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ON THE ORIGIN OF GLOW PEAKS IN *EUGLENA* CELLS, SPINACH CHLOROPLASTS AND SUBCHLOROPLAST FRAGMENTS ENRICHED IN SYSTEM I OR II

P. V. SANE, T. S. DESAI, V. G. TATAKE and GOVINDJEE*

Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay 400 085, India

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Abstract—The origin of glow peaks (thermoluminescence) was investigated in isolated spinach chloroplasts and *Euglena* cells by pretreatment with various concentrations of 3-(3,4 dichlorophenyl)-1,1-dimethylurea (DCMU)†, different light intensities, and after mild heating at various temperatures. Experiments are also reported on subchloroplast fractions enriched in pigment systems I (PSI) or II (PSII) (prepared under conditions to reduce destruction of membranes by excessive detergent contact).

These results provide the following, most likely, suggestion for the origin of glow peaks: (1) *Z* peak originates in metastable states; it is insensitive to DCMU, temperature (320–328 K), and appears only when other peaks are saturated ($10\,Wm^{-2}$). (2) Peak I involves the use of a reducing entity *A* (plastoquinone) beyond *Q* (the primary electron acceptor of pigment system II, PSII), or, of a high "*S*" state (charge accumulator) of oxygen evolving system; its intensity is dramatically reduced by low concentrations ($1\,\mu M$) of DCMU, and, there is more of it in PSII than in PSI particles. (3) Peak II is due to reaction of Q^- with the "*S*" states of the oxygen evolving system; its intensity increases upon the addition of low concentrations of DCMU, at the expense of peak I; it is most sensitive to mild heating, and there is more of it in PSII than in PSI particles. (4) Peak III was not studied here as it was not resolved in most of our preparations. (5) Peak IV is from both pigment system I and II; it is sensitive to heating (>50°C), is somewhat sensitive to DCMU, and is present in both PSI and PSII particles. (6) Peak V is from PSI; it is least sensitive to mild heating, and it is enriched in PSI particles.

The present studies have extended our knowledge regarding the origin of glow peaks in spinach chloroplasts and *Euglena* cells; in particular, the involvement of the charge accumulating "*S*" states of oxygen evolution (for peaks I and II) and of system I (for peak V) are emphasized in this paper.

INTRODUCTION

Delayed light emission from photosynthetic organisms was discovered by Strehler and Arnold (1951) and thermoluminescence (TL) by Arnold and Sherwood (1957). Although delayed light emission has been studied extensively (see Fleischman and Mayne, 1973; Lavorel, 1975), TL has not been thoroughly investigated. Arnold and Azzi (1968, 1971), Rubin and Venedictov (1969), and Shuvalov and Litvin (1969) were able to resolve the different TL bands observed in plant and algal cells to some extent. Using 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU), an electron transport inhibitor, they tried to explain the origin of TL bands; only 303 K peak survived DCMU treatment. Arnold and Azzi also heat-treated their samples and used mutants lacking pigment system (PS) I or II (PSII). They could not observe 267, 303 and 325 K peaks in the mutant lacking PSII, whereas regular samples and PSI-lacking mutant contained all three peaks. More recently, Sane *et al.* (1974), Desai *et al.* (1975), Lurie and Bertsch (1974a, b) and Ichikawa *et al.* (1975) studied the glow curves (TL bands) of leaves, algal cells and chloroplasts in greater detail by using several inhibitors of photosynthetic electron transfer, preferential excitation of one of the two photosystems and other treatments with known effects on electron transport. A different approach to the study of thermoluminescence was employed by Mar and Govindjee (1971) and Jursininc and Govindjee (1972). They studied light emission from chloroplasts and algal cells by temperature jump (also see Malkin and Hardt, 1973). With the exception of Shuvalov and Litvin (1969), Desai *et al.* (1975) and Sane (1975) who attributed one or more glow peaks to pigment system I, other workers have suggested that all the TL bands originate only in pigment system II (PSII).

In this communication, we report our studies on the different glow peaks and provide experiments with subchloroplasts preparations enriched in pigment system I and II (prepared under conditions so as to reduce destruction of membranes by excessive detergent contact), and on chloroplasts and *Euglena* cells under various pretreatments (light intensity, mild

*On sabbatical leave from the University of Illinois at Champaign-Urbana; reprint requests should be sent to Govindjee, Department of Botany, 289 Morrill Hall, University of Illinois, Urbana, IL 61801, U.S.A.

