REGULATION OF EXCITATION TRANSFER BY CATIONS: WAVELENGTH–RESOLVED FLUORESCENCE LIFETIMES AND INTENSITIES AT 77 K IN THYLAKOID MEMBRANES OF PEA CHLOROPLASTS

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Received 23 May 1979

1. Introduction

In cation-depleted chloroplast membranes (thylakoids) at 77 K, the ratios of intensities of chlorophyll *a* (Chl *a*) fluorescence emitted at 685 and 695 nm (F685 and F695) to that at 730 nm (F730) are decreased by the addition, at room temperature, of low concentrations ($\simeq$ 10 mM) of NaCl [1–3], and increased by low concentrations of $MgCl_2$ or high concentrations ($\simeq$ 100 mM) of NaCl [1–5]. These phenomena have been reviewed by Barber [6] and Williams [7]. The above-mentioned changes in fluorescence intensities could be the consequence of changes in Chl *a* fluorescence efficiencies and/or changes in the populations of the fluorescent species. The former could come about from changes in the rate constant for excitation transfer from photosystem (PS II) to PS I [4,8], also known as 'spill-over' or 'redistribution', or in the rate constant of some other radiationless transition [9,10]. The changes in fluorescence efficiencies (ϕ) can be directly measured by changes in lifetimes (τ) as $\tau = \phi\tau_0$, where τ_0 is the intrinsic lifetime of fluorescence. The purpose of this work is to investigate by lifetime measurements whether or not the cation-induced changes in relative fluorescence intensities at 77 K reflect changes in the quantum efficiencies of fluorescence emitted by PS II and/or PS I. We found that changes in fluorescence intensities are much greater than changes in lifetimes (and thus quantum efficiencies). These differences could be explained on the basis of changes in the excitation energy transfer from PS II to PS I, and of changes in the population of fluorescent species.

2. Materials and methods

Sucrose-washed thylakoid membranes were prepared from pea (*Pisum sativum*) chloroplasts as described previously [11]. Concentrated thylakoid suspensions (1–1.5 mg Chl/ml) in 100 mM sucrose were stored in liquid nitrogen until used. Dilutions were made with 100 mM sucrose containing 0.4 mM Tris–NaCl at pH 7.6 in all experiments, with the final pH of the sample in the range of 7.0 ± 0.2.

Fluorescence lifetime was measured by the phase-delay method using a mode-locked He–Ne laser (λ = 632.8 nm; modulation frequency = 75 MHz; average output intensity = 40 mW/cm^2) as described by Merkelo et al. [12]. Fluorescence was detected with a RCA 7102 photomultiplier (S-1 photocathode response) protected with appropriate optical filters, and the lifetime was calculated from the relation $\tau = \tan \Delta\phi/2\pi f$, where $\Delta\phi$ = phase-delay between the incident light and fluorescence, and f = modulation frequency. Relative fluorescence intensities at 77 K

Results presented at the 22nd Annual Meeting of the Biophysical Society and the American Physical Society, March 27–30, 1978, Washington, DC (abst. no. TU-PM-B9 (1978) Biophys. J. 21, 108a)

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were measured separately with the spectrofluorometer described by Shimony et al. [13]. Fluorescence was excited with a tungsten projection lamp through an interference filter with maximum transmission at 636 nm (half-bandwidth, 8 nm) and detected with an EMI 9558B photomultiplier (S-20 response) through a Corning CS 2-59 glass filter and a monochromator. Other experimental details are given in the legends of the tables.

3. Results

The Chl *a* fluorescence lifetimes measured at 77 K at the maximum fluorescence level (denoted below by subscript M), when all reaction centers are closed, are presented in table 1. For sucrose-washed thylakoids suspended in cation-free medium the fluorescence lifetimes (τ) are 0.4 ns for emission at 686 nm, to be referred to as $\tau(F686_M)$, 0.8 ns for $\tau(F695_M)$, and 2.0 ns for $\tau(F730_M)$. Additional measurements at 680 nm, $\tau(F680_M)$, give the same results as for $\tau(F686_M)$. The addition of 10 mM NaCl (denoted by Na^+) to salt-depleted thylakoids does not change $\tau(F686_M)$ or $\tau(F695_M)$, but slightly (7 ± 4%) increases $\tau(F730_M)$. Subsequent addition of 10 mM $MgCl_2$ ($Na^+ + Mg^{2+}$), however, increases $\tau(F686_M)$ by 40 ± 19% and $\tau(F695_M)$ by 29 ± 10%, compared to the Na^+ sample, but has no significant effect on $\tau(F730_M)$.

Next, the maximum fluorescence intensities at 690 and 730 nm were measured, and the ratio $F690_M/F730_M$ obtained. For comparison, the lifetime-ratios $\tau(F686_M)/\tau(F730_M)$ and $\tau(F695_M)/\tau(F730_M)$ were also calculated from the values in table 1. The results summarized in table 2 show that upon addition of 10 mM NaCl to salt-depleted thylakoids both $\tau(F686_M)/\tau(F730_M)$ and $\tau(F695_M)/\tau(F730_M)$ remain relatively constant, going from 0.21 to 0.20 and 0.38 to 0.36, respectively, while $F690_M/F730_M$ decreases by ~26%, from 0.31 to 0.23. However, the subsequent addition of 10 mM $MgCl_2$ leads to an increase in all three ratios with the largest change in the intensity-ratio – 109% from 0.23 to 0.48 for $F690_M/F730_M$ compared to 45% from 0.20 to 0.29 for $\tau(F686_M)/\tau(F730_M)$ or 31% from 0.36 to 0.47 for $\tau(F695_M)/\tau(F730_M)$.

