

Chapter 19

Summary and Outlook

We have arrived at the end of our story. It is a highly provisional end; but then, the picture only started to unfold itself three decades ago. Until then, only two fundamental facts were known about the mechanism of photosynthesis: (1) that it consists of a photochemical stage and an enzymatic "dark" stage, with the latter imposing a "ceiling"—a maximum rate at which the overall reaction can proceed in strong light (F. F. Blackman, 1905); and (2) that the dark reaction requires about 10^{-2} sec for its completion (at 20°C), and has a maximum yield (as measured after a strong, "saturating," light flash) corresponding to about one molecule of O_2 produced by a single flash per 2500 molecules of chlorophyll present. These observations (Robert Emerson and W. Arnold, 1932) were interpreted (Hans Gaffron and K. Wohl, 1936) as indicating the existence of "photosynthetic units" of $2500/n$ chlorophyll molecules ($n =$ a small number—perhaps 4 or 8), containing a single enzymatic "reaction center."

The first spurt in the recent progress of understanding of the mechanism of photosynthesis followed the discovery of the long-lived radioactive carbon isotope, ^{14}C , by Sam Ruben and Martin Kamen in 1940. In the hands of Melvin Calvin and Andrew Benson, ^{14}C became a master tool for unraveling the complex enzymatic mechanism of carbon dioxide transformation into carbohydrate. This development still goes on, but the two main facts—the pivotal roles of PGA (phosphoglyceric acid)

and RDP (ribulose diphosphate), were established already in 1949 and 1952, respectively.

Another flood of new knowledge has begun to spread concerning what is, perhaps, the most exciting aspect of photosynthesis—its primary photophysical and photochemical process. From the empirical picture of a “photosynthetic unit,” there developed a theoretical concept of *excitation energy migration*—either among identical molecules, by a “first order” (or “exciton”) resonance mechanism, or between different molecules, by a “second order” (“Förster”) resonance mechanism. This migration makes it possible for quanta absorbed by any pigment molecule within a unit to initiate a sequence of enzymatic reactions in a single common “reaction center.”

Photosynthesis now appears as a tripartite reaction. One part of it is the *evolution of oxygen from water*, leaving behind an unknown reduced compound ZH (Fig. 5.4). The second is the “*uphill*” transfer of *H-atoms* (or electrons) from ZH to the—also not definitely known—primary hydrogen acceptor, X. The third part is *reduction of CO₂* by XH, leading to hexose and starch. (This third part is the one elucidated by the carbon tracer experiments.)

The light quanta captured by the pigments are used to perform the second, energy-storing step. In other words, light energy is utilized in photosynthesis for an *oxidation-reduction reaction against the gradient of chemical potential*.

We now have several reasons to believe that the photochemical, energy-storing stage in photosynthesis itself consists of *two separate photochemical steps*, I and II, connected by an enzymatic reaction sequence; the two are sensitized by two separate pigment systems, “PSI” and “PSII.”

Most convincing in this connection are two sets of observations. The first is Robert Emerson’s measurements (with Charlton Lewis) of the *quantum requirement of photosynthesis* (1938–42); they suggested that a minimum of *eight* quanta is needed to transfer *four* hydrogen atoms (or electrons) from H₂O to CO₂, reducing the latter to the carbohydrate level, and liberating one molecule of oxygen. His observation, in 1943, of the *red drop* of this efficiency at the long-wave end of the visible spectrum, and discovery (with R. Chalmers and C. Cederstrand in 1957) that the rate of oxygen evolution in the region of the “red drop” is enhanced when illumination with far-red light is supplemented by a

beam of higher frequency light, led to the conclusion that two consecutive four-quanta processes (two consecutive uphill transfers of four electrons or H-atoms), are involved in photosynthesis, and that they are brought about by two "pigment systems," containing a slightly different assortment of pigments (Fig. 13.9). The two systems must operate at the same rate for photosynthesis to run with maximum efficiency; the "red drop" occurs when absorption in one pigment system (PSII) becomes much weaker than that in the other (PSI). It disappears when additional illumination improves the absorption in PSII (*enhancement effect*).