†Abbreviations used: Chl: chlorophyll; DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP: 2,6-dichlorophenol indophenol; PSI: photosystem I; PSII: photosystem II, TL: thermoluminescence.

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heating, and DCMU addition). These results allow us to provide new information on the origin of various glow peaks (see Abstract).

MATERIALS AND METHODS

C-type chloroplasts (see Hall, 1972) were isolated from spinach leaves according to Sane *et al.* (1970), and were resuspended in 0.05 *M* potassium phosphate buffer (pH 7.8) containing 0.01 *M* KCl.

Fractionation of chloroplasts into PS II and PS I enriched samples was accomplished by using Anderson and Boardman's procedure (1966) in a modified way to obtain a PS I enriched fraction sedimenting at 40,000 × *g*. The chlorophyll-to-digitonin ratio used was 1:6.5. The contact time of digitonin to thylakoid membranes was reduced to 1.5 h from 2.5 h and 0.4 *M* sucrose was used to protect the membranes from damage. After incubation with digitonin for 30 min, the chloroplast suspension was immediately centrifuged for 10 min at 1000 × *g*. The supernatant was recentrifuged for 30 min at 10,000 × *g* to yield system II enriched pellet, and system I enriched supernatant. The latter was centrifuged at 40,000 × *g* for 20 min to yield system I enriched pellet. The relative enrichment of system I and II was judged by the Chl *a*/Chl *b* ratio, fluorescence spectra at 77 K, and system I and II activities in fractions prepared under identical conditions.

0.5 and 0.8% digitonin and 0.1 and 0.15% Triton were used in experiments involving the effects of detergents on chloroplast thermoluminescence.

Chlorophyll (Chl) which ranged from 0.25 to 0.9 mg per 0.5 mℓ of suspension was determined according to Arnon (1949).

Studies on the effect of different light intensity on the glow peaks were carried out by using a 500 W tungsten lamp placed at different distances from the sample. The incident energies on the sample were measured by a precalibrated photocell. For studying the effect of pre-heating on TL, the *Euglena* cell suspension (in a thin wall test tube) was kept for 5 min at room temperature, immersed in a Dewar flask containing water of appropriate temperature for 2 min in the dark, then allowed to cool down to room temperature in the dark and used subsequently for TL studies.

The stainless steel planchet containing the sample of the photosynthetic material was illuminated for 1 min with a tungsten (microscope) lamp while being cooled to 77 K (illumination intensity, 10 Wm^{-2}). The planchet containing the illuminated sample was transferred to the planchet holder of the cryostat (Tatake *et al.*, 1971) maintained at 77 K. Glow peaks were measured by the equipment and procedure described by Desai *et al.* (1975). For best resolution of peaks, the rate of heating was 10 K per min.

RESULTS AND DISCUSSION

In the following description, we have adopted the designation given by Desai *et al.* (1975) for the TL peaks observed in leaves. The relative proportions of different peaks, as calculated by areas under particular peaks, are different in chloroplasts (Fig. 1, Table 1) as compared to leaves (Desai *et al.*, 1975). Peak II at 261 K is practically absent in chloroplasts, but is quite prominent in leaves. On the other hand, peak I, with a maximum at 236 K, is quite prominent in chloroplasts, but is very small in leaves (Desai *et al.*, 1975). Peak III (maximum, 283 K) is not well resolved in chloroplasts but peak IV (maximum 298 K) is relatively large.

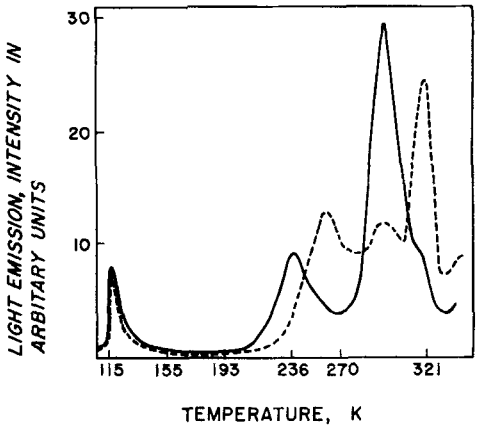

Figure 1. Glow curves of spinach chloroplasts. Control (——) and in the presence of 0.1 m*M* DCMU (– – –).

