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Molecular Aspects of Some Photobiological Receptors*

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Abstract

The photobiological receptors of phototactic, phototropic, and photomorphogenic responses of various organisms have been described in terms of spectroscopic, photophysical and photochemical properties which may be relevant in elucidating the energy transduction mechanism(s) in photobiology. The photoreceptors discussed include carotenoids, flavins, stentorin and phytochrome. Although the molecular modes of their photobiological action still remain largely unexplained, it is possible to suggest several primary molecular processes of the photoreceptors in eliciting responses of various organisms to light.

I. Introduction

It is almost inconceivable to describe "biology" without "photochemistry." One of the most fundamental processes in nature involves interactions between plants (and some bacteria) and light, i. e., photosynthesis. In addition, there are quite a few forms of interaction between biological systems and light, and as such these photoresponses of many organisms represent the fascinating subject of study in photobiology and photochemistry.

The aim of the present essay is to suggest various molecular mechanisms involving selected photobiological receptors on the basic of spectroscopic and photochemical properties of their electronic excited states. At the outset, it should be pointed out that many of our models developed are speculative and their biological applicability remains to be tested. Nonetheless, it is felt that the models proposed would stimulate further experiments which may lead to a better understanding of the primary mechanism (s) of energy transduction in photoresponsive organisms.

II. Molecular Relaxation Processes of Photoreceptors

Responses of *Euglena*, *Phycomyces*, morning glories, corn seedlings and many others to light are triggered by their respective photoreceptors which absorb light(analogous to rhodopsin which is the photoreceptor in animal eyes). In order to understand the molecular mechanisms involved in the primary photoprocess of light response events of organisms, it is useful to describe how the photoreceptors absorb (or detect) and utilize

In "Nong Hwahak Hwoejee", The Professor C.Y.Lee 60th Birthday Commemmorative Issue, 1977.
* This paper is dedicated to Professor Chun-Yung Lee who inspired me to the study of biochemical science.

the absorbed light energy in effecting the light-response behaviors of the organisms. Clearly, the light response or sensory transduction is an energy conversion process; light absorbed by the photor eceptors is efficiently converted to a chemical or biological form of energy which can trigger phototropism, phototaxis, etc.

The photoreceptor bound to a protein or membrane absorbs a quantum of light. The light-energized photoreceptor generated relaxes to the ground state via several modes of relaxation processes. Such relaxation modes can be coupled to dynamic as well as static conformational changes of protein and/or membranes to which the photoreceptor is bound. The coupled relaxation may then lead to affect the membrane potential, the structural specificity of the photoreceptor relative to its effector site, and regulation of growth regulators or hormones, etc.

We envision several coupling modes between a photoreceptor's electronic relaxation and the macromolecular conformation. Molecular relaxation of the excited photoreceptor includes the following possible schemes.

(a) **Photophysical processes.** Internal conversion, intersystem crossing and fluorescence from the lowest electronic excited state are not by themselves useful for effecting conformational changes of protein or membrane. However, the fluorescence can be followed as a probe to monitor the photoreceptor microenvironment and its dynamics.

(b) **Acid-base equilibria.** The pK_a of acidic and basic centers of photoreceptors can change dramatically upon their light excitation. Aside from the obvious implication in the light-induced proton gradient and transmembrane potential, changes in pK_a in the excited state (pK_a^*) may also induce static and dynamic conformational changes of interacting proteins and/or membrane.

(c) **Excited state dipole moment.** The perm anent dipole moment of a photoreceptor usually changes upon light excitation, entailing reorientation of the photoreceptor and/or surrounding residue dipoels of the protein or membrane.

(d) **Polarizability.** The polarizability, both isotropic and anisotropic,of photoreceptor changes upon excitation. Because of its instantaneous evolution and disappearance in concert with the light absorption process, utility of the polarizability change as a driving force for conformational change is limited, although the anisotropic polarizability may have a significant effect on the microenvironmental dynamics of chromophore binding site.

(e) **Conformational change and isomerization.** Electronic excitation can bring about conformational changes in the photoreceptor chromophore. Photoisomerization in rhodopsin is well known.[1] These chromophore changes will often be accompanied by conformational changes of the interacting protein and/or membrane. In this connection, photo-induced viscosity changes of polymer solution arising from the photoisomerization of bound azo-dyes serves as a good illustration.[2]

(f) **Photodissociation of the photoreceptor.** Photodissociation equilibria of molecular complexes are well known. The bound photoreceptor may temporarily dissociate in the excited state or in metastable state,resulting in conformational changes in the protein or membrane matrix. The reverse(association) is also possible(c. f., binding of phytochrome P_{fr} produced from the excitation of P_r form; *vide infra*). The dissociation may be brought about after photo reactinos such as in the photoisomerization of 11-*cis* retinal rhodopsin to *all-trans* retinal and opsin.[1]

(g) **Photoredox reactions.** The photoredox reaction has a direct implication on the membrane potential.

III. Experimental

Materials described in the essay have been obtained as reported in various publications from this laboratory. The absorption and fluorescence spectra were recorded on a Cary 118C spectrophotometer and Perkin-Elmer Hitachi MPF3 or single-photon counting high resolution spectrofluorometer. The circular dichroism spectra were

recorded on a JASCO-20 ORD-CD spectropolarimeter equipped with the photoelastic modulator-PAR lock-in amplifier/phase detector(Model 121). The nanosecond fluorescence lifetimes were measured on an SLM phase/modulation fluorometer, as described recently.[3]

IV. Carotenoids as Photoreceptor

A number of action spectra for photoresponses of certain organisms retemble the absorption spectra of carotenoids, the most recent identification of the action spectrum in terms of a carotenoid being the photoinduced carotenoid biosynthesis in *Neurospora crassa*.[4] In the case of phototaxis in *Euglena*[5] and phototropism in *Phycomyces*[6,7] and *Avena* coleoptile[8], the photoreceptor is likely to be a flavoprotein. In our previous paper,[9] it was concluded that carotenoids are a less likely candidate for a photorecepter than flavoprotein in these photoresponses. Among other reasons, the short lifetime due to efficient internal conversion($S_1 \rightsquigarrow S_0$) was regarded as the most critical barrier for a carotenoid or carotenoprotein to overcome as a photoreceptor. This viewpoint still holds, as far as the primary photoreceptor for sensory transduction is concerned.Additionally, the action spectra show a significant contribution at 350—380 nm which is not present in carotenoid spectra. However, it is possible under certain circumstances for carotenoids to act as either a primary or secondary photoreceptor. We discuss these possibilities below.

