Binary oscillation of the thermoluminescence of chloroplasts preilluminated by flashes prior to inhibitor addition

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1. INTRODUCTION

On the acceptor side of PS II a two-electron acceptor, B (also called 'R') functions between the primary acceptor, Q, and the plastoquinone pool (PQ) [1,2]. The semireduced state of this acceptor, B^-, is very stable [3] but in the fully reduced state, B^{2-}, it transfers an electron pair to PQ within 10 ms [4]. Addition of diuron-type inhibitors causes a backflow of electrons from B^- to Q indicating that the inhibitor strongly shifts the equilibrium $QB^- \rightleftharpoons Q^-B$ to the right [1,5–8]. As a result of the change in the equilibrium constant between Q and B, in a sequence of pre-illuminating flashes followed by the addition of DCMU, the redox state of Q oscillates with a periodicity of 2 reflecting the redox state of B present before DCMU addition. Consequently, in dark-adapted chloroplasts the prompt and delayed fluorescence yields, which are partly controlled by the redox state of Q, exhibit a binary oscillation as a function of the number of pre-illuminating flashes given before DCMU addition [1,5,9,10].

The aim of this study was to investigate whether the intensity of TL of isolated chloroplasts after applying pre-illuminating flashes followed by the addition of different PS-II inhibitors is also controlled by the redox state of B prior to the addition of an inhibitor.

It was found that when ~50% of the B pool is in semi-reduced state the amplitude of TL oscillates with a periodicity of 4 with maxima occurring at flash numbers 2, 6 and 10. After a long-term dark adaptation of chloroplasts when a major fraction of the B pool is oxidized, the period-4 oscillation is superimposed with another oscillation of period-2.

These results indicate that after flash pre-illumination followed by the addition of an inhibitor the TL yield is determined by the redox states of the oxygen-evolving system and that of the B pool prior to inhibitor addition. In a sequence of pre-illuminating flashes the binary oscillation of the TL yield reflects the binary oscillation of the redox state of B.

2. MATERIALS AND METHODS

Intact chloroplasts were obtained from enzymatically isolated mesophyll protoplasts of maize as in [11]. The chloroplasts were suspended in a medium containing 0.4 M D-sorbitol, 10 mM NaCl and 50 mM Tris-HCl buffer (pH 7.4) with 100 μg chl/ml. The suspension contained 30% ethylene glycol to prevent the distortion of the glow curves by the solid–liquid phase transition of water [12]. Before the TL measurements the samples were frozen and thawed twice in the dark to ensure penetration of inhibitors into the chloroplasts. The measurement of TL was done over − 80 to +80°C using an apparatus similar to that in [13]. Samples were illuminated by white light at an intensity of 10 W/m^2 for

Abbreviations: atrazine, 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine; B, secondary acceptor of photosystem II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNOC, 4-6-dinitro-*o*-cresol; PS II, photosystem II; Q, primary acceptor of photosystem II

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2 min during a period of continuous cooling from +20°C to −60°C. In flash experiments samples were subjected to 1–12 saturating xenon flashes (General Radio, Stroboslave, 3 μs, 0.5 J) at 1 s intervals; 10 s after the last flash the different inhibitors were added, the suspension was mixed for 20 s in dark and cooled down quickly to −40°C. The samples were then heated in the dark at 20°C/min and TL was measured.

3. RESULTS AND DISCUSSION

Thermoluminescence originates from charge recombination between positively-charged donors and negatively-charged acceptors of PS II which were formed during the photosynthetic charge-separation process [12,14,15]. DCMU, atrazine, DNOC and orthophenanthroline although having very different chemical structures, all block the electron transport between the primary acceptor, Q, and the secondary acceptor, B, resulting in accumulation of electrons on Q [16–19]. Therefore in the presence of these inhibitors, following illumination of chloroplasts, the negative charges participating in the generation of TL originate from the reduced primary acceptor, Q^- [20].

Fig.1 shows the glow curves of isolated chloroplasts which were treated by DCMU, orthophenanthroline, atrazine and DNOC. The peak positions of the TL bands were at higher temperatures than those published earlier [21] because the rate of heating was higher in the present experiments. Since DCMU, orthophenanthroline, atrazine and DNOC inhibit the reoxidation of the reduced one-electron acceptor Q^- by B the excitation of chloroplasts by a saturating single flash given after inhibitor addition can fill the entire electron pool available before the site of action of these inhibitors [16]. Further flashes will not increase the amount of negative charges which could contribute to the radiative charge recombination giving rise to TL. Fig.2A indeed shows that the intensity of TL of dark-adapted chloroplasts which were treated by DCMU is almost constant regardless of the number of exciting flashes (- - -).

