ENHANCEMENT OF LUMINISCENCE IN CHLOROPLASTS BY ELECTRON TRANSPORT INHIBITORS

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Received November 17, 1971

SUMMARY: DCMU*, NQNO and BDHB caused pronounced enhancement of the triggered luminiscences and of the delayed light in chloroplasts. The enhancement induced by DCMU was higher at lower levels and at shorter periods of the preillumination and decayed during the few seconds of the dark period after the preillumination. These findings support the possibility that the enhancement is caused by an increase of the emission yield from the excited state, as shown by the parallel enhancement of the variable fluorescence. However, a reservation to this explanation is the absence of emission enhancement at short exposures of high preillumination intensity, in difference from the variable fluorescence that shows considerable enhancement.

INTRODUCTION

The isolated chloroplasts are capable of various emission processes after the end of their preillumination: (a) delayed light (1), (b) light emitted as a result of various triggering processes like pH transitions, salt addition and temperature jump(2,3,4,5).

Previous reports indicate that the electron transfer inhibitor, DCMU, inhibits the fast component of the delayed light (6,7), as well as the triggered lumniscences (2,3,5,8). Clayton, however, observed that under particular conditions (weak exciting light and low DCMU concentrations) the delayed light is stimulated rather than inhibited(9).

This communication reports similar enhancement effects of DCMU and of other inhibitors - NQNO and BDHB (10) on the delayed light and on the triggered luminiscences. Quite pronounced enhancements of the emission (up to $\sim$ x 5 of the control) were caused by the effect of these inhibitors, under appropriate conditions.

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*Abbreviations: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; NQNO, 2-n-nonyl-4-hydroxyquinoline-N-oxide; BDHB, butyl-3,5-diiodo-4-hydroxy benzoate.

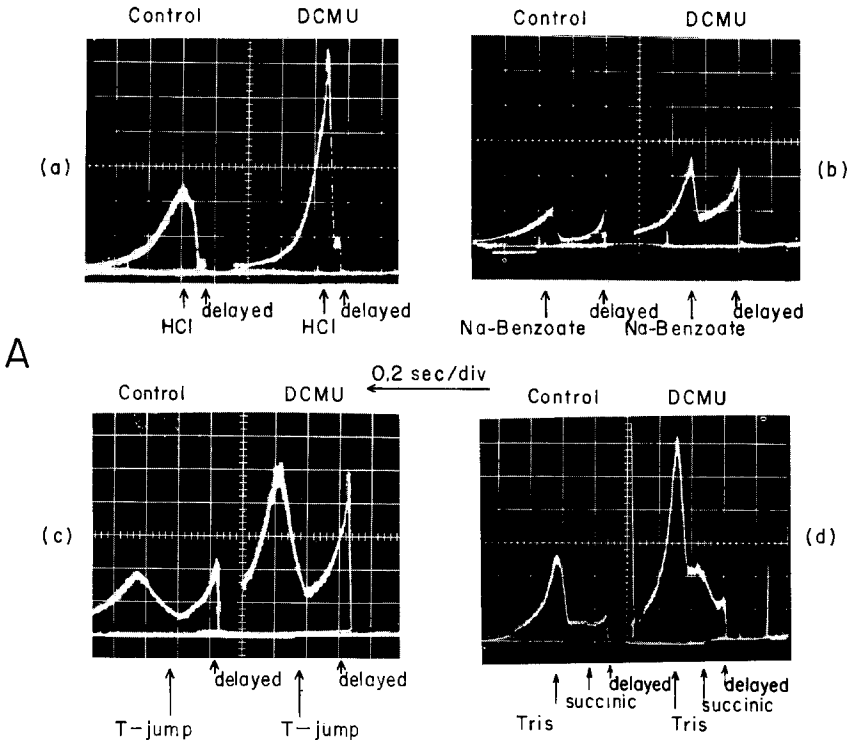
METHODS

Chloroplasts were prepared by a standard procedure (11) and stored at liquid nitrogen temperature (12). The luminescence measurements were carried out by an instrument described in full elsewhere (13). The delayed light emitted by the chloroplasts was measured at 22 msec after the end of the preillumination. The different types of induced luminescences were triggered as described before (13, 14). Two kinds of preillumination were used: (A) light from a continuous lamp (slide projector). This was passed through a $CuSO_4$ solution and a cut-off glass filter ($\lambda > 530$ nm), which transmitted a band between 530 and 600 nm. Exposure period was determined by a camera shutter (shortest time was 1/125 sec). (B) In several experiments we used for preillumination an electronic flash (xenon), $\sim$ 50 μ-sec duration, which was filtered by a cut-off glass ($\lambda > 530$ nm). The inhibitors were used at final concentrations of $5 \cdot 10^{-6}$ M and added 1 minute before preillumination.

