LINEAR DICHROISM AND ORIENTATION OF PHEOPHYTIN, THE INTERMEDIARY ELECTRON ACCEPTOR IN PHOTOSYSTEM II REACTION CENTERS

Irene B. GANAGO, Vyacheslav V. KLIMOV, Alexander O. GANAGO, Vladimir A. SHUVALOV and Yuri E. EROKHIN

Institute of Photosynthesis, USSR Academy of Sciences, Poustchino, 142292, USSR

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1. Introduction

Specific molecular organization of reaction centers which provide the high quantum efficiency of light-induced charge separation in photosynthesis has been intensively studied by polarized light spectroscopy [1–10]. Orientation of electric transition dipole moments corresponding to the primary electron donors and acceptors in the reaction centers of photosynthetic bacteria and photosystem I of higher plants has been analyzed on the basis of linear dichroism (LD) and photoselection data [1–6].

The transition dipole of the 820 nm absorption band of the oxidized primary electron donor, chlorophyll $P680^{+}$, in the reaction center of photosystem II (PS II) lies almost parallel to the thylakoid membrane plane [7]. Here, we report LD measurements of light-induced absorbance changes related to photoreduction of pheophytin, the intermediary electron acceptor of PS II, in oriented subchloroplast particles.

Light–dark difference absorption spectra of pheophytin photoreduction in reaction centers of PS II reported here, consist of 2 components:

(1) Corresponding to light-induced reduction of pheophytin itself with an absorption band near 680 nm and the Q_y transition dipole almost perpendicular to the membrane plane. The Q_x transition dipole of the pheophytin molecule is approximately parallel to thylakoid membrane plane.

(2) Representing a blue shift of a chlorophyll absorption band centered at 680 nm and polarized approximately parallel to the membrane, which has been tentatively ascribed to P680 or another chlorophyll molecule within or near to the reaction center of PS II.

2. Materials and methods

Sub-chloroplast particles enriched in PS II reaction centers were prepared as in [8]. The procedure involved treatment of pea chloroplasts with detergents digitonin (0.4%) and Triton X-100 (0.1%), and the particles were sedimented by centrifugation at 20 000 × g [8]. This preparation is designated DT-20. Samples were oriented in polyacrylamide gel as in [4]. A rectangular sample was squeezed 1.5-fold along its x-axis and elongated in z-direction, while the y-size was kept constant. Light–dark absorbance changes, ΔA, were measured with the apparatus in [8]. A non-polarized beam of saturating actinic red light passed through the sample along its x-axis; the monochromatic, linearly polarized measuring beam was propagated in y-direction. Two values of absorbance changes, ΔA_x and ΔA_z, were registered when the electric vector of the measuring light beam was parallel to the x-axis of the sample or to its z-axis, respectively [5].

Light-induced accumulation of reduced pheophytin in the reaction center of PS II is observed at low redox potential, E_h, when the electron acceptor Q is chemically reduced in the dark [8]. To fullfil these conditions, the polymerized samples were pre-incubated at 2°C in buffer solutions with dithionite at $E_h \approx -450$ mV as in [9].

3. Results and discussion

Absorption spectra of oriented DT-20 measured in linearly polarized light were quite similar to those measured [10] in oriented chloroplasts. Comparison of the signs of LD values in DT-20 and in chloroplasts showed that the Q_y transition dipoles of chlorophyll

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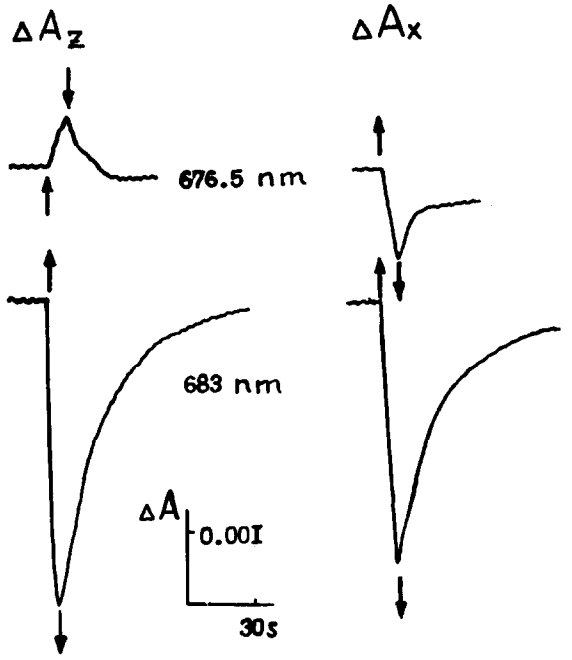