(a) Retinal Schiff's Bases. Retinal Schiff's bases occur as the vision photoreceptor chromophore (11-*cis* retinal) in rhodopsin.[1] In addition, bacteiorhodopsin also utilizes the retinal Schiff's base as the photoreceptor for generating proton pump, ATP production and phototaxis in *Halobacterium halobium*.[10a,b] These are two examples of well established photoreceptors of the short chain carotenoid.

Retinal(Fig. 1, 1) fluoresces at low temperature with $\phi_f = 0.017$, λ_{ex} at 410nm.[11,12] Poliyenes show anomalously long fluorescence lifetime in contrast to the predicted behavior according to the relation[13] $\tau_f^0 = \tau_f^{obs}/\phi_f$. This is also true for retinal.[12] However, rhodopsin does not fluoresce even at low *temperature. This is apparently due to the fast*-phototransformation of rhodopsin to bathorhodop-

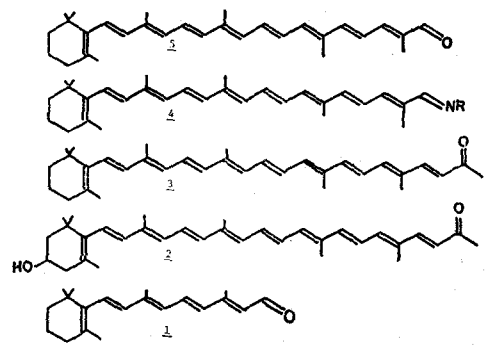


Fig. 1. Structure of Carotenoids. 1, all-*trans*-retinal; 2, reticulataxanthin; 3, citrana xanthin; 4, β-apo-8′-carotenal Schiff's base; 5, β-apo-8′-carotenal.

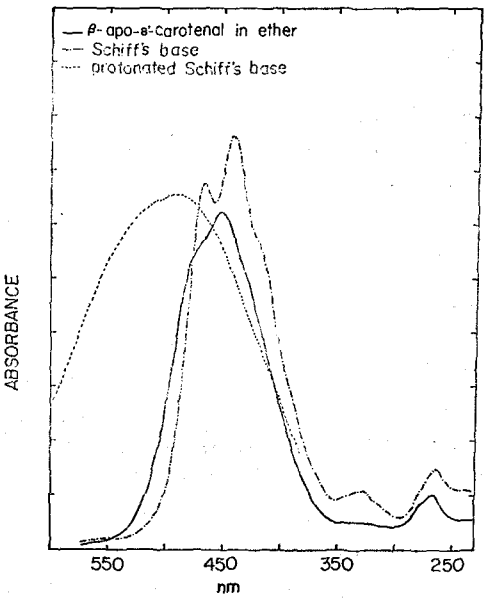


Fig. 2. Absorption spectra of β-apo-8′-carotenal (—); neutral Schiff's base(-·-·-) and protonated Schiff's base (···) in ether at room temperature.[46] The method for preparation of Schiff base and the calcu lated transition energies, oscillator strengths, polarizations and excited state dipole moments of these species are available elsewhere.[24]

sin, which is formed with a rate constant greater than 10^{11} sec.$^{-1}$ [14]

Bacteriorhodopsin emits weak fluorescence in aqueous solution[15-17] at room temperature($\phi_f=2\times10^{-5}$)[17] and at 77K($\phi_f=3\times10^{-4}$)[17]. We have also measured the fluorescence spectrum of bacteriorhodopsin(gift from Prof. W. Stoeckenius) in ethylene glycol:water (1 : 1) mixture at 77K. The fluorescence lifetime was found to be$<$50 psec, which is shorter than the subnanosecond time sesolution of the phase-modulation spectrofluorometer. Alfano *et al.*[17a] and Hirsch *et al.*[17b] reported $\tau_f=40\pm5$ psec at 90K and 15 psec at physiological temperature respectively.

It is unlikely that the weak emission from the purple membrane is of the $A_g^- \longrightarrow A_g^+$ origin, but the emission is probably from a relaxed B_u state on the proein. Furthermore, its lifetime is not intrinsically affected by the chromophore-chromophore exciton interaction in the purple membrane.[17,18]

It is clear from the above consideration of the lifetime and primary photoprocesses that the retinal Schiff's base of rhodopsin and bacteriorhodopsin act as a highly efficient photoreceptor for energy transduction elicited by the coupling of the photoisomerization and membrane receptor potential and by the proton pump-ATP formation, respectively. In considering carotenoids as possible photoreceptors, it is assumed that neither phototropic nor phototactic (blue+near UV) action spectra of plants and microorganisms can be readily resolved with retinal, because free retinal and retinal Schiff's base (neutral form) absorb maximally at 360—400 nm rather than at 400—500 nm and that the protonated Schiff's base bound to protein absorbs at $\lambda_{max}>480$ nm.* Furthermore, there is no definite evidence that blue lightresponsive(phototropic and phototactic) organisms produce retinal for their photobiology.

