Part I

RESEARCH IN PHOTOSYNTHESIS: BASIC DEVELOPMENTS TO ABOUT 1960

Part I describes major developments in our understanding of photosynthesis during the past three centuries, especially from about 1930 to 1960. We shall trace the evolution of our views of two basic aspects of the subject: the primary photochemistry and the basic chemical patterns that surround this central process, and the roles of the pigments that absorb light energy and initiate the photochemistry.

The membranous structures that support photosynthesis in plants and in photosynthetic bacteria are described in Part II. It is satisfying to be able to visualize these structures while studying the physical and chemical processes that they mediate. To this end the reader is invited to examine the pictorial introduction to Part II while progressing through Part I.

The last chapter of Part I is an extended digression into molecular spectroscopy and optics applied to biology, to serve as preparation for the more detailed subsequent material.

1 The chemical nature of photosynthesis

1.1 Early history

An approximate overall equation for photosynthesis in green plants is

$$6CO_2 + 6H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} C_6H_{12}O_6 + 6O_2 \qquad (1.1)$$

where $C_6H_{12}O_6$ represents a simple sugar. Let us see how the components of this reaction became recognized during the past three centuries.

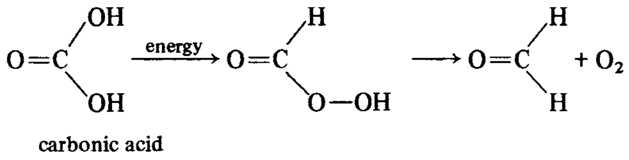
About 1650 van Helmont grew a willow tree, starting with a 5-lb tree in 200 lb of sandy soil. Some 5 years later the tree weighed 570 lb, and the soil weighed 199 lb. Well before the enunciation of the law of conservation of matter (by Lomonosov in 1748 and Lavoisier in 1760), van Helmont guessed that most of the weight of the tree must have come from the water that had been added to the soil. He knew nothing of the role of carbon dioxide.

A century later Bonnet recorded that leaves submerged in water developed gas bubbles when placed in the sun. He was not likely to have been the first to see this, and he had no idea that a chemical process was involved. But the next three decades brought a remarkable growth in our knowledge of chemistry, and especially in the study of gases. In 1771 Joseph Priestley showed that a mouse could not live in a container with air that had been "burned out" by a candle flame, but that a spray of mint, perhaps chosen because of its fresh smell, restored the air so that after a few days a candle could burn again, or a mouse could live for a time. Thus Priestley showed that a plant could produce oxygen, or, in the language of his time, could dephlogisticate the air.

It remained for Ingenhousz to show, in 1779, that plants need their green parts and light in order to freshen the air, and that at night they spoil the air, just as a mouse or candle does (by respiration; formally the reverse, in the dark, of Reaction 1.1). Then in 1782 Senebier pointed out that plants need "fixed air," or carbon dioxide, to dephlogisticate (oxygenate) the air. Ingenhousz suggested in turn, in 1796, that this carbon dioxide was the source of all the organic matter in a plant. And finally in 1804 de Saussure, aware of the law of conservation of matter, confirmed van Helmont's guess that most of the weight of a plant comes from water (and from CO_2).

By that time all the ingredients of Reaction 1.1 had become implicated in the life of plants. It remained only for Robert Mayer, who in parallel with Joule had developed the law of conservation of energy, to point out in 1845 that the energy taken up as sunlight was stored, in part, as chemical energy in the organic matter, represented in Reaction 1.1 as sugar. And it remained for organic chemists in the early twentieth century to characterize the essential green pigment, chlorophyll.

These chemists, notably R. Willstätter and A. Stoll, naturally tried to imagine how chlorophyll could mediate the process of photosynthesis. Guided only by notions of chemical plausibility, Willstätter and Stoll suggested that the reactants CO_2 and H_2O, which together form carbonic acid, are somehow bound to chlorophyll and rearranged when the chlorophyll absorbs light and goes into a state of greater energy. The rearrangement could create an unstable organoperoxide which would release oxygen:



(1.2)

In this view the first stable organic product of photosynthesis would be formaldehyde! All efforts to detect even a trace of formaldehyde in illuminated plant tissues failed. More significantly, this line of speculation suggested no new experiments or approaches to the problem. Then in the 1920s the microbiologist C. B. van Niel began to formulate a new, fruitful, and essentially correct view of the photochemical process of photosynthesis.

