

## Chapter 18

# Photophosphorylation

### HIGH ENERGY PHOSPHATE

High energy phosphate is a "portable battery charger," an almost omnipresent depository for storage and withdrawal of measured amounts of energy needed for various life processes. It was early speculated that in photosynthesis, too, some light energy may be stored in this form. Some even suggested that formation of high energy phosphate may be the main, if not the only, mechanism of energy storage in photosynthesis. However, in this form the hypothesis is implausible. The high yield of energy utilization of photosynthesis seems incompatible with a mechanism in which the energy quanta provided by light (about 40 Kcal/einstein each), are first broken into "phosphate quanta" (about 8 Kcal/mole), to be reassembled later as "oxidation-reduction quanta" (about 120 Kcal/mole). However, high energy phosphate may well play an accessory role in photosynthesis. In reaction scheme 14.4, over 90% of the energy of photosynthesis is stored in the two "uphill" oxidation-reductions; but some ATP is formed as a side product in the intermediate enzymatic reaction chain, and used later to make certain endergonic follow-up reactions possible.

What is "high energy phosphate"? It is an ester of phosphoric acid that liberates 7-8 Kcal per mole upon hydrolysis, whereas the hydrolysis of "ordinary" or "low energy" phosphate esters is slightly endothermic.

Hydrolysis is the breaking of a chemical bond by water, with the

water radicals (H) and (OH) attaching themselves to the open ends of the broken bond; examples are the hydrolysis of a C—C bond:

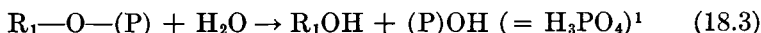


where  $R_1$  and  $R_2$  are two organic radicals; and hydrolysis of a C—O bond, such as

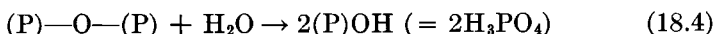


Hydrolyses do not change the reduction level (Chapter 5) of the hydrolyzed compounds, and, therefore, have only relatively small values of  $\Delta H$ .

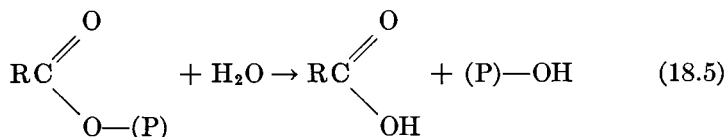
Hydrolysis of a phosphate ester is similar to reaction 18.2, except that one (or both) organic radicals,  $R_1$  and  $R_2$ , are replaced by univalent phosphate radicals, (P) =  $H_2PO_3$ , for example:



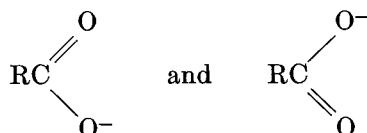
or



The “high energy phosphates” differ from other phosphate esters because the products of their hydrolysis are stabilized by a kind of *resonance* that did not exist before hydrolysis. For example, in the case of a carboxylic acid, the hydrolysis of its phosphate is given by:



This reaction leads to a carboxyl group, which is stabilized by resonance. This is easiest to illustrate on the example of its anion, which can exist in two “resonating” forms with the same energy content:



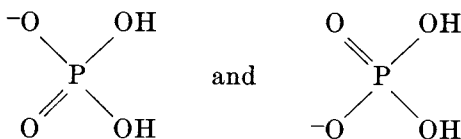
differing only by distribution of electrons. It is a general rule that the existence of such “resonating” structures contributes to the stability of a molecule. Resonance stabilization of the carboxyl group makes carboxyl

<sup>1</sup> At other places in this book  $P_i$  is used, instead of  $P(OH)$ , for  $H_3PO_4$ .

phosphates "high energy phosphates," compared to (for example) alcohol phosphates,  $RCH_2O(P)$ , because free alcohol formed by its hydrolysis is *not* stabilized by resonance:

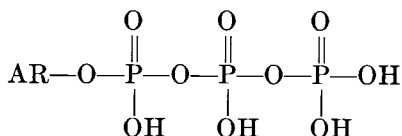


Phosphate esters of the type (18.4) also are "high energy" phosphates, because free phosphoric acid is stabilized by a resonance similar to that present in carboxylic acids. For example, in the case of a univalent phosphate anion,  $H_2PO_4^-$ , the resonance is between the forms

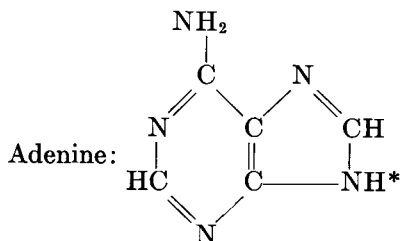


which differ only by electron distribution.

In metabolic processes, the most common high energy phosphate is *adenosine triphosphate* (ATP):



where AR is an organic base called adenosine, consisting of a nitrogenous organic base, adenine, A (see below), to which a pentose sugar (ribose, R) is attached in the position marked by asterisk.



Why the principal biological energy carrier has to be attached to a specific organic base, why it includes a pentose sugar, and why it contains three phosphate radicals in a row, we don't know; the "high energy" character is not dependent on any of these characteristics. They may be important, however, from the point of view of enzymatic specificity,

permitting convenient coupling of the conversion of ATP to ADP + (P)OH with an oxidation-reduction.

In mitochondria, a series of oxidation-reduction catalysts (including several cytochromes) are arranged in such a way that the transfer of an electron from one of them to the next one can be coupled with the formation of an ATP molecule from ADP and (P)OH. In this way, about one half of the free energy liberated in a whole series of oxidation-reductions is stored in ATP molecules. The latter diffuse away, to be hydrolyzed into ADP + (P)OH when and where energy is needed.

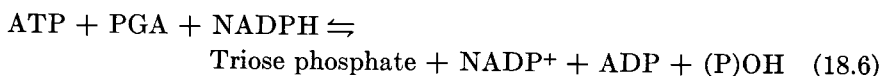
Recently, a theory became popular according to which the coupling of a redox reaction with ATP-synthesis occurs by the redox reaction establishing an H<sup>+</sup>-ion concentration difference between two sides of a membrane; and the ATP formation occurring at the cost of the free energy of this gradient. (This is the so-called "chemiosmotic" theory of phosphorylation, proposed by the British chemist, P. Mitchell in 1961.) Experiments by André T. Jagendorf (then at Johns Hopkins University in Baltimore) showed that some ATP can, in fact, be formed by such a mechanism at the cost of an artificially established H<sup>+</sup> gradient. It remains, however, to be shown whether this mechanism of ATP formation can be as efficient as the ATP-synthesis actually is in respiration and in photosynthesis.

Earlier in the reaction sequence of respiration (before the cytochromes come into play) oxidation of phosphoglyceraldehyde (= triose phosphate), by nicotinamide adenine dinucleotide, to phosphoglyceric acid, PGA, also is coupled with conversion of ADP to ATP. The free energy available in the oxidation of the couple RCOOH/RCHO (with a potential of about -0.5 volt) by the couple NAD<sup>+</sup>/NADH (with a potential of about -0.35 volt) is neatly stored in this coupled reaction. (The free energy available is  $0.5 - 0.35 \text{ eV} = 0.15 \text{ eV} = 3.5 \text{ Kcal per electron}$ , or 7.0 Kcal for the two electrons involved in oxidation of an aldehyde to an acid—which is just enough to synthesize one molecule of ATP.)

## ATP-NEED IN PHOTOSYNTHESIS

The now widely accepted scheme of photosynthesis (Chapter 17) suggests that NADPH (reduced nicotinamide dinucleotide phosphate) and

ATP (adenosine triphosphate), supplied by the photochemical primary processes, produce a reversal of the reaction described at the end of the preceding section, that is, *reduce* phosphoglyceric acid (PGA) to phosphoglyceraldehyde (triose phosphate) and hydrolyze ATP to ADP and (P)OH. The "reducing energy" of NADPH and the hydrolysis energy of ATP together make the reaction 18.6 run in the desired direction.