RESULTS AND DISCUSSION

Oscilloscopic pictures of typical luminescence events are given in Fig. 1A, 1B, 1C. Each photograph shows in parallel the luminescence peaks of a control and of an experiment with an inhibitor. The enhancement is defined as the ratio between two luminescence peaks, corresponding to an experiment with an inhibitor and to the control. DCMU enhanced all types of luminescence considered (Fig.1A (a), (b), (c)), except that in the case of the acid-base transition (Fig. 1A (d)), where the enhancement has been obtained only after preillumination with a short flash, but was absent after preillumination from the continuous lamp. In agreement with Clayton's observation with DCMU, the delayed light preceeding the triggered luminescences was amplified by the three inhibitors (Fig. 1A, 1B, 1C). NQNO enhanced all the investigated luminescences in a similar manner as DCMU (Fig.1B). A somewhat different pattern was obtained with BDHB. This inhibitor did almost not increase the HCl, the T-jump and the acid-base luminescences (Fig. 1C, (a), (c), (d)). However, the sodium benzoate luminescence (b) and the delayed light were amplified, to the extent observed with the other inhibitors.

The pattern of the effect of the preillumination time (t_1) on the enhancement induced by DCMU is illustrated in Fig. 2, for the delayed light and for the succinic acid induced luminescence; the enhancement of the fluorescence yield is also given for a comparison. The largest degrees of enhancement were caused by the shortest preillumination periods, at which the luminescence responses themselves were relatively small. With increasing preillumination times the enhancement effect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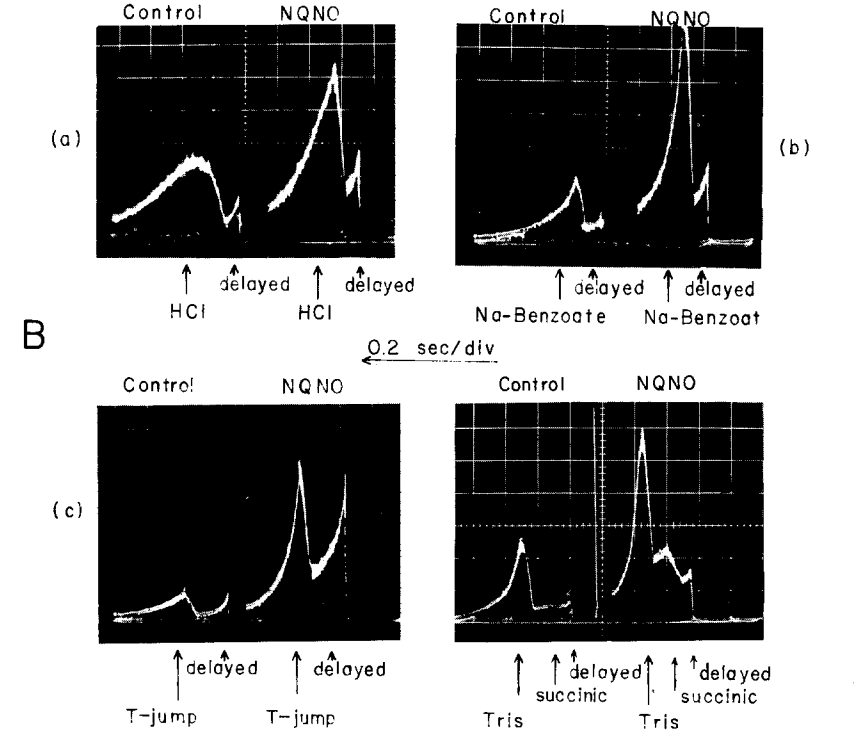
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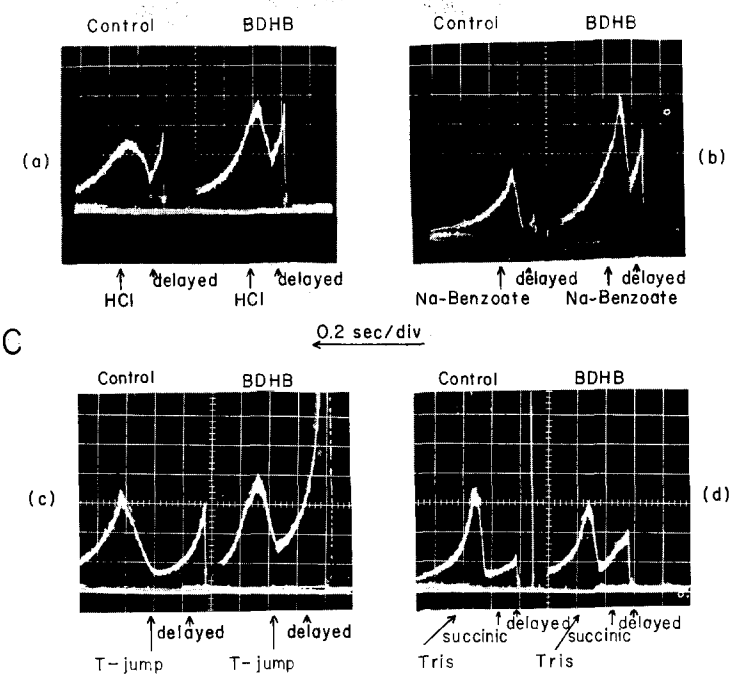