It is generally known that chloroplasts in intact leaves have a higher efficiency of electron transport than is observed in isolated broken chloroplasts; this is due to many causes, including the loss of some component between *Q* and P700 (the reaction center chlorophyll of PS I) or to loss of ferrodoxin. (For a review on electron transport chain and on the use of artificial electron acceptors and donors, see Avron, 1975). The rates of PS II reaction, as monitored by 2,6 dichlorophenol indophenol (DCPIP) reduction in chloroplasts isolated by our procedure, were ~120 μmol DCPIP reduced/mg chl. h. At the same time, PS I rates, monitored as oxygen uptake with methylviologen (MV) as the electron acceptor and $DCPIPH_2$(DCPIP + ascorbate) as the donor in the presence of DCMU, were ~100 μmol of oxygen taken up/mg chl. h. However, the rates of electron flow from H_2O to $NADP^+$ (in the presence of added ferredoxin and reductase) were much lower (30–40%). It is, therefore, likely that a possible breakage of link between *Q* and P700 in our chloroplast preparations could permit accumulation of reducing equivalents in plastoquinone (*A*), which in the leaf is kept efficiently oxidized by PS I. Thus, if peak I involves back reaction around PS II involving A^-, this peak would be

Table 1. Effect of different concentrations of DCMU on the intensity of glow peaks from spinach chloroplasts

	DCMU concentration, *M**				
Peak	Control	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Z	46	42	42	42	42
I	182	92	60	—	—
II	absent	32	60	312	288
IV	422	404	400	180	184
V	88	108	128	240	280
Total luminescence	738	678	690	774	794

*In all the experiments 0.45 mℓ of chloroplasts containing 900 μg chlorophyll were used. To this 0.05 mℓ of the required concentration of DCMU solution was added to obtain various final concentrations of this inhibitor.

expected to predominate in chloroplasts. This seems possible because in DCMU-treated chloroplasts (where reduction of A by Q^- is blocked), peak II appears at the cost of peak I (see next section).

Effect of different concentrations of DCMU

Different concentrations of DCMU were used to test how the reducing side of photosystem II is involved in glow peaks. A DCMU solution was initially made up to 0.1 M in 100% alcohol, and this was further diluted as necessary for addition to the chloroplast suspensions. Alcohol concentrations in the 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} M DCMU solutions were 0.01, 0.1, 1 and 10%, respectively. We observed that 1% or less of alcohol had no effect on photochemical activities and on glow peaks; data for [DCMU] > 10^{-3} M have been omitted because of alcohol effects.

Table 1 and Fig. 1 summarize the effects of different concentrations of DCMU on the glow peaks of spinach chloroplasts. Addition of DCMU to a final concentration of 1 μM reduces the intensity of peak I and causes an appearance of peak II. Increasing the concentration of DCMU to 10 μM further increases peak II with an equivalent decrease in peak I. At this concentration, peak V (maximum, 321 K) is also substantially increased accompanied by a decrease in peak IV. At 0.1 mM DCMU (as well as at 1 mM) peak I is completely lost, peaks II and V are dominant, and peak IV is substantially reduced (43% of control; Fig. 1).

The total luminescence (see last horizontal row of Table 1) at concentrations of DCMU ranging from 1 μM and 1 mM is not very different from that in the control. This indicates that the decrease and increase in different peak intensities, induced by DCMU, is at the cost of each other. This could be interpreted to mean that the different peaks observed during TL arise from the same sources produced during freezing in light, and that the different peaks represent various routes through which back reactions of reduced acceptors with oxidized donors take place. At lower DCMU concentrations (up to 10 μM) the yields of peaks I + II and IV + V remain almost constant, indicating the possibility that peaks I and II are related to each other just as peaks IV and V are. However, at 1 and 10 μM DCMU, a much more dramatic effect on the ratio of peaks I and II, than on peaks IV and V, is observed. At higher DCMU concentrations (0.1 and 1 mM) the total yields of I + II and IV + V are different. The ratio of yield in peaks I + II to that in peaks IV + V is 0.23 for 1 mM DCMU, but increases to 0.74 for 0.1 mM DCMU.

