CARBON FIXATION

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Summary

The energy in sunlight is introduced into the biosphere by a process known as photosynthesis. Photosynthesis occurs in green plants, seaweeds, algae, and some bacteria. These organisms are sugar factories, producing millions of new glucose molecules per second. Each year, photosynthesising organisms produce about 170 billion tons of extra carbohydrates, about 30 tons for every person on Earth. Photosynthesis is a physicochemical process which produces the reduced carbon and energy required for the survival of virtually all life on Earth, as well as the molecular oxygen necessary for the survival of oxygen consuming organisms, which include bacteria, plants and animals. The overall equation for photosynthesis looks very simple, but the truth is that a complex set of physical and chemical reactions must occur in a coordinated sequence for the fixation of carbon from the atmosphere into carbohydrates. Photosynthesis is a two-stage process. The first stage consists of the light dependent reactions, and requires the direct energy of light to make energy carrier molecules that are used in the second stage. The light independent reactions occur when the products of the light dependent reactions are used to form carbohydrates. Some photosynthetic bacteria can use light energy to extract electrons from molecules other than water.

The amount of carbon dioxide removed from the atmosphere each year by oxygenic photosynthetic organisms is massive. However, more carbon dioxide is still released

into the atmosphere than is taken up by photosynthesis so the net global carbon dioxide concentration is increasing. Little is known about the impact of such drastic atmospheric and climatic changes on plant communities and crops. Current research is directed at understanding the interaction between global climate change and photosynthetic organisms.

1. Introduction

The Sun provides the majority of the energy for nearly all life on Earth. The energy in sunlight is introduced into the biosphere by a process known as photosynthesis. Photosynthesis occurs in green plants, seaweeds, algae, and certain bacteria. These organisms are essentially sugar factories, producing millions of new glucose molecules per second. Plants use much of this glucose as an energy source to build their fruits, seeds, flowers and leaves. They also convert glucose to cellulose, the structural material used in plant cell walls. Most plants produce more glucose than they use, however, and they store it in the form of starch and other carbohydrates in their leaves, stems, and roots. The plants can then draw on these reserves for extra energy or building materials. Herbivores introduce the energy stored in the carbohydrates into the animal food chain. Each year, photosynthesising organisms produce about 170 billion tons of extra carbohydrates, about 30 tons for every person on Earth.

Photosynthesis is a physicochemical process. Plants, algae and photosynthetic bacteria use light energy to drive the synthesis of organic compounds, thus converting light energy into chemical energy. In plants, algae and some types of bacteria, the photosynthetic process results in the removal of carbon dioxide from the atmosphere that is used to synthesize carbohydrates (oxygenic photosynthesis) and the release of molecular oxygen. Other types of bacteria use light energy to create organic compounds but do not produce oxygen (anoxygenic photosynthesis). Photosynthesis produces the reduced carbon and energy required for the survival of virtually all life on Earth, as well as the molecular oxygen necessary for the survival of oxygen consuming organisms, which include bacteria, plants and animals. In addition, the fossil fuels currently being burned to provide energy for human activity were produced by ancient photosynthetic organisms. Although photosynthesis occurs in microscopic cells and organelles, the process has an immensely important and far-reaching impact on the earth's atmosphere and climate. More than 10% of the total atmospheric carbon dioxide is reduced to carbohydrate by photosynthetic organisms annually. The vast majority of the reduced carbon is then returned to the atmosphere as carbon dioxide by animal, microbial and plant metabolism, and by the combustion of biomass in natural fires or by anthropological processes. In turn, the performance of photosynthetic organisms depends on the earth's atmosphere and climate. The escalation in the amount of atmospheric carbon dioxide released from anthropogenic sources is certain to have an extreme impact on the performance and competition of photosynthetic organisms over the next few decades. Knowledge of the physicochemical process of photosynthesis will become even more important for understanding the relationships between living organisms and the atmosphere and the balance of life on earth.

