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ON THE MUTUAL ORIENTATION OF PIGMENTS IN PHOTOSYSTEM I PARTICLES FROM GREEN PLANTS

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SUMMARY

The mutual orientation of pigments in Photosystem I reaction centers from spinach is evaluated by polarized photochemistry. The photoinduced linear dichroism of the absorption changes of chlorophyll a_I at 701 nm is studied as function of the excitation wavelength. The Photosystem I reaction center particles contain about 100 and if depleted about 40 chlorophylls, respectively. To prevent their rapid Brownian rotation they were immobilized on DEAE-Sephadex.

The excitation spectrum of the linear dichroism reveals a high degree of order between the long axis of β-carotene and the Q_y transition moments of those chlorophyll *a* molecules absorbing at the red end of the spectrum. The latter are the most endangered ones for destructive oxidation via their triplet state. Hence, the location of β-carotene in close proximity to and in parallel with these chlorophylls seems to be most favourable for the protective role of β-carotene within the antennae system I. It is observed that the dichroic ratio of the absorption changes of chlorophyll a_I does not exceed a figure of $\frac{4}{3}$, which characterizes a circularly degenerate system, even at far red excitation (724 nm). This will hit selectively those few chlorophyll *a* molecules with their peak absorption at about 700 nm (including the photooxidizable dimer). We conclude, if the dimer is the only species peaking at 700 nm then the two chlorophyll *a* within the dimer have their *y*-axes oriented perpendicular to each other. If there are some antennae in addition to the dimer, the *y*-axes of all chlorophyll-*a* peaking at 700 nm form a star which accounts for the circular degeneracy of absorption.

INTRODUCTION

Optical polarization spectroscopy has contributed some understanding of the average orientation of antennae pigments in the thylakoid membrane and on the orientation of the photochemically active chlorophylls. The orientation of these pigments could be inferred from linear dichroism or from fluorescence polarization

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since any absorption band or fluorescence band of a dye is polarized along given axes within the molecule. In chlorophyll *a* the major absorption bands are polarized in plane of the porphyrin ring along either of two mutually perpendicular axes (for an illustration, see inset in Fig. 2).

Linear dichroism studies on single *Euglena* chloroplasts [1] and on macroscopically oriented spinach chloroplasts [2–4] revealed that the *y*-axis of those antennae chlorophylls absorbing at the red end of the spectrum (above 680 nm) are oriented almost coplanar to the thylakoid membrane. In contrast to this no such preference was observed for the *y*-axis of the other chlorophyll *a* and of the chlorophyll *b* molecules. The carotenoids were found to be more or less parallel to the membrane, also. In the region of the chlorophyll *a* Soret bands a complex pattern of orientations was observed with a pronounced preference for a high inclination (higher than 35°) against the membrane [3]. These features were confirmed by fluorescence polarization [4–7]. While the above-cited studies on the static linear dichroism of all antennae did not discriminate between antennae attributable to either Photosystem I or Photosystem II, the technique of polarized photochemistry reveals the mutual orientation within each of these systems. In previous papers we reported on the linear dichroism of the flash-induced absorption changes of chlorophyll a_I (*P*-700) observed on originally isotropic suspensions of chloroplasts after excitation by linearly polarized light [8–10]. Based on these photoselection experiments we concluded that the major transition moments of chlorophyll a_I (700, 683 and 432 nm) are inclined at only less than 25° to the thylakoid membrane. For the Q-band region this was reconfirmed at much higher resolution in experiments with oriented chloroplasts [11, 12]. This paper presents an extension of this work aimed at two questions: (1) Which is the mutual orientation of the antennae within close proximity to the reaction center in Photosystem I particles? (2) What can be learned about the internal structure of the photochemically active chlorophyll *a* complex from photoselection experiments involving decreasing amounts of antennae chlorophyll? These questions were tackled by experiments with immobilized but isotropically distributed reaction center I particles [13, 14].

MATERIALS AND METHODS

Preparation of Photosystem I particles depleted of cytochromes. Chloroplasts were prepared by homogenization of portions of about 60 g of spinach leaves in a blender running at low speed for 7–10 s. The medium at 200 ml contained 0.4 M sucrose, 10 mM NaCl, 10 mM tricine, pH 8, and 10 mM sodium ascorbate. The homogenate was filtered through gauze and centrifuged for 7 min at 4000 rev./min (RC2-B Sorvall, GSA rotor). The precipitate was suspended in 10 mM tricine, pH 8, and centrifuged at 11 000 rev./min for 5 min. The pellet was dispersed by a glass-teflon homogenizer in a medium containing 0.4 M sucrose, 5 mM $MgCl_2$, 10 mM NaCl, and 10 mM tricine, pH 8. The chlorophyll concentration was adjusted to 1 mg/ml. Digitonin was added as a 10 % solution in water to yield a final concentration of 1 %. After incubation at 0 °C for 1 h the mixture was centrifuged at 11 000 rev./min for 10 min. The pellet was discarded. Solid NaCl and 10 % digitonin solution was added to give a final concentration of 0.1 M and 1.5 %, respectively. After overnight incubation in the refrigerator the mixture was centrifuged at 11 000 rev./min for 10 min, the pellet was discarded and the supernatant was centrifuged at 49 000 rev./min for 1 h with the 60 T II rotor in a Beckman centrifuge. The pellet was homogenized in a medium containing 0.4 M sucrose, 10 mM NaCl, and 10 mM tricine, pH 8, at a chlorophyll concentration of 3 mg/ml. If frozen at −70 °C this preparation can be stored for at least 2 years. For further details, see ref. 15.

