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SPECTROSCOPIC PROPERTIES OF *PISUM SATIVUM* IMMOBILIZED PHOTOSYSTEM I

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The isolated Photosystem I (PS I) complex from pea Pisum Sativum, immobilized in the poly(vinyl alcohol) film shows energy dissipation by heat in the β-carotene absorption region by means of the photoacoustic spectroscopy. The linear dichroism measurements of the oriented photosynthetic pigments of the PS I complex indicate two forms of β-carotene molecules with max. at around 490 nm and 500 nm. In the thermal deactivation takes part only long wavelength form. The 730 nm fluorescence maximum, characteristic only for chlorophyll a bind to the reaction center protein, has higher intensity when PS I complex is in more rigid environment (e.g. film).

INTRODUCTION

The co-operation of two types of reaction centers, Photosystem I (PS I) and Photosystem II (PS II) is necessary for oxygenic photosynthesis of plants. The knowledge of the reaction center complex organisation is of fundamental importance for the study of the efficient conversion of light energy into chemical (redox) energy.

Information about absorption and fluorescence transition moments of the pigment molecules, with respect to the geometrical longest axis of the complex, can be obtained from polarized absorption and fluorescence spectroscopy of oriented complexes. The spectroscopic studies by linear dichroism have given an information of the molecular orientation of the small PS I particles. Linear dichroism (LD) and fluorescence polarization (FP) studies generally have shown the orientation of the chlorophyll-protein complexes (ellipsoidal in shape) in the thylakoid membrane (Biggins & Sveykovsky, 1980; Haworth, Tapie, Arntzen & Breton, 1982b; Tapie, Havorth, Herro & Breton, 1982; Tapie, Acker, Arntzen, Choquet, Deleplarie, Dinner, Wollman & Breton, 1984; Szito, Zimanyi & Faludi-Daniel, 1985). Data of the relationship between pigments and protein are not very clear except that Q_y transition of the longer wavelength absorbing chlorophyll is more highly oriented than that of chlorophyll a (Chl a) absorbing at shorter wavelength (Haworth, Tapie, Arntzen & Breton, 1982a) and that β-carotene molecules are oriented parallel to these longer wavelength forms (Junge, Schaffernicht & Nelson, 1977). Apparently extraction of β-carotene affects small PS I subunits and the state of longer wavelength-absorbing chlorophyll a (Chl a) forms (Białek-Bylka & Brown, 1986).

The PS I reaction center research is covered well by recent papers (Thonberg, Morishige, Anandan & Peter, 1991; Golbeck & Bryant, 1991; Golbeck 1992, Evans & Nugent, 1993; Schubert, Klukas, Krauss, Saenger, Fromme & Witt, 1995). The X-ray crystallography data has shown similarity between the quinone-type bacterial reaction center (RC) (Deisenhofer, Epp, Sinning & Michel, 1995) and the Fe-S type PS I RC (Schubert *et al.*, 1995) in the arrangement of the prosthetic groups which are responsible for the primary electron-transfer reactions.

Absorption of the light by the PS I induces the electronic excitation of pigments. This excitation is channelled by energy transfer to P-700, which gets excited and becomes able quickly transfer an electron to the primary acceptor causing a stabilisation of the charge separation. The yield of the excitation energy transfer between β-carotene and Chl a molecules is not influenced by the incorporation of the PS I particles in isotropic poly(vinyl alcohol) - PVA film (Białek-Bylka, Szkuropatov, Kadosznikov & Frąckowiak, 1982; Białek-Bylka & Brown, 1986; Białek-Bylka, Brown & Manikowski, 1987). Accounting for the very low yield of the fluorescence of carotene (van Grondelle, 1985; van Grondelle & Amesz, 1986; van Grondelle, Dekker, Gillbro & Sundstrom, 1994) and an efficiency of energy transfer from β-carotene to Chl a around 30% (Białek-Bylka & Brown, 1986), the rest of the excitation energy is for photochemistry and thermal deactivation (TD) of β-carotene and Chl a molecules.

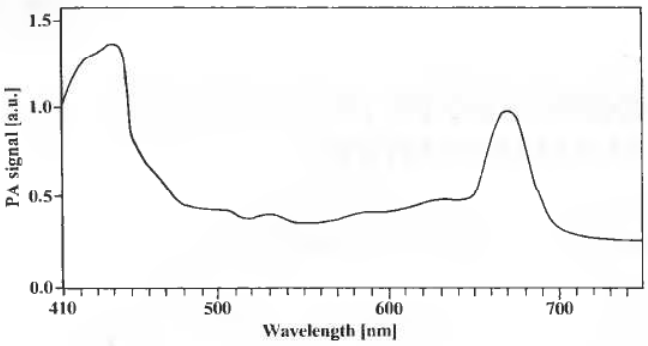

Fig. 1a Room temperature photoacoustic spectrum of the PS I in the PVA film.

