PHOTOSYNTHESIS BY ISOLATED CHLOROPLASTS

VIII. PHOTOSYNTHETIC PHOSPHORYLATION AND THE GENERATION OF ASSIMILATORY POWER

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SUMMARY

Photochemical ATP formation by isolated chloroplasts was coupled with a reduction of ferricyanide or TPN. Esterification of two moles of orthophosphate was coupled with the formation of two moles of $TPNH_2$ and the *evolution* of one mole of oxygen.

The addition of catalytic amounts of FMN, vitamin K or phenazine methosulfate to the TPN phosphorylating system suppressed $TPNH_2$ accumulation as well as oxygen evolution and greatly increased the light-dependent ATP formation.

A revised general scheme is presented for photosynthesis by isolated chloroplasts.

INTRODUCTION

Isolated chloroplasts have long been known[1] to be capable of carrying out an evolution of oxygen (Eqn. 1) under the influence of light in the presence of a non-physiological electron acceptor such as, for example, ferricyanide (Hill reaction)

$$2\ Fe^{+3}Cy + 2\ H_2O \xrightarrow[\text{chloroplasts}]{\text{light}} 2\ Fe^{+2}Cy + \tfrac{1}{2}\ O_2 + 2\ H^+ + H_2O \qquad (1)$$

The recently discovered capacity of isolated chloroplasts for a light-induced synthesis of ATP from ADP and orthophosphate was observed under conditions when molecular oxygen was neither consumed nor evolved[2–6]. There was no indication that photosynthetic phosphorylation is compatible with a simultaneous oxygen evolution, such as characterizes a Hill reaction. But more recent experiments[7] have shown that these two photochemical reactions are linked, as represented by Eqn. 2.

$$2\ Fe^{+3}Cy + 2\ H_2O + ADP + P \xrightarrow[\text{chloroplasts}]{\text{light}} 2\ Fe^{+2}Cy + \tfrac{1}{2}\ O_2 + ATP + H_2O \qquad (2)$$

The purpose of this article is to present the evidence linking photosynthetic phosphorylation to the Hill reaction and, what is physiologically more significant, to a reduction of TPN accompanied by oxygen evolution[7] (Eqn. 3).

Abbreviations: ATP, ADP, adenosine triphosphate and diphosphate, respectively; FMN, riboflavin phosphate (flavin mononucleotide); TPN, triphosphopyridine nucleotide; DPN, diphosphopyridine nucleotide; $TPNH_2$, reduced TPN; tris, tris(hydroxymethyl)aminomethane.

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In reaction 3 the evolution of oxygen is accompanied by a conversion of light energy into chemical energy which is apportioned between two compounds, ATP and $TPNH_2$, henceforth referred to jointly as "assimilatory power"[7]. The formation of the two components of assimilatory power marks the photochemical phase of photosynthesis. Carbon assimilation is accomplished in the dark, at the expense of assimilatory power, by water-soluble enzymes which are readily extracted from chloroplasts[5,8].

$$TPN + 2\,H_2O + ADP + P \xrightarrow[\text{chloroplasts}]{\text{light}} TPNH_2 + ATP + \tfrac{1}{2}\,O_2 + H_2O \qquad (3)$$

METHODS

The methods used in this investigation were those described in the preceding article[9]. Further details are given in the tables and in the legends to the figures.

RESULTS

ATP formation coupled with ferricyanide reduction

Fig. 1 shows the esterification of orthophosphate coupled with oxygen evolution and the reduction of ferricyanide by illuminated chloroplasts. Of the Hill reagents tried ferricyanide was found to be compatible with photosynthetic phosphorylation to a far greater degree than quinone. The results illustrated by Fig. 1 provide experimental support for the stoichiometry of the coupled phosphorylation shown in Eqn. 2. One mole of oxygen is evolved and 2 moles of orthophosphate are esterified in a transfer of four electrons to ferricyanide.

The rate of oxygen evolution in the Hill reaction coupled with phosphorylation was higher than in a conventional Hill reaction without phosphorylation (Eqn. 1). As shown in Fig. 2 the Q_{O_2} (mm^3 O_2 evolved/mg chlorophyll/h) for ferricyanide reduction unaccompanied by phosphorylation was 1500; by coupling ferricyanide reduction with phosphorylation the Q_{O_2} was increased to 2800.