The overall equation for photosynthesis looks very simple. However, the truth is that a complex set of physical and chemical reactions must occur in a coordinated sequence for the fixation of carbon from the atmosphere into carbohydrates. Plants require almost 30 different proteins to work within a complicated membrane structure to produce a single molecule of a sugar such as sucrose. One of the topics of research into photosynthesis is to investigate the molecular processes that use light energy to drive carbohydrate synthesis and the mechanism of photosynthesis, and another focuses on understanding the structure of the photosynthetic components. The research involves several disciplines, including biochemistry, biophysics, chemistry, molecular biology, physics, physiology and structural biology.

Photosynthesis is a two stage process. The first stage consists of the light dependent reactions (light dependent process or light reactions), and requires the direct energy of light to make energy carrier molecules that are used in the second stage. The light independent reactions (light independent process or dark reactions) occur when the products of the light dependent reactions are used to form C-C covalent bonds of carbohydrates. The light independent reactions can usually occur in the dark, if the energy carriers from the light process are present. Recent evidence suggests that a major enzyme of the light independent reactions is indirectly stimulated by light, thus the term dark reactions is somewhat of a misnomer. The light dependent reactions occur in the grana and the light independent reactions take place in the stroma of the chloroplasts. An overview of the entire photosynthetic process is shown in Figure 1.

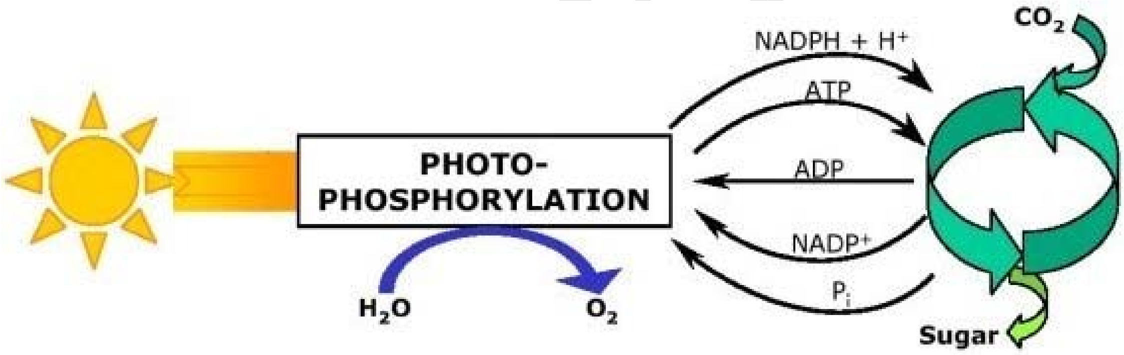

Figure 1. Overview of the whole photosynthetic process.

An English chemist and clergyman was the first person to show that plants release a type of gas that allows combustion. Joseph Priestley performed experiments in the 1770s using a candle burning in a closed vessel until the flame went out. He then placed a sprig of mint in the chamber and after several days showed that the candle could burn again. His experiments demonstrated that plants release oxygen into the atmosphere, although nothing was known about molecular oxygen at that time. Even now, more than two centuries later, one of the most active areas of photosynthetic research is investigating the mechanism plants use to produce oxygen. A Dutch physician named Jan Ingenhousz continued Preistley’s work, and demonstrated that only the green parts of plants could release oxygen, and that sunlight was necessary for photosynthesis. At around the same time, two workers from Switzerland, a plant physiologist and chemist, Nicolas Théodore de Saussure, and Jean Senebier, a naturalist and botanist showed that water is required and discovered that carbon dioxide is also required for photosynthetic growth. Much later, a German physician and physicist named Julius Robert von Mayer proposed that photosynthetic organisms convert light energy into chemical free energy in1845.