Fig.1. Light-induced absorbance changes in DT-20 particles measured at 676.5 nm and 683 nm in linearly polarized light with its electric vector parallel to the stretching direction (ΔA_z) and to the squeezing direction (ΔA_x) of the gel sample. The arrows ↑ and ↓ indicate turning the actinic light beam on and off, respectively. Chlorophyll was 17 μg/ml, optical path-length 20 nm; room temperature; illumination time, 7 s.

molecules were approximately parallel to the z-axis of a gel sample, and it was concluded that the thylakoid membranes were aligned predominantly parallel to the z-axis of the sample. This conclusion enabled us to estimate the orientation of the transition dipoles which correspond to absorption bands in light–dark difference spectra of DT-20 regarding the thylakoid membrane plane.

Fig.1 shows that in oriented DT-20 samples the light–dark absorbance changes associated with accumulation of reduced pheophytin were essentially different for two different polarizations of the measuring beam. Fig.2 shows the spectra of reversible light-induced absorbance changes in the oriented DT-20 preparations. Similar results were obtained with PS II samples prepared using detergent Deriphat 160.

Spectra ΔA_x and ΔA_z differ in several respects. A narrow band, which appears near 674 nm, is observed only in the ΔA_z spectrum. In the Q_y-transition region 675–695 nm, the bleaching band in the ΔA_x spectrum is shifted by 2–3 nm with respect to the band in

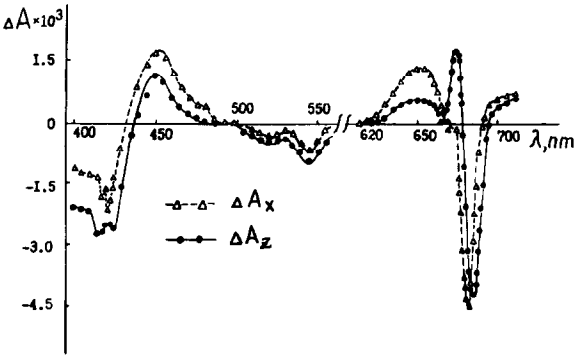

Fig.2. Spectra of reversible light-induced absorbance changes measured in linearly polarized light in oriented DT-20 preparations. See fig.1 and text for details.

the ΔA_z spectrum. A broad absorption band which appears at 630–660 nm (it was tentatively ascribed to formation of the pheophytin anion-radical, $Ph^{\cdot -}$) dominates in the ΔA_x spectrum. The dichroic value:

$$P = \frac{\Delta A_z - \Delta A_x}{\Delta A_z + \Delta A_x}$$

was constant over the latter band, the value $P = -0.42 \pm 0.01$ was calculated from 20 measured points. In the Q_x-transition region of pheophytin (a characteristic bleaching band near 545 nm) the dichroic value was positive, $P = +0.18 \pm 0.02$. From 570–620 nm, the magnitudes of both signals (ΔA_x and ΔA_z) were $<1 \times 10^{-4}$; this part of the spectra is not shown in fig.2. In the blue region of 400–490 nm the various bands displaying absorbance increases and decreases were considerably overlapped.

The dramatic changes of dichroic value in the 660–695 nm region indicate that the measured spectra resulted from an overlap of at least 2 components with different polarizations. We assumed that the number of components was exactly 2 and calculated their bandshapes using the decomposition technique proposed by Alentsev (see [11]) and applied to polarization measurements in [3]. Component 1, characterized by the constant dichroic value interval ($P_1 = -0.42 \pm 0.01$) of the spectra in fig.2 can be excluded according to [3,11]. The bandshape of the second component is shown in fig.3a. We could approximate its dichroic value $P_2 = +0.2 \pm 0.1$ using the 685–690 nm region of the measured spectra. Then