These results indicate that the electron transport system of chloroplasts functions more effectively when it is coupled to the synthesis of ATP. The Hill reaction by itself (Eqn. 1) may thus be regarded as an uncoupled photophosphorylation, *i.e.* as a measure of photochemical electron transport which is proceeding without its associated phosphorylation reaction. The Hill reaction would thus be analogous to those electron-transport reactions studied in particulate systems of animal origin in which oxidation has been uncoupled from its normally associated phosphorylation—for example, in the KEILIN-HARTREE preparations[10] or in the electron-transport particles of GREEN[11].

ATP formation coupled with TPN reduction

A light-induced ATP synthesis coupled with the formation of a reductant would clearly be of great importance for CO_2 fixation by isolated chloroplasts if the reductant formed were a physiological substance of a strong reducing potential, such as a pyridine nucleotide, rather than ferricyanide as formed in reaction 2. VISHNIAC AND OCHOA[12], TOLMACH[13] and ARNON[14] have already shown that, in light, isolated chloroplasts can reduce pyridine nucleotides and simultaneously evolve oxygen, if this photochemical reaction is coupled with a "pulling reaction" *i.e.* an enzyme system

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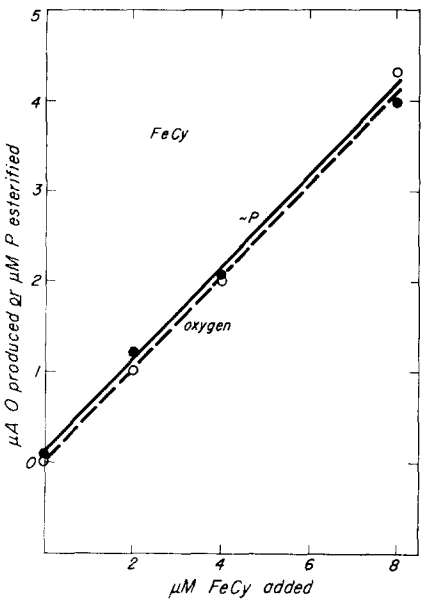

Fig. 1. Photophosphorylation and oxygen evolution with ferricyanide. The stoichiometric relation between moles of ferricyanide reduced, moles of orthophosphate esterified, and atoms of oxygen produced was 2:1:1. The reaction mixture contained, in a final vol. of 3 ml, "broken" chloroplasts (P_{18}) containing 0.1 mg chlorophyll and the following in μmoles: tris, pH 8.3, 80; $MgCl_2$, 5; ADP, 10; $[^{32}P]K_2HPO_4$, 10; NaCl, 35; and $K_3Fe(CN)_6$ as indicated. The reaction was run at 15° for 18 min, at which time the reduction of ferricyanide was complete. Gas phase, nitrogen. KOH in center well of vessel. Illumination as described previously[24].

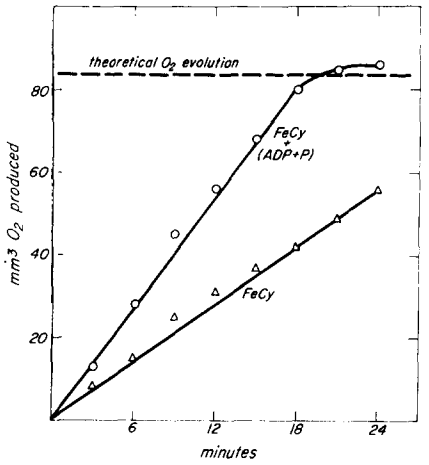

Fig. 2. Effect of phosphate acceptor system on the Hill reaction with ferricyanide. The reaction mixture contained, in a final vol. of 3 ml: "broken" chloroplasts (P_{18}), containing 0.1 mg chlorophyll; and the following in μmoles: tris, 80; NaCl, 35; $K_3Fe(CN)_6$, 15. The vessel with the phosphate acceptor system received in addition (in μmoles): ADP, 10; K_2HPO_4, 10; and $MgCl_2$, 5. Oxygen evolution was measured manometrically at 15°. Gas phase, nitrogen; KOH in the center well of the vessel.

capable of utilizing at once the newly-formed reduced pyridine nucleotide. There was no suspicion, however, that photochemical reduction of pyridine nucleotides by chloroplasts could be accompanied by a *formation* of ATP. On the contrary, several schemes were postulated[15,16,17], but which later experiments failed to support[18], envisaging a photochemical reduction of pyridine nucleotides by chloroplasts as requiring a *consumption* of ATP formed by previous photochemical events.

