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New proposal for structure of special-pair chlorophyll

(photosynthesis/chlorophyll–protein interactions/photo-oxidized chlorophyll/photo-reactive chlorophyll/chlorophyll–ligand interactions)

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ABSTRACT **A new model is proposed for the structure of the special pair of chlorophyll *a* molecules believed to correspond to the P700 species in green plants and algae. The proposed model, although admittedly speculative, is based upon exciton-theoretical considerations and on *in vitro* infrared and visible absorption spectra of a 700 nm absorbing ethanol adduct of chlorophyll *a*. In the new model, two chlorophyll *a* molecules are held together by (*a*) two strong ring V keto C=O···H—O (or keto C=O···H—N, or keto C=O···H—S) hydrogen bonds and by (*b*) π–π van der Waals stacking interactions between the two chlorophyll *a* macrocycles. Macrocycle stacking provides the intermolecular π–π overlap necessary to promote the (experimentally observed) delocalization of the unpaired electron of the *in vivo* radical over two chlorophyll π systems. The new model provides an explicit role for the participation of protein in the formation of the chlorophyll special pair. The *in vitro* system yields an electron spin resonance signal indistinguishable from that of oxidized P700 in *Chlorella vulgaris*.**

The primary events (for a recent review see ref. 1) of photosynthesis take place in a photosynthetic unit (2–4) where a large number of chlorophyll (Chl) molecules act cooperatively as an antenna to absorb visible light and to transfer the electronic excitation so produced to a photoreaction center or trap (5). In the excited trap, an electron is ejected from a special pair of chlorophyll molecules, Chl_{sp} (6), thereby creating a radical, $Chl^{\cdot}_{sp}$, in which the unpaired electron is delocalized over the π-systems of both macrocycles. The $Chl^{\cdot}_{sp}$ of photosystem I in green plants (i.e., the radical of P700) has a characteristic Gaussian electron spin resonance (ESR) signal with a free-electron *g*-value of 2.0025 and a signal width (i.e., twice the Gaussian standard deviation) of 7 gauss (1 gauss = 10^{-4} tesla) (7). Evidence for the participation of just two chlorophyll molecules in sharing the *in vivo* unpaired electron comes from ESR (7) and electron nuclear double resonance (ENDOR) spectroscopy. A comparison of the signal width for the monomeric Chl radical *in vitro* with that of the P700 *in vivo* radical shows that the latter is reduced relative to the former by a factor of approximately $1/\sqrt{2}$. Theory predicts that the signal should be narrowed by a factor of approximately $1/\sqrt{N}$ when an unpaired electron is spread equally over *N* Chl molecules (7). Both *in vivo* ESR (7) and ENDOR evidence (8) supports a two-molecule species for the *in vivo* P700 radical. P700 bleaches upon chemical oxidation or photo-oxidation primarily at about 700 nm, and secondarily at about 680 nm; concomitantly there is an apparent increase in absorption at about 690 nm (9–12). It is generally believed that the chlorophyll species that bleaches and the chlorophyll species corresponding to the *in vivo* radical are one and the same.

The structure of *in vivo* Chl_{sp} has not been experimentally established. However, two models have been proposed for its structure in green plants. These are the Katz and Norris model (6) and the Fong model (13). In the Katz and Norris model (Fig. 7C of ref. 6) two Chl *a* (see ref. 6 for structure) molecules are linked together via a water molecule that simultaneously coordinates (on the side of the macrocycle opposite to the carbomethoxy group) to the Mg of one of the Chl *a* molecules and forms hydrogen bonds to both the ring V keto C=O and the carbomethoxy C=O of the other Chl *a* molecule. The macrocycle planes are depicted as being parallel with only a translation required to superimpose one Chl *a* onto the other. The Chl *a* molecules are environmentally nonequivalent and their π-systems cannot be in contact (π–π contact occurs at $\leq$3.6 Å separation between macrocycle planes) because of the presence of the bulky carbomethoxy group between the macrocycle planes at the point of intermolecular contact. In the Fong variant (Fig. 1 of ref. 13), the two Chl *a* molecules are held together by two water molecules, each of which coordinates to the Mg of one Chl *a* molecule and simultaneously hydrogen bonds to the carbomethoxy C=O of the other Chl *a* molecule. The entire structure has C_2 symmetry. We estimate from molecular models that the separation between the macrocycle planes in the Fong structure is approximately 5.6 Å; thus, the π-systems of the two macrocycles in this model are also not in contact. In the Fong model the ring V keto C=O is not bound or used in special pair formation in any way.

