

## FLUORESCENCE AND DELAYED LIGHT EMISSION IN TRIS-WASHED CHLOROPLASTS

Prasanna MOHANTY, Barbara Zilinskas BRAUN and GOVINDJEE

*Department of Botany, University of Illinois, Urbana, Illinois 61801, USA*

Received 19 November 1971

Revised version received 12 December 1971

### 1. Introduction

Yamashita and Butler [1] have shown that extraction of chloroplasts with a high concentration of Tris (0.8 M, pH 8.0) eliminates flow of electrons from water to nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>)\* or other Hill oxidants. They suggest that washing with Tris causes a block on the water side of the electron transport chain. Addition of electron donors such as reduced phenylene diamine (PDA) restores the electron flow to NADP<sup>+</sup>. This donation of electrons by PDA is sensitive to the herbicide diuron (DCMU), a well known inhibitor of photosystem II (PS II) reactions. Measurements of chlorophyll *a* (Chl) fluorescence in Tris-washed chloroplasts (without any added donor) show a very low level of fluorescence, but the addition of DCMU increases the yield of fluorescence [1]. One could suggest that the low yield of Chl fluorescence in Tris-washed chloroplasts is due to "trickling" of electron flow from some endogenous donor (cf. [1]). However, if this were so, we should expect the fluorescence yield to slowly rise and reach the maximum yield at longer times. But, this never happens. No satisfactory explanation was available for this peculiar behavior of fluorescence

yield in Tris-washed chloroplasts. In this communication, we report results of experiments designed to evaluate the cause of the low fluorescence yield in Tris-washed chloroplasts. We have considered three possible hypotheses (i) an increased cyclic flow of electrons from Q<sup>-</sup> (the reduced primary electron acceptor of photosystem II, PS II) to Z<sup>+</sup> (the oxidized primary electron donor of PS II), (ii) a larger spill-over of excitation energy from PS II to PS I, and (iii) the donor Z and the acceptor Q remain simultaneously in the oxidized state. Our results favor the last hypothesis. Our results also support the earlier suggestion that DCMU inhibits the flow of electrons on the Q side of photosystem II, Q being the primary electron acceptor of this system [2].

### 2. Experimental methods

Chloroplasts were prepared from market spinach. Tris extraction was made according to Yamashita and Butler [1]. The method for measuring Chl fluorescence was essentially that of Munday and Govindjee [3] and for the delayed light emission (DLE) that of Jursinic and Govindjee [4]. For measurements of the recovery of fluorescence transients, a dark period of 2 min was interposed. Hill activity was assayed by measuring the photoreduction of dichlorophenol indophenol (DCPIP) in saturating light; Chl concentration was determined according to Arnon [5]. Other details are given in the legends of the tables.

#### \* Abbreviations:

A: intersystem intermediate; Chl: chlorophyll; DCMU, Diuron: 3-(3,4-dichlorophenyl)-1,1 dimethylurea; DCPIP: dichlorophenol indophenol; DLE: delayed light emission; NADP<sup>+</sup>: nicotinamide adenine dinucleotide phosphate; PDA: phenylene diamine; Q: primary electron acceptor of system II; TMPD: N, N, N', N' - tetramethyl-p-phenylenediamine; Z: primary electron donor of system II.

Table 1

Relative changes of fluorescence yield (final levels) upon the addition of DCMU in dark and in light, and also upon the addition of 5 mM MgCl<sub>2</sub>.

Treatment	Normal chloroplasts	Tris-washed chloroplasts
I. None	1.04 ± 0.04	0.65 ± 0.05
10 μM DCMU added in the light	1.26 ± 0.05	0.80 ± 0.04
10 μM DCMU added in the dark	1.30 ± 0.05	1.25 ± 0.05
II. None	0.96 ± 0.04	0.70 ± 0.04
5 mM MgCl <sub>2</sub>	1.82 ± 0.06	1.20 ± 0.05

Three ml samples containing 45 μg of Chl in 0.05 M Tris-HCl buffer pH 7.8. Fluorescence yield was measured at 685 nm. The sample was excited with blue light (Corning filters: C.S. 4-72 and C.S. 3-73). The intensity of exciting light was ~ 10 K ergs cm<sup>-2</sup> sec<sup>-1</sup>. 10 μM DCMU, when added in light, was injected from a syringe after 3 min of illumination. 5 mM MgCl<sub>2</sub> was added in the dark. (For this experiment, Tris-buffer was 0.005 M). Fluorescence yield values given here represent the average of 5 measurements.