Long conjugated carotenoids possess very short-lived excited states so that the primary photoprocess necessary in the sensory photoreception act cannot be very efficient.[9] However, there are ways te overcome this difficulty, as discussed in section (b) below.

(b) Carotenals and Carotenones. Symmetric carotenoids without functional groups at the ends of the conjugated chain, which comprise the majority of carotenoids in nature, are not likely to be the photoreceptor pigment due to their lack of photoreactivity (e.g., inefficient ϕ_{isc} and photoisomerization) and extremely shoft lifetime.[9] However, there are naturally occurring carotenoids with at least one carbonyl end,[19] particularly apo-carotenals in plants.[20-23]

In order to examine how apo-carotenals and carotenones can possibly function as the primary photoreceptor, we studied the spectroscopic properties of selected carotenoids. Fig. 2 shows the absorption spectra of apo-carotenal and its Schiff's base. It can be seen that the Schiff's base has the most likely absorption spectrum in fitting several phototropic and phototactic action spectra (with resolution in the blue light region), except for the lack of a strong near UV peak. The latter difficulty can be resolved if the carotenoid is isomerized to the *cis* isomer. The protonated Schiff's base of this carotenal shows a λ_{max} shifted too far to the long wavelength region. Reticulataxanthin (and citranaxanthin) shows similar spectral characteristics (Fig. 3).

Our principal objective in preparing Schiff's base and protonated Schiff's base was to attempt to observe fluorescence. Blatz and Pippert[26]have shown that the retinylic carbonium ion is fluorescent and shows a very small Stokes shift(1,300 cm^{-1} as opposed to 9,000cm^{-1} for the parent

* The absorption spectrum ($\lambda_{max}\sim400$nm) of all-*trans* retinal in isopentane at 77K is significantly red shifted relative to the room temperature spectrum.[24] In general, the low temperature absorption spectra mimic those of the protein bound polyenes.[9] In some cases (e.g., beta-lactoglobulin-retinol complex), binding to protein is more effective in shifting λ_{max} and resolving the vibronic-bands.[25]

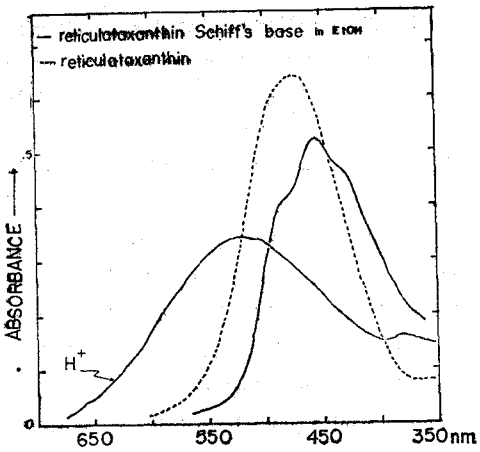


Fig. 3. Absorption spectra of reticulataxanthin (···), Schiff's base (—) and protonated Schiff's base in ethanol at room temperature.[46]

retinol), indicating that when bond alternation is reduced the Frank-Condon forbidden nature of the transition is also reduced. Our theoretical study[24] of these molecules revealed that aside from the excited state dipole moment(μ^*) of the protonated form, they differ very little from their neutral parents. No fluorescence was observable from carotenals or their Schiff bases.

The dipole moment for the ground state (^{1}A) and first excited singlet (^{1}B) state of the Schiff's bases and protonated forms have been calculated (Fig. 4). It can be seen that the dipole moment of the ^{1}B state of the protonated form is several times larger than that of the ^{1}A state. In the unprotonated form the change in dipole moment is also significant, though smaller in absolute magnitude. Increase in the pK_a of the Schiff's base nitrogen upon excitation accompanies the dipole moment increase. Based on these results, the following scheme for the primary photoprocess of a carotenal Schiff's base photoreceptor is proposed (Fig. 5).

This scheme is applicable for consideration to those blue light photoresponse systems with action spectra lacking the near UV peak in which a flavoprotein photorceptor is less likely for one reason or another. In Fig. 5, the approximate

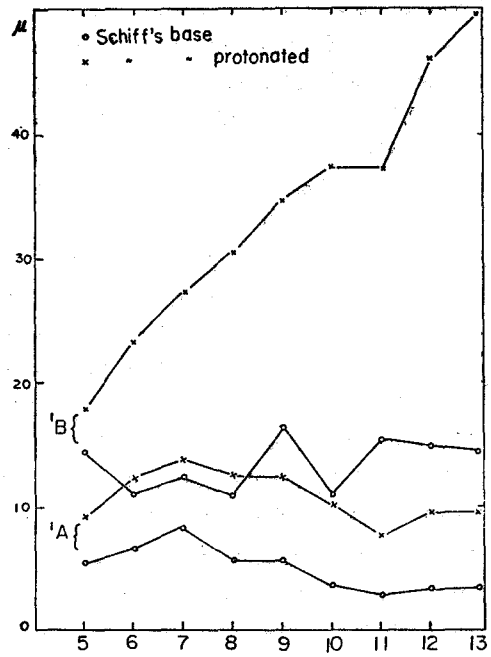


Fig. 4. Ground and lowest singlet state dipole moments for polyene Schiff's bases of 5 to 13 double bonds calculated by the P-P-P method. The ordinate is in Debye units.[46] ○—Schiff's base ×—protonated Schiff's base □—azlactone^{1}A state △—azlactone ^{1}B state

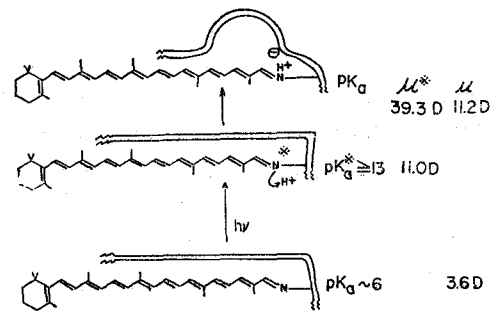


Fig. 5. The light induced proton uptake and conformational change of membrane(schematic). Approximate pK_a values and dipole moments (calculated) are shown.

pK_a values based on the present π-electron density calculation and pK_a value of 6.95 for all-*trans*-retinal Schiff's base[27] are also given.

