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Molecular Topology of the Photosynthetic Light-Harvesting Pigment Complex, Peridinin–Chlorophyll a–Protein, from Marine Dinoflagellates†

Pill-Soon Song,* Prasad Koka, Barbara B. Prézelin,‡ and Francis T. Haxo

ABSTRACT: The photosynthetic light-harvesting complex, peridinin–chlorophyll a–protein, was isolated from several marine dinoflagellates including *Glenodinium sp.* by Sephadex and ion-exchange chromatography. The carotenoid (peridinin)–chlorophyll a ratio in the complex is estimated to be 4:1. The fluorescence excitation spectrum of the complex indicates that energy absorbed by the carotenoid is transferred to the chlorophyll a molecule with 100% efficiency. Fluorescence lifetime measurements indicate that the energy transfer is much faster than fluorescence emission from chlorophyll a. The four peridinin molecules within the complex appear to form two allowed exciton bands which split the main absorption band of the carotenoid into two circular dichroic bands (with negative ellipticity band at 538 nm and positive band at 463 nm in the case of peridinin–chlorophyl a–protein complex from *Glenodinium sp.*). The fluorescence polarization of chlorophyll a in the complex at 200 K is about 0.1 in both circular dichroic excitation bands of the carotenoid chromophore. From these circular dichroic and fluorescence polarization data, a possible molecular arrangement of the four peridinin and chlorophyll molecules has been deduced for the complex. The structure of the complex deduced is also consistent with the magnitude of the exciton spliting (ca. >3000 cm^{-1}) at the intermolecular distance in the dimer pair of peridinin (ca. 12 Å). This structural feature accounts for the efficient light-harvesting process of dinoflagellates as the exciton interaction lengthens the lifetime of peridinin (radiative) and the complex topology increases the energy transfer probability. The complex is, therefore, a useful molecular model for elucidating the mechanism and efficiency of solar energy conversion in vivo as well as in vitro.

The marine dinoflagellates *Glenodinium sp.* and *Gonyaulax polyedra* contain a peridinin–chlorophyll a–protein complex (PCP)[1] which apparently acts as a photosynthetic light-harvesting accessory pigment (Prézelin and Haxo, 1976). The pigment complex (mol wt 35 500 and 34 500 for *Glenodinium sp.* and *Gonyaulax polyedra* PCP's, respectively) contains four carotenoids and one chlorophyll a molecule per protein. The dinoflagellate *Amphidinium carterae* (Plymouth 450) also contains PCP (mol wt 39 200), but its apoprotein (mol wt 31 800) is associated with nine peridinin and two chlorophyll a molecules (Haxo et al., 1976). These complexes show an efficient energy transfer from peridinin to chlorophyll a, as measured by fluorescence excitation spectroscopy (Haxo et al., 1972, 1976; Prézelin and Haxo, 1974, 1976; Prézelin, 1975).

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[1] Abbreviations used are: PCP, peridinin–chlorophyll a–protein complex; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; CD, circular dichroism; ORD, optical rotatory dispersion.

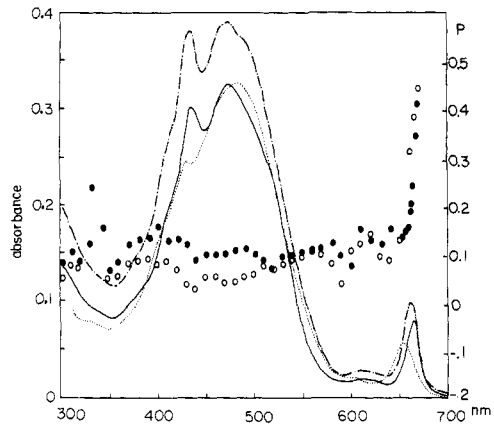

FIGURE 1: Absorption spectra of native (—) and denatured (···) peridinin-chlorophyll a-protein (PCP) from *Glenodinium sp.* and *G. polyedra* (- · -). The spectrum of polarization of fluorescence excitation (excitation band-pass 0.5 nm and emission at 675 nm, 2 nm bp) of PCP: (●) *Glenodinium sp.* and (○) *G. polyedra* in Tris-glycerol (1:4) at 200 K, recorded on the single-photon counting spectrofluorometer. Absorbance at the peridinin and chlorophyll peaks were kept at 0.3 and 0.07 (for *Glenodinium sp.*) and 0.16 and <0.05 (for *G. polyedra*), respectively, in order to avoid self-depolarization due to chlchl energy transfer.

