Topic 9. Exercises on Photosynthesis

The pathways of photosynthesis and respiration are quite different. However, at the global level, one is perfectly complementary to the other as the end products of respiration are the reagents for photosynthesis. The oxygenated air that sustains all animal life is due to photosynthesis. This fact was only discovered in the late eighteenth century when Joseph Priestly found that plants could "fix" air exhausted by fire and/or respiration to allow a mouse to survive in a closed jar.

It is worth considering the overall reactions of photosynthesis and respiration.

Respiration	$C_6H_{12}O_6 + 6\ O_2$	--->	$6CO_2 + 6\ H_2O$
Photosynthesis	$6CO_2 + 6\ H_2O$	--->	$C_6H_{12}O_6 + 6\ O_2$

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I. Where Photosynthesis Occurs in Plant Cells

While some parts of respiration occur outside the mitochondrion, all parts of photosynthesis occur in the chloroplast. Inside the chloroplast is an internal system of membranes called **thylakoids**, some of which are clustered into stacks called **grana.** The space not occupied by these membranes is called the **stroma**.

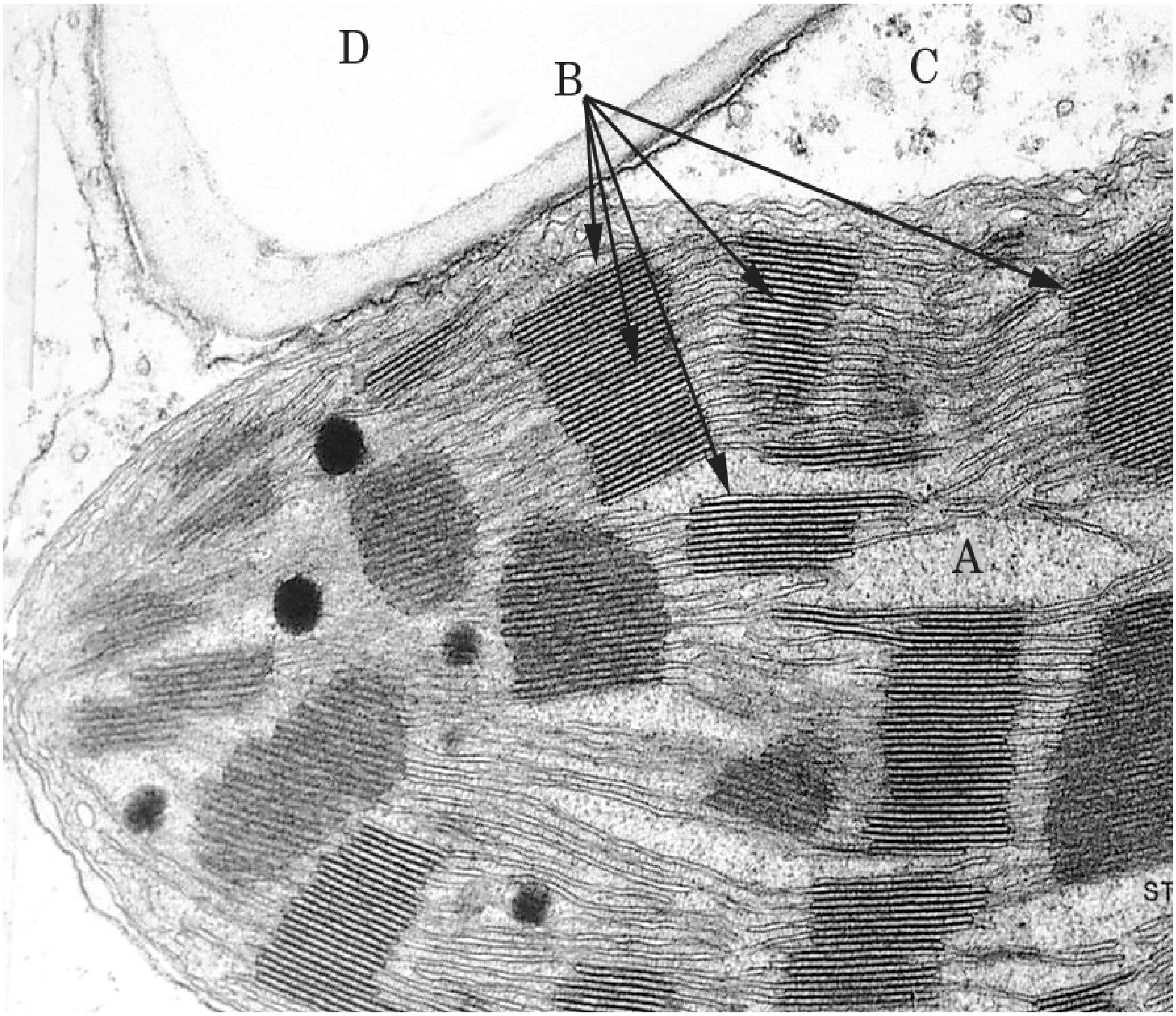
The Light Dependent Reactions

The **light reactions** require the spacial structure generated by the intact thylakoids. The pigment systems of **light reaction I and II** are both bound to these membranes and are each coupled with an electron transport chain. As in the mitochondrion, these electron transport chains generate a hydrogen ion concentration across a boundary which is harnessed to generate **ATP**. The ultimate electron receptor in the process is not oxygen, as in the mitochondrion, but **NADP+** which generates **NADPH.**

