C_4 AND CAM PHOTOSYNTHETIC PATHWAYS

Type of life science: Photosynthesis
Other fields of study: Biochemistry, botany, cell biology (cytology), evolutionary biology, plant anatomy, and plant physiology

Photosynthesis is the process by which plants remove carbon dioxide from the atmosphere and use it to produce sugar. Three different biochemical pathways have evolved for accomplishing this task.

Principal terms

BIOCHEMICAL PATHWAY: a series of chemical reactions in cells which leads to the formation of a product

BUNDLE SHEATH CELLS: cells in the interior of the leaf that surround the specialized conducting tissue known as the vascular system

CARBON DIOXIDE: a gas present in the atmosphere in minute amounts that is the primary source of carbon for the photosynthetic production of sugar; a molecule of carbon dioxide is composed of one carbon atom and two oxygen atoms

CHLOROPLASTS: structures within the cells of leaves which contain the green pigment chlorophyll and are the site of sugar production in photosynthesis

ENZYMES: specialized proteins present in most cells which are responsible for catalyzing chemical reactions

FIXATION: the process by which an enzyme attaches carbon dioxide onto an organic molecule in photosynthesis

MESOPHYLL CELLS: photosynthetic cells distributed throughout the leaf

PHOSPHOENOLPYRUVATE CARBOXYLASE: an enzyme present in C_4 plants and CAM plants which incorporates carbon dioxide into an organic compound, usually malic acid

PHOTOSYNTHESIS: the process that takes place in chloroplasts within the cells of green plants, which uses the sun's energy and carbon dioxide to generate sugar and oxygen

RIBULOSE BISPHOSPHATE CARBOXYLASE: the enzyme responsible for incorporating carbon dioxide into sugar in all plants

Summary of the Phenomenon

Photosynthesis is the physiological process whereby plants use the sun's radiant energy to produce organic molecules such as sugar. The backbone of all such organic compounds is a skeleton composed of carbon atoms, and plants use carbon dioxide from the atmosphere as their carbon source.

Different plant species use three biochemical pathways of carbon acquisition. The overwhelming majority of plants use a single reaction to attach carbon dioxide from

the atmosphere onto an organic compound, a process referred to as carbon fixation. This process takes place inside specialized structures within the cells of green plants known as chloroplasts. The enzyme that catalyzes this fixation is ribulose bisphosphate carboxylase, and the first stable organic product is a three-carbon molecule. This three-carbon compound enters a biochemical pathway leading to sugar formation. Such plants are referred to as C_3 plants because the first product made with carbon dioxide is a three-carbon molecule.

For many years, scientists thought that this series of actions was the only way plants photosynthesized. In the early 1960's, however, researchers studying the sugarcane plant discovered a biochemical pathway that involved incorporation of carbon dioxide into organic products at two different stages. First, carbon dioxide from the atmosphere enters the sugarcane leaf, and fixation is accomplished by a different enzyme, phosphoenolpyruvate carboxylase. This carbon dioxide fixation step takes place within the cell solution known as the cytoplasm, not inside the chloroplasts. The first stable product is a four-carbon organic compound that is an acid, usually malate. Sugarcane and other plants with this photosynthetic pathway are known as C_4 plants.

In C_4 plants, this photosynthetic pathway is tied to a unique leaf anatomy known as "kranz" anatomy. This terms refers to the fact that in C_4 plants the cells that surround the water- and carbohydrate-conducting system (known as the vascular system) are densely packed with chloroplasts. In all other plants, these cells, known as the bundle sheath cells, lack chloroplasts. This kranz anatomy plays a major role in C_4 photosynthesis.

In C_4 plants, the initial fixation of carbon dioxide from the atmosphere takes place in cells near the outer edge of the leaf, adjacent to the stomatal pores, which open and close to allow carbon dioxide into the leaf. These cells are known as the mesophyll cells. After the carbon dioxide is fixed into a four-carbon organic acid, the malate is transferred through tiny tubes from these cells to the specialized bundle sheath cells in the interior of the leaf. Inside the bundle sheath cells, the malate is chemically broken down into a smaller organic molecule, and carbon dioxide is released. This carbon dioxide then enters the chloroplast of the bundle sheath cell and is fixed a second time with the enzyme ribulose bisphosphate carboxylase and continues through the C_3 pathway to produce sugar.

