

REMARKS ON THE LONG-WAVE-LENGTH LIMITS OF PHOTOSYNTHESIS AND CHLOROPHYLL FLUORESCENCE

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The photosynthetic quantum yield of *Chlorella* and other green algae is, in general, independent of the wavelength of the light absorbed by the chloroplast pigments. Two exceptions to this rule have been found. First, light absorbed by β -carotene is less efficiently used for photosynthesis than that absorbed by the chlorophylls; and, second, there is a rapid decline of the quantum yield with increasing wave length from 6850 Å on. According to Emerson and co-workers,¹ this so-called long-wave-length limit of photosynthesis begins very near the maximum of the red absorption band of chlorophyll *a*. More recently, Duysens² found that the fluorescence yield of these algae, i.e., of their chlorophyll *a*, has the same long-wave-length limit as photosynthesis and that the curve of fluorescence yield versus wave length is very similar to the corresponding curve of the photosynthetic quantum yield.

In blue-green algae and, particularly, in red algae the quantum yields depend strongly upon the pigment which absorbs the light. Blinks and Haxo³ studied these algae very carefully. In general, they found that light absorption by β -carotene or by chlorophyll *a* contributes very little to the process of photosynthesis and gives an unusually small yield of fluorescence of chlorophyll *a*. The long-wave-length limit of both the photosynthesis and the fluorescence-yield curves is shifted considerably to shorter wave lengths and coincides with the beginning of the absorption bands of the phycobilins. Blinks interpreted these results by assuming that most of the chlorophyll *a* molecules in these algae neither participate in photosynthesis nor are able to fluoresce. Light absorbed by these chlorophyll *a* molecules is presumably lost by dissipation as heat. Such behavior would be expected, for instance, if most of the chlorophyll were present in a form of colloidal particles. Since, according to Blinks, only a few chlorophyll *a* molecules are photochemically active and able to fluoresce, these must be situated near enough to the phycobilins that excitation energy can be transferred from the phycobilins with a high yield. Blinks's picture is thus in agreement with the results of French⁴ and of Duysens,³ that excitation energy always migrates to the pigment whose absorption spectrum extends farthest to the red, i.e., in algae, to chlorophyll *a*.

In an important paper published recently in these PROCEEDINGS, Emerson *et al.*⁵ have shown that Blinks's assumption that the major part of chlorophyll *a* is entirely inactive cannot be correct. Rather, they found in *Chlorella* that light of wave length longer than the limit can be utilized for photosynthesis with the same quantum yield as light of shorter wave length, provided that the long-wave-length irradiation is supplemented by a short-wave-length irradiation. In a second paper, presented at the April, 1958, meeting of the National Academy (and made available to the present author through the kindness of Professor Emerson), Emerson *et al.* show that the same is true for blue-green and for red algae. They conclude that two types of excited states of dyes play a role in photosynthesis and that they

differ in the amount of energy they contain. The low-energy type might be used for a step of low-energy demand, the higher may have sufficient energy to promote the high-energy, as well as the low energy, step. Long-wave-length absorption is supposed to form the excited states of lower energy; shorter-wave-length absorption, on the other hand, will produce excited states of higher-energy content. The two types of excitation levels are ascribed by Emerson to two different plant pigments. According to him, the lower one in *Chlorella* is the first excited singlet state of chlorophyll *a*, and the higher one is the first excited singlet state of chlorophyll *b*. Indeed, evidence presented by the authors proves that light energy absorbed by chlorophyll *b* in *Chlorella* is much more efficient as a supplementary source of radiation than is light absorbed by chlorophyll *a*, even if its wave lengths are shorter than the long-wave-length limit. In blue-green and in red algae, the corresponding excitation of a phycobilin replaces that of chlorophyll *b*.

The requirement that the first excited singlet levels of two different dyes each be photosynthetically active implies abandoning the idea that excitation energy is always transferred to chlorophyll *a*; it offers no explanation for the behavior of the fluorescence yield of chlorophyll *a*.