The aim of the present work is to deduce a spectroscopically consistent molecular topology of the PCP complex which is conducive to the efficient energy transfer from peridinin to chlorophyll a. Energy transfer from carotenoids to chlorophyll is very inefficient, or lacking, in solution at both low (12 K) and room temperatures (Song and Moore, 1974; Moore, 1975). This is apparently due to the extremely short lifetime ($\sim 10^{-14}$ s) of the excited sirglet state (^1B) of carotenoids (Song and Moore, 1974). Therefore, it is of interest to understand how the highly efficient energy transfer from carotenoids (e.g., peridinin) to chlorophyll is carried out in vivo. Because of its well-defined molecular stoichiometry, PCP is an ideal system for studying the relationship between the molecular topology and the mechanism of energy transfer.

Experimental Section

Materials. Peridinin-chlorophyll a-protein complexes from *Glenodinium sp., Gonyaulax polyedra,* and *Amphidinium rhyncocephaleum* were isolated and purified as described elsewhere (Prézelin, 1975; Prézelin and Haxo, 1976). Freeze-fried samples of PCP (*Glenodinium sp.*) were dissolved in 2 mM Tris buffer, pH 8.4. The Tris buffer was prepared from Trizma-HCl and Trizma base (from Sigma Chemical Co.) in double-distilled water. The PCP complexes from *G. polyedra* and *A. rhyncocephaleum* were kept in saturated sucrose solution and were dialyzed against Tris buffer at 4 °C prior to use. Chlorophyll a was isolated from spinach according to Strain and Sherma (1969). Glycerol and other solvents of spectroquality were obtained from Matheson, Coleman and Bell.

Methods. Absorption spectra were recorded on a Cary 118C spectrophotometer at room temperature. Corrected luminescence emission and excitation spectra were measured on a Model MPF-3 Perkin-Elmer spectrofluorometer. High resolution emission and excitation spectra and polarization at 200 K were measured on a monophoton-counting spectrofluorometer designed in this laboratory and described elsewhere (Moore and Song, 1973; Mantulin and Song, 1973).

Fluorescence polarization with respect to emission and excitation were measured by the method of photoselection at 200 K on solutions of PCP in 2 mM Tris buffer-glycerol (1:4, v/v) mixture. Denatured PCP was obtained by boiling the PCP solution with 1% sodium dodecyl sulfate for 2 min. Polarization of denatured PCP at 200 K was recorded on solutions of PCP in the same (1% sodium dodecyl sulfate-glycerol) mixture. For the low temperature polarization measurements, the PCP in Tris buffer (or with 1% sodium dodecyl sulfate) and glycerol was sealed in a selected Pyrex glass tubing of 6-mm diameter and about 45-mm in length so as to fit into the sample mount of a Cryocooler optical chamber (Model 20, Cryogenic Technology, Waltham, Mass.). The sample was then allowed to equilibrate for 1 h at 200 K before proceeding with the polarization measurements.

The circular dichroic (CD) spectra were recorded on a JASCO-20 CD-ORD spectropolarimeter which was modified to enhance the signal-to-noise ratio by replacing the Pockel cell and associated circuitry with a Morvue photoelastic modulator (PEM-3) and lock-in amplifier (PAR Model 121).

The fluorescence lifetimes were measured at room temperature with an SLM-Model 480 phase-modulation spectrofluorometer, utilizing continuous light (from a 1 kW Xe arc, Oriel Optics) that has been sinusoidally modulated. Lifetimes of fluorescence from chlorophyll a in PCP were measured at frequencies of 10 and 30 MHz. To insure homogeneous emission a 0.63-μm red filter was used in the emission light path (Oriel Optics).

Results

Figure 1 shows the absorption spectrum of PCP (*Glenodinium sp.*) in Tris-glycerol (1:4). The complex was found to be stable even after repeated freeze (200 K)-thaw cycles. The molar ratio of peridinin to chlorophyll a in the PCP complex has been estimated to be 4:1 from Figure 1 (based on a peridinin molar extinction coefficient of 8.44×10^4 M^{-1} cm^{-1} (Prézelin and Haxo, 1976)). The effect of sodium dodecyl sulfate denaturation is to shift the chlorophyll Q_y and peridinin B bands to the blue and red, respectively. Figure 1 also shows the absorption spectrum of the PCP of *G. polyedra* which is essentially identical to that of *Glenodinium sp.*, the only apparent difference being the stronger Soret band of chlorophyll a in the former.

