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RELATION BETWEEN SLOW DELAYED LIGHT EMISSION AND ACID-BASE TRIGGERED LUMINESCENCE IN CHLOROPLASTS

YUICHIRO NISHIZAKI

Institute for Agricultural Research, Tohoku University, Sendai 980 (Japan)

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SUMMARY

Acid-base triggered luminescence in relation to slow delayed light emission (> 3 s) was studied in chloroplasts. After analyzing their time courses, the acid-base induced luminescence curve was found to return to the original curve of delayed light emission. Peaks of the acid-base triggered luminescence induced after various darkness periods following preillumination decreased parallel to the time course of delayed light emission without base treatment. 3-(3,4-Dichlorophenyl)-1,1-dimethylurea enhanced both the delayed light emission and acid-base induced luminescence, while carbonyl cyanide *m*-chlorophenylhydrazone inhibited both. Several photophosphorylation uncouplers inhibited the acid-base induced luminescence without any substantial effect on the delayed light emission. It is concluded that the acid-base triggered luminescence is not caused by the reversion of electrons from remote intermediates on the reducing side of Photosystem II. The possibility of the presence of an activation pathway for the acid-base triggered luminescence which differs from that of the delayed light emission is also discussed.

INTRODUCTION

It has been found that stimulation of delayed light emission from chloroplasts can be induced by any number of physical and chemical treatments [1–7]. The delayed light emission is attributed to back reactions of the primary electron acceptor and donor of Photosystem II [8]. Although the rate of this back reaction is thought to be stimulated by pH transitions, salt addition, electric field applications, etc., the relation of acid-base triggered luminescence to delayed light emission remains uncertain. Hardt and Malkin [5] indicated that the delayed light emission at 22 ms seems to have separate precursors from the acid-base and other triggered chemiluminescence. However, little work has been done to elucidate the dependence of acid-base triggered luminescence on delayed light emission with slow decay.

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

In this study, simultaneous measurements of the kinetics of delayed light emission (> 3 sec) and acid-base induced luminescence were made under several conditions to determine the correlation between light emissions.

MATERIALS AND METHODS

Chloroplasts were prepared from market spinach as described earlier [9] and suspended in preparation medium diluted to one half (0.2 M sucrose, 10 mM Tricine and 5 mM NaCl, pH 7.8). The chloroplasts were stored at 0 °C before use. The chlorophyll was determined following the method of Arnon [10].

Luminescence was measured with a Model 2000 Integrating Photometer (SAI Technology Co., ATP Photometer) and recorded on a Yokogawa 3047 pen recorder. When necessary, integrated counting of light emission was performed by selecting the photometer readings.

Chloroplasts containing 0.08–0.1 mg chlorophyll in 0.1 ml were added to a glass vial containing 0.1 ml of 10 mM succinate and 10 mM Tris and then mixed by shaking (pH 4.2). The vial was inserted immediately into the photometer and the chloroplasts were illuminated for 20 s with 669 nm light of 3340 ergs · cm^{-2} · s^{-1}, obtained from an incandescent light source with an interference filter. The photometer shutter was open 3 s after preillumination and then the delayed light emission was recorded.

At various times during the dark period, following preillumination, 0.2 ml of a base solution containing 0.1 M Tris · HCl was injected into the vial with an automatic pipette to bring the chloroplasts to pH 8.4. Since a linear relationship between the total output of triggered luminescence and the peak was established [5], the height of the spike of the induced luminescence superimposed upon the delayed light emission in the recorder tracing was used as the quantitative measure of the acid-base induced luminescence in this paper. All experiments were made at room temperature of about 20 °C.

RESULTS

Time courses of delayed light emission and acid-base triggered luminescence

Fig. 1 shows the recordings of typical time courses of delayed light emission and acid-base triggered luminescence. The decay of delayed light emission during the dark period, from about 6 to 30 s, apparently followed first order kinetics (Fig. 1, inset). The acid-base induced luminescence decreased when the dark period was lengthened prior to the addition of base solution. When the two curves are superimposed, the decay of delayed emission after the transient burst of triggered luminescence was found to be scarcely affected by the emission of triggered luminescence. Though slight decreases of the delayed light emision, probably due to the dilution of the chloroplast suspension and/or the changes of pH, were sometimes observed after the emission of induced luminescence, it may be concluded that the acid-base induced luminescence is independent of delayed light emission.