A direct reduction of pyridine nucleotides by illustrated chloroplasts, as measured by the accumulation of the reduced product, was first demonstrated by SAN PIETRO AND LANG[19]. It soon became clear that, as shown in Fig. 3, catalytic amounts of chlorophyll were capable of reducing substrate amounts of TPN but only in the presence of a soluble TPN-reducing factor, readily extractable from chloroplasts and having properties characteristic of proteins[20]. An aqueous extract of chloroplasts (*CE*) has served in previous experiments[20] as the source of the TPN-reducing factor. This factor which has recently been further purified under the name of photosynthetic pyridine nucleotide reductase[21], has not been found necessary, under our conditions, for the photochemical reduction of ferricyanide or quinone.

Table I shows that the reduction of substrate amounts of TPN by illuminated chloroplasts was accompanied by an evolution of oxygen in accordance with Eqn. 4.

$$TPN + 2\,H_2O \xrightarrow[\text{chloroplasts} + CE]{\text{light}} TPNH_2 + \tfrac{1}{2}\,O_2 + H_2O \qquad (4)$$

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TABLE I

EQUIVALENCE OF TPN REDUCTION AND OXYGEN EVOLUTION BY ILLUMINATED CHLOROPLAST PARTICLES IN THE PRESENCE OF EXCESS CHLOROPLAST EXTRACT (*CE*)

The reaction mixture contained, (in addition to TPN) in a final vol. of 3 ml, "broken" chloroplasts (P_{1s}), containing 0.1 mg chlorophyll; tris, pH 8.3, 80 μmoles; NaCl, 35 μmoles; and *CE* extracted from chloroplasts containing 2 mg chlorophyll. The reaction was run to completion at 20° under nitrogen, with KOH in the center well of the manometer vessel. After centrifuging off the particles, reduced TPN in the supernatant fluid was measured spectrophotometrically at 340 mμ. O_2 evolution was measured manometrically.

μmoles TPN added	*μmoles TPN reduced*	*μatoms oxygen evolved*
0	0.1	0.2
1.0	0.9	1.2
2.0	1.9	2.2
3.0	2.9	3.1
4.0	4.1	4.2

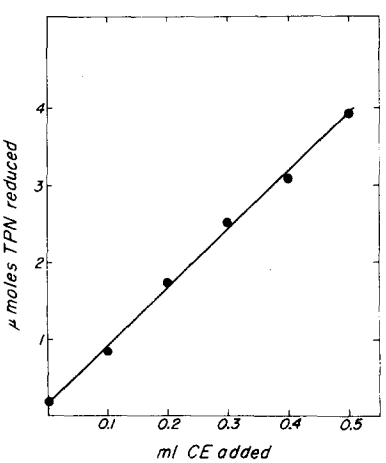


Fig. 3. Light-dependent reduction of TPN by washed chloroplast particles as a function of added chloroplast extract. The reaction mixture contained, in a final vol. of 3 ml.: "broken" chloroplasts (P_{1s}), containing 0.1 mgm, chlorophyll) tris, pH 8.3, 80 μmoles; NaCl, 35 μmoles; TPN, 6 μmoles; and chloroplast extract as indicated. 1 ml chloroplast extract was obtained from a quantity of chloroplasts containing 2 mg chlorophyll. The reaction was run to completion at 20° under nitrogen, with KOH in the center well of the manometer vessel. Illumination as described elsewhere[24]. After centrifuging off the particles, reduced TPN in the supernatant fluid was measured spectrophotometrically at 340 mμ.

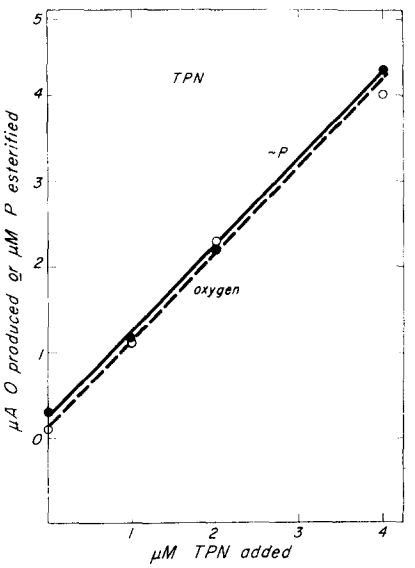
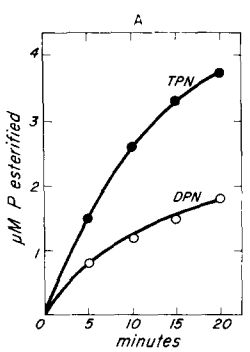


