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Chlorophyll Fluorescence Characteristics of Photosystems I and II from Grana and Photosystem I from Stroma lamellae

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With 5 figures

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Summary

Comparative fluorescence investigations of the pigment system I from grana and stroma revealed significant differences in the organization of chlorophyll in the two fractions as (1) the degree of polarization of fluorescence of system I from grana was only 50–60 % of that of system I from stroma; (2) the ratio of fluorescence intensity at 685 nm (F685) to 735 nm in system I from grana (free from detergent solubilized chlorophylls) was about twice that in system I from stroma; (3) the system I from stroma contained a higher concentration of a chlorophyll *a* form absorbing at 705 nm (Chl *a* 705) than the system I from grana (GASANOV and FRENCH, 1973). A drastically reduced but almost equal fluorescence transient and 1 second delayed light emission were observed in the two fractions. Analysis of the slow delayed light emission characteristics of the various fractions (system I from grana and stroma, and system II from grana) revealed differences in their kinetics. Fluorescence data on pigment system II from grana are also presented for comparison. As compared to system I from grana, system II from grana has a 7 fold higher ratio of F685/F735, a 60 fold higher intensity of 1 second delayed light intensity, about four fold higher variable chlorophyll fluorescence, and almost equal degree of polarization of fluorescence. No evidence for system II from stroma is reported.

Introduction

Photochemical properties of pigment system I from stroma lamellae, and of a mixture of pigment systems I and II have been studied extensively in the past (see BOARDMAN, 1968). However, only recently it has been possible to separate pure pigment systems I and II from grana and compare system I of grana with that from stroma. For example, ARNTZEN et al. (1972) fractionated French-press treated particles with digitonin; they obtained, on chlorophyll basis, 60 % photosystem II and 40 % photosystem I from grana membranes. Photosystem I particles, obtained from stroma or grana membranes, were quite similar with regard to eleotron transport activity, P700 content, ultrastructure appearance and ultrafiltration characteristics. However, the stroma photosystem I fragments did not recombine with the grana photosystem II fraction and reconstitute electron transport activities from diphenyl carbazide to $NADP^+$, as did the grana photosystem I fraction. Furthermore, the

stroma photosystem I sample had slightly higher chlorophyll (Chl) *a*/Chl *b* ratio and P700/Chl *a* than grana photosystem I. On the basis of the P700 content, SANE et al. (1970) had earlier suggested that the photosystem of stroma has a smaller photosynthetic unit than the grana photosystem I. GASANOV and FRENCH (1973) have shown, by analyzing the absorption spectra of these fractions at – 196° C, that system I preparations from stroma and grana are similar but not identical, the former containing more longwavelength forms of Chl *a*. In this paper, we present our comparative investigations, on system I and II from grana and system I from stroma, of the emission spectra, Chl fluorescence induction, degree of polarization of Chl fluorescence and the delayed light emission. (Care was taken to avoid any artifacts resulting from the effect of the detergent in our experiments.) Our results show that the fluorescence properties of system I from grana, although similar to that from stroma, are significantly different; there is a lower degree of polarization of fluorescence, lower ratio of longwavelength to short wavelength fluorescence bands and lower content of Chl *a* 705 in system I from grana than from stroma.

Materials and Methods

Chloroplasts were isolated from spinach leaves by homogenizing in phosphate buffer (0.05 M phosphate, 0.4 M sucrose and 0.01 M NaCl, pH = 7.8). The chloroplast pellet was resuspended in a solution containing 150 mM KCl and 50 mM Tricine-KOH buffer (pH = 7.8) and used for isolating the particles corresponding to photosystems I and II from grana*) and photosystem I from stroma, as previously described (ARNTZEN et al., 1972; GASANOV and FRENCH, 1973). Detergent was removed in two ways (1) by a resuspension of the «particles» and differential centrifugation at 144 × g for 30 mins; or (2) by a second sucrose density gradient separation after resuspension of the «particles». Chlorophyll concentration was determined according to ARNON (1949).

Emission spectra, at 77° K, were measured as described by CHO and GOVINDJEE (1970a, 1970b). Emission spectra, reported here, were corrected for the spectral variation of the observation monochromator and the photomultiplier (EMI 9558B). These spectra were plotted as relative quanta per unit wavelength. Other details of measurements are given in the legends of figures and table 1.

