CARBON 13/CARBON 12 RATIOS IN PHOTOSYNTHESIS

Type of life science: Photosynthesis
Other fields of study: Biochemistry, botany, evolutionary biology, and plant physiology

The ratio of carbon 13 to carbon 12 isotopes in plant tissues is a good indicator of the photosynthetic pathway in many terrestrial plants.

Principal terms

C_3 PLANTS: plants with the photosynthetic pathway that has only a single carbon dioxide fixation, with the initial product of carbon fixation being a three-carbon molecule

C_4 PLANTS: plants with the photosynthetic pathway that has two carbon dioxide fixation steps, the first producing a four-carbon molecule known as malate; malate is moved to bundle sheath cells on the interior of the leaf and broken down to provide carbon dioxide for the C_3 pathway of photosynthesis, which is restricted to the bundle sheath cells

CAM PLANTS: plants with two carbon dioxide fixation steps, one occurring at night and the other occurring during the day; CAM (Crassulacean acid metabolism) plants open their stomatal pores at night and fix carbon dioxide into malic acid, which is stored overnight in the cell vacuole; during the day, the stomata are closed and the malic acid is broken down, giving off carbon dioxide for photosynthesis

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

CARBON FIXATION: the process whereby an enzyme attaches carbon dioxide onto an organic molecule in photosynthesis

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

PHOTOSYNTHESIS: the process, occurring in chloroplasts within the cells of green plants, that 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

Atoms are the basic unit of natural elements such as carbon. Although the atom

is the smallest unit having the properties of its element, atoms are composed of subatomic particles, protons, neutrons, and electrons being the most important. All atoms of a given element have the same number of protons, but some atoms may have more neutrons than other atoms of the same element. These different atomic forms are called isotopes of the element, and in nature there is usually a mixture of isotopes.

Carbon has an atomic number of 6, meaning that it has six protons. Most carbon atoms have six neutrons. Since the atomic weight is largely determined by the mass of the protons plus neutrons, this isotope is called carbon 12; it is the most common form of carbon, accounting for about 99 percent of the carbon in nature. Most of the remaining 1 percent of carbon consists of atoms of the isotope carbon 13, with seven rather than six neutrons; thus, this isotope is heavier than carbon 12. A third isotope, carbon 14, is present in the environment in minute quantities but is not very stable. Consequently, it may decay spontaneously, giving off radiation, and thus it is a radioactive isotope. Carbon 12 and carbon 13 are considered stable isotopes.

Stable isotopes are measured on a mass spectrometer, an instrument that separates atoms on the basis of their mass differences. Initially, the plant material is combusted, and the carbon dioxide given off is analyzed by the mass spectrometer for the ratio of carbon 13 to carbon 12 isotopes. This ratio is compared to the ratio of carbon 13 to carbon 12 in an internationally accepted standard and is expressed as the difference between the sample and the standard minus one. This number is multiplied by one thousand and expressed as a "per mil." In plant matter, this number is always negative. The more negative the ratio of carbon 13 to carbon 12, the less carbon 13 there is present.

There are three biochemical pathways of carbon acquisition used by different plant species, and the ratio of carbon 13 to carbon 12 in plant tissues is often a useful means of distinguishing the photosynthetic pathway being used. One group of plant species, the largest, photosynthesizes by attaching carbon dioxide obtained from the atmosphere onto an organic compound in a single carbon fixation step. This reaction is catalyzed by an enzyme known as 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.

Some plants, such as corn and sugarcane, however, have two carbon fixation steps. Atmospheric carbon dioxide is fixed by the enzyme phosphoenolpyruvate carboxylase, and the first product is a four-carbon organic acid; these plants are known as C_4 plants. This product is moved to the interior of the leaf and broken down, and the carbon dioxide molecule is released within specialized cells known as kranz-type bundle sheath cells. Within these cells the carbon dioxide molecule is fixed a second time, and the C_3 enzyme ribulose bisphosphate carboxylase and glucose are produced.

A third group of plants, known as CAM (for Crassulacean acid metabolism) plants, open their stomatal pores on the leaf surface at night; carbon dioxide enters and is fixed with the C_4 enzyme phosphoenolpyruvate carboxylase. The organic acid produced is stored in the cell overnight. During the day, the stomata are closed and the acid is broken down; carbon dioxide which is released is fixed by the C_3 enzyme, and glucose is produced.

In terrestrial plants, carbon isotope ratios of photosynthetic tissues vary from −8 per mil to −15 per mil in C_4 plants and from −20 per mil to −35 per mil in C_3 plants. CAM plants may range from C_4-like to C_3-like in their carbon isotope ratios. For atmospheric carbon dioxide, the ratio of carbon 13 to carbon 12 is about −8 per mil, and thus C_4 plant tissues have slightly less carbon 13 than the air, while C_3 plants have much less carbon 13 than the air. In other words, during photosynthesis plants tend to discriminate against the carbon dioxide molecule formed from the carbon 13 isotope and more readily fix the carbon 12 isotope of carbon dioxide; this discrimination against carbon 13 is more pronounced in C_3 plants.