Figure 2 shows the fluorescence excitation spectrum of PCP (*Glenodinium sp.*). The ratio of excitation at the chlorophyll Q_y to the peridinin B is nearly identical (~ 4) when the excitation of the former (667 nm) was corrected. Since absorbance at the peridinin maximum (476 nm) is less than 0.3 and the excitation ratio is essentially unchanged at a lower absorbance of 0.16, the excitation spectrum (Figure 2) indicates efficient (100%) energy transfer from the peridinin excited state to chlorophyll a Q_y. Similarly, PCP's from *G. polyedra* and *Amphidinium rhyncocephaleum* show a 100% energy transfer within experimental error (Figure 2). The low temperature, high resolution (0.4 nm) excitation spectra recorded on a single-photon counting spectrofluorometer also show no significant alteration of the structure of PCP's, as judged by the excitation ratio (data not shown). Denaturation completely abolishes the intra-complex energy transfer, as shown in Figure 3, although some peridinin and chlorophyll molecules are still bound to the apoprotein (see CD and fluorescence polarization data; vide infra). The low temperature (200 K) excitation spectra of the PCP's also show no evidence of energy transfer.

Figure 4 shows the CD spectrum of PCP (*Glenodinium sp.*). The expanded CD spectrum of the chlorophyll Q_y band shows no indication of intermolecular interaction between chlorophylls or between PCP's. The CD spectra of PCP's from *G.*

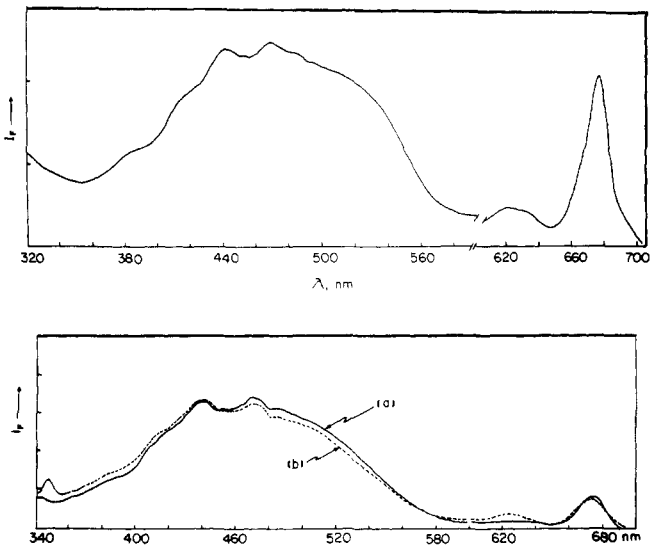

FIGURE 2: (Top) The corrected fluorescence excitation spectrum of PCP (*Glenodinium sp.*) at room temperature. The spectrum above 600 nm was not automatically corrected by the MPF3 spectrofluorometer. (Bottom) The corrected fluorescence excitation spectra of (a) PCP from *G. polyedra* and (b) *A. rhyncocephaleum* in Tris-glycerol (1:4) at room temperature. Absorbance at the peridinin band was kept at <0.3, so that direct comparison with the corresponding absorption spectra is meaningful.

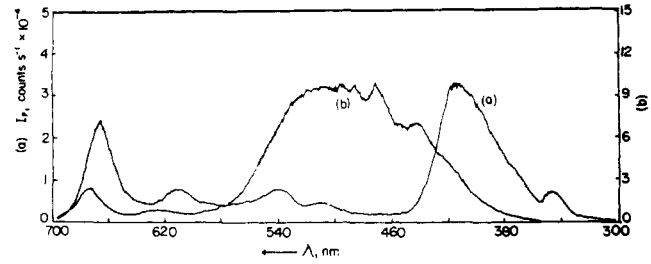

FIGURE 3: Fluorescence excitation spectra of (a) denatured and (b) native PCP (*Glenodinium sp.*) recorded on the single-photon counting spectrofluorometer. Excitation band-pass 0.4 nm.

