rather than reducing NADPH.
- This **cyclic photophosphorylation** produces ATP in the absence of oxygen evolution. (Fig. 22.12 and 22.22)

Proton stoichiometry is not made clear. If the cytochrome complex pumps 2 protons per electron, that amounts to 4 protons per NADPH. Since reduction of CO_2 occurs in the stroma, there is no need to export the ATP for that purpose, so 3 protons per ATP corresponds to about 1.3 ATP per pair of electrons.

Slide 23

Detailed Architecture of Photosynthetic Reaction Centers

- Deisenhofer, Michel and Huber shared the 1984 Nobel Prize for solving the structure of the reaction center from *Rhodopseudomonas viridis*
- This bacterium contains $\mathbf{P_{870}}$, a bacterial pheophytin, and quinones analagous to the plant pigment systems. (Fig. 22.16)
- It catalyzes a cyclic photophosphorylation in the bacterial membrane. (Fig. 22.17)

Slide 24

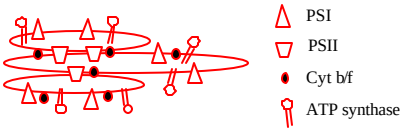
Architecture of PSI and PSII

- Core structure of PSII is probably similar to the *R. viridis* complex. (Fig 22.19)
  - Note the additional antenna chlorophyll proteins, and a cytochrome complex
- X-ray structure of PSI from the cyanobacterium *Synechococcus elongatus* has been solved.
  - Probably a good model for other PSI complexes.
  - Essential features in Fig 22.20

Slide 25

Summary of Light Reaction

- Figure 22.21 provides an overall summary of the complexes and their relationship in the thylakoid membrane.
- Your text does not discuss the lateral organization, which looks something as follows:



PSI and ATP synthase are exposed to the stroma, while PSII is buried in the stacked regions of the grana. The cytochrome chain is spread throughout the thylakoid membrane.

Slide 26

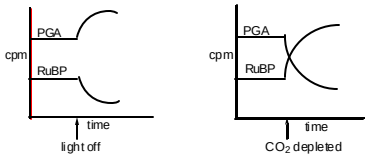
The Dark Reaction Early Experiments

- Use of $^{14}CO_2$ by Melvin Calvin's group was one of earliest applications of radioisotope tracers.
- Incubated *Chlorella* with $^{14}CO_2$ for varying times, extracted cells, separate products by paper chromatography.
  - Earliest labeled product is 3-phosphoglycerate (3-PGA)
  - Longer incubations produced sugar diphosphates, especially ribulose-1,5-bisphosphate (RuBP)

Slide 27

The Dark Reaction Early Experiments, con't.

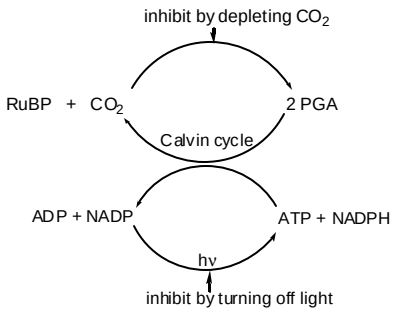
- "Cyclic" nature of the dark reaction illustrated by steady state labeling with $^{14}CO_2$.
- Then the reaction is blocked by turning off the light or depleting the CO_2.



In steady state labeling the cells are exposed to $^{14}CO_2$ over a period of time until all products are labeled to the same specific activity.

Slide 28

Cyclic Nature of the Dark Reaction



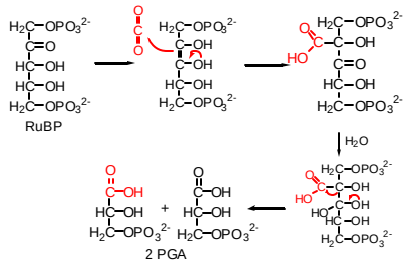
Slide 29

CO_2 Fixation

- Catalyzed by **Rubisco**
  - *Ribulose-1,5-bisphosphate carboxylase*
- Most abundant protein on earth
  - 15% of chloroplast protein
  - Found in stroma
- 8 large (55 kD), 8 small (15 kD) subunits
  - Diagram of X-ray structure (Fig 22.23)
- Enzyme bound carboxylated intermediate is cleaved into two PGA molecules (Fig 22.24)