The implication of the scheme shown in Fig.

5 is that a proton pump(uptake) can be produced by electronic excitation of the chromophore, which then leads to the membrane potential change induced by conformational changes of the chromophore and/or membrane due to the increased dipole moments of the excited state Schiff's base and its protonated form(Fig. 4). The longer carotenal Schiff's bases are more suited to induce the conformational changes owing to the larger dipole moment of the excited state(Fig.4). Thus, the proposed mechanism is analogous to the well established proton pump (H^+ release) of the purple membrane of *H. halobium*[10] and the luciferin-luciferase system.[28] The proton release pump is not likely with the presumed carotenal Schiff's base photoreceptor, as the absorption spectra of the protonated Schiff's bases are at $\lambda \geq 550$ nm region inconsistent with the blue light photoresponse.

There is no experimental evidence for or against the occurence of carotenals and carotenones as the photoreceptor. However, carotenoproteins have been isolated from various sources.[29] Furthermore, it is interesting to note that all well established photoreceptors of light signal amplifying response systems are covalently bound(e.g., phytochrome, phycocyanin, phycoerythrin, rhodopsin, bacteriorhodopsin, etc.). We therefore propose that a search for carotenoid Schiff's bases as photoreceptors of the blue light responses be made,as long as the near UV maximum is absent in the action spectra corrected for the screening effect of near UV absorbing materials.

Carotenoid Schiff's bases must still overcome the extremely shoft lifetime of the excited singlet state ($<10^{-13}$sec)[9] in order for them to be an efficient photoreceptor. Thus, carotene, carotenals, carotenones and their Schiff's bases do not emit fluorescence even at 12K (measuring on a single photon counting spectrofluorometer), in contrast to retinal and its Schiff's base[99] which fluoresce with relatively large quantum yields (greater ahan 10^{-2}). Nonetheless,it is conceivable that the combined effects of a fast primary photoprocess and the excited state lifetime as mdified by the Schiff's base linkage, binding to protein or membrane, and exciton interactions[30] among nearest neighbor chromophores can be operative. In this connection, the fluorescence lifetime of all-*trans* retinol is lengthened upon binding to beta-lactoglobulin by a factor of 4.3 at room temperature(τ_f=2.8 nsec for free retinol in ethanol; τ_f=11.8±0.3 nsec for the bound retinol in phosphate buffer).[25] The retinol binding to the protein also brings about a dramatically resolved absorption spectrum.[25]

(c) Carotenoids as the Secondary Photoreceptor. The problem of short lifetime of the excited state incarotenoids as a photoreceptor can be partly overcome by exciton interactions, as shown recently in the photoreceptor pigment complex composed of 4 peridinins, 1 chlorophyll *a* and 1 protein isolated from marine dinoflagellates.[30] Energy transfer (Figs. 6 and 7) from the exciton state of the secondary photoreceptor(i.e., carotenoid) to the excited state of the primary photoreceptor molecule (i.e., chlorophyll *a*) can account for the phototactic action spectra of several dinoflagellates.[31] Fig. 6 shows the molec-

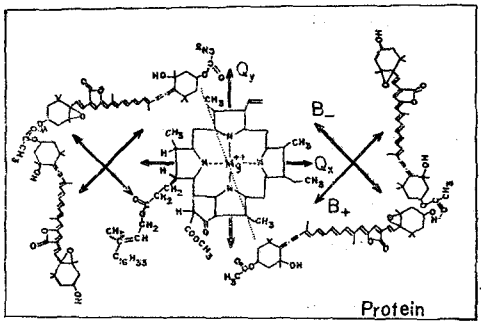


Fig. 6. A probable molecular topography of chlorophyll *a* and peridinins based on relative orientations of transition moments (double arrows) of Q_y(fluorescence) and B^+(exciton) transition.[30] The calculated polarization axis of the $^1Q_y \leftarrow A$ transition deviates by 18° from the C_{2v} —assumed Q_y axis (unpublished SCF MO CI data). If the calculated Q_y axis is adopted, the two pairs of peridinins should also be rotated by 18°.

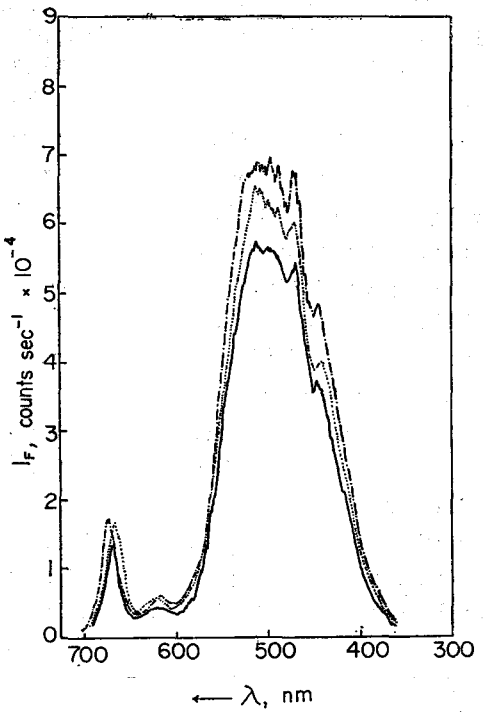


Fig. 7. Fluorescence excitation spectra of PCP's (-·-:*Gl. sp.*, ···: *A. rhyncocephaleum*, —; *G. polyedra*) in Tris-glycerol(1 : 4) at 200 K, recorded on the single-photon counting spectrofluorometer with an excitation bandpass of 0.4nm. It can be seen that the absorption of light at 440 —550 nm by peridinins efficiently leads to the fluorescence emission from chlorophyll *a*.

ular topography of the peridinin-chlorophyll-protein complex. Energy transfer from the dimeric peridinin exciton state to chlorophyll a occurs with the maximum efficiency(100%), as determined by the fluorescence excitation spectra (Fig. 7).