The Light Independent Reactions

The **Calvin Cycle**, which fixes carbon, occurs in the stoma. It requires the reducing potential (NADPH) and the energy (ATP) provided by the light reaction, but is not, itself, dependent upon the structure of the internal membranes of the chloroplast (other than to keep the whole mix together). To produce sugars and to evolve oxygen, the light reaction and the Calvin Cycle must work in tandem. While the light reaction can produce ATP without the Calvin Cycle, it cannot split water without the regeneration of NADP+ from NADPH accomplished by the Calvin Cycle. The Calvin Cycle, in turn, cannot fix CO_2 without a constant supply of ATP and NADPH from the light reaction.

Consider the figure



1. Where do the light reactions occur? __________

2. Where does the Calvin Cycle occur? __________

II. The Necessity of Chlorophyll for Photosynthesis.

To ‘eat’ light as plants do, it must first be absorbed. As plants are universally green due to chlorophyll, it seems obvious that a green pigment is necessary for photosynthesis. However, correspondence doesn’t constitute proof of causality. The following is a simple exercise to provide evidence of that relationship. In the exercise we will consider the hypothesis

Chlorophyll is necessary for photosynthesis.

At the side bench is a variety of *Coleus* with variegated leaves. There are two obvious pigments to be found in various regions of its leaves. Observe leaves on the plant, and note that some leaves have areas with...

- just chlorophyll colored green;
- just anthocyanin colored red;
- regions of overlap, and
- regions with neither which are white.

Because chlorophyll is not found everywhere in the leaf, we can use this plant to evaluate whether chlorophyll is necessary for photosynthesis. To do so, however, we require a method for determining photosynthetic activity.

One approach is to consider where in the leaf starch forms. Starch is derived from sugars produced by photosynthesis and we can detect starch using iodine in I_2KI.

Procedure: Work in pairs.

1. Take a leaf and draw it. Clearly indicate where chlorophyll and anthocyanin are located and where they overlap.
2. Boil the leaf in water to remove the water soluble anthocyanin pigment. Make a second drawing clearly showing where the chlorophyll is located.
3. Boil the leaf in alcohol to remove the chlorophyll pigmentation.
4. Carefully place the bleached and brittle leaf on a watch glass and flood it with I_2KI.
5. Again draw the leaf clearly indicating where the purple stained starch is located.

Drawing 1	Drawing 2	Drawing 3
Untreated leaf	Anthocyanin removed	Stained with I_2K

Discussion:

Are your results consistent with the hypothesis?

____

Consider a second hypothesis:

Anthocyanin is necessary for photosynthesis.

Do your results support that statement?

____

Can you reject the second hypothesis?

____

Have you “proved” the first hypothesis?

____

III. The Light Harvesting Pigments of Photosynthesis

In the following activities you will observe specifically which colors of light are absorbed by a pigment extract from banana leaf, and, hence, which colors are used by photosynthesis.

IIa. The Spectrum Viewer

Work with a partner

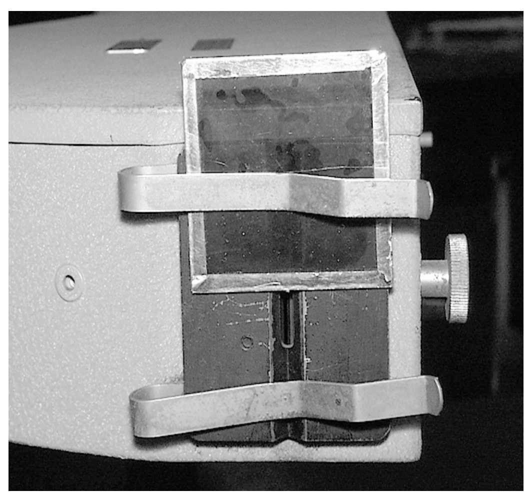
See the illustration of the spectrum viewer on the next page. These are located on the side bench. Look through the front opening. If the light behind the viewer is on and is properly aligned with the opening at the back, you should view a spectrum of colors projected to the right. Note that this spectrum is projected onto a scale. The scale is to be read in nanometers. Note that each color is projected at the point on the scale corresponding to that color’s wavelength in nanometers.

Spectrum Viewer Illustrations

Spectrum Viewer

Look to the right to view the scale

The spectrum from the light source will be projected against a scale in nanometers.



