Excitons and Their Migration

In photochemical reactions in condensed systems, intermediary steps can occur between light absorption and the primary photochemical reaction: the photochemical processes of energy or charge transfer. Either the excited electron, or the hole it leaves in the absorbing molecule, may move, to be found at the end of the excitation period in a site far removed from the original locus of excitation. If the electron and the hole travel separately, the final effect is separation of charges—which can be also described as internal oxidation-reduction.

The electron-hole pair is often referred to as an exciton. When the excited electron and the hole are associated in a single molecule, the (perhaps superfluous) term “intramolecular exciton” is used. Figure 1 illustrates the different modes of transfer of intramolecular and intermolecular excitons (1).

The binding energy of the exciton may be so small that it can dissociate under the influence of thermal motion, or under the pull of an applied electric field (photoconductivity). If impurities are present in the system, or adsorbed on the surface, they may trap the electron, or the hole, or both.

Recently, it has been pointed out that the concentration of pigment molecules in the photosynthetic organelles is so high as to justify approaching photosynthesis from the point of view of phenomena in the solid state, making the above considerations relevant.

Structure of Chloroplasts

All chloroplasts contain lamellae—flat or involuted, thin extended layers, about 20 μ thick. They are found also in chloroplast-free cells of the blue-green algae. The lamellae either fill out the whole body of the chloroplast (lamellar chloroplasts), or form cylindrical grana (granular chloroplasts). Figures 2 and 3 show both types of chloroplasts in the same plant. Figure 3 shows that in granular chloroplasts, the lamellar structure extends into the intergranular stroma; the grana are merely approximately cylindrical volumes in which the lamellae are denser and more numerous.

The high optical density of the lamellae in preparations fixed with osmic acid (as in figs 2 and 3) is due to higher concentration of precipitated osmium; and this in turn seems to be due to the presence of lipids. Consequently, the lamellae must contain the greatest concentration of lipids, while the stroma is more predominantly proteinaceous. In granular chloroplasts, absorption and fluorescence microscopy confirm that the (lipophilic) pigments are located in the grana.

Chlorophyll, whose molecule consists of a flat, slightly polar, colored conjugated ring system (chlorin) and a long, colorless tail (phytol), should accumulate on interfaces between the hydrophilic and the hydrophobic layers. Thomas and co-workers (2) compared the number of chlorophyll molecules in the chloroplasts of 8 species with the total surface areas of their lamellae. The results varied between 1.8 μ and 3.8 μ per chlorophyll molecule, while the amount of chlorophyll per chloroplast varied by a factor of 5 x 103.

Artificial chlorophyll surface layers (3, 4) exist in two forms: crystalline and amorphous. In the first form, the area requirement is about 0.45 μ2 (suggesting a 2-molecular layer), and the red absorption
Fig. 1. Transfer of intramolecular and intermolecular excitons. ○, hole; ●, electron; and *, excited electron. Top row, creation (1) and transfer (2), (3) of intramolecular exciton—Förster-Heller-Marcus mechanism. Center row, same—Wannier mechanism. Bottom row, conversion of intra- into intermolecular exciton (1); its transfer (2), (3); and dissociation (4).

band lies at 735 mµ; in the second form, the area requirement is 1.06 mµ², and the red absorption band lies at 678 mµ.

In both types of artificial surface layers, the chlorophyll ring system must be stacked obliquely, since the actual area of this system—which would be required for the molecules to lie flat on the interface—is about 2.4 mµ². This is close to the average found by Thomas et al for chloroplasts. The position of the absorption band in vivo—at 675 to 680 mµ (fig 4)—shows the absence of crystalline, and supports the possible presence of amorphous monolayers.

The electron micrographs suggest the existence of flat chambers enclosed between pairs of lamellae in grana (fig 3). One can suggest that one product of the photochemical reaction (e.g., the reduction intermediate) is trapped in the chamber, while the other—the O₂ precursor—escapes on the outside. Lamellar chloroplasts, containing no such chambers, also are capable of photosynthesis; but some evidence suggests that their lamellae tend to associate in pairs; so that a similar separation mechanism could also operate in them.