A second argument for the two-step concept of the primary photochemical process was derived by Robert Hill and F. Bendall in 1960 from consideration of the oxidation-reduction potentials of the cytochromes (Fig. 14.4). The finding (by L. N. M. Duysens, J. Ames, and B. M. Kamp in 1961) that light absorbed by one pigment system (PSII—specifically, by the phycobilins in red algae) causes the reduction, and light absorbed by another pigment system (PSI—specifically, by chlorophyll *a* in red algae) causes the oxidation of certain cytochromes provided one of the best pieces of evidence for two light reactions in photosynthesis. (Cytochromes, long known to play a key role in respiration, were found in 1952, by R. Hill and co-workers, to be present in all photosynthesizing cells.) Hill and Bendall suggested that one pigment system (PSII) sensitizes the oxidation of water (normal redox potential, +0.8 volt) by a reduced cytochrome (normal potential, about 0.0 volt), and the other (PSI), the oxidation of a reduced cytochrome (with a normal potential of about +0.4 volt) by an organic oxidant with a normal potential of about -0.4 volt, such as oxidized pyridine nucleotide (and indirectly, by carbon dioxide).

A great amount of work was devoted recently to the enzymatic reaction sequence connecting the two photochemical processes—a "downhill" electron transfer from $E_0' = 0.0$ volt to $E_0' = +0.4$ volt. Several components apparently involved in this reaction sequence have been identified. Among these are plastoquinone and plastocyanin, in addition to two cytochromes, cytochrome *f* and (probably) cytochrome b_3 ; and an as yet mysterious "compound Q." Difference spectroscopy has led to tentative identification of the "energy traps" (reaction centers) postulated in the two pigment systems (P700 in PSI identified by Bessel Kok in 1956, and P690 in PSII, identified in H. T. Witt's laboratory in 1967).

From electron microscopy, the conclusion was drawn that photosynthetic units probably are real structural elements of the chloroplasts (rather than, what was a priori a possibility, merely statistical ratios between the numbers of pigment molecules and those of enzymatic reaction centers in them). They are now thought to consist of about 200–300 chlorophyll molecules. Each unit includes accessory pigments (such as chlorophyll *b*, carotenoids, or phycobilins), several varieties of chlorophyll *a*, and a single reaction center (“energy trap”). Light energy absorbed by a pigment molecule anywhere in the photosynthetic unit is transferred (by the above-mentioned resonance mechanisms) to this trap, where it can be utilized for the energy-storing primary photochemical reaction. Units of one type (PSII), which bring about the oxidation of ZH and reduction of a cytochrome, contain most of the accessory pigments (including chlorophyll *b* in green plants, and phycobilins in red and blue-green algae), slightly more of a “short-wave form” of chlorophyll *a* (Chl *a* 670), and slightly less of “long-wave” form, Chl *a* 680 than units of type PSI, and a “trap,” P690; while units of the second type (PSI), which bring about the oxidation of a cytochrome and the reduction of X, contain much less accessory pigments, somewhat less Chl *a* 670, and somewhat more of the long-wave form of Chl *a* (Chl *a* 680). They also contain a Chl *a* form absorbing at still longer waves (Chl *a* 695), and a “trap,” P700.¹

In light reaction II, a strong oxidant (Z) is produced, that can oxidize H₂O to O₂, and an intermediate reductant (such as a reduced cytochrome *b*₃); in light reaction I, a strong reductant is produced that can reduce CO₂ to carbohydrate, and an intermediate oxidant (such as oxidized cytochrome *f*). Formation of high energy phosphate, ATP, is coupled to the reaction connecting light stages II and I—reaction between ferri-cytochrome *f* in PSI and the ferrocytochrome *b*₃ in PSII. This reaction seems to involve, in addition to the two cytochromes, several other intermediate catalysts. A back reaction in PSI also seems able to produce ATP; but this can occur only when photosynthesis runs at less than full maximum quantum efficiency, because it requires a part of the products of reaction I to be lost for the overall oxidation-reduction process.

Two products of the light reactions—high energy phosphate, ATP, and a strong reductant (such as NADPH)—act together to reduce CO₂

¹ In red and blue-green algae, Chl *a* 670 and Chl *a* 680, are largely replaced, in PSII, by phycobilins.

to the carbohydrate (CH_2O) level. In this reaction sequence, as elucidated by Calvin and co-workers, CO_2 is first added to a five-carbon sugar phosphate (ribulose diphosphate), thus producing two molecules of phosphoglyceric acid (PGA); these are then reduced to two molecules of a three-carbon sugar (triose) by NADPH (or another equally potent, or even more potent, photochemically generated reductant), with the help of the hydrolysis energy of ATP. Two molecules of the so-formed triose are transformed into one molecule of a six-carbon sugar (fructose or glucose). The CO_2 -“carrier,” ribulose diphosphate, needed for the next cycle, is regenerated from additional molecules of the synthesized triose, by another series of enzymatic reactions (Calvin-Benson cycle).

