ON THE ORIENTATION OF CHLOROPHYLL-a_I IN THE FUNCTIONAL MEMBRANE OF PHOTOSYNTHESIS

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1. Introduction

Light induced redox reactions in photosystem I in photosynthesis of green plants are indicated by absorption changes with maxima at 430 nm and 705 nm. These negative going absorption changes have been attributed to the rapid photooxidation of a special chlorophyll named *P* 700 [1] or chlorophyll-a_I [2].

The extremely rapid oxidation of this pigment [3] contributes to the extremely rapid generation of an electric potential difference across the functional membrane of photosynthesis [4–6]. Thus the reaction path of the oxidation is likely to be vectorial in the membrane. We studied the orientation of the porphyrin ring of chlorophyll-a_I in the membrane, hoping for a better understanding of the structural conditions of the electric potential generation.

Several models for the location and the orientation of this pigment have been discussed in the literature, However, it is obvious that they could not be substantiated by the present resolving power of electron microscopy and X-ray scattering for photosynthetic membranes. Moreover, model studies on the orientation of chlorophylls in bimolecular lipid membranes [7, 8] cannot be used for conclusions by analogy as to the orientation of chlorophyll-a_I, which is distinguished from the bulk chlorophylls by its reactivity and its spectroscopic properties.

In principle it should be possible to obtain the desired information from studies on the linear dichroism of this pigment in membranes which are oriented with respect to the lab system. Various attempts to orient the submicroscopic inner membranes of chloroplasts have been reported. The most recent ones were based on the orienting effect of high magnetic field strength [9–11] and dielectric surfaces [12], respectively. These studies revealed a strong linear dichroism of those chlorophyll-*a* molecules with absorption maxima at wavelength greater than 680 nm. It was concluded that the transition moments ($\lambda \geqslant 680$ nm) of these chlorophylls are all planar in the membrane [12]. On the other hand, there was less or practically no dichroism of the chlorophyll absorption bands at wavelength below 680 nm, leading to the conclusion that the corresponding transition moments were isotropically, or at the 'magic angle', oriented in the membrane [12].

Although these studies yielded information as to the orientation of the bulk chlorophylls, their resolution was too low to detect any dichroism resulting from chlorophyll-a_I which accounts for a fraction of only a few per millilitre of the total chlorophyll. To resolve this contribution one has to study the linear dichroism of the light induced absorption changes of this pigment. We did so on aqueous suspensions of isolated spinach chloroplasts. The difficulty that the membranes in such a suspension are oriented at random with respect to the lab system has been overcome by selection of an oriented ensemble by excitation of photosynthesis with a linearly polarized light flash. This photoselection technique has been applied, recently, in studies on the rotational mobility of rhodopsin [13] and the cytochrome oxidase [14] in the inner membranes of retinal rods and mitochondria, respectively. Photoselection studies with chloroplasts being somewhat more intriguing, the principle of this technique will be reviewed briefly: Irradiation of an isotropic suspension of chromophores with a linearly polarized flash of light

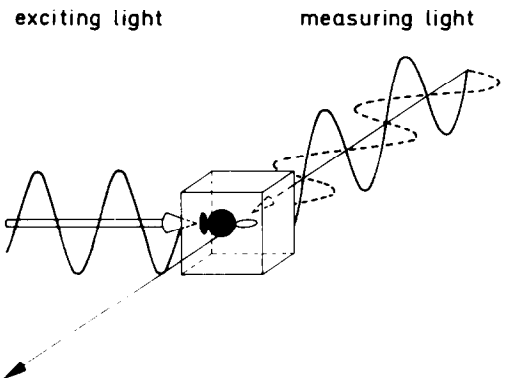

Fig. 1. The geometry in photoselection experiments. The orientation of the E-vector of the exciting and the measuring beam is indicated by sinoidal lines: Full circle, membrane orientations which lead to the excitation of in plane oriented oscillators; open circle; membrane orientation which does not lead to excitation.

causes a preferential excitation of those chromophores with their transition moments in parallel with the E-vector of the exciting flash. If the chromophores undergo photochemical reactions, absorption changes become observable, the extent of which depends on whether the measuring beam is polarized in parallel or perpendicular to the exciting one (see fig. 1). Photoinduced linear dichroism will be diminished or even abolished by two effects: i) resonant energy transfer between chromophores with different orientations before the onset of the photochemical reaction; ii) rapid rotation of the chromophores with respect to the lab system.