The arrangement of chlorophyll in monomolecular layers was confirmed by Goedheer's (5) study of the (relatively weak) dichroism and the (pronounced) birefringence dispersion of large chloroplasts. According to Goedheer, his observations suggest that chloroplasts contain very thin (0.2 to 0.6 mµ thick) pigment layers, which are obviously monomolecular. The axes of the chlorophyll molecules seem to be only very imperfectly oriented, thus accounting for the weakness of the dichroism. Goedheer suggested that this may be due to the monolayer covering not a flat, but a grained surface. Spherical macromolecules can, in fact, seen on the surface of lamellae (6). They have a diameter of 7 to 10 mµ, and thus a surface of 150 to 300 mµ² (fig 5). This should be enough to accommodate 150 to 300 adsorbed chlorophyll molecules, if the sphere could be covered uniformly—which is somewhat difficult if the macromolecules are incorporated in lamellae.

The hypothetical picture of protein macromolecules carrying 200 or 300 molecules is similar to the empirical picture of phycobilins as protein macromolecules (mol. weight ≈ 280,000) carrying 50 to 100 chromophores (S. and M. Brody (7)). The main difference may be in the additional association of the chlorophylls with lipoids (probably through the hydrophobic phytol tail), which prevents the extraction of chlorophyll macromolecules with water.
III. States of Chlorophyll in the Chloroplasts

Various evidence indicates that chlorophyll in the chloroplasts is present in at least two forms (see e.g., Krasnovsky et al (8) and Smith et al (9)). It has been suggested that one is monomeric and another aggregated. Apparently discordant measurements of life-time and of the quantum yield of fluorescence (in Chlorella) by Brody (10) and Latimer (11) can be reconciled by the assumption that about one fourth of the chlorophyll is in the fluorescent state (with a yield of approximately 10%), while three fourths is non-fluorescent (giving an average yield of 3%). Other observations (Brody (12)) make it appear likely that one non-fluorescent form of chlorophyll in vivo has an absorption band near 705 mμ, and a fluorescence band at 720 mμ (which appears only at low temperatures).

The monomeric and polymeric, fluorescent and non-fluorescent forms of chlorophyll in vivo probably mean different states in a monolayer—possibly distinguished by the density of the monolayer, or the kind of molecules with which it is associated. Franck (13) concluded, from kinetic considerations, that two types of chlorophyll molecules in vivo are characterized by contact with water, or with non-polar molecules, respectively. The former are fluorescent; no free electrons can occur in this hydrated part of the monolayer. The lipid-protected part could be non-fluorescent, and could support the existence of free electrons. According to Platt (14), the non-fluorescent ππ excited state must be lower than the fluorescent ππ state in lipid-bound, and higher than the latter in the hydrated form, thus accounting for the latter's capacity for fluorescence (fig 6).

Franck suggested that for the primary photochemical process in photosynthesis, 2 excited molecules are needed, one of which must be in the ππ excited state, while the other can be in the ππ state.

IV. Energy and Charge Transfer in Chloroplasts

The problem of energy transfer in photosynthesis arose in 1936, when Gaffron and Wohl (15) interpreted the results of Emerson and Arnold's flashing light experiments (showing that the maximum yield of O2 per brief flash (10⁻⁵ sec) is about 1 molecule of O2 per 2000 molecules of chlorophyll), by the assumption that about 2000 chlorophyll molecules act as a photosynthetic unit, an energy catch-basin associated with a single reduction site, in which the photochemical process takes place. The number 2000 must be reduced (perhaps, by a factor of 8) because the liberation of 1 molecule of O2 requires several (perhaps 8) elementary photochemical processes. The photosynthetic unit is therefore most likely to consist of about 250 chlorophyll molecules. This makes it possible to consider identifying the unit with the pigment-bearing macromolecules in the lamellae, which according to section II, may carry 100 to 300 chlorophyll molecules.

Gaffron and Wohl said that cooperation between the many light-absorbing chlorophyll molecules and a single reaction center in a unit can be attributed to the migration, either of energy-rich particles generated at each chlorophyll molecule, or of energy quanta. The second picture has fascinated workers in photosynthesis in the last 20 years, without a definite answer being found as to its relevance.

Goedheer (5) observed a very low fluorescence polarization of phycobilin, suggesting that the excitation energy moves among the differently oriented chromophores attached to the macromolecule. In analogy, one could expect energy exchange also between chlorophyll molecules attached to a common macromolecule. It may perhaps take in several adjacent macromolecules, thus accounting for the rela-

Fig. 2 (left). Chloroplasts (Zea mays). The 3 chloroplasts on the left are granular, the 2 on the right are lamellar. (After Vatter.)