Fig. 4. Stoichiometry of oxygen evolution and ATP-formation resulting from the photochemical reduction of TPN. The relation between moles of TPN reduced, moles of orthophosphate esterified and atoms of oxygen produced is 1:1:1. The reaction mixture contained, in a final vol. of 3 ml, "broken chloroplasts" (P_{1s}) containing 0.25 mg chlorophyll, chloroplast extract (*CE*) equivalent to 2 mg chlorophyll, and the following in μmoles: tris, pH 8.3, 90; $MgCl_2$, 5; ADP, 10; [^{32}P]K_2HPO_4, 10; NaCl, 35 and TPN as indicated. The reaction was run at 15° for 33 min, at which time the reduction of TPN was complete. Gas phase, nitrogen.

In the presence of ADP and orthophosphate reaction 4 was found to be coupled with the formation of ATP[7]. The reaction between TPN reduced, oxygen evolved, and orthophosphate esterified is shown in Fig. 4. One mole of orthophosphate was esterified for each mole of TPN reduced and half a mole of oxygen produced. This stoichiometric relation, expressed in Eqn. 3, was observed only under carefully controlled experimental conditions which included the omission of ascorbate, washing of chloroplasts and dialysis of the TPN-reducing factor. Unlike cyclic photophosphorylation[9], the phosphorylation accompanying TPN reduction was found to be independent of added ascorbate.

Under our experimental conditions ATP formation linked with oxygen evolution occurred preferentially with TPN rather than DPN[20] (*cf.* [19,21]). A comparison between the effects of TPN and DPN is given in Fig. 5.

Reaction 3 represents a new type of photosynthetic phosphorylation. In the type described previously[2-5,22,23], not accompanied by the evolution of oxygen, it was



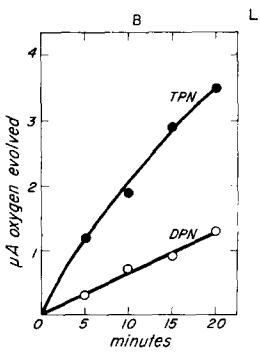


Fig. 5. The effect of TPN versus DPN on photosynthetic phosphorylation (A) accompanied by oxygen evolution (B). The reaction mixture contained 4 μmoles of TPN *or* DPN respectively. KOH in the center well of vessel. Other components and conditions as indicated in legend to Fig. 4.

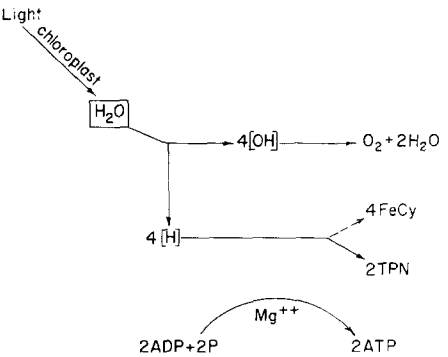


Fig. 6. Diagram representing the generation of "assimilatory power" and the evolution of oxygen by isolated chloroplasts. The components of assimilatory power are $TPNH_2$ and ATP. In a nonphysiological variant of this reaction ferricyanide (FeCy) can replace TPN.

envisaged that all the absorbed light energy was trapped in the pyrophosphate bonds of ATP. In the formation of assimilatory power (reaction 3) only part of the light energy absorbed by chlorophyll is used for ATP formation; the remainder is used for the formation of a reductant $TPNH_2$, which provides the "hydrogens" needed for the conversion of CO_2 to sugars.

The stoichiometry of reaction 3 is the same as of reaction 2: the evolution of one mole of oxygen and the synthesis of 2 moles of ATP accompanies the transfer of 4 electrons or the generation of 4 hydrogen equivalents which are required for the reduction of one mole of CO_2 to the level of carboxydrate (–CHOH). Reaction 3, the generation of assimilatory power, would thus be in accord with the well-known photosynthetic ratio O_2/CO_2 of 1, also observed with isolated chloroplasts, when CO_2 is assimilated to the level of carbohydrates[24]. Reaction 2, the phosphorylation coupled with ferricyanide reduction, may thus be viewed as a non-physiological model for the generation of assimilatory power. A general scheme based on these findings is shown in Fig. 6.