We propose to avoid the contradictions between the conclusions of Blinks and of Emerson by the following alterations: (1) In Blinks's assumptions, we replace the idea of an abnormally high probability of radiationless transitions from the first excited singlet state to the ground state of the bulk of the chlorophyll *a* by that of an abnormally high transition probability from excited singlet to metastable triplet state of chlorophyll *a*. (2) Instead of Emerson's conclusion that the first singlet excitation states of two different dyes are utilized for the photochemical steps of photosynthesis, we make use of the conclusion—on which the present author has based his theory⁶ of the photochemical steps of photosynthesis—that a co-operation between two different quantum states of chlorophyll *a* is needed for photosynthesis, i.e., between chlorophyll *a* molecules in their first excited singlet state with others in their lowest metastable triplet state. For information about the two proposed ways of visualizing such a co-operation, the reader is referred especially to the *Handbuch* article and the paper of Brugger and Franck.⁶ Both theoretical pictures discussed there lead to the conclusion that optimal quantum yields can be expected only if the number of singlet states used for photosynthesis is at least as great as that of the metastable triplet states. It follows that any condition which leads to an unusually high proportion of metastable states depresses the yield of fluorescence and photosynthesis. The questions are: Does irradiation of *Chlorella* with long-wave-length red light produce mainly metastable states, and, if so, why should this be the case for light of all wave lengths absorbed by chlorophyll *a* in blue-green and red algae?

In the following paragraphs we propose answers to these questions, which are based on spectroscopic evidence that two types of electronic systems participate in the light-absorption act of the chlorophylls; one of these electronic systems has the quality of producing, under irradiation, metastable states in excess.

Chlorophyll molecules possess—as do a number of other aromatic compounds—in addition to the normal set of quantum states of the π -electrons, other sets which correspond to transitions of a non-bonding electron into unoccupied orbits of the π -electronic system.⁷ Pairs of non-bonding electrons are situated in certain chem-

ical groups connected with, or adjacent to, the conjugated ring system. In chlorophyll, two nitrogen atoms of the pyrrole rings and the oxygen of the C=O group of Ring V contain such non-bonding electrons. Since the chlorophylls have a very extended system of conjugated bonds, the energy difference between singlet excitation in the $n-\pi$ and $\pi-\pi$ system is very small. Consequently, each band of the absorption spectrum of the chlorophyll contains contributions of the $\pi-\pi$ and $n-\pi$ transitions. The two types of transitions differ in several ways. The $n-\pi$ bands are weaker and more narrow than the $\pi-\pi$ bands; the transition moments of the two electronic systems are perpendicular to each other; the $n-\pi$ singlet levels possess an abnormally strong admixture of triplet character, whereby the probability of radiationless transitions between singlet and metastable triplet becomes so high that the fluorescence intensity is lowered to an exceedingly small value; the $\pi-\pi$ levels shift to lower-energy values with rising dielectric constant of the solvent (red shift); the energy of the $n-\pi$ levels, on the other hand, rises with the dielectric constant (blue shift).

The enumerated particular qualities of the $n-\pi$ levels explain, according to Platt and to Kasha,⁷ the discovery of Livingston⁸ that chlorophylls *a* and *b* are practically non-fluorescent in dry benzene (non-polar solvent). If small quantities of water are added, the fluorescence yield rises to values nearly as high as are observed in alcohols and other polar solvents. Platt and Kasha concluded that in benzene the first excited $n-\pi$ singlet lies below the $\pi-\pi$ singlet level. Thus the fluorescence is quenched because the excitation energy will be transferred to the lowest singlet state, in this case an $n-\pi$ singlet, which, for the reasons mentioned above, shows no fluorescence. Small additions of water are supposed to reverse the order of the $n-\pi$ and $\pi-\pi$ singlets. The $\pi-\pi$ level becomes the lower one because of the blue shift of the $n-\pi$ level and the red shift of the $\pi-\pi$ level.

Platt's and Kasha's explanation is undoubtedly correct; however, it is necessary to extend somewhat the analysis to remove certain apparent difficulties. They are as follows: (1) The sensitivity of the fluorescence in benzene toward water is exceedingly high. One water molecule per chlorophyll molecule seems to be sufficient to allow the fluorescence to reappear. Furthermore, the water must have a direct or indirect influence on the magnesium atom of the chlorophyll because removal of the magnesium destroys the effect. (2) The presence of small concentrations of water changes the absorption spectrum of the chlorophyll. It enhances considerably the F -value of the long-wave-length band of the chlorophylls. Other polar molecules do cause similar effects but only if they are present at much higher concentrations. (3) Platt's and Kasha's assumption implies that the phosphorescence yield of chlorophyll is high because the quenching of the fluorescence is assumed to be caused by the high probability of radiationless transitions into the metastable triplet state. The observation shows, on the other hand, only a very weak phosphorescence in chlorophyll *b* and none in chlorophyll *a*.