Preparation of Photosystem I reaction center particles (PS-I-RCP). The Photosystem I particles depleted of cytochromes were treated with Triton X-100 and chromatographed on DEAE-cellulose column as previously described [13, 14]. The first 30 ml of the green fraction that was eluted from the column were collected. This fraction was applied on a gradient from 5 to 20 % sucrose in 25 mM Tris · HCl, and 0.2 % Triton X-100 and centrifuged for 18–24 h in a swinging bucket rotor (Beckman 4C) at 25 000 rev./min. The lower green bands were recollected and portions of 1 ml were frozen under liquid nitrogen. They can be stored for more than 6 months.

Immobilization and depletion of Photosystem I reaction center particles. To suppress rotational mobility of Photosystem I reaction center particles 0.5–1 ml of the above preparation (chlorophyll about 0.2 μM/ml) was applied to a DEAE-Sephadex A-50 column (1 × 10 cm). The Sephadex was equilibrated with 10 mM Tris · HCl, pH 8. Then the column was washed with 4 ml of 20 mM Tris · HCl. For partial depletion of antennae chlorophyll, if intended, it was washed before with 20 ml containing 10 mM Tris · HCl and 1 % Triton X-100. The upper green part of the column with the reaction center particles attached to the DEAE-Sephadex was transferred into an optical absorption cell ($2 \times 2 \times 4.5$ cm^3) which was filled up with DEAE-Sephadex to yield a final volume of 15 ml. The optical experiments were carried out under addition of redox agents, 6.6 mM sodium ascorbate, and 1.3 μM *N*-methylphenazonium sulfate.

Linear dichroism of absorption changes in photoselection. PS-I-RC particles were filled into an absorption cell with 2 cm optical path. The cell was mounted into a rapid kinetic spectrophotometer (see refs. 16 and 17). The sample was excited by a short Xenon flash (Osram XIE 200, half-time of duration 15 μs). The exciting light was filtered through interference filters at various peak wavelength with a half-width of transmission between 10 and 13 nm (Schott, Dr. Anders). The energy of the exciting flash impinging on the cuvette (area of illumination about 4 cm^2) after having passed through the interference filter and a polaroid sheet was variable between 0.05 and 0.2 mJ. Depending on the wavelength this flash excited between 3 and 15 % of the reaction centers, only. Non-saturating excitation is a prerequisite for photoselection from an isotropic ensemble. The measuring light was filtered through a monochromator (half-width 3 nm). It passed a rotatable polarizer before impinging on to the absorption cell. The intensity of the measuring light was gated by a photoshutter, to avoid steady oxidation of chlorophyll a_{I} before the flash. This was important to avoid prepolarization by the measuring light of the only slowly reversible chlorophyll a_{I} in the immobilized particles. To improve the signal to noise ratio the intensity of the measuring light was set as high as possible. However, its intensity times the opening time of the photoshutter (i.e. the energy per gate of about 200 ms) was kept approximately five times lower than the excitation energy of the flash. Changes in absorbance were recorded. Signals were excited repetitively and averaged on a Nicolet on line computer. The electrical bandwidth was limited by the dwell time per address of the analogue to digital converter of the computer which was set at 1 ms. As previously [9] the geometry of the optical set up was such that the exciting and the measuring light impinged on to the sample perpendicular to each other, both propagation vectors being horizontally. The electric vector of the exciting light was kept polarized in the vertical throughout the experiments, while the polarization of the measuring light was varied between vertical and horizontal, thus giving rise to changes of absorption denoted $\Delta A_{\parallel}$ and $\Delta A_{\perp}$, respectively. To avoid apparent dichroism from scattered flash light or from polarized fluorescence no extra polarizer was placed between the sample and the photomultiplier. The depolarization of light by scattering in the slightly turbid Sephadex suspension as measured by a pair of Glan-Thompson prisms was below 1 % throughout the spectral range from 400 to 700 nm. Limited by the beam divergence the polarization of the exciting flash light was $P = (I_v - I_h)/(I_v + I_h) \geqslant 90\,\%$. Throughout the article reference will be made to the theory of photoselection without further explanation. This theory is represented in an article by Albrecht [18]. Its application to various biological chromophores was recently reviewed by Hofrichter and Eaton [19]. We will discuss the linear dichroism in terms of the dichroic ratio of absorption changes: $\Delta A_{\parallel}/\Delta A_{\perp}$. The relationship of this expression to other ones used in the current literature is straightforward. Other expressions to measure the linear dichroism are: the polarization $P = (\Delta A_{\parallel} - \Delta A_{\perp})/(\Delta A_{\parallel} + \Delta A_{\perp})$ and the anisotropy parameter $r = (\Delta A_{\parallel} - \Delta A_{\perp})/(\Delta A_{\parallel} + 2\Delta A_{\perp})$.