The key features of plant photosynthesis were known by the middle of the nineteenth century. It had been demonstrated that plants could use light energy to make carbohydrates from carbon dioxide and water. The empirical equation representing the net reaction of photosynthesis for oxygen evolving organisms can be written as follows:

$$CO_2 + 2H_2O + \text{Light Energy} \rightarrow [CH_2O] + O_2 + H_2O \qquad (1)$$

$[CH_2O]$ represents a carbohydrate.

A large input of light energy is required for the manufacture of a carbohydrate (e.g. glucose, a six-carbon sugar) from carbon and water. The standard free energy for the reduction of one mole of carbon dioxide to the level of glucose is +478 kJ/mol. The standard free energy for the synthesis of glucose is +2,870 kJ/mol. Because glucose is often an intermediate product of photosynthesis, the net equation of photosynthesis can also be written as:

$$6CO_2 + 12H_2O + \text{Light Energy} \rightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O. \qquad (2)$$

Early scientists studying photosynthesis thought that carbon dioxide was split by light energy, and they concluded that the O_2 released by plants came from carbon dioxide. Cornelis van Niel, working in the 1930s, knew that some photosynthetic bacteria released sulfur instead of oxygen and that these bacteria could use hydrogen sulfide (H_2S) instead of water for photosynthesis. Many workers around that time, including Van Niel, concluded that the O_2 released during photosynthesis comes from the oxidation of water and that photosynthesis depends on electron donation and acceptor reactions. Van Niel's research into the comparison of plant and bacterial photosynthesis lead him to propose the general equation of photosynthesis that applies to plants, algae and photosynthetic bacteria:

$$CO_2 + 2H_2A + \text{Light Energy} \rightarrow [CH_2O] + 2A + H_2O. \qquad (3)$$

2A represents O_2 in oxygenic photosynthesis.
A represents fumarate or elemental sulfur (S^0) in anoxygenic photosynthesis.

In anoxygenic photosynthesis, which occurs in some types of photosynthetic bacteria, the electron donor can be an organic hydrogen donor such as succinate (in which case, A is fumarate) or an inorganic hydrogen donor, such as H_2S (in which case A is elemental sulfur). Ruben and Hill and Scarisbrick provided experimental evidence that molecular oxygen came from water in the early 1940s, by demonstrating oxygen evolution in the absence of carbon dioxide in illuminated chloroplasts and by using ^{18}O enriched water.

The biochemical fixation of carbohydrate from carbon dioxide is a reduction reaction that involves the rearrangement of covalent bonds between oxygen, carbon and hydrogen. Energy rich molecules that are produced by the light-driven electron transfer reactions provide the energy for the reduction of carbon. Melvin Calvin, Andrew Benson and James Bassham in the late 1940s and 1950s elucidated a series of biochemical reactions that cause carbon reduction to occur in the dark. Most of the intermediate steps that result in the production of carbohydrate were identified using the radioisotope ^{14}C. Calvin was awarded the Nobel Prize for Chemistry in 1961 for this work, and the set of reactions was named the Calvin Cycle.

In the 1950s it was discovered that plants (D. Arnon and co-workers) and photosynthetic bacteria (A. Frenkel) use light energy to produce adenosine triphosphate (ATP). Adenosine triphosphate is an organic molecule that serves as an energy source for many biochemical reactions. The primary photochemical reaction of photosynthesis was shown to be an oxidation/reduction reaction that occurs in a protein complex (the reaction center) by L Duysens. It was discovered over the next few years that plants, algae and cyanobacteria require two reaction centers, PS II and PS I, operating in series. Several groups, such as those of L Duysens, Horst Witt and Robert Hill carried out instrumental research at this time. Johann Deisenhofer, Hartmut Michel, Robert Huber and co-workers were then awarded the Nobel Prize for Chemistry in 1988 for work in which they determined the structure of the reaction centre of the purple bacterium *Rhodospeudomonas viridis*. Most of the proteins required for the conversion of light energy and electron transfer reactions of photosynthesis are found in membranes.