1.2 Photosynthesis as an oxidation-reduction process

C. B. van Niel was schooled in the study of comparative biochemical patterns among microbes. When he began to study photosynthesis in the 1920s, it was known that many types of bacteria can grow at the expense of light energy. The photosynthesis of these bacteria differs in several ways from that of green plants and algae:

1. The essential pigments, bacteriochlorophylls, are analogous to but chemically distinct from the chlorophylls of green plants.
2. No oxygen is evolved in bacterial photosynthesis.
3. In order for photosynthetic bacteria to assimilate carbon dioxide, they must be provided with a reducing substance. Among different

species of photosynthetic bacteria the list of such substances can include H_2, H_2S, and a variety of simple organic compounds such as alcohols and fatty acids. Generically this "oxidizable substrate" can be called H_2A. If it is an organic compound, it may serve both as a source of carbon (in place of CO_2) and as a reducing substance.

Van Niel noted that for many examples of bacterial photosynthesis the overall reaction could be approximated by

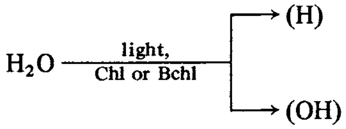
$$CO_2 + 2H_2A \xrightarrow[\text{bacteriochlorophyll}]{\text{light,}} (CH_2O) + H_2O + 2A \qquad (1.3)$$

where (CH_2O) represents stored organic matter. If the storage product is carbohydrate, (CH_2O) could denote one-sixth of a molecule of sugar. For green plants and algae the overall reaction (Reaction 1.1) could be rewritten, dividing by 6 and adding one H_2O to each side:

$$CO_2 + 2H_2O \xrightarrow[\text{chlorophyll}]{\text{light,}} (CH_2O) + H_2O + O_2 \qquad (1.4)$$

The similarity between Reactions 1.4 and 1.3 suggested to van Niel that green-plant photosynthesis is a special case in which water serves as the oxidizable substrate, and O_2 is the oxidation product. This gives green plants and algae a great advantage over the photosynthetic bacteria: Water is a ubiquitous and plentiful substrate.

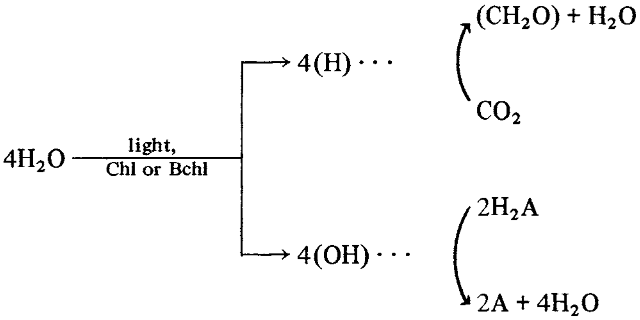
Comparing these equations, van Niel proposed that photosynthesis should be seen as a coordinated pair of oxidizing and reducing processes: H_2A is oxidized to A, and CO_2 is reduced to (CH_2O). From these insights he developed a simple and elegant view of the photochemical part of photosynthesis: The light reaction, mediated by chlorophyll or bacteriochlorophyll (henceforth abbreviated Chl and Bchl), generates a pair of "primary" oxidizing and reducing entities. These in turn bring about the coordinated oxidation of H_2A and reduction of CO_2, respectively. Lacking specific knowledge, van Niel wrote the light reaction as a splitting of water into an oxidizing fragment denoted (OH) and a reducing fragment denoted (H):



$$H_2O \xrightarrow[\text{Chl or Bchl}]{\text{light,}} \begin{cases} \to (H) \\ \to (OH) \end{cases} \qquad (1.5)$$