In the present paper we propose a new model for the structure of Chl_{sp} based upon exciton-theoretical considerations and on the *in vitro* visible absorption and infrared spectra of a 700 nm absorbing Chl *a*–ethanol adduct.

700 nm absorbing chlorophyll *a*–ethanol adduct

Brody and Brody (14) observed the formation of about 700 nm absorbing Chl *a* species in ethanol that fluoresces at about 720 nm. We have taken a new look at this species because of the close similarity between the position of its major visible absorption peak and the position of the major absorption peak of P700 *in vivo*. The visible absorption spectrum of a 0.094 M solution of Chl *a* in dry toluene with a 1.5 molar ratio of ethanol (about 0.14 M ethanol) at 298 K, 273 K, 247 K, 224 K, and 175 K is shown in Fig. 1. As the temperature is lowered there is a shift from a predominance of a species that absorbs near 670 nm to a species that absorbs near 700 nm. The C=O stretch region of the infrared spectrum of a 0.064 M Chl *a* solution in dry toluene with a 1.5 molar ratio of ethanol (about 0.096 M ethanol) at 298 K (spectrum A) and 183 K (spectrum B) is shown in Fig. 2. At the higher temperature, the 670 nm species predominates, and at the lower temperature, the 700 nm species predominates. As the temperature is lowered, there is very little change in the area of the ester C=O absorption, and the ester peak shifts only slightly from 1737 cm^{-1} to 1733 cm^{-1}. On the other hand, the transformation of the 670 nm → 700 nm species

Abbreviations: Chl, Chlorophyll; Chl_{sp}, Chlorophyll special pair; $Chl^{\cdot}_{sp}$, Chlorophyll special pair radical; R = phytyl; R′ = H, ethyl, or protein; ESR, electron spin resonance; ENDOR, electron nuclear double resonance; Bchl, Bacteriochlorophyll; $Bchl_{sp}$, bacteriochlorophyll special pair.

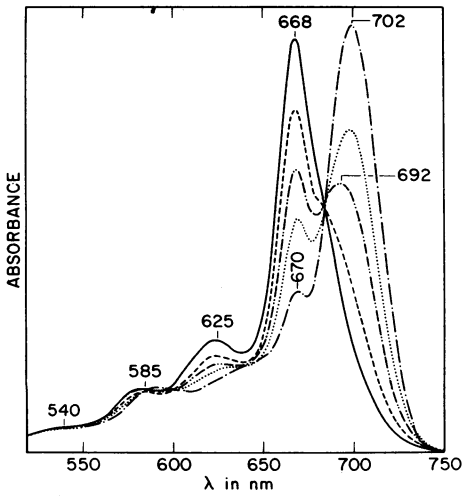

FIG. 1. Visible absorption spectrum of 0.094 M chlorophyll *a*, 0.14 M ethanol in toluene at: 298 K ——; 273 K, - - -; 247 K, – -·- –; 224 K, ···; and 175 K, —·—. The absorbance scale covers the range from 0 to 1.0.

is accompanied by a large decrease in area of the free keto C=O absorption, which shifts from 1697 cm^{-1} to 1686 cm^{-1}. A new peak at 1658 cm^{-1} also appears in the low temperature spectrum and this peak is logically assigned to bound keto C=O (15). From the infrared data we conclude that in the 670 nm species both of the ester carbonyls and the keto C=O do not experience coordination interactions and are unbound, but that in the 700 nm species, while ester carbonyls are still unbound, the keto C=O is bound and is either coordinated to Mg or is strongly hydrogen bonded.

