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Biochimica et Biophysica Acta 1777 (2008) 379–387
Biochimica et Biophysica Acta BBA
www.elsevier.com/locate/bbabio

Seasonal changes of excitation energy transfer and thylakoid stacking in the evergreen tree *Taxus cuspidata*: How does it divert excess energy from photosynthetic reaction center?

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Received 13 August 2007; received in revised form 23 January 2008; accepted 24 January 2008
Available online 11 February 2008

Abstract

Photosystems must efficiently dissipate absorbed light energy under freezing conditions. To clarify the energy dissipation mechanisms, we examined energy transfer and dissipation dynamics in needles of the evergreen plant *Taxus cuspidata* by time-resolved fluorescence spectroscopy. In summer and autumn, the energy transfer processes were similar to those reported in other higher plants. However, in winter needles, fluorescence lifetimes became shorter not only in PSII but also in PSI, indicating energy dissipation in winter needles. In addition, almost the same fluorescence spectra were obtained with different excitation wavelengths. In contrast, the fluorescence spectrum showed a large difference due to excitation wavelength in spring needles. The fluorescence spectrum of spring needles in 550-nm excitation showed similar spectra to that of winter needles, however, red-chlorophyll fluorescence was not observed in chlorophyll excitation. These observations suggest that some complexes with some kind of red-shifted carotenoid and red-chlorophyll unlink from the core complex in spring. Seasonal changes of excitation energy dynamics are also discussed in relation to changes in thylakoid stacking.

Keywords: Seasonal change; Energy transfer; Quench; Time-resolved fluorescence; Evergreen plant; Thylakoid stacking

1. Introduction

In the natural environment, plants encounter stressful conditions such as high light, drought, salt and low temperatures. Under these conditions, photosynthetic activity decreases due to the inactivation of CO_2 fixation enzymes and photodamage of photosystems [1–3]. Decrease in photosynthetic activity must be accompanied by dissipation of absorbed light energy to protect against further photodamage [4].

Evergreen plants retain chlorophyll (Chl) under freezing conditions. Under these conditions, light energy is harvested by chlorophyll but the energy cannot be used for photosynthesis [5]. Therefore they protect themselves against freezing and photodamage using many strategies, including dissipation of light energy channeled into Chls depending on seasons [6]. Several mechanisms to prevent photodamage in winter have been reported previously. Chloroplasts of *Taxus* congregated together in the centers of the cells during winter, whereas they were localized adjacent to plasma membranes in summer, which may serve to control the amount of light absorption [7]. In case the excess light energy induces formation of reactive oxygen species, evergreen plants minimize damage using the antioxidants glutathione and α-tocopherol [8]. Dissipation of absorbed light energy before it arrives at reaction centers is especially important for evergreen plants because CO_2 fixation is completely inhibited and absorbed light energy cannot be used for photosynthesis. Ottander et al. focused on photosystem II (PSII) organization and pigment composition in the evergreen *Pinus sylvestris*, and revealed the losses of chlorophyll, of the D1-protein of the PSII reaction center (RC) and of PSII light-

Abbreviation: PSI, photosystem I; PSII, photosystem II; RC, reaction center; LHC, light-harvesting complex; Chl, chlorophyll; FDAS, fluorescence decay-associated spectrum; ELIP, Light-induced stress protein; TRFS, time-resolved fluorescence spectra

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doi:10.1016/j.bbabio.2008.01.008

harvesting-complex (LHCII) proteins in winter. Much of the remaining chlorophyll was reorganized in aggregates that quench excitation energy efficiently [9]. Steady-state fluorescence spectra revealed the presence of the 705-nm and 715-nm fluorescent forms in winter leaves of *Eucalyptus pauciflora* and *Amyema miquelii* [10,11]. These forms coincide structurally with a loss of Chl and an increase in energy-dissipating carotenoids and were assumed to be involved in energy dissipation. However, the details of energy transfer and quenching processes in evergreen species are still unknown.

Time-resolved fluorescence spectroscopy is a useful tool to reveal these processes because energy transfer is expressed as peak-shifts or peak-alternations of fluorescence spectra [12], and the energy quenching can be monitored by the rapid dissipation of fluorescence peaks [13]. In this study, we examined the seasonal changes in excitation relaxation dynamics in needles of *Taxus cuspidata* by means of a picosecond time-resolved fluorescence spectroscopy. PSII fluorescence of winter needles was quite small and its lifetimes were twice shorter than that of summer needles, indicating the efficient quenching of excitation energy and low PSII level. PSI was also modified to dissipate absorbed light energy. Energy migration and dissipation are discussed in relation to the structural change in chloroplast.