Fig. 3 (right). Chloroplast structure (Zea mays). Cross section of a granular chloroplast, showing intergranular lamellae. (After Vatter.)
Figure 4. Absorption curves of 2 types of chlorophyll surface layers on water (arrow indicates the position of the bands in solution). (After Jacobs et al.)

tively large number (at least 250) molecules in the unit; however, in this case, only one out of several macromolecules could carry an enzymatic reaction center.

Plant cells need a high concentration of pigment to provide sufficient light absorption; but they do not have enough space to provide a separate enzymatic conveyor belt for each chlorophyll molecule. A high ratio (chlorophyll):(enzyme) is possible because even in direct sunlight, a chlorophyll molecule absorbs quanta only about once every 0.1 second, while enzymes can handle their substrates in a small fraction of this time. Excitation energy migration in the photosynthetic unit provides an elegant solution to the problem of how to utilize the light-absorbing capacity of several hundred pigment molecules to feed a single enzymatic conveyor belt.

It has been asked (Franck and Teller (16)) whether enough time is available between light absorption and energy dissipation to permit effective energy transfer in the unit. If the transfer is by random walk, considerably more than 250 transfers will be needed to assure effective delivery of the quantum to the reaction center in a 250-molecule unit. (On the other hand, it may be sufficient to conduct the quantum into the neighborhood of the center, rather than to a single specific pigment molecule). The natural life-time of chlorophyll in the Chl* excited state is 15 m\(\mu\) sec. (Brody and Rabinowitch (10)). With 3% fluorescence yield (Latimer, Bannister and Rabinowitch (11)), the actual life-time is 0.5 m\(\mu\) sec; with 10% yield, 1.5 m\(\mu\) sec. A thousand energy transfers during this period means a visiting time of 0.5 to 1.5 \(\times 10^{-3}\) m\(\mu\) sec. The width of the absorption band of chlorophyll in vivo suggests undisturbed coupling with intramolecular vibrations, and this requires electronic excitation to last \(\gg 10^{-4}\) m\(\mu\) sec. Comparison of the 2 figures (0.5 to 1.5 \(\times 10^{-3}\) m\(\mu\) sec available and \(\gg 10^{-4}\) m\(\mu\) sec needed) shows just about enough time for the postulated number of visits.

The range of effective migration could be extended if one could assume that fluorescence is limited by transfer of 'Chl*' into the metastable 'Chl-state (which is plausible), and that energy migration can continue by the Wigner mechanism 'Chl' + 'Chl' \(\rightarrow\) 'Chl' + 'Chl'. Resonance transfer in the triplet state was demonstrated by Terenin and co-workers (17) in frozen hydrocarbon solutions. However, it requires an overlapping of the electron clouds of the exchange partners to make their total spin a significant invariant, and what we know about the chlorophyll monolayers in vivo may not justify this assumption.

The mechanism of energy migration considered so far corresponds to figure 1 (a), (b)—the migration of an intramolecular exciton. It may be asked whether the formation and transfer of intermolecular excitons (fig 1 (c)), with separation of charges, also is possible. This was postulated by Arnold (18) to account for light-induced electron trapping in dried chloroplast layers, and by Commoner et al (19), and Calvin et al (20), to account for light production of unpaired spins, revealed by paramagnetic resonance. However, the postulate that light absorption in the chloroplast leads to electron transfer into a conductance hand, or at least, to the formation of a loosely bound intermolecular exciton, meets with difficulty. The absorption band of chlorophyll in vivo is very similar to that in vitro; this suggests that the excitation leads to the same intramolecular excited state. Simultaneous hole-electron transfer can then occur as an "afterthought" because of resonance between chlorophyll molecules. A similar delayed transfer of the electron without the hole—i.e., conversion of an intramolecular into an intermolecular exciton, indicated in figure 1 (c)—could occur only if the energy level of the second is below that of the first, which seems unlikely. (See note added in proof). It is, however, possible that charge separation occurs when the migrating exciton encounters an impurity, or a chlorophyll mole-

Figure 5. Granular surface of a lamella (after Steinmann).
cule in a special state, which can serve as a “trap” for either the electron or the hole.

Since the quantum yields of the observed creation of trapped electrons (Arnold) and of free electron spins (Commoner, Calvin) appear to be low, these may follow exceptional absorption acts, e.g., in chlorophyll molecules immediately adjacent to traps. Furthermore, effects observed with dried chloroplasts may well be due to aggregates formed in drying. S. Brody (21) observed that illumination of crystalline chlorophyll does lead to abundant production of free spins.

Franck suggested that free electrons may be viable in the water-free “protected” parts of the chlorophyll layers, but Franck’s mechanism of photosynthesis (13) does not require their occurrence.