RA Marcus received the Nobel Prize in Chemistry in 1992 for work on the theory of electron transfer reaction in chemical systems. A key element in photosynthetic carbon fixation is the electron transfer within and between simple organic molecules and protein complexes. The electron transfer reactions are very fast and highly specific.

2. Carbon fixation in higher plants

The energy that drives photosynthesis originates in the sun, where the fusion of hydrogen converts mass to heat. Over time, the heat energy reaches the sun's surface, where some of it is converted to light that reaches the Earth by black body radiation. Plants absorb a small fraction of the visible light reaching the Earth. Photosynthetic organisms transform light energy into chemical energy in a form that can last for hundreds of millions of years (e.g. as fossil fuel) through a series of energy transducing reactions. The photosynthetic process in plants and algae occurs in small organelles known as chloroplasts, inside the cells. More primitive photosynthetic organisms, for example, anoxygenic photosynthetic bacteria and oxygenic cyanobacteria, prochlorophytes lack organelles.

The photosynthetic reactions are traditionally divided into two stages—the "light dependent reactions," which consist of electron and proton transfer reactions and the “light independent reactions” or "dark reactions", which consist of the biosynthesis of carbohydrates from carbon dioxide. The light dependent reactions occur in a complex membrane system (the photosynthetic membrane) that is made up of protein complexes, electron carriers, and lipid molecules. The photosynthetic membrane is surrounded by water. Protein complexes embedded in the photosynthetic membrane have an asymmetrical arrangement which allows some of the energy released during electron transport to create an electrochemical gradient of protons across the membrane.

In plants, the photosynthetic process occurs inside organelles called chloroplasts, which are found in certain cells. Chloroplasts provide the energy and reduced carbon needed for plant growth and development, while the plant provides the chloroplast with carbon dioxide, water, nitrogen, organic molecules and minerals necessary for the chloroplast biogenesis. Specialised leaf cells can contain 50 or more chloroplasts each. Chloroplasts are defined by an inner and an outer membrane and are usually shaped like a convex lens (Figure 2), although many different shapes and sizes can be found in plants. The inner membrane acts as a barrier, controlling the flux of organic and charged molecules in and out of the chloroplast. Water passes freely through the membranes, as do other small neutral molecules like carbon dioxide and O_2. There is some evidence that chloroplasts were once free living bacteria that invaded a non-photosynthetic cell long ago. They have retained some of the DNA necessary for their assembly, but much of the DNA necessary for their biosynthesis is located in the cell nucleus. This enables a cell to control the biosynthesis of chloroplasts within its domain.