An explanation of these deviations from expectations becomes possible if one considers that the chlorophylls should have three metastable states, namely, one $\pi-\pi$ metastable triplet state, one $n-\pi$ state involving the oxygen atoms of the CO groups, and another $n-\pi$ state connected with non-bonding electrons of the nitrogen atoms of the pyrrole rings. The last-mentioned metastable triplet state is the most interesting because it is connected with the problems 1 and 2. Two of the four ni-

trogen atoms are at each moment bound to the magnesium atom, while each of the two remaining nitrogens has an unshared pair of electrons. The magnesium bonds resonate quickly between nitrogen pairs. Therefore, the magnesium atom must be situated at the center of the four nitrogen atoms. We interpret the high sensitivity against water by the assumption that one water molecule becomes attached to two of the nitrogens by hydrogen bonds. That binds this water molecule relatively strongly and enhances the force by which the non-bonding electrons are held in their ground state. Therefore, one water molecule attached to a chlorophyll is sufficient to shift the first excited $n-\pi$ singlet level considerably to a higher-energy value. Furthermore, it causes a stoppage of the resonance of the magnesium bonds. These bonds will stay with one pair of nitrogen atoms, which will attract the magnesium atom, and it is therefore drawn away from the center, and that changes the charge distribution of the conjugated ring system. The great sensitivity of the fluorescence against water is then an indication that in dry benzene the $n-\pi$ singlet connected with the nitrogen is the one which lies below the $\pi-\pi$ singlet. Its blue shift by contact with water raises the fluorescence yield; the change of absorption spectrum of chlorophyll is a by-product.

For the third problem, we propose a solution based on the results of Kanda and Sponer⁹ with other aromatic compounds. If we apply these to the case of the chlorophylls, we come to the conclusion that the two metastable triplet states of the $n-\pi$ electronic system must lie considerably higher in the energy scale than the metastable $\pi-\pi$ state. Therefore, three different phosphorescence spectra might be expected, of which the spectrum emitted by $\pi-\pi$ transitions from the metastable state to the ground state has the longest wave lengths. However, radiationless transitions between the higher $n-\pi$ triplet and the lower one will be exceedingly quick and the same will be true at room temperature for the radiationless transitions from both $n-\pi$ triplets to the metastable $\pi-\pi$ triplet. That quenches the emissions of the two higher levels, and only the phosphorescence spectrum which has the longest wave lengths remains. That is in agreement with Livingston's and with Linschitz's results,¹⁰ who observed in their studies of the absorption spectrum of the metastable state at room temperature that only one metastable state was present. However, if the temperature is very low, the rate of the internal transitions between $n-\pi$ and $\pi-\pi$ triplets is expected to become slow enough to permit a weak $n-\pi$ phosphorescence. The reason for such a delay of the radiationless transitions would be a small "heat of activation" needed to make the transitions possible between quantum states with radiation moments perpendicular to each other. That has been observed, too, by Kanda and Sponer⁹ in other aromatic compounds.

We therefore ascribe the weak phosphorescence of chlorophyll *b* with a wave length $<1 \mu$ observed by Becker and Kasha at -195°C .⁷ to the $n-\pi$ system and assume that a much stronger phosphorescence spectrum with wave lengths $>1 \mu$ would have evaded observation on account of the insensitivity in this wave length (compare R. Livingston's⁸ last quotation) region of the photomultiplier system used. It is of interest that Tollin and Calvin¹¹ recently observed under special conditions (afterglow of dried chloroplasts) an emission spectrum of chlorophyll *a* in this long-wave-length region. However, experiments with chlorophyll dissolved in organic solvents are still needed to clarify this situation further.

We now have to answer the question how the described experiments with $\pi-\pi$

and $n-\pi$ transitions of chlorophyll in vitro can be used to explain the observations of Emerson and of Blinks of the long-wave-length limit of photosynthesizing cells.

All chloroplast pigments are adsorbed at proteins. They are at least partly sandwiched between alternating layers of lipoprotein and protein having less lipoidic character. This has been shown by electron-microscopic observations. Studies of the afterglow of chlorophyll in plant cells and chloroplasts (Strehler and Arnold),¹² together with Arnold's and Sherwood's observations¹³ of a weak electronic photoconductivity of dried chloroplast smears, gave special information about the situation of chlorophyll a and its surroundings. The bulk of it must be adsorbed as a monomolecular layer in a kind of crystalline order, and it must be protected against contact with liquid water and its solutes by a lid of fatlike protein. If these conditions were not fulfilled, neither the observed conductivity nor the recombination connected with light emission having the fluorescence spectrum of chlorophyll a would be possible. For these and further deductions on afterglow, the reader is referred to the paper of Brugger and Franck.⁶

