THE FRAGMENTS OF THE PHOTOSYNTHETIC ELECTRON TRANSFER CHAIN IN MODEL SYSTEMS

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ABSTRACT In this paper the recent research from our laboratory is reviewed. Short fragments of the photochemical electron transfer chain of photosynthesis were reproduced in aqueous detergent solutions or in organic solvents. The function of photosystem I is reproduced in a ternary system of chlorophylls, electron donors (dienols, sulfhydryl compounds, hydrazine, etc.), and electron acceptors (viologens, nicotinamide-adenine dinucleotide [NAD], flavines, etc.). Chlorophyll-photosensitized reduction of viologens in some cases is activated by oxygen at the expense of active reductants formed during the photosensitized oxidation of an initial electron donor (thiourea). Chlorophyll-photosensitized oxidoreduction of cytochromes is activated by flavines, viologens, vitamin K derivatives, and some other redox systems (cofactors of cyclic photophosphorylation). The primary mechanism of the reactions studied depends on the reversible chlorophyll photooxidoreduction. In binary systems, chlorophyll (monomeric or aggregated) and electron donor or electron acceptor, reversible photoreduction or photooxidation is observed. Irreversible bacteriochlorophyll oxidation leads to the formation of chlorophyll and protochlorophyll analogues; irreversible protochlorophyll photoreduction results in chlorophyll-like pigment appearance. The photodisaggregation of chlorophyll was observed. The models of photosystem II studied were the photochemical oxygen evolution in aqueous solutions of electron acceptors (ferric compounds, quinone), photosensitized in the near UV part of the spectrum by inorganic semiconductors (tungsten, titanium, and zinc oxides). All reactions described are based on electron (hydrogen) transfer photosensitized by pigment system.

INTRODUCTION

In photosynthesis the light energy absorbed by chlorophyll is utilized in an oxidation-reduction process against a gradient of chemical potential. The light energy conversion proceeds in the photosynthetic electron transfer chain where photochemical reactions of excited chlorophyll molecules are coupled with the work of biochemical systems. The reconstruction of simple fragments of the electron transfer chain may facilitate the study of the molecular mechanism of light energy conversion.

Any type of electron transfer in which unexcited partner molecules are involved may be used to release the energy of chlorophyll excitation in the form of stored chemical energy. In the binary system (excited chlorophyll–unexcited partner molecule) either member of the pair might be reduced or oxidized. In the triple system (reductant-chlorophyll-oxidant), photosensitization might be observed with the excited chlorophyll being the intermediate electron carrier. Thus, the action of light absorbed by chlorophyll is to transfer an electron from reductant to oxidant.

Excited States

According to some authors, the absorption of a quantum of light by a molecule of chlorophyll leads to the singlet excited state ($S + h\nu \rightarrow S^*$) which is converted into a triplet metastable molecule ($S^* \rightarrow T$) capable of absorbing a second light quantum. The formation of excited triplets ($T + h\nu \rightarrow T^*$) is probable only at very high light intensities, and such a mechanism of summation of the energy of two light quanta is very improbable in photosynthesis which requires rather low intensities of light.

The pioneering work of Terenin in 1943 suggested the peculiar role of triplet molecules in photochemistry to be that of chemically reactive biradicals. Now, vast experimental data have accumulated showing that photochemistry of "monomeric" chlorophyll and dyes in solution may be considered mainly as the chemistry of triplet excited molecules (1).

The unstable intermediates of photochemical reactions, carrying the energy of light quanta, are very short-lived, and their study requires special methods; it is easier, however, to study the more stable products formed from these labile intermediates. In this paper, we discuss our work with the models of photosystems I and II of photosynthesis; the previous work of our laboratory has already been reviewed in references 2–5.

MODELS OF PHOTOSYSTEM I

Here, an electron donor with a redox potential (Eo') close to that of the cytochromes and an electron acceptor with Eo' close to that of ferredoxin are used. As an electron acceptor, we used methylviologen with an Eo' of -0.43 v. In aqueous solutions of the detergent Triton X-100, it is possible to observe chlorophyll-photosensitized reduction of viologens, flavines, and some other dyes, using one of the following as an electron donor: ascorbate, cysteine, glutathione, thiourea, hydrazine, etc. (6, 7). The study of these photoreactions is possible by immediate spectrophotometric measurements in the Thunberg tube. For instance, after illumination with red light of an aqueous detergent solution of chlorophyll, cysteine, and methylviologen, a blue-reduced viologen appears, which is reversibly oxidized after admission of air. This type of reaction requires anaerobiosis, as oxygen reacts rapidly with the reduced forms of the acceptors listed above. Recently, Brune and San Pietro (8) have studied chlorophyllin-catalyzed photoreduction of viologen dyes.