It has been demonstrated earlier (Etienne, 1974) that only at very high concentrations, DCMU interferes directly with the "S" charge accumulator states involved in O_2 evolution. (For a review on "S" states, see Joliot and Kok, 1975.) Since lower concentrations decrease peak I, it could be concluded that for the appearance of this peak reduction of the plastoquinone pool (A) by Q^- is essential, because DCMU is known to block electron flow from Q^- to A (Duysens, 1972). Peak I therefore may represent the reaction of some reduced intermediate beyond Q with oxidized donors of PS II (S states). The increase in peak II at these low concentrations of DCMU may then indicate that it is due to recombination of Q^- with the oxidized donors of PS II. Furthermore, in the presence of DCMU, only one turnover of reaction center II is allowed—preventing the formation of S_3 and S_4 states of oxygen evolving system; peak I, therefore may alternatively be due to the recombination of Q^- with S_3. Relatively higher amounts of DCMU (0.1 mM) are needed to drastically decrease peak IV. Thus, this could also involve the "S" states, but considering the high amount of chlorophyll used in the experiment, the ratio of DCMU/Chl was not high enough for this effect of DCMU on "S" states to take place. (Perhaps due to some direct effects of DCMU on system I, this peak was affected.)

Effect of heat treatment on the glow curves

Heat treatment was used to identify the involvement of "S" states in glow peaks as mild heating is known to destroy these states. For these studies *Euglena* cells were used. Table 2 shows the effect of heating the cells (to different temperatures) on the glow

Table 2. Effect of preheating *Euglena* cells to different temperatures on the intensity of different glow peaks

		Temperature (°C)				
Peak	Control*	47	49	50	52	55
Z	22	19 (86%)	21 (96%)	19 (86%)	21 (96%)	17 (77%)
I	←		Not detectable		→	6
II	149	85 (55%)	80 (53%)	78 (52%)	46 (31%)	10 (7%)
III	124	120	– – – – – – – – merged with IV – – – – – – – –			
IV	302	290 (96%)	196 (64%)	120 (39%)	65 (21%)	40 (13%)
V	54	59 (109%)	41 (76%)	34 (63%)	32 (59%)	16 (30%)
Total luminescence	651	570 (88%)	338 (52%)	251 (39%)	164 (25%)	89 (14%)

*Percent of control are given in parentheses.

peaks. The data for temperatures between 22 and 45°C are not presented since there is little effect on the yield of different peaks in this temperature range. Peak II is most sensitive to heating, since its intensity was reduced to about 50% in a sample heated to 47°C; this treatment did not change the intensity of other peaks. Thus, this peak involves "*S*" states. Heating the cells to 49 or 50°C considerably reduced the peak IV intensity. Peak III is not resolved from peak IV in samples heated to these temperatures, and its intensity is also very much reduced. Heating the cells to 52°C further reduces the intensity of both peak II and IV. Thus, we cannot yet exclude the participation of "*S*" states for peak IV, although greater sensitivity of peak II, as compared to peak IV (noted above), to mild heating suggests that peak II involves "*S*" states; peak IV may be from both PS I and PS II (see below). Peak V is much more stable to heating. These data do not prove, by themselves, that peak V is from PS I, since it could be from a recombination of Z^+ (or $P680^+$) (P680 and *Z* are the primary and secondary electron donors of PS II.) and Q^-; its intensity increased with DCMU addition.

Comparing the yields of different peaks in cells heated to 52°C with the control, peak II is reduced to 31%, peak IV to 22%, whereas peak V is reduced to only 50%. Emission intensity after heating at 55°C is 7% for peak II, 13% for peak IV and 30% for peak V. The total luminescence (last row of Table 2) decreases with increased heating; for a sample heated to 55°C it is about 15%. The above data clearly demonstrate that peak II is most sensitive to heating whereas peak V is most resistant. These observations are consistent with the conclusion that peak II originates in PS II involving "*S*" states, and peak V does not.

