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Effects of electron transport inhibitors on millisecond delayed light emission from chloroplasts

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SUMMARY

When electron transport in chloroplasts is blocked after the plastoquinone pool, the delayed light emission is not inhibited. This is in sharp contrast to the situation when the transport of electrons to plastoquinone is blocked. Thus the inhibition of the oxidation of plastohydroquinone by dibromothymoquinone (a plastoquinone antagonist) or by KCN (a plastocyanin inactivator) does not produce a corresponding inhibition of delayed light emission whereas inhibition of the reduction of plastoquinone by 3-(3,4-dichlorophenyl)-1,1-dimethylurea or *o*-phenanthroline does. It is suggested, therefore, that the plastoquinone pool may serve as an electron reservoir used in the generation of delayed light.

Chloroplasts (unfragmented, naked lamellae) were prepared by the method of Saha *et al.*[1] from leaves of spinach (*Spinacea oleracea* L.). Cyanide-treated chloroplasts were prepared by incubating the chloroplasts at 0 °C for 90 min in a 30 mM KCN solution buffered at pH 7.8 as described by Ouitrakul and Izawa[2]. Dibromothymoquinone was prepared from thymoquinone as outlined by Carstanjen[3]. The delayed light emission was measured 3.0 to 3.5 ms after the end of each illumination period. Each illumination period lasted for 30 ms and was followed by 30 ms of dark (16 hz). Unless otherwise noted the intensity of the delayed light emission was determined after the chloroplasts had been in the flashing light for 30 s, within which time a pseudo-steady state emission was achieved. The design of the mechanical phosphoroscope used in this study has been described by Priestley and Haug[4]. The actinic light used was obtained by passing the beam from a Xenon lamp through 6 cm of a saturated $CuSO_4$ solution. The intensity of this light was varied with neutral density filters.

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

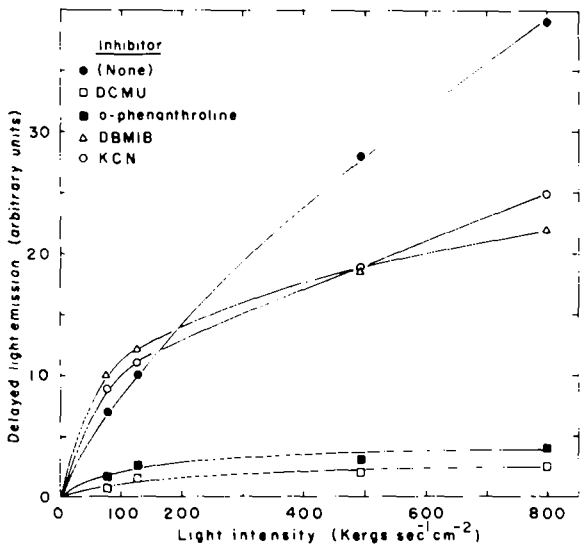

Fig.1. Effects of various electron transport inhibitors on millisecond delayed light emission at varied light intensities. The reaction mixture (2 ml) contained 0.1 M sucrose, 0.04 M Tricine–NaOH buffer (pH 8.2), 2 mM $MgCl_2$, 50 μM methylviologen and chloroplasts equivalent to 30 μg chlorophyll. Final concentrations of inhibitors were: DCMU, 1 μM; *o*-phenanthroline, 0.1 mM; and dibromothymoquinone (DBMIB) 0.5 μM. For KCN treatment and other conditions see paragraph for methods.

The effects of four different electron transport inhibitors on delayed light emission are compared in Fig. 1. In each instance the electron transport (methylviologen reduction) was inhibited by more than 95%. It can be seen that the inhibitors belong to two distinct classes with regard to their effects on the millisecond delayed light. *o*-Phenanthroline and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), both of which are thought to block electron transfer between Photosystem II, and the plastoquinone pool[5,6], nearly abolish the delayed light[7,8] regardless of the intensity of the actinic light. In contrast, the plastocyanin inhibitor KCN (ref.2) and the plastoquinone antagonist dibromothymoquinone[9] inhibit delayed light emission only moderately at high actinic light intensities, and actually increase delayed light emission at low actinic light intensities.

To approximately the extent shown by the data given for dibromothymoquinone in Fig. 2, the prompt fluorescence of the chloroplasts is enhanced by all of these inhibitors. This can be readily explained, as originally proposed by Duysens and Sweers[6], if we assume that under such conditions the primary electron acceptor of Photosystem II(Q) stays reduced(Q^-) and therefore the photochemistry (Q reduction) which would ordinarily absorb a large part of the excitation energy is prevented. It seems reasonable to suppose that this increase in the efficiency with which excited chlorophyll fluoresces is in large part responsible for the actual increase in delayed light emission seen at low light intensities with dibromothymoquinone or KCN.

