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Spectral properties of antenna pigment–protein complexes studied by hole-burning spectroscopy

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Persistent spectral hole-burning fluorescence spectra of photosystem II core antenna at 4.2 were measured. The obtained hole widths were interpreted in terms of fast excited energy transfer within pigment–protein complexes. The energy transfer efficiency, the inhomogeneous distribution function and hole-burning mechanism are discussed with respect to the role of the surrounding protein environment (polymer, glass). The observed light induced hole filling is also interpreted as a result of efficient energy transfer.

1. Introduction

The photosynthetic antenna systems are composed of multi-molecular pigment–protein complexes with a primary function of the transfer of absorbed light energy to the photochemical reaction center. Particular pigment–protein complexes of the antenna system are generally characterized by different absorption maxima and thus form a "ladder" system for heterogeneous and directed excited energy transfer (EET) [1]. Within one pigment–protein complex the pigment molecules are specifically bound to polypeptides which determine the positions, environments, orientations and spacings of the pigment molecules (three-dimensional organisation of the pigment for energy transfer). It is assumed that energy is transfered between individual pigment molecules of a specific pigment–protein complex within the order of picoseconds [2]. The inner antenna of photosystem II (PS II) of cyanobacteria and higher plants consists of two chlorophyll-protein complexes denoted as CP 43 and CP 47 with the number of chlorophyll-a (Chl-a) molecules varying between 20 and 50.

The application of hole-burning (HB) spectroscopy in the study of photosynthetic systems offers an independent method for determining excited state lifetimes of particular pigment–protein complexes. The hole widths δ_{HB} obtained in HB are related to the total relaxation time which, in the presence of fast EET, is predominantly determined by the excited state lifetime (population decay time) T_1 [3,4]:

$$\delta_{HB} = (\pi c T_1)^{-1}.$$

Next to relaxation dynamics, persistent spectral HB (PSHB) can serve as a tool for determining the inhomogeneous distribution function (IDF) of pigment (Chl-a) molecules in the protein matrix. The phenomenon of light induced filling of the originally burnt hole is also a demonstration of efficient intermolecular EET.

The aim of this report is to compare spectroscopic and functional properties of PS II core antenna in two different forms: 1) in native PS II particles in buffer/glycerol glass, and 2) isolated in polyacrylamide gel by non-denaturating electrophoresis.

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2. Experimental

Photosynthetically active PS II particles were isolated from cyanobacterium *Synechococcus elongatus* as described in detail in ref. [5]. PS II particles contain active RC and both CP 43 and CP 47 proteins of the core antenna. Non-denaturating SDS polyacrylamide gel electrophoresis was performed as described in ref. [3]. The CPa2 zone of the gel is a mixture of CP 43 and CP 47 proteins.

Fluorescence (F) spectra were excited with a pulsed nitrogen laser pumped dye laser (Lambda Physik FL 1000) at 440 nm. A ring dye laser (Spectra Physics 380C, DCM) pumped by a CW Ar ion laser was used for burning. The samples were quickly cooled in the dark to 4.2 K in a liquid helium cryostat. The luminescence was resolved using a double grating monochromator and detected with a photomultiplier together with a boxcar integrator. Spectra were processed with a personal computer (deconvolution of the spectral width of the monochromator slit).

3. Results and discussion

The hole widths at 4.2 K were determined as the zero extrapolated values in the burning fluence experiments (0.05–15 J cm^{-2}). The δ_{HB} values observed in native PS II particles (1.7 cm^{-1}) were found to be about 60% higher than those in gel isolated CPa2 samples (1.0 cm^{-1}). The corresponding T_1 values of 6.2 and 10.6 ps, respectively, document slower EET in chlorophyll-protein complexes in the gel. An analogous 20–40% EET slow-down in the gel isolated antenna was observed on in vivo antennae in thylakoid membranes [6]. During the process of gel electrophoretic isolation of antenna complexes, conformational changes in the whole antenna proteins can occur. These spatial changes provide changes in mutual distances and optimal orientation of antenna chlorophylls which result in a slowing of EET within the antenna and/or a spectral shift of fluorescence maxima. The HB experiments performed on materials isolated by means of the same electrophoretic method from different sample preparations show that the observed conformational changes are fully reproducible. Despite the loss of photosynthetic activity in gel, the application of gel electrophoretic isolation, especially on antenna complexes, is thus fruitful.

Spectral manifestation of the HB mechanism (blue shifted antihole and HB efficiency are very similar for both materials (PS II particles and CPa2) although the protein surrounding environments are significantly different: glass (buffer/glycerol) versus polymer (polyacrylamide gel). Non-photochemical HB mechanism thus predominantly reflects photoinduced changes in the pigment-protein complex conformation.

The inhomogeneous distribution function determined from the relative depths of saturated zero-phonon holes (ZPH) at different burning wavelengths [3] characterizes pigment–protein in-

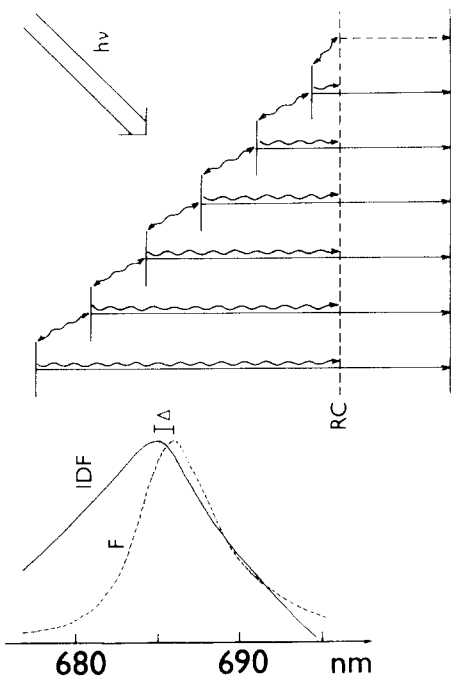

Fig. 1. Schematic model of excited energy transfer (EET) within one pigment–protein complex together with the fluorescence (F) spectrum and the inhomogeneously broadened distribution function (IDF). Full lines in energy scheme represent energy levels and F transitions of antenna Chl, dashed lines correspond to the RC, wave lines depict EET; the F and IDF maxima differences is $\Delta = 23$ cm^{-1}.

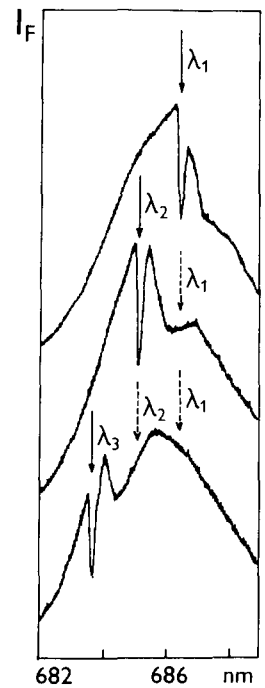

Fig. 2. Light-induced hole filling (LIHF) in fluorescence spectra of core antenna pigmenmt-protein complex CPa2 at 4.2 K; λ_1, λ_2, λ_3 – burning wavelengths; solid arrows – last burns; dashed arrows – previous burns.

teractions (fig. 1). Together with the IDF fig. 1 shows the corresponding F spectrum and the related energy scheme. The observed shift between the F and IDF maxima (~ 23 cm^{-1}) fits well with the red shift (~ 20–22 cm^{-1}) between ZPH and the phonon side-band hole observed in ref. [3]. The energy scheme in fig. 1, showing particular steps of EET within one antenna pigment-protein complex, corresponds well with the observed IDF.

The phenomenon of light induced hole filling (LIHF) was observed in the gel isolated antenna complex. Figure 2 shows a series of three successive PSHB spectra: the top curve represents the original hole burnt at λ_1; after an additional burn at λ_2 ($\lambda_2 < \lambda_1$) the original hole (at λ_1) completely (100%) disappears (middle curve); bottom curve – the experiment is repeated with $\lambda_3 (\lambda_3 < \lambda_2 < \lambda_1)$ and the previous hole (at λ_2) is again fully erased. This effect was observed over the whole range of the IDF ($675 < \lambda < 695$ nm) for $\lambda_1 - \lambda_2 \cong 30$ cm^{-1}. On the other hand, for $\lambda_1 < \lambda_2$ only approximately 10% LIHF occurred. A similar effect was measured on Rhodamin 640 in polyvinylalcohol [7] where a 12% and 6% hole recovery for higher and lower energy shifted successive burns, respectively, was reported and explained by a model that introduces intrinsic and extrinsic two level systems (TLS). The 6% LIHF was the main argument against an EET interpretation of the rhodamine 640 spectra where conditions for efficient EET are rather bad. The results for the photosynthetic antenna complexes (especially the significant difference of 100% and 10% LIHF) cannot be interpreted merely using the TLS model of ref. [7]. The 10:1 LIHF ratio can be explained as a result of downward intermolecular EET as suggested in fig. 1. During the successive burn at higher energy (with respect to the original hole) part of the burning energy is transfered to the nearest vicinity of the original hole where direct recovery of previously burnt molecules can occur. The LIHF in the opposite spectral direction is one order less efficient, due to a downward EET.

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