The *Z* peak remains unaffected by heating. This result confirms our earlier observations (Sane *et al.*, 1974). (See next section for further discussion of *Z* peak.)

Dependence on intensity

The studies on the effect of different light intensities during freezing on the glow curves were carried out using *Euglena* cells, chloroplasts and leaf samples. In all cases the trends observed were similar. Figure 2 shows the behavior of different peaks when different light intensities were used during freezing of *Euglena* cells. The yields of peaks II, IV and V increase with light intensity and saturate. Peak V, however, saturates at relatively lower intensities. Saturation of all except the *Z* peak (discussed below) was obtained at $\sim 5\,Wm^{-2}$. At the lowest intensities all the peaks, except the *Z* peak, are observed. At low intensities, the peaks II and V are of almost equal intensity, but peak IV is prominent. At high intensities, peak II is about twofold higher than peak V. If one examines the contribution of each peak to the total luminescence (Table 3), it is seen that at the lowest intensities used, peak II contributes approximately 5% but at

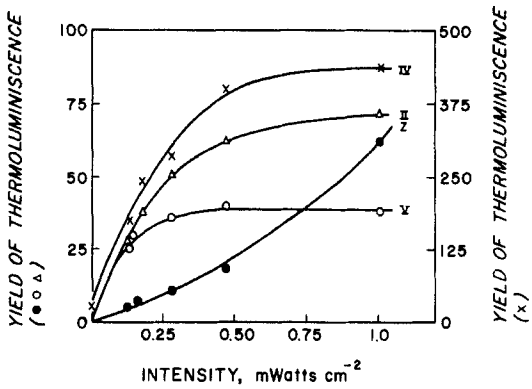

Figure 2. Effect of different light intensities on the yield of different glow peaks (thermoluminescence, TL) in *Euglena* cells. The amplifier gain for *Z* peak (●) was ten times that of peaks II (△) and V (○). The zero on the intensity axis represents values for a sample relaxed in the dark for 5 min.

$1.4\,Wm^{-2}$ and up, its contribution remains constant between 10–14%. The contribution by peak IV at all light intensities remains around 75–80%. Peak V at the lowest intensities accounts for about 20% of the total but at all other intensities its contribution again remains low and constant (around 8–12%). These data indicate that the contribution by different glow peaks to the total luminescence is not very different at different light intensities.

Shuvalov and Litvin (1969) proposed that their component IV originates in PSI, since this component was excited by 700 nm light. This conclusion was questioned by Lurie and Bertsch (1974a) on the grounds that component IV saturates at much lower intensities than other components. Desai *et al.* (1975) have also attributed peaks IV and V to PS I since both these peaks could be excited by far red light. The data presented above show that the saturating intensities for different peaks are not very different, and at any rate even at very low light intensities all the peaks are present. Thus, association of peak IV and V with PS I cannot be ruled out on the basis of light saturation curves for different peaks. It is likely that far red light, which is poorly absorbed,

Table 3. Percent contribution by different glow peaks to the total luminescence from *Euglena* cells frozen in presence of light of different intensities

	Intensity in Wm^{-2}							
Peak	0.3	1.3	1.4	1.6	1.8	2.9	4.7	10.3
Z	0.0	0.2	0.2	0.2	0.2	0.3	0.3	1.2
II	5.0	11.7	11.9	12.7	9.8	13.7	11.6	14.0
IV	74.1	77.7	76.3	74.9	80.1	76.3	80.6	77.3
V	20.9	10.4	11.6	12.2	9.9	9.7	7.5	7.5
Total luminescence*	167.4	239.5	259.5	307.1	389.7	372.5	535.8	512.6

*Total luminescence was calculated by adding areas under all the glow peaks.

would give us low light intensity results, i.e. high IV peak and about equal II and V peaks.

The "*Z*" peak is very small at low intensities but starts increasing when the other peaks are saturated (Fig. 2). At higher intensities, when the light saturates photosynthetic reactions, excess energy may be diverted to the metastable states of chlorophylls or pheophytins leading to the possibility that the *Z* peak is from metastable state.