There is no certainty that the enzymatic mechanism of CO_2 -reduction to sugar, developed by Calvin and co-workers, is unique. Rather, as in the case of the reverse process, that of cellular respiration, several alternative enzymatic pathways may exist. One such mechanism, involving phosphoenolpyruvic acid as primary carbon dioxide absorber (instead of ribulose phosphate) was proposed recently (1966) by M. D. Hatch and C. R. Slack for corn, sugar cane, and some other higher plants. Such alternative pathways may account for occasional appearance of compounds not in the Calvin-Benson cycle, such as malic acid, among early heavy-labeled products in C^* -tracer experiments.

One may ask whether the suggested two-step mechanism of the primary photochemical process also allows variations. Undoubtedly, under certain conditions, only one of the two systems is operative, while the other idles, or is inhibited (or, in the case of exclusive excitation of one pigment system, is not involved at all). One or both stages may take part in bacterial photosynthesis, and in “artificial” variants of the natural process, such as the Hill reaction.

Even leaving all possible variants aside, the above-described tripartite mechanism of photosynthesis is not yet established beyond all challenge; only future experiments can decide which parts of it will survive and which will have to be modified or abandoned.² Nevertheless, compared to the total ignorance that had prevailed before 1940 (when the photosynthetic cell appeared as a magic black box, taking up CO_2 , H_2O ,

² Alternative formulations include schemes in which one light reaction makes ATP, and the other NADPH, and schemes in which both light reactions produce oxidants and reductants, but the two oxidants somehow cooperate to oxidize H_2O and the two reductants cooperate to reduce CO_2 .

and light and pouring out sugar and oxygen), the development of this mechanism represents an impressive advance achieved in the last quarter of a century.

Important questions that will have to be tackled in the future include the detailed physical mechanism of energy migration in the photosynthetic units, the physicochemical mechanism of the primary photochemical steps, and the details and alternatives of the enzymatic mechanism. The latter include, in particular, the coupling of ATP synthesis and hydrolysis with oxidation-reductions, the function of cytochromes and quinones in the intermediate enzymatic reaction sequence, and the—all but unknown—mechanism of oxygen evolution. The locations of the components of the two systems in the chloroplast structure (which we now know to be lamellar) remain to be established; so is the whole “topochemistry” of the three stages of the overall process. Promising in that connection are experiments by Boardman and Anderson (1966) suggesting the possibility of *mechanical separation* of PSI and PSII or at least, their substantial enrichment in certain fractions of chloroplast material.

The ultimate possibility of *in vitro photosynthesis* is beckoning. Ideally, this should take the form of combined storage of light energy and synthesis of organic matter from inorganic materials; more modestly, but still very importantly, one can aim at finding a way to store efficiently light energy as utilizable chemical energy in a relatively simple (inorganic or organic) chemical system.

The *evolutionary history* of photosynthesis remains a fascinating question. Has chemosynthesis preceded it on earth? Did bacterial photosynthesis, utilizing relatively unstable “energy-rich” hydrogen sources, such as H_2S or H_2 , in turn precede “true” photosynthesis (which makes use of the most abundant, but also the most reluctant hydrogen donor on earth, water)? This hypothesis fits the widely accepted Oparin-Haldane theory of the origin of life on earth, which postulates the early existence on earth of a reducing atmosphere (an atmosphere containing H_2 , H_2S , and hydrocarbons, but no O_2). Plant metabolism is supposed to have first enriched the air with oxygen. However, bacterial photosynthesis (and bacterial chemosynthesis), as they occur now, may well represent not the survival of early forms of life, but relatively recent products of adaptation of early photosynthetic organisms to a “lazy” way of life in energy-rich, reducing media.

Could life have developed on other cosmic bodies in a different way from that which it had followed on earth? We have every reason to assume that the same chemical elements are present throughout the universe. Can one imagine life based on elements other than carbon, oxygen, and hydrogen? Could, for example, silicon substitute for carbon, to make biological materials viable at much higher temperatures outside the "biological range" of temperatures on earth? Could ammonia function, at low temperatures, as a substitute of water? Such speculations are tempting, but unlikely. The capacity of silicon to imitate carbon in the formation of complex molecules is quite limited, even if in recent years, the chemistry of silicones has become an important branch of industrial chemistry.

If life *must* be based on the elements C, H, and O, it can only exist between 0 and 100°C—that means, only on moderately warm cosmic bodies. Also, it can only develop on planets not much lighter than the earth, since otherwise the originally available hydrogen would have dissipated in space soon after the birth of the planet.

Altogether, there are many fascinating problems left related to, or arising from, the chemical and physical mechanisms of photosynthesis, its evolution, its control, and its imitation outside the living cell.