A quantum of light which is absorbed by an antennae pigment of the photosynthetic appartus 'visits' about 100 other pigments via resonance transfer before being trapped by a photochemical reaction center [15]. Thus, we would not expect any dichroism to occur, if the antennae pigments were oriented at random in the membrane. However, as the 680 nm transition moments of chlorophyll-*a* lie in the plane of the membrane, resonant energy transfer among these transition moments will converse some information on the polarization of the exciting beam. In this respect it is important to note, that resonant energy transfer, if mediated by Förster-[16] or a weak exciton mechanism [17], which is probable for the antennae system [15, 18], occurs into the direction of longer wavelength. This ensures that a quantum, once absorbed by one of the in plane oriented oscillators at the long wavelength end of the spectrum, does not leave the oriented ensemble. Thus, photoselection is likely to work on chloroplasts despite the efficient resonant energy transfer. However, instead of favourably oriented single transition moments, membrane orientations are selected by the polarized flash. For resonant transition moments which are planar in the membrane the exciting beam selects those membrane orientations, which are illustrated by solid circles in fig. 1.

Our experiments with chloroplasts at excitation wavelength above 680 nm revealed a photoinduced dichroism of the α-band (705 nm) and the Soret (430 nm) of chlorophyll-a_I. The dichroic ratio of the absorption changes $\Delta A_{\parallel}/\Delta A_{\perp}$ was greater than 1.2. This, under reasonable assumptions leads to the conclusion that the orientation of the porphyrin ring with respect to the membrane is almost planar.

2. Experimental

Broken chloroplasts were prepared from market spinach according to the method given in ref. [19]. The preparation was stored under liquid nitrogen until use. A typical value for the noncyclic phosphorylation rate of these chloroplasts: 45 nM/μM chl. x sec.

Chloroplasts were suspended at an average chlorophyll concentration of 10 μM in the following standard reaction medium: 3 mM tricine (pH 8); 20 mM KCl; 3 mM NH_4Cl; 1 mM $MgCl_2$; 3 μM benzyl viologen. The 2 cm absorption cell was mounted into a rapid kinetic spectrophotometer. Light reaction activity was induced by a short flash of light (half-time 15 μsec). For photoselection the flash energy was attenuated to yield excitation of only about 20% of the chlorophyll-a_I. The exciting light was passed through an absorption filter with a short wavelength cut-off (10%) at 690 nm (RG8/3, Schott). The exciting light was linearly polarized via a polaroid sheet. The geometry and the E-vector polarization of the exciting and the measuring beam are illustrated in fig. 1.

Before impinging on the absorption cell the measuring light beam was passed through an interference filter (IL-type, Schott) centered at 430 nm and 705 nm, respectively, and a rotatable polaroid sheet.

Changes in transmission on excitation were recorded.

The signals were induced repetitively by periodical flashes and averaged in a CAT 1000 computer for improvement of the signal to noise ratio [20]. The electrical bandwidth of the whole detection system was determined by the dwell time per address of the averaging computer, which in our experiments was 125 μsec.

As there was spectral overlap between the excitation at $\lambda > 680$ nm and the measuring light at 705 nm special care had to be taken to avoid artifacts caused by relaxation of the photomultiplier in response to bursts of scattered flash light. Flash burst artifacts were eliminated by two means: i) placing a very sharp cut-off interference filter (705 nm, $\Delta\lambda = 4$ nm, Dr. Anders KG) on the cathode of the photomultiplier; ii) increasing the intensity of the measuring beam up to 6000 erg/cm^2/sec. Under these conditions the photomultiplier (EMI 9558 BQ) was run at 4 dynodes, only. The absence of flash burst artifacts has been checked by measuring absorption changes under conditions where both light reactions were made inoperative (addition of 30 μM DCMU + 10 mM ferricyanide). No apparent absorption changes have been observed under these conditions.

Depolarization by light scattering in the turbid suspension was less than 5% as measured with a pair of Glan–Thompson prisms.