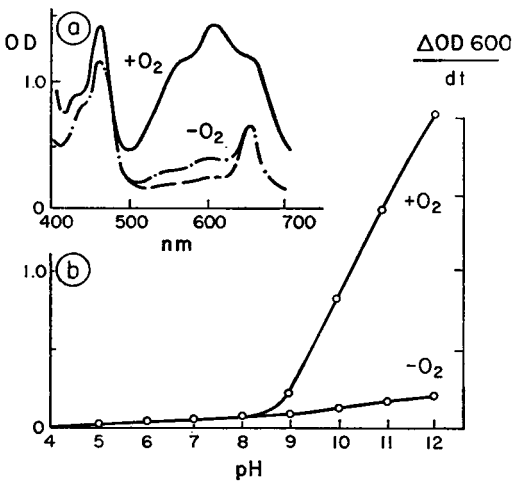

FIGURE 1

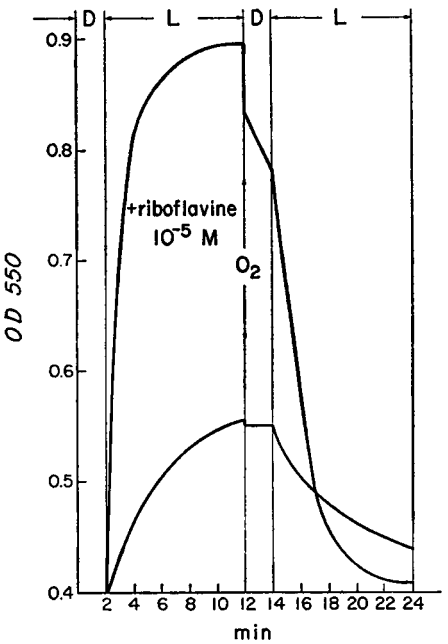

FIGURE 2

FIGURE 1 Chlorophyll-photosensitized reduction of methylviologen in 0.5% aqueous solution of Triton X-100 in the presence of thiourea. (*a*) Absorption spectrum at pH 10; solid curve, $+O_2$; dashed curve, $-O_2$, before illumination; dashed-dot curve, $-O_2$, after illumination (9, 10). (*b*) Dependence of reaction on pH; abscissa is rate of change in absorbance (OD) at 600 nm; $-O_2$, *in vacuo*; $+O_2$, in air.

FIGURE 2 Chlorophyll-photosensitized oxidoreduction of cytochrome *c* (OD change at 550 nm) in aqueous 0.5% solution of Triton X-100 in the absence and in the presence of 10^{-5} M riboflavine or riboflavine-5′-phosphate. L, light; D, dark (11).

When thiourea was used as an electron donor, we could not observe photoreduction of viologen under anaerobic conditions (Fig. 1); but paradoxically, in the presence of atmospheric oxygen, illumination of the system thiourea-chlorophyll-viologen (avoiding stirring of the Thunberg tube cuvette!) caused a high rate of photoreduction of viologen (9). After stirring with air, the reduced viologen is oxidized. This reaction is enhanced at a pH of the medium more basic than 8. The study of this phenomenon revealed that photosensitized thiourea oxidation proceeds in the zone of illumination which leads to local anaerobiosis (9, 10). The photooxidation product of thiourea is a strong reducing agent capable of reducing viologen in the dark (or in a photosensitized reaction) and is also capable of photoreducing chlorophyll. The stimulation of photoreduction of viologen by oxygen was observed most clearly in the case of thiourea; in the case of cysteine and glutathione, the reaction proceeds after oxygen evacuation, but without this procedure the sensitized viologen reduction is more efficient.

The question is, Why does the photoreaction not proceed backwards immediately?

One possible explanation is that the primary "one-electron" intermediate undergoes immediate dismutation and that the reaction of the more stable "two-electron" products requires activation energy. In the case of the intact photosynthetic electron transfer chain, such a demand is fulfilled at the terminals of the chain where rather stable two-electron reduced pyridine nucleotides are formed and the oxidation of water molecules leads to gaseous oxygen evolution.

Chlorophyll-Photosensitized Oxidoreductions of Cytochrome

This reaction was studied in an aqueous solution of Triton X-100 containing oxidized cytochrome *c* and chlorophyll. Under red light illumination *in vacuo*, cytochrome reduction is observed; after admission of air, illumination caused ferrocytochrome oxidation (11, 12).