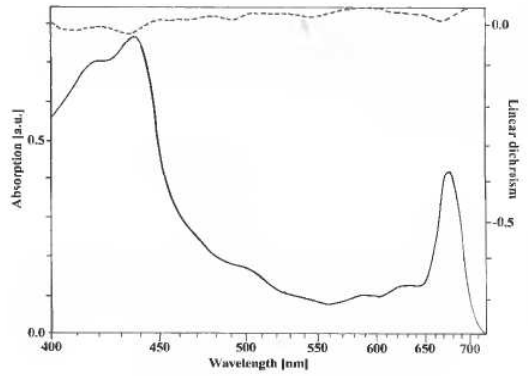

Fig. 1b. Room temperature spectra of the PS I in the PVA film: —— absorption, - - - LD.

EXPERIMENTAL

Preparation of Samples.

The PS I complex was prepared from a pea *Pisum Sativum* according to the method of Bengis and Nelson (1975). The Chl a content was 15 μm/ml and PVA content was 15%. The film preparation and stretching was according to Fiksiński and Frąckowiak (1980). The PVA film was drying about 7 days. The particles were mixed with 10% w/v aqueous PVA solution. The film was stretched mechanically to as much as four times of its initial length ($\Delta l/l = 300\%$). Thickness of the film was 240 μm.

Spectral measurements.

The photoacoustic spectra were measured according to Frąckowiak, Erokhina, Balter, Lorrin, Szurkowski and Szych (1986) at the modulation frequency of 11 Hz. The samples were thermally thin and optically transparent and the acoustic signal was proportional to the absorption Rosencweig (1980). Carbon black as a reference sample was used.

Absorption spectra were measured with M40 Specord Spectrophotometer equipped with polarizers. The absorption of the light polarized parallel $A_{\parallel}$ and perpendicular $A_{\perp}$ with respect to the direction of the film stretching were measured. Also absorption (A) of the natural (unpolarized) light by the sample (in the unstretched film) was measured.

The fluorescence spectra were measured with Hitachi 850 fluorometer. The exciting wavelength was seated at 440 or 460 nm (±7 nm). The fluorescence emission was observed at 90°.

RESULTS AND DISCUSSION

The photoacoustic spectrum of the PS I complex in the PVA isotropic film is shown in the Fig. 1a. Comparing the absorption (Fig. 1b) and photoacoustic (Fig. 1a) spectra one can see some differences in the energy dissipated as a heat for the pigments of the PS I complex. The amplitude of the PAS in the red spectral region is smaller than that in the blue part of the spectrum, because there exists the efficient internal conversion between the S_2 and S_1 states of the excited chlorophyll molecules. The longwavelength band of the β-carotene (around 500 nm) is seen well in the photoacoustic spectrum. Taken into account that the yield of the

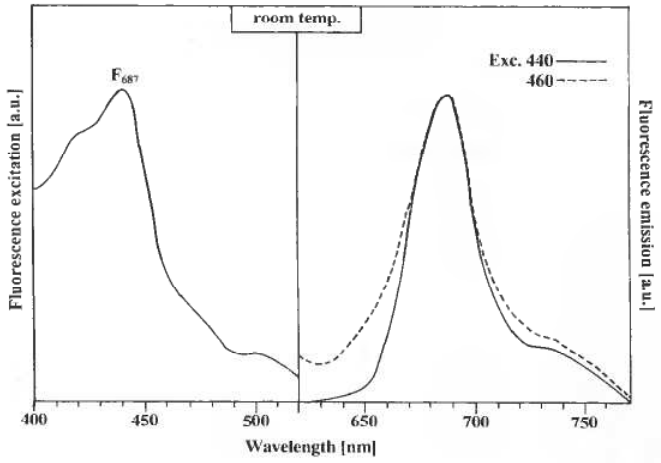

Fig. 2. Room temperature steady state fluorescence emission spectra: curve — of the PS I complex in the PVA film, curve - - - of the free pigments extracted from the PS I complex and incorporated into PVA film (excitation at 440 nm).

fluorescence of β-carotene is very low van Grondelle (1985) and that the efficiency of the energy transfer from β-carotene to Chl a in PS I complex in both, buffer (Białek-Bylka *et al.*, 1982) and in the PVA film (Białek-Bylka & Brown, 1986) is around 30%. The energy dissipated by heat and used for photochemistry is around 70%.

Linear dichroism measurement (Fig. 1b) of the PS I complexes from pea were used to study the orientation of the absorbing dipoles of the pigment molecules with respect to the longest dimension of the complex. Absorption spectrum of the PS I complex in the PVA film is shown in Fig. 1b (in the upper part of the picture). The signal amplitude and shape of the LD curve depend on the orientation degree of the complex (Havorth, Arntzen, Tapie & Breton, 1982). The LD signal (Fig. 1b) around zero was observed in the range of the Chl b absorption (around 460-470 nm). The amplitude of the positive band at 698 nm in the LD spectrum depends on the state (reduced or oxidised) of the PS I RC. From our room temperature LD spectra (698 nm band is considerable diminished) seems that PS I complex preparation in the PVA film is in the oxidised state. The LD signal of PS I RC is superposition of the LD signals of three photosynthetic pigments: Chl a, Chl b and β-carotene present in this PS I complex. The absorption band at around 460-470 nm belongs to Chl b, at around 500 nm to β-carotene and around 420,440 and 680 nm to Chl a absorption (Goedheer, 1969; 1972; Havorth *et al.*, 1982 a). There is a high degree of order between the long axis of β-carotene and Q_y transition moments of Chl a molecules absorbing at the red end of the spectrum (Junge *et al.*, 1977). Location of β-carotene in the close proximity to and in parallel with Chl a molecules seems to be the most favourable for a protective role of β-carotene in the photosynthetic reaction centre complex. According to Białek-Bylka, Hiyama, Yumoto and Koyama (1996) study the 15-*cis* configuration of β-carotene, recently found in the PS I RC, is suggested for the photoprotective function in the reaction center of PS I.