RESULTS

Fig. 1 shows the absorption changes of chlorophyll a_I at 701 nm under excitation at 487 nm with a single flash of light at time zero. The traces were run for parallel and for perpendicular polarization of the exciting light with regard to the measuring one. The upper traces show the dichroic difference obtained by subtracting the lower two traces. It is evident that no dichroism is observed, if PS-I-RC particles are just suspended in aqueous solution where they are free to carry out rapid Brownian rotation. According to Tao's [20] rule of thumb for the rotational relaxation time of

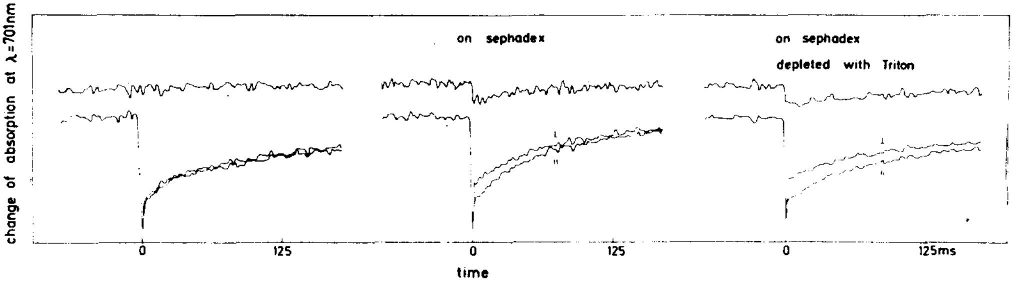

Fig. 1. Linear dichroism of the absorption changes at 701 nm observed with Photosystem I reaction center particles at excitation at 487 nm. Lower traces: Flash-induced absorption changes for parallel and for perpendicular polarization of the exciting flash and the measuring light. Upper traces: The difference of the absorption changes from the lower traces. Left: PS-I-RC particles suspended in buffer medium. Middle: PS-I-RC particles immobilized on DEAE-Sephadex. Right: Chlorophyll-depleted particles on DEAE-Sephadex. The extent of the absorption changes is about $\Delta I/I = 10^{-3}$. The traces were obtained by averaging over 64 repetitions at a frequency of 0.4 Hz. The optical bandwidth of the measuring light was 3 nm, the electrical integration time was 1 ms.