According to Vernon (13), flavines enhance cytochrome photoreduction in chloroplasts; in our model systems, the reaction was strongly enhanced by flavines (riboflavine, flavine mononucleotide, flavine-adenine dinucleotide), viologens, menadione, and some redox systems that are cofactors of photophosphorylation (Fig. 2). The mechanism of activation is probably due to the action of compounds listed above as auxiliary redox systems between chlorophyll and cytochrome.

To reveal the molecular mechanism of photosensitized reactions, interaction between excited chlorophyll and partner molecules (i.e., electron donors and acceptors) was studied. The reversible transformations of chlorophyll under the action of light were revealed in the pioneering works of E. Rabinowitch (14) and R. Livingston (15, 16).

Excited Chlorophyll–Electron Acceptor Reaction

Reversible Oxidation. As early as 1937, Rabinowitch and Weiss (17) observed that the reaction of chlorophyll with ferric ions is activated by light. Linschitz and Rennert (18) described the photochemical interaction of chlorophyll with quinone in ethyl ether–isopentane, and ethyl alcohol (EPA) at liquid nitrogen temperature; Tollin and Green (19) found the electron spin resonance (ESR) signal of semiquinone when illuminating this system.

The reversible oxidation of chlorophyll and its analogues is easy to observe in viscous ethyl alcohol–glycerol solutions in the presence of electron acceptors at −70°C (20, 21), but in the case of the bacteriochlorophyll-ubiquinone system, we observed the reversible reaction at room temperature (22). After the light is switched off, the reaction goes backwards (Fig. 3). The oxidized intermediate of chlorophyll has an absorption maximum at 470 nm, and that of bacteriochlorophyll, at 430 nm. Essentially, illumination by blue light in the absorption band of the intermediates considerably accelerates the back reactions (22).

$$\mathrm{Chl} + \mathrm{Ox} \underset{\text{blue } h\nu}{\overset{\text{red } h\nu}{\rightleftharpoons}} \cdot\ \mathrm{Chl}^{+} + \cdot\ \mathrm{Ox}^{-}.$$

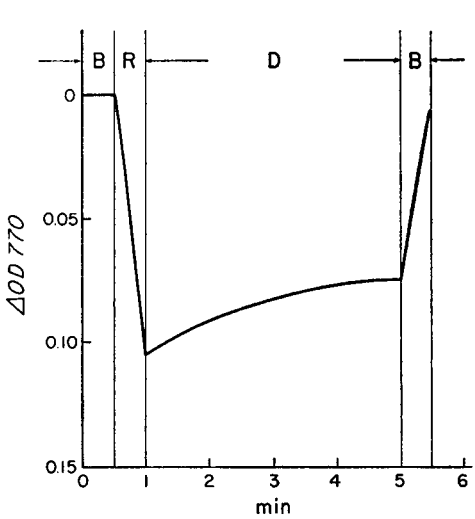

FIGURE 3

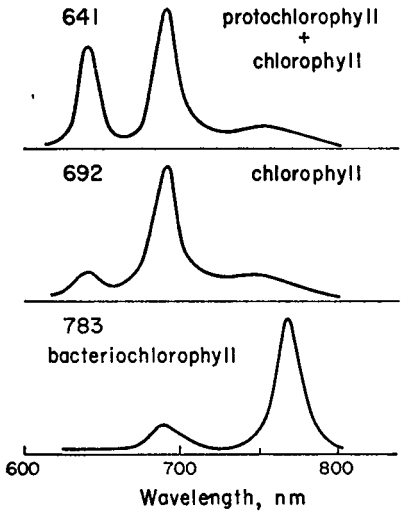

FIGURE 4

FIGURE 3 Reversible photooxidation of bacteriochlorophyll (OD change at 770 nm) by ubiquinone in alcohol-glycerol mixture. D, dark; action of red (R) or blue (B) light (22). FIGURE 4 Fluorescence spectra of bacteriochlorophyll photooxidation (by *o*-quinone) products in toluene (24). Ordinate, absorbance or optical density in relative units; products are chlorophyll and protochlorophyll.

This phenomenon explains the paradoxical effect of blue light inactivity during pigment photooxidation.

Studying reversible chlorophyll photooxidation, Evstigneev had demonstrated in the system pigment-quinone that the reaction proceeds most strongly in acid media, and that the potential of the inert platinum electrode is changed to the positive direction, probably because of cation radical pigment formation having electrode activity (see reference 23). It must be pointed out that the photopotential caused in this system by red light is quenched under the action of blue light (22).