If the molar extinction ratio of the primary to the secondary photoreceptors is greater than 1 : 10 and maximum energy transfer is assumed, the measured action spectra of blue-light responses would resemble the absorption spectra of carotenoids (as the secondary photorceptor) with little (due to screening) or no action peak at the absorption maximum of the primary photorceptor. This is analogous to the photosynthetic action spectrum which is mostly contributed by the antenna chlorophylls, as the contribution of the reaction center chlorophylls is not readily discernible in the gross action spectrum. Such a possibility should be explored in order to establish whether some of the blue light responses are triggered by a carotenoid photoreceptor (in an exciton-like assembly, interacting among carotenoids and/or between carotenoids and an unidentified primary photoreceptor).

Finally, it should be noted that the idea for energy transfer from the secondary to the primary photoreceptors has been previously proposed by Briggs.[32] Krinsky[33] critically reviewed the function of carotenoids in blue light response systems. The present author's view is that carotenoids are far less likely to be the primary photoreceptor in the blue light incuced sensory transduction than flavins, notwithstanding possible models described in section IV(b).[9,39] In fact, non-covaleltny bound carotenoids can be ruled out as the photoreceptor for blue light responses for which the action spectral maxima occur in the 450 and 360 nm regions, as even *cis*-carotenoids with sufficiently high absorbance at 350 nm[9,45,46] are not able to overcome the kinetic difficulty imposed by the short excited state lifetime.[9] This difficulty may still prevail for carotenoid Schiff's bases to act as the *primary* photoreceptor, though not entirely dismissible until a search is made for them. However, it is more likely that carotenoid plays a role as a secondary photoreceptor in several blue light responsive organisms (e.g., phototaxis in dinoflagellates[31] and in *Chlamydomonas reinhardi*.[47]).

V. Flavins as Photoreceptor

Recent reports[7,34] indicate a strong support for flavin as the photoreceptor in phototropism. The action spectrum of phototaxis for ***Euglena*** can also be best resolved in terms of flavin.[35-38] The relative merits of flavins vs. carotenoids as the photoreceptor have been discussed in detail from the view point of primary photophysics and photoreactivity of these molecules.[9,39] It was

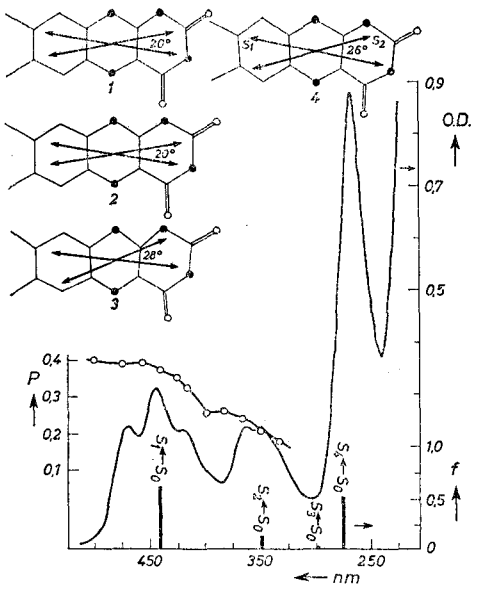


Fig. 8. Absorption spectrum (—), polarized fluorescence excitation spectrum (-o-o-) in ethanol at 77 K, and calculated polarizations of two absorption bands at 350 and 445 nm. Calculation 1 based on assumed geometry, and 2, 3, 4 based on observed geometries.[101]

concluded that the flavin meets various requirements for being the blue light response photoreceptor much better than doep carotenoid.[9,40]**

Fig. 8 shows the absorption spectrum of riboflavin at 77 K. The low temperature spectrum usually mimics the absorption spectrum of chromophore tightly bound to the protein.[9,25,39]

In addition, flavin photochemically mediates blue light induced absorbance changes as the result of photoreduction of cytochrome *b* in photoresponsive fungi with the familiar 450 nm+near UV action spectrum,[41-23] implicating the likelihood of a flavin photoreceptor. However, blue light induced absorbance changes attributable to the photoreduced cytochrome *b* may not be the primary photoprocess responsible for the blue light response behavior of organisms.[44] In this section, we examine probable mechanistic aspects of flavin as the phototropic and phototactic photoreceptor.

(a) **Photophysical Requirements of Flavin as the Photoreceptor.** With the notable exceptions of D-amino acid oxidase[48] and lipoate dehydrogenase[49] most flavoproteins are non-fluorescent or only very weakly fluorescent. This is most probably due to strong interactions between FMN or FAD and aromatic residues, particularly trp. Flavoproteins of this type are not suitable as the photoreceptor, since the excited state is effectively quenched via static and/or dynamic quenching processes.In fact, the highly efficient intersystem crossing in free flavins[39,49] is almost completely suppressed in flavoproteins.[50]

From the brief consideration given above, it is suggested that flavins in the phototropic and phototactic photoreceptors not be bound at a site where the excited state is quenched via static charge transfer interactions. Thus, the flavoprotein photoreceptor is likely to be fairly fluorescent particularly when the photorecptor functional unit is disrupetd (e.g., isolated or frozen), It is aniticipated that the photoreceptor in a fully operational form *in vivo* is non-fluorescent or very weakly fluorecent owing to the efficient primary photoprocess responsible for triggering blue light responsive sensory transduction. In this connection, a covalently bound flavin is of special interest. Flavins covalently bound to proteins are well known.[51] In the following spection, we consider possible primary photoprocesses of the flavin

** Hartmann and Unser[97] proposed that blue light response action spectra represent neither flavin nor carotenoid as the photoreceptor on the basis of an elaborate analysis of the photoreceptor dichroism for the "low irradiance movement" of the *Mougeotia* chloroplast. They argue that phytochrome is the active pigment for the blue light response. This conclusion implies that the primary photoprocess initiates directly from an upper electronic excited state and that highly efficient radiationless transitions to the lower electronic states do not occur, as is predicted from the time tested theories and experiments on radiationless processes of organic molecules. Zurzycki[98] has satisfactorily resolved the blue+near UV action spectrum for chloroplast movement in *Funaria* in terms of two transition moments for flavin as the photoreceptor.

photoreceptor.