Discrimination against carbon 13 is attributable to the great mass of this isotope. One consequence is that carbon 13 does not diffuse as readily to the site of photosynthesis as does carbon 12, which accounts for some small level of discrimination against carbon 13 in all plants. The major difference in carbon isotope ratio between C_3 and C_4 plants, however, results from a difference in discrimination between the primary carboxylation enzyme. In C_3 plants, the carboxylating enzyme ribulose bisphosphate carboxylase results in a −27 per mil discrimination against carbon 13. In C_3 plants, this enzyme is present in the cells adjacent to the stomatal pores and thus obtains carbon dioxide more or less directly from the atmosphere. Since carbon 13 is discriminated against, it will tend to accumulate, but it readily diffuses out of the leaf when stomatal pores are open. In C_4 plants, on the other hand, the initial carbon-fixing enzyme, phosphoenolpyruvate carboxylase, discriminates very little against carbon 13. In C_4 photosynthesis, the secondary carbon-fixing enzyme, ribulose bisphosphate carboxylase, is sequestered in the interior of the leaf in the bundle sheath cells, and the carbon dioxide it fixes is derived from the breakdown of the C_4 fixation product. As the ribulose bisphosphate carboxylase enzyme discriminates against the carbon dioxide molecules made of carbon 13, this heavier isotope accumulates within the bundle sheath cells and diffuses out very slowly. As the carbon 13 form of carbon dioxide accumulates in the bundle sheath cells, the higher concentration of carbon 13 will overcome the discrimination by the enzyme; in effect, the enzyme will be forced to fix the carbon 13 isotope, and thus discrimination is minimal. C_4 plant tissues consequently have less negative carbon 13/carbon 12 ratios.

In typical CAM photosynthesis, the atmospheric carbon dioxide is fixed at night by the enzyme phosphoenolpyruvate carboxylase, and, as in C_4 plants, this enzyme discriminates very little against carbon dioxide molecules made from the carbon 13 isotope. During the day, the C_4 fixation product is broken down, and the carbon dioxide that is released is fixed into glucose with the ribulose bisphosphate carboxylase enzyme. This enzyme will discriminate against carbon 13, but since the stomata are closed during the day, the carbon 13 will accumulate within the leaf and eventually be fixed; consequently, little discrimination occurs. Such CAM plants have carbon 13/carbon 12 ratios similar to those of C_4 plants. Some CAM plants, however, will open their stomatal pores for varying lengths of time during the day or switch to strictly C_3 photosynthesis during certain times of the year. In these plants, the carbon 13/carbon 12 ratio will be more similar to that observed for C_3 plants.

In aquatic plants, the carbon 13/carbon 12 ratio does not indicate the photosynthetic pathway. C_3 aquatic plants frequently will have carbon isotope ratios very similar to that of the source carbon from the water: Because the enzyme ribulose bisphosphate carboxylase discriminates against carbon 13, carbon 13 tends to accumulate in the layer of water around the leaf. Because the diffusion of gases in water is very slow, the plant will eventually be forced to fix the carbon 13. Other aspects of the aquatic environment also influence the carbon isotope ratio of aquatic plant tissues.

Methods of Study

C_3 and C_4 plants are readily distinguished without elaborate biochemical studies. It should be noted, however, that a simple anatomical examination for kranz-type bundle sheath cells is another easy method for determining the presence of C_4 photosynthesis. In CAM plants, the carbon isotope ratio reflects the degree to which carbon fixation is restricted to solely nighttime carbon uptake.

For aquatic plants, the method of measuring carbon 13/carbon 12 ratios reveals very little about the photosynthetic pathway; however, it can reveal other characteristics of the aquatic environment. For example, if two plant species both utilize the C_3 photosynthetic pathway and both are dependent on only carbon dioxide for photosynthesis (some plants can utilize bicarbonate dissolved in the water), one can determine the degree of resistance the water plays in the uptake of carbon dioxide. Plants growing in relatively stagnant water will tend to have less negative carbon isotope ratios compared to plants from fast-moving streams: In stagnant water, the resistance of the water layer around the leaf will inhibit the diffusion of carbon dioxide. As the C_3 enzyme discriminates against the carbon 13 form, this isotope will accumulate and eventually be fixed.

The carbon isotope method has been utilized to investigate photosynthetic pathways in fossil plants. Organic matter from fossils has been analyzed for the carbon 13/carbon 12 ratio and shown to possess the C_3 or C_4 pathway. In one study, a fossil cactus was demonstrated to possess the C_3 pathway, but at another point in time, thousands of years later, the carbon isotope ratio of the cactus was much less negative, indicating a switch to the CAM pathway.

