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Minireview

Visualization of excitation energy transfer processes in plants and algae

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Abstract

Development of the time-resolved fluorescence spectroscopy in the pico-second time range and its application to the energy transfer processes in many photosynthetic organisms is reviewed here. This method enabled visualization of energy transfer processes by three-dimensional expression of fluorescence spectra and discrimination of kinetic components and spectral components. The second generation of the ultrafast fluorescence spectroscopy is the femto-second (fs) fluorescence up-conversion, and this has enabled analyses of the transfer processes from carotenoids to chlorophylls with a resolution of less than 100 fs. For future progress, a further development of the spectroscopy is indispensable as well as structural data at atomic resolution.

Prehistory of the time-resolved fluorescence spectra

Photosynthesis is driven by absorption of light by the antenna system and subsequent transfer to the reaction center (RC), followed by charge separation in the RC. (See R.K. Clayton, this issue, for a historical perspective.) The number of antenna molecules is far greater than that of electron carriers in RC; thus the light energy absorption by antenna will be a limiting factor for growth under a low light environment, leading to competition with other photosynthetic organisms.

Energy transfer is one of the relaxation processes of excited molecules: it takes time to proceed; in other words, energy transfer is essentially a time-dependent phenomenon. Energy transfer processes have been investigated mainly by fluorescence spectroscopy because fluorescence directly reflects the fate of the excited molecules. However, analyses of fluorescence as a time-dependent phenomenon were not common in the field of photosynthesis until the mid-1970s, even though this kind of analyses is necessary to understand the processes and mechanisms of energy transfer.

Fluorescence lifetimes of Chl *a in vivo* and *in vitro* were first measured independently by two groups in the late 1950s. Steven Brody and Eugene Rabinowitch (1957) at Urbana, Illinois, and O.D. Dmetrievsky, V.L. Ermolaev and A.N. Terenin (1957) in the Soviet Union measured lifetimes by the flash method and the phase fluorometery, respectively (see S. Brody, this issue, for his personal story). During the following years, several investigators, including the research groups of E. Rabinowitch and Govindjee, confirmed and extended the measurements. Early events on the development of lifetime measurements are summarized in a review by Govindjee and Jursinic (1979).

Phase fluorometry has been used to analyze fluorescence lifetimes, but the time resolution of the apparatus is limited to the hundred-picosecond region, and when multiple components are present, discrimination of those components is not necessarily easy by this system. On the other hand, measurements by the flash method have given direct information on the energy transfer process and fluorescence quantum yield. Several new results were obtained by using a hydrogen lamp at the University of Illinois, Urbana-Champaign by S. Brody. Innovation of a laser and a detector sys-

tem led to a suitable system that analyzed fluorescence kinetics at fixed wavelengths. Henri Merkelo, Govindjee and co-workers, also at Urbana, Illinois (1969) introduced the mode-locked He–Ne laser to measure the fluorescence lifetime with a resolution of 80 ps. On the basis of the fluorescence rise and decay curves at several wavelengths responsible for individual pigments, George Porter et al. (1978) and Geoff Searle et al. (1978) described the energy transfer processes in the red alga *Porphyridium cruentum*. However, decay kinetics at one wavelength does not necessarily correspond to one component because there is often heterogeneity and overlap of the spectral component in the *in vivo* system. Thus, it is important to analyze consistency between spectral components and kinetic components, as was done by Tomoko Yamazaki et al. (1994). Time-resolved fluorescence spectrum (TRFS) is a key method for this analysis.

In 1981, Iwao Yamazaki and coworkers at the Institute of Molecular Science, Okazaki, Japan, started to construct the apparatus for the TRFS with a time resolution lower than 10 ps and the spectral resolution greater than 1.5 nm (Toshiro Murao et al. 1982) by using the single photon counting method. The light source was an Ar^+ laser-excited dye laser tunable for wavelengths longer than 550 nm by choosing dyes appropriate for the specific wavelength regions (Figure 1). The system had an excitation pulse duration of approximately 6 ps and the spectral region for monitoring fluorescence was between 550 and 750 nm. An output of the dye laser was stabilized for more than several hours by a feedback circuit to make it a suitable light source for the TRFS. A fluorescence decay curve at one wavelength was monitored with a micro-channel plate (MCP) photomultiplier, which detected light pulses of widths shorter than 60 ps (Yamazaki et al. 1985b). This ensured the time resolution down to 6 ps by the convolution method. Fluorescence decay curves were measured for a certain time (usually longer than 30 s) at one wavelength and the monochromator was scanned to measure at another wavelength. After collection of all decay curves in a certain wavelength region, TRFS was obtained by reconstructing on a microcomputer after correcting the spectral sensitivity of the measuring system. This system was applied to energy transfer processes of many photosynthetic organisms.