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Improbability of lipoic acid as the primary reductant in photosynthesis

CALVIN and his associates[25] have assigned to lipoic (thioctic) acid a key role in photosynthesis as a compound concerned in the primary conversion into chemical energy of the light quanta absorbed by chlorophyll. They have proposed[26] that the reduced lipoic acid (dithiol form) could in turn reduce DPN or TPN. Lipoic acid could thus fulfil the role of hydrogen carrier in the water-splitting reaction leading to the formation of $TPNH_2$ (reaction 3).

Reactions in which lipoic acid is a cofactor are very sensitive to arsenite inhibition; the inhibition can be reversed by the addition of dithiols but not by monothiols, as shown by PETERS *et al.*[27]. They and GUNSALUS[28] observed inhibition of the pyruvic oxidation system by concentrations of arsenite of the order of $3 \cdot 10^{-5}\ M$; the inhibition was reversed by the dithiol BAL (2,3-dimercaptopropanol) but not by the monothiol glutathione. In the chloroplast system $2 \cdot 10^{-3}\ M$ arsenite failed to inhibit the TPN reduction and its coupled phosphorylation or the corresponding ferricyanide system (Table II). It seems unlikely therefore that lipoic acid is a cofactor in the light reactions of chloroplasts. The TPN reduction and its coupled phosphorylation are sensitive, however, to other sulfhydryl inhibitors as evidenced by their inhibition by *p*-chloromercuribenzoate.

The improbability of lipoic acid as a participant in photosynthetic phosphorylation is also indicated by the results of GELLER[29]: $10^{-2}\ M$ arsenite failed to inhibit phosphorylation by illuminated particles of *Rhodospirillum rubrum*.

TABLE II

INSENSITIVITY TO ARSENITE ($2 \cdot 10^{-3}\ M$) OF PHOTOPHOSPHORYLATION AND OXYGEN EVOLUTION RESULTING FROM THE PHOTOREDUCTION OF TPN OR FERRICYANIDE BY ISOLATED CHLOROPLASTS

The reaction mixtures contained either 4 μmoles TPN or 8 μmoles $K_3Fe(CN)_6$. KOH in center wells of each vessel. Other conditions as described in legends to Figs. 4 and 1, respectively.

Treatment	*μmoles P esterified*	*μmoles oxygen evolved*
TPN, control	4.2	4.3
TPN, arsenite added	4.1	3.5
Ferricyanide, control	3.8	4.4
Ferricyanide, arsenite added	4.3	4.3

Cyclic photophosphorylation

In measuring the generation of assimilatory power the phosphorylation coupled with the reduction of TPN was accomplished without adding either flavin mononucleotide (FMN) or vitamin K, both of which have previously been identified as cofactors of photosynthetic phosphorylation[6,30]. The additional of small amounts of either FMN or vitamin K altered the system profoundly. The results are shown in Figs. 7 and 8.

Phosphorylation was sharply increased, whereas oxygen evolution and the accumulation of reduced TPN were abolished. The most direct explanation of these results is that the addition of catalytic amounts of either FMN or vitamin K brought about additional phosphorylation accomplished at the expense of energy liberated by the reoxidation of $TPNH_2$ by [OH], the oxidized product of photodecomposition of water (*cf.* [31]).

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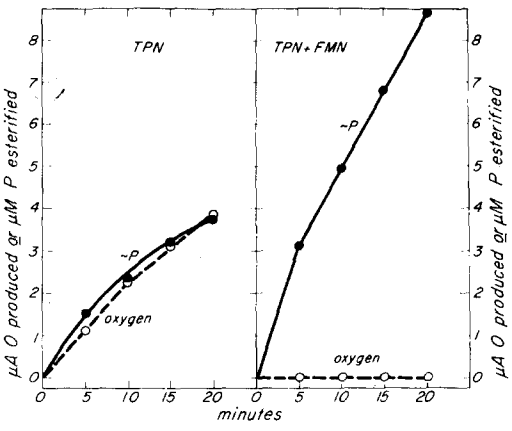

Fig. 7. Photophosphorylation and oxygen evolution with TPN in the presence and absence of FMN. The reaction mixture was the same as that described in Fig. 4, except that 15 μmoles K_2HPO_4, 15 μmoles ADP, and 4 μmoles TPN were used in each vessel. In the "TPN + FMN" series, 0.1 mole FMN was added.