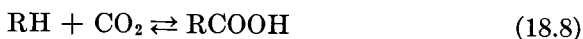


In the presence of the two enzymes that catalyze this reaction (triose dehydrogenase and kinase), reaction 18.6 will run from left to right, or from right to left, depending on relative concentrations of the reactants.

It was mentioned in Chapter 17 that another reaction, postulated in the Calvin cycle, also was found to require ATP—the introduction of a second phosphate group into ribulose monophosphate, to form ribulose diphosphate.

The Calvin cycle thus requires *three* ATP molecules to reduce *one* molecule of  $\text{CO}_2$ —one to form ribulose diphosphate, and thus to produce *two* molecules of PGA (see Eq. 17.4), and *two* to convert these two PGA molecules into two triose molecules. The Hatch—Slack pathway (see p. 239 in Chap. 17) requires an additional ATP.

Hydrogenation of *two* PGA molecules is needed because we must move *four* hydrogen atoms from  $\text{H}_2\text{O}$  to  $\text{CO}_2$  to produce one  $\text{O}_2$  molecule. Theoretically, this could be done in two steps—first, reducing a molecule of an acid,  $\text{RCOOH}$ , to an aldehyde,  $\text{RCHO}$ , and then reducing the latter to an alcohol,  $\text{RCH}_2\text{OH}$ . The second step requires considerably less energy than the first one ( $E_0'$  is  $-0.5$  volt for the first step, and only  $-0.3$  volt for the second one). However, according to Calvin's scheme, nature has chosen the hard way—that of reducing *two*  $\text{RCOOH}$  groups (in two PGA molecules). The excess energy stored in this way is regained, according to this scheme, in the dismutation associated with carboxylation, when ribulose diphosphate is converted to two PGA molecules (Eq. 17.4). In this way, the "waste" of light energy implied in the reduction of two carboxyls (instead of one carboxyl and one carbonyl), reveals itself as a clever trick. Ordinary carboxylations



are endergonic and cannot run from left to right without supply of outside energy. Reaction (18.8), runs spontaneously from right to left (except under very high partial pressure of carbon dioxide, not available in nature). But reaction 17.4 is exergonic because dismutation is involved. By storing extra energy in two reduced carboxyl groups, nature found a way to utilize light energy indirectly for the carboxylation step.<sup>2</sup>

It was mentioned before that the primary acceptor of electrons in light reaction I may be an unknown compound (X), rather than the known intermediates, NADP<sup>+</sup>, or ferredoxin. If PGA is reduced directly by XH (with an  $E_0'$ -value as low as  $-0.6$  eV), the need for ATP can be eliminated. This may reduce the amount of ATP needed for the Calvin cycle to the one molecule needed for the phosphorylation of RMP to RDP.

Chloroplasts do produce ATP in light; this observation is to be discussed below. Whether the ATP yield obtainable in this way is high enough to permit the Calvin cycle to run in its original version (requiring 3 ATP per 4 electrons), or is sufficient only for the modified cycle (requiring 1 or 2 ATP's), remains an open question.

## PHOTOSYNTHETIC PHOSPHORYLATION

Association of photo- and chemosynthesis with the conversion of adenosine diphosphate (ADP) and inorganic phosphate (P)OH into adenosine triphosphate (ATP) has been often suggested. Actual ATP formation from ADP and (P)OH was first observed in chemosynthetic bacteria: It was also noted in photosynthesizing cells by O. Kandler as uptake of (P)OH, and by B. Strehler (as ATP production revealed by firefly luminescence). Later (in 1954) it was found by D. Arnon, M. B. Allen, and F. R. Whatley (in Berkeley) in illuminated chloroplast prep-

<sup>2</sup> However, it may be that this is not at all the way it works. In some recent modifications, the Calvin cycle is supposed to involve the formation of only one PGA molecule, so that only one reaction reduces a carboxyl group in PGA, while the other may involve the reduction of a carbonyl; if both reductions use NADPH as reductant, ATP should be needed only in the "difficult" carboxyl reduction, and not in the "easy" carbonyl reduction; this would reduce the ATP requirement from 3 to 2. The above-suggested ingenious solution of the problem of "how to make carboxylation exergonic," will then have to be given up.