This observation was further confirmed by directly comparing the photometer readings of total emission from 15 to 75 s after termination of the preillumination with and without the acid-base triggered luminescence. As shown in Table I, depending on the times of the treatment, the extent of total luminescence was clearly in-

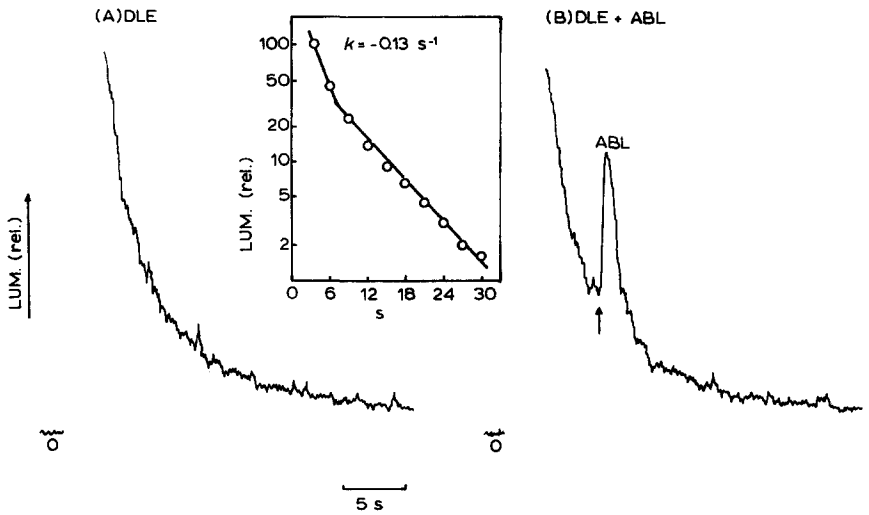

Fig. 1. Time courses of (A) the delayed light emission (DLE) and (B) acid-base induced luminescence (ABL) superimposed on the DLE. Preillumination was ended at 0. The arrow shows when the base solution was added to the acidified chloroplast suspension (pH 4.2). Final pH was 8.4. The decay of DLE obeys apparent first order kinetics, as shown in the inset.

TABLE I

PHOTOMETER COUNTINGS OF TOTAL EMISSION WITH AND WITHOUT ACID-BASE INDUCED LUMINESCENCE

Countings were started and ended at 15 and 75 s, respectively, and base solutions were added at 16, 40 and 70 s after the end of preillumination.

Luminescence	Total counting*	
	−DCMU	+DCMU
Experiment 1		
DLE**	66	70
DLE+ABL*** (at 16 s)	82	87
DLE+ABL (at 40 s)	76	78
DLE+ABL (at 70 s)	70	73
Experiment 2		
DLE	48	
DLE+ABL (at 16 s)	69	

* Relative values. The countings with DCMU cannot be directly compared with those in the absence of DCMU because of different sensitivity of the apparatus.

** DLE: delayed light emission.

*** ABL: acid-base induced luminescence.

creased by the acid-base transition. This means that the amount of induced luminescence was not compensated by a subsequent decrease of the delayed light emission. The above results suggest that acid-base triggered luminescence has different precursors from

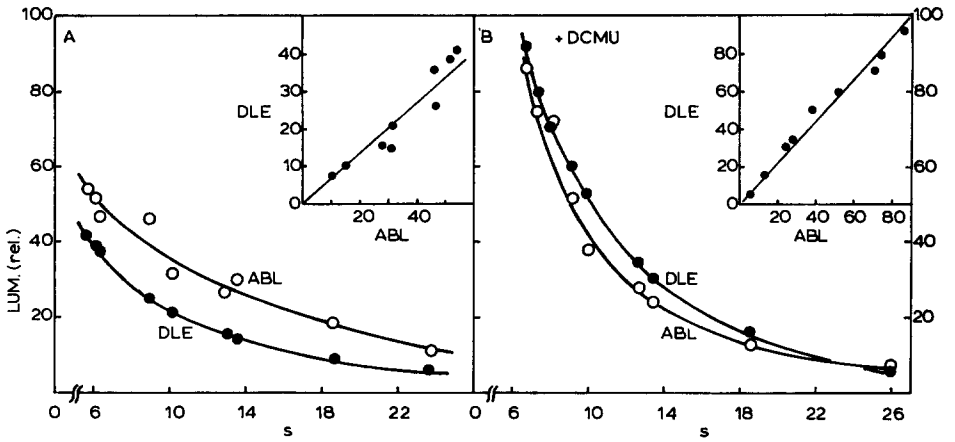

Fig. 2. Decay of delayed light emission (DLE) and acid-base induced luminescence (ABL) when the base solutions were added at various darkness periods in the absence (A) and in the presence (B) of DCMU. Experimental conditions were the same as in Fig. 1. DCMU concentration, $1.4 \cdot 10^{-5}$ M in the base stage.

delayed light emission, or at least, the former does not consume the same fraction of precursor as the latter.