To summarize, in C_4 plants, carbon dioxide is fixed twice: once in the mesophyll cells, near the stomatal pores, and a second time in the interior of the leaf, in the bundle sheath cells. The two carboxylase enzymes are distributed differently within the leaf; the C_4 enzyme phosphoenolpyruvate carboxylase is restricted to mesophyll cells, and the C_3 enzyme ribulose bisphosphate carboxylase is restricted to the bundle sheath cells.

This double-carbon fixation pathway confers a greater photosynthetic efficiency on C_4 plants over C_3 plants, because the C_3 enzyme ribulose bisphosphate carboxylase is highly inefficient in the presence of atmospheric levels of oxygen. In order for the enzyme to operate, carbon dioxide must first attach to the enzyme at a very particular location known as the active site; however, oxygen is also able to attach to this active site and prevent carbon dioxide from attaching, a process known as photorespiration. As a consequence, there is an ongoing competition between these two gases for attachment at the active site of the ribulose bisphosphate carboxylase enzyme. At any given time, the "winner" of this competition is largely dictated by the relative concentrations of these two gases. When a plant opens its stomatal pores, the air that diffuses in will be at equilibrium with the atmosphere, which is 21 percent oxygen and 0.04 percent carbon dioxide. Since water vapor will always diffuse out, most plants face certain desiccation if the stomata are left open continuously. When these pores are closed, the concentration of gases will change. As photosynthesis proceeds, carbon dioxide will be consumed and oxygen generated. Since one molecule of oxygen will be generated for every molecule of carbon dioxide consumed, the relative change in oxygen will be minor compared to the change in the concentration of carbon dioxide. When the concentration of carbon dioxide drops below 0.01 percent, oxygen will out-compete carbon dioxide at the active site and no net photosynthesis occurs. C_4 plants, however, are able to prevent photorespiration from inhibiting photosynthesis, because the phosphoenolpyruvate carboxylase enzyme is not inhibited by oxygen. Thus, when the stomatal pores are closed, this enzyme continues to fix carbon inside the leaf until it is consumed.

A third photosynthetic pathway, known as crassulacean acid metabolism (CAM), exists in succulents such as cactus and other desert plants. These plants have the same two carbon-fixing steps as are present in C_4 plants, but, rather than being spatially separated between the mesophyll and bundle sheath cells, CAM plants have both carbon-dioxide-fixing enzymes within the same cell. These enzymes though are separated temporally in their activity. Just as kranz anatomy is unique to C_4 plants, CAM plants are unique in that the stomatal pores are open at night and largely closed during the day.

The biochemical pathway of photosynthesis in CAM plants begins at night. With the stomatal pores open, carbon dioxide diffuses into the leaf and into mesophyll cells, where it is fixed by the C_4 enzyme phosphoenolpyruvate carboxylase. The product is different in charge from that of a C_4 plant, and thus it is referred to as malic acid rather than malate. The fate of this malic acid is identical to that of C_4 plants; namely, it will be broken down, and the resulting carbon dioxide will be utilized by the C_3 enzyme to produce glucose. This process will not, however, occur in the dark, because glucose production requires light energy. Consequently, the malic acid is transported from the cytoplasm of the cell to a large storage bag known as the vacuole, but it never leaves the cell. The vacuole will accumulate malic acid through most of the night. During the waning phases of the dark period, the vacuole will fill up, and malic acid will begin to accumulate in the cytoplasm outside the vacuole. As it does, the pH of the cell solution surrounding the phosphoenolpyruvate enzyme will become acidic, causing the enzyme to stop functioning for the rest of the dark period.