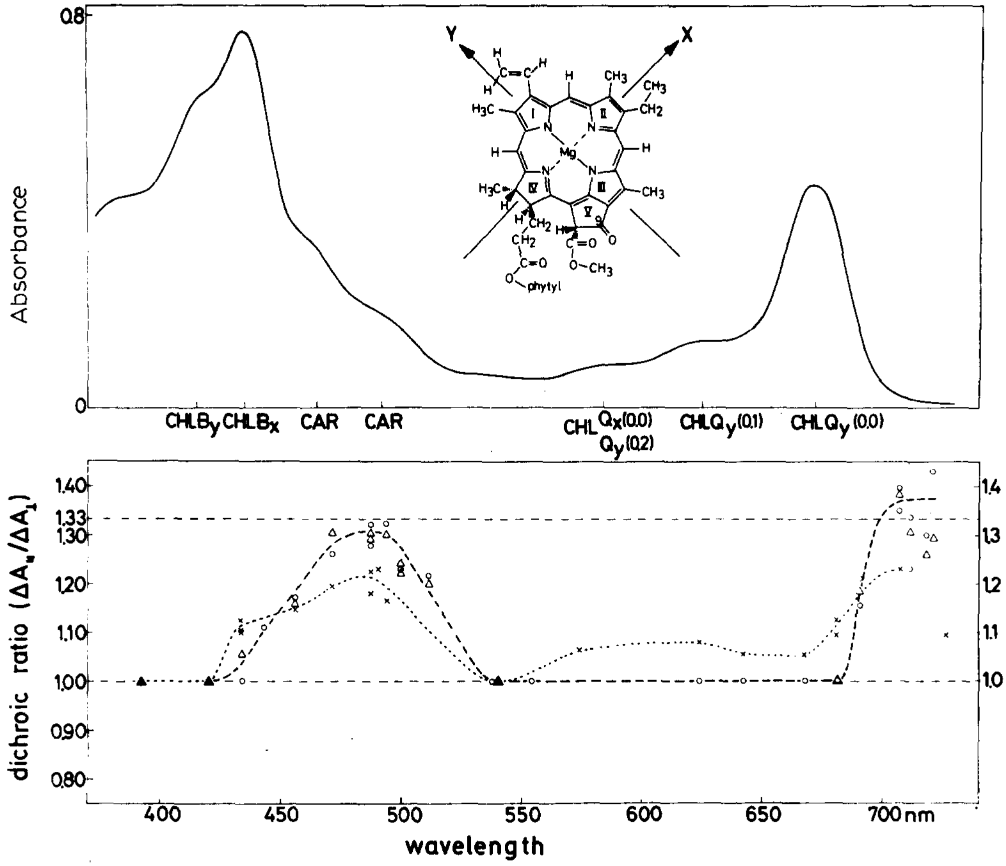

Fig. 2. Absorbance and excitation spectra of the photoinduced linear dichroism at 701 nm. Inset: The conventional axes assignment of chlorophyll *a*. Upper curve: Absorption spectrum of Photosystem I reaction center particles. Lower curves: Excitation spectrum of the photoinduced linear dichroism at 701 nm for PS-I-RC particles (crosses), for depleted PS-I-RC particles (circles) and for depleted particles additionally fixed with glutaraldehyde (triangles).

globular proteins the particles with an approximate molecular weight of $3 \cdot 10^5$ (Nelson, N. and Bengis, C., unpublished) are expected to relax in 80 ns if suspended in a medium at the viscosity of water. This, however, would not have been resolved in our experiments with an integration time of 1 ms.

Fig. 2 shows that linear dichroism appears after immobilization of the particles on Sephadex (middle and right). Additional treatment with the cross-linking agent glutaraldehyde had only small effect on the dichroic ratio. The dichroic ratio was greater in depleted particles than in the original ones. Fig. 2 shows the absorption spectrum of the particles (above) and the excitation spectrum of the dichroic difference of the absorption changes of chlorophyll a_I at 701 nm (below). The inset gives the structure of chlorophyll *a* and the conventional assignment of axes. As a biochemical analysis of PS-I-RC particles [13] revealed 98 chlorophyll *a*, 2 chlorophyll *b*, 13 β-carotene and no further carotenoids, the peaks and shoulders in the absorption spectrum of the particles can be assigned to chlorophyll *a* and to β-carotene as indicated. The attribution of the chlorophyll bands and shoulders to *x*- and *y*-polarized transitions is based on experimental and theoretical work which was recently reviewed by Sauer [21] (but see refs. 22 and 23 too). The lower part of Fig. 2 gives the excitation spectrum of the flash-induced dichroic ratio of the absorption changes at 701 nm. The crosses were obtained for the original particles (about 100 chlorophylls per center) and the circles for depleted particles (about 40 chlorophylls per center). The triangles were obtained for depleted particles treated with glutaraldehyde. The major features of the excitation spectrum are as follows: (1) The dichroic ratio does not exceed markedly a figure of $\frac{4}{3}$, not even at far red excitation. (2) There are several subsets of chlorophyll *a* molecules revealing different degrees of order (as evident from different dichroic ratios) relative to chlorophyll a_I. (3) The highest dichroic ratios and hence the highest degree of order is exhibited by the Q_y transition moments of those antennae chlorophyll *a* absorbing above 690 nm and by the β-carotene molecules. (4) No dichroic ratio smaller than 1 is observed at any wavelength. (5) Depletion sharpens the discrimination between the unordered and the ordered antennae.

DISCUSSION

On the internal structure of the chlorophyll a_I complex

One of the most striking features of the excitation spectrum of the linear dichroism (Fig. 2) is that the dichroic ratio does not exceed a figure of $\frac{4}{3}$, not even at an excitation wavelength of 724 nm. The following arguments make us believe that excitation at 724 nm selects the photochemically active dimer chlorophyll a_I plus possibly antennae chlorophyll *a* peaking at the same wavelength as the former (698 nm): In reasonable approximation the absorption spectrum of monomeric chlorophyll *a* in solution was found to be composed of Gaussian bands [24]. Deconvolutions of the complex absorption spectrum of chloroplasts in the spectral region of the Q_y bands of chlorophyll *a* gave no indication for a deviation from Gaussian lineshapes of the various physically different types of chlorophyll *a* in vivo (e.g. refs. 25 and 26). In analogy we may rely on the assumption that Photosystem I particles absorb light like an ensemble of Gaussian pigments. Gaussian pigments are distinguished by strong discrimination in their tail region. We evaluated the relative probability for exciting two Gaussian pigments as function of the excitation wavelength (see Table I). The peak wavelength of one pair of pigments were assumed to be spaced 10 nm apart, the ones of the other pair 20 nm apart. The half-widths (FWHM) were assumed to be rather wide (about 17 nm). As evident from Table I the discrimination at excitation at 725 nm is 240 : 1 for pigments peaking at 700 and 690 nm while it is $5 \cdot 10^5 : 1$ for pigments at 700 and 680 nm. Inspection of the absorption spectrum of PS-I-RC particles in Fig. 2 shows that most of the chlorophyll *a* molecules absorb around 680 nm while much less around 690 nm. This is in agreement with computer deconvolutions of chloroplast or fragmented chloroplast absorption spectra [25, 26]. These deconvolutions did not produce any evidence for components absorbing at longer wavelength than the photochemically active species chlorophyll a_I. This together with the high discrimination of Gaussians makes us believe that excitation at 724 nm hits pigments absorbing at the same wavelength as chlorophyll a_I. It is unknown how many chlorophyll *a* molecules per reaction center give rise to an absorption peak at 700 nm. It is generally accepted,