Irreversible Photooxidation. In photosynthetic organisms, most pigments differ in the degree of "semi-isolated," double-bond reduction according to the following sequence: bacteriochlorophyll-chlorophyll-protochlorophyll (chlorophyll *c*). We shall consider the oxidation of the most reduced pigment-bacteriochlorophyll. After interaction *in vacuo* with fresh *o*-quinone solution (obtained by pyrocatechol oxidation with Ag_2O), some chlorophyllous pigment is formed in the dark. Illumination in the bacteriochlorophyll absorption band leads to a nearly complete conversion into chlorophyllous pigment (see Fig. 4), which, in turn, is converted into protochlorophyllous pigments by subsequent illumination with red light (in the "chlorophyll" absorption band) (24).

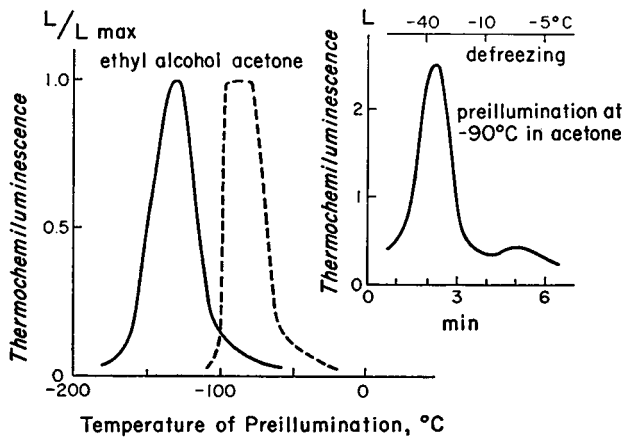

FIGURE 5 Thermochemiluminescence of chlorophyll; dependence on the temperature of preillumination. Right curve, kinetics of thermoluminescence after chlorophyll illumination in acetone at −90°C; L/L max, ratio of luminescence intensity to the maximum luminescence intensity observed (27–29).

Excited Chlorophyll–Oxygen Interaction

Along with products of irreversible destructive chlorophyll photooxidation, intermediates are formed which regenerate some chlorophyll (bacteriochlorophyll) after interaction with reductants (25, 26). After the illumination of chlorophyll in the presence of oxygen, chemiluminescence arises as a result of the transformation of high energy chlorophyll intermediates (peroxides) (27). When the chlorophyll solutions were illuminated at low temperatures, a thermoluminescence was observed upon warming or after thawing (labeled "defreezing" in the figure; see Fig. 5); this thermoluminescence was due to subsequent reactions of primary chlorophyll peroxide (28, 29). Electron donors, especially thiourea, strongly quench this photochemiluminescence, thus indicating their interaction with chlorophyll peroxide. On the other hand, the oxidants, the electron acceptors, quench the chemiluminescence by their interaction with excited singlet and triplet chlorophyll. There are the following main steps of the reaction mechanism: triplet excited chlorophyll interacts with oxygen forming the primary photoperoxide; the "dark" stages of primary peroxide transformations lead to the appearance of singlet excited molecules which emit the chemiluminescence quanta.

Excited Chlorophyll–Electron Donor Reactions

Reversible Photoreduction. All the chlorophyll analogues and derivatives possessing hydrogen, magnesium, or zinc in the center of the porphyrin ring are liable to this reaction as revealed in 1948 (reviews 2–5). The ability to react does not depend on the degree of semi-isolated, double-bond reduction; the reaction proceeds in the case of bacteriochlorophyll, too. The primary reversible step is the electron

addition to the system of circularly conjugated double bonds of the porphyrin molecules; the back reaction proceeds in the dark accompanied by initial pigment regeneration. In the primary step of the reaction, an ion-radical pair is formed as registered by ESR measurements and the polymerization technique.

$$\cdot\text{Chl}\cdot + \text{Red} \rightarrow \cdot\text{Chl}^- + \cdot\text{Red}^+.$$

The photoreduction is often accompanied by pheophytin formation due to easier pheophytinization of photoreduced intermediates.

In an aqueous solution of Triton X-100 containing dissolved thiourea and atmospheric oxygen, the reversible photoreduction of chlorophyll is observed. At pH 9, the red-photoreduced chlorophyll is formed; upon stirring with air, the initial green chlorophyll is regenerated. The red-reduced chlorophyll reacts easily with various electron acceptors in the dark. (I wish to mention that we have seen a peculiar case when photoreduction of unexcited chlorophyll was carried out by excited [at 365 nm] reduced pyridine nucleotide analogues [30].)