Calvin and co-workers (20) believe that photochemical separation of charges, followed by electron and hole migration across the lamella can separate the oxidation products from the reduction products in photosynthesis. It was often pointed out by this author that this separation is the real crux of photosynthesis; in non-living photochemical processes, high energy intermediates often are produced, but tend to disappear rapidly by recombination. However, the pigment layers in chloroplasts probably are monomolecular; therefore, all that is needed to separate the oxidation product from the reduction product, is for them to be produced on different sides of this layer. They could then be drained into alternate interstices, without the need for more electron conductance than is made possible by the aromatic structure of chlorin.

Electron and hole migration in the plane of the lamella is a different matter. One could conceive of it as helping to initiate reactions in 2 different enzymatic sites—the reduction of an appropriate intermediate (such as TPN), and the generation of free O₂. However, this mechanism would be less efficient in preventing recombination; and it, too, is made unlikely by the above-mentioned spectroscopic evidence.

To sum up, we suggest, as a working hypothesis, that migration of an intramolecular exciton is the most important type of energy migration in the chloroplast, bringing excitation energy close to the enzymatic center, located in each, or in each few, macromolecules in the lamellae.

We still need to specify the location of the different types of chlorophyll, as well as of the accessory pigments, particularly in view of Emerson’s observations (22) suggesting their distinct photochemical functions. In the case of the phycobilins, it is likely that their macromolecules also lie in the lamellae. The carotenoids (other than the photosynthetically highly efficient fucoxanthol of brown algae), must be distributed in a different way; if Platt’s picture (23) of them as “electron pipelines” between excited chlorophyll molecules and electron acceptors (or donors) is correct, some carotenoids must be associated with the enzymatic reaction centers.

V. Photochemical Function of Chlorophyll

There are other, chemical data which require fitting into the picture. These are findings by Krasnovsky and co-workers (24), Rabinowitch and Weiss (25) and Bannister (26) of the capacity of chlorophyll for reversible photochemical reduction and oxidation, suggesting that chlorophyll sensitizes photosynthesis by undergoing one, or both, of these reactions. From the studies of chlorophyll-sensitized reductions of various oxidants by ascorbic acid in pyridine, it appears (Evestigneev and Gavrilova (27), Bannister (26)), that chlorophyll is first reduced to an unstable product (a free radical?), which can reduce such unwilling oxidants as riboflavin; if not immediately reoxidized, it is then transformed into the more stable, probably valence-saturated, reduced form, pink in the case of chlorophyll a (eosinophyll). Examination of difference spectra of illuminated Chlorella cells by Coleman and Rabinowitch (28) suggested—but did not prove—that this last product may accumulate in cells—up to 1/300 of total chlorophyll when photosynthesis becomes light-saturated. The similarity of this figure and the size of the unit suggests that chlorophyll molecules associated with the reaction centers may be the ones to become reduced (perhaps, only to unstable radicals as long as no light saturation occurs, and then to saturated eosinophyll molecules).

This picture of the photophysical and primary photochemical stages in photosynthesis is speculative, and may prove wrong in parts or in toto. It seemed, however, tempting to try to develop such a picture from the data accumulated by recent research in various laboratories.

The experimental work in part utilized in this paper was carried out in the Photosynthesis Laboratory of the Botany Department at the University of Illinois by E. E. Jacobs, S. S. Brody, M. Brody, P. Latimer, and T. T. Bannister, with the assistance of the Office of Naval Research and continuous ready and invaluable help of the late Dr. Robert Emerson. The electron micrographs were obtained by A. Vatter at the Electron Microscope Laboratory of the University.

Note added in proof: Rosenberg (Faraday Society Discussion on Energy Transfer, April, 1959) pointed out that, in some molecular melts, strong photoconductivity is observed without the appearance
of a new absorption band leading to a conductance level; such conductance could result, for example, from coalescence of the metastable triplet levels of adjoining molecules, so that transfer into the triplet state, commonly postulated to precede the primary photochemical act, becomes identical with transfer into a conductance band. It remains to be seen, however, whether such processes can occur with high efficiency in non-crystalline chlorophyll monolayers.

BIBLIOGRAPHY

1. See, for example, Halbleiterprobleme, Vol. I, article 1 (M. Volz), and Vol. IV, articles 1 (H. Haken), and 1 a (W. Schottky).

* Many of these available in translations by E. Milner, from the Crerar Library, Chicago.