This was admittedly a mere formalism. There was no evidence that H_2O enters into the light reaction, and the primary oxidizing and reducing equivalents "(OH)" and "(H)" might just as well have been symbolized "+" and "−" or "X^+" and "Y^-." We shall retain van Niel's notation for the present, and come to grips later with the actual identities of the primary products.[1]

Reaction 1.5 could then be elaborated as follows:



(1.6)

Four reducing equivalents are needed to convert one CO_2 to (CH_2O), and four oxidizing equivalents to convert $2H_2A$ to 2A (or $2H_2O$ to O_2 in the case of green plants and algae). This view of photosynthesis had several implications:

1. The O_2 evolved by green plants comes entirely from H_2O and not from CO_2.
2. Neither the assimilation of CO_2 nor the evolution of O_2 (more generally, the oxidation of the substrate H_2A) is a part of the primary photochemistry.
3. The function of the substrate is to react with the primary oxidizing entity, so as to preserve an overall oxidation–reduction balance.
4. The photochemical act, which distinguishes photosynthesis from other ways of life, is a separation of oxidizing and reducing entities. These primary photoproducts carry some of the light energy into further chemical reactions, and coordinate the parallel processes of CO_2 reduction and H_2A oxidation.

We shall trace van Niel's formulation through a number of revisions. But his view of the photochemistry as a separation of oxidant and reductant, driven by light and mediated by Chl or Bchl, remains as a foundation of our current descriptions of photosynthesis.

1.3 Digression: qualitative aspects of oxidation and reduction

An understanding of oxidation and reduction is crucial to an understanding of photosynthesis. Oxidation and reduction are defined as the loss or gain of electrons, respectively:

$$X \xrightleftharpoons[\text{reduction of } X^+]{\text{oxidation of } X} X^+ + e^- \qquad (1.7)$$

$$Y^- \xrightleftharpoons[\text{reduction of } Y]{\text{oxidation of } Y^-} Y + e^- \qquad (1.8)$$

$$\mathrm{X} + \mathrm{Y} \underset{\substack{\text{X}^+\text{ oxidizes Y}^-\text{,} \\ \text{Y}^-\text{ reduces X}^+}}{\overset{\substack{\text{X reduces Y,} \\ \text{Y oxidizes X}}}{\rightleftharpoons}} \mathrm{X}^+ + \mathrm{Y}^- \tag{1.9}$$

An atom or molecule with high affinity for additional electrons acts as an oxidant; one that tends to release electrons acts as a reductant. These descriptions are of course relative to the surroundings of the atom or molecule. Thus, in Reaction 1.9 if the reaction proceeds spontaneously to the right, Y is a stronger oxidant than X; Y can take electrons away from X. Conversely if the state "$\mathrm{X} + \mathrm{Y}$" is more stable than the state "$\mathrm{X}^+ + \mathrm{Y}^-$," the reaction will go spontaneously to the left, and energy would be needed to drive it to the right. If photosynthesis begins with a reaction of the general form $\mathrm{X} + \mathrm{Y} \xrightarrow{\text{light, Chl}} \mathrm{X}^+ + \mathrm{Y}^-$ (a variation of van Niel's symbolism), one can imagine that part of the energy of light is used in driving the reaction, and becomes stored because "$\mathrm{X}^+ + \mathrm{Y}^-$" has more energy than "$\mathrm{X} + \mathrm{Y}$." We shall consider the energetics of oxidation and reduction at a quantitative level in Section 4.3.

In an aqueous environment, with H^+ ions (protons) ever present, the entities involved in oxidation and reduction may bind or release protons; for example,

$$\mathrm{Y} + e^- \longrightarrow \mathrm{Y}^- \qquad \text{followed by} \qquad \mathrm{Y}^- + \mathrm{H}^+ \longrightarrow \mathrm{YH} \tag{1.10}$$

If YH is more stable than the dissociated form $\mathrm{Y}^- + \mathrm{H}^+$ at the prevailing concentration of H^+, the reduction step in Reactions 1.10 will be followed rapidly by the protonation step, giving an overall reaction

$$\mathrm{Y} + e^- + \mathrm{H}^+ \longrightarrow \mathrm{YH} \tag{1.11}$$

This behavior is shown by many organic molecules at neutral pH. Thus the gain of an electron, if accompanied by protonation, is effectively the gain of an H atom. In such cases oxidation and reduction can be described in terms of H transfer instead of electron transfer:

$$\mathrm{A} + \mathrm{BH} \xrightarrow[\text{oxidation of B}]{\text{reduction of A,}} \mathrm{AH} + \mathrm{B} \tag{1.12}$$

