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Long-wavelength chlorophyll forms in Photosystem I from pea thylakoids

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Abstract

Absorption maximum positions of three LW Chl forms in pea chloroplasts were estimated using 77 K excitation spectra of fluorescence detected in their maxima (720, 732 and 746 nm). The 705, 714 and 723 nm components were revealed in the second derivative plots of the excitation spectra. The same maxima were found in normalized excitation spectra obtained with dividing excitation spectra by absorption spectrum. It was confirmed that the observed maxima belong to absorption of LW fluorescing Chl forms. The same maxima were displayed in an action spectrum of P700 oxidation measured at room temperature. It confirms the energy transfer from LW Chl forms to P700. Close to 50% efficiency of bulk Chl forms in both excitation of LW fluorescence and P700 oxidation was found. Analysis of the shape of normalized excitation spectra suggests that there is no energy exchange among LW Chl forms. Their location and physiological role are discussed.

Introduction

Photosystem I (PS I) is one of two photosystems coupled in electron transport in oxigenic photosynthesis. Peculiarities of PS I structure specify functional activity in various stages of the process beginning with the earliest such as energy transfer, its trapping and primary photochemical transformation. PS I complex from higher plants consists of two main parts: core and light-harvesting complex, LHC I. The latter has not been found in cyanobacteria. Two pigmented protein subunits produced by PsaA and PsaB chloroplast genes form PS I core (Goldbeck 1992). Protein composition of the latter in higher plants and cyanobacteria is similar and a lot of knowledge concerning biochemical composition arose from genetically studied cyanobacteria. Beside PsaA and PsaB encoded subunits PS I core contains 9 subunits in cyanobacteria and 11 in chloroplasts which are the cofactors of the electron transport system (Chitnis 1996). 65–90 Chl molecules bound with PS I core (Mullet et al. 1980; Bassi and Simpson 1987; Turconi et al. 1994) and Chls of RC, P700, are situated in two subunits consisting of two sequential and structural domains. They are an N-terminal, periferal, antenna-binding domain and a C-terminal, central RC-domain (Schubert et al. 1988).

About 110 Chl molecules are bound with LHCI (Bassi and Simpson 1987) which consists of three pools, LHC–730, LHC–680A and LHC–689B, formed by polypeptides coded by 4 nuclear genes of *cab* family (Knoetzel et al. 1992). It was shown that 8 LHC I subunits are arranged near PS I core (Boekema et al. 1990). Therefore the native PS I complex consisting of PS I core and LHC I pool contains in total 200Chl/P700. Chl molecules in LCH I and PS I core form inhomogeneous light-harvesting system containing various Chl aggregates. They cause the fine structure of absorption and fluorescence bands. Analysis of the structure was performed by derivative spectroscopy method and deconvolution of complex spectral contour on individual components (Gasanov and French 1973; Kochubey and Guliev 1980; Kochubey et al. 1984; Kochubey and Ruban 1988; Croce et al. 1996). Measurements of T-dependence of fluorescence intensities in spectrally resolved components

give information concerning energy exchange in light-harvesting antenna. However, the most detail information rises from time-resolved spectroscopy recently developed (Holzwarth 1991).