Slide 30

Rubisco Mechanism



Slide 31

Ribulose-1,5-Bisphosphate Regeneration

- Regeneration of RuBP from PGA requires energy in form of NADPH and ATP.
- Reaction pathway is called the **Calvin-Benson** cycle.
- Overall net reaction converts 3 CO_2 to a triose phosphate. (Or 6 CO_2 to a hexose phosphate)

$$3\ C_5 + 3\ CO_2 \rightarrow 6\ C_3$$

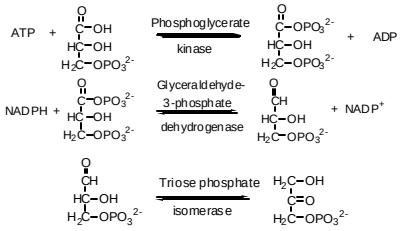
$$5\ C_3 \rightarrow 3\ C_5$$

$$\text{net:}\quad 3\ CO_2 \rightarrow C_3$$

Slide 32

Calvin Benson Cycle

- First step is reduction of PGA to triose-3-P
  - Requires 1 ATP and 1 NADPH per PGA

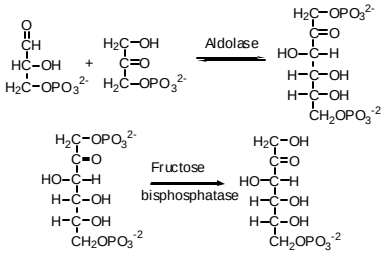


Note similarity of these reactions to those in glycolysis. NADPH is the reductant for the dehydrogenase, though. The three steps are all at equilibrium, and one gets an equilibrium mixture of the triose phosphates glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.

Slide 33

Calvin Benson Cycle, con't.

- Continuing on to the synthesis of hexoses:

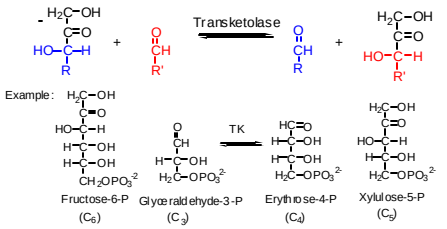


Aldolase is reversible, acting near equilibrium. It is essentially like the enzyme in glycolysis, except it has a broader specificity. The phosphatase is irreversible, acting far from equilibrium.

Slide 34

Calvin Benson Cycle, con't.

- Next comes a series of "shuffling" reactions, catalyzed by a new enzyme, **transketolase.**

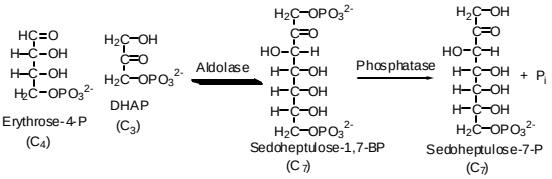


Transketolase has a fairly broad specificity for both acceptor and donor. R is a sugar chain with the last hydroxyl phosphorylated, but it can be 2, 3, or 4 carbons. Note the configuration of the third carbon is always L.

Slide 35

Calvin Benson Cycle, con't.

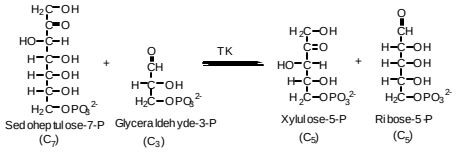
- Aldolase can then condense erythrose-4-P with DHAP



Slide 36

Calvin Benson Cycle, con't.

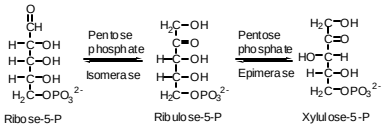
- The shuffling continues:



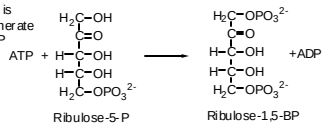
Slide 37

Calvin Benson Cycle, con't.

- Xylulose-5-P and Ribose-5-P are converted to Ribulose-5-P



Finally, an ATP is needed to regenerate Ribulose-1,5-BP



Slide 38

Overall Stoichiometry of the Calvin Benson Cycle

- Fig. 22.25 summarizes the reactions we have just gone through.
- Table 22.1 shows an overall net reaction in producing hexoses as:

  $6\ CO_2 + 18\ ATP + 12\ NADPH \rightarrow glucose + 18\ ADP + 12\ NADP^+$
- You should be able to use the reactions we have just detailed to show the following stoichiometry is also possible:

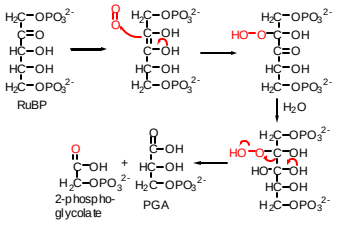
  $3\ CO_2 + 9\ ATP + 6\ NADPH \rightarrow triose\text{-}P + 9\ ADP + 6\ NADP^+$

Either stoichiometry shows that each CO_2 fixed requires 2 NADPH and 3 ATP. Remember the NADPH was used to reduce two 1,3-bisphosphoglycerates, 2 ATP's were needed to form the bisphosphoglycerates, and 1 ATP was needed to phosphorylate ribulose-5-phosphate.

