TRIGGERED-LUMINESCENCE IN DARK ADAPTED *CHLORELLA* CELLS AND CHLOROPLASTS

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1. Introduction

Luminescence in higher plants results from the recombination of an electron-hole pair which has been separated through the photochemical functioning of the System II center and stabilized on the primary electron donor and acceptor. In principle, it should be possible, by feeding charges to the primary donor and acceptor at the expense of exogeneous redox substances, to make them recombine at the reaction center and produce luminescence. Such a 'chemiluminescence' has never been observed in higher plant cells or chloroplasts (but proved to be possible in bacterial chromatophores (see Fleischman and Mayne [1]). This probably was owing to a difficult access of exogeneous chemicals to the sites of the primary donor and acceptor. A way to naturally circumvent this difficulty occurred to us following the work of Velthuys and Amesz [2] who hypothesized that, in addition of Q, the acceptor side of System II had a secondary carrier B – seel also Bouges-Bocquet [3] – which, in the presence of DCMU, experiences a negative shift of its redox potential which brings it lower than that of Q. If some B^- (reduced) is stable in dark-adapted material, addition of DCMU should induce the reduction of Q and luminescence should be triggered by recombination of the pair carried by Q^- and positive charge on S_1, i.e.

$$S_1 Q B^- \longrightarrow S_1 Q^- B \longrightarrow S_0 Q B + h\nu$$

Incidentally, this would also indicate that S_1 is not essentially different from the other S states as a luminescence substrate (Lavorel [4]). The above scheme, with S_1 replaced by S_2 or S_3, has indeed been verified by observing a stimulation of luminescence when adding DCMU a few seconds after preillumination with one or two flashes [5].

We have been able to observe such a DCMU-triggered luminescence. Following the same reasoning, we have also found that H_2O_2 was a very active agent for chemiluminescence (presumably because of its oxidizing action on the S side of centers). It is necessary to point out at the start that, according to all known criteria, the material we use is completely relaxed and that our results in no sense duplicate the numerous observations on triggered luminescence (acid-base, temperature jump, salt, etc.) which was always recognized to require prior illumination and thus bespoke unrelaxed pools of charges of photochemical origin.

2. Methods

Algae (*Chlorella pyrenoidosa*) and chloroplasts (spinach) were handled as described previously [6,7]. Luminescence was detected with a very simple set-up: a cylindrical vessel (diameter 3.5 cm) containing 50 ml of suspension (chlorophyll approx. 50 $\mu g\ ml^{-1}$) was placed in front (at 5 cm) of a photomultiplier (EMI 9558B) with interposed red filters (Wratten 70, Corning 2–64), inside a light-tight cardboard box. The photomultiplier was cooled at –20°C and operated at 1250 V; the anode current – in the 10^{-9}–10^{-10} A range – was recorded after amplification (Lemouzy Picoampéremètre). The suspension was stirred by bub-

Abbreviations: 3-(3,4-dichlorophenyl) -1,1-dimethylurea.

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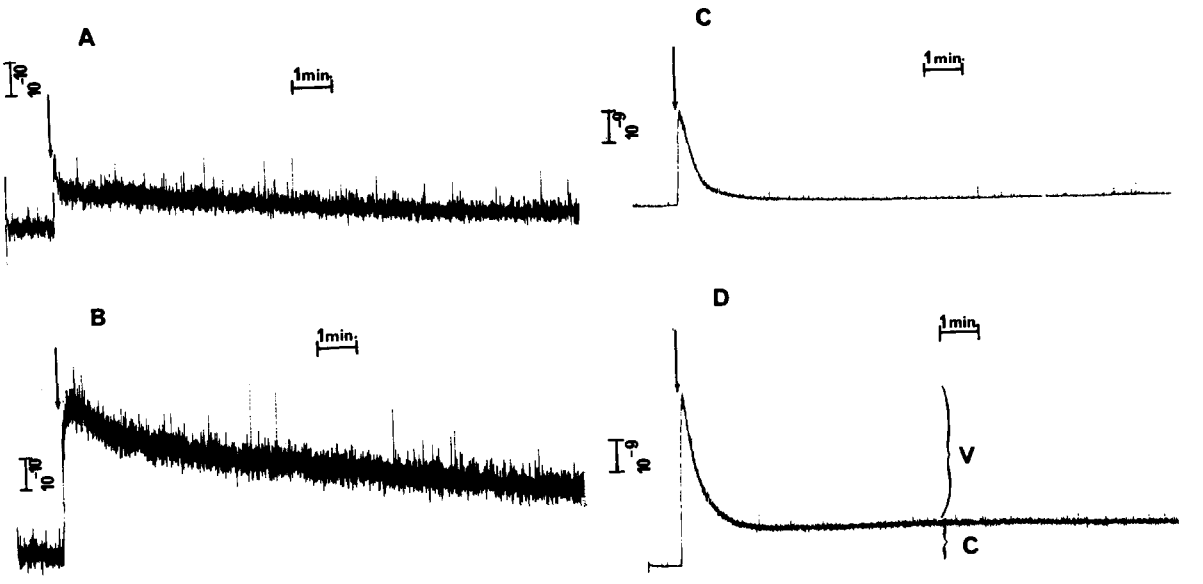