In this connection it is instructive to ask, for example, whether the conversion of two molecules of acetic acid to one of succinic acid,

$$2\mathrm{CH_3{\cdot}COOH} \longrightarrow \mathrm{HOOC{\cdot}CH_2{\cdot}CH_2{\cdot}COOH} \tag{1.13}$$

would represent a net oxidation or reduction, or neither. In this example the product has the same number of carbon and oxygen atoms as the reactants, but it has two H atoms fewer. The reaction involves a net oxidation, the removal of two H atoms (two electron equivalents). For a balanced reaction, 2H should be appended to the right side of Reaction 1.13.

As a second example, consider the relative levels of reduction of the two molecules $CH_2OH \cdot CHOH \cdot CH_2OH$ (glycerol) and $CH_3 \cdot CH_2 \cdot CH_2OH$ (propanol). The addition or removal of water from a molecule can be taken as neutral with respect to oxidation or reduction; so we can subtract H_2O as we like from these formulas, bringing them down to "skeleton" formulas of $CH \cdot C \cdot CH$ for glycerol and $CH_3 \cdot CH_2 \cdot CH$ for *n*-propanol. We can then see that *n*-propanol is four H atoms (or four electron equivalents) "more reduced" than glycerol. Another help in evaluating skeleton formulas is to say that the removal of an oxygen atom is equivalent to the insertion of two H atoms, because the same thing could be done by inserting two H atoms and then removing H_2O. The presence of an O atom thus counts for two oxidizing equivalents. Then by counting the number of O atoms (two oxidizing equivalents each) and H atoms (one reducing equivalent each), scaled to equal numbers of C atoms, one can rank the various metabolic products of photosynthesis as to their degree of reduction. This approach is useful in understanding the relationships between external nutrients and the products of their metabolic assimilation, by requiring an overall oxidation–reduction balance.

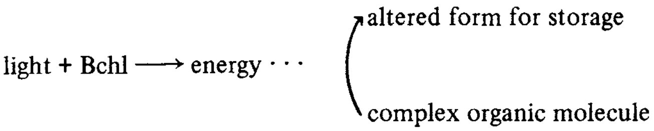
1.4 Modifications of van Niel's representation of photosynthesis

Some of the implications of van Niel's formulation (see the text following Reaction 1.6) received experimental scrutiny during the 1930s and 1940s. The idea that the O_2 evolved by green plants and algae comes not from CO_2 but from H_2O, as suggested by Reaction 1.4, could in principle be tested by means of the stable isotope ^{18}O. One could suspend algae in ^{18}O-labeled water with unlabeled CO_2, or vice versa, and examine the isotopic composition of the evolved O_2. Unfortunately this experiment can give ambiguous or misleading results for a variety of reasons. Oxygen can be exchanged between CO_2 and H_2O through the repeated formation and dissociation of carbonic acid, H_2CO_3. Exchange is generated by respiration (concurrent with photosynthesis), which makes new CO_2 out of O_2 and previously stored carbon compounds. There can be internal pools of O_2, CO_2, and H_2O that do not mix rapidly with the external medium. The chemistry of O_2 evolution, even if written simply as $2H_2O \longrightarrow O_2 + 4H^+ + 4e^-$, might involve CO_2 or bicarbonate in some secondary way. The first reports of this kind of experiment seemed to support van Niel's view, but with more repetitions and variations the results became ever more inconclusive, and there the matter rests.