Slide 39

Rubisco Also Has Oxidase Activity

- Its full name is *ribulose bisphosphate carboxylase/oxygenase*



Slide 40

Photorespiration

- The oxidase activity leads to **photorespiration** which is wasteful of ATP and NADPH
  - The phosphglycolate is converted to glycolate, which is oxidized to **glyoxalate** in peroxisomes, and can be converted to glycine or used in the glyoxalate cycle. Two glycines condense to form serine and CO_2 (See Fig 22.29)
- Regulation of Rubisco insures that it is only active when the light reaction is running.

Slide 41

Regulation of Rubisco

- Rubisco is activated by Mg^{2+} and by carbamylation:
  $R\text{-}NH_2 + CO_2 \rightarrow R\text{-}NH\text{-}COOH$
- Carbamylation is promoted by high pH and by CO_2
- The light reaction raises the pH of the stroma (Fig 22.27) as well as increasing $[Mg^{2+}]$.
  - An activase also participates in converting rubisco to a form that can be carbamylated, and the activase is also only active in the light.

Remember that the electrical component of the proton gradient is neutralized by Mg^{2+} transport from the thylakoid lumen to the stroma. The book describes also how ribulose-BP inhibits by binding to the inactive, non-carbamylated form of the enzyme, and the activase promotes its removal so carbamylation can occur. The requirement of CO_2 for activation insures that there will be CO_2 around to compete with O_2 and inhibit photorespiration.

Slide 42

Regulation of the Calvin Cycle

- In addition to the Rubisco regulation, several enzymes are activated by reduction of disulfide bonds.
  - The bonds are reduced by **thioredoxin**, which in turn is reduced by ferredoxin or NADPH (See Fig 22.28)
- The enzymes so activated include **fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase,** and **ribulose-5-P kinase**
  - (These are the irreversible steps of the pathway)
  - The pathway is active only when light stimulates PSI.

Although the Calvin Cycle is referred to as the "dark reaction", it does not occur in the dark. Light must be present to drive the light reaction for the Calvin Cycle to be activated.

Slide 43

Starch versus Sucrose Synthesis

- Triose-P in the stroma is converted to glucose-6-P, which is activated to ADP-glucose, the precursor for starch synthesis.
  - Therefore starch is made and stored in the chloroplast
- Triose-P can exit the chloroplast to the cytoplasm, where it can be converted to hexoses as well, but here the hexoses are converted to sucrose.
  - Therefore sucrose is made in the cytoplasm.

The regulation of diverting triose-P in one direction or another is complex, and involves among other things the antiport exchange of triose-P with P_i across the chloroplast membrane. The book does not cover this topic, so we won't get involved in its details. Fructose-2,6-BP plays a role in the process, though, just as we will see it playing a role in regulation of glycolysis and gluconeogenesis in liver and muscle. (Its concentration decreases during active photosynthesis, causing the rate of glycolysis to drop and diverting the triose-P in the cytoplasm to sucrose synthesis).

Slide 44

C-4 Pathway
aka Hatch and Slack Pathway

- At high temperatures, photorespiration becomes more of a problem.
- Tropical plants have developed a further protection by using a CO_2 delivery system that increases the CO_2 concentration.
- CO_2 is "fixed" as oxaloacetate in mesophyll cells.

  $PEP + CO_2 \rightarrow OAA$
- Synthesis of PEP "costs" 2 ATP

  $Pyruvate + P_i + ATP \rightarrow PEP + AMP + PP \rightarrow 2P_i$

Sugar cane and corn are C-4 plants. CO_2 is fixed by the enzyme PEP carboxylase. PEP is made by the enzyme pyruvate-phosphate dikinase, with an unusual mechanism described on page 739. Hydrolysis of two phosphate anhydride bonds are necessary for the reaction to be spontaneous.

Slide 45

C-4 Pathway, con't.

- OAA is reduced to malate, which is carried to the bundle sheath cells where malic enzyme releases CO_2, and can build up the concentration of CO_2 to more effectively compete with O_2. (Fig 22.30)
  - (In some plants, OAA is converted to aspartate instead)
- Also O_2 does not diffuse as readily into the bundle sheath cells.

Slide 46

CAM Plants

- CO_2 and O_2 enter plants through pores called **stomata**. Water leaves the same way.
- Succulent plants such as *Crassulaceae* in arid regions do not open their pores during the day, so CO_2 can only enter at night.
- Malate is synthesized as in C-4 plants, but is stored in vacuoles until daylight, when the CO_2 is released for incorporation by the Calvin-Benson pathway.
- This process is referred to as *crassulacean acid metabolism* (CAM).