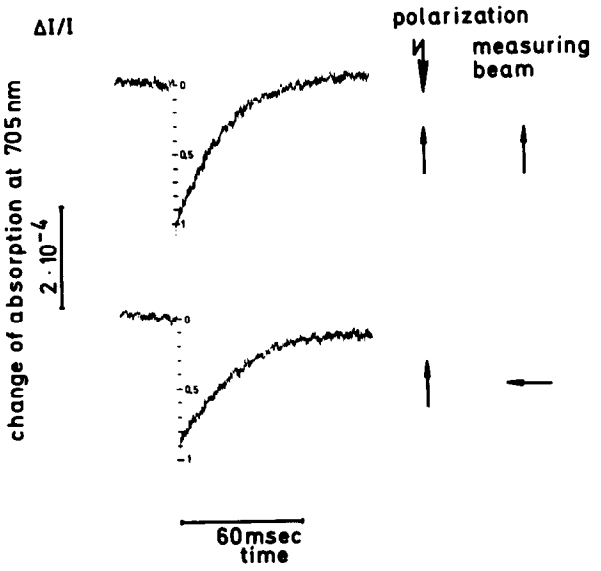

Fig. 2. Flash induced absorption changes of chlorophyll-a_I at 705 nm: Above, exciting and measuring beam polarized in parallel to each other; below, exciting and measuring beam perpendicular to each other; standard reaction medium; T = 20°C; viscosity, 1 cPoise, average over 320 signals; repetition rate 4 Hz; intensity of the measuring beam: 600 erg/cm^2/sec, excitation at wavelength greater than 690 nm, 20% of saturation.

3. Results

The absorption changes of chlorophyll-a_I at a wavelength of 705 nm are depicted in fig. 2. They were induced by a short flash of linearly polarized light of nonsaturating energy (20% saturation); As indicated in fig. 2 the E-vector polarizations of the exciting and the measuring beams were parallel to each other for the upper trace and perpendicular for the lower one. Comparison of the two traces shows that the extent of the absorption changes differs depending on the relative polarizations. The dichroic ratio of the absorption changes; $\Delta A_{\parallel}/\Delta A_{\perp} = 1.19$. The same ratio was detected if the excitation energy was lowered furthermore. On the other hand no dichroism was observed on excitation with saturating flash energy.

The absorption changes at 430 nm are depicted in fig. 3. Here the extent is 12% of that observed at saturating flash energies. Again photoinduced dichroism is obvious with a ratio $\Delta A_{\parallel}/\Delta A_{\perp} = 1.3$. The absorption changes at 430 nm and 705 nm, which indicate a reaction of the same pigment, have the same relaxation time if measured under similar conditions. Comparison of figs. 2 and 3 shows that the relaxation is slower at 430 nm. This was due to the fact that the experiments at 430 nm have been run at lower temperature. We varied the temperature and the viscosity (Ficoll was added to the standard reaction medium) in order to find out whether the measured dichroic ratios were diminished by the rotation of whole membranes in the suspension.

The rotational relaxation time of photosynthetic membranes can be estimated from Perrin's formula [21]: $\tau = (2\eta r^3/3kT)$, wherein r is the radius of the approximating sphere, and η the viscosity of the medium. To estimate a lower limit for the rotational relaxation time we took one thylakoid stack as the smallest rigid unit. Such a stack can be approximated by a sphere with a radius of 2500 Å units. In a 1 cPoise medium (water at 20°C) the corresponding

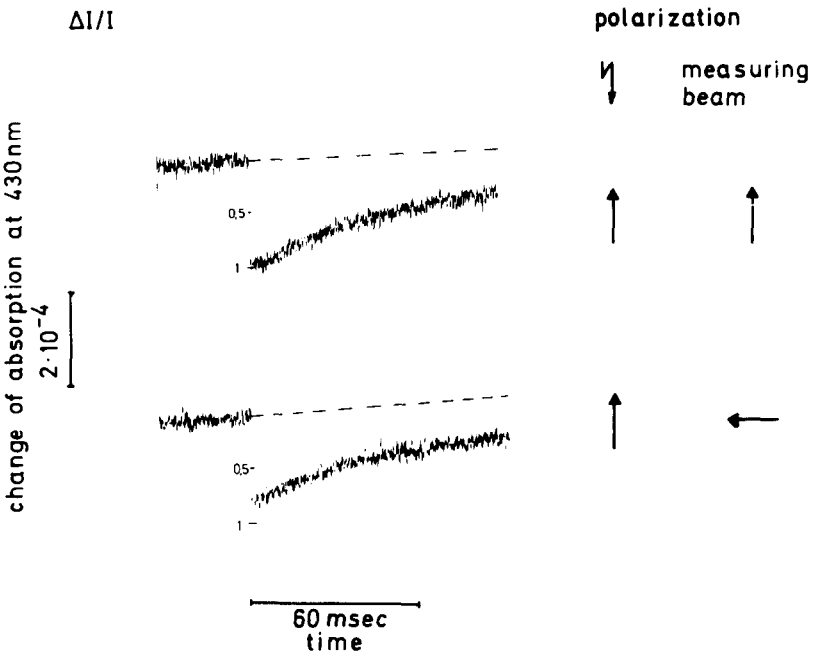

Fig. 3. Flash induced absorption changes of chlorophyll-a_1 at 430 nm. Polarization as in fig. 2: Standard reaction medium + 13% Ficoll (Pharmacia), T = 2°C; viscosity, 15 cPoise; average over 3200 signals (4 changes of the cuvette); repetition rate 4 Hz; intensity of the measuring beam: 1000 erg/cm^2/sec, excitation at wavelength greater than 690 nm, 12% saturation.