Fig. 1. Enhancement of induced luminescences and of delayed light (d.l.) by DCMU, NQNO and BDHB, compared to control experiments. (Enhancement ratios are given in parenthesis after the experimental conditions of each photograph). In all experiments the chlorophyll concentration was ≈ 60 µg/ml.

A. Effect of DCMU $5 \cdot 10^{-6}$ M: (a) HCl induced luminescence. (Injection of 1/4 ml 0.01 N HCl to 1 ml chloroflasts suspension). Time of preillumination t_I = 1 sec; preillumination intensity I = 0.4 nE (nano Einstein) $cm^{-2}sec^{-1}$. (d.l.- 2.5; HCl - 2.5). (b) Sodium benzoate induced luminescence, (injection of 0.25 ml 0.35 M sodium benzoate); t_I = 1/2 sec; I = 4.3 nE cm^{-2} sec^{-1}. (d.l. - 2.4; sodium benzoate - 2.4). (c) T-jump induced luminescence (injection of 1 ml water at 93°C into the sample at 24°C); t_I = 1/8 sec; I = 13 nE cm^{-2} sec^{-1}. (d.l. - 2.1; T-jump = 2.7). (d)Acid base induced luminescence (triggered by injecting successively 0.25 ml 0.02 M succinic acid and 0.25 ml 0.1 M tris base). Preillumination with a flash of 50 µsec duration. (d.l. - 1.7; tris - 2.4). The succinic acid luminescence peaks are not clearly defined in this photograph and also in photographs (d) of B and C.

B. Effect of NQNO $5 \cdot 10^{-6}$ M: (a) HCl luminescence; t_I = 1/15 sec; I = 6 nE cm^{-2} sec^{-1}. (d.l. - 1.3; HCl - 3.3). (b) Sodium benzoate luminescence; t_I = 1/15; I = 6 nE cm^{-2} sec^{-1}. (d.l.-1.2; sodium benzoate-3.3). (c) T-jump luminescence; t_I = 1/2 sec; I = 3.5 nE cm^{-2} sec^{-1}. (d.l. - 4.1; T-jump - 4.4). (d) Acid-base luminescence, experimental details as for A (d). (d.l. - 2.5; tris-2.3).

C. Effect of BDHB $5 \cdot 10^{-6}$ M: (a) HCl luminescence; t_I = 1/15; I = 6 nE cm^{-2} sec^{-1}. (d.l. - 1.4; HCl - 1.6). (b) Sodium benzoate luminescence; t_I = 1/15; I = 6 nE cm^{-2} sec^{-1}. (d.l. - 4.2; sodium benzoate - 2.0). (c)T-jump luminescence; t_I = 1/8 sec; I = 13 nE sec^{-1}. (d.l. > 2.5; T - jump 1.2). (d) Acid-base luminescence; experimental details as for A (d). (d.l. - 2.1; tris - 0.9).

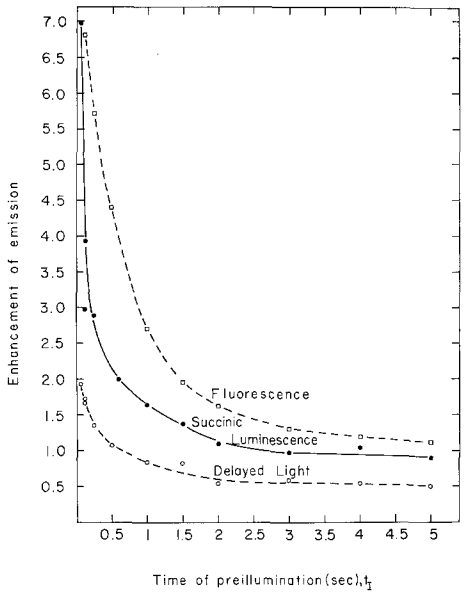

Fig. 2. Enhancement of emission by DCMU vs. time of preillumination t_I. $I \simeq 6.6$ nE cm^{-2} sec^{-1}; the succinic induced luminescence was triggered 0.25 sec after the preillumination period by injection of 0.25 ml 0.02 M succinic acid. The enhancement of fluorescence is defined as the ratio between two variable fluorescence induction heights, at any preillumination time, corresponding to an experiment with the inhibitor and to the control.

diminished and finally levelled off.