polyedra and *A. rhyncocephaleum* are also shown (Figure 5). The most important feature in these CD spectra is that the peridinin absorption band is split into two approximately conservative positive and negative ellipticity bands (463 and 538 nm for *Glenodinium sp.* PCP, 464 and 541 nm for *G. polyedra* PCP, and 462 and 545 nm for *A. rhyncocephaleum* PCP). Denaturation completely destroys the CD band splitting. Chlorophyll a in the denatured PCP also remains bound to the apoprotein, since the CD minima in Figure 5 are significantly different (686, 673, and 665 nm for native, denatured PCP of *Glenodinium sp.* and free chlorophyll a, respectively) and fluorescernce polarization remains at near the maximum value.[2]

In order to determine the orientations of the two components of the exciton transition moments with respect to the Q_y transition moment of chlorophyll a, fluorescence polarization with respect to the chlorophyll fluorescence was measured. Figure 1 shows the spectra of polarization of fluorescence excitation of PCP's from *G. polyedra* and *Glenodinium sp.* The *A. rhyncocephaleum* PCP shows a similar polarized excitation

[2] Although the spectral shape of polarization of the fluorescence excitation for denatured PCP complexes was found to be similar to that of chlorophyll a in solution, the polarization degree was consistently higher throughout the spectrum. One likely cause for this effect is the scattered light in the denatured PCP samples.

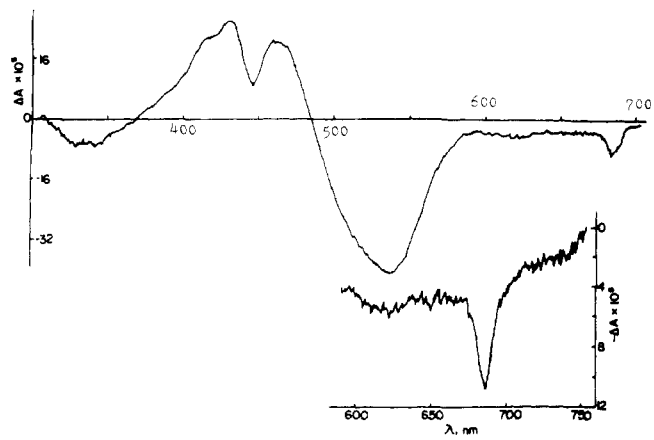

FIGURE 4: Circular dichroic (CD) spectrum of PCP (*Glenodinium sp.*) in Tris-glycerol (1:4) at room temperature; absorbance 0.3. The insert shows the CD spectrum for the visible band of chlorophyll a of the PCP complex at an expanded scale.