Therefore, the bulk of the chlorophyll a in the protected chlorophylls cannot be directly connected with the photochemical processes of photosynthesis. To make the excitation energy of the protected chlorophylls available for photosynthesis, the excitation energy must be transferred to the small percentage of chlorophyll a molecules which are exposed to the water and its solutes. Such transfer can be performed in an efficient way because the contact with water causes a red shift of the singlet and triplet levels, provided that they belong to the $\pi-\pi$ electronic system. A more detailed analysis reveals that the excitation energy of the lowest excited singlet, as well as of the $\pi-\pi$ metastable triplet, can be transferred to the few exposed chlorophylls. If all the protected chlorophylls are situated in a surrounding which lifts the $n-\pi$ singlet levels above the $\pi-\pi$ singlets, this transfer should be so quick that practically only singlet excitation migrates from the protected to the unprotected chlorophylls. If, on the other hand, the influence of the surroundings puts the $n-\pi$ singlet levels lower than the $\pi-\pi$ levels, the transfer to the $n-\pi$ singlet will be the fastest process in the protected chlorophylls, followed immediately by radiationless transitions into the metastable states. Thus, in this case, only metastable excitation energy is transferred from the protected to the exposed chlorophylls.

A model in which only transfer of excitation energy from protected to unprotected chlorophyll a is taken into account would then, in the first of the two cases, give high photosynthetic and fluorescence yields and no dependence on wave length because both singlet and triplet energy is transferred. In the second case the rates would be zero at all wave lengths because only triplet energy is transferred. More probable than this model, with its "all-or-nothing," response would be one which leads to the alternative of photosynthetic rates lower than optimal at all wave lengths or to a good yield at short wave lengths and a low one at the longest wave-lengths of the chlorophyll a absorption. The condition for the first alternative would be that one of the two $n-\pi$ singlet levels lies very little above the $\pi-\pi$ singlet level. In that case thermal fluctuations at room temperature would cause very frequent transitions from the $\pi-\pi$ to the $n-\pi$ singlet, with the result of lowering the yields.

The second alternative would occur if, in one part of the protected chlorophylls, both $n-\pi$ singlets lie high enough above the $\pi-\pi$ singlet to avoid thermal transitions,

while another part is in a surrounding where the two $n-\pi$ singlets or one of them lies below the $\pi-\pi$ singlet. The contribution of this latter part of the chlorophyll to the yields would then be small; and, since the $n-\pi$ absorption band corresponding to this part of the transition lies at the long-wave-length side, low yields would occur only in this long-wave-length region. Our present knowledge of the structure of the chloroplasts is not sufficient to predict the exact positions of the $n-\pi$ and $\pi-\pi$ levels of the protected chlorophylls. We do know, however, that they should be influenced by the polarity of the proteins themselves and by the moisture they contain. In estimating these influences, we have to take into account that most of the hydrogen bonds of the protein itself are utilized for protein-protein interlinking and thus might be only to a small part available for linkage with the non-bonding electrons. The hydrogen of the water molecules might not be available at all places. If the water concentration is small in the protein, only a few number of molecules might be found in the right positions to form a bridge between two nitrogen atoms. Our conclusions are that great differences in the water content of protein can be caused by small variations in the structure of the chloroplasts in different species of algae. They can be made responsible for the whole variety in the transfer of excitation energy from protected to exposed chlorophylls, as is indicated by the differences in the occurrence of the long-wave-length limit. In some algae a difference in the yields might occur if light of different wave lengths were absorbed by chlorophyll *a*. In others, light of all wave lengths absorbed by chlorophyll *a* may give low yields (etc.).

Differences of the quantum yields are also possible if, under the influence of changes in the external conditions, the content of water in the protein should change. That seems to be a plausible interpretation of the observations of Seybold and Egle¹⁴ that in non-aquatic algae, living on the bark of trees, a lowering of the humidity of the air causes a lowering of quantum yields of photosynthesis and fluorescence.