The finding that the effect of inhibitors on delayed light emission is critically dependent on the position of the block relative to the plastoquinone pool suggests that the

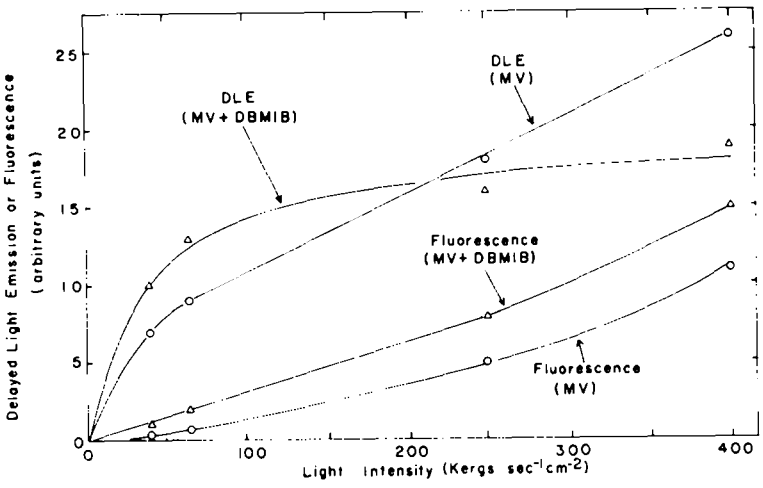

Fig. 2. Effects of dibromothymoquinone (DBMIB) on millisecond delayed light emission (DLE) and prompt fluorescence at various light intensities. The basic composition of the reaction mixture was as in Fig. 1 with methylviologen (MV) as electron acceptor. The arbitrary units for DLE are unrelated to and much smaller than the fluorescence units. The delayed light intensity was not corrected for fluorescence intensity.

plastoquinone pool plays an important role in the generation of delayed light. Since it seems likely that the delayed light emission results from a back reaction of the primary reductant of Photosystem II, Q^-, with the primary oxidant, Z^+ (ref. 10), it also seems likely that the reducing equivalents necessary for the regeneration of Q^- in the dark come from the plastoquinone pool.

Delayed light emission, in the presence or absence of inhibitors such as dibromothymoquinone or KCN, remains sensitive to uncouplers such as gramicidin. Therefore it is reasonable to suggest that the reverse flow of electrons from the reduced plastoquinone to Q which takes place immediately after the light is turned off, is driven by a membrane potential. Such a membrane potential may be generated in the light by plastoquinone reduction and/or by ion transport associated with plastoquinone reduction. Indeed, a proton translocation reaction associated with the electron transport span water→plastoquinone has been recently demonstrated[11]. This role of membrane potential in delayed light emission as a source of activation energy for the return of electrons to a reaction center has been stressed by Fleischmann[12] and Crofts *et al.*[13].

The use of dibromothymoquinone in studies of either delayed light emission or prompt fluorescence requires caution. In its oxidized form this inhibitor (> 2 μM) quenches chloroplast fluorescence and the fluorescence of chlorophyll solutions (data not presented), perhaps by a direct chemical interaction with the chlorophyll. Thus delayed light emission, prompt fluorescence, and the quantum efficiency of electron transport[14] are all greatly lowered if too much of the oxidized inhibitor is used. See Fig. 3. This effect is still further complicated by the fact that the inhibitor is readily reduced by the chloroplasts[14,15], and (above pH 8.0) reoxidized by molecular oxygen[14]. In its reduced

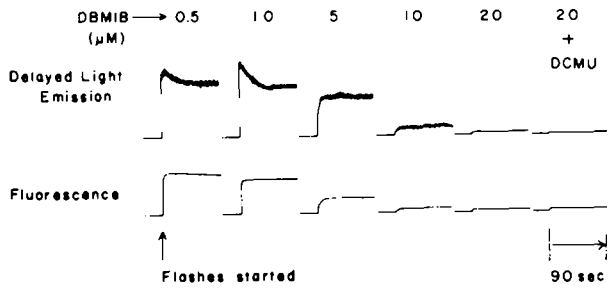

Fig. 3. Effects of increasing concentrations of dibromothymoquinone (DBMIB) on delayed light emission and prompt fluorescence. The basic composition of the reaction mixture was as in Fig. 1. The light intensity was 400 kergs$\cdot s^{-1} \cdot cm^{-2}$.

form the inhibitor continues to inhibit electron transport, but not to quench fluorescence. Therefore, very complex changes in the fluorescence and delayed light emission may be encountered if too high a concentration of the inhibitor is used and if the redox level of the medium is not under control.

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