ORIENTATION OF THE PRIMARY DONOR CHLOROPHYLL OF PHOTOSYSTEM II IN CHLOROPLAST MEMBRANES

P. MATHIS, J. BRETON, A. VERMEGLIO and M. YATES

Service de Biophysique, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, BP 2, 91190 Gif-sur-Yvette, France

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1. Introduction

During the last years there has been a great advance in the knowledge of the structure of photosynthetic membranes, largely based on studies of purified reaction centers, and on detailed investigations of the asymmetry of the membranes and of the orientation of different components relatively to the membrane plane. In this respect it has been shown that the long wavelength transition moment of P700 (or Chl a_I), the trap of Photosystem I, was oriented nearly parallel to the membrane plane [1–3]. In this report we take advantage of the recent observation [4] of an absorption band peaking at 825 nm of the oxidized trap of Photosystem II (named Chl^{+}_{II}) for studying the orientation of this trap. We conclude that, like for P700, the long wavelength transition moment of Chl_{II} is predominantly parallel to the thylakoid membrane plane.

2. Materials and methods

Chloroplasts were prepared from freshly picked young spinach leaves by a 5 sec mild grinding in a blendor, in a sucrose (0.4M)–Tris (20 mM, pH 8.0)–KCl (20 mM) buffer. The juice was filtered on a nylon cloth and centrifuged at 2000 *g* for 1 min. The pellet was resuspended in a mixture of buffer and glycerol (1–2, by volume). The suspension was kept in ice for less than 4 h.

Chloroplasts contained in a cell (1 mm thick) were oriented in a 12 kG magnetic field, cooled and transferred in a Dewar flask. The detailed technique for trapping at low temperature the oriented chloroplasts is described elsewhere (A. Vermeglio, J. Breton and P. Mathis, manuscript in preparation). The measurement of flash-induced absorption changes was as previously described [4], except that the cuvette was perpendicular to the measuring beam and the exciting flash (λ = 600 nm, provided by a dye laser, Electrophotonics model 33) was made nearly colinear with the measuring beam. The cell was excited from the front side. Before falling on the cuvette, the measuring beam was polarized with a Polaroid sheet (type HN7). The temperature in the cuvette was $-170 \pm 10^{\circ}$C.

In each experiment a cuvette received successively 4 saturating flashes, separated by 25 sec. Each series consisted of an even number of experiments. In half of the experiments the absorption change (ΔA) was measured with the measuring beam vertically polarized for the 1st and the 4th flash, and horizontally polarized for the 2nd and the 3rd flash. In the other half of the experiments the reverse order was used. This procedure was chosen in order to correct for the evolution of the magnitude of ΔA in a series of successive flashes [4]. In two sub-groups of the memory of a Didac 4000 multichannel analyzer we stored the signals corresponding to the two directions of polarization of the measuring beam. We present the average absorption change per flash.

In the magnetic field the thylakoid membranes are oriented predominantly perpendicular to the field [5,6]. The position of the cuvette in the magnet and then in the flash spectroscopy equipment was such that the oriented membranes were vertical

and parallel to the axis of propagation of the light of the measuring beam. When this one was vertically polarized we measured the absorption change mostly in the plane of the lamellae ($\Delta A_{\parallel}$); when it was horizontally polarized, we measured the absorption change mostly perpendicular to the lamellar plane ($\Delta A_{\perp}$).

3. Results

Excitation of the chloroplasts suspension by the laser flashes induces an absorption increase at 825 nm. A semi-log plot of the absorption recovery after the flash indicates a fast exponential decay with $t_{1/2}$ = 2.8 ms followed by a slowly decaying phase, whose magnitude decreases from 100 to 70 (arbitrary units) in 40 msec. It has been shown previously [4] that the rapid phase is due to the absorption of the oxidized primary donor of Photosystem II (Chl^{+}_{II}) and that the slow phase to its counterpart in Photosystem I ($P700^{+}$).

In the figure we present, for a typical series of experiments, the absorption changes for both polarizations of the measuring beam ($\Delta A_{\parallel}$ and $\Delta A_{\perp}$, traces (a) and their difference ($\Delta A_{\parallel} - \Delta A_{\perp}$, trace (b). The traces (c) emphasize the property that the absorption changes have the same kinetics for both orientations: the difference ($\Delta A_{\parallel} + \Delta A_{\perp}$) can be nearly superposed, after magnification, with ($2\Delta A_{\parallel} + \Delta A_{\perp}$) which is a best evaluation of the average absorption change. The dichroic ratio

$$D = \frac{\Delta A_{\parallel}}{\Delta A_{\perp}}$$

was found to be similar for both phases:

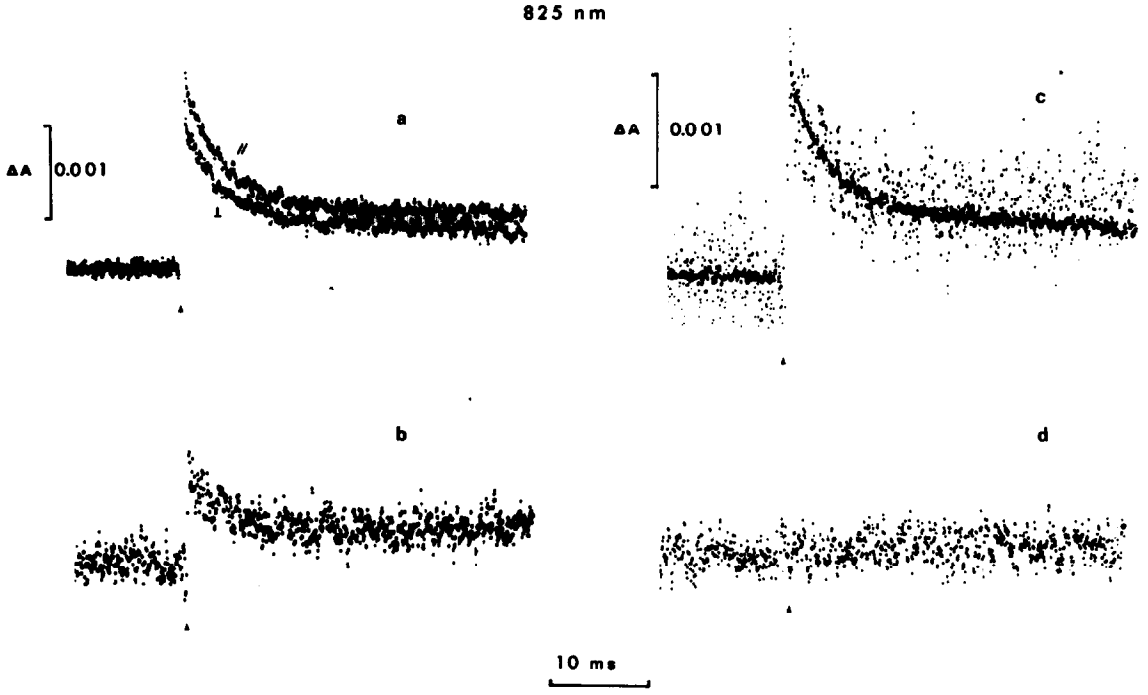

Fig.1. Absorption increase at 825 nm induced by a laser flash in a suspension of chloroplasts (chlorophyll concentration: 0.8 mg·ml^{-1}) at −170°C. Trace (a) Average of 12 experiments with the magnetic field perpendicular to the direction of the measuring beam. The electric vector of the measuring beam is vertical (or parallel to the chloroplasts membrane plane, $\Delta A_{\parallel}$) or horizontal (or perpendicular to the chloroplasts membrane plane, $\Delta A_{\perp}$). trace (b) difference ($\Delta A_{\parallel} - \Delta A_{\perp}$) from traces (a), expanded X 2. trace (c) the same difference (expanded X 11) compared with ($2\Delta A_{\parallel} + \Delta A_{\perp}$). The scale holds for the sum. trace (d) equivalent to trace (b), from 4 experiments with the magnetic field parallel to the direction of the measuring beam.

D (fast phase) = 1.32 ± 0.05
D (slow phase) = 1.40 ± 0.05

As a control we conducted the same experiments with chloroplast membranes oriented perpendicular to the axis of propagation of the measuring beam. In this case there is no difference in the absorption changes measured with the polarizer set in the two different positions (trace (d)). In another control, with the usual geometry, we measured the dichroic ratio for the absorption decrease due to the oxidation of P700, at 703 nm; we found D = 1.4.

4. Discussion

The transitions which are observed at 825 nm belong to the chlorophyll radical-cation of the primary donor of Photosystem I ($P700^+$) and of Photosystem II (Chl^+_{II}). For the radical-cation of bacteriochlorophyll it has been found, by molecular orbital calculations, that the long-wavelength transition has the same orientation, in the plane of the tetrapyrrole ring, as the Y transition of the non-ionized molecule [7]. The same property holds probably for chlorophyll *a*, a prediction which receives some support from the identical dichroic ratio which is measured, with oriented chloroplasts, for the long-wavelength absorption band of P700 at 703 nm and for the absorption band of its cation, around 820 nm (present results; J. Breton, manuscript in preparation; A. Vermeglio, J. Breton and P. Mathis, manuscript in preparation).

From our experiments we can therefore conclude that the Q_y transition moment of the primary donor chlorophyll of Photosystem II has the same orientation, relatively to the membrane plane, as the long-wavelength transition of P700, i.e. nearly flat [1–3]. The dichroic ratios that we measured at 825 nm (1.32 for Chl a^+_{II}, 1.40 for $P700^+$) are pretty low, indicating a poor orientation of the membranes and/or of the transition moments relatively to the membrane plane. In an independant study it has been found, however, that in low temperature measurements, there are a series of factors which contribute to a poor orientation of the membranes, detected by a decreased dichroic ratio for both P700 and the antenna pigments (A. Vermeglio, J. Breton and P. Mathis, manuscript in preparation). The main loss of orientation is due to the addition of glycerol which is necessary for obtaining a clear glass.
We presume that the same factors affect the orientation of the membranes in these experiments and that the low value of the dichroic ratio is not inconsistent with the proposal that the Q_y transition moment of Chl_{II} lies nearly flat in the thylakoid membrane.
In this discussion it is implicitly supposed that Chl_{II} is a monomeric chlorophyll. It has been proposed that it is a dimeric form [8], in which case our conclusion would probably apply to both molecules in the dimer. As for P700, this might however add some degree of uncertainty to our conclusions.

References

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