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ENERGY TRANSFER IN CYANOBACTERIAL (*SYNECHOCOCCUS ELONGATUS*) PHOTOSYSTEM I AND II

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The energy transfer study in the pigment-protein complexes enriched in PS II and PS I of cyanobacterium *Synechococcus elongatus* Nag. f. thermalis Geitl. in the polyvinyl alcohol (PVA) films were performed in order to obtain information about the function of carotene in the complexes with different pigment and protein composition. Our results suggest efficient energy transfer between carotenoids and long-wavelength forms of chlorophyll a molecules in both pigment-protein complexes of PS I and PS II. Such type of energy transfer (to the long-wavelength chlorophyll a forms) can be observed only in a case of the pigment-protein complexes inside the membrane (in our case in an artificial PVA membrane). This data are in a good agreement with the synthetic β-carotene - chlorophyll a - protein energy transfer study in the model system with the same artificial membrane.

INTRODUCTION

The photosynthesis (the process whereby light energy is converted into chemical potential energy and conserved in the form of ATP and NAD(P)H, which are utilized in the biosynthetic pathway of organic molecules) of cyanobacteria, higher plants and algae proceeds in two photosystems, i.e. photosystem I (PSI) and photosystem II (PSII).

Photosystem II is unique because it generates a strong oxidant which eventually oxidizes water to form atmospheric oxygen. That is why the photochemical process conducted by these organisms is called "oxygenic photosynthesis".

Cyanobacterial PS II differs from other oxygenic photoautotrophs primarily in the region of the external light-harvesting apparatus: this is mainly composed of phycobilins (Gantt, 1981; Glazer, 1984; Zilinskas & Greenwald, 1986). The procaryotic organism like thermophilic cyanobacterium Synechococcus elongatus contains no chlorophyll b (chl b) (a light harvesting complex of the LHCP type was not found in this species).

Since our samples do not contain chl b (in samples with chl b the electronic absorption spectral region of the chl b is highly overlapped with carotenoid spectral region) they are very useful for the energy transfer study between carotenoids (mainly b-carotene) and different chlorophyll a forms existing in the PS I and PS II complexes.

Another more distinct difference is in the peripheric oxygen-evolving complexes (Ono, Satoh & Katoh, 1986). The core structure of the PS II fully corresponds to the known X-ray structure of purple photobacteria, (Deisenhofer, Epp, Miki, Huber & Michel, 1985; Michel & Deisenhofer, 1988).

In the photosynthetic systems carotenoids carry out the functions of harvesting, transfering and dissipating light energy (Mathis & Schenck, 1982; Young & Britton, 1993; Cogdell, 1978; Cogdell & Frank, 1987; Koyama, 1991; Frank & Cogdell, 1993; Siefermann-Harms, 1987). The presence of carotenoids and their close association with chlorophylls is a prerequisite for oxygenic photosynthesis, because carotenoids desensitize the chlorophyll triplet state, which would otherwise result in the formation of highly reactive, singlet oxygen lethal to all forms of life (Franck & Cogdell, 1993). Carotenoids quench chlorophyll triplets very effectively by the electron exchange mechanism (Dexter, 1953). The light-harvesting function of carotenoids is generally acknowledged; they participate in the process of radiant energy capture and transport as auxiliary pigments (Goedheer, 1979; Mathis & Schenck, 1982).

The aim of this paper was to find out which particular forms of the chlorophyll a in the PS I and PS II complexes participate in the acceptance of

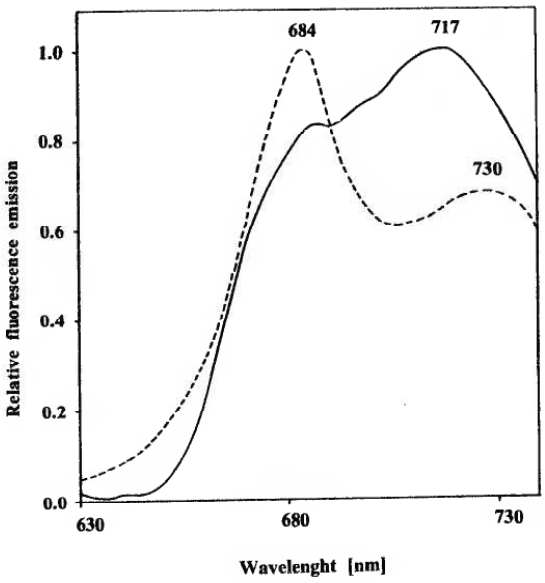

Fig.1. Fluorescence emission spectra of PS I (—) and PS II (- - -) in PVA films (normalized at max. fluorescence intensities). Excitation wavelength, 500 nm.