The degree of polarization of chlorophyll fluorescence was measured by a procedure similar to that of MAR and GOVINDJEE (1972). Delayed light emission in the 1–10 sec. time range was measured with an apparatus described by JURSINIC and GOVINDJEE (1972).

Results and Discussion

1. Fluorescence Emission Spectra of Fractions Free of Detergent-Solubilized Chlorophylls

The low temperature (77° K) fluorescence spectra of the chloroplast fractions and sub-chloroplast fractions ([Chl], 15 $\mu g\, ml^{-1}$) are shown in Figure 1 (also see table 1). As is already known, the fluorescence spectrum of grana membranes after they were

*) Reproducible results were obtained by incubating grana fractions having 0.3 to 0.5 mg Chl/ml in 0.6 to 1 % digitonin for 30 min. at 4° C.

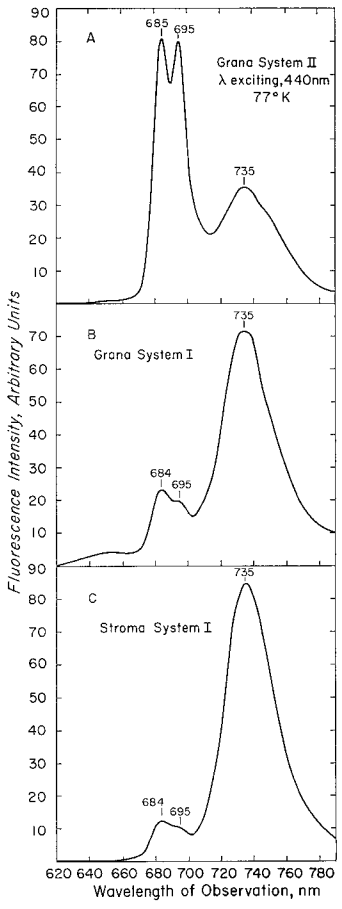

Fig. 1: Emission spectra of chlorophyll fluorescence at 77° K in chloroplast fractions (system II from grana, system I from grana, and system I from stroma). [Chl], 5 μg ml^{-1}; exciting wavelength, 440 nm.

passed through a needle valve (labeled P-10K, no detergent) had three maxima at 685 nm, 695 nm, and 735 nm, with relatively high intensity of short wavelength bands of fluorescence; the ratio of F685/F735 (fluorescence intensities at the wavelengths indicated in nm) at 77° K was 1.7 (cf. with chloroplast fragments and with system I protein complex and purified system II particles, see GOVINDJEE and YANG (1966) and MOHANTY et al. (1972)). The fluorescence spectrum of the fraction which corresponds to pigment system II from grana, from which detergent-solubilized chlorophyll was removed by differential centrifugation (labelled, F-2G1), showed a higher ratio (2.3) of F685/F735. This ratio is lower than that obtained for system II

Table 1: Comparison of the ratios of the short-wavelength to the long-wavelength emission in the different fractions (as estimated from emission spectra at room and liquid nitrogen temperatures).

	Ratios			
	F680/F710 (298° K)		F685/F735 (77° K)	F695/F735 (77° K)
Fraction	440*)	485	440	440
P-10K	6.8	7.0	1.47	1.40
P-144K	3.2	3.4	0.15	0.15
P-144KI	2.6	3.1	0.14	0.11
F-2G	6.6	7.4	2.10	2.20
F-2GI	6.6	7.2	2.30	2.10
F-1G	7.6	7.1	3.30	1.20
F-1GI	3.8	4.2	0.32	0.27
F-2D	6.3	6.9	2.00	2.00
F-1D	4.7	5.5	0.49	0.50

*) – Excitation wavelength in nm
P-10K – grana membranes after passing through needle valve.
P-144K – fraction from stroma, corresponding to system I.
P-144KI – the same fraction after digitonin treatment and removal of digitonin by centrifugation.
F-2G – fraction from grana after digitonin treatment of grana membranes and sucrose gradient isolation, corresponding to system II.
F-2GI – the same fraction after separation of the solubilized chlorophyll by differential centrifugation.
F-1G fraction from grana after digitonin treatment of grana membranes and sucrose gradient isolation, corresponding to system I.
F-1GI – the same fraction after separation of the solubilized chlorophyll by differential centrifuation.
F-2D – fraction from digitonin treated grana membranes by differential centrifugation, corresponding to system II.
F-1D – fraction from digitonin treated grana membranes by differential centrifugation, corresponding to system I.