Fig.1. Luminescence intensity as a function of time after addition of either DCMU or H_2O_2 in dark-adapted *Chlorella*. (A) DCMU (final concentration 2.10^{-5}M) added at time marked by the arrow after a dark incubation of 20 min. (B) same as A, except that dark incubation (14 min) was in presence of H_2O_2 0.03%, KCN 10^{-4} M. (C) H_2O_2 (final concentration 0.06%) after a dark incubation of 20 min with KCN 10^{-4}M. (D) same as C, except that dark incubation (35 min) was in presence of DCMU 2.10^{-5}M. Note change in vertical scale (photomultiplier current, Ampere) from A, B to C, D. Chlorophyll 50 μg ml^{-1}. Room temperature (20–25°C).

bling a gas steam (10 1/hr); addition of chemicals was made manually by expelling the content of a 10 ml syringe in the vessel through a length of plastic tubing. An electronic flash (G. E. Stroboslave) with blue filter (Corning 4–96) placed opposite to the photomultiplier window with respect to the vessel center could be fired for control experiment (with water in the vessel the flash gave a negligible artifact).

3. Results and discussion

3.1. *DCMU-Triggered luminescence*

Fig.1A shows the effect of the addition of DCMU (final concentration 2.10^{-5}M) to a dark adapted (20 min) suspension of *Chlorella*. The signal is not due to the accompanying addition of ethanol (1% final concentration). It is known that within this time in darkness deactivation of the S states is complete: S_2 and S_3 which are associated with the decay of photo-induced luminescence do not survive longer than a few minutes [8]. In fact we have checked that the DCMU-triggered luminescence, expressed as the sum of light ΣL ($=\int_0^\infty L\,dt$, L = luminescence intensity) is essentially independent of the time spent in darkness in the relaxed state (Table 1). We have attempted to calibrate the emission by comparison with ΣL following a flash (in order to illuminate most of the suspension 10 flashes 1 second apart were given and the lumin-

Table 1
DCMU-triggered luminescence

Condition	ΣL	
	Chlorella	Chloroplasts
Dark time = 10 min	3.6	6
20 min	3.6	1.2
40 min	6.4	0
10 flashes (no DCMU)	1450	280
10 flashes (+ DCMU)	720	–

ΣL is the light sum emitted following addition of DCMU (final concentration 10^{-5}M). Unit is arbitrary (= 5.10^{-9} Coulomb delivered from the photomultiplier). Each line represents a different experiment with a fresh aliquot of biological material. Chlorophyll 50 μg ml^{-1} – Room temperature (20–25°C).

escence signal recorded down to the 10^{-10} A level); it is seen (Table 1) that the DCMU-triggered luminescence is only a small fraction of the normal luminescence: 3–4‰ if compared to the control without DCMU; twice as much if compared to the control with DCMU. These figures are very crude estimates.

From several lines of evidence, it has been ascertained that this emission is basically of the same origin as the normal one. It is abolished by previous heating of the suspension (5 min at 50°C) and pretreatment with low (5.10^{-5} M) and high (10^{-2} M) concentration of NH_2OH. The above treatments are known to disconnect or destroy the water splitting component of System II [9]; the effect of NH_2OH at low concentration would additionally point to a reduction of S_1 to S_0 as the explanation of the phase shift in O_2 or luminescence sequences [8,10].

H_2O_2 has been proposed as an artificial electron donor to System II [11,12]; in fact, it is also possible that it directly interacts with the S states as an oxidant ($S_i \rightarrow S_{i+1}$). We were thus led to try a DCMU-triggering after dark incubation of the algae with H_2O_2 (in the presence of 10^{-4} M KCN to prevent H_2O_2 dismutation by the endogeneous catalase). As shown on fig.1B, this treatment results in an approx. 10-fold stimulation of the DCMU-triggered luminescence.

We have found that chloroplasts are also able to emit light under the above conditions; however this ability seems to be lost after 40 min darkness (Table 1). An explanation of this different behaviour could possibly be that, contrary to *Chlorella* cells, chloroplasts are able to completely oxidize their plastoquinone pool in darkness (see Diner [13]).