When the sun rises, the stomata will close and photosynthesis by the C_3 enzyme

(ribulose bisphosphate carboxylase) will quickly deplete the atmosphere within the leaf of all carbon dioxide. At this time, the malic acid will be transported out of the vacuole to the cytoplasm of the cell. There it will be broken down, and the carbon dioxide will enter the chloroplast and be used by the C_3 enzyme ribulose bisphosphate carboxylase to produce glucose; thus, photosynthesis is able to continue with stomatal pores closed.

Crassulacean acid metabolism derives its name from the fact that it involves a daily fluctuation in level of acid within the plant and that it was first discovered to be common in species within the Stonecrop plant family, which carries the scientific name Crassulaceae. The discovery of this photosynthetic pathway dates back to the 1960's. The observation that succulent plants become very acidic at night, however, dates back to at least the seventeenth century, when it was noted by taste that cactus is sour in the morning and bitter in the afternoon.

There are two distinctly different ecological environments where CAM plants may be found. Most are terrestrial plants typical of deserts or other harsh, dry sites. In these environments, the pattern of stomatal opening and closing provides an important advantage for surviving arid conditions: When the stomata are open, water is lost; however, the rate of loss decreases as the air temperature decreases. By restricting the time period of stomatal opening to the nighttime, CAM plants are extremely good at conserving water.

The other ecological setting where CAM plants are found is in certain aquatic habitats. At first, when this location was discovered, it seemed quite odd, since in these environments conserving water would be of little value to a plant. It was found, however, that there are aspects of the aquatic environment which make CAM photosynthesis advantageous. In shallow bodies of water, the photosynthetic consumption of carbon dioxide may proceed at a rate in excess of the rate of diffusion of carbon dioxide from the atmosphere into the water, largely because gases diffuse several times more slowly in water than in air. Consequently, pools of water may be completely without carbon dioxide for large parts of the day. Overnight, carbon dioxide is replenished, and aquatic CAM plants take advantage of this condition to fix the plentiful supply of carbon dioxide available at night and store it as malic acid. Hence, during the day, when the ambient carbon dioxide concentration is zero, these plants have their own internal supply of carbon dioxide for photosynthesis. Thus, two very different ecological conditions have selected for the identical biochemical pathway.

These three photosynthetic pathways adequately describe most of the species of terrestrial plants, although there exists much variation. For example, there are species that appear in many respects to have photosynthetic characteristics intermediate to C_3 and C_4 plants. Other plants are capable of switching from exclusively C_3 photosynthesis to CAM photosynthesis at different times of the year. Photosynthesis by aquatic plants appears to present even more variation. C_3-C_4 intermediate plants seem to be relatively common compared to the terrestrial flora, and several species have C_4 photosynthesis but lack kranz anatomy.

Methods of Study

The primary method of studying the biochemical pathway of photosynthesis involves the use of radioactive carbon dioxide. If a plant leaf is exposed to an atmosphere in which some of the carbon dioxide molecules have the radioactive carbon 14 isotope, then a portion of the products produced will also be radioactive. If one wants to determine the first product produced by carbon fixation, then the leaf would be exposed for a brief period (a second or less) to the radioactive carbon dioxide. In order to stop photosynthesis at this point, the leaf is immediately put in boiling alcohol or liquid nitrogen. An extract of the cells is then separated by chromatography and/or electrophoresis. Both techniques separate the organic molecules on a two-dimensional sheet or plate according to differences in size, electrical charge, and other characteristics. To determine which product is radioactive, a process known as autoradiography is used. This process involves attaching a special type of unexposed film to the plate and allowing the radioactively labeled compound to expose the film by emitting energy similar to light. After developing, places on the film that were exposed will appear as dark spots. By doing similar separations with known radioactively labeled compounds, a map of where each compound occurs on the plate can be used to identify the radioactively labeled compounds. To elucidate other steps in the pathway, leaves are exposed to the radioactive carbon dioxide for increasingly longer periods of time.