Archaeologists have made use of this technique to examine dietary habits of ancient populations. The carbon isotope ratio of a food plant will affect the carbon isotope ratio of the tissues and bones of the organism that consumed the plant. Consequently, remains of a prehistoric human population with a diet concentrated on a C_3 plant, such as beans, will be distinguishable from a population that depended largely on a C_4 plant, such as corn.

This technique has been used for other "food web" studies as well. For example, nectar-feeding ants in tropical rain forests may forage on CAM or non-CAM species. The difference will be reflected in a difference in the carbon isotope ratio of the ants. The distinctive carbon isotope ratio of C_3, as opposed to C_4, plants is reflected in the diet of predators that prey on insects, which in turn feed on one photosynthetic type or the other.

By examining the carbon isotope ratios of feces collected from the large grazing fauna of East Africa, scientists have been able to estimate the proportion of C_3 plants utilized in their diet. There are three levels of temporal resolution available by examining the isotope ratios of different tissues of these grazers: Fecal-matter carbon isotope ratios are indirect indicators of immediate dietary consumption; soft tissues reflect the recent history of grazing; and bone collagen gives an integrated view of the lifetime grazing pattern.

In ecosystem studies, it is often of interest to know the source of carbon deposited into streams or the soil. The ratio of carbon 13 to carbon 12 has been used to distinguish the sources of carbon in studies of this sort.

The method has also been used to distinguish other physiological attributes. For example, it is a useful technique for evaluating the water-use efficiency of plants with the C_3 pathway. The explanation for this lies in the fact that C_3 plants differ from one another in carbon isotope ratio, based on the extent to which the stomatal pores remain open. Since water vapor will always diffuse out, most plants face 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. Species, and populations of the same species, differ in the degree to which photosynthesis can be maintained with stomata closed, and this affects the amount of carbon dioxide fixed per quantity of water lost—that is, the water-use efficiency. The longer photosynthesis continues with stomata closed, the greater the probability of carbon 13 being fixed, despite its being discriminated against by the ribulose bisphosphate carboxylase enzyme. As a consequence, if one measures the average concentration of carbon dioxide within the leaf and the carbon 13/carbon 12 ratio, one can estimate the relative water-use efficiency of different photosynthetic tissues.

The method of measuring the carbon 13/carbon 12 ratio is also useful for understanding aspects of metabolism other than photosynthesis. For example, when carbohydrates are converted into other compounds, such as lipids, there is further discrimination against the carbon 13 isotope. Consequently, the carbon 13/carbon 12 ratio of carbohydrates differs from that of lipids. This fact has been used to determine the substrate for respiration by plant and animal tissues. For example, the flowers of *Philodendron selloum* are capable of generating heat up to 40 degrees Celsius above ambient temperature. By measuring the carbon 13/carbon 12 ratio of the carbon dioxide molecules released during respiration, scientists can show that this phenomenon results from the "burning," or respiration, of lipids rather than carbohydrates, the normal substrate for respiration in plants.

Context

Measuring the carbon 13/carbon 12 ratio is an important tool because it allows scientists to investigate a phenomenon that otherwise would require much more detailed studies. To assign a plant to the C_3 or C_4 photosynthetic type, one can do biochemical studies involving the determination of organic products which are produced during photosynthesis utilizing radioactive carbon dioxide molecules. The distinction of photosynthetic type can also be obtained by determining the carbon isotope ratio of the photosynthetic tissues. The advantages of this approach are that very small samples, on the order of a few milligrams, are all that are needed. Additionally, dried specimens, of any age, can be used.

For plants with CAM photosynthesis, the carbon isotope ratio can reveal the extent to which the plants employ this pathway on a year-round basis. The alternative would be to study the CAM plant on a year-round basis. For some plants in very isolated, out-of-the-way places, this may not be done easily.

In food web studies, the carbon 13/carbon 12 ratios can provide information that would otherwise be unavailable. For archaeologists interested in the diets of prehistoric populations, the carbon isotope technique may be the only form of evidence available in some cases. Even if food remains are present with the fossils, they may reveal very little about the relative quantities of each food item consumed by the ancient population. The carbon isotope ratio, on the other hand, will give an integrated measure of the relative quantities of C_3 versus C_4 foot items in the diet. If C_4 plants were not included in the diet, however, this method would not be of use.

In the study of water-use efficiency, there are two advantages of this method. First, measuring the carbon 13/carbon 12 ratio, and internal carbon concentration, is relatively simple compared to the detailed studies of photosynthesis and transpiration. Second, the isotope ratio provides an integrated measure of water-use efficiency over the period during which the tissue was laid down. Such a measure may be of greater use to a physiological ecologist than a single measurement in time.

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Jon E. Keeley

Cross-References

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