3.2. *H_2O_2-Triggered luminescence*

Since the plastoquinone electron pool is not empty in dark-adapted *Chlorella* cells, as evidenced by far-red stimulation of the O_2 burst (see Joliot [14]), it was of interest to see whether this electron pool could recombine with positive charges produced by the postulated oxidizing action of H_2O_2 on the S states. fig.1C demonstrates such a H_2O_2-triggered luminescence. It is about 7 times stronger (as ΣL) than the DCMU-triggered luminescence. The following observations point to a limited pool of negative charge as the origin of this emission (Table 2, 1): the same amount of light ΣL is evolved independently of H_2O_2 concentration; furthermore following prior triggering by H_2O_2, a new addition of H_2O_2 produces almost no additional luminescence.

The origin of the electron pool thought to be responsible could possibly be clarified by subjecting the same sample to successive DCMU- and H_2O_2-triggering (fig.1D). Under this condition, it is seen that the H_2O_2-triggering evolves a burst of light similar to that without DCMU-pretreatment. There is however

Table 2
H_2O_2 – and DCMU-triggered luminescence

	Condition	Material	ΣL	$t_{1/2}$ (s)	Dark incubation time (min)
1	H_2O_2 0.03%	*Chlorella*	65		20
	H_2O_2 0.06%				
	1st addition		72		20
	+ 2nd addition		< 5		37
2	H_2O_2 0.06%	*Chlorella*	25	20	20
	DCMU 4 10^{-5} M		6		20
	+ H_2O_2 0.06%		27 (V) 460 (C; during 1 hr)	20	45
3	H_2O_2 0.06%	*Chloroplasts*	3		20
	DCMU 4 10^{-5} M		0.6		20
	+ H_2O_2 0.06%		25		46

See Table 1. Each number corresponds to experiments performed on different days. Each line is for a fresh sample, except when the + sign is shown in 'condition' meaning that the addition follows the treatment of the preceding line. V = variable. C = constant (see text).

a remarkable difference: this burst (V in fig.1D) seems to be superposed unto a constant level of emission (C in fig.1D). The later has been monitored up to 2 hr after the DCMU-H_2O_2 treatment without being exhausted; the total ΣL (V + C) is now of the same order of magnitude as the flash induced ΣL. The fact that the variable component has similar characteristics (ΣL, $t_{1/2}$, see Table 2,2) with or without prior DCMU-triggering argues against the plastoquinone electron pool being the likely explanation. The double triggering experiment (DCMU/H_2O_2 or H_2O_2/DCMU above) suggests that various configurations such as S_1QB^- and $S_0Q\,B^-$ coexist in the dark-adapted state: $S_1Q\,B^-$ would be discharged after addition of DCMU and similarly $S_0Q\,B^-$, after being transformed to S_0Q^-B by H_2O_2. Noting that the DCMU-triggered luminescence is always smaller than the H_2O_2-triggered one, we are also wondering whether the species responsible for the DCMU-triggered emission could not be $S_2Q\,B^-$ rather than $S_1Q\,B^-$ [15].

The sustained emission (C) in the DCMU/H_2O_2 experiment is difficult to explain. Heat inactivation only partly abolished it, while completely removing the variable part. We are wondering whether some catalase like action of the System II (or System I) center could not be invoked, since H_2O_2 participates in two redox equilibria of opposite character: oxidizing (E'_0 = 1.35 V) and reducing (E'_0 = 0.202 V). We have checked that dismutation of H_2O_2 by catalase alone does not evolve any light detectable in our apparatus.

Chloroplasts behaved similarly (Table 2, 3) and responded more to H_2O_2 than to DCMU-triggering. However double triggering (DCMU/H_2O_2) gave the largest amount of light with no constant, long-lasting emission (in fact, a possible corresponding aspect is found in that the decay has two distinct phases with very different half-lives).

4. Conclusion

Clearly, in the dark-adapted, completely relaxed state, the photosynthetic apparatus is found to keep positive and negative trapped charges, probably residing in the carriers close to the System II centers, which can be induced to recombine with emission of light through chlorophyll singlet states. It is not known whether these charges are the remains of anterior photosynthetic electron flow or are permanently created by some metabolic processes. The difficulty of calibrating the light emission in terms of electron-hole pairs – a notorious pitfall in the field, even for the photoinduced luminescence – does not permit so far to correlate quantitatively this emission with the concentration of centers in their various dark-adapted configurations. Further experiments are planned in view of a better characterization of this phenomenon and understanding of its significance.

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