particles, prepared by Huzisige's method (Mohanty et al., 1972), as the latter may have lost the long wavelength form af chlorophyll due to a sonication step in their method (see Bazzaz and Govindjee, 1973). The ratio of F685/F735 from stroma system I [P-144K (untreated with detergent) or P-144KI (first treated with detergent, and then detergent removed by differential centrifugation)] was 0.14 ± 0.01, but the same ratio for grana system I from which detergent solubilized chlorophyll was removed by differential centrifugation (labelled F1GI) was 0.32, suggesting that the system I of grana is «different» from system I of stroma. However, we considered the possibility that this difference could be partly or wholly due to contamination by system II of grana. In order to explain the ratios obtained, we would have to predict that system I from grana is a mixture of 2 parts system II to 1 part system I. We know, however, from independent measurements on delayed light

emission, fluorescence induction and photochemical activities (not reported here) that this is not the case as our results agree with those of ARNTZEN et al. (1) in all other respects. Thus, we conclude that there is a significant difference between the fluorescence characteristics of system I from grana and stroma. A very low value of F685/F735 in the stroma fraction also indicates that stroma may not contain any system II.

2. Effect of Detergent on Fluorescence Spectra

The effect of detergent treatment my be seen by comparing the ratio of F685/F735 in grana system II with detergent (F2G) and those from which detergent was initially removed (F2GI); this ratio was the same (2.1 ± 0.2) in both samples. (The method of preparation is, perhaps, such that detergents are automatically removed from system II particles.) If stroma system I was treated with detergent, and then detergent removed, the F685/F735 was the same as that for untreated stroma system I (0.14 ± 0.01). Thus detergent treatment *per se* does not cause any effect. However, the preparation of system I from grana is contaminated with detergent solubilized chlorophylls: the first peak is shifted to 682.5 nm (Fig. 2), the ratio F682.5/F735 is very high (2.9), and the ratio of the quantum yield of fluorescence of F1G to that of F1GI is about 2. Removal of detergent solubilized chlorophyll leads to the reduction

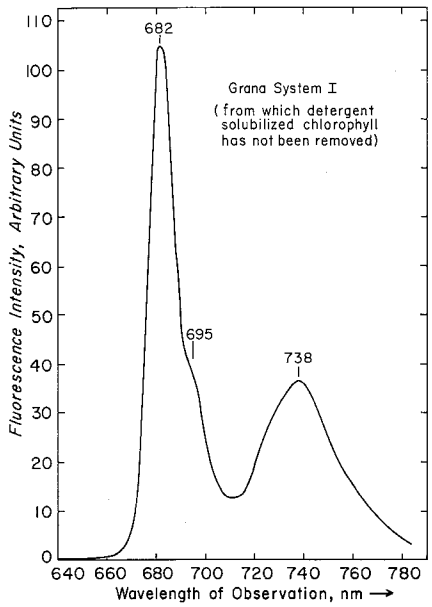

Fig. 2: Emission spectra of system I from grana from which solubilized chlorophyll has not been removed. Other conditions as in Fig. 1.

of the ratio to 0.32. Thus, we must be careful of analysis of particles in which detergent solubilized chlorophylls are not removed from system I (prepared after detergent treatment). We speculate that this may be one of the reasons why OGAWA and VERNON (1969) observed a high F685/F735 in their system I preparations. The explanation given by these authors that this is due to reduced energy transfer to long wavelength forms of Chl *a* may have to be modified.

3. Degree of Polarization of Fluorescence

The polarization (p) of Chl fluorescence, as excited by 632.8 nm, is 3.3 ± 0.1 % in system I from stroma free of detergent solubilized chlorophyll, but it is only 1.6 ± 0.1 % in system I from grana (also free of detergent solubilized chlorophyll) (table 2). The p value for system II from grana is 2.0 ± 0.01 %. These data indicate

Table 2: Degree of polarization of fluorescence in the different sub chloroplasts particles (see legend of table 1). Excitation wavelength, 632.8 nm; Corning filter CS-2-64 before the measuring photomultiplier; calculations using equations in MAR and GOVINDJEE (1972).