Fig. 2 A shows a parallel decay of delayed light emission and acid-base induced luminescence when base solutions were added at various darkness periods. The amount of acid-base induced luminescence was measured by subtracting the recording trace heights of delayed light emission from the peaks of acid-base triggered luminescence taken when the base solutions were added. The correlation between two light emissions was approximately linear (Fig. 2, inset). The results suggest that the ability of the chloroplasts to emit triggered luminescence decays during the dark period approximately follows first-order kinetics, in accordance with the results of Hardt and Malkin [5].

Effect of inhibitors

As shown in Fig. 3, considerable enhancement of both the delayed light emission and acid-base induced luminescence was observed when DCMU, an electron transport inhibitor on the reducing side of Photosystem II, was added prior to preillumination. Triggered luminescence was similarly superimposed on the delayed light emission as in Fig. 1 (see also Table I). The decay of delayed light emission during the dark period from 6 to 30 s was also found to follow apparent first order kinetics with a similar decay rate as in Fig. 1 (Fig. 3, inset). Fig. 2B (shown above) indicates a similar parallel decay of both kinds of luminescence in the presence of DCMU. This implies that DCMU enhances both emissions without modifying their correlation and that acid-base induced luminescence is not caused by the reverse movement of electrons (through the site of DCMU inhibition) from remote intermediates located on the reducing side of the Photosystem II.

The inhibitor CCCP, which blocks electron transport on the oxidizing side of Photosystem II [11], almost completely inhibited both triggered luminescence and delayed light emission (Fig. 4C).

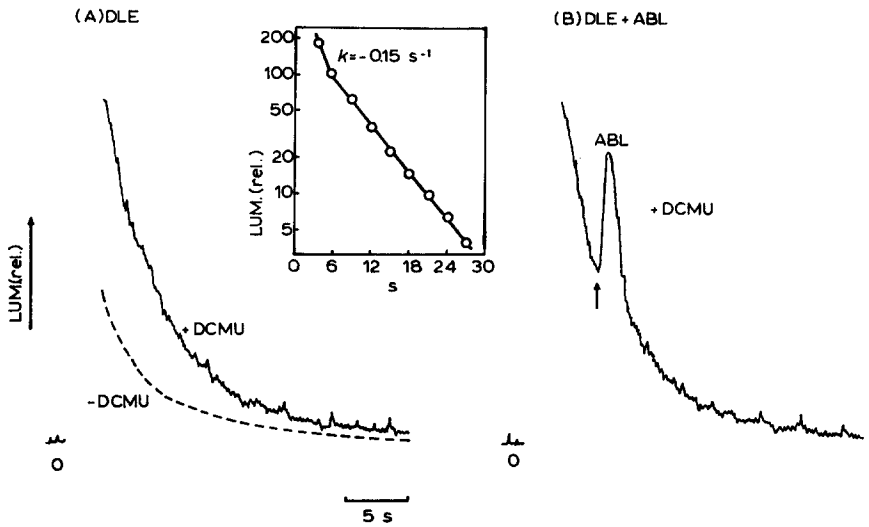

Fig. 3. Enhancement of the delayed light emission (DLE) and acid-base induced luminescence (ABL), when DCMU was added at the start of the experiments. Decay curve of the DLE without DCMU is represented by the dotted line. The decay of the DLE is found to obey first order kinetics, as in Fig. 1. DCMU concentration was $1.4 \cdot 10^{-5}$ M in the base stage.

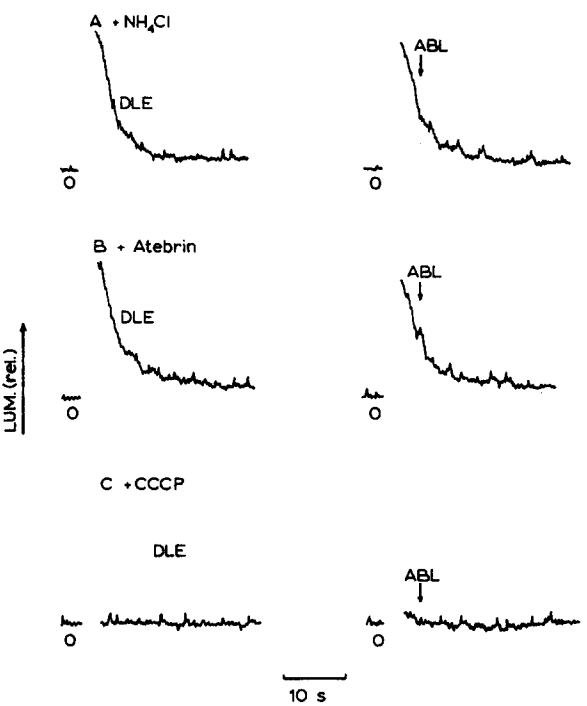

Fig. 4. Effects of NH_4Cl, Atebrin, and CCCP on the delayed light emission (DLE) and acid-base induced luminescence (ABL). Concentrations of the reagents in the base stage were: NH_4Cl, 10 mM; Atebrin, $1.2 \cdot 10^{-4}$ M; CCCP, $1 \cdot 10^{-5}$ M.