Evidence for the reactions studied was revealed in most cases by spectral measurements; the photopotentiometric measurements facilitated the study of primary photoproducts (23). The potential reactivity of triplet states of chlorophyll is often proved by variation of the redox potential of the partner molecule. It is not yet clear whether it is possible for an excited or photooxidized chlorophyll to accept an electron from a water molecule (31).

Irreversible Photoreduction. We would expect that analogous to the stepwise oxidation presented above there may also be successive photoreduction from protochlorophyll → chlorophyll → bacteriochlorophyll; it is well known that during illumination of etiolated leaves protochlorophyll is transformed into chlorophyll with high quantum yield. In solutions of protochlorophyll, however, the reversible photoreduction by ascorbic acid is seen; an intermediate which absorbs maximally at 470 nm appears, and a chlorophyllous product is formed usually with negligibly low quantum yield. When pyridine treated with sodium is used as a solvent, the photoreduction of protochlorophyll by ascorbic acid results in irreversible chlorophyll-like pigment formation with a yield of up to 20% (32). In this case the sodium-dipyridyl formed probably may act as an intermediate redox system which facilitates the reduction of the semi-isolated double bond of chlorophyll.

In the course of chlorophyll photoreduction, there are formed several "shortwave" intermediates including a pink-photoreduced form (having an absorption band at 520 nm), but not bacteriochlorophyll. Unlike chlorophylls, the photoreduction of porphyrins in acid media leads to chlorin and possible bacteriochlorin, coming probably from intermediate isomerization (33).

Mechanism of Sensitized Electron Transfer

The mechanism of reactions studied does not involve energy transfer from excited pigment molecules to the reacting molecules, because according to present knowledge

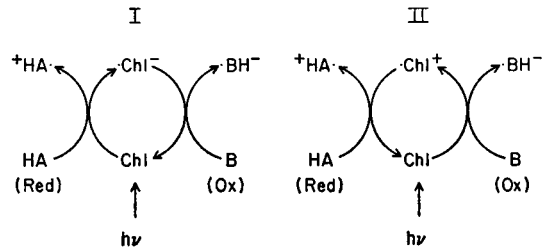

FIGURE 6 The scheme of primary mechanism of chlorophyll-photosensitized oxidoreductions. *hν*, light reactions; others, dark reactions.

most of the electron acceptors and donors used do not possess singlet or triplet levels situated lower than the S^* or the T levels of chlorophyll, a necessary prerequisite of energy transfer. This is probably the case, however, when carotene or tetracene inhibits the photoreactions of chlorophyll.

The experiments indicate that the elementary mechanism of reactions studied involves primary photooxidation or photoreduction of the sensitizing pigment accompanied by a dark stage of interaction between the primary photoproducts and electron donors or acceptors (Fig. 6). These ideas were proposed in primary form by E. Baur, J. Franck, and R. Livingston. The domination of a given type of primary photoreaction depends on the nature of the medium, the properties of reactants, and the pH, as shown in the experiments of Evstigneev (34) and Seely (35).

Along with the involvement of photoreduced and photooxidized pigments in the system of sensitized reactions, there is a possibility of participation of semioxidized electron donors and semireduced electron acceptors in the intermediate reactions; the photooxidation of thiourea results in the appearance of very active reducing substances capable of reducing, in the dark, viologens, safranine, and other electron acceptors. During photosensitized oxidation in the presence of oxygen, two elementary reactions compete: photoreduction of chlorophyll by the electron donor and moloxide formation with subsequent dark reactions of these active photoproducts.

MODELS OF PHOTOSYSTEM II

This photosystem corresponds to the Hill reaction with dichlorophenol indophenol (DCPIP) as oxidant; photooxidation of water molecules is coupled with a suitable reduction. The action of solvents, detergents, and ultrasonic treatment on the ability of chloroplasts to do the Hill reaction, photophosphorylation, and light-induced pH changes was studied in our laboratory. The photophosphorylation inhibition usually coincides with inhibition of the light-induced pH change, both of which require an intact membrane, while the ability to do the Hill reaction is much more stable to damage. We tried to determine the conditions of self-assembly of active chloroplast structure after damage by organic solvents. Under action of ethyl ether on chloroplasts, Hill activity is fully suppressed, and chlorophyll disaggregation is seen by