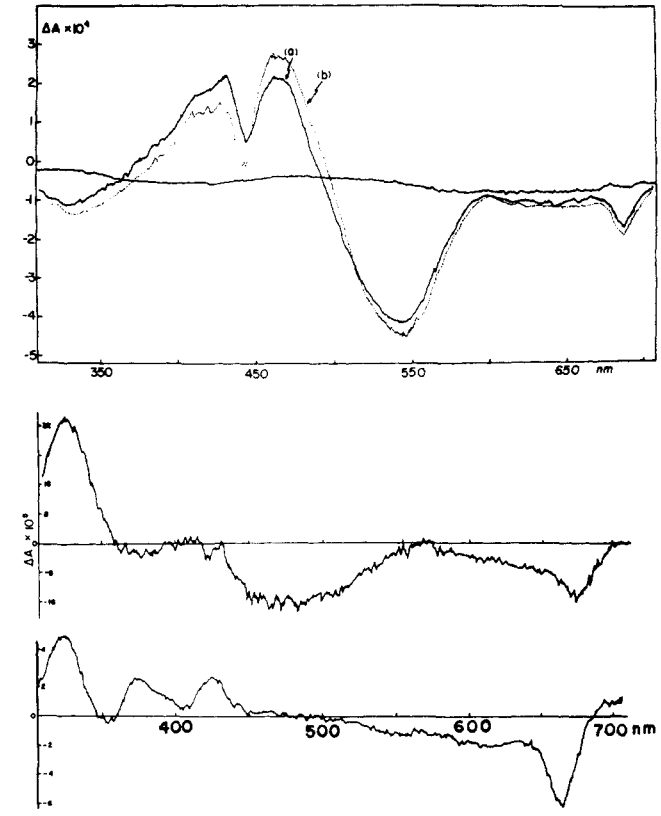

FIGURE 5: (Top) CD spectra of PCP from (a) *G. polyedra* (absorbance 0.16) and (b) *A. rhyncocephaleum* (absorbance 0.29) in Tris-glycerol (1:4) at room temperature. The baseline for the CD cell and solvent is also indicated. (Middle) CD spectra of denatured PCP (*Glenodinium sp.* OD_{475} 1.5) in Tris-glycerol (1:4). (Bottom) Chlorophyll a in ether.

spectrum, as shown in Figure 6, although the polarization degree for the positive CD band (*cf. Figure 5*) is somewhat lower than the other PCP's. These results indicate that the orientations of both components of the peridinin CD bands are 45–50° with respect to the Q_y axis of chlorophyll a. Denaturation causes substantial distortions of the transition moment orientations, as the spectrum of polarization of fluorescence excitation is modified (not shown), and fluorescence excitation is now predominantly due to chlorophyll itself without sensitization by peridinins within the complex.[2]

Figure 7 shows the fluorescence spectrum of PCP (*Gleno-*

TABLE I: Fluorescence Lifetimes (τ_F) of the *Glenodinium sp.* Peridinin-Chlorophyll a-Protein Complex in Tris-Glycerol (1:4) at 298 K.[a]

Phase τ_F (ms)	Demodulation τ_F (ms)	Modulation frequency (MHz)
5.38	5.48	30
5.52	5.72	10

[a] Using four different modes of measurements; phase tan ϕ and demodulation $(\frac{1}{2}\pi fm^{-2} - 1)^{1/2}$, where ϕ is the phase shift, f is the modulation frequency, and m is the attenation or demodulation ratio which equals cos ϕ. The percent standard deviation of lifetime values is 2.7.

dinium sp.) recorded at 200 K and at high resolution. The emission maxima are 675, 672, and 672 nm for the PCP's of *Glenodinium sp., G. polyedra,* and *A. rhyncocephaleum,* respectively. Fluorescence lifetimes were measured by phase and modulation (Table I). The average fluorescence lifetime of the PCP complex (excited at the peridinin band) is essentially the same as that of free chlorophyll (5.33 ns in ether) or PCP-bound chlorophyll (excited at a chlorophyll absorption band). Since all four lifetime measurements (phase and modulation at 10 and 30 MHz) yield consistent values (average 5.53 ± 0.15 ns, each value being the average of 10 ~ 20 measurements), the fluorescence decay of PCP is exponential. The PCP from *G. polyedra* emits fluorescence with a lifetime of 4.20 ± 0.2 ns (average of 20 phase and modulation measurements at 30 MHz).

Discussion

It is clear from the results summarized so far that PCP's show very efficient energy transfer from the peridinin excited state to the chlorophyll Q_y state (cf. Figure 2). Furthermore, efficient energy transfer requires a specific molecular topology of the complexes, as suggested by the denaturation effect studies (Figures 1, 3, and 5). We now discuss the relationship between the energy transfer efficiency and molecular arrangement of the PCP complexes on the basis of the results obtained.

First, we examine whether the critical distance (R_0) for Förster's energy transfer mechanism (Förster, 1949, 1959) for the peridinin-chlorophyll a in PCP's is sufficient to explain the observed results. Long, conjugated carotenoids are nonfluorescent (Song and Moore, 1974). Peridinin in denatured PCP's showed no fluorescence on our single-photon counting spectrofluorometer even at 17 K, indicating that the fluorescence quantum yield of peridinin is less than 10^{-5}-10^{-4} (limitation of the instrument). This means that the mean lifetime of the peridinin excited state (^{1}B) is on the order of less than or equal to 10^{-14}-10^{-13} s. This is indeed too short a lifetime to transfer the excitation energy to chlorophyll a. Critical distances corresponding to these lifetimes are calculated to be 5.8 ~ 8.6 Å, assuming that a hypothetical fluorescence spectrum of peridinin is roughly a mirror image of its absorption spectrum (Moore and Song, 1973; Song et al., 1976).