relaxation time is 8 msec, while it is 120 msec at 15 cPoise as in fig. 3. At least in the latter case rotation of the membranes will not lead to a decrease in the apparent dichroism at the time resolution of our experiments.

The absence of any detectable dichroic relaxation even in the experiments at lower viscosities (fig. 2) leads to the conclusion that broken chloroplasts are made up of several units of the size of one thylakoid stack, which are quite rigidly interconnected with each other, in agreement with electron microscopic results. At 430 nm we found the same dichroic ratio in experiments at viscosities of 1 cPoise and 15 cPoise, respectively.

The dichroic ratios at the two wavelengths were different for different chloroplast preparations. This will be specified in a subsequent paper it is sufficient to note that the dichroic ratio for both bands of chlorophyll-a_1 were always greater than 1.15.

The rapid absorption changes of P-430 [22] did not come into play at the relatively low time resolution of our experiments.

4. Discussion

A quantitative interpretation of the observed dichroic ratios as to the orientation of chlorophyll-a_1 in the membrane has to rely on assumptions on the in plane arrangement of the antennae transition moments ($\lambda > 680$ nm). It is obvious that the degree of photoselection depends on whether or not resonant energy transfer between the absorption and the final trapping of a quantum of light leads to a perfect circular distribution of the absorbed linear polarization. Two conditions which together are sufficient for this being so are: i) several resonant energy transfers before trapping, even at excitation wavelength greater than 680 nm; ii) an at least 3-fold symmetry axis for the orientation of the transition moments ($\lambda > 680$ nm) of the resonant antennae chlorophylls.

A great number of transfers has been found for excitation into the Soret band of the antennae chlorophylls [15], as mentioned above. This makes several transfers at excitation with $\lambda > 680$ nm probable, although there is no basis for a quantitative estimate,

yet. Likewise, we have only probability arguments for the fulfillment of the second condition. Studies on the plane structure of photosynthetic membranes by electron microscopy [23–25] and X-ray scattering [26] have revealed either a rectangular or an irregular geometry. Thus we regard a chlorophyll arrangement in large linear arrays as improbable.

If we assume a perfect circular distribution of the incident linear polarization via resonant energy transfer, then the antennae system ($\lambda > 680$ nm) around each photochemical reaction center is equivalent to a single circularly degenerate absorption dipole. In this case the theory of photoselection predicts a maximum dichroic ratio $\Delta A_{\parallel}/\Delta A_{\perp} = 4/3$, if the transition moment under observation lies in plane of the circle [27].

The dichroic ratios of about 1.2 or greater we have observed for both absorption bands of chlorophyll-a_I are then compatible with an inclination of the corresponding transition moments of less than than 15° to the plane of the membrane. The transition moments of the α-band and the Soret are approximately perpendicular to each other in plane of the porphyrin ring [28–29]. Thus it follows that the porphyrin ring of chlorophyll-a_I is only slightly inclined to the membrane, almost planar.

The relevance of such a configuration with respect to the mechanism of the rapid electric potential generation will be discussed in a subsequent paper.

The above experiments revealed a photoinduced dichroism of chlorophyll-a_I at a 100 msec time scale without need for treatments which may hinder the Brownian rotation of molecules in membranes. This contrasts with the outcome of similar experiments with the cytochrome oxidase in the inner membrane of mitochondria. There, photoinduced dichroism at the msec time scale was observed, only, if the rotation of this large enzyme (mol. wt 140 000) was hindered, for instance by glutaraldehyde fixation [14]. There are two alternative interpretations for the experimental outcome in the case of chloroplyll-a_I: either chlorophyll-a_I is embedded in a relatively large rigid domain and thus immobilized in the photosynthetic membrane or it is free to rotate around a single axis, which then has to be normal to the membrane and thus to the porphyrin plane as well. The latter possibility would not influence the photoinduced dichroism, as circular degeneracy is already imposed by resonant energy transfer. Since resonant energy transfer requires a tight packing of chlorophyll molecules we would rather favour the first possibility.

Acknowledgements

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