There are other techniques for classifying a particular plant as C_3, C_4, or CAM. Generally, CAM photosynthesis can be ruled out if photosynthetic tissues do not show a difference in concentration of acids between morning and evening. Tissue can be tested by determining the amount of some type of base, such as sodium hydroxide, required to bring a volume of tissue extract to a particular pH (for example, pH 7.0). In terrestrial plants, C_4 photosynthesis can be ruled out if the leaf lacks kranz anatomy. C_3 and C_4 plants can also be distinguished by the measurement of the ratio of the two carbon isotopes carbon 13 and carbon 12. This technique may also identify CAM plants, but, because some species switch between CAM and C_3, it is not always definitive. Because of certain characteristics of the aquatic environment, this isotope method will not distinguish photosynthetic pathways in aquatic plants.

For quantitative studies such as determining the rate of photosynthesis in C_3, C_4, and CAM plants, radioactive carbon dioxide may be used in certain instances, such as studies of aquatic plants, but it is seldom the appropriate technique for terrestrial plant photosynthesis studies. The best technique involves placing a leaf within an enclosed clear plastic chamber that is gas-tight. The leaf is irradiated with high-intensity lamps, and the temperature and humidity within the chamber are controlled. A gas stream with a known concentration of carbon dioxide is passed over the leaf in the chamber and then into an infrared spectrophotometer. Since carbon dioxide absorbs in the infrared portion of the radiation spectrum, the level of carbon dioxide remaining in the gas stream can be determined, and thus one can determine the rate at which the leaf consumes carbon dioxide. Since photosynthesis is very

sensitive to small changes in carbon dioxide concentration, a more sensitive technique maintains the carbon dioxide concentration in the gas stream constant and determines the amount needed to keep the carbon dioxide concentration constant within the chamber.

Context

The vast majority of land plants possess the C_3 pathway of photosynthesis. This predominance reflects the fact that the ribulose bisphoshate carboxylase is a very primitive enzyme that evolved more than three billion years ago. At that time, the earth's atmosphere was quite different from today's atmosphere in that the ratio of carbon dioxide to oxygen was much greater. Consequently, photosynthesis was not inhibited by oxygen. As the level of oxygen increased in the atmosphere, oxygen inhibition became an increasingly important limitation to photosynthesis. Over the millennia, however, ribulose bisphosphate carboxylase evolved into an extremely complex protein—apparently so complex that mutations which would prevent oxygen from binding at the active site, and thus increase photosynthetic efficiency, were not possible without destroying other aspects of the enzyme function.

The only way this oxygen inhibition could be overcome was through evolution of a second carbon-fixing enzyme, namely, the C_4 enzyme phosphoenolpyruvate carboxylase. By sequestering the C_3 enzyme ribulose bisphosphate carboxylase away in the interior of the leaf, C_4 plants have overcome oxygen inhibition. As a consequence, C_4 plants, including sugarcane, corn, and sorghum, have the highest photosynthetic rates and are considered to be among the most productive crops. The evolution of the C_4 pathway is thought to be a relatively recent phenomenon, probably having developed within the last hundred thousand years. Today, it is found in only a fraction of all land plants, but in many distantly related species; thus, it appears as though this pathway has evolved independently in more than one plant lineage.

A question often asked is why the C_4 plants have not outcompeted C_3 plants to become the dominant photosynthetic type. To understand why, one must consider all the factors that limit photosynthesis, particularly temperature, light, and water availability. In many environments, such as the shady understory of a forest, light is the most limiting of these factors. As a consequence, the rate of photosynthesis is low, and thus it may be only infrequently that the carbon dioxide level in the leaf drops to less than 0.01 percent where oxygen inhibition is important. In a very moist meadow environment, plants may be able to keep their stomatal pores open continuously and thus seldom reduce the carbon dioxide very far below atmospheric level. In general, C_4 photosynthesis is most likely to provide an advantage to photosynthesis in relatively dry, hot, high light environments, and as a consequence this photosynthetic pathway is most abundant in tropical grasslands and becomes progressively less common toward higher latitudes.