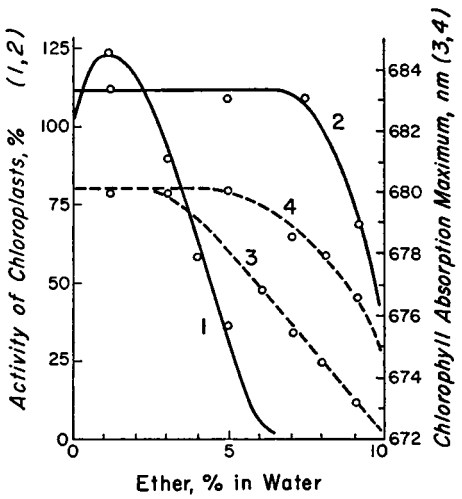

FIGURE 7 The action of diethyl ether on the Hill reaction and chlorophyll absorption maximum in chloroplasts. 1, Hill reaction activity in the presence of ether; 2, after ether removal by evacuation; 3, absorption maximum with ether; 4, after ether removal by evacuation (36).

absorption spectra measurements. After ether removal by evacuation, the Hill activity is restored (see Fig. 7), and the primary state of chlorophyll (680 nm absorption maximum) reversibly appears (36). Similar effects were observed under the action of dimethyl sulfoxide and methanol on chloroplasts. If the solvent concentration is above some critical value, the damage is irreversible. It seems that under the action of solvents, local solubilization of pigments in lipoproteidic lamellar structures proceeds and the removal of solvent causes the self-assembly of primary structure; however, we cannot yet reconstruct the Hill reaction when chlorophyll is extracted from the chloroplasts' structure.

We succeeded in creating inorganic models of the Hill reaction (37, 38). Zinc, titanium, and tungsten oxides were used as photosensitizers in water solutions of oxidants, ferric ammonium sulfate, ferricyanide, or *p*-benzoquinone. When illuminated by long wave UV in the region of the absorption band of the photosensitizer, the model system evolved molecular oxygen with quantum yields of up to 1% (39).

The stoichiometry of the reaction studied was the same as that of the Hill reaction:

$$2Fe^{+++} + H_2O \rightarrow 2Fe^{++} + 2H^+ + \tfrac{1}{2}O_2 .$$

The following primary mechanism of reaction was proposed. After light absorption, the electron of the semiconductor lattice is shifted into the conducting zone, leaving behind a hole (electron vacancy). The water molecules (or hydroxyl ions) and ferric ions are probably absorbed on different active centers of semiconductor particle surface. The following elementary acts probably take place:

$$Fe^{+++} + e^- \rightarrow Fe^{++},$$

$$OH^- + \oplus \rightarrow OH.$$

Subsequent OH radicals recombine at the semiconductor surface, which results in oxygen evolution. In the case of WO_3, the mechanism may be complicated by photochemical cleavage of WO_3, leading to lower oxide formation which gives a dark reaction with added oxidant. The realization of models described above becomes possible because the semiconductor particles function not only as photosensitizers but also as catalysts which promote recombination of primary photoproducts. Metzner (40) described similar reactions using silver chloride sensitized by the absorbed dyes excited by visible light.

Aggregated Chlorophylls

The question is, What mechanism of electron transfer operates in living cells? The elucidation of this problem requires knowledge of the state of pigments in the photosynthetic organisms. The bulk of chlorophyll in a chloroplast exists in aggregated forms closely bound to the lipoids and proteins of the lamellae and enzymatic partners of the electron transfer chain. The bulk of pigment acts as an antenna absorbing light quanta and transferring the energy of excitation to an active center, the latter probably is a type of pigment aggregate. The study of the photochemical properties of the aggregated pigment is thus important.

Self-Assembly of Pigment Molecules in Aggregates. The state of pigments in cells differs from that in solution. Usually the absorption and fluorescence spectra of pigments in vivo are situated at longer wavelength regions as compared with those of the dissolved pigments. This phenomenon is particularly pronounced in the case of bacteriochlorophylls. The spectral effects of aggregation were studied in the laboratories of E. Rabinowitch, C. S. French, and S. Brody.