Fluorescence polarization ratio $FP = F_{\parallel} / F_{\perp}$ (where: $F_{\parallel}$ and $F_{\perp}$ are fluorescence intensity components emitted parallel and perpendicular, respectively to the orientation axis which is in the plane of the film) calculated at the fluorescence maximum of the room temperature polarized fluorescence emission spectra (spectra not shown) of PS I complex in the PVA anisotropic film equals 1.3. This value is similar to the PS I data *in situ*, with characteristic fluorescence emission parallel greater than perpendicular one (Ganago, Garab & Faludi-Daniel, 1983; Szito *et al.*, 1985; Białek-Bylka & Brown, 1990).

The linear dichroism and fluorescence polarization ratios are analogous indications of the orientation of the pigments by assuming that a more intensive absorption parallel to the membrane plane corresponds to a more intensive fluorescence emitted in the same direction (Tapie *et al.*, 1984). This assumption is valid in the case of the individual molecules and usually is not very much correct for a membrane in which a lot of pigment molecules participate in the absorption. But only the lowest excited states of the interacting molecules will fluoresce and also one can assume that β-carotene molecules practically no fluoresce. The PS I pigment-protein complex is not a very simple sample. It consists of the three photosynthetic pigment molecules: Chl a, Ch b and β-carotene, with highly overlapping absorption spectra, why

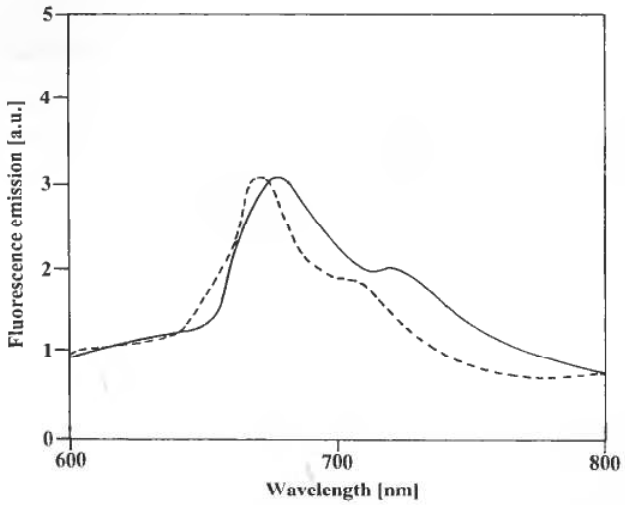

Fig. 3. Room temperature fluorescence: emission (on the right side of figure; excitation at 440 and 460 nm) and fluorescence excitation (on the left side of the figure; emission detected at 687 nm) spectra of the PS I complex in the buffer.

one should not expect and account for a very good correlation between the LD and FP data.

Fluorescence emission spectra of the PS I complex in the PVA film (room temperature spectra) and free pigments (extracted from pigment-protein complex) also in the PVA film are shown in Fig. 2. In the free pigment sample the long wavelength part around 730 nm is almost missing and the red band maximum is shifted towards shorter wavelengths.

There is almost always some free pigment in the PS I preparation, because of the denaturation process during thawing of the sample which has to be stored in the liquid nitrogen prior to the usage in the experiment. The fluorescence emission and excitation spectra (room temperature spectra) of the PS I complex in the buffer before incorporation into the film are shown in the Fig. 3. Fluorescence excitation spectrum shows the energy transfer from Chl b and β-carotene to the Chl a molecules, as it is in the intact complex. From the comparison between the fluorescence emission spectrum of the fresh preparation in the buffer (Fig. 3) and after immobilization in the PVA film (Fig. 2) one can conclude that incorporation of the PS I complex into PVA film does not change quality of the complex. The intensity of the long wavelength steady state fluorescence emission at the 730 nm (characteristic for PS I complex) is higher for the complex in the more rigid environment (PVA film) than in the buffer (compare Fig. 2 & 3). Such effect was already observed in the spinach PS I Białek-Bylka and Brown (1986)

The PS I complex (from pea *Pisum Sativum*, in poly(vinyl alcohol) film) study shows: 1) the energy dissipation by heat in the long wavelength β-carotene absorption region, 2) the two forms of β-carotene molecules with max. at around 490 nm and 500 nm, and 3) the long wavelength fluorescence band around 730 nm, characteristic only for PS I Chl a bound to the reaction center protein, which intensity is higher for the complex in the more rigid environment (e.g. film) than in the buffer.

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