LOW-TEMPERATURE FLUORESCENCE DECAY AND ENERGY TRANSFER IN PHOTOSYNTHETIC UNITS

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1. Introduction

Certain progress in the understanding of primary energy conversion mechanisms in photosynthesis has been achieved through the use of picosecond lasers. Particularly, the energy transfer processes in the two photosystems (PS) of green plants have been investigated by means of fluorescence decay measurements. However, sometimes picosecond techniques give unusually short lifetimes for chlorophyll (Chl) in vivo as compared to those obtained with conventional methods. Although this discrepancy has been explained by considering exciton annihilation quenching of fluorescence under large excitation density [4] and normal lifetimes were obtained using single pulses of moderate energy [5,6], the ordinary photon counting technique retains its importance as a rather sensitive method [7]. Similar to recent in vitro investigations [8,9], this method enabled us to adjust the wavelength of recording (λ_f) at the emission maxima of selected Chl in vivo spectral forms and to follow the energy transfer between different forms.

Most of the Chl fluorescence lifetimes (τ_f) in vivo have been measured at room temperature or at 77 K, except for some data collected at 23 K in [10]. In [11] we reported the τ_f of chloroplasts and their fractions measured at 4.2 K in order to exclude the processes requiring thermal activation. Since the fluorescence yield (ψ_f) of Chl in chloroplasts is known to change at <77 K [12], we expanded this study to the region 4.2–77 K.

2. Materials and methods

Subchloroplast fragments were obtained with digitonin by a modified method [13,14]. The light fraction (obtained at 145 000 × g in 0.06 M sucrose–phosphate buffer at pH 7.0) consisted of PS I particles from intergranal thylakoids (Chl a to Chl b concentration ratio ≈6). The heavy fraction (20 000 × g) was used without further purification from granal PS I.

In fluorescence measurements we used dried thin layers of suspensions deposited on glass platelets. The low-temperature spectra of these samples proved to be identical to those of corresponding frozen suspensions. Control measurements of τ_f on suspensions gave similar results.

The experimental set-up was essentially as in [8]. The excitation was performed by a discharge between tungsten electrodes in air operating at 6 kV with ~1 kHz frequency. The emission of the flash in conjunction with a broadband blue filter had its maximum output near 400 nm. The recording system consisted of a DFS-24 double monochromator with 1 nm bandpass and a photon counting system including a Schlumberger 7117 time-to-amplitude converter. The decay curves were computer-fitted with one exponential term using a deconvolution procedure taking into account the flash profile [7,9]. Temperatures from 4.2–50 K were controlled and measured by an automatic thermocouple system.

3. Results and discussion

Similar to earlier low-temperature data [15],

several subbands with their maxima at 681, 685, 696 and 734 nm could be distinguished in the fluorescence spectra of chloroplasts and PS II and PS I particles Fluorescence decay curves were measured monitoring emission on these four maxima (in [11] it was found that τ_f was constant over the long-wavelength band from 725–750 nm)

In fig 1 some typical decay curves at 10 K are presented In fig 2 the τ_f values obtained by the single-exponential fit are shown as a function of temperature At room temperature [1,2,7] and at 90 K [16] more rapidly decaying (subnanosecond) components were observed, which were assigned to the open PS II reaction centres (RC II) [6] Our samples were pre-illuminated under the 442 nm cw Cd-laser beam (~0 1 W/cm^2), so that RC II may be considered as mostly closed

An interesting feature in fig 2a is the dependence of τ_f on the emission wavelength λ_f within the PS II main fluorescence band This dependence, which becomes more prominent by lowering the temperature, can be interpreted as a result of energy transfer between different Chl forms A step-wise transfer to lower energy forms reduces τ_f values for the short-wavelength forms serving as excitation energy donors for the long-wavelength ones A similar phenomenon was demonstrated for Chl *a* in ether solutions at low temperatures [9], where starting from 10^{-4} M Chl *a* concentrations an increasing dependence of τ_f on λ_f

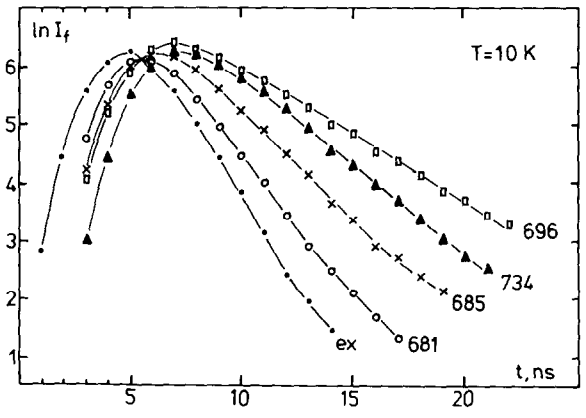

Fig 1 Time dependence of the fluorescence intensity (in arbitrary units) of PS II fraction recorded at 681, 685 and 696 nm and of PS I fraction at 734 nm, compared with the excitation pulse profile (ex) T = 10 K