We observed that pigments in solid films and colloids reveal the types of aggregation seen in living cells and perfectly reproduce the spectra of pigments in organisms (see 2–5). Thus, solid films of chlorophyll *a* (obtained by evaporation of ether solution) have an absorption maximum at 680 nm just as do chloroplasts (41–43); in water vapor at 20°C, a shoulder appears at 705–715 nm; heating it up to 50°C leads to a crystalline form absorbing at 740–745 nm. The vapors of methanol, ethanol, and aqueous solutions of acetaldehyde, formaldehyde, and ammonia produced the same effect at room temperature, probably because the bonding with magnesium atoms of chlorophyll makes "bridges" between the pigment molecules in aggregates. The solid films of bacteriochlorophyll usually possess two absorption maxima at 800 and 860 nm; in the vapors of water, alcohols, or acetaldehyde containing ether, a long wave maximum at 900–920 nm appears. The films of *Chlorobium* chlorophyll (bacterioviridin) display the same long wavelength shift (740–760

nm); dissolved pigment absorbs at 665 nm as chlorophyll *a*. The farnesol "tail" in the bacterioviridin molecule, being shorter than phytol in chlorophyll, facilitates aggregation. Similar effects are seen in low temperature fluorescence spectra of aggregated pigments. A study of the fine structure of absorption and luminescence spectra of aggregated pigments in cells and in solid films has shown their similarity; the detailed study of this problem was undertaken by Litvin and coworkers (44).

Thus, the chemical structure of pigment molecules and the presence of low molecular additives determine the mode of pigment molecular arrangement in aggregated structures in chloroplasts and chromatophores. The gradual conversion from monomeric to aggregated chlorophyll forms in the course of greening of etiolated leaves was studied at great length in our laboratory with the aid of absorption and luminescence spectra measurements. The specificity of action of light quanta on aggregated pigments, as compared with photochemistry of monomeric forms which dominate in solutions, remains to be elucidated.

Photochemistry of Aggregated Pigments

Photosensitization. In 1946 in our work with Brin, the photosensitizing activity of crystalline phthalocyanines was revealed. Photosensitizing action of aggregated pigments is most convenient to study in the case of such irreversible reactions as photoreduction of azo dyes or cytochromes. Preferential formation of a different pattern of aggregates, having absorption bands at 680 and 740 nm, was found in aqueous colloidal solutions of chlorophyll and bacterioviridin. The measurements of quantum yield of methyl red photoreduction had shown the equal effectiveness of both forms (45). Similar results were obtained with bacterioviridin colloids; however, the quantum yield of photosensitization by monomeric forms is much higher.

Photodisaggregation. We proposed that destructive bleaching of aggregated pigments in chloroplasts proceeds via intermediate disaggregation. That phenomenon was observed most clearly in the case of illumination of leaves of pigment-deficient maize mutants (Fig. 8) (46). The illumination of leaves *in vacuo* results in a strong enhancement of fluorescence; after the admittance of air, a quick decrease of fluorescence results because of the bleaching of monomeric chlorophyll. To observe disaggregation it is necessary to avoid oxygen to prevent bleaching of monomeric chlorophyll. The photodisaggregation phenomenon was seen also in model systems, by illumination of aggregated pheophytin in a water-acetone mixture; the effect was observed only in the presence of oxygen, probably being a special case of pheophytin oxidation (Fig. 9) (44).

Elementary Photoprocesses in Aggregates. Terenin and Kholmogorov (48) observed an ESR signal in solid chlorophyll films in the presence of water and oxygen. It was not then possible to see the triplet signal in chloroplasts and solid pig-

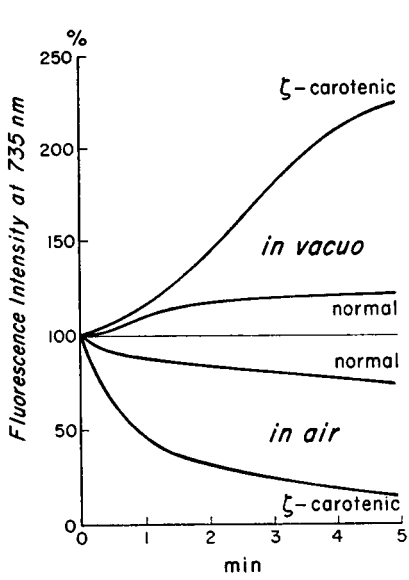

FIGURE 8

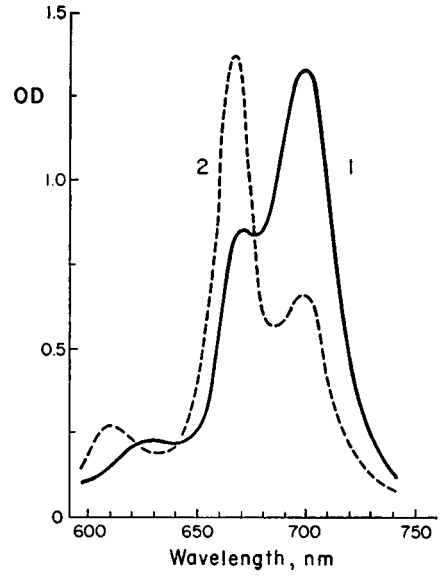

FIGURE 9

FIGURE 8 Photodisaggregation and photooxidation of chlorophyll in normal and mutant (ζ-carotenic) maize leaves (46).