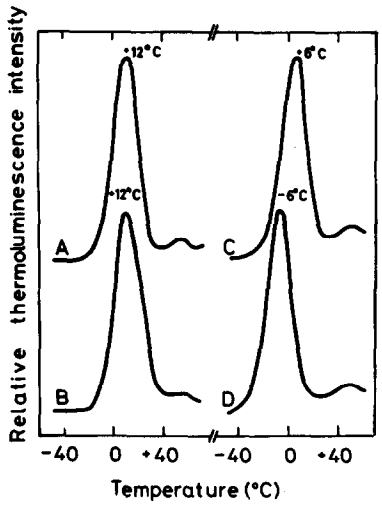

Fig.1. Effect of chemically different photosystem II inhibitors on the glow curve of isolated chloroplasts: (A) 10 μM DCMU; (B) 2.5 mM orthophenanthroline; (C) 25 μM atrazine; (D) 180 μM DNOC.

The situation is completely different when the flashes are given before DCMU addition. In this case the oxidation of the reduced Q by B is not inhibited and occurs in < 1 ms [9].

When TL is measured immediately after isolation of chloroplasts, the B pool is in the steady-state distribution, that is B/B^- is ~ 1 [10,22]. After excitation of the sample either by an even or uneven number of flashes 50% of the B pool is in the half-reduced semiquinone state, B^-. Mixing the sample with DCMU after the last pre-illuminating flash induces a back-transfer of electrons from B^- to Q [1,22]. Due to the steady-state distribution of B/B^- present before DCMU addition the amount of reduced Q does not depend on the number of pre-illuminating flashes. Consequently, the oscillation of TL emission is determined by the 4 successive charge accumulation states of the water-splitting system, as can be concluded from the large period-4 oscillations of the TL yield (fig.2A, ——). Assuming that immediately after isolation of chloroplasts the major part of the oxygen-evolving enzyme is in S_1 state [23] the maxima after the 2nd, 6th and 10th pre-illuminating flashes suggst that the S_3 state is the positively charged state which participates in the generation of TL after DCMU addition [21].

At room temperature after DCMU addition the reduced Q is probably quickly reoxidized by charge recombination of Q^- with states S_2 and S_3 of the oxygen-evolving enzyme [3,8,24]. To inhibit the relaxation of PS II by the backflow of electrons from

Q^- to the S states the samples are excited and DCMU is added at a much lower temperature (– 5°C) than the peak temperature of the TL band characteristic of DCMU-treated chloroplasts. Thus the electrons and holes remain trapped until the temperature of the sample is increased during TL measurements.

The amount of B oxidized in the dark increases during dark adaptation of chloroplasts and after an even number of flashes the majority of electrons are transferred via B^{2-} to the PQ pool. Since after DCMU addition these are not available for radiative charge recombination the intensity of TL decreases after an even number of flashes (fig.2B).

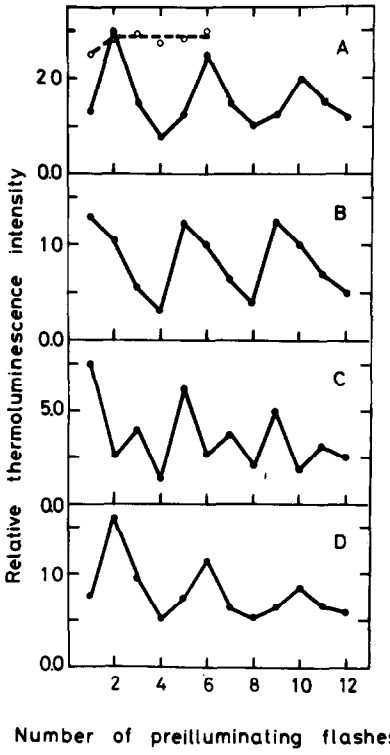

Fig.2. Oscillation of the intensity of the main TL band after a variable number of pre-illuminating flashes. Flash excitation of the samples occurred at – 5°C and it was followed by the addition of 10 μM DCMU. (A) (——) flash excitation was provided immediately after isolation of chloroplasts; (- - -) TL of dark-adapted chloroplasts excited after DCMU addition. (B) chloroplasts were stored for 2 h in the dark at +6°C before flash excitation and DCMU addition; (C) as (B) except that chloroplasts were stored for 6 h; (D) chloroplasts stored for 6 h in the dark were pre-illuminated by continuous white light of 10 W/m^2 for 1 min and kept in the dark for 2 min before flash excitation.

As a result of long-term dark adaptation of chloroplasts (6 h) the B pool is almost completely oxidized and B/B^- becomes >2.3 [3,22]. Following an uneven number of flashes ~75% of the B pool finds itself in the half-reduced semiquinone state, B^-. The singly reduced secondary acceptor B^- is very stable in the dark [3,22,25]; whereas after an even number of flashes B^- is reduced to B^{2-} which is rapidly reoxidized to B by the plastoquinone pool. Therefore the redox state of B oscillates with a periodicity of 2 between the fully oxidized state and the singly reduced state. In harmony with the binary oscillation of the redox state of the B pool prior to DCMU addition, the redox state of Q and consequently the amplitude of the oscillation of TL also shows an oscillation with a periodicity of 2 as a function of the number of pre-illuminating flashes (fig.2C).

Pre-illumination of long-term dark-adapted chloroplasts by weak continuous light for 1 min shortly before flash excitation of TL restores the steady-state distribution of 50%/50% of B/B^-. In this