2. Materials and methods

2.1. Preparation of samples

Second-year needles were sampled from *T. cuspidata* growing on the campus of the Hokkaido University and used for electron microscopy as previously reported [7]. For fluorescence measurements, sampling was carried out on July 31 (hereafter, referred to as 07/31 or summer), November 7 (11/07 or autumn), December 30 (12/30 or winter), and March 31 (03/31 or spring) and the needles were frozen in liquid nitrogen immediately after harvest, and stored at −70 °C until measurements. Fluorescence spectra of intact needles were measured by the surface-reflection technique to prevent changes in spectral properties during chloroplast isolation, because winter chloroplasts have specific intraorganelle conditions such as redox state [14,15].

2.2. Fluorescence spectroscopy

Steady-state fluorescence spectra were measured using a fluorescence spectrometer (Hitachi F-4500, Japan). Time-resolved fluorescence spectra (TRFS) and fluorescence decay curves were measured with a picosecond time-correlated single-photon counting system [16]. The light source was a Ti:Sapphire laser (Coherent MIRA 900, USA) and the excitation wavelength was 425 nm, which excites both Chl *a* and Chl *b*. We employed a microchannel plate photomultiplier (Hamamatsu R3809, Japan) as a detector, combined with a monochromator (Nikon P-250, Japan). The time step was 2.6 ps/ch or 52 ps/ch. Excitation laser intensity was set to give fluorescence signals of less than 10,000 c/s around fluorescence peak wavelengths, and in this condition samples did not suffer damage from the laser excitation with a repetition rate of 2.9 MHz. All measurements were carried out at −196 °C with a custom-made Dewar system. After deconvolution with an instrument function, the time resolution was improved to approximately 3 ps.

2.3. Electron microscopy

Electron microscopy was performed following procedures described in a previous paper [7]. Sampling was carried out on October 28, November 22, December 6, December 16 of 2004, and January 6, February 1, March 7, March 14, March 17, March 22, March 25, March 28, April 18, May 16, June 20, July 19, August 22, and September 12 of 2005. About 10 micrographs of chloroplasts (magnification 5000-10,000) were used to obtain an average stacking number of thylakoids on each date. Grid lines with an interval of 550 nm were drawn perpendicular to the plane of thylakoid membrane and the number of membranes per grana stacks was counted along the grid lines. These values were distributed from 1 to more than 10, and reflect overall thylakoid stacking in chloroplasts. We calculated an average stacking number on each date.

3. Results and discussion

3.1. Steady-state fluorescence spectra

To reveal the energy migration and quenching efficiency, we first measured the steady-state fluorescence spectra of the needles in various seasons. The solid lines in Fig. 1 show steady-state fluorescence spectra when the needles were excited at 440 nm (Chl *a* excitation) at −196 °C. For summer and autumn needles, three fluorescence peaks at approximately 685,

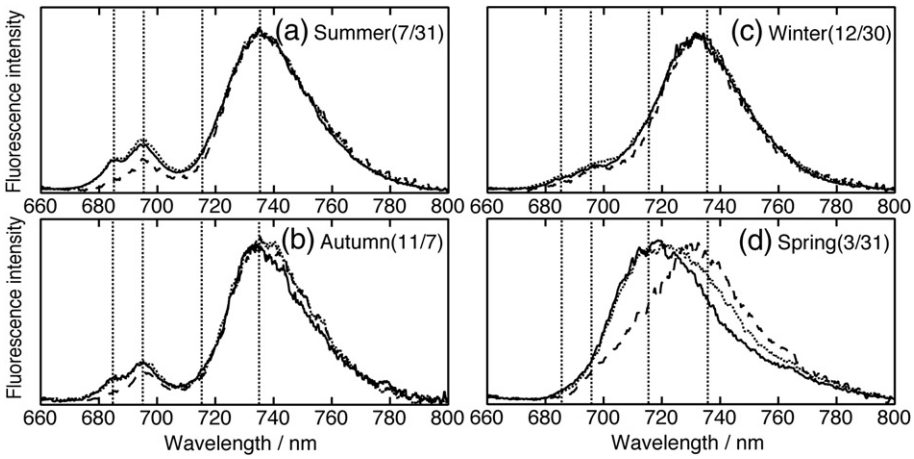

Fig. 1. Steady-state fluorescence spectra of intact needles normalized at peak intensities. Excitation was performed at 440 nm for Chl *a* excitation (solid line), 480 nm for Chl *b* excitation (dotted line), 550 nm for carotenoid excitation (broken line). All measurements were performed at −196 °C. Vertical dotted lines indicate 685, 695, 715, and 735 nm.