TABLE I

RELATIVE PROBABILITY OF EXCITATION FOR TWO PAIRS OF PIGMENTS WITH DIFFERENT PEAK WAVELENGTH OF ABSORPTION

It was assumed that the absorption profile be Gaussian in the frequency space: $A(v) = A(v_0)\cdot\exp(-0.5((v-v_0)/\delta)^2)$. The width parameter δ was assumed to be 150 cm^{-1} which, at the given wavelength, implies a bandwidth in wavelength of about 17 nm. Ant, antennae; $\mathrm{Chla_I}$, chlorophyll a_I; exc, excitation.

λ_{exc}(nm)	Discrimination			
	λ(ant) 690 nm	$\lambda(\mathrm{Chla_I})$ 700 nm	λ(ant) 680 nm	$\lambda(\mathrm{Chla_I})$ 700 nm
700	$1:2.6$		$1:6\cdot10^{1}$	
705	$1:6.6$		$1:3.5\cdot10^{2}$	
710	$1:1.7\cdot10^{1}$		$1:2.5\cdot10^{3}$	
715	$1:4.2\cdot10^{1}$		$1:1.4\cdot10^{4}$	
720	$1:1\cdot10^{2}$		$1:8.3\cdot10^{4}$	
725	$1:2.4\cdot10^{2}$		$1:5.3\cdot10^{5}$	
730	$1:5.8\cdot10^{2}$		$1:2.9\cdot10^{6}$	
735	$1:1.4\cdot10^{3}$		$1:1.6\cdot10^{7}$	
740	$1:3.2\cdot10^{3}$		$1:9.2\cdot10^{7}$	

however, that the long known bleaching of *P*-700 [27] is attributable to the oxidation of a dimer of chlorophyll *a*, with the unpaired electron delocalized over the two porphyrin rings [28]. Because of our lack of information on the actual number of pigments at 700 nm we will split the discussion into two cases:

(I) The chlorophyll *a* dimer be the only species absorbing at 700 nm. Then excitation at 724 nm hits the two Q_y transition moments in the dimer likewise and causes their bleaching, which is measured. It is noteworthy that excitation of one chlorophyll *a* will produce bleaching of both chlorophylls (or their half bleaching in the time average). This follows from the EPR- [28] and ENDOR- [43] detected rapid delocalization of the unpaired electron. According to the theory of photoselection [18] the only configuration of the two strongly coupled transition moments which accounts for the observed dichroic ratio of $\frac{4}{3}$ is the one where they are mutually perpendicular*.

(II) In contrast to case I we will now assume that the absorption at 700 nm is caused by the photochemically oxidized dimer plus some antennae. To make the situation differ as much as possible from case I we will furthermore assume that the two Q_y transition moments in the dimer are parallel to each other. Moreover we note that absorption of a quantum by one chlorophyll *a* out of the antennae set produces bleaching of the dimer, while the antennae remain unchanged at the time resolution of our experiments. Absorption of one quantum by the dimer itself will produce its oxidation and hence bleaching as well. (If the antennae shifted or bleached their absorption peak in consequence to the oxidation of the dimer, then the situation becomes similar to case I, however, now for two groups of parallel transition moments instead of two moments, only.) If there are only two groups of transition moments, each by itself oriented in parallel, the two ones of the dimer as well as the ones of the

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* This conclusion is not affected, if the band at 700 nm was the result of exciton interaction within the dimer or between the dimer and antennae.

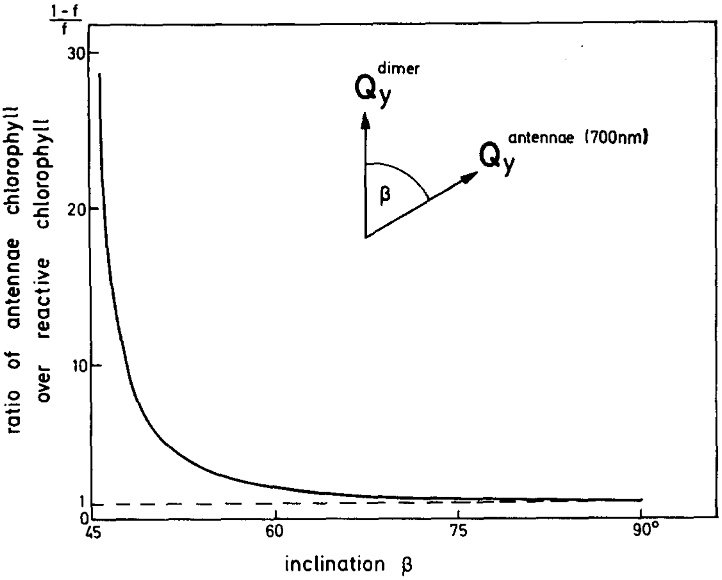

Fig. 3. The dependence of the proportion of antennae chlorophylls over chlorophyll in the oxidizable dimer on the angle between their respective Q_y transition moments under the assumption of an observed dichroic ratio of 4/3 (for details, see text and Appendix).