Fractions	Polarization, %
P-10K	2.2
P-144	3.3
P-144KI	3.4
F-2G	2.0
F-1G	2.2
F-1GI	1.6
F-2D	1.9
F-1D	2.0

that either the Chl molecules are more oriented in stroma system I (supported by the finding that they have slightly more long wavelength forms of Chl *a* from absorption spectra (GASANOV and FRENCH, 1973) and from action spectra of Chl *a* fluorescence, (not shown) or that the size of the photosynthetic unit is smaller in stroma system I (as suggested by SANE et al. (1970)). The two effects are not mutually exclusive.

4. Fluorescence Induction

System II from grana showed a clear chlorophyll fluorescence transient. Systems I from both grana and stroma showed the almost absence of fluorescence induction (Fig. 3). A very small variable fluorescence was, however, observed in both the systems I; this was only a quarter of that in system II. We are not sure whether this is not partially due to a small contamination with system II or is solely a real transient associated with system I.

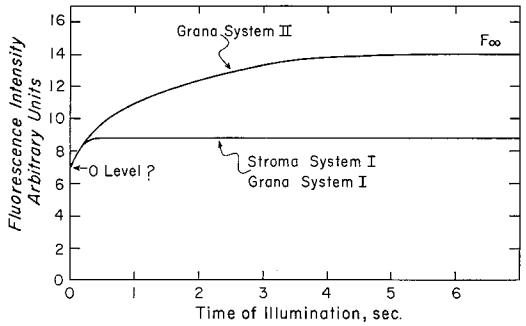

Fig. 3: Yield of chlorophyll fluorescence as a function of time of illumination in chloroplast fractions (system II from grana, system I from grana, and system I from stroma).

5. *Delayed Light Emission*

In stroma system I, the delayed light emission (DLE), 1 sec after the cessation of illumination, was about only 3 % of that in system II from grana (Fig. 4; table 3). The grana system I had equivalent (or even lower) delayed light emission – about only 1.5 % of that in system II from grana. The low intensities of DLE observed in system I do not allow us to judge with certainty whether the observed differences in intensities in system I from grana and stroma are significant. However, it is clear that DLE is not higher in system I from grana suggesting a very low contamination by system II, if any. Furthermore, the addition of 10^{-5} DCMU and 10^{-4} M NH_2OH decreased the DLE by 90 % (system I from stroma), by 80 % (system I from grana), and by 70 % (system II from grana). In view of the larger errors in system I measurements, we consider that these decreases are almost equivalent. Thus, these data would support the concept that all of the 1 sec DLE is from system II, and the small amount of DLE in system I is due to almost equal but small contamination (less than 5 %) of system II in both systems I (from grana and stroma). Such a small contamination would suggest that only a portion of variable fluorescence in system I fraction is from system I.

Fig. 5 shows a plot of $L^{-1/2}$ versus time after illumination for the various preparations, where L is the luminiscence intensity. It is clear that curve is linear in

Table 3: Maximal yield of delayed light emission from different fractions (arbitrary units).

Fractions	Control	+ 10^{-5} DCMU	+ 10^{-5} DCMU + 10^{-4} NH_2OH
P-10K	37.0	33.0	8.5
P-144K, P144KI	3.2	2.7	0.4
F-1G, F1GI	1.5	0.7	0.2
F-2G, F2GI	88.0	90.0	24.0