The Förster vertical distance is given by:

$$R_0^6 = \frac{(8.79 \times 10^{-25})\kappa^2\phi_P}{n^4\bar{\nu}^4} J_{PC} \qquad (1)$$

where ϕ_P is the peridinin fluorescence quantum yield, n is the refractive index, $\bar{\nu}$ is an average frequency in cm^{-1}, and J_{PC}

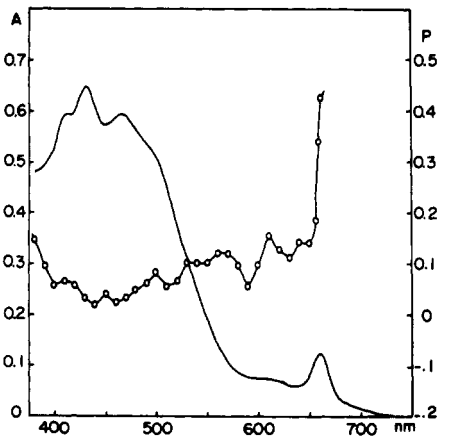

FIGURE 6: The spectrum of polarization of fluorescence excitation (excitation band-pass 0.4 nm) of PCP from *A. rhycocephaleum* in Tris-glycerol at 200 K.

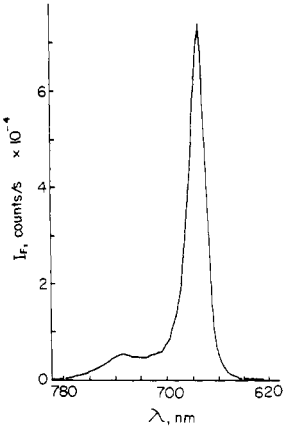

FIGURE 7: The fluorescence emission spectrum of PCP (*Glenodinium sp.*) in Tris-glycerol (1:4) at 200 K, recorded on the single-photon counting spectrofluorometer (emission band-pass 0.5 nm).

is the spectral overlap integral between the peridinin fluorescence and chlorophyll absorption bands. The orientation factor, κ^2, was assumed to be $\frac{2}{3}$ (a value for random orientations of dipoles involved). Although PCP and peridinins within the PCP are not rotating fast enough, the excitation polarization over the peridinin absorption band approaches zero (Figure 1), resulting from the specifically fixed orientations of peridinins (vide infra). This coincidentally satisfies the κ^2 value for random orientations (for review see Dale and Eisinger, 1974).

For 100% efficient energy transfer in the PCP's (see Figure 2), therefore, the distance between all four peridinin molecules and chlorophyll must be substantially less than the 5.8-8.6 Å calculated here. Since the rate-limiting step in the energy transfer process measured is the emission from the chlorophyll a Q_y state and thus the rate constant for the transfer of energy from peridinin to chlorophyll a only needs to be greater than 2×10^8 s^{-1}, these considerations based on the lifetime and critical distance of monomeric peridinins and chlorophyll a are contradictory to the efficient energy transfer in PCP's. It should also be noted that eq 1 based on the Förster transfer mechanism is valid only if the rate of transfer from peridinin to chlorophyll a is much less than that of the vibrational relaxations in peridinin. To resolve the above question, lack of fluorescence and the short lifetime of free peridinin must be effectively overcome in the PCP's. Initially, we suspected that the main absorption band (B ← A) of peridinin in native PCP's

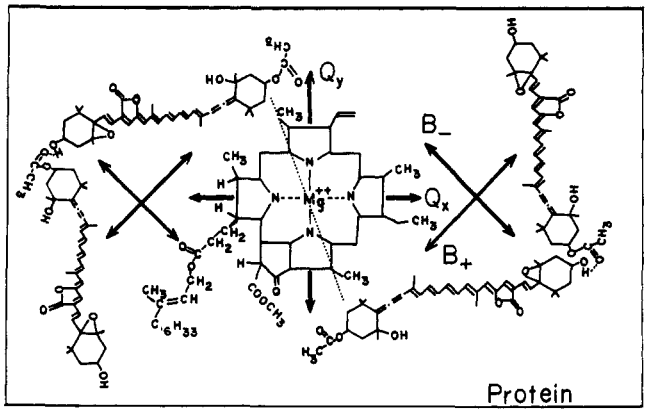

FIGURE 8: A probable molecular arrangement of chlorophyll a and peridinins based on relative orientations of transition moments (double arrows) of Q_y (fluorescence) and B^+ (exciton) transitions. The calculated polarization axis of the $^1Q_y \leftarrow A$ transition deviates by 18° and from the $2C_2$, 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°.