The uncouplers NH_4Cl and Atebrin added prior to preillumination inhibited only acid-base induced luminescence as shown in Fig. 4, A and B. Although the delayed light emission began to decay relatively quickly in NH_4Cl, Atebrin had scarcely any effect on delayed light emission. Also, Triton X-100 only inhibited luminescence induced by acid-base transition and produced a somewhat slow decay of delayed light emission (data not shown).

Simultaneous addition of DCMU and NH_4Cl (or Atebrin) showed an enhancement of delayed light emission and an inhibition of triggered luminescence similar to that of additions of each reagent separately.

Phlorizin, one of the energy transfer inhibitors [12], did not suppress either emission under concentrations below 5 mM (at the base stage), but above 10 mM it suppressed both partially. Almost complete inhibition of acid-base induced luminescence occurred at a concentration above 20 mM (data not shown). Thus, the coupling factor CF_1 does not seem to have a direct bearing on either luminescence.

DISCUSSION

Three possibilities can be considered for the time course of delayed light emission and acid-base induced luminescence (Fig. 5): (a) the acid-base induced luminescence (plus delayed light emission) decays according to a curve which moves parallel to the original curve of delayed light emission following the initial emission increase (Fig. 5A). This suggests that there is an increase in the amount of some common precursors for both emissions by the acid-base transition. (b) After a burst of triggered luminescence, the emission time course exceeds the original curve of delayed light emission and remains beneath it (Fig. 5B). This may be caused by the induced luminescence being directly stimulated at the rate of back reaction by the same precursor as the delayed light emission's, thus consuming a fraction of the precursor. The total amount of triggered luminescence must be compensated afterwards by a corresponding decrease of delayed light emission. (c) After the burst of acid-base induced luminescence, the emission time course returns to the original curve of delayed light emission (Fig. 5C). A pathway to activation for induced luminescence which differs from that for delayed emission is suggested in this case. This may be accomplished by the presence of a different precursor for triggered luminescence or a different state of the same precursor.

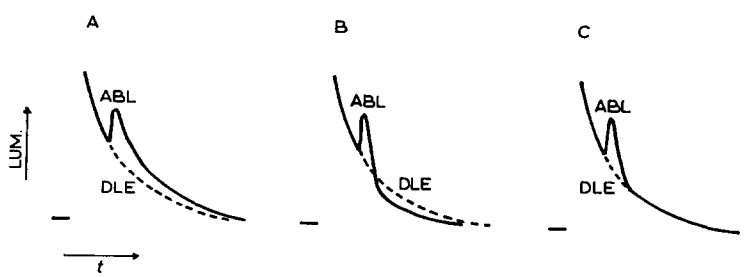

Fig. 5. Three possibilities on the time courses of the delayed light emission (DLE) and acid-base induced luminescence (ABL) which are dependent on the ways of activation to ABL. For explanation, see text.

The time courses of Figs. 1 and 3 show clearly that (c) is the correct evaluation under the experimental conditions of the present paper. A similar time course for delayed light emission and a stimulated emission by KCl in the presence of valinomycin has been reported by Barber [13]. Table I indicates further that the acid-base induced luminescence was not caused by process (b). On the other hand, an extra luminescence signal with a time course similar to (a) has been demonstrated by Arnold and Azzi [6] when applying an external variable electric field across a preilluminated chloroplast suspension.

As shown in Figs. 3 and 4, both the delayed and triggered luminescence were inhibited by CCCP and enhanced by DCMU. In this system, then, light emission caused by the reversion of electrons from remote intermediates located on the reducing side of the Photosystem II must be excluded. The precursor for the emission would accumulate at the direct proximate of system II by the flow of electrons from the oxidizing side during preillumination.

Although the presence of the same precursor for the delayed and stimulated light emission might be assumed by the fact that DCMU enhanced both the light emissions and by the decay of the ability of the chloroplasts to emit acid-base induced luminescence (parallel to that of delayed light emission). However, the fact that triggered luminescence was highly sensitive to uncouplers that had scarcely any effect on delayed light emission (Fig. 5) clearly rules out this assumption. The enhancement of delayed light emission and the inhibition of acid-base triggered luminescence in the presence of both DCMU and uncouplers further suggests the presence of some precursor for the triggered luminescence which differs from that for the delayed light emission.