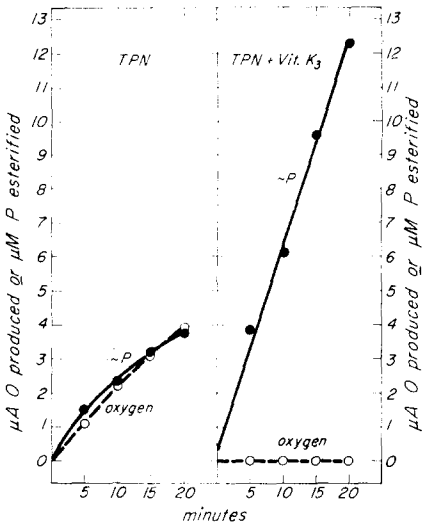

Fig. 8. Photophosphorylation and oxygen evolution with TPN in the presence and absence of vitamin K_3. Experimental conditions were the same as those described in Fig. 7. In the "TPN + vit. K_3" series, 0.2 mole vitamin K_3 (menadione) was added.

Expressed in another way, the addition of either FMN or vitamin K has brought about a recombination of the photodecomposition products of water, [H] and [OH], and a conversion into ATP of all the light energy originally trapped in the "water-splitting" reaction. Under these conditions CO_2 reduction cannot occur since the hydrogens needed for the reduction of CO_2 become a part of a reconstituted water molecule instead of a newly-formed sugar molecule. This type of light-induced phosphorylation is the same as was reported previously under the general name of photosynthetic phosphorylation[2-5,22,23]. It has now been designated by the more specific name of cyclic phosphorylation[7] (Eqns. 5 and 6) to distinguish it from the "primary" phosphorylation associated with the generation of assimilatory power when only part of the captured light energy is converted into ATP (Eqn. 3).

$$2\,H_2O \xrightarrow[\text{chloroplasts}]{\text{light}} 2\,[H] + 2\,[OH] \qquad (5)$$

$$2\,[H] + 2\,[OH] + n\,ADP + n\,P \longrightarrow n\,ATP + 2\,H_2O \qquad (6)$$

Another explanation of the observed effects of FMN and vitamin K on the primary phosphorylation is possible, and is being tested experimentally. It would limit phosphorylation only to the electron-transfer step coupled with TPN reduction and would explain the increased phosphorylation which follows the addition of FMN or vitamin K (Figs. 7 and 8) as resulting from a more rapid turnover of the TPN rather than from the activation of additional phosphorylation sites.

The marked increase in phosphorylation accompanied by a total abolition of oxygen evolution and $TPNH_2$ accumulation shown in Figs. 7 and 8 occurred on adding 0.1 μmole FMN or 0.2 μmole vitamin K (in a final volume of 3 ml). However, the addition of even extremely minute amounts of either FMN or vitamin K had a

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measurable effect on reaction 3. Table III shows that the addition of as little as 0.0002–0.0005 μmole FMN or vitamin K increased ATP formation without appreciably depressing oxygen evolution (and the corresponding $TPNH_2$ accumulation). Similar effects were observed on adding small amounts of phenazine methosulfate (Table IV).

As is discussed more fully elsewhere[32], the addition of small amounts of one of these catalysts of photosynthetic phosphorylation had an influence on carbon assimilation. Concentrations of FMN and vitamin K which suppressed oxygen evolution (and $TPNH_2$ accumulation) also suppressed reductive CO_2 fixation, but the minute concentrations of these catalysts which increased ATP formation without markedly depressing oxygen evolution also increased reductive CO_2 fixation.

TABLE III

EFFECT OF FMN AND VITAMIN K_3 ON PHOSPHORYLATION AND OXYGEN EVOLUTION LINKED TO TPN REDUCTION

KOH in center well of manometer vessel. Other conditions as described in legend to Fig. 4.