### 3. Results and discussion

In the absence of DCMU, the yield of fluorescence in Tris-washed chloroplasts is very low and is close to the level f<sub>0</sub> (see table 1 and [1]). Addition of DCMU in dark enhances the fluorescence yield to a high level. In normal chloroplasts without DCMU, the maximum fluorescence yield is the same as that in chloroplasts treated with 10 μM DCMU. In the latter case, however, there is a fast rise to a maximum level (see [6]). We have observed that the fluorescence level in DCMU treated (in dark) Tris-washed chloroplasts is slightly lower than in the DCMU poisoned normal chloroplasts. We have not explored the cause of this difference. In our samples, Tris-washed chloroplasts could not reduce DCPIP with H<sub>2</sub>O as donor, while normal chloroplasts reduced the dye at a rate of 150 μmoles/mg Chl/hr. However, with diphenylcarbazide (DPC) as electron donor, the Tris-washed chloroplasts could reduce DCPIP at a rate of 50 μmoles/mg Chl/hr. These results confirm that our test system is the same as used by previous investigators [1]. We now consider the three possibilities mentioned in the Introduction.

Table 2

Relative intensities of delayed light emission (1 sec) in the presence and absence of DCMU.

Treatment	Normal chloroplasts	Tris-washed chloroplasts
None	70 ± 5	76 ± 5
10 μM DCMU	41 ± 5	41 ± 5
20 μM DCMU	45 ± 5	48 ± 5
10 μM DCMU + 1 mM NH <sub>2</sub> OH	0	0

Chloroplast suspension: 2 ml of samples containing 40 μg Chl in 0.05 M Tris-HCl buffer pH 7.8 was illuminated for 15 sec with blue light (Corning filter: C.S. 4-96). The intensity of the exciting light was ~ 10 K ergs cm<sup>-2</sup> sec<sup>-1</sup>. DLE was measured after ~ 1 sec of the cessation of the illumination. All additions were made in the dark. DLE values given above represent an average of 10 measurements except in the case of 20 μM DCMU where the value of 2 measurements is given.

#### 3.1. Increased cyclic flow

The low fluorescence yield in Tris-washed chloroplasts could be caused by an accelerated cyclic flow of electrons from Q<sup>-</sup> to Z<sup>+</sup>, as suggested by Rosenberg et al. [7]. This suggestion further requires that DCMU intercepts this cyclic electron flow in order to cause the high fluorescence yield seen in the presence of DCMU, with the consequent assignment of DCMU acting on the water side of photosystem II.

It has been recently suggested that the slow component of delayed light emission (DLE) originates from the back recombination reaction between Z<sup>+</sup> and Q<sup>-</sup> [8–10]. If Tris washing were to induce a strong back recombination between Z<sup>+</sup> and Q<sup>-</sup> and DCMU were to inhibit this flow [7], we would expect (1) a higher intensity of DLE in Tris-washed chloroplasts than in normal and (2) complete elimination of DLE in the presence of DCMU. We conducted experiments to check the above predictions (table 2). The intensity of DLE (sec) in Tris-washed chloroplasts was found to be approximately the same as in normal chloroplasts. Addition of 10 μM DCMU considerably lowers the intensity of DLE in both cases. This lowering of DLE by DCMU has been reported in intact algal cells and in isolated chloroplasts [4, 10]. It is noteworthy, however, that in the presence of 10 μM DCMU the intensity of DLE is

approximately the same in both cases. Increasing the DCMU concentration to 20  $\mu\text{M}$  did not lower the DLE any further. However, addition of 1 mM  $\text{NH}_2\text{OH}$ , which is known to feed electrons very close to PS II, completely abolished DLE. The elimination of the slow component of DLE by the addition of both  $\text{NH}_2\text{OH}$  and DCMU has been shown to occur both in intact algal cells and in isolated chloroplasts and has been interpreted to be due to the inhibition of the back reaction between oxidized donor  $Z^+$  and reduced acceptor  $Q^-$  of PS II [9, 11, 12]. If the back recombination reaction between  $Z^+$  and  $Q^-$  is indeed the source of slow component of DLE, our results suggest that DCMU does not inhibit it. Thus, our results in table 2 indicate that Rosenberg et al.'s hypothesis [7] for the low fluorescence yield in Tris-washed chloroplasts and for the site of action of DCMU is inconsistent with our data.

We have further confirmed that DCMU does not inhibit the back flow of electrons from  $Q^-$  to  $Z^+$  in Tris-washed chloroplasts by measuring the restoration of the fluorescence transient. One would expect that the fluorescence rise from a low level to a high level could not be repeated, as in case of hydroxylamine in the presence of DCMU [11, 12], if DCMU somehow were to inhibit the back reaction between  $Q^-$  and  $Z^+$  in Tris-washed chloroplasts. Our results, however, show that the fluorescence transient could be repeated in DCMU-treated Tris-washed chloroplasts just as in case of normal chloroplasts. Artificial electron donors including  $\text{NH}_2\text{OH}$  [11, 12], and TMPD plus ascorbate [13] inhibit this dark restoration of the transient. Since low fluorescence yield does not seem to be induced by the cyclic flow of electrons from  $Q^-$  to  $Z^+$ , we considered the remaining two possibilities.