New structural model for the special pair

A schematic representation of the basic structure of the Chl_{sp} model is shown in Fig. 3. In this structure, two Chl *a* molecules are held together with C_2 symmetry by two linking R′—O—H ligands, each of which is simultaneously coordinated (via oxygen lone pairs) to the Mg of one Chl *a* and hydrogen bonded to the keto C=O of the other Chl molecule. In this structure the distance between the Mg atoms is about 8.9 Å and the separation between the macrocycle planes is about 3.6 Å. Each Mg is displaced 0.4 Å from the macrocycle plane on the side of the macrocycle opposite to that of the carbomethoxy group. The Mg-to-ligand oxygen coordination bond length is about 2.1 Å. The ligand O—H to keto C=O hydrogen bond is linear and is approximately along the direction of one of the sp^2 lone pairs on the keto C=O; the O···O distance in this hydrogen bond is about 2.7 Å. Many of the structural features in this model were chosen to be compatible with the Strouse (16) x-ray crystal structure for ethyl chlorophyllide $a \cdot 2H_2O$, where ethyl chlorophyllide molecules (see ref. 16 for molecular structure) interact through π–π stacking and strong ring V keto C=O··· H—O— — —Mg hydrogen bonds. In the x-ray structure, the Mg is displaced 0.4 Å on the carbomethoxy side of the macrocycle, while in the structure of Fig. 3 the Mg is displaced 0.4 Å on the side of the macrocycle away from the carbomethoxy group. We see no compelling reason why the Mg should not displace with almost equal probability to either side of the macrocycle in the absence of secondary interactions. In the x-ray structure, the strongly hydrogen-bonded chlorophyllide molecules are related by translational symmetry, not C_2 symmetry. Porphyrin macrocycles typically stack at separations of 3.4 Å to 3.6 Å in crystals; this distance is the optimum van der Waals contact between the π-systems of adjacent molecules.

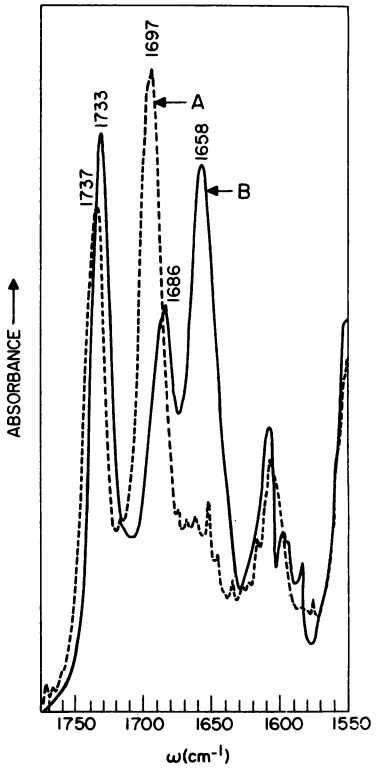

FIG. 2. Difference infrared absorption spectra of 0.064 M chlorophyll *a*, 0.096 M ethanol in toluene against toluene at 298 K (spectrum A) and 183 K (spectrum B).

In the structure of Fig. 3 (π–π stacking at 3.6 Å) there will be some orbital overlap between the two π-systems in the ring III and ring V regions. We wish to point out that although we have depicted (Fig. 3) the special pair as being held together by two R′—O—H ligands, based on *in vitro* experiments similar to those shown in Fig. 1, any of the following combinations may work just as well: R′—O—H, R′—O—H; R′—O—H, R′—S—H; R′—O—H, R′—NH_2; R′—S—H, R′—S—H; R′—S—H, R′—NH_2; R′—NH_2, R′—NH_2. Thus, the special pair model of Fig. 3 could be formed *in vivo* by any of the following moieties commonly found on or near proteins: water, the side chains of the natural amino acids arginine (N—H), cysteine (S—H), hydroxylysine (O—H or N—H), hydroxyproline (O—H), lysine (N—H), serine (O—H), threonine (O—H), and tyrosine (O—H).