FMN or vit. K_3 added (μmoles)	*FMN system*		*Vit. K_3 system*	
	P esterified μmoles	*O_2 evolved μatoms*	*P esterified μmoles*	*O_2 evolved μatoms*
none	5.6	3.6	5.6	3.6
0.0002	6.5	4.2	6.1	3.6
0.0005	7.4	3.8	7.5	2.9
0.001	7.9	3.3	8.0	2.3
0.003	8.4	1.2	9.6	0.9
0.01	9.0	0.4	10.0	0.9

TABLE IV

EFFECT OF PHENAZINE METHOSULFATE ON PHOSPHORYLATION AND OXYGEN EVOLUTION LINKED TO TPN REDUCTION

KOH in center well of manometer vessel. Other conditions as described in Legend to Fig. 4

Phenazine methosulfate added (μmoles)	*P esterified (μmoles)*	*O_2 evolved (μatoms)*
none	4.5	4.0
0.003	6.1	4.0
0.01	9.0	3.2
0.1	10.0	0.8

TPN and cyclic photophosphorylation

The finding that the addition of either FMN or vitamin K (Figs. 7 and 8) converted the primary phosphorylation into the cyclic type, fitted well with the previously recognized role of TPN as a catalyst of cyclic photophosphorylation[20]. The conversion of the primary phosphorylation into the cyclic type could be explained as a lengthening of the electron or hydrogen transport chain (Fig. 6) through the oxidation of the reduced TPN by FMN or vitamin K_3. In this connection the role of TPNH diaphorase found in chloroplasts by AVRON AND JAGENDORF[33] merits consideration.

However, as reported in the preceding article[9], further investigation of the cofactors of photosynthetic phosphorylation led to the tentative distinction between two pathways of cyclic photophosphorylation, one catalyzed by FMN and the other

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by vitamin K. The distinction was based in part on differential sensitivity to two inhibitors, *o*-phenanthroline and dinitrophenol, and in part on the greater and more consistent dependence on TPN of the FMN pathway (at low concentrations of FMN) than of the vitamin K pathway. The possibility was suggested therefore that TPN is not normally a component of the vitamin K phosphorylation system but only of the FMN pathway of cyclic photophosphorylation. Support for this tentative conclusion was again found in inhibition experiments with *o*-phenanthroline and dinitrophenol. The photophosphorylation accompanying TPN reduction (Eqn. 3) and the cyclic photophosphorylation with FMN were more sensitive to these two inhibitors than cyclic photophosphorylation with vitamin K. (See Table X in [9])

Based on these conclusions, the effect of FMN in converting the assimilatory power reaction to cyclic photophosphorylation (Fig. 7) can be visualized as catalyzing a reconstitution of water accomplished through a transfer of hydrogen from $TPNH_2$ to [OH]. It was suggested that in catalyzing this process FMN probably acts jointly with a cytochrome system and that additional phosphorylations accompany this lengthening of the electron chain (see Fig. 5 in [9]). Added vitamin K (and phenazine methosulfate) also suppressed the assimilatory-power reaction (*cf.* Fig. 8 and Table IV) again probably through a reconstitution of water, but no mechanism for this effect can be usefully proposed at this time.

DISCUSSION

The results of this investigation demonstrated in isolated chloroplasts a direct connection among three, previously known only as separate, photochemical events: evolution of oxygen, formation of ATP and formation of a reductant, $TPNH_2$, capable of reducing CO_2. The reaction in which all the three events occur (Eqn. 3) summarizes the basic feat of photosynthesis in green plants: the conversion of light into useful chemical energy accompanied by an evolution of molecular oxygen.

The conversion of light into chemical energy is fundamentally independent of CO_2 assimilation: this is clearly seen in isolated chloroplasts in which the two "energy-rich" compounds formed at the expense of light energy, ATP and $TPNH_2$, can be made to accumulate in substrate amounts and their accumulation is a direct result of the energy conversion process. In the living cell these compounds are present in catalytic amounts and hence the trapped light energy is usually, although perhaps not always[7], stored by coupling their formation with carbon assimilation. *In vivo*, the photochemical phase of photosynthesis is therefore tightly bound to carbon assimilation whereas in isolated chloroplasts the two are readily separated. Assimilatory power can be generated first in the light and used later for CO_2 assimilation in the dark. The key problem of photosynthesis, the conversion of light into useful chemical energy, may thus be studied in isolated chloroplasts independently of the dark enzymic reactions responsible for the synthesis of carbohydrates.

A physical separation of the light and dark phases of photosynthesis by isolated chloroplasts has recently been accomplished[34]. Substrate amounts of assimilatory power were first generated in the light, in the absence of CO_2. At the end of the light reaction $[^{14}C]CO_2$ was supplied in the dark, and, after an incubation period, its total fixation was measured. The newly-formed radioactive compounds were identified by paper chromatography and radioautography. The two phases when carried out

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consecutively, yielded, essentially, the same final photosynthetic products as the continuously illuminated complete chloroplast system[34].