### 3.2. Massive spill-over of excitation energy from PS II to the weakly fluorescent PS I

In this case, DCMU must intercept this spill-over in order for us to explain the high yield in DCMU.

To test this hypothesis we compared the 77° K emission spectra of both normal and Tris-washed chloroplasts (see [14] for methods and interpretations). If the spill-over of energy from PS II to PS I would have been facilitated by Tris-washing, we would have seen a preferential increase of the long-wavelength fluorescence band (system I) and a de-

crease of the short wavelength band (system II) in the Tris-washed sample. This did not happen; the F730/F685 ratios were the same. Furthermore, addition of 5 mM  $\text{MgCl}_2$  to the Tris-washed chloroplasts did not increase the yield to a greater extent than in normal chloroplasts (table 1). Therefore, we believe that no significant increase in the spill-over of excitation energy from PS II by PS I is induced by Tris-extraction.

### 3.3. Donor ( $Z$ ) and acceptor ( $Q$ ) remain in the oxidized state

In this case, the Tris-washed chloroplasts, unlike the control chloroplasts, cannot reduce all of the pool of the intersystem intermediates due to the block in the supply of electrons from water.

In this proposal, one assumes that  $Q$  is initially reduced by  $Z$ , but reduced  $Q$  is reoxidized by the neighboring electron acceptors ( $A$ ) (while  $Z^+$  is not reduced as Tris washing blocks the flow of electrons from  $\text{H}_2\text{O}$  to  $Z^+$ ). This implies that in strong light both the donor ( $Z$ ) and the acceptor ( $Q$ ) remain simultaneously in the oxidized form in Tris-washed but not in normal chloroplasts. If oxidized  $Q$  quenches the fluorescence [2], the fluorescence will remain low. If this explanation is correct, one will not observe a rise in fluorescence yield if DCMU is injected during illumination, but one will expect an increase in the yield if DCMU is added in the dark. Our results (table 1) show that in the case of Tris-washed chloroplasts the yield of Chl fluorescence does not significantly increase upon the addition of 10  $\mu\text{M}$  DCMU in light. The slight increase that we observe here is low as compared to the marked increase in the yield upon the addition of the same amount of DCMU added in the dark. We do not expect an increase in the fluorescence yield in the normal chloroplasts by the addition of DCMU as the maximum yield in the presence and the absence of DCMU is the same. Thus, our data suggest that the low fluorescence yield in Tris-washed chloroplasts is simply due to a loss of ability of the donor to reduce the pool of intersystem intermediates.

### Acknowledgements

We thank Dr. Rosenberg for discussions and for

the preprint of his paper. We are grateful to National Science Foundation for continued support. B.Z.B. thanks NASA for a fellowship.

## References

- [1] T. Yamashita and W.L. Butler, *Plant Physiol.* 43 (1968) 1978.
- [2] L.N.M. Duysens and H. Sweers, in: *Studies in Microalgae and Photosynthetic Bacteria*, ed. J. Ashida (Univ. of Tokyo Press, 1963) p. 353.
- [3] J.C. Munday, Jr. and Govindjee, *Biophys. J.* 9 (1969) 1.
- [4] P. Jursinic and Govindjee, *Photochem. Photobiol.*, in press.
- [5] D.I. Arnon, *Plant. Physiol.* 24 (1949) 1.
- [6] R. Govindjee, Govindjee, J. Lavorel and J-M. Briantais, *Biochim. Biophys. Acta* 205 (1970) 361.
- [7] J. Rosenberg, S. Sahu and T. Bigat, *Biophys. J.*, in press.
- [8] J. Lavorel, *Progr. Photosyn. Res.* 2 (1969) 883.
- [9] W.T. Stacy, T. Mar, G. Swenberg and Govindjee, *Bio-physic. Soc. Abstracts* 10 (1970) 203a; *Photochem. Photobiol.* 14 (1971) 197.
- [10] T. Mar and Govindjee, *Biochim. Biophys. Acta* 226 (1971) 200.
- [11] P. Bennoun, *Biochim. Biophys. Acta* 216 (1970) 357.
- [12] P. Mohanty, T. Mar and Govindjee, *Biochim. Biophys. Acta* 253 (1971) 213.
- [13] P. Homann, *Biochim. Biophys. Acta* 245 (1971) 129.
- [14] N. Murata, *Biochim. Biophys. Acta* 189 (1969) 171.