Specific peculiarity of PS I complexes is the presence of LW Chl forms or red pigments (RPs) called so because they are first revealed by intensive LW fluorescence band in low temperature spectra (Goedheer 1965). This emission was characterized by strong temperature dependence of yield and lifetime (Avarmaa et al. 1979; Kochubey and Guliev 1980; Tusov et al. 1980; Paschenko et al. 1981; Pellegrino et al. 1983; Mukerii et al. 1989; Turconi et al. 1993), and anomalous great bandwidth. T-dependence of fluorescence yield was explained by the interaction of LW fluorescent Chl forms with reaction centers (Kochubey et al. 1977; Pellegrino et al. 1983; Kochubey and Ruban 1988; Jia et al. 1992; Werst et al. 1992). Great bandwidth is caused by inhomogenity of 735 nm band firstly revealed by some structural details in its contour (Govindjee and Yang 1966; Borisov and Il'ina 1969) and later by derivative spectroscopy (Kochubey and Guliev 1980) and by deconvolution of the contour (Koucherov and Kochubey 1979; Kochubey and Ruban 1988; Croce et al. 1996). Separation of LHCI–PS I complex into LHC I and PS I-core possessing fluorescence bands at 720 and 735 nm, respectively, originated from the idea that those bands were the main components of F735 (Mullet et al. 1980). Their bandwidths were approximately the same as that of F735 in the spectrum of native PS I complex, and their strong temperature dependence remained. Later three components were supposed to be in F735 of LHCI–PS I complex with the maxima at 720, 732 and 746 nm for pea (Kochubey and Ruban 1988) and at 720, 730 and 742 nm for maize (Croce et al. 1996). The bandwidth of the components was about 3 times smaller than that of the F735 in the LHCI–PS I spectrum.

The nature and the role of RPs in energy transfer and their physiological role was not entirely clear until now. Their number, absorption and fluorescence properties and location in PS I antenna need to elucidate. The number of RPs is supposed to be highly species-dependent (Gobets et al. 1998). One pool of RPs for *Synechocystis* PCC 6803 and two pools of RP for *Synechococcus elongatus* were revealed (Gobets et al. 1998; Palsson et al. 1996). Presence of three RP pools was supposed for pea and maize PS I on the basis of three component structure of F735. Absorption maxima of RPs were determined in cyanobacterium absorption spectra at 708 nm for *Synechocystis* PCC 6803 and 708 and 719 nm for *Synechococcus elongatus* (Palsson et al. 1996). For higher plants RP absorption maximum positions were estimated using bandwidths of F735 subbands. Three maxima at 704, 712 and 723 nm and two those at 705 and 715 nm were reported for pea (Kochubey and Ruban 1988; Trissl et al. 1993) and 714, 725 and 738 components were revealed for maize (Croce et al. 1996). The discrepancies in these data may be species-dependent or caused by distortion arises from the procedure of absorption maxima determination. Absorption maxima positions in (Kochubey and Ruban 1988) and (Croce et al. 1996) were calculated using characteristics of fluorescence subbands obtained by deconvolution of wide fluorescence band at 735 nm. Under deconvolution the greatest error is produced by a halfwidth approximation (Kochubey 1986). In the present study we verify RP absorption maximum positions with other approaches using excitation spectra of fluorescence detected in subband maxima and divide these spectra by absorption spectrum of PS I particles. As was shown by us earlier (Kochubey 1986), the highest intensity in such normalized spectra is reached at the wavelength corresponding with the absorption maximum of the Chl form, the fluorescence of which is detected, while intensities in the region of absorption of the Chl forms with non-resonance energy exchange are lower. Comparing of the intensities in RP own-absorption region and absorption of Chls sensibilizing of LW fluorescence allows us to estimate energy transfer efficiency.

Action spectra of P700 oxidation and quantum yield of P700 oxidation measured by various authors demonstrated differences, especially in LW region (Hiyama 1985; Palsson et al. 1998). Accurate measurements of the shape of action spectrum of P700 oxidation and this spectrum divided by absorption spectrum allowed to confirm energy transfer from RPs to reaction centers and to estimate the efficiency of this transfer as well as the transfer from bulk Chls. Discrepancy in LW region in the data of various authors is discussed.

There are contradicting opinions concerning the location of RPs. They are supposed to be bound with LHC I (Mullet et al. 1980; Bassi and Simpson 1987; Jennings et al. 1998; Knoetzel et al. 1998) or with PS I core (Trinkunas and Holzwarth 1994; Gill and Wittmershaus 1999). Some authors assume that F720 pool is located in PS I core and that 735 nm fluorescence is only partially emitted by LHC I (Turconi et