(b) Molecular Relaxation Processes Relevant to the Photoreception. The phototropic equilibria in flavins are predicted to change upon light excitation.[52] Thus, the basicity of N_1 in the groundstate of flavin decreases, while that of N_5 increases significantly upon excitation either to S_1 or T_1 state.[52]The pK_a changes upon excitation of flavins have been studied by several authors.[53-57] It is conceivable that the pK_a values of the nitrogen centers in flavin change upon binding to protein and/or membrance. The flavin binding apparently increases the pK_a values of tyr residues of the Shethna flavoprotein appreciably.

Light excitation of the flavin photoreceptor not only affects transiently its own pK_a's but those of amino acid residues. Both the proton gradient across the photoreceptor bound membrane[59] and conformational change of the membrane protein can be induced by the blue light excitation. In addition, a moderate increase in the permanent dipole moment occurs upon excitation.(to S_1 state) of flavin (by 3.8 D[60] or by 1.5—2.0 D [61]). The blue light induced reorientation of flavin and protein residue dipoles may lead to the generation of photocurrent through the photoreceptor bound membrane, as has been suggested in a model study.[59]

Although photo-induced transmembrane potential and its electrical stimulus of the phototactic effector are operative in principle,the phototropic response of higher plants (e.g., corn) may not involve such an electrical stimulus as the primary trigger. Thus, it has been found that auxins translocate laterally across a transversely polarized coleoptile before the transverse electrical poteential appears.[62-64] In the following section, we propose a plausible mechanism for phototrop ism.

(c) The Photochemical Mechanism of Photo tropism. Galston[40,65] showed that auxin is phot ooxidized by flavin. The action spectrum for the photooxidation of auxin in an etiolated pea homogenate suggested its possible identity as the photoreceptor with the phototropic action spectrum.[66] Subsequently, further photochemical studies of flavin-sensitized oxidation of auxin have been carried out *in vitro*.[67]

Auxin is apparently laterally translocated in coleoptiles from the lighted to the shaded side without significant photooxidation of auxin.[8,64,70,71] Previously, we have proposed a photochemical scheme whereby auxin bound to a macromolecule is released for translocation induced by the photoexcitation of the flavin photoreceptor.[39] This model accounts for the lateral auxin transport in coleoptiles without photodecomposition. We now attempt to further refine this model by considering specific photoprocesses which may be responsible for the phototropic transduction of blue light energy.

We assume that the concentration of the flavin photreceptor is considerably less than that of auxin. Let us also assume that auxin is either bound to a protein or membraneous compartment analogous to the acetyl choline receptor sites on synaptic memberane. This assumption is experimentally supported.[72,73] To trigger release of the bound auxin, the excited flavin photoreceptor changes the conformation of the auxin-bound protein and/or membrane, resulting in release of auxin in a non-stoichimeric ratio (i.e., the number of auxin released is much greater than the number of photoreceptors excited, and the photosignal is thus amplified). This scheme is analogous to the rhodopsin-induced release of the visual excitation transmitter (Ca^{++}) from the rod outer segment discs.[74]

We propose that the mose effective way to trigger the auxin release is by photoreduction of the flavin photoreceptor itself by a hydrogen donor. The reduced flavin is non-planar[75] and its binding to protein can be either vacated or modified to the extent that the photoreceptor membrane undergoes a conformational transition favorable for auxin release. Among such hydrogen donating substrates as amino acids,[76] NADH,[77] menadiol diphosphate[49] and other artificial donors for the photoreduction of flavin, auxin was found to be by far the best donor in terms of the quantum yield.[39,68,69] Furthermore, auxin photoo-

xidation is nearly oxygen-independent,[69] in contrast to other hydrogen donors examind. The photoreaction by either singlet or triplet flavin is, therefore, faster than or comparable in its rate to the quenching of the singlet or triplet flavins by oxygen(at least under the conditions of experiment, i.e., excess auxin conc.). For these reasons, we envision auxin itself to act as the photoreducing agent of the flavin photoreceptor in order to trigger the auxin release from the membrane or membraneous compartment as the primary photoact in phototropism. A schematic diagram for this mechanism is illustrated in Fig. 9. Work is in progress to test this hypothesis by searching for flavin-bound membrane vesicles prepared in the presence of auxin.

It should be noted that the above mechanism does not result in significant photodecomposition of auxin, since the concentration of the flavin photoreceptor is much lower than that of auxin. This model also accounts for thenecessary amplification factor to effect the phototropic curvature, as discussed previously.[8,39] It is noteworthy that phototropic auxin transport in coleoptiles is oxygen dependent.[8,72,78] This could be accounted for by the reoxidation of the photoreduce flavin photoreceptor by oxygen, which could also give rise to the post auxin transverse electrical potential.[62-64]

The photochemical mechanism proposed here may well be applicable to the fungus phototropism in which auxin is replaced by some other electron dinors for the photoreduction of the flavin photoreceptor.

