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ANALYSIS OF THE KINETICS OF THE CATION-INDUCED INCREASE IN PHOTOSYSTEM II FLUORESCENCE IN ISOLATED THYLAKOIDS

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A hypothesis is put forward, namely that the fluorescence quenching by transfer to Photosystem (PS) I depends upon the distance between PS II and PS I according to Förster's law and it is observed that the rate of increase in distance is proportional to the inverse of the square of the distance between the two photosystems.

Introduction

Markwell et al. [1] demonstrated that all chlorophylls are localized in chromoprotein complexes. Thus, no exciton transfer between photosynthetic units can occur via free chlorophylls embedded within the lipid matrix of the membrane. At low monovalent cation concentrations, thylakoids are unstacked and the PS I and PS II complexes are homogeneously distributed within the membrane. Screening of negative charges on the membranes by cations induces stacking and the lateral segregation of PS I and PS II. This segregation is, as suggested by Barber and Chow [2] and Anderson [3], the simplest explanation for the decrease in PS II to PS I exciton transfer.

However, there is no direct correlation between stacking or partitioning of PS I and PS II markers as seen by freeze fracture, and changes in PS II fluorescence obtained by addition of various cation concentrations [4,5]. Fluorescence changes are more sensitive to divalent cations than the other structural modifications. Wollman and Diner [5], by comparing the variations of scattering with those of fluorescence, confirmed the above results. They showed that addition of cations did not yield identical kinetics for these phenomena. Changes in the orientation of the pigments may also be considered as Biggins [6] observed a cation-induced change in linear dichroism of thylakoids at wavelengths which could correspond to pigments associated with the light-harvesting complex, changes which could be due to variations in orientation. Therefore, the cation-induced structural change responsible for spillover variations is still questionable.

Here instead of considering the steady states, reached at various cation concentrations, we analyzed the kinetics of the fluorescence increase upon $MgCl_2$ addition. The analysis is based on the hypothesis that the quenching of PS II by transfer to PS I decreases as the distance between them increases according to Förster's law. It has been observed that the rate of separation of the two photosystems is proportional to the inverse of the square of the distance between the two photosystems.

Abbreviations: PS, photosystem; Chl, chlorophyll DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Tricine, *N*-tris(hydroxymethyl)methylglycine.

Materials and Methods

Broken pea or spinach chloroplasts were isolated as previously described [7] and resuspended in a medium containing: 10^{-2} M Tricine (pH 7.8), 10^{-2} M NaCl, 0.4 M sorbitol, 10^{-5} M DCMU and 10^{-6} M gramicidin D *. The 3 ml sample (chloroplasts equivalent to 1–10 μg Chl/ml) was contained in a $1 \times 1 \times 4$ cm cuvette. Magnetic stirring of the sample enabled the mixing of a few microliters of the $MgCl_2$ solutions to take place in less than 0.3 s. The excitation of the suspension by blue light was obtained using a Corning 4-96 filter. The fluorescence, emitted at 90°, was collected by a light pipe and selected using red filters (Corning 2-64 plus Wratten 92).

The fluorescence changes, upon $MgCl_2$ additions, were recorded with a signal averager (Interzoom, SEIN), then stored on tape and the kinetics analyzed using a Hewlett-Packard 9845 computer. Measurements were performed at 20°C.

Fluorescence measurements in a 1 cm^2 cuvette are very sensitive to the heterogeneity of the incident light, reabsorption and scattering of the sample. Thus, the chloroplast concentration has to be as low as possible to minimize these effects. Furthermore, as the scattering of the chloroplast suspension increases upon cation addition, the kinetics of the fluorescence enhancement will be modified by a time-dependent decrease in incident intensity from the front to the back of the cuvette. This is shown in Table I, where changes of transmission at 480 nm, induced by addition of 10 mM $MgCl_2$ to chloroplast suspensions, are presented for various chloroplast concentrations. The measurements were done using a Cary 14 spectrophotometer.

From Table I, the change of incident intensity is almost negligible for 1 μg Chl/ml and the kinetics of the fluorescence changes upon cation addition will be almost unaffected by variations of incident intensity. For 5 and 10 μg Chl/ml the kinetics are significantly different from those obtained with 1 μg Chl/ml. At 10 μg Chl/ml we observe the same kinetics as those published by Rubin et al. [8]. However, we believe these kinetics are perturbed by light scattering.