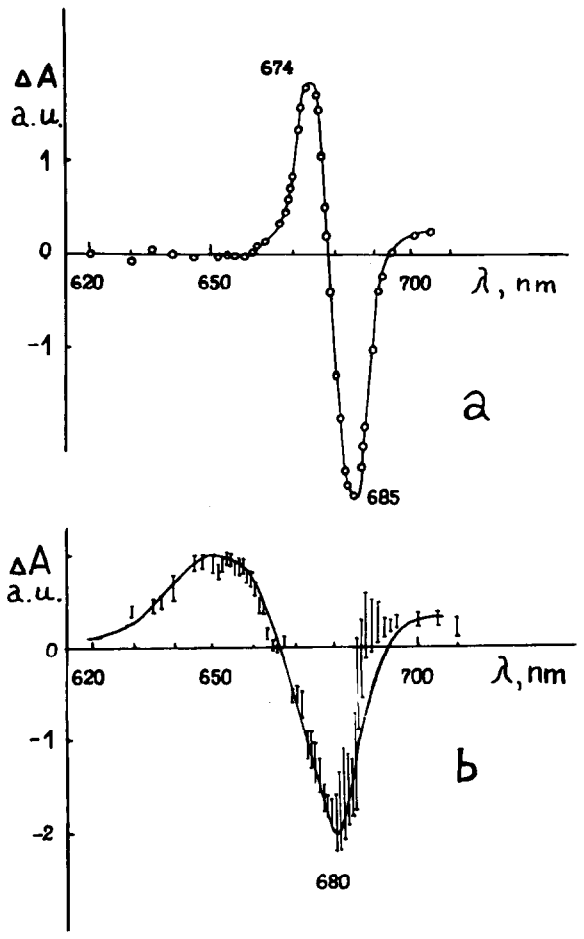

Fig.3. Calculated components of the light–dark absorption spectra shown in fig.2: (a) bandshape of the component with dichroic value P_2 = +0.2 ± 0.1; (b) bandshape of the component with dichroic value P_1 = −0.42 ± 0.01 (vertical bars) and the difference absorption spectrum corresponding to formation of the pheophytin anion-radical ($Ph^{\cdot -}$) in solution [12] shifted by 17 nm to longer wavelengths (solid line). See the text for details.

the bandshape of component 1 could be calculated; it is depicted in fig.3b by vertical bars (the height of each bar corresponds to the error in P_2 value estimate). The solid line in fig.3b represents the difference spectrum measured upon reduction of pheophytin to form an anion-radical in solution in [12]. (Note that the latter spectrum is shifted to longer wavelengths by 17 nm.) The bandshape of the latter spectrum is quite similar to that of the component 1 in fig.3b by vertical bars. We conclude that the component 1 (P_1 = −0.42 ± 0.01) corresponds to the difference absorption spectrum of light-induced reduction of pheophytin in reaction centers of PS II. Its extremum is near 680 nm. The diversity in both peak positions and half-bandwidths of the 2 spectra in fig.3b can be ascribed to difference in molecular interactions and environments in solution and in reaction centers of PS II.

Component 2 (fig.3a) (P_2 = +0.2 ± 0.1) comprises a bleaching band near 685 nm and a developing band near 674 nm. It resembles the part of the difference absorption spectrum observed upon light-induced reduction of bacteriopheophytin in reaction centers of photosynthetic bacteria ascribed to a short-wavelength shift of bacteriochlorophyll absorption band [2,3]. We may assume that in PS II preparations the component 2 (fig.3a) corresponds to a blue shift of an absorption band centered at 680 nm, with the transition dipole approximately parallel to the thylakoid membrane plane. This band may belong to a chlorophyll which either is a constituent part of the PS II reaction center (presumably, P680 pigment) or is located in close vicinity to the reaction center of PS II. The dichroic value of +0.14 ± 0.02 for the $P680^{\cdot +}$ absorption band in [7] is similar to P_2 estimated here. The Q_y and Q_x absorption bands of the measured spectra (fig.2) correspond to monomeric pheophytin [15]. Hence it is possible to estimate orientation of the pheophytin molecule with respect to the thylakoid membrane plane. Since the Q_y transition dipole is almost perpendicular to the membrane plane (P_1 = −0.42 ± 0.01), and the Q_x dipole is approximately parallel to the membrane (P = 0.18 ± 0.02), the macrocycle plane of the pheophytin molecule is approximately perpendicular to the membrane. A similar orientation of the bacteriopheophytin molecule has been observed in bacterial membranes [1–6,13,14].

Thus, arrangement of the systems involving (bacterio)pheophytin and (bacterio)chlorophyll in reaction centers of PS II and photosynthetic bacteria are very similar. This supports the proposal for the common evolutionary origin of the photosystems of green plant PS II and purple photosynthetic bacteria (see [8,15]).

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