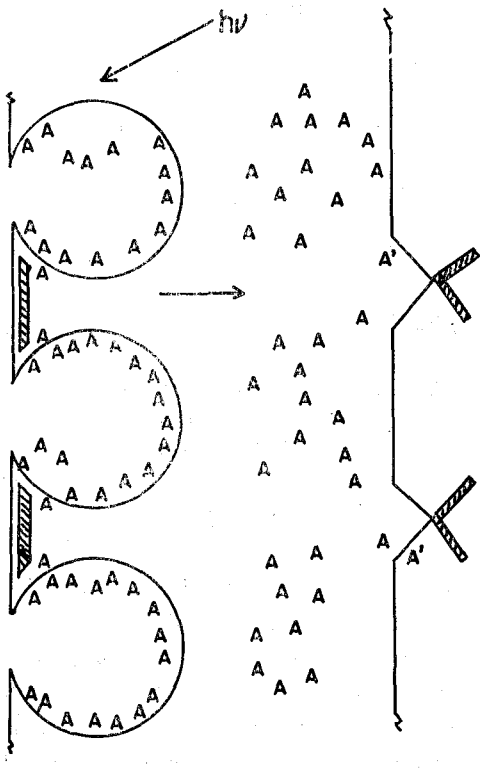


Fig. 9. Photo-induced release of auxin(schematic). A, auxin; ≈≈≈, flavin photoreceptor; ≈≈≈, photoreduced flavin photoreceptor; A', photoxidized electron donor (e.g., auxin photoproduct). The solid line represents membrane.

VI. Stentorin

Stentorin is the blue green pigment in stentor which acts as the photoreceptor for the photop hobic response of this organism.[79,80] The chromophore structure of stentorin is apparently identical with the antidepressant hypericin,[81] which is also a powerful natural photodynamic sensitizer and it belongs to the *meso*-naphthodianthrone group of compounds (Fig. 10).

A recent configuration analysis calculation[81] suggests that there is a significant charge transfer-(a) from the hexahydroxynaphthodianthrene ring

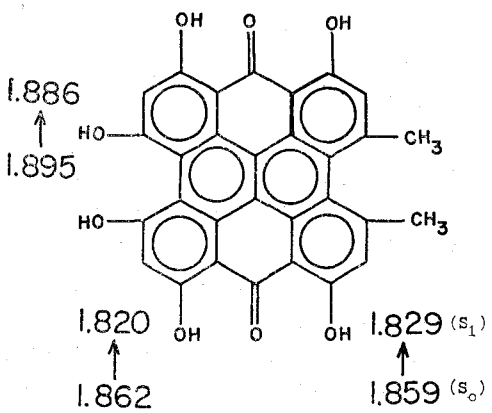


Fig. 10. The stentorin chromophore, hypericin, shows lowering of pK_a upon excitation to S1 in termsof π-electron densities on the hydroxyl oxygens.

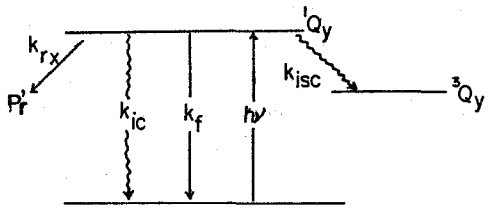
to the quinoid carbonyl groups and (b) from the hydroxyl groups to the ring in the singlet excited state. This charge redistribution is reflected in the decrease in π-electron density on the hydroxyl groups upon excitation of stentorin (Fig. 10). Such a charge decrease implies that the hydroxyl protons become much more acidic in the excited state. From the data shown in Fig. 10, we estimate that the pK_a of stentorin (for 1- and 6-OH) is reduced by about 6 units upon excitation, analogous to the case of luciferin.[28]

Stentorin does not provide a large dipole moment upon excitation(0.4 D and 1.9 D for the ground and excited π, π^* states, respectively; calculated by the P-P-P SCF MO CI method). This photoreceptor molecule is also rigid, so that a light induced conformational change of neither the chromophore nor the membrane to which stentorin is bound is possible. Therefore, the most likiely trigger mechanism for *Stentor* is a proton pump generated by the excitation of the photoreceptor. The phototactic role of the proton pump in *Halobacterium*[10b] is an illustrative example for this type of sensory transduction mechanism. The proposed proton pump is also consistent with the receptor potential in vacuoles of *Stentor* which is elicited by light.[79]

VII. Phytochrome

Unlike the vision photoreceptor rhodopsin, the phototransformation of phytochrome from P_r to P_{fr} forms appears not to be directly coupled to an effector system for light energy transduction. Instead, light merely transfroms physiologically "inactive" P_r to "active" P_{fr} which then activates a photomorphogenic effector system (e.g.,binding to membrane at the P_{fr} receptor site).[82]

Nonetheless, there are certain similarities between the vision photoreceptor and the photomorphogenic receptor, particularly in their primary photoprocesses. Both photoreceptors are responsible for the efficient detection of light stimulus at about the same level of light intensity (i.e., plants can detect $\sim 3\times10^4$ photons/cm^2/sec) for eliciting a morphogenic responses). The high sensitivity of the photoreceptors requires that the primary photoprocesses be fast and efficient. In fact, rhodopsin phototransforms to bathorhodopsin with the rate constant of $\geq 2\times10^{11}$ sec^{-1},[14] and the primary photoprocess of P_r is equally fast, if not faster. This is illustrated in Fig. 9, Fig. 11 illustrates the kinetics of the primary photoprocess of P_r on the basis of fluorescence lifetime and quantum yields of large mol. wt. phytochrome.[83-85] Details of the phototransformation kinetics and intermediates can be found elsewhere.[86-90]



$$\tau_F = \tau_F^{\circ} \times \Phi_F \simeq 10^{-12} \sim 10^{-11}\ s$$

$$(\tau_F^{obs} \leq 18\times10^{-11}\ s)$$

$$k_f + k_{isc} + k_{ic} \leq 2.3\times10^{9}\ s^{-1}$$

$$k_{rx} \simeq 10^{11} \sim 10^{12}\ s^{-1}$$

$$P_r \xrightarrow{h\nu} {}^1P_r \xrightarrow{10^{11}\sim10^{12}\,s^{-1}} P_r' \longrightarrow \text{lumi-R}$$

vs.