Clayton has shown that the amplifying effect of DCMU on the delayed light is related to a similar enhancement effect on the prompt fluorescence (9). In the explanation of this relationship he used the idea, first promoted by Lavorel (15), that the delayed light intensity is proportional to the yield of the variable fluorescence. Lavorel's equations are: $F = \Phi \cdot I$, $L = \Phi \cdot J$, where F and L are the intensities of the fluorescence and of the delayed light respectively, Φ is the yield of the variable fluorescence, I is the rate of light absorption and J is the rate of excitation to the singlet state of chlorophyll from the unknown precursors of luminescence. The DCMU amplification is explained by an increase of Φ (9). The increase is caused by the blocking of the electron transfer, which drives the photochemical traps into a "closed" form and dissipates the excitation energy as fluorescence.

In a similar way, we also tried to explain the enhancement of the various luminescences, by the effect of the inhibitors on Φ. During the preillumiation time, t_I, a reaction goes on which "closes" the reaction centers ($Q \rightarrow Q^-$)), manifesting itself in the large increase of the fluorescence yield. This reaction

proceeds much faster in presence of DCMU, which blocks the reaction of Q^- with the secondary electron acceptors. Therefore, for equal short t_l, Φ in presence of DCMU is much larger than Φ for the control. As t_l is increased this difference diminishes, and at sufficient light intensity, Φ for the control ultimately becomes equal with that of the inhibited sample. This explains why the enhancement decreases with t_l

In conformity with the above explanation our results on the DCMU amplification show, at least qualitatively, that the decay of the enhancement effect at longer t_l is similar to that of the variable fluorescence (Fig. 1). The inhibition of the luminescences by DCMU at long t_l may result from the effect of DCMU on J; an effect which is probably not very significant, since complete inhibition is not achieved even at high concentrations of DCMU (cf. ref. 3). In a similar way the inhibitory effect of DCMU on the delayed light at high light intensities was explained by Wraight and Crofts (17) by its effect on J. However, their work does not report any amplification effect.

The enhancement of the inhibitors was quenched at higher preillumination levels (Fig. 3). For the delayed light this is in conformity with Clayton's results (9). These results appear complementary to the dependence of the emission enhancement on t_l, and may be caused, as explained above, by the smaller ratio Φ (+ inhibitor)/ Φ (-inhibitor), obtained at higher t_l.

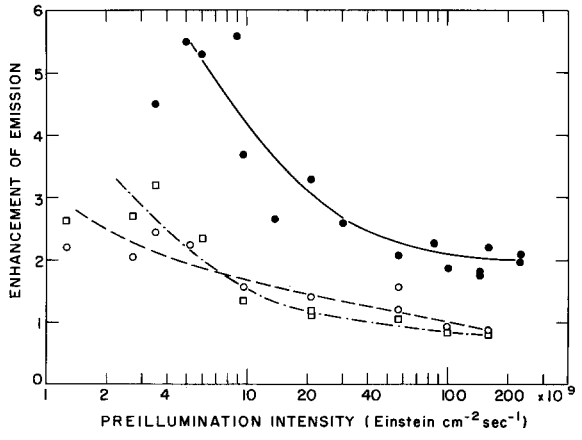

Fig. 3. Enhancement of emission, by DCMU, vs. preillumination intensity I. t_l = 1/8 sec; t_D = 0.25 sec. The light intensity was varied by using neutral density filters. Enhancement of succinic acid luminescence (o). Enhancement of sodium-benzoate luminescence (●). Enhancement of the delayed light preceeding the sodium-benzoate luminescence (□). (Details of injection as in the previous figures).

The above results elucidate that the inhibitory effects of DCMU on the acid-base luminescence (2), on the acid salt types (3, 8) and on the T-jump type, reported previously by other investigators, were probably obtained because of long t_I's or of high intensity of preillumination.

