Photosynthesis In A C_3 Versus A C_4 Grass

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At the beginning of class you are expected to turn in a brief summary of the lab and the answers to the 2 questions posed in the introduction (in green).

Introduction

You are now familiar with the C_3 pathway i.e. the Calvin cycle. Not long after Calvin and his colleagues used the newly available $^{14}CO_2$ to fully elucidate what we know today as the Calvin cycle. Other researchers around the world started feeding $^{14}CO_2$ to their particular plant of interest. In many cases the ^{14}C label quickly appeared in a 4 carbon sugar, not the 3 carbon PGA that Calvin had initially seen it in. The first person to publish this observation was Hugo Kortschak, who was working in the Hawaiian Sugar Planters Research Lab. In sugar cane he found the radio label from $^{14}CO_2$ ended up in malate. By 1969 Hal Hatch and Rodger Slack also working with sugar cane at the Colonial Sugar Refining Co. in Brisbane Australia had elucidated the complete pathway we know today as C_4 photosynthesis. C_4 is one of the 2 major carbon concentrating mechanisms the other being CAM.

List 3 major differences between CAM photosynthesis and C_4.

For many years the C_4 pathway was called the Hatch-Slack pathway. C_4 Photosynthesis occurs in only ≈ 1% of characterized species and is known to occur in both monocots and dicots, but it is especially prevalent in the grasses. The grasses of most economic interest that are C_4 are corn, sugarcane, and sorghum.

Are rice and wheat C_3 or C_4?

C_4 photosynthesis is not something that occurs instead of C_3 photosynthesis i.e. the Calvin cycle, rather C_4 is fancy "front end" that has evolved as a carbon pump to provide CO_2 to the Calvin cycle. In fact because of this division of labor C4 plants have chloroplasts in 2 different cell types that are close to each other (fig 1). The initial CO_2 capture occurs in mesophyll cells and the resulting four carbon sugar diffuses to the bundle sheath cell where the CO_2 is released and is used by rubisco to feed into the Calvin cycle. As you know in C_3 photosynthesis CO_2 is initially captured by rubisco. In C_4 photosynthesis CO_2 in the form of HCO_3^- is initially captured by the enzyme phosphoenolpyruvate carboxylase (PEP carboxylase) which has a much higher affinity than rubisco. PEP carboxylase catalyzes the reaction of HCO_3^- and phosphoenol pyruvate (3C) to form oxaloacetate (4C) (OAA). OAA is quickly converted to malate and the malate is transported from the mesophyll cell to the bundle sheath cell where the enzyme NADP malic enzyme converts the malate to pyruvate releasing a CO_2 which is then recaptured by rubisco. The pyruvate moves back into the mesophyll cell where it is converted to phosphoenol pyruvate (PEP) by the enzyme pyruvate-orthophosphate dikinase. This highly energetic step uses one phosphate from ATP to phosphorylate the pyruvate and transfers another one to a free phosphate forming pyrophosphate (PP).

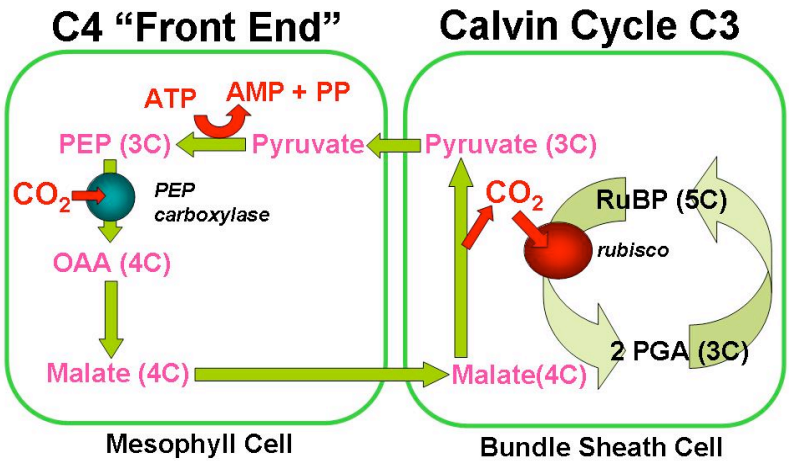

Figure 1. The Generalized C_4 cycle

Because the bundle sheath cells are relatively gas tight due the unique structure of the cell walls in C_4 plants the C_4 cycle acts as a CO_2 pump concentrating CO_2 at the site of rubisco. Because CO_2 levels are so high at the active site of rubisco the wasteful oxygenation side reaction, called photorespiration, is suppressed in favor of solely carboxylations. Since C_4 eliminates the costly and wasteful photorespiration the question arises why was C_4 selected for in so few plants? The answer lies in the cost of C_4 to the plant. The pyruvate dikinase enzyme uses 1 ATP for every CO_2 consumed and worse yet the ATP had 2 phosphates removed to form AMP. The road back from AMP to ATP is an energetically costly one.