$$Rh \xrightarrow{h\nu} {}^1Rh \xrightarrow{\geq 2\times10^{11}\,s^{-1}} BRh \longrightarrow \text{lumi-Rh}$$

Fig. 11. The electronic relaxation processes (internal conversion, fluorescence and intersystem crossing) and the primary photoprocess (k_{rx})of phytochrome(P_r). Kinetic parameters are shown for P_r and rhodopsin for comparison.

Since the ratio of the oscillator strengths of the visible band (Q_y) and near UV (Soret analog) band is not altered significantly in going from P_r to P_{fr}, the gross π-electron conformation of the chromophore must remain essentially identical (Fig. 12).[91] This raises the question as to the nature of the structural change a ccompained by the $P_r \leftrightarrow P_{fr}$ phototransformation, since only the P_{fr} from may specifically find at a receptor site.[92] It also appears that the three dimensional structure of P_r and P_{fr} is not significantly different.[93,94]

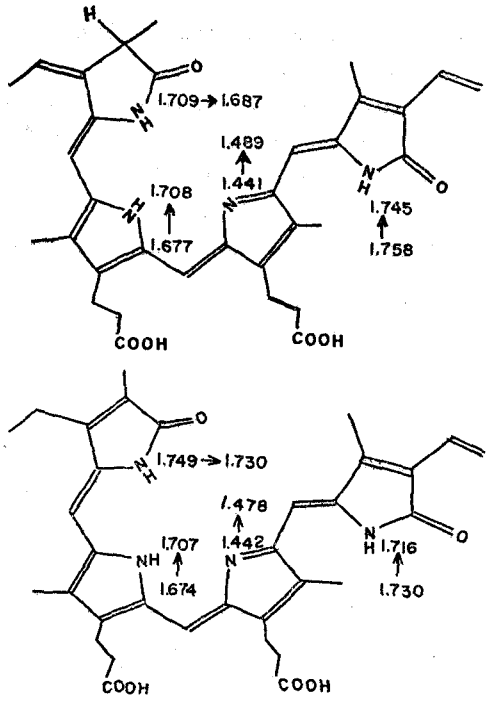


Fig. 12. The calculated conformations of P_r (top) and P_{fr} (bottom) consistent with spectroscopic data. Changes in π-electron density at each nitrogen occur upon excitation to the lowest excited singlet state (Q_y).

However, there is evidence for conformational changes in the local chromophore binding site.[94]

In order to investigate the local changes in the P_r and P_{fr} binding sites, we measured CD spect ar of both small and large phytochromes.[91] The rotational strength for the 500nm CD had is 5.95×10^{-40} for large P_r. The visible CD band at 670nm is storongly negative ($R=-1.51\times10^{-39}$). These induced CD signals in P_r (binding site involves one or more trp residues[91]) are nearly lost upon phototransformation to P_{fr}. This implies that the chromophore binding site in P_{fr} is considerably more flexible or even vacated compared to that of P_r. This local conformational change could account for the enhanced accommodation of P_{fr} at the receptor site.

We now examine a possible role of the excited state for the transformation of P_r to P_{fr}. Fig. 12 shows the π-electronic charge density of nitrogens upon excitation of phytochrome to the lowest excited singlet state (Q_y). The charge redistribution shown in Fig. 12 implies that the acidity of NH protons and the basicity of the ring C nitrogen substantially increase upon excitation. It is thus possible that an ionic form of P_r can be produced in the excited state[95] which subsequently relaxes to a metastable intermediate and eventually to P_{fr}. Changes in the microscopic electric field present on the protein and in the pK_a values of amino acid residues near the chromophore binding site could accompany the light induced ionic equilibria for P_r.*

Alternatively, the phototautomerism at ring A may account for the $P_r\rightarrow P_{fr}$ prototransformation.[96] Although the structure of P_{fr} is yet to be firmly established, our spectroscopic study[91] and theoretical treatments[81] are more consistent with the Siegelman mechanism than other models exmined (anion formation, photoisomerization, etc.). However, a definitive choice between the two phototransformation mechanisms proposed here cannot be made at the present.

Finally, it should be pointed out that, while the *cis-trans* type photoisomerization for $P_r\rightarrow P_{fr}$ is not consistent with the present result (Fig. 12), intermediates during the phototransformation of phytochrome may well invlove twisting around single and/or double bonds of the chromophore. We are now examining this possibility with large mol. wt. phytochrome in our laboratory.

VIII. Epilogue

The science of photobiology, particularly with regard to the modes of action of the photoreceptors for light responsive plants and microorganisms, can blossom only when physicochemical mechanisms and photobiological response data

* The calculated dipole moments of P_r and P_{fr} are such that microenvironmental changes of the binding site are not likely; i.e., 12.9D for S_0 state and 15.4D for S_1 state of P_r and 12.1D for S_0 state of P_{fr}.

can be satisfactorily corroborated. In this essay, I have proposed a number of speculative physicochemical mechanisms which, I hope, will stimulate further experiments in order to make contributions toward explaining the photobiological responses. No apologies will be made if I should be informed of the stupidity of some of the ideas expressed in this essay; I would rather thank the reader for pointing out the stupidity and thus for enlightening the author. However, I do make apologies to those whose pertinent references may have been overlooked, as this essay was prepared hastily to meet the deadline on a rather short notice.

Acknowledgements.

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