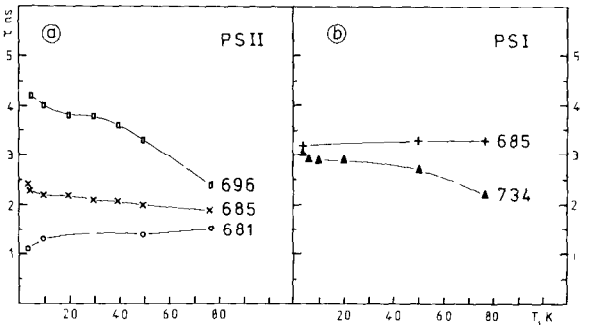

Fig 2 Fluorescence lifetimes of PS II (a) and PS I (b) fractions of pea chloroplasts at different wavelengths in dependence on the temperature

was observed The effectiveness of heterotransfer between native Chl forms has also been demonstrated by spectral measurements [17] and supported by model calculations [18]

Our results are in agreement with the tripartite model [19], a modified scheme of which (see also [5]) is presented in fig 3 The excitation energy collected by the light-harvesting (LH) system of PS II (*F*681 and *F*685) is rapidly transferred to RC II antenna Chl *F*696 tightly bound to Chl *P*680 In closed centres excitation can migrate from *P*680 back to antenna Chl, as deduced from the fluorescence induction measurements [19]

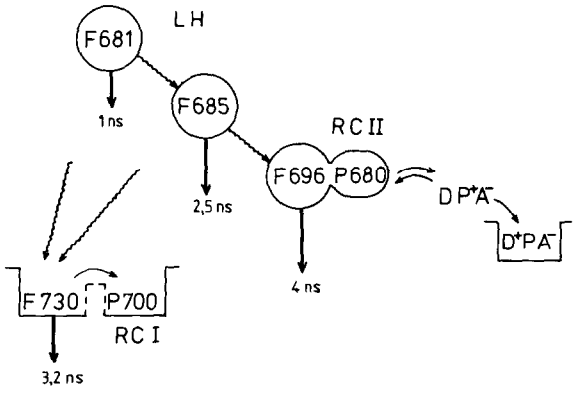

Fig 3 Tentative scheme of excitation energy transfer (wavy arrows) in the photosystems II and I of chloroplasts (based on the model in [19]) $P \equiv P680$, PS II reaction centre Chl, D, electron donor, A, acceptor, *P*700, PS I reaction centre Chl Fluorescence lifetimes of different Chl forms at 4 2 K are shown

In the case of energy transfer between randomly distributed molecules the decay of donor fluorescence should be non-exponential. The absence of remarkable non-exponentiality of the time dependences in their decaying tails (fig.1). may be owing to two factors. First, within a certain pigment system Chl molecules may be positioned in regular arrays [18]. Second, since PS II Chl forms *F*685 and *F*696 serve simultaneously as energy donors and acceptors, the shortening of the 'donor' lifetime by the Förster mechanism is partly compensated by the increase of the lifetime of the 'acceptor' [9]. This also explains the smallness of the relative shifts in the maxima of the corresponding decay curves (fig.1). On the other hand, a noticeable time delay can be observed in the PS I emission at 734 nm. This indicates that *F*730* functions mainly as an excitation acceptor in PS I. Invariability of τ_f within the long-wavelength band of PS I supports the conclusion about the mobile character of excitons in this pigment system [4].

The longer τ_f obtained at 681 and 685 nm for PS I fraction (fig.2b and [11]) as compared with those for PS II at the same λ_f values show PS I probably has its own LH system. However, a doubt remains if this difference is not produced by fragmentation, since large τ_f values for the short-wavelength band of PS I do not afford effective transfer to *F*730. The excitation donor for *F*730 should have low fluorescence yield and short lifetime; it cannot therefore, be identified within our experimental data.

An appreciable increase of the PS II τ_f values at 685 nm and 696 nm is observed when temperature is lowered from 20–4 K, whereas the increase seems to proceed at still lower temperatures (fig.2a). Such dependence gives evidence that in PS II there exist non-radiative processes with very low thermal activation barriers. One of the possible reasons could be the freezing out to the photon-assisted (i.e., vibrationally activated) part of the energy transfer rates. In contrast, for PS I the τ_f value at 734 nm shows only slight changes from 4.2–50 K, while an essential decrease of τ_f takes place between 90–300 K [10,16], in accordance with the deep energy traps found for RC I [19].

The increase of τ_f values for Chl in vivo with decreasing temperature agrees with the observations [3,5,10] and correlates with the simultaneous increase of quantum yield ψ_f [12,20]. However, it should be pointed out that there need not be exact proportionality between τ_f and ψ_f values: in heterogeneous systems strongly fluorescent species give the dominating contribution to the signal and one effectively detects the weighted lifetime, which is further affected by the excitation migration.

Summarizing the results of the present study, it may be noted that the use of very low temperatures and selective recording in the lifetime measurements enabled us to follow a distinct irreversible energy transfer in chloroplast photosystems. Appreciable differences are found between the time-dependent characteristics of PS I and PS II, respectively.

* Although the exact position of this band slightly depends on sample preparation, we use the standard notation *F*730

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