The real benefit to the plant from C_4 photosynthesis comes at high temperature. As the temperature increases rubisco gets more and more "sloppy" and catalyzes more oxygenations, i.e. photorespiration increases. So at low temperatures < 25°C C_4 plants have no real advantage over C_3 plants in fact in many cases they are at a disadvantage due to the increased ATP used by C_4. At high temperatures, however C_4 plants are at an advantage. Under these conditions the energy consumed by C_4 is less than the energy that would have been consumed by photorespiration. Under these hot conditions water loss can be a real problem for the plant. Because the PEPcarboxylase has such a high affinity for HCO_3^- and is saturated at ambient CO_2 concentrations C_4 plants are able to reduce their stomatal aperture thus minimizing water loss without incurring a loss in CO_2 fixation. It has been estimated that about 30% of global photosynthesis by terrestrial plants comes from C_4 plants growing in savannahs. From the 12 most rapidly growing crop or pasture plants 11 are C_4 and 8 of 10 worst weed species world-wide are C_4 (Heldt 1997).

Procedure:

You will work in 2 groups to accomplish all the tasks. However, each student is responsible for all the data in the lab notebook and submitting your own report. So make sure you get all the data. If you don't write it down yourself copy it from your partner later. Because we only have two IRGA's to measure photosynthesis we will have to take turns. I want to make sure everyone has a chance to "drive". We will be making an A - C_a curve. A is for assimilation which is a term used by plant biologists to mean the photosynthetic rate. C_a is the concentration of CO_2 in the air surrounding the leaf. We will be comparing A C_a curves for 2 agronomically important grasses wheat (C_3) and corn (C_4). One group will start with corn and the other with wheat. Once you have finished making all your measurements with one plant move on to the next.

1. Before you clamp the 6400 onto your leaf make sure your conditions that are set are appropriate for the measurement you wish to make. What is the CO_2 level? (set to the highest CO_2 level you will measure) Are the lights on and what is the light level? What is the humidity? What is the temperature in the chamber? What is the flow rate?

2. Carefully place a leaf into the chamber, try to fill as much of the chamber as possible to ensure a good signal. Once the leaf is in the chamber gently clamp the leaf in place. Make sure you did not break the petiole.

3. While watching the instrument panel allow the plant to acclimate for 5 – 10 minutes. While you are waiting for the leaf to acclimate keep a careful eye on the leaf temperature, chamber temperature and dew point. We do not want to allow the dew point to climb above the chamber temperature. Why would we not want the dew point to rise above the chamber temperature? If the dew point does start to climb close to the chamber temperature you can make some adjustment to lower the humidity.

a. If you have a good CO_2 signal i.e. the difference between the reference and sample CO_2 is greater than 5 ppm you can raise the flow rate.

b. If the flow rate is already at max and/or the CO_2 signal is low you can scrub the incoming air to remove water vapor and lower the reference dew point.

c. If 1 and 2 fail you can always raise the chamber temperature.

You also will want to "Match" the reference and sample IRGA's to make sure they have not drifted apart. Matching allows the sample air stream that has passed over the leaf to pass through both the sample and the reference IRGAs. When this happens both IRGA's should read the same, if they do not you can "match" them so that they do. This is the equivalent of taring or zeroing a balance before you weigh something.

4. Watch the photosynthetic rate, is it climbing, falling or relatively stable. When you are satisfied that the photosynthetic rate is relatively stable match the IRGA's one last time and make a measurement. Now decrease the CO_2 level, allow to acclimate for 5 minutes and take another reading. Repeat this until you have measured the photosynthetic rates at all the CO_2 levels listed below. Make sure to record the sample CO_2 level, this is the C_a, the transpiration rate and the conductance.

Reference CO_2 levels 1200, 1000, 800, 600, 500, 400, 300, 200, 100, 50, 30, 0 (ppm)

5. When you are finished measuring assimilation rates for a particular plant carefully unclamp the chamber. If the plant leaf did not fill up the entire chamber you will need to measure the leaf area so that you can correct the assimilation rates to reflect the actual area of leaf that was photosynthesizing. If you are unsure how to measure the leaf area ask your instructor or TA for advice.

6. Prepare a graph with assimilation versus C_a with corn and wheat on the same graph and compare them. The apparent compensation point is the point where photosynthesis is exactly balanced by photorespiration + respiration. Where would this be on your graph? Mathematically estimate the apparent CO_2 compensation point using some of the data you collected.

Other Considerations

If photorespiration is so wasteful why hasn't a better rubisco been selected for?
Hint: Think about earth's CO_2 levels on a geologic time scale.

There is currently a world wide effort to genetically engineer C_4 into rice. What do you think are some of the biggest challenges in doing this? Tell me what you think, but search the literature and the web and see what the scientific consensus is, it's okay to "cheat" on this question.

Given that CO_2 levels are rising do you think C_4 plants will continue to be at an advantage? Again search the literature and see what others have to say on this point.

To Turn In

There is no formal lab report for this lab but please prepare the following graphs and answer the following questions

1. A line graph showing A versus C_a for corn and wheat
2. What is the apparent compensation point for corn and wheat? How did you determine this? (if you are unsure how to do this please come and see me)
3. A line graph showing how the conductance changes with CO_2 concentration in corn and wheat
4. Where does the carbon to make the CO_2 that plants are giving off under photorespiratory conditions come from?
5. Answer all questions in the Section above labeled "Other Considerations"