We now have to discuss why—in those cases in which light absorbed by chlorophyll *a* gives low yield at all wave lengths—absorption acts in accessory plant pigments may give high yields in spite of the fact that the energy absorbed by the latter pigments is transferred to chlorophyll *a*. In these cases we are forced to the conclusion that the excitation energy acquired by the accessory pigments is preferentially transferred to the unprotected chlorophylls instead of to the protected ones. That becomes possible if the distance between the location of the accessory pigments to the unprotected chlorophylls is smaller than that to the protected ones. Indeed, such a situation has a great probability because we know that accessory pigments cannot be indiscriminately interspersed into the layer of the protected chlorophyll *a* molecules. Otherwise they would disturb the crystalline order of the latter to such an extent that no electron conductivity and no occurrence of after-glow could result. Thus we assume that, for instance, the phycobilins are absorbed outside from the lid of protecting fat and are situated nearer to the position of the unprotected chlorophylls. On the other hand, that is not expected to be the case for β -carotene. This pigment is supposed to be adsorbed at the lid of the fat itself and thus should transfer its excitation energy to its direct neighbors, the protected chlorophylls. It is therefore understandable that light absorbed by β -carotene has not higher and sometimes lower efficiency than light absorbed by chlorophyll *a*

itself. Chlorophyll *b* is supposed to disturb much less the crystalline order of the protected chlorophyll *a* molecules; thus it might be partly located under the lid of fat. However, the observations of Emerson, Chalmers, and Cederstrand make it probable that the greater part of chlorophyll *b* is adsorbed at the protein in locations nearer to the unprotected chlorophyll *a* molecules than to the protected ones.

On the basis of the preceding discussion, it is simple to fit into our scheme the most important part of the results of Emerson *et al.*, i.e., the influence of supplementary irradiation by light of shorter wave lengths on the photosynthetic quantum yield of light of longer wave lengths than the limit. According to our thesis, the latter produces predominantly chlorophylls excited to the metastable states. If long-wave-length irradiation is added to the illumination by shorter wave lengths, the concentration of metastable excited chlorophylls, respectively, that of their photochemically produced photosynthetic intermediate, will rise to a higher steady-state value than this concentration would be if the total number of light quanta had been offered in the form of short-wave-length light. That has the effect of enhancing above normal the utilization for photochemical purposes of singlet energy produced by the short-wave-length irradiation. Thereby, it lowers the production of metastable states by the short-wave-length irradiation and lowers the fluorescence yield. In brief, the simultaneous irradiation with long- and short-wave-length light shifts the main chore of producing the needed singlet excitation to the short-wave-length irradiation, while the light of long wave length provides most of the metastable excitation. The over-all effect is that the combined irradiations with both light sources adjust the production rates of singlet and metastable excited chlorophyll *a* molecules in such a way that again equal number of singlets states and of metastable states are utilized for photosynthesis.

In summarizing, we state that the discoveries of Emerson *et al.* strongly support the concept that two quanta are needed to achieve the utilization of one hydrogen atom and one OH radical of a water molecule for photosynthetic purposes. Furthermore, it strongly supports the main hypothesis of our theory that a cooperation between the first excited singlet state of chlorophyll and its metastable triplet state is a necessary condition for the process of photosynthesis.

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CRYSTAL STRUCTURE AND INFORMATION THEORY

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I. *Introduction.*—From the standpoint of “information theory” as set forth by Shannon,¹ Shannon and Weaver,² Brillouin,³ and others and more particularly from the viewpoint of “description mechanics,”⁴ the study of crystal structures using X-ray data involves the consideration of three models. These models have a point-by-point relationship with one another as follows: (1) the crystal is called the “describee,” (2) the diffraction pattern is a “code” which is to be deciphered, and (3) the proposed structure (or solution) is a “replica” which should have a direct similarity to the describee.

The basic assumption in this type of study is that no more information can be gained than is contained in the diffraction pattern. The “descriptive capacity” of the crystal as a describee and of the diffraction pattern as a code can be computed, and thus the adequacy of the diffraction pattern in describing the crystal can be estimated. The methods for making such estimations will be illustrated in the following paragraphs through the use of a one-dimensional diffraction pattern.

II. *The Describee.*—Following the methods of description mechanics, we first draw a line whose length represents the length a of a single crystallographic unit cell in the x direction. Then we mark the distance a into N equal intervals which we call “description boxes.” These description boxes each have dimension $\Delta x = a/N$. This distance, Δx , represents the accuracy with which we wish to locate the atoms in the unit cell. If we know from other sources that there are M identical atoms per unit cell, then the descriptive capacity of the crystal is the number of ways of placing M atoms in N boxes with no more than one atom per box. If we were dealing with point atoms, the descriptive capacity ω_c' would be

$$\omega_c' = \frac{N!}{M!(N-M)!} \quad (1)$$

But, since atoms occupy volumes that greatly exceed the limits of accuracy, another