According to the recombination hypothesis [8, 17], luminescence is the radiative decay of a singlet exciton resulting from recombination of a pair of charges within the activated reaction center. Luminescence is thus the reversal of the early photochemical charge separation. If the precursors (or states) described above are regarded as those charge separations, delayed light emission and triggered luminescence under the time range of this experiment can be tentatively explained on the basis of speculative models [8, 14, 15] as shown schematically in Fig. 6. During preillumination, light energy absorbed by Photosystem II is stabilized in at least two forms of precursors, $^{+}ZChlQ^{-}$ and ZChlQH, which are not interconvertible (stabilized at different sites ?) and have different energy levels. Boltzmann activation of

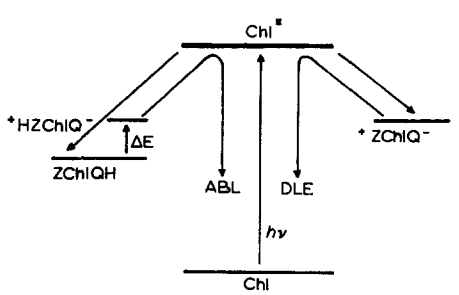

Fig. 6. Schematic explanation of the relation between the delayed light emission (DLE) and acid-base induced luminescence (ABL) in the present experiments. A part of both $^{+}ZChlQ^{-}$ and $^{+}HZChlQ^{-}$ (and all of ZChlQH without the ABL) will be deactivated with no light emission.

recombination of $^{+}ZChlQ^{-}$ brings the reaction center chlorophyll into the excited state Chl* resulting in a delayed light emission. The acid-base transition raises the energy level of ZChlQH alone by ΔE, reducing the activation energy "barrier" resulting in the formation of $^{+}HZChlQ^{-}$ [15] and in the light emission (triggered luminescence). The energy level of ZChlQH would be lower than that of $^{+}ZChlQ^{-}$ since no emission appeared unless activated by the acid-base transition. An activation pathway to Chl* from ZChlQH through $^{+}ZChlQ^{-}$ could not explain the time course of Fig. 5C.

Stabilization of the precursor might be connected to some dynamic states (e.g., electroosmotic ones) of the thylakoid membrane which are activated by the acid-base transition. It is possible that similar processes would operate in other various triggered luminescence, e.g., triggered by salts [13, 16] according to their time courses.

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REFERENCES

1 Mayne, B. C. and Clayton, R. K. (1966) Proc. Natl. Acad. Sci. U.S. 55, 494–497
2 Mayne, B. C. (1966) Brookhaven Symp. Biol. 19, 460–466
3 Miles, C. D. and Jagendorf, A. T. (1969) Arch. Biochem. Biophys. 129, 711–719
4 Barber, J. and Kraan, G. P. B. (1970) Biochim. Biophys. Acta 197, 49–59
5 Hardt, H. and Malkin, S. (1971) Photochem. Photobiol. 14, 483–492
6 Arnold, W. and Azzi, J. (1971) Photochem. Photobiol. 14, 233–240
7 Ellenson, J. L. and Sauer, K. (1976) Photochem. Photobiol. 23, 113–123
8 Lavorel, J. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 223–317
9 Nishizaki, Y. (1975) Plant Cell Physiol. 16, 589–594
10 Arnon, D. I. (1949) Plant Physiol. 24, 1–15
11 Kimimura, M., Kato, S., Ikegami, I. and Takamiya, A. (1971) Biochim. Biophys. Acta, 234, 92–102
12 Carmeli, C. (1969) Biochim. Biophys. Acta, 189, 256–266
13 Barber, J. (1973) in Ion Transport in Plants (Anderson, W. P., ed.), pp. 191–204
14 Clayton, R. K. (1967) Arch. Mikrobiol. 59, 49–58
15 Kraan, G. P. B., Amesz, J., Velyhuys, B. R. and Steemers, R. S. (1970) Biochim. Biophys. Acta, 223, 129–145
16 Lurie, S. and Malkin, S. (1975) in Proceedings of the Third International Congress on Photosynthesis, 1974 (Avron, M., ed.), pp. 83–91, North-Holland, Amsterdam
17 Lavorel, J. (1975) in Proceedings of the Third International Congress on Photosynthesis, 1974 (Avron, M., ed.), pp. 145–148, North-Holland, Amsterdam