FIGURE 9 Pheophytin photodisaggregation in acetone-water. 1, initial spectrum; 2, after illumination (47).

ment films by ESR measurements; in the same experimental condition, the chlorophyll solutions reveal a clear triplet signal (49). Many years ago Terenin and Nelson found photoconductivity in solid films of chlorophyll. Litvin and Zvolinsky (50) demonstrated that the quantum yield of photoconductivity is higher in "long wave" quasi-crystalline forms than in the "shortwave" ones; the same phenomenon was observed in the films of chloroplasts and bacterial chromatophores. Studying the photoeffect on the boundary of solid pigment film and solution, Evstigneev and Terenin (51) had found that the photopotential depends on the nature of dissolved substances. The effect was studied in detail by Evstigneev (52). Based on these experiments, one can present the mechanism of the photosensitizing action of aggregated forms that would be similar to the action of inorganic semiconductors: an electron acceptance by a molecule on the phase boundary equilibrated by an electron donation by the donor molecule to the "hole" that also migrates to the phase boundary. This elementary mechanism is complicated with the energy migration from shortwave to long wave pigment forms.

Reversible Photoreactions of Aggregated Pigments. We studied reactions of aggregated pigments with electron donors and acceptors in solid films and water colloid solutions. The solid films of chlorophylls possess very low fluorescence at room temperature; freezing to liquid nitrogen temperature leads to enhancement of the long wave fluorescence of aggregated forms. *In vacuo*, the solid films possess

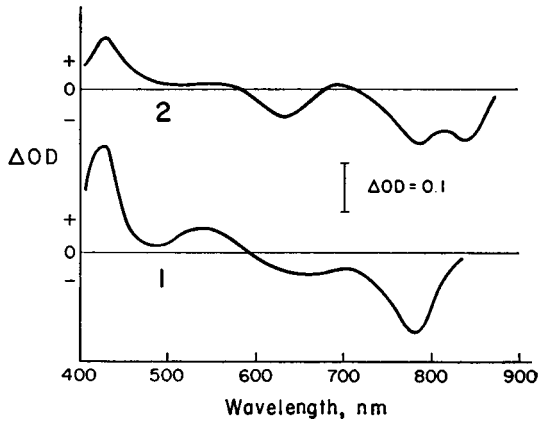

FIGURE 10 Reversible photooxidation of aggregated bacteriochlorophyll by *p*-benzoquinone in alcohol-glycerol. Differential absorption spectra: 1, after 2 min of illumination; 2, after 12 min of illumination (54).

luminescence at room temperature; oxygen causes the quenching of this long wave luminescence. This quenching, however, is seen under the action of the light beam exciting the fluorescence, and it lasts several seconds; this quenching is not a physical one but it is due to a photochemical reaction with oxygen (53). The most active reaction occurs in the case of long wave pigment forms that are possibly arranged without any close packing of pigment molecules.

In a viscous alcohol-glycerol (1:9) solution, having an excess of glycerol, the aggregates of bacteriochlorophyll and chlorophyll dominate, remaining in equilibrium with some monomers. The illumination of solutions containing *p*-benzoquinone had shown the possibility of reversible photooxidation of aggregates; intermediates correspond, by their absorption spectra, to the photooxidized monomer (Fig. 10) (54). The mechanism of the reaction is not yet clear; perhaps the intermediate stage is pigment photodisaggregation. It is possible that energy migration from monomeric to aggregated pigments is active.

Thus, all the data presented demonstrate that monomeric and aggregated pigments that are excited are able to undergo reversible oxidoreduction and sensitization of oxidoreductive reactions, modeling the fragments of photosynthetic electron transfer chain. The question arises as to whether these properties of pigments underlie their action in photosynthesis. The study of photochemical pigment transformations in vivo by the aid of differential spectroscopy and flash techniques has revealed spectral changes usually ascribed to triplet state formation, and oxidoreductive and acid-base transitions involved in electron and proton transfers. It is usually proposed that these changes take place in the reaction centers: the long wave pigment aggregates bound to proteins and lipids. These changes are coupled with the redox transformations of biocatalysts: cytochromes, quinones, etc. The results on the study of chlorophyll photochemistry in isolated systems are in agreement with the findings

in the in vivo systems, but the ultimate elucidation of the mechanism of primary photoreactions in photosynthesis requires much more effort.

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