The enhancement decayed slowly as a function of a dark period (t_D) interposed between the preillumination and the triggering operation (Fig. 4A). In comparison, the ratio Φ (+ DCMU)/ Φ (-DCMU) was also measured, as a function of t_D (Fig. 4B). This ratio did not show any significant variation during t_D, except perhaps for a small increase at the beginning. Therefore, the decay of the enhancement can be only explained by a decay in the ratio J (+ DCMU)/J (-DCMU) as t_D is increased which means perhaps that J decays faster in presence of DCMU.

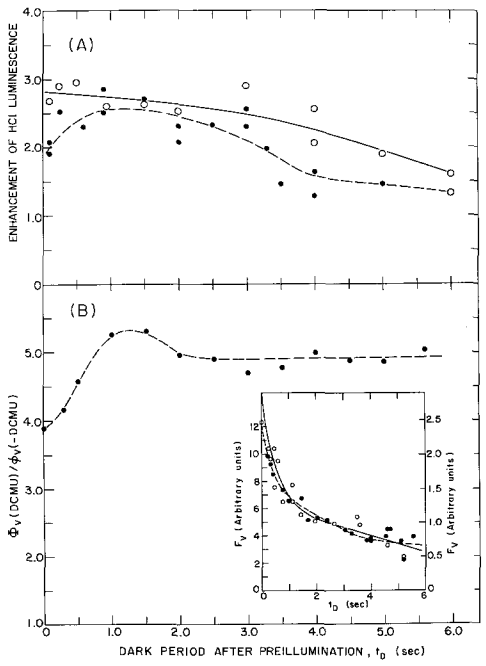

Fig. 4. A. Enhancement of HCl luminescence by DCMU vs. dark period after preillumination t_D. I = 13.5 nE cm^{-2}; sec^{-1}; t_I = 1/8 sec. The two curves correspond to two different chloroplast preparations (details of injection as in Fig. 1A (a)). B. Insert: dependence of the variable fluorescence Fv on the dark period t_D. Left scale (●), + DCMU; right scale (o), - DCMU. In this experiment the fluorescence yields were measured at the end of the dark period. The variable fluorescence was obtained by substracting the initial level Fo, of the fluorescence induction curve. I = 13.5 nE cm^{-2} sec^{-1}; preillumination period t_I = 1/8 sec. Main figure : dependence of Φ (+ DCMU)/ Φ (-DCMU) on t_D, as calculated from the smooth lines of the insert.

There is, however, one observation which leads us to question the correctness of the above theory. Figure 5 shows the results of an experiment in which I was varied, but t_I was decreased as I was increased, in such a manner as to keep the product $I \cdot t_I$ constant. Consequently, the total amount of quanta absorbed during the preillumination was the same. In spite of this, the enhancement decreased markedly with increasing I. By checking the fluorescence induction curves, we concluded that in this experiment the enhancement of Φ was the same ($\sim$ 3.5) at all the light intensities used. We also expected a similar enhancement for the delayed light and succinic acid luminescence measured in this experiment, but this was not

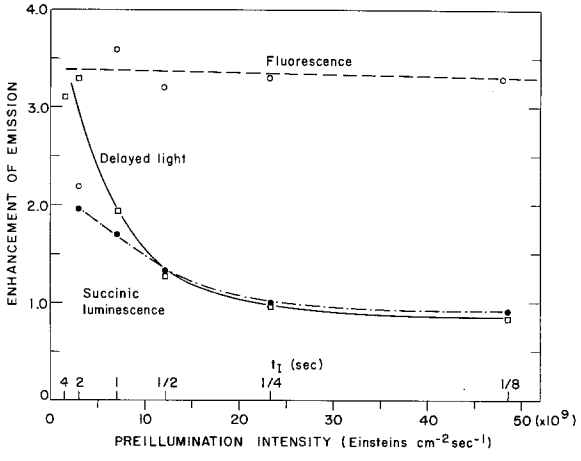

Fig. 5. Enhancement of emission vs. preillumination itensity I, at constant $I \cdot t_I$. The light intensity was varied using neutral density filters. t_I was varied as described in Methods. Succinic acid luminescence was triggered as in Fig. 2.

observed. It may be argued here we had an effect on J, which decreased as the light intensity was increased for the inhibited sample, so as to cancel the stimulation in Φ . However, this does not explain how J was influenced by the light intensity in spite of the fact that $I \cdot t_I$ was kept constant. This is in difference to the fluorescence induction which obeyed the $I \cdot t_I$ law. Further experiments are underway to solve this problem.

ACKNOWLEDGMENTS : One of the authors (H. H.) wishes to thank Dr. I. Gutstein for help in preparing this manuscript.

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