The Chl_{sp} model is consistent with the infrared spectrum (Fig. 2) of the *in vitro* about 700 nm absorbing Chl *a*–ethanol adduct to the extent that the ester groups are unbound and the ring V keto C=O groups are bound. From Fig. 3 it can be seen that the four ester C=O functions are brought together in the same general region of space, leading to the speculation that they might provide binding sites for electrophiles such as metal ions, water hydrogen atoms, appropriate amino acid side chains of protein, etc. Coordination of a system containing a metal ion at this site might provide a route for electron transfer in or out of Chl_{sp}. The N1–N3 axes of the two molecules in the model are aligned very nearly parallel. This leads to strong interaction between the Q_y transition densities on the two molecules, as the Q_y transition dipole vector is approximately parallel to the N1–N3 axis.

Application of exciton theory to the special pair model

An exciton theory for Chl aggregates that simultaneously treats environmental shifts and transition density shifts of electronic

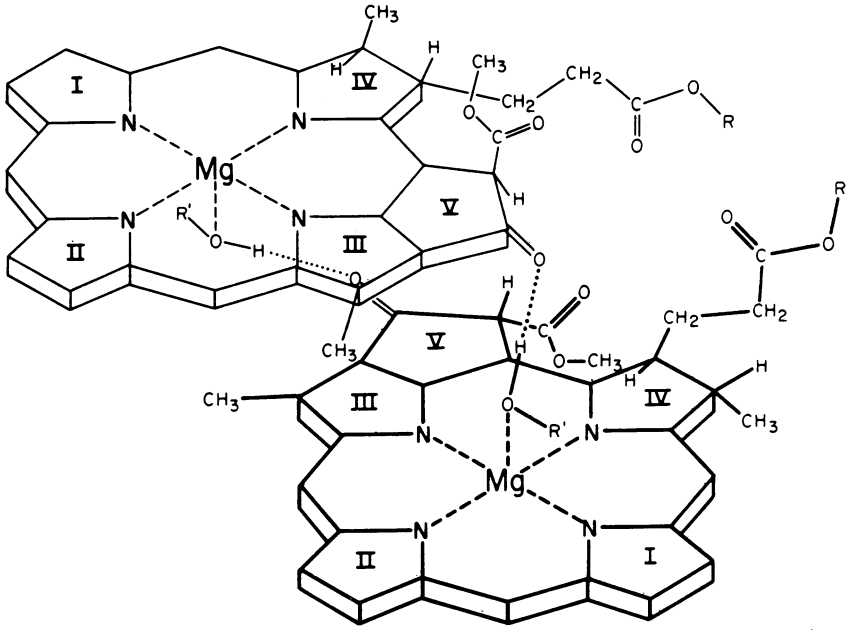

FIG. 3. Schematic representation of the proposed structure of special-pair chlorophyll *a*. For clarity the groups attached to rings I and II are not shown. R = phytyl and R′ is H, ethyl, or protein.