The studies of Ichikawa *et al.* (1975) have emphasized that the *Z* peak, designated as *Zv*, occurs at different temperatures depending upon the temperature of illumination. Our studies (data not shown) confirm these observations. An explanation based on the temperature of illumination, given by Tatake *et al.* (1976) for another system, seems plausible for the *Zv* peak. Excitation and emission spectra (data not shown) suggest that this peak originates in chlorophylls although pheophytins cannot be ruled out. The metastable states mentioned above may produce the *Z* peak either by direct decay to ground state, or via singlet state. Phosphorescence data of Krasnovsky *et al.* (1975) show a peak at ~960 nm, but the emission peak for the *Z* peak is at 740 nm (Sane *et al.*, 1974). This would favor the latter hypothesis. It is also possible that emission at 740 nm is due to phosphorescence from pheophytins, not from chlorophyll *a*. The complexity of the *Z* peak is further emphasized by the appearance of a peak, labelled *Zn*, in photosynthetic bacteria which is suggested to originate in Mg-protoporphyrin IX (Govindjee *et al.*, 1976).

Luminescence from PS II *and* PS I *enriched chloroplast fractions obtained by digitonin treatment*

Table 4 shows the yield of different peaks obtained in D-0 (chloroplast suspension treated with digitonin), D-10 and D-40 fractions (samples obtained by centrifugation at 10,000 × *g*, and 40,000 × *g*, respectively). We chose the D-40 fraction rather than D-144 as a PS I enriched fraction for the following reasons: (a) the yield in terms of chlorophylls of D-40 fraction is higher; (b) D-40 fraction obtained by the modified procedure of Anderson and Boardman (see Materials and Methods) is quite enriched in PS I relative to PS II (see below); (c) in case of the D-144 fraction, the digitonin stays too long with the lamellar fragments causing damage to the PS I fraction. This, we have observed, destroys the luminescence properties (see below). (In our fractions D-10 and D-40, solubilized chlorophylls are not present, since they are removed when the supernatant is decanted.)

The enrichment of D-40 and D-10 in system I and system II, respectively, was evident from (a) a higher ratio (4.3) of Chl *a*/Chl *b* in the former than in the latter (2.3); (b) a lower ratio (1.04) of F695/F730 (where F695 and F730 are fluorescence intensities at 77 K) in D-40 than in D-10 (2.36) particle; and (c) an approximately two fold higher activity of system I reaction (electron flow from $DCPIPH_2$ to methylviologen) in D-40 than in D-10.

Table 4. Percent contribution by different TL peaks to the total luminescence in PS I and PS II enriched fractions from spinach chloroplasts

	Fraction		
Peak	D-0	D-10	D-40
I + II	11.7	12.2	3.2
IV	14.6	32.7	23.8
V	73.7	55.1	73.0
Total luminescence	171	156	63

Table 4 shows that D-0 and D-10 are more or less similar with the exception that D-10 has (relatively) a higher peak IV and a lower peak V. This suggests that peak IV is from PS II and peak V from PS I. In D-0 and D-10, peaks I and II contribute about 12%; this is reduced to only 3% in D-40. As compared to D-10, D-40, which is a PS I enriched fraction, shows a very much reduced total luminescence as expected. Peak V seems to account for practically all (73%) the luminescence observed in D-40 fraction; this is reduced to 55% in D-10.

The negligible yield of peak I and II (3%) in a PS I enriched fraction and the fact that practially all the luminescence originates in peak V along with observations noted above indicate that peak I and II may be associated with PS II whereas peak V may be associated with PS I. Again, the source of peak IV is not clear; it could originate from either PS I or PS II or, most likely, both.

In what follows, we suggest possible reasons why Lurie and Bertsch (1974a) were unable to see any luminescence from PS I enriched fraction. First, we have observed that incubation of chloroplasts with detergent for a longer time or with a higher detergent-to-chlorophyll ratio has an adverse effect on the luminescence, as discussed below. Note that in our experiments we had obtained a PS I-enriched fraction (Chl $a/b = 4.7$) quickly by manipulating the fractionation procedure such that extensive damage to PS I by the detergent was minimized. Furthermore, the presence of 0.5 *M* sucrose, in our preparation, minimizes the destructive effect of digitonin (see Hauska and Sane, 1972). Secondly, the chlorophyll concentrations used in our study were an order of magnitude higher than those used by Lurie and Bertsch (1974a).