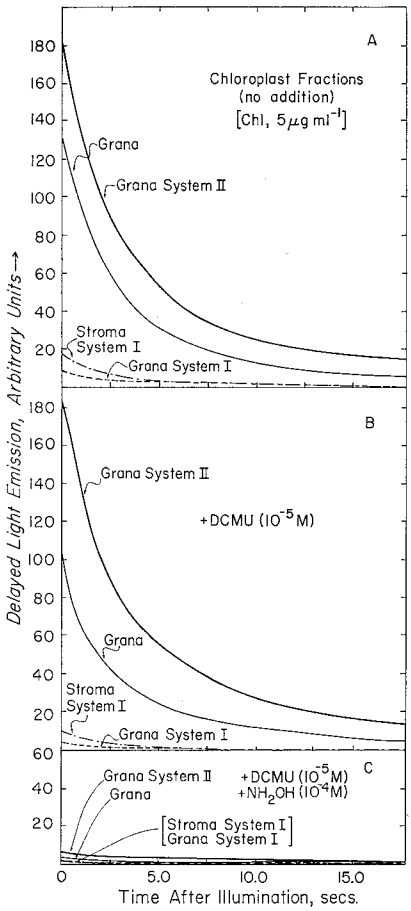

Fig. 4: Delayed light emission in chloroplast fractions (system II from grana, system I from grana, and system I from stroma) as a function of time after illumination. A: no addition, B: 10^{-5}M DCMU; C: 10^{-5}M DCMU + 10^{-4}M NH_2OH.

almost all cases confirming the second order character of luminiscence at room temperature in the 1–6 sec range (see JURSINIC and GOVINDJEE, 1972, for results on DCMU-poisoned chlorella cells). From the slopes of the L-½ versus time curves, one can calculate the apparent rate constants of the reaction leading to luminiscence. Clearly, these rate constants are 3–5 fold higher for system I (from both grana and stroma) than for system II fractions (table 4). This might indicate that one is dealing with, at least, two components of delayed light emission. Further work is needed to analyse these data as has been recently done by ITOH and MURATA (1973).

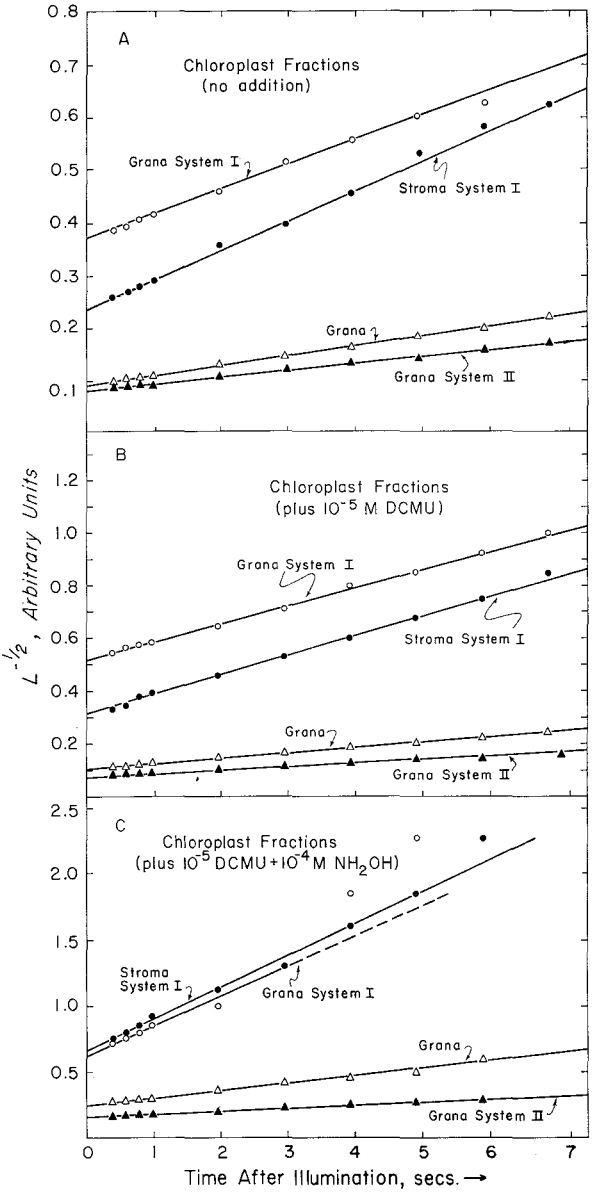

Fig. 5: $L^{-1/2}$ versus time after illumination. L = luminescence intensity; A: no addition, B: 10^{-5}M DCMU; C: 10^{-5} DCMU + 10^{-4}M NH_2OH.

Table 4: Slopes of $L^{-1/2}$ versus time curves (in $sec.^{-1}$).

	Control	+ 10^{-5} M DCMU	+ 10^{-5} M DCMU + 10^{-4} M NH_2OH
Grana	0.016	0.016	0.042
Grana System II	0.011	0.011	0.012
Grana System I	0.038	0.054	0.38
Stroma System I	0.045	0.058	0.20

[Chl], 5 μg m^{-1} suspension.

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