antennae, then the observed dichroic ratio implies the following relationship (see Appendix): $(1-f)/f = -\cos^{-1}(2\beta)$ where $(1-f)/f$ is the ratio of the numbers of transition moments (antennae over dimer), and β is the angle of inclination of the antennae against the Q_y axes within the dimer. This relationship is derived in Appendix. The functional dependence is plotted in Fig. 3. It is evident that at least two antennae chlorophylls ($(1-f)/f = 1$) are required to account for the observed dichroic ratio of $\frac{4}{3}$. These have to be oriented perpendicular to the dimer with their Q_y transitions. If there are four antennae, the angle is still 60°. If there are six antennae the angle is 55°. This, however, can be considered as an upper limit already, as a total of eight chlorophylls absorbing at 700 nm per reaction center is not compatible with the deconvolutions of the absorption spectra [25].

Thus our photoselection data lead us to two alternative possibilities for the construction of the reaction center. If the photooxidized dimer is the only species absorbing at 700 nm the Q_y transition moments of the two chlorophylls are mutually perpendicular. If there are antennae in addition to the dimer, then the y-axes of the chlorophylls in the dimer may be in parallel, but this implies that the y-axes of the antennae be almost perpendicular to the former.

Mar and Gingras [29] obtained very similar results in photoselection studies with isolated reaction centers from *Rhodospirillum rubrum*. Exciting (870 nm) and observing (900 nm) the same broad band they observed a dichroic ratio of the absorption changes of $\frac{5}{3}$, only. This contrasted sharply with the very high fluorescence polarization ratio ($\frac{3}{1}$) reported for similar preparations [30]. Mar and Gingras [29] concluded that there are two (or two sets of) transition moments, which fluoresce independently from each other but interact in producing the oxidation of a bacteriochloro-

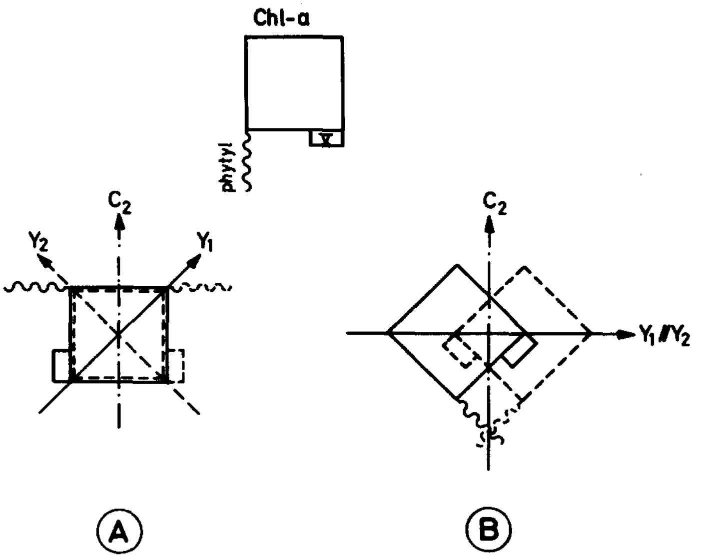

Fig. 4. Simplified representation of proposed structures for the oxidizable chlorophyll *a* dimer. (A) Structure related to the one proposed by Fong [34] with the *y*-axes highly inclined. (B) Structure related to the one proposed by Shipman et al. [35] with the *y*-axes only little inclined.

phyll dimer. They concluded as to a rather large inclination between the two (groups of) transition moments.

How do these results fit into the current hypotheses for the internal structure of the reaction centers in green plants (Photosystem I) and bacteria? In both systems there is evidence that the primary electron donor is a dimer of two chlorophyll molecules [28], the unpaired electron symmetrically delocalized over two porphyrin rings. In both systems there is evidence for exciton interaction within the absorption band involving the dimer [31, 32]. Structural models for the dimer were proposed based on model studies on chlorophyll aggregates in vitro [33–35]. Their common features are an almost coplanar orientation of two porphyrin rings with electron donor groups on ring V of one chlorophyll interacting with the central magnesium of the other. The more recent models by Fong [34] and Shipman et al. [35] are to be preferred for their C_2 symmetry which seems to be necessary to account for the symmetrical distribution of the unpaired electron over the two porphyrin rings. Their major difference lies in the inclination of the Q_y transitions. This is illustrated in Fig. 4. While the *y*-axes are inclined at 60° in Fong's model [34] they are almost in parallel in the one by Shipman et al. [35]. It is certainly premature to reject one or the other model based on the above photoselection work. However, it is evident that such a discrimination can be obtained once the number of those chlorophyll *a* molecules is known, which have their peak absorption at 700 nm (or 697 nm in particles) in contrast to the majority of antennae chlorophyll *a* which peak at shorter wavelength. This number is under present investigation. Under the assumption that the photooxidizable dimer is the only species peaking at 700 nm the photoselection data are compatible neither with Fong's model [34] for the dimer nor with the one by Shipman et al. [35]. While the former with an inclination between the *y*-axes of 60° would cause a dichroic ratio of 1.6

the latter with the two y-axes in parallel would cause a ratio of 3. If, however, there are antennae peaking at 700 nm, too, neither model can be ruled out. Then one has to conclude that all y-axes (special antennae plus dimer) are arranged such (e.g. forming a regular star) that circular degeneracy results.