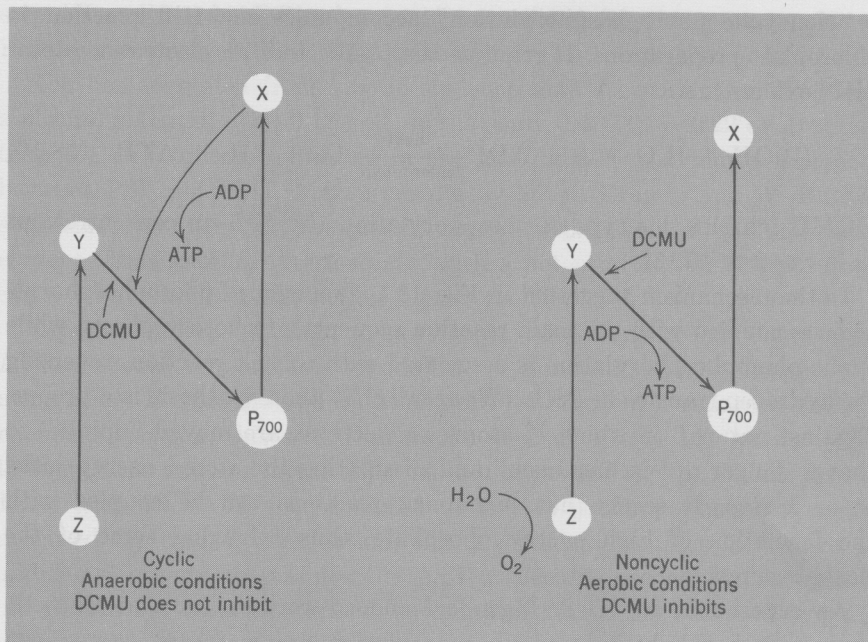
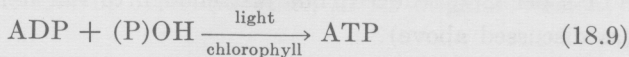


FIG. 18.1 Cyclic and noncyclic photophosphorylation. (Symbols have the same meaning as in Fig. 14.4; DCMU = 3-(3',4' dichlorophenyl)1,1 dimethyl urea.)

erations, and by A. Frenkel (in Minneapolis) in illuminated chromatophores from purple bacteria.

Arnon introduced a distinction between two kinds of photophosphorylation, which he called "cyclic" and "noncyclic." The first one is not coupled with on-going oxidation-reduction; the second one is (see Fig. 18.1).

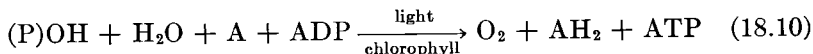
In chloroplasts, cyclic phosphorylation occurs most efficiently when the Hill reaction is prevented; in whole cells, in the absence of photosynthesis.<sup>3</sup> Under these conditions, ATP formation is the only net photochemical change:



Well-known poisons of photosynthesis (such as 3-(3',4' dichlorophenyl)1,1 dimethyl urea, DCMU), do not inhibit this kind of phosphorylation.

<sup>3</sup> Or when photosynthesis runs with less than the maximum yield. Cytochrome *b<sub>6</sub>* is an intermediate in this reaction.

“Nencyclic photophosphorylation” accompanies the Hill reaction in chloroplast preparations. It requires the presence of an electron acceptor (Hill oxidant), A.



DCMU inhibits this type of phosphorylation (by 50% in concentrations as low as  $2 \times 10^{-7}M$ ).