Meanwhile two other implications of van Niel's model did receive clear support. Both the assimilation of CO_2 and the evolution of O_2 could be separated experimentally from the photochemical process in green plants and algae. R. Hill was making experiments with suspensions of chloroplasts, the green subcellular bodies that could be released from leaves by disrupting the tissue. He hoped to demonstrate complete photosynthesis in these suspensions, and he enjoyed a partial success. The chloroplasts did not convert CO_2 to sugar, but they did show a light-dependent evolution of O_2 if a suitable oxidizing agent had been added. The list of satisfactory "Hill oxidants" included Fe^{3+}, $Fe(CN)_6{}^{3-}$ (ferricyanide), and benzoquinone. These substances appeared to act as substitutes for CO_2, becoming reduced as a consequence of the light reaction. This *Hill reaction* (or chloroplast reaction, as Hill preferred to call it) seemed to show that the assimilation of CO_2 could be separated from the rest of photosynthesis. The evolution of O_2 could be coupled to the reduction of chemicals other than CO_2.

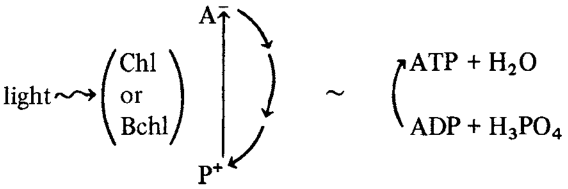
Just as CO_2 could be replaced by other reducible substances on the "reducing side" of van Niel's picture, so could the "oxidizing side" be modified, with the oxidation of hydrogen gas replacing the conversion of H_2O to O_2. This was shown by H. Gaffron in studies with certain green algae. If the algae were incubated in the dark in the absence of O_2, they acquired the ability to behave like photosynthetic bacteria. Using H_2 as the substrate "H_2A," they could assimilate CO_2 in the light without evolving O_2. This adaptation became lost in the light; the algae soon reverted to their normal O_2-evolving photosynthesis during illumination. But the potentiality had been shown: In these algae the evolution of O_2 could be separated from the rest of photosynthesis. The oxidizing product of the light reaction, "(OH)" in van Niel's early symbolism, could react with H_2O (giving O_2) *or* with H_2. The reducing product could drive the reduction of CO_2 *or* of a variety of other reducible substances, the so-called Hill reagents.

Ironically it was Gaffron who launched the strongest attack on van Niel's scheme in the 1930s and 1940s. Gaffron noticed that in some cases of bacterial photosynthesis the assimilation of carbon involved relatively little overall chemical change. Complex organic nutrients were assimilated, in a light-dependent reaction, with only minor alterations to convert them to storage products. If these cases were to be forced into van Niel's mold, one would imagine that the nutrient, acting as the substrate H_2A, is oxidized, perhaps even to the level of CO_2. These oxidation products are converted to storage products, drawing on the reducing entity formed by the light reaction. Gaffron argued that such a pattern is needlessly complicated, and suggested a simpler scheme of the general form



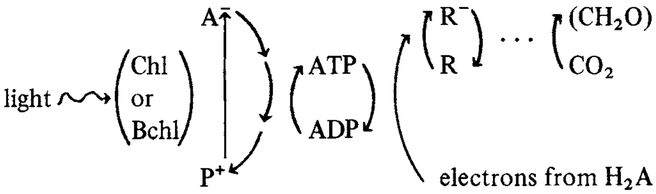
$$\text{light} + \text{Bchl} \longrightarrow \text{energy} \cdots \left(\begin{array}{l} \nearrow \text{altered form for storage} \\ \\ \searrow \text{complex organic molecule} \end{array}\right. \tag{1.14}$$

This was before the word "energy" in Reaction 1.14 had been identified with adenosine triphosphate, ATP. When the chemical patterns of ATP formation began to emerge, in the hands of F. Lippman, O. Meyerhof, and others, it became possible to bring van Niel's picture of photosynthesis into harmony with that proposed by Gaffron. Initially van Niel held that any reaction between the primary photochemical products, such as a recombination of (H) and (OH) to regenerate H_2O, would be wasteful. The function of H_2A was to prevent such a reaction. But in the light of new knowledge one could visualize such a "back-reaction" as a means of transmuting and storing chemical energy. One could imagine that the primary reducing and oxidizing entities interact through a controlled sequence, with reducing equivalents (electrons or H atoms) cycling through a specific series of carriers from primary reductant to primary oxidant. The energy released in this exergonic process is captured, in part, through a coupled conversion of ADP to ATP (the mechanism of this energy coupling will be discussed in Chapter 10). Representing the photochemistry as the transfer of an electron from a donor P to an acceptor A, producing A^- and P^+ rather than van Niel's original (H) and (OH), one can write