transition energies has recently been derived (17). Both of these shifts are operational in our model of Chl_{sp} (Fig. 3), where the combined effects of strong hydrogen bonding to the keto C=O and π–π stacking give rise to an environmental red shift of the Q_y transition, and the approximately parallel alignment of the Q_y transition dipoles gives rise to a transition density splitting of the Q_y transitions. Because detailed transition density distribution information is not available for Chl *a*, a point transition dipole approximation is used for our exciton calculations. In the Chl_{sp} model, the Q_y transition dipole vectors (directed along N1–N3) form an angle of about 180° with each other, are separated by about 8.9 Å (assuming the point dipoles are centered midway between N1 and N3) and form an angle of about 24° with the line between their centers. To apply exciton theory it is necessary to have dipole strengths for the interacting transitions. From Gaussian deconvolutions of the visible absorption spectrum of Chl *a* in diethyl ether we estimate the total dipole strength of the Q_y transition to be 30.5 debye^2, while the dipole strength of the $Q_y(0,0)$ vibronic component alone is 22.7 debye^2. The strong coupling approximation applies in exciton theory whenever the transition density splitting, 2T, is much greater than the energy difference (about 1160 cm^{-1}) between the (0,0) and (0,1) vibronic components, and weak coupling applies when the transition density splitting is much less than the energy difference between the (0,0) and (0,1) vibronic components. For the strong coupling approximation the total Q_y dipole strength is used to calculate the transition dipole–transition dipole interaction energy and for the weak coupling case, only the dipole strength of the $Q_y(0,0)$ component is used. Because 2T/1160 of about 0.5 is not much less than unity for our special pair model, neither the weak coupling nor the strong coupling case strictly applies and we have chosen to use an intermediate value (22.7 + 30.5)/2 = 26.6 debye^2 for the dipole strength in the intermediate coupling case. Using this dipole strength, we compute the transition dipole–transition dipole interaction energy, T, to be 292 cm^{-1}. Therefore, if our special pair absorbs at 700 nm (14,286 cm^{-1}), it follows from exciton theory (17) that the Q_y transition of each monomer is environmentally red-shifted to 686 nm (14286 + 292 = 14578 cm^{-1}) in the special pair. Transition density interactions then split the Q_y transition of the monomers into an exciton transition at 700 nm with almost all of the Q_y dipole strength from both monomers and an exciton transition at 672 nm (14578 + 292 = 14870 cm^{-1}) with almost no dipole strength. We can ask if the environmental shift to 686 nm is consistent with the known absorption maximum at 735 nm (13605 cm^{-1}) found for the case of hydrated methyl chlorophyllide *a* monolayers (18), where molecules interact *via* π–π stacking and keto C=O···H—O— — —Mg interactions similar to those of the Chl_{sp} model in Fig. 3. We assumed that the structure of the methyl chlorophyllide *a* monolayers is identical to the structure of the hydrogen-bonded sheets (ab crystal plane) found in the x-ray crystal structure of ethyl chlorophyllide *a*·2H_2O (16). These crystalline layers or sheets have one molecule per unit cell and for the special case of a crystal with one molecule per unit cell the environmental shift and transition density shift are additive. We computed the transition density shift by picking a molecule in the crystalline sheet and then calculating the pairwise sum of the interaction energies between its point transition dipole and the point transition dipoles of all the other molecules in the sheet. For the crystalline sheet, 2T/1160 of about 1.7 is not much greater than unity, so we assume that intermediate coupling applies. Using a dipole strength of 26.6 debye^2, we compute the transition density shift, T, to be 975 cm^{-1}. Therefore, the monomers in the crystalline sheet have their Q_y transition environmentally shifted to 13605 + 975 = 14580 cm^{-1} (686 nm), the same environmental shift needed to make the Chl_{sp} model absorb at 700 nm! We conclude that under the assumption of intermediate coupling, a 700 nm absorption maximum for the Chl_{sp} of Fig. 3 is consistent with the experimentally observed 735 nm absorption maximum for the hydrated monolayer.

Is C_2 symmetry important for the special pair?