Inclination of Photosystem I antennae relative to chlorophyll a_I at 700 nm

The more pigments are involved, the more ambiguous becomes a theoretical interpretation of photoselection data. Different configurations of several pigments may produce the same dichroic ratios. Nevertheless we will illustrate the mutual orientation of antennae and of the transition moment of chlorophyll a_I in terms of a "virtual angle of inclination" which is defined in the inset in the upper right of Fig. 5. It is assumed that Photosystem I including chlorophyll a_I behaves like a circularly degenerate system. This assumption is based on our previous photoselection work with oriented chloroplasts [36] and on the evidence presented in the foregoing section. We will assume that the antennae transition moments are located on a cone the axis of which is perpendicular to the plane containing the transition moment of chlorophyll a_I at 700 nm. The "virtual angle of inclination" is the angle this cone forms with the plane perpendicular to its axis (see Fig. 5, inset). Fig. 5 describes the dependence of the dichroic ratio in photoselection in dependence on the virtual inclination. An inclination at the magic angle, 35.26° against the plane, causes a dichroic ratio of 1 which is equivalent to the one for an isotropic distribution of the antennae. The virtual inclination of the x- and the y-polarized transitions of the majority of antennae chlorophylls

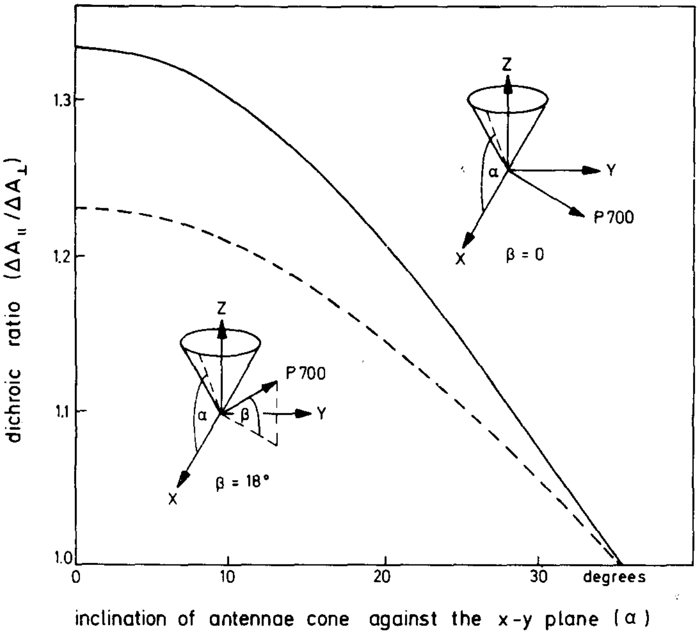

Fig. 5. The dependence of the dichroic ratio in photoselection experiments on the inclination of antennae pigments with their transition moments lying on a cone. The observed transition moment is located in the plane perpendicular to the symmetry axis of the cone (solid line) or inclined at $\beta = 18°$ to this plane (broken line).

is about 30° in the original Photosystem I particles while it is at the magic angle in the depleted particles. In contrast to this the Q_y transitions of chlorophyll *a* above 690 nm and the long axes of β-carotene are virtually inclined at 18° to the plane in the original particles while at zero degree in the depleted ones. These results are in agreement with those from studies on the static linear dichroism of oriented chloroplasts (e.g. ref. 3). The latter revealed that the carotenoids and the Q_y transitions of chlorophyll *a* above 680 nm are oriented almost parallel to the thylakoid membrane. (According to our previous photoselection work [8, 9] and the one from Breton's lab [11, 12] chlorophyll a_I is nearly parallel to the plane of the membrane.) However, it is new that β-carotene belongs to these pigments which are closest to Photosystem I. One can only speculate about the purposes for the location of β-carotene near Photosystem I. Besides acting as antennae β-carotene molecules may fulfil two tasks: (1) It has been proven that β-carotene protects triplet excited chlorophyll *a* in chloroplasts from irreversible photooxidation [37–40]. (2) It has been suggested that β-carotene may act as an electron conductor across the thylakoid membrane. This suggestion was based on experimental evidence from photovoltaic effects in artificial lipid bileaflet membranes doped with chlorophyll *a* and β-carotene [41].