(Figures 1 and 6) was split into two components, one blue-shifted maximum and another red-shifted shoulder relative to that of denatured PCP (cf. Figure 1). The shoulder is most prominent in PCP from *A. rhyncocephaleum*. This has been confirmed by the CD spectra (Figures 4 and 5) which clearly show a splitting of the peridinin band into two approximately equal ellipticity bands of opposite signs. The baseline is such (cf. Figure 5) that the rotational strengths, as calculated by integrating over both CD bands in a wavenumber scale, are approximately equal, indicative of exciton interactions between two peridinin molecules. Thus, four peridinin molecules in PCP's form two sets of dimeric excitons. Analogously, Philipson and Sauer (1972) and Olson et al. (1973) were able to resolve oligomeric exciton components of the photosynthetic reaction center bacteriochlorophylls on the basis of CD band splitting.

Since denaturation destroys the two component CD spectra of the PCP's without fully dissociating peridinins from the apoprotein (i.e., induced CD of peridinin seen in Figure 5), the dimeric exciton of peridinins seems to be one of the unique structural features associated with the efficient energy transfer in native PCP's. Fluorescence polarization data shown in Figures 1 and 6 suggest that the orientations of both exciton components corresponding to CD maximum and minimum (Figures 4 and 5) are approximately the same, i.e., 45–50° with respect to the polarization axis (Q_y) of the chlorophyll fluorescence. Figure 8 shows a possible molecular arrangement of the PCP which satisfactorily accommodates all of the data presented.

It should be mentioned that the apoproteins of PCP's are relatively small molecules, with the total chromoprotein molecular weights in the range of 35 000–40 000 (Prézelin, 1975; Prézelin and Haxo, 1976). In order to accommodate four peridinins and one chlorophyll a according to the proposed arrangement (Figure 8), the apoprotein is either nonspherical, ellipsoidal, or provides a crevice to maintain the molecular arrangement similar to that shown in Figure 8. In other words, it is not necessary that the suggested molecular topology in Figure 8 be coplanar, as long as both the exciton transition moment vectors are 45–50° from the Q_y axis. Alternatively, one can envision a molecular arrangement in which each corner of the chlorophyll chlorin ring is in contact with peridinin, thus accounting for the polarization data of fluorescence excitation shown in Figures 1 and 6. However, this arrangement is inconsistent with CD data previously discussed and it also does not help to resolve the kinetic consideration involving the short lifetime of peridinin as the energy donor.[3]

The geometric arrangement of peridinins and chlorophyll a in the PCP's (Figure 8) is only *relative* and we must await a crystallographic determination for the absolute molecular topology. However, not only the relative orientations of peridinins and chlorophyll a deduced are consistent with observed data, but an approximate distance between two peridinins of the dimeric exciton can be estimated from the magnitude of spliting. Thus, the maximum and minimum CD bands of peridinin from Figure 4 and 5 yield an exciton split of 3000–3300 cm^{-1}, corresponding to a distance of 12–13 Å between the *centers of mass* of two peridinin transition moment vectors which lie along the long axis of each molecule.

There are other geometries for four closely coupled oscillators that result in only two allowed transitions in absorption and in CD (cf. Kasha, 1963; Kasha et al., 1965). For example, the rectangular tetrameric array formed by two dimer pairs in Figure 8 (closing the distance between them and stacking the four carotenoids above the chlorophyll plane) would result in two allowed and two forbidden exciton transitions. The lowest energy exciton state is forbidden in such a tetrameric array, and its radiative lifetime will be substantially longer than that of monomeric or dimeric exciton state of peridinin. However, the mean lifetime could still be very short. The tetrameric array is also consistent with the polarization of fluorescence excitation observed, if we rearrange the allowed transition dipoles (ca. 45° with respect to Q_y) and forbidden components are assumed to be either spherical or planar oscillator.