Table 1
Effects of cations on chlorophyll *a* fluorescence lifetime at 77 K

Sample	Lifetime, τ, nanoseconds[a]		
	$\tau(F686_M)$[b]	$\tau(F695_M)$	$\tau(F730_M)$
Salt-depleted	0.42 ± 0.04	0.77 ± 0.04	2.02 ± 0.04
+ 10 mM NaCl	0.43 ± 0.04	0.77 ± 0.03	2.16 ± 0.08
+ 10 mM NaCl + 10 mM $MgCl_2$	0.60 ± 0.06	0.99 ± 0.07	2.10 ± 0.07

[a] The results presented are the mean for five separate measurements from three different batches of chloroplasts preparations. The uncertainties in the mean values of τ are standard errors. The experimental precision for each measurement was about 10 ps, and the same trend for the cation effects was observed in every set of samples

[b] Fluorescence was detected through inteference filters: at 686 nm (half-bandwidth, HB, 6.8 nm) for $\tau(F686_M)$, at 695 nm (HB, 6.3 nm) for $\tau(F695_M)$, and at 730 nm (HB, 8.4 nm) for $\tau(F730_M)$, in combination with a Schott RG5 longpass filter (thickness, 3 mm)

Sucrose-washed thylakoids were diluted with 100 mM sucrose containing 0.4 mM Tris–HCl at pH 7.6 to a final chlorophyll concentration of about 25 μg/ml and pH 7.0 ± 0.2; samples were frozen in a 1 mm cuvette by submerging in liquid nitrogen in a Dewar flask and fluorescence measured from the front surface

Table 2
Effects of cations on the short- to long-wavelength chlorophyll *a* fluorescence lifetime- and intensity-ratios at 77 K

Sample	Lifetime-ratios[a]		Intensity-ratios[b]
	$\tau(F686_M)/\tau(F730_M)$	$\tau(F695_M)/\tau(F730_M)$	$F690_M/F730_M$
Salt-depleted	0.21	0.38	0.31
+ 10 mM NaCl	0.20	0.36	0.23
+ 10 mM NaCl + 10 mM $MgCl_2$	0.29	0.47	0.48

[a] Fluorescence lifetime values in table 1 are used
[b] Relative fluorescence intensities at 77 K were measured with the spectrofluorometer described by Shimony et al. [13]. For each sample, a 0.5 ml aliquot of thylakoid suspension ([Chl] ~ 25 μg/ml) was adsorbed onto two layers of cheesecloth, held down by a Teflon ring in a Dewar flask furnished with a flat optically clear bottom, and frozen in liquid nitrogen. The monochromator bandpass was 10 nm

4. Discussion

For the present discussion it will be taken that both F680 and F686 monitor mainly fluorescence associated with Chl *a* complexes of the light-harvesting Chl *a/b* complex (Chl LH) and of the other antenna complex of PS II [14–16], F695 mainly with the Chl *a* present in the core antenna complex of PS II (Chl a_{II}), and F730 mainly with the Chl *a* antenna complex of PS I (Chl a_I) (see [8,17–20]).

With the above picture, the results in table 1 show that the lifetime of fluorescence from PS II is unaffected by Na^+ addition to salt-depleted thylakoids, but is increased by 30–40% upon subsequent addition of Mg^{2+}. In the case of PS I fluorescence, the lifetime is slightly increased (~7%) by Na^+ addition, after which it is unaffected by the presence of Mg^{2+}. These findings show that the lifetimes of PS I and PS II fluorescence can be independently affected by monovalent and divalent cations. Thus, the efficiencies of fluorescence from PS I and PS II are cation-dependent, with the largest effect observed in the increase in PS II fluorescence lifetime upon Mg^{2+} addition. This effect is also seen as the increase in the lifetime-ratios $\tau(F686_M)/\tau(F730_M)$ and $\tau(F695_M)/\tau(F730_M)$ (see table 2) upon addition of Mg^{2+} to the Na^+ sample, since $\tau(F730_M)$ is essentially unaffected by Mg^{2+} (see table 1).

A comparison of the cation-induced changes in the PS II/PS I fluorescence lifetime-ratios with the corresponding intensity-ratios in the Na^+ and Na^+ + Mg^{2+} samples shows obvious differences. The addition of Na^+ to sucrose-washed thylakoids shows no significant effect on the PS II/PS I lifetime-ratio but a ~26% decrease in the corresponding intensity-ratio. Subsequent addition of Mg^{2+} to the Na^+ sample leads to a 30–45% increase in the PS II/PS I lifetime-ratio but a larger (109%) increase in the intensity-ratio. Since the energy transfer from PS II to PS I is expected to be short (~140 ps, cf. Campillo et al. [21]) compared to the PS I lifetime (~2 ns, table 1), any change in energy transfer from PS II to PS I would affect the fluorescence intensity-ratio to a greater extent than the lifetime-ratios. For instance, assuming no other effects, an inhibition of energy transfer from PS II to PS I could increase the PS II fluorescence intensity and lifetime, and decrease the intensity of PS I fluorescence but not its lifetime, leading to a greater increase in the PS II/PS I fluorescence intensity-ratio than the lifetime-ratio (last two lines in table 2). In this case, of course, both the excited state population and the fluorescence efficiency of PS II are increased by inhibiting the PS II to PS I energy transfer pathway Although the sensitization of PS II is slightly greater ($\leqslant$ 20%) in the Na^+ + Mg^{2+} sample than the Na^+ sample (cf. [11,19,22]), a detailed analysis in

terms of various models of the light harvesting apparatus [23] reveals that part of the lifetime- and intensity-ratio discrepancies must be accounted for by the Mg^{2+} inhibition of 'spill over'.

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