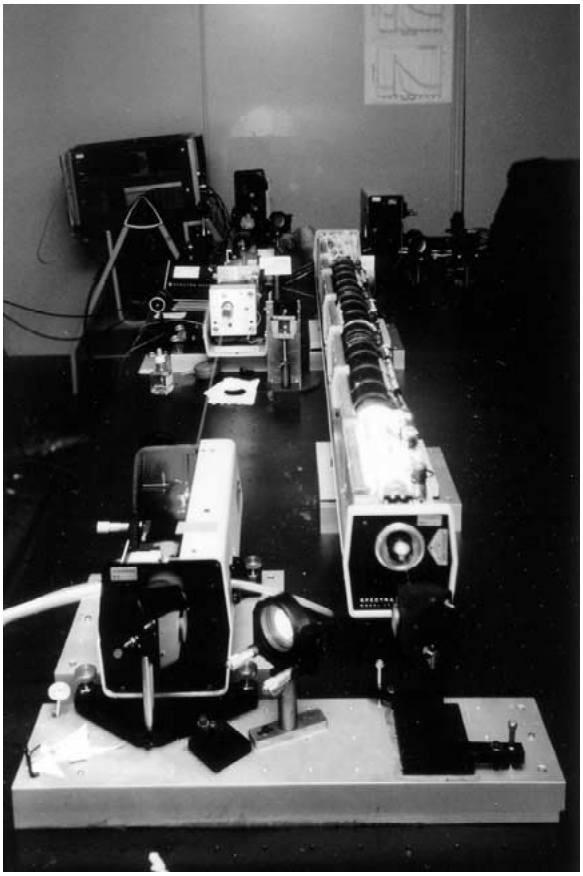

Figure 1. The original laser system for the time-resolved fluorescence spectra. An Ar+ ion laser (green beam, right) excited a dye-laser (red beam, left) to generate a pulse for the time-resolved fluorescence spectra (I. Yamazaki and his co-workers, unpublished). For a color version of this figure, see section in the front of the issue.

Application to the *in vivo* system

In 1983, Yoshihiko Fujita and I started to collaborate with Yamazaki's group for analyses of the energy transfer processes of photosynthetic organisms. At first, we selected intact cells of the red alga *P. cruentum* and a cyanobacterium *Anacystis nidulans* (renamed *Synechococcus* sp. PCC 6301) for the following two reasons. (1) There are at least three emitters in the systems: phycoerythrin (PE) (only for *P. cruentum*), phycocyanin (PC), allophycocyanin (APC), and chlorophyll (Chl) *a*; thus, these were suitable systems to test the validity and the limit of our experimental set-up, and (2) by using intact cells we avoided the use of any kind of detergents (needed for making pigment system particles), which could introduce an artifact that interfered with the transfer

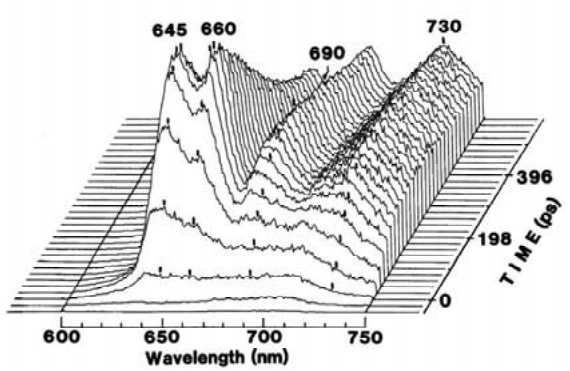

Figure 2. A bird's-eye view of the time-resolved fluorescence spectra at −196 °C (77 K) of intact cells of *Anabaena variabilis* (M-3). Excitation wavelength was 580 nm with pulse duration of 6 ps to excite phycocyanin (PC). Decay curves were measured in the wavelength region from 600 to 750 nm, and spectra were reconstructed on a microcomputer. Samples were frozen in a growth medium containing 15% polyethylene glycol 4000 to obtain homogeneous ice. Tic marks indicate the respective wavelengths to detect easily the shift of fluorescence peaks. Peak-shift within phycobiliproteins and also Chl *a* was clearly resolved. (Mimuro 1990).

Figure 3. I. Yamazaki.