Only push the filters and pigment extract half way down the opening at the back

Procedure:

1. Take color filters and push them half way down the opening at the back as you view the projected spectrum (see illustration). Note, if properly positioned you will still view the total spectrum of the light source on the bottom of the scale while also viewing the light transmitted through the filter at the top.

   Are the filters totally effective?

   - With the red filter do you see colors other than red? _______
   - With the green filter do you see colors other than green? _______
   - With the blue filter do you see colors other than blue? _______

2. Take a test tube of pigment extract, and, while viewing the projected spectrum, push the tube half way down the opening in the back. Carefully note that there are two regions where the pigment absorbs the light most completely. If you cannot discern these regions, the pigment may be worn out and will need to be replaced. If you have trouble here ask your TA for help!

   - The two regions of maximum absorption corresponds to what two colors?

   - The two regions of maximum absorption corresponds to what two ranges of wavelengths?

IIb. The Spectrophotometer:

Work in pairs. The spectrophotometer is on the side bench. Instructions for its use are provided by the instrument, and your TA is available to assist.

With a spectrophotometer, we can quantify what you have just observed with the spectrum viewer. Each pair of students will measure the transmittance of light through a solution of pigment extract at each of the wavelengths given in the table. **Transmittance** is simply a measure of the portion of light not absorbed as passes through a sample. From your observations with the spectrum viewer it should be clear that this value will vary with color. The data table below will dictate your choice of wavelengths. Measure transmittance at each of these wavelengths. Record your readings below, and graph the data on the following page. In class, check with other students to verify that you didn't make mistakes. Turn in this table and graph in discussion.

Wave Length (nanometers)	Transmittance
400	
425	
450	
475	
500	
525	
550	
575	
600	
625	
650	
675	
700	
725	

1. Do the results in Part IIb. Correspond to you observations in IIa?

2. Are they consistent with the information given in your book about the action spectrum of photosynthesis?

Name ________________________________

IIc. Fluorescence of Chlorophyll

If the light energy absorbed by chlorophyll is not transferred to a reaction center, then the energy is lost either as heat, or as fluoresced light, or some combination of the two. View the demonstration of fluorescence at the front bench. Do not confuse this fluoresced light with the transmitted light viewed by the spectrum viewer.

What emits the green light viewed through the spectrum viewer?

____

What emits the red, fluoresced, light?

____

IId. Isolating Pigments Using Paper Chromatography

<u>Work in groups of two</u>

The glassware required for this activity is at the front of your bench. The chromatography paper and solvent, and the pigment extract for use in this activity are at the front bench.

<u>Procedure</u>:

1. Take a piece of chromatography paper, roll it into a cylinder, and staple it in three places (see illustration on the next page).

2. Remove the 500 ml lipless beaker from the larger of the two petri dishes (see illustration). Take the smaller petri dish and add enough pigment extract to cover the bottom.

3. Stand the paper cylinder in the smaller dish until the pigment has moved about 1.5 cm up the cylinder.

4. Set the paper aside for 5 minutes to dry.

5. Load the paper two additional times (repeat "c" and "d" twice).

6. After loading the paper with pigment the third time, allow to dry at least 10 minutes. Then **return the pigment extract to the stock bottle**; wipe the small petri dish with a tissue, half fill that dish with chomotography solvent and place it in the center of the larger petri dish; sit the paper cylinder in the pigment extract, and cover with the 500 ml lipless beaker.
7. If you properly dried your paper, you should quickly see the chromatography solvent move through the previously loaded pigment band with the immediate result that bands of pigment separate from each other.

Chromatography Glassware

Chromatography Paper with smaller and larger petri dishes

Explanation: These pigments are separating by their physical affinity for water (determined by the extent of their molecular polarity). Water is bound to the fibers in the paper, and as the chromatography solvent moves up the cylinder of paper, it acts as a drag on the pigment molecules. The extent of this drag is directly related to each molecule's attraction to water. At the top of the cylinder you should see a deep yellow band made up of the carotinoid pigment, carotene. This layer moves freely with the moving solvent front. It is non polar with no affinity for water. The next pigment down, represented by one or two yellow bands, is the carotinoid pigment, xanthophyll. Next going down the cylinder, is a green band made up of Chlorophyll a, and the lowest band is made up of the pigment with the greatest affinity for water, Chlorophyll b.

Which of these pigments are integrated into the reaction centers of both photosystems I and II?

____

Which act as accessory pigments in the antenna complexes of photosystems I and II?

____

Which serve to protect the photosystems from photo oxidative damage?

____

Which is the plant source for vitamin A in our diet?

____

Discussion Topics for Your Consideration

1. Consider the argument that the first instance of organisms polluting their environment were the Cyanobacteria releasing noxious diatomic oxygen.

2. How has the presence of oxygen gas made life on land possible?

3. As oxygen is cycled between respiration and photosynthesis, in what molecular forms is it found?

4. What came first, aerobic respiration or photosynthesis?

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