As resonant energy transfer within the antennae in chloroplasts involves energy dissipation, it is directed towards pigments at longer wavelength. If the trap is closed, the quantum will reside most probably in the subset of chlorophyll *a* antennae absorbing at the red end of the spectrum. Hence this subset is the most endangered one for photooxidation via the triplet state. As the protective action of β-carotene involves resonant energy transfer from chlorophyll *a* to β-carotene, the most favourable configuration between these two types of pigments is the one with the long axis of the carotene in parallel and in close proximity to the *y*-axis of chlorophyll *a* (above 690 nm). The photoselection data are compatible with this configuration. For the suggested electron conducting role of β-carotene, however, one would rather expect that β-carotene be oriented perpendicular to the porphyrin ring of chlorophyll a_I, which is oriented almost parallel to the thylakoid membrane [8, 9, 11, 12].

One feature of the excitation spectrum of the photoinduced linear dichroism (Fig. 2, below) is in seeming discrepancy to observations by Breton and coworkers [2, 3]. We did not detect any dichroic ratios smaller than 1, while Breton and coworkers reported negative dichroism for the majority of the *x*-polarized transitions of chlorophyll *a* in the Soret region [2, 3]. It is conceivable that these chlorophyll *a* molecules with their *x*-axes inclined at more than 35° to the plane of the thylakoid membrane and hence to chlorophyll a_I belong to Photosystem II or if they serve Photosystem I they are only loosely coupled to the reaction center and lost during preparation of Photosystem I particles.

APPENDIX

On the superposition of linear dichroism from two components in photoselection

The subsequent derivation applies to the case mentioned in Discussion. A photoselection experiment is carried out where two types of species will be excited, say a dimer with parallel transition moments plus antennae inclined at an angle β to the dimer (see inset in Fig. 5). Both types of excitation shall produce absorption changes resulting from the dimer, only. Any transformation occurring transiently within the antennae is not time resolved in the experiment. The observed linear dichroism of the absorption changes of the dimer will therefore result from two types of excitation, the one of the dimer directly (superscript d) and the one of the dimer via the antennae (superscript a). The observed absorption changes are composite of these two contributions (superscript t for total).

Due to the additivity of absorption changes the total dichroic difference is:

$$\Delta A^{\mathrm{t}}_{\parallel}-\Delta A^{\mathrm{t}}_{\perp} = \Delta A^{\mathrm{d}}_{\parallel}+\Delta A^{\mathrm{a}}_{\parallel}-(\Delta A^{\mathrm{d}}_{\perp}+\Delta A^{\mathrm{a}}_{\perp}) \tag{1}$$

This is equivalent to:

$$\Delta A^{\mathrm{t}}_{\parallel}-\Delta A^{\mathrm{t}}_{\perp} = \{[(\Delta A^{\mathrm{d}}_{\parallel}-\Delta A^{\mathrm{d}}_{\perp})/(\Delta A^{\mathrm{d}}_{\parallel} + 2\Delta A^{\mathrm{d}}_{\perp})]\cdot(\Delta A^{\mathrm{d}}_{\parallel}+2\Delta A^{\mathrm{d}}_{\perp})\}+\{\ldots{}^{\mathrm{a}}\} \tag{2}$$

If we define the fraction of absorption changes contributed by direct excitation of the dimer f:

$$f=(\Delta A^{\mathrm{d}}_{\parallel}+2\Delta A^{\mathrm{d}}_{\perp})/(\Delta A^{\mathrm{d}}_{\parallel}+2\Delta A^{\mathrm{d}}_{\perp}+\Delta A^{\mathrm{a}}_{\parallel}+2\Delta A^{\mathrm{a}}_{\perp}) = (\Delta A^{\mathrm{d}}_{\parallel}+2\Delta A^{\mathrm{d}}_{\perp})/(\Delta A^{\mathrm{t}}_{\parallel}+2\Delta A^{\mathrm{t}}_{\perp}) \tag{3}$$

and the polarization anisotropy r:

$$r = (\Delta A_{\parallel}-\Delta A_{\perp})/(\Delta A_{\parallel}+2\Delta A_{\perp}) \tag{4}$$

we obtain:

$$r^{\mathrm{t}} = fr^{\mathrm{d}}+(1-f)r^{\mathrm{a}} \tag{5}$$

The proportion of absorption changes caused by the antennae $(1-f)$ over the ones by the dimer (f) then becomes:

$$(1-f)/f = (r^{\mathrm{d}}-r^{\mathrm{t}})/(r^{\mathrm{t}}-r^{\mathrm{a}}) \tag{6}$$

Eqn. 6 is equivalent to Weber's summation law [42]. In our above case the polarization anisotropies are (see Ref. 18): $r^{\mathrm{d}} = 0.4$ equivalent to a dichroic ratio of $\frac{3}{1}$, $r^{\mathrm{a}} = (3\cos^2\beta-1)/5$ and $r^{\mathrm{t}} = \frac{1}{10}$ which corresponds to the observed total dichroic ratio of $\frac{4}{3}$. Inserting these data into Eqn. 6 we obtain the following relation between the proportion of antennae over dimer and the angle of inclination between them:

$$(1-f)/f = -1/\cos 2\beta \tag{7}$$

This relation is plotted in Fig. 5.

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