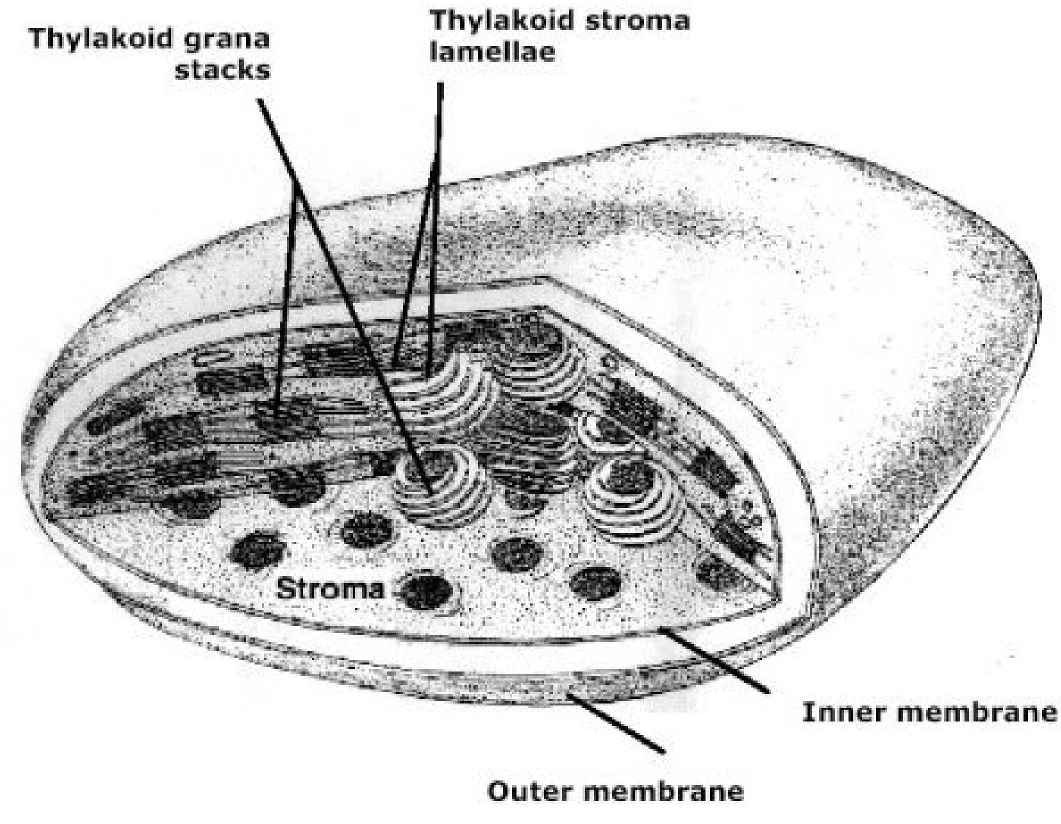

Figure 2. Chloroplast structure.

Inside the chloroplast there is a complicated membrane system, known as the photosynthetic or thylakoid membrane, which contains most of the proteins required for the light dependent reactions. The photosynthetic membrane is composed mainly of glycerol lipids and protein. The proteins required for the fixation and reduction of carbon dioxide are located outside the photosynthetic membrane in the surrounding water. The photosynthetic membrane is vesicular, defining a closed space with an outer water space (stromal phase) and an inner water space (lumen). The organization of the photosynthetic membrane can be described as groups of stacked membranes, interconnected by non-stacked membranes that protrude from the edges of the stacks (Figure 2).

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Biographical Sketches

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She obtained a BSc(Hons) in Environmental Biology from the University of Wales, Swansea in 1995. She then joined the School of Water Sciences at Cranfield University as an MScs student and stayed on to complete her PhD in wastewater biotechnology in 1999. Dr Burgess then worked as a post-doctoral research officer in the School of Water Sciences until moving to Rhodes University as a research fellow in 2002. Dr Burgess' interests range through all aspects of biotechnology for wastewater, solid waste and air pollution bioremediation.

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He was born in 1968 and obtained his BSc (1989), BSc Hons (Biochemistry) (1990), MSc (Biochemistry) (1992) and PhD (Biochemistry) (1996) at the University of Port Elizabeth, South Africa. During his under- and postgraduate training he was awarded several NRF, MRC and UPE bursaries, as well as a Van Der Biji Scholarship (ESKOM). During his postgraduate training he was also appointed as a Supplementary Instruction Leader (Feb to Nov 1993) and as a Lecturer (contract) in the Department of Biochemistry and Microbiology (July 1995 to Sept 1996). He worked at The Ludwig Maximilians University in the Surgical Clinic and Policlinic, Munich, Germany during October 1996, and was then appointed as a Postdoctoral Fellow/Chief Scientific Officer in the Departments of Chemical Pathology and Biochemistry at The University of Cape Town, South Africa from June 1997 to Dec 1999. In January 2000, he was appointed to the post of Lecturer in Biochemistry at the Department of Biochemistry, Microbiology and Biotechnology at Rhodes University, Grahamstown, South Africa. His research interests are in the field of Environmental Enzymology and Bioremediation. He is currently actively involved in conducting research on enzymes involved in the anaerobic digestion of wastewater sludges, the teaching of undergraduate students and supervision of post graduate students.