(1.15)

The energy of ATP can then be used for all the needs of the living cell, including the metabolic conversions implied in Reaction 1.14. Among other things, the energy of ATP can drive electrons from a weaker reductant to a stronger one ("reductive dephosphorylation"). Then even the coupled oxidation of "H_2A" and reduction of CO_2 can be driven by the energy of ATP:

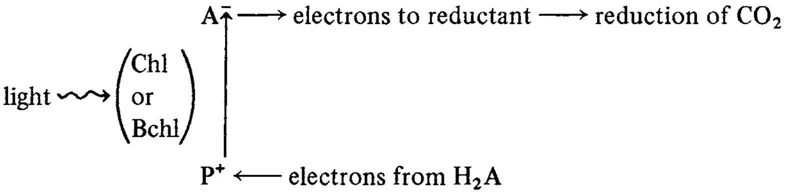


(1.16)

In this scheme R^- is a strong reductant that can mediate CO_2 reduction.

This primitive representation will be amended and amplified as we proceed. For the present we should note that the overall chemical result is exactly the same as that of van Niel's original scheme. Light generates a separation of primary oxidant and reductant, H_2A is oxidized, and CO_2 is reduced. There is the added flexibility that ATP can be used for purposes other than the transfer of electrons from H_2A to R. For example, the scheme of Reaction 1.16 can embrace the less specific suggestion of Gaffron shown by Reaction 1.14. Reaction 1.16 also puts the role of H_2A in a new light. This substrate is not needed in the sense originally visualized by van Niel, to get rid of the primary oxidant and prevent a recombination between primary oxidant and reductant. Instead, H_2A can be drawn upon only as needed, to introduce reducing equivalents and maintain as overall redox balance between nutrients and storage products. Maximum metabolic flexibility is allowed if we accept both possibilities, the cyclic pattern of Reaction 1.16 and the noncyclic pattern of van Niel's initial formulation.

Noncyclic:



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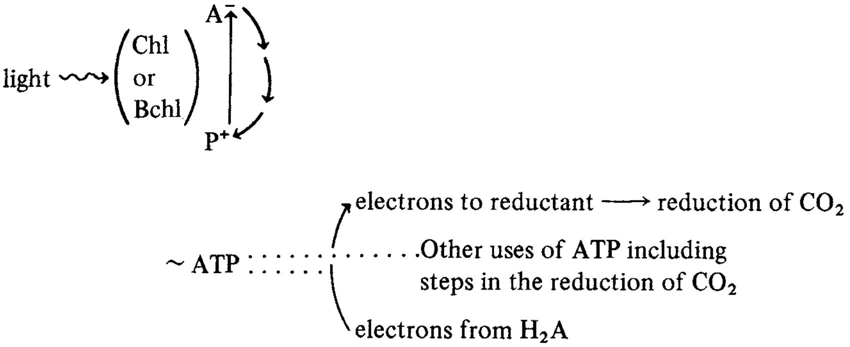
Cyclic:



(1.18)

We shall see that both patterns have their place, and that the formation and the utilization of ATP are coupled to noncyclic patterns of electron flow as well as to cyclic patterns.

Gaffron's studies of the assimilation of organic nutrients by photosynthetic bacteria, which led to the viewpoint expressed by Reaction 1.14, were given more concrete form by Doudoroff, Stanier, and their collaborators about 1960. They found that organic compounds are stored in photosynthetic bacteria in two major forms, the counterparts of starch in green plants: a polymer of β-hydroxybutyric acid, $(\text{—}CH_2\cdot CHOH\cdot CH_2\cdot CO\text{—})_n$, and the carbohydrate glycogen, $(C_6H_{10}O_5)_n$. If we subtract H_2O from these formulas until no oxygen atoms remain, and scale each of the resulting "skeleton formulas"