The structural model proposed here (Fig. 3) has C_2 symmetry if the R–X–H (X = O, N, S) moieties binding the special pair are identical and if the conformations of the groups attached to the two Chl *a* molecules are identical. The Fong model (13) also has C_2 symmetry and Fong has argued (13) that the C_2 symmetry results in an antisymmetric lowest triplet exciton state

from which radiationless transitions to the ground state are both spin and symmetry forbidden. According to Fong, this forbiddenness allows the lowest triplet exciton state to live long enough to be upconverted by incoming singlet excitation from the antenna. However, Fong has apparently overlooked the significance of the fact that the first order splittings between triplet states that are degenerate at zeroth order are generally quite small compared to kT at room temperature. We feel that it is unreasonable to expect that coherence between very weakly coupled triplet excitations on the two molecules can be maintained over a long enough time to allow for upconversion from a coherent antisymmetric triplet exciton state; the coherency should be quickly broken by intermolecular collisions. We feel, therefore, that the arguments by Fong for C_2 symmetry as a way to promote upconversion via a long-lived triplet state are not meaningful. As far as we know, no convincing experimental evidence has been published that indicates light conversion in photosynthesis proceeds to a significant extent by way of upconversion (usually called singlet-triplet annihilation) as suggested by Franck and Rosenberg (19) and Fong (13).

In the Chl_{sp} model (Fig. 3) C_2 symmetry implies the equivalence of the two Chl *a* molecules with respect to each other and this equivalence along with the intermolecular π–π overlap present in our model (but not present in either of the two previously proposed models) promotes delocalization of the unpaired electron in $Chl_{sp}^{\cdot}$ over the π systems of both Chl *a* molecules. This delocalization has been established by ESR and ENDOR spectroscopy (7, 8) of the *in vivo* radical arising from P700. We conclude that C_2 symmetry is probably not important in itself, but that the near-equivalency of the environments of the two Chl molecules and close π–π stacking are of considerable importance.

A P700-like ESR signal from an *in vitro* system

A solution of 1 mM Chl *a*, 1.5 mM ethanol, and 100 mM tetranitromethane (water may have been present in the tetranitromethane) in toluene was prepared in the dark. Tetranitromethane functions as an electron acceptor to photo-oxidize Chl *a*. After slow cooling of the solution to 100 K the electronic transition spectrum was taken and a single major peak at 698 nm was observed. The solution was cooled slowly from room temperature to 96 K in the dark in an ESR tube and then irradiated with 700 nm light (300 W Eimac xenon arc lamp, Baird Atomic B-3 700 nm interference filter); a 7.5 gauss wide Gaussian signal was observed. When the temperature was slowly raised to 145 K the signal remained Gaussian and its width decreased to 7.1 gauss. Significantly, the ESR signal from this *in vitro* system is practically indistinguishable in shape, width, and temperature dependence from the ESR signal arising from photooxidation of P700 in *Chlorella vulgaris* (7, 8).

Conclusions

Our new structural model for the special pair (Fig. 3), while admittedly speculative, has several attractive features, of which perhaps the most important is that it can in principle be generated by protein–Chl interactions. Nucleophilic groups in amino acid side chains can serve the same purpose as does water or ethanol, butylamine, or ethylmercaptan in the laboratory to form low temperature Chl species absorbing near 700 nm (20). We have attributed the origin of the red shift to a combination of π–π stacking, strong hydrogen bonds to ring V keto carbonyls, and transition density–transition density interactions. In our model the two Chl *a* molecules are equivalent (or nearly so) and this accounts quite nicely for the experimentally observed delocalization of the unpaired electron over the π-systems of two Chl molecules.

The structure of Fig. 3 may also be a good model for the special pair in bacterial reaction centers. However, bacteriochlorophyll (Bchl) molecules can also be stacked and held together *via* acetyl C═O···H—O— — —Mg interactions involving the acetyl group in Bchl that replaces the vinyl group in ring I of Chl *a*. The proteins characteristic of bacterial reaction centers (21) contain a large number of amino acid residues from the group serine, lysine, cysteine, arginine, threonine, and tyrosine, and any pair from this group or any one of them plus water should be capable of forming $Bchl_{sp}$. The model srructure of Fig. 3 is sufficiently open in the region of R′ to allow plenty of space to accommodate the remainder of the amino acid side chain and nearby amide units of the polypeptide backbone.

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