CAM photosynthesis in terrestrial plants is of particular significance in arid environments because photosynthesis occurs with minimal water. CAM plants are absent, however, from some arid environments, such as Death Valley, which remains so warm at night that the opening of stomata at night does not result in sufficient savings of water. Other arid environments, such as the shrubby chaparral of California, are so dense with growth that the CAM plants are shaded out. CAM plants cannot compete in these shrubby environments: Growth rates are too slow, a result of the fact that total daytime photosynthesis is limited by the amount of malic acid that can be stored in the vacuole.

Thus, it is curious that the same biochemical pathway, with two carbon-dioxide-fixing steps that are spatially separated in the leaf in C_4 plants and temporally separated in activity in CAM plants, would have generated such totally different characteristics.

Bibliography

Berry, Joseph A. "Adaptation of Photosynthetic Processess to Stress." *Science* 188 (May 9, 1975): 644-650. This article focuses on examples of plant adaptations to environmental stresses. Examples of optimal conditions for C_4 plants are provided, and advantages of different photosynthetic pathways under different conditions are discussed.

Edwards, Gerald E., Maurice S. B. Ku, and Russell K. Monson. "C_4 Photosynthesis and Its Regulation." In *Photosynthetic Mechanisms and the Environment*, edited by J. Barber and N. R. Baker. New York: Elsevier Science Publishing, 1985. In-depth examination of the physiology of photosynthesis in C_4 plants. Describes range of variation present in biochemical pathways of different groups of C_4 plants.

Keeley, Jon E. "Photosynthesis in Quillworts: Or, Why Are Some Aquatic Plants Similar to Cacti?" *Plants Today* 1 (July/August, 1988): 127-132. Gives a historical account of the discovery of Crassulacean acid metabolism in the aquatic plants known as quillworts. Discusses the ecological significance of this pathway in an aquatic habitat.

Kluge, Manfred, and Irwin P. Ting. *Crassulacean Acid Metabolism*. New York: Springer-Verlag, 1978. One of the early reviews of CAM photosynthesis.

Osmond, C. Berry, Klaus Winter, and H. Ziegler. "Functional Significance of Different Pathways of CO_2 Fixation in Photosynthesis." In *Physiological Plant Ecology II: Water Relations and Carbon Assimilation*, edited by Otto L. Lange et al. New York: Springer-Verlag, 1982. This chapter reviews the physiological and ecological characteristics of the three photosynthetic pathways in terrestrial plants.

Pearcy, Robert, Olle Bjorkman, Martyn M. Caldwell, Jon E. Keeley, Russell K. Monson, and Boyd R. Strain. "Carbon Gain by Plants in Natural Environments." *Bioscience* 37 (January, 1987): 21-29. Describes how different photosynthetic characteristics affect carbon fixation by plants from different environments, and examines the advantages and disadvantages inherent in the different photosynthetic pathways.

Pearcy, Robert W., and James Ehleringer. "Comparative Ecophysiology of C_3 and

C_4 Plants." *Plant, Cell and Environment* 7 (1984): 1-13. A review article that relates the physiological significance of C_4 photosynthesis to plant performance in nature. The article compares the physiological effects of the C_3 and C_4 pathways on photosynthesis and then discusses their performance in contrasting environments.

Winter, Klaus. "Crassulacean Acid Metabolism." In *Photosynthetic Mechanisms and the Environment*, edited by J. Barber and N. R. Baker. New York: Elsevier Science Publishing, 1985. This chapter is a review of all knowledge pertaining to Crassulacean acid metabolism in land and aquatic plants, with a discussion of the evolution of this photosynthetic pathway.

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Cross-References

Anaerobic Photosynthesis, 87; The Calvin-Benson Cycle, 316; Carbon 13/Carbon 12 Ratios in Photosynthesis, 330; Chloroplast Reaction Sites, 424; Chromatographic Analysis of Chloroplast Pigments, 453; Photosynthetic Light Absorption, 2073; Photosynthetic Light Reactions, 2080.