Neither Ichikawa *et al.* (1975) nor Lurie and Bertsch (1974) presented data on the unfractionated chloroplast suspension incubated with the detergent. The detergents, in addition to separating grana from stroma lamellae solubilize several of the membrane components (see Park and Sane, 1971). The lipids of the membrane are replaced by the detergent molecules and, additionally, have effects on the photochemical activities. Since the TL phenomena are dependent upon an active electron transport and an organized membrane structure, treatment with detergents would be expected to change the TL character-

Table 5. Effect of different concentrations of digitonin and triton on the TL peaks of spinach chloroplasts

Treatment*	Peaks (intensity as a percent of control) I + II	IV + V	Total luminescence
Control	100	100	100
0.5% digitonin	144	69	79
0.8% digitonin	70	19	29
0.1% Triton	78	78	78
0.15% Triton	63	26	33

*The digitonin to chlorophyll ratio in case of 0.5% digitonin and 0.8% digitonin was 6.5:1 and 13:1, respectively. In case of triton the detergent to chlorophyll ratio for 0.1 and 0.15% triton was 1.3:1 and 2.6:1, respectively.

istics of the chloroplasts. In Table 5 we provide data for the effect of digitonin and Triton on the TL peaks at two different detergent-to-chlorophyll ratios. When the detergent-to-chlorophyll ratio was increased to two-fold from a ratio which had reduced the total luminescence yield to about 80%, the yield went down to 30% in the case of both detergents. An interesting feature of the effect of increased detergent concentration was its differential effect on the peak intensity of I + II as compared to IV + V. At higher concentrations of digitonin, the yield of I + II declined by only 30%, whereas the decrease in luminescence intensity for the peaks IV + V was as high as 80%. A similar effect was observed in the case of higher Triton concentration. The effect observed for higher detergent concentrations can also be observed if the chloroplasts are in contact with a lower concentration of the detergent but for a longer time (data not shown). The preferential destructive effect of detergent on peaks associated with PS I (i.e. IV and V) was expected since Wehrmeyer (1962) had demonstrated, by electron microscopy, that detergents affect stroma lamellae first and then grana. We know from the work of Sane *et al.* (1970) that PS II is restricted to grana membranes in grana-containing chloroplasts. Since the PS I fraction arises from stroma lamellae solubilized by the detergents (Park and Sane, 1971), it is expected that its luminescence properties (also see Gasanov and Govindjee, 1974) will be affected more. On this basis, we argue that the inability of Lurie and Bertsch (1974a) to observe any luminescence from a PS I fraction could be due to excessive damage to PS I TL by the detergent.

Data of Arnold and Azzi (1968) on mutants of the alga *Scenedesmus* seem also to contradict our conclusion that a system I glow peak could be observed. These authors reported the presence of only three glow peaks in Bishop's mutant No. 8 (inactive in system I) and none in mutant No. 11 (inactive in system II). We consider it likely that in these early measurements, the detection of glow peaks may not have been optimum, especially because of high heating rates (3°C/s) used. Arnold and Azzi reported the disappearance of their peaks *A* and *C* by DCMU addition. Their peak C appears to be equivalent to our peak IV. Thus, we believe Arnold and Azzi did not look at a peak equivalent to our peak V in their mutant work. It is, therefore, necessary that glow peaks in the above-mentioned mutants be measured under our experimental conditions before this work is taken to contradict our conclusions.

Another question that arises from the data obtained thus far is: if peaks IV and V are associated with PS I, how could the luminescence from these two peaks be more than from those peaks associated with PS II, since most of the delayed light emission is assumed to originate in PS II? First, a larger portion of peak IV may originate from PS II. Second, we would expect a significant amount of TL associated with PS I under our experimental conditions. The TL associated with PS I occurs at 321 K (48°C). Most delayed light emission and fluorescence measurements which suggest that light emission is mainly from PS II have been done at room temperature. Preliminary fluorescence and delayed light emission measurements in our laboratories indeed show that the ratio of PS I (710 nm) to PS II (685 nm) emission (fluorescence and delayed light emission) is high at higher temperatures (e.g. 320 K) (data to be published). We do not know why, however, PS I emission is high at very low (77 K) and at very high temperatures.

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