TABLE I

CHANGES IN TRANSMISSION OF A CHLOROPLAST SUSPENSION IN A 1 cm SQUARE CUVETTE UPON $MgCl_2$ ADDITION (10 mM) AT 480 nm

Chlorophyll concentration (μg/ml)	% transmission		$+Mg^{2+}/-Mg^{2+}$
	$-Mg^{2+}$	$+Mg^{2+}$	
1	91	90	0.990
5	60.8	57.5	0.946
10	37	33	0.905

Results and Discussion

It has been shown that cations modulate PS II fluorescence mainly by changing exciton transfer from PS II to PS I [9,10]. If F and F_q are, respectively, the PS II maximum fluorescence yield in the absence and presence of this exciton transfer, then:

$$F = \frac{kf}{kf + kd} \tag{1}$$

$$F_q = \frac{kf}{kf + kd + k(\mathrm{II} \rightarrow \mathrm{I})} \tag{2}$$

Where kf, $k(\mathrm{II} \rightarrow \mathrm{I})$ and kd are, respectively, rate constants of PS II deactivation through fluorescence, PS II to PS I transfer and other pathways.

According to Förster, $k(\mathrm{II} \rightarrow \mathrm{I})$ will depend upon the overlap between the emission spectra of the exciton donor and the absorption spectra of the acceptor, the orientation of their corresponding dipoles and their distance R. Murata [9] has shown that cations do not induce changes of the absorption spectrum or the PS II emission spectrum. If we assume that there is no change of orientation, then:

$$k(\mathrm{II} \rightarrow \mathrm{I}) = K \cdot \frac{1}{R^6} \tag{3}$$

Combining Eqns. 1–3 we obtain:

$$\frac{F}{F_q} - 1 = \frac{K}{kf + kd} \cdot \frac{1}{R^6} \tag{4}$$

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* To obtain complete unstacking before $MgCl_2$ addition, the incubation time in the above medium was at least 30 min.

In the absence of Mg^{2+} $F_q = 1/2\ F$, then for this condition:

$$R^6 = R_0^6 = \frac{K}{kf + kd} \tag{5}$$

We showed previously [10] that in the absence of Mg^{2+}, approximately half of the PS II excitons are transferred to PS I. Wollman et al. [11] have used a distance of 5 nm between PS II units and we will use this distance for PS II and PS I for a probability of transfer of 50%. This value may be an overestimation as the overlap between PS II fluorescence and PS I absorption is larger than for PS II units.

The Eqn. 4 becomes:

$$\frac{F}{F_q} - 1 = \left(\frac{R_0}{R}\right)^6 = \left(\frac{5}{R}\right)^6 \tag{6}$$

The kinetics of the fluorescence increase upon $MgCl_2$ addition (Fig. 1) can be converted using Eqn. 6 to the kinetics of R increase (Fig. 2).

If the separation occurs in a viscous medium, then its rate dR/dt will be directly proportional to the force A which monitors the increase in distance between the two photosystems.

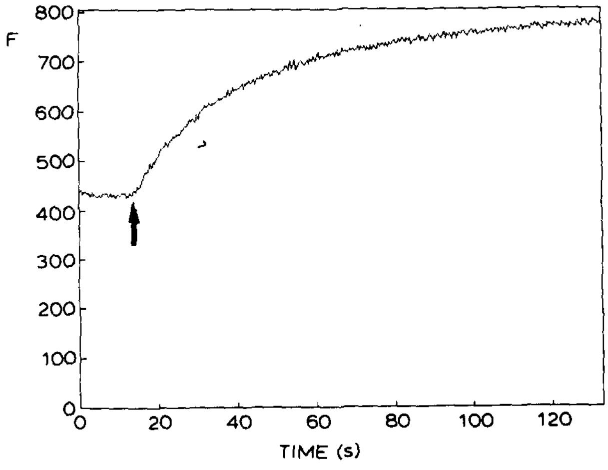

Fig. 1. Kinetics of PS II fluorescence increase upon 5 mM $MgCl_2$ addition. 1 μg Chl/ml. The arrow indicates the addition of $MgCl_2$. The maximum fluorescence yield corresponds to the upper limit of the graph. The fluorescence was measured during 400 s.