In the mechanism suggested in Fig. 18.1, “nencyclic” photophosphorylation is coupled with the main reaction sequence of photosynthesis, while cyclic photophosphorylation is associated with a back reaction, reversing the hydrogen transfer in PS I. (We recall that photosynthesis is a process “against nature,” in which H-atoms or electrons are moved “uphill,” so that a danger of “falling back” and dissipating the stored energy lurks at each step. It seems that such back reactions can be coupled with the formation of high energy phosphate, thus salvaging some of the wasted energy.)

An experiment by A. T. Jagendorf and co-workers (now at Cornell) suggested that light-induced phosphorylation occurs in two steps: one photochemical, the other dark. They preilluminated chloroplasts and transferred them quickly (within seconds) into another vessel (kept in darkness), containing ADP, (P)OH and  $Mg^{++}$ . The synthesis of ATP now took place. Light must have produced some long-lived high energy intermediate, capable of synthesizing ATP from ADP and P(OH) in darkness.

As shown in Fig. 18.1, at least one molecule of ATP can be formed as a “free premium” in the passage of two electrons through the main oxidation-reduction chain, without requiring a diversion of quanta. Since the main process uses 8 quanta to send four H-atoms from  $H_2O$  to  $CO_2$ , only two ATP molecules can be obtained in this way per  $CO_2$  reduced. This is not enough to make the Calvin cycle (or the Hatch and Slack pathway; see p. 239) run in its original version, requiring 3 (or 4) ATP's per  $CO_2$  reduced; but just enough to run it in the amended way (as discussed above).

If one postulates that ATP is formed in photosynthesis by the “chemi-osmotic” mechanism, utilizing the energy of several electron transfers (each liberating less energy than is needed to form one ATP), then the enzymatic redox reaction sequence that follows the photochemical



step  $[X \rightarrow \text{Fd} \rightarrow \text{NADP}]$  also could be utilized for this purpose, even if it liberates only 0.25 eV per electron.

Kok has suggested from kinetic evidence that the oxidant of PSII has a normal potential of +0.18 volt, so that only  $0.4 - 0.18 = 0.22$  eV (rather than 0.4 eV) become available in the intermediate enzymatic sequence between PSI and PSII. Measurements by W. A. Cramer and W. Butler suggested, however, that the correct value is -0.03 volt; they also found evidence that another intermediate, with a potential of -0.27 volt, may exist, the available energy is thus 0.44 or even 0.67 eV.

Experimentally, quantum requirements of 6 have been found per ATP-molecule formed, for "cyclic" and "noncyclic" photophosphorylation alike. If photophosphorylation were to occur in a separate photochemical reaction, this would mean the need of 12 (or 18) quanta just to supply the two (or three) ATP molecules needed for the Calvin cycle. Even with two ATP's contributed as a side product when 8 quanta are used for oxidation-reduction, this mechanism would still require 6 additional quanta to produce the ATP molecules needed for the original Calvin cycle—a requirement incompatible with the observed overall quantum requirement of 8! (Answers to these questions may be found only when simultaneous, precise quantum yield measurements for ATP production and oxygen evolution will be made.)

These calculations emphasize the uncertainty of the widely accepted assumption that the photochemical process in photosynthesis supplies both the NADPH and all the ATP needed for the Calvin cycle. This assumption is based on two sets of facts: (a) chloroplasts *do* contain sufficient amounts of  $\text{NADP}^+$ , and *can* convert it in light to NADPH, and *do* transform ADP to ATP; and (b) enzymatic mechanisms *can* convert carbon dioxide to carbohydrates, if enough NADPH and ATP is supplied. However, to be certain that a model mechanism suggested for photosynthesis is truly adequate, one has to prove that it can run, under natural conditions, with the high efficiency characteristic of natural photosynthesis—and this proof is as yet missing for the ATP-requiring steps in the Calvin cycle (at least, in its original version).