The separation of the light and dark phases of photosynthesis in isolated chloroplasts was aided by the association of each phase with a different portion of the chloroplast. The dark phase of photosynthesis was found to be associated solely with the water-soluble chlorophyll-free extract[8,5]. The light phase was, as would be expected, localized in the "grana" fraction which contains the chlorophyll pigments[32]. However, the light phase appears to involve, in addition to the "grana", some "stroma" factors. At least one of these, the pyridine nucleotide-reducing factor[20,21], must be present if the generation of assimilatory power is to occur in the light.

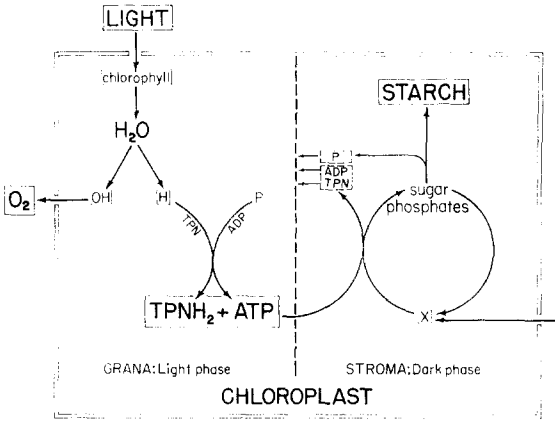

Fig. 9. Scheme for photosynthesis by isolated chloroplasts. In the light phase, photolysis of water, catalyzed by "grana", results in the evolution of oxygen and the generation of assimilatory power comprising two components: $TPNH_2$ and ATP. In the dark phase assimilatory power is used by the enzymes in the "stroma" for the assimilation of CO_2 in reactions independent of light.

A diagrammatic scheme of photosynthesis by chloroplasts, based on these findings, is shown in Fig. 9. The concept of photosynthesis by chloroplasts represented by Fig. 9 shares certain similarities with, but also differs from, the earlier one which it now replaces[4,5,22]. It is similar in regarding the chloroplast as a complete photosynthetic unit containing multienzyme systems divided into three main groups, each controlling an increasingly complex phase of photosynthesis: photolysis of water, photosynthetic phosphorylation and CO_2 fixation. CO_2 fixation remains, as before, at the apex of this hierarchy and requires the participation of all three groups of enzymes. But photolysis of water is now no longer regarded as resulting *either* in the synthesis of ATP *or* in the reduction of CO_2. ATP synthesis is *coupled* with the formation of the reductant ($TPNH_2$) required for CO_2 fixation. Thus the same light quanta which accomplish the reduction of TPN also bring about the synthesis of ATP, and generate the assimilatory power needed for the conversion of CO_2 into carbohydrates or analogous end products of photosynthesis.

The coupling of ATP synthesis with TPN reduction simplified the concept of CO_2 assimilation by chloroplasts. The oxygen evolved bears a stoichiometric relation ($2\ TPNH_2 : O_2$) to the $TPNH_2$ used in CO_2 reduction (Eqn. 3). If the ATP generated during the TPN reduction step (Eqn. 3) is sufficient for CO_2 assimilation it is no longer necessary to visualize a competition for light energy between photosynthetic phosphorylation and CO_2 fixation[5]. The generation of the two components of assimilatory power, $TPNH_2$ and ATP, would go up and down simultaneously, in accordance with the rate of CO_2 fixation. If, however, reaction 3 cannot by itself supply enough ATP for carbon assimilation then it could be supplemented, to a varying degree, by cyclic

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photophosphorylation (Eqn. 5). It is assumed that the cell has suitable regulatory mechanism for keeping the two reactions in balance.

The large body of experimental evidence gathered now in several different laboratories on photosynthesis by isolated chloroplasts has been obtained almost entirely with chloroplasts from one species, spinach. It seemed desirable to test the validity of the principal conclusions with chloroplasts from other species. This has now been done by WHATLEY *et al.*[35]. ATP formation coupled with the photochemical reduction of TPN or ferricyanide and CO_2 assimilation to the level of carbohydrates has been carried out with isolated chloroplasts from pokeweed*, sugar beets, sunflower, tobacco, and *Tetragonia expansa*.

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