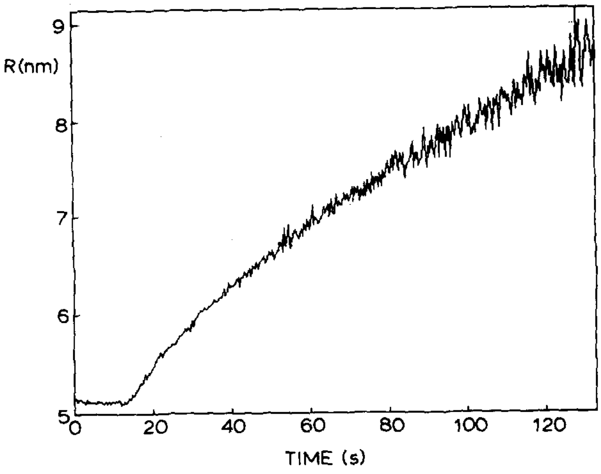

Fig. 2. Kinetics of R variations upon 5 mM $MgCl_2$ addition deduced from Fig. 1 according to Eqn. 6.

Then:

$$f \cdot \frac{dR}{dt} = A \tag{7}$$

where f is the friction coefficient.

It is observed that dR/dt decreases with time, i.e., when R increases. This is similar to coulombic interactions.

If we use this analogy then:

$$A = \frac{k}{R^2} \tag{8}$$

where k is a constant.

Then from Eqns. 7 and 8:

$$dR/dt = k'/R^2 \tag{9}$$

where $k' = k/f$.

Integration of Eqn. 9 gives:

$$R^3 = 3k't + R_0^3 \tag{10}$$

Fig. 3 shows a plot of R^3 vs. time; a linear relationship is obtained which fits Eqn. 10.

Fig. 1 shows the kinetics after 5 mM $MgCl_2$ addition. From the slopes obtained after addition of 2, 5 and 10 mM $MgCl_2$, k' values were determined, their values being 0.96, 1.47 and 2.30, respectively. In this concentration range the same

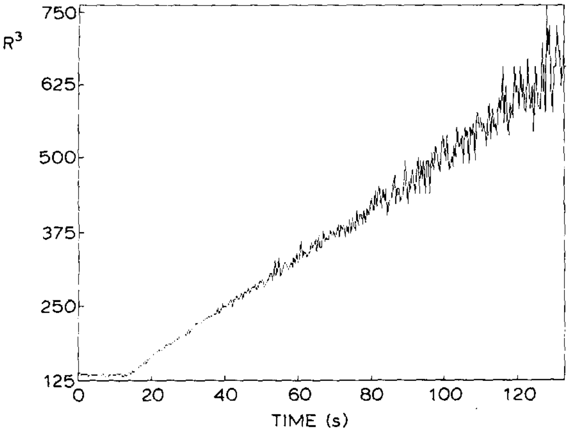

Fig. 3. Kinetics of R^3 (nm^3) deduced from Figs. 1 and 2 according to Eqn. 9.

maximum enhancement of fluorescence is obtained. The kinetics at $MgCl_2$ concentrations higher than 10 mM were not studied because, as previously observed by Wollman and Diner [5], an additional quenching develops.

It is not surprising that k' increases with $MgCl_2$ concentration; indeed, Barber [12] showed that Ψ_0, the negative surface potential, decreases as the divalent cation level increases within this concentration range (Fig. 6A of Ref. 12).

It is well known that the concentration required to saturate the increase in scattering and in particle segregation is larger than that needed to saturate the fluorescence increase [4,5] and that the first phenomenon takes much more time than the fluorescence increase [13]. This agrees with our analysis, which points out that a smaller increase in distance than those observed in totally stacked chloroplasts is required to cancel the PS II → PS I exciton transfer. We suggest that the closer the two adjacent membranes of two thylakoids are appressed the stronger is the energetic repulsion (A) which monitors the increase of distance between PS I and PS II.

As stated by Barber and Chow [2], 'the picture that emerges for thylakoid stacking to occur involves an interplay between heterogeneity of charge distribution on the membrane surface and van der Waals' forces. Electrostatic screening of the charged protein complexes gives rise to migration of these particles so as to allow net attraction between adjacent membranes at less polar regions and membrane repulsion at the more electrically charged regions'.

Apparently, the fluorescence increase is much faster than stacking and the segregation of PS I and II in the stromal and granal zones. It is possible to describe the fluorescence increase by the same mechanism as Barber and Chow have proposed but on a smaller scale. When cations are added there is an immediate attraction of opposite membranes (this will correspond to the rapid phase of scattering [5]. As the membranes approach each other there will be a distinct attraction, of the van der Waals' type, between the PS II of opposite membranes. In contrast, the PS I (negatively charged) will tend to be repelled from these zones of attraction. These opposed movements of PS I and PS II, though of a weak nature, are sufficient to suppress all PS II → PS I ($1/R^6$) exciton transfer. The greater separation and the regrouping of PS II in regions takes place later, the fluorescence no longer being sensitive to it.

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