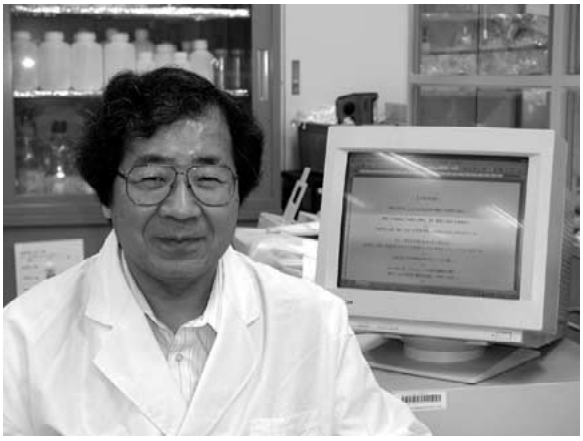

Figure 4. M. Mimuro in his laboratory.

sequence and/or kinetics. In 1984, we published the TRFS of intact cells of the red alga *P. cruentum* at physiological temperature (Yamazaki et al. 1984); the time-dependent red-shift of fluorescence maximum, in the order of PE, PC, APC and Chl *a,* was clearly shown and a peak shift was completed within 300 ps after excitation. This was the first clear visualization of energy transfer processes in photosynthetic organisms with a time resolution of 5 ps. Kinetic analysis of individual pigments indicated that the processes could be described by the Förster equation, i.e., the rise and decay kinetics of the four kinds of pigment were proportional to the square root of time. The same results were obtained for *A. nidulans* (Yamazaki et al. 1984) and *Tolypothrix tenuis* (Mimuro et al. 1985). These led to a new aspect in analyses of the excitation energy transfer processes almost 30 years after the measurements in the research laboratory of E. Rabinowitch (Brody and Rabinowitch 1957; Giiti Tomita and Rabinowitch 1962).

Energy transfer process in intact cells of a cyanobacterium *Anabaena variabilis* (M-3) was investigated at −196 °C (77 K). Low-temperature spectroscopy and three-dimensional presentation ensured a high spectral resolution of the transfer processes (Figure 2; Mimuro 1990). (Figures 3 and 4 show photographs of Yamazaki and Mimuro, respectively.) The overall energy flow process was the same as described for *A. nidulans*; however, the red-shift of the fluorescence peak was detected within one species of phycobiliprotein, Photosystem (PS) II Chl *a* (at approximately 690 nm) and PS I Chl *a* (at approximately 730 nm) species. It is natural to assume that in phycobilisomes, there are several types of chromophores in PC or APC whose energy levels are different; thus, the red-shift within the one molecular species indicated presence of the energy migration or a minor transfer pathway. At physiological temperature, this process was regarded as an equilibration of energy distribution among antenna molecules. Energy difference among pigments engaging in these processes is

small; thus at physiological temperature, these processes might act as an energy reservoir. Description of an equation for energy transfer including these minor processes has not yet been shown. However, the energy transfer processes in the physiological condition include forward as well as backward processes; thus, it is important to describe the whole transfer processes instead of analyzing the main downhill transfer pathway. This discussion is also applicable to the energy migration in PS I and/or PS II Chl *a*.

The TRFS method was applied to many systems in addition to cyanobacteria and red algae: purple bacteria (Keizo Shimada et al. 1989; Yoshinobu Nishimura et al. 1993), aerobic purple bacteria (*Erythrobacter* sp.) (Shimada et al. 1990), green filamentous bacteria (*Chloroflexus aurantiacus*) (Mimuro et al. 1989, 1994a), Chl *d* containing cyanobacterium (*Acaryochloris marina*) (Mimuro et al. 1999), cryptomonad (*Cryoptomonas* sp.) (Mimuro et al. 1998), dinoflagellates (*Protogonyaulax tamarensis*) (Mimuro et al. 1990), green alga (*Chlorella pyrenoidosa*) (Yamazaki et al. 1985a), spinach chloroplasts (Mimuro et al. 1987), isolated LHC II of green alga (*Bryopsis maxima*) (Katsumi Nakayama et al. 1994), and D_1–D_2-cyt b_{559} complex (Mimuro et al. 1988). We also succeeded in obtaining the time-resolved fluorescence polarization spectra (Danuta Fraçkowiak et al. 1989; Mimuro et al. 1994b); these spectra were complementary to the TRFS obtained earlier.

Energy transfer processes are affected by the composition of the antenna system, for example, a ratio of PS I/PS II. Y. Fujita et al. (1994) reviewed changes in the PS I/PS II ratio of cyanobacteria in relation to light intensity and light quality. Their achievements were honored in an special issue of *Photosynthesis Research* (Mimuro et al. 1997). Analyses of the energy transfer processes on cells with different PS I/PS II ratios may provide a new aspect of energy transfer processes and distribution of excitation energy between two pigment pools.