We favor the two dimer pair model over the rectangular (or square) tetramer on the basis of the observed data including magnitude of the exciton split (cf. the main transition dipole of peridinin is extremely large) and small perturbation of the peridinin absorption spectrum. Since the chlorophyll π-electron cloud and apoprotein are not symmetric, it is also unlikely that two forbidden exciton states of the tetramer are so rigidly forbidden that *both* absorption and CD intensities are practically zero.

It is likely that exciton resonance significantly lengthens the lifetime of the peridinin excited state from which energy transfer occurs. It is also probable that non- or near-degenerate exciton couplings between the peridinin and chlorophyll states (Q_x and less likely with $B_{x,y}$ and Q_y states) contribute to the efficient energy transfer. Clearly, more detailed studies on the mechanism of energy transfer in PCP's are desirable, and work along this line is now in progress. Nevertheless, the present study provides a useful model for understanding the light-harvesting processes of plants in general and dinoflagellates in particular. This system is also useful for the elucidation of energy transfer processes in model monolayer systems which undoubtedly involve carotenoid–carotenoid interactions (exciton type and other forms of interactions: e.g., Sineshchekov et al., 1972; Strauss and Tien, 1973).

Acknowledgment

The technical assistance of Mr. Robert D. Fugate is greatly appreciated.

[3] It is not entirely necessary that carotenoids form excitons to be able to transfer energy to chlorophyll. For example, it is conceivable that one carotenoid forms a coupled oscillator with either or both chlorophyll Q_y and $B_{x,y}$ states which are near degenerate with B state of carotenoid. In this case, the energy transfer rate is determined by the magnitude of coupling between the electronic states within the manifold of complex.

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Equilibrium between Six- and Five-Coordinated Hemes in Nitrosylhemoglobin: Interpretation of Electron Spin Resonance Spectra†

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ABSTRACT: Nitrosylhemoglobin without inositol hexaphosphate, which has the quaternary oxy structure, shows an electron spin resonance (ESR) spectrum similar to that of a synthetic nitrosylheme with piperidine in the sixth coordination position. Nitrosylhemoglobin with inositol hexaphosphate, which has the quaternary deoxy structure, shows an ESR spectrum which is a composite of that of the nitrosylheme with piperidine and of a synthetic nitrosylheme in which the sixth coordination position is empty.

In the absence of organic phosphates, the allosteric equilibrium of nitrosylhemoglobin A, as of other low spin compounds of hemoglobin A, is biased strongly toward the quaternary oxy structure. However, nitrosylhemoglobin A is unique among these compounds in allowing inositol hexaphosphate (IHP[1]) to switch the equilibrium to the deoxy structure (Cassoly, 1974; Salhany, 1974; Perutz et al., 1976). The transition is accompanied by marked changes in electronic, infrared, and ESR spectra; the latter were first demonstrated by Rein et al. (1972), but could not then be interpreted. The clue to their interpretation came from Maxwell and Caughey's (1976) study of the infrared NO stretching frequencies in nitrosylhemoglobin. They found that without IHP nitrosylhemoglobin exhibits a single ^{14}NO stretching frequency at 1615 cm^{-1}, similar to that of the six-coordinated 1-methylimidazole Fe(II)-NO protohemedimethyl ester. Addition of IHP caused the intensity of this band to be halved and a new band at 1668 cm^{-1} to appear. This frequency corresponds to that given by the five-coordinated Fe(II)-NO protohemedimethyl ester. These results indicate the coexistence of two distinct chemical species: one in which the iron is linked to N_ϵ of the proximal histidine and to the NO nitrogen and another in which the bond to the proximal histidine is either broken or severely stretched.

Figures 1a and 1b show the ESR spectra of nitrosylhemoglobin with and without IHP. They are rhombic. Without IHP strong resonances appear at $g = 1.97$, 2.03, and 2.06; in addition there are indications of hyperfine splitting. On addition of IHP, strong hyperfine splitting at g_z is superimposed on this spectrum. The spectra of [^{15}N]nitrosylhemoglobin with IHP published by Maxwell and Caughey (1976) show marked hyperfine splitting also at g_x and g_y.

We shall now compare these spectra with those of synthetic heme derivatives published by Wayland and Olson (1974). The six-coordinated Fe(II)-NO tetraphenylporphinepiperidine shows a rhombic ESR spectrum with strong resonances similar

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[1] Abbreviations used: ESR, electron spin resonance; IHP, inositol hexaphosphate.