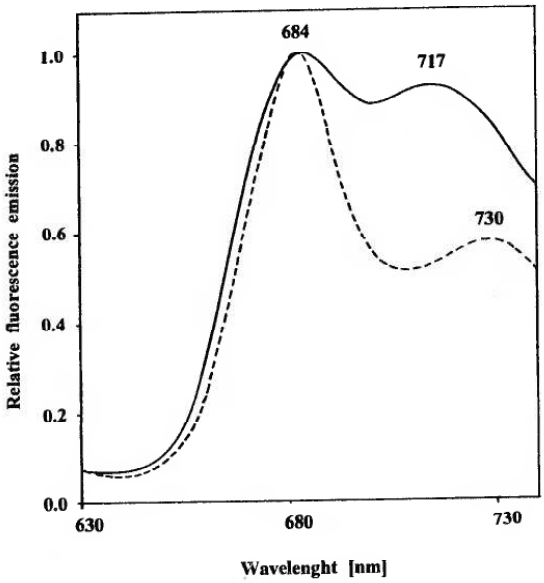

Fig.2. Fluorescence emission spectra of PS I (—) and PS II (- - -) in PVA films (normalized at max. fluorescence intensities). Excitation wavelength, 429 nm.

the excitation energy transfer from β-carotene molecules.

EXPERIMENTAL

Preparation of samples

Thylakoid membranes from cyanobacterium *Synechococcus elongatus* Nag. f. thermalis Geitl., were extracted by the detergent dodecyl-β, D-maltoside (DM) (Sofrova, Plazakova & Hladik, 1987). Detergent (DM) extract contains mainly chlorophyll (absorption region around 430 nm and 670 nm) and some phycobilins (absorption around 600 nm). The step of detergent (DM) extract purification by the centrifugation in the sucrose density gradient separates carotenoids and phycobilins from chlorophyll zones.

The chlorophyll zones, denoted upper (U) and lower (L) are enriched in PS II and PS I, respectively and further separation can be done by the ion exchange high performance liquid chromatography (HPLC) (Sofrova, Kucera & Hladik, 1992).

The cyanobacterial photosystem II was embedded in films of polyvinyl alcohol (PVA) (Siódmiak & Frąckowiak, 1972) in order to preserve the integrity and activity of the isolated pigment-proteins (Bialek-Bylka, Brown & Manikowski, 1987).

Spectral measurements

Absorption spectra were measured with a M40 Specord (Carl Zeiss) spectrophotometer.

Fluorescence emission spectra were measured with a home made fluorometer connected with a computer. Emission spectra were corrected for the sensitivity of the detection system. Fluorescence was collected from the front surface of the sample film placed at a 30° angle to the emission beam and at a 60° angle to the actinic beam. The resolution of the system was 2 nm. All measurements were done at room temperature.

RESULTS AND DISCUSSION

In order to discuss the possible role of the carotenoid states in energy transfer to chlorophyll a it is important to consider a good matching of the energy levels for both the dipole allowed (Foerster, 1948, 1967) mechanism and the electron exchange model of Dexter (1953). It seems that matching of the energy levels for energy transfer from donor (β-carotene) to acceptor (chlorophyll a) one can obtain considering sequential energy transfer from shorter-wavelength to the longer-wavelength forms of chlorophyll a (for chlorophyll forms see Brown, 1983). The detection of the fluorescence in naturally occurring carotenoids is very difficult due to the very low fluorescence quantum yield (Cos-

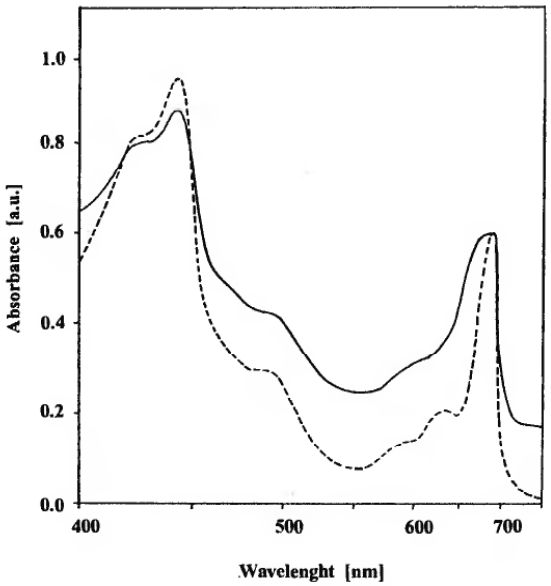

Fig.3. Absorption spectra of PS I (——) and PS II (- - -) in buffer (normalized at 680 nm).

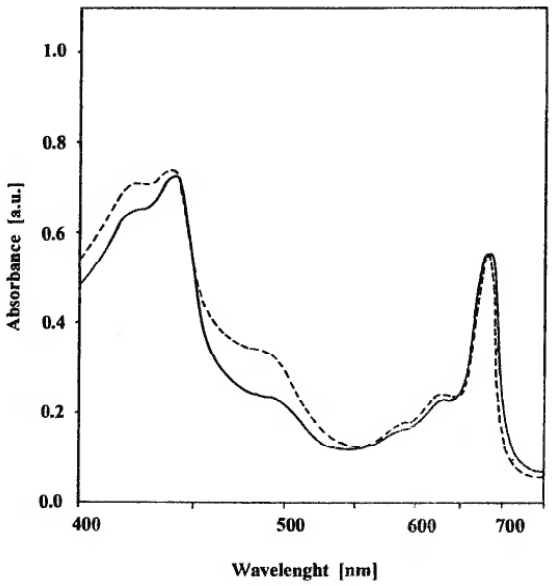

Fig.4. Absorption spectra of PS I (——) and PS II (- - -) in PVA films (normalized at 680 nm).

grove, Guite, Burnell & Christensen, 1990; Katoh, Nagashima & Mimuro, 1991; Mimuro & Katoh, 1991; Andersson, Gillbro, Asato & Liu, 1992; De Coster, Christensen & Gebhard, 1992).

The β-carotene (11 double bonds) lifetimes of the emission from the 2^1Ag and 1^1Bu states are in the range of 3-10 ps (Wasielewski & Kispert, 1986; Shreve, Trautman, Owens & Albrecht, 1991; Noguchi, Kolaczkowski & Arbour, 1989; Hashimoto & Koyama, 1990; Andersson, Gillbro, Asato & Liu, 1992) and 200-250 fs at room temperature (Shreve, Trautman, Owens & Albrecht, 1991).

The emission yield can be expressed as ratio of the lifetime and the natural radiative lifetime of the molecular state. The natural radiative lifetime can be estimated by integrating the absorption profile of the emitting state, and has been estimated to be about 10^{-9} s for carotenoids such as β-carotene (Gillbro & Cogdell, 1989; Shreve, Trautman, Owens & Albrecht, 1991). When this value is used, the lifetime of β-carotene can be estimated to be 200 fs. This value agrees well (taking into consideration the error in quantifying such a low fluorescence yield) with data from direct kinetic measurements of Shreve *et al.* (Shreve, Trautman, Owens & Albrecht, 1991).

For the discussion of the carotenoid-chlorophyll energy transfer it is important to remember that the 1^1Bu state decays into the 2^1Ag state in a few hundred femtoseconds and then the 2^1Ag state lives for a few picoseconds.

Since β-carotene is practically nonfluorescent, the interaction of β-carotene and chlorophyll a forms can be detected by the chlorophyll a fluorescence emission. Some amount of the energy directed to the longer-wavelength chl a forms can be released as fluorescence (Brown, 1983).

In the complexes of PS I and PS II the main carotenoid is β-carotene, which was found in peripheral regions of the complex as well as in close vicinity of the reaction centers of the two photosystems (core or sub-core complexes) (Siefermann-Harms, 1985; Lichtenthaler, 1987; Brody, 1988).

According to our results (Fig. 1), in the case of PS I and PS II the energy from β-carotene is transferred to the chl a forms with max. around 717 nm and 730 nm, respectively. Comparing Fig. 1 - excitation in the range of β-carotene absorption and Fig. 2 — excitation in the range of chl a absorption, one can see a very efficient energy transfer from β-carotene to the 717 nm form of chl a in the case of PS I complex and a much less efficient energy transfer to the 730 nm chl a form in the case of PS II complex.

These results agree well with those of the higher plant PS I preparations which have been depleted

of antenna pigments (Burton, Chow & Jordan, 1984). It is well known from the literature (Mullet, Burke & Arntzen, 1980) of higher plants that chl b (which is also missing in our cyanobacterial PS I preparation) and β-carotene (Bialek-Bylka & Brown, 1986) affect the state of the longer wavelength-absorbing forms of chl a. The long wavelength emission maximum is blue-shifted in the chl b-less barley mutant compared with the wild type.

These data are in a good agreement with our energy transfer study in model systems (Bialek-Bylka, Shkuropatov, Kadoshnikov & Frackowiak, 1982) and in the artificial carotenoid-chlorophyll - protein complexes (Bialek-Bylka, 1992).

Fig. 3 shows absorption spectra of PS I & PS II complexes in buffer. In a case of PS I in buffer one can observe strong aggregation. According to the literature (Guikema & Sherman, 1981; Takahashi, Koike & Katoh, 1982) the PS I complex has a distinct tendency to form high-molecular aggregates or their degradation products.

In order to eliminate the aggregation problem, complexes were immobilized in PVA films (see Fig. 4).

Carotenoids in photosynthetic systems have two functions of light-harvesting and photo-protection (Frank & Cogdell, 1993). Natural selection of the carotenoid configurations (15-*cis* for photoprotective and all-*trans* for light-harvesting) to carry out these functions has been found in purple photosynthetic bacteria (Koyama, 1991) and in higher plants (Białek-Bylka, Tomo, Satoh & Koyama, 1995).

A second role for β-carotene is that of an acceptor of excess triplet energy, thereby protecting chlorophyll a from photooxidation. In the higher plant photosystems, which are very similar to cyanobacterium PS I and PS II, the β-carotene is the only carotenoid present in an isolated core complex of PSII (Satoh, 1993) and it is in the 15 *cis* configuration (Bialek-Bylka, Tomo, Satoh & Koyama, 1995).

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