Difference between the pump-probe absorption method and time-resolved fluorescence spectrum

It is well known that there is another method to monitor the time behavior of antenna pigments: the pump-probe transient absorption method. Since this method monitors the difference absorption, signals include bleach of the ground state, formation of the excited state, and stimulated emission. These sometimes make the discrimination of signals difficult, even though the time resolution is much better for this method. Compared with this situation, it is rather simple that the TRFS monitors fluorescence, which reflects the excited state. The best experimental condition is a combination of these two methods because they are complementary to each other. However, unfortunately, it is not yet a common practice, in most laboratories, to combine both these methods.

TRFS takes the back seat when the fluorescence up-conversion method is adopted (J. Shah 1988). By this method, fluorescence is not directly measured, but fluorescence and a gated pulse hitting on the surface of a non-linear crystal generates the sum frequency of two pulses (called the up-converted signal) and this signal is detected. This method enables the investigators to monitor the excited state with a time resolution equivalent to that by the transient absorption method; it seems that both the techniques provide essentially the same information. We have presented below an analysis of the excited state dynamics of carotenoids in pigment–protein complexes and in organic solvents.

Energy transfer from carotenoid to chlorophyll *a* in algae

Carotenoids are one set of the antenna pigments in photosynthesis (for reviews on the photochemistry of carotenoids, see Frank et al. 1999). Even though the energy influx from carotenoids is regarded to be minor, some photosynthetic organisms such as photosynthetic bacteria, brown algae, diatoms, and some species of green algae live on absorption of blue light in their habitats (for a historical account, see Govindjee 1999). Duysens (1952) classical doctoral thesis, and an earlier historical minireview by H. Dutton (1997) discuss the earliest results on this topic.

Energy transfer mechanism from carotenoids to Chl *a* depends on the energy transfer pathway. The transition to the lowest singlet excited (S_1) state of carotenoids is shown to be one-photon forbidden; therefore, when energy transfer occurs from the S_1 state, a dipole–dipole interaction is not applicable (Mimuro and Tetzuya Katoh 1991) because a dipole moment of the S_1 state is not expected; the Dexter interaction (i.e., the electron exchange mechanism between a excited donor molecule and an acceptor molecule in the ground state both located almost within the van der Waals contact) is a possible mechanism. When transfer occurs from the S_2 state, a

dipole–dipole interaction is applicable. Independent of these assumptions, we predicted theoretically that the dipole–dipole interaction would play an essential role in the energy transfer from carotenoids (Hiroyoshi Nagae et al. 1993), and this was proved as described below. We noticed that efficient antenna carotenoids contain a keto-carbonyl group at the end of the conjugated double bond, and in organic solvents these carotenoids show optical properties (fluorescence from the S_1 state, Mimuro et al. 1992; and a long lifetime of the S_1 state, Mimuro et al. 1993) that very different from carotenoids without a keto-carbonyl group. These led us to measure the energy transfer processes in the peridinin-Chl *a*-protein (PCP) isolated from the dinoflagellate *Alexandrium tamarense* (Seiji Akimoto et al. 1996). Peridinin is unique in its molecular structure. We showed that the energy transfer occurred only from the S_1 state of peridinin to the S_1 state of Chl *a*. This was the first *direct* proof for the energy transfer from carotenoids to Chl. An asymmetrical structure of π-electron system of peridinin and a long lifetime of the S_1 state indicate that the S_1 state is partially allowed, leading to the idea that the dipole–dipole interaction would be responsible for energy transfer. Since the crystal structure of PCP is known (Hoffman et al. 1996), energy transfer processes among the 10 pigments in PCP will be clearly resolved in the near future. In the case of photosynthetic bacteria, the energy transfer between the S_2 states was proved (M. Ricci et al. 1996); thus, dipole–dipole interaction is the key mechanism for energy transfer. If the up-conversion technique had not been applied to this system, a direct proof for the transfer process would have remained difficult. Our approach to this problem, therefore, was significant.

Application of the up-conversion technique will also be useful in understanding the excited state dynamics of Chl and carotenoids in photosynthesis. Even though our understanding is not yet complete and the subject is much debatable, development of the optical technique is essential for further comprehensive understanding of energy transfer processes. A combination of molecular structure in the atomic resolution and ultra-fast spectroscopy is the minimum requisite for analyses of the energy transfer mechanism, as shown by the history of development of this field. Survey for a new type of photosynthetic organisms is also important as shown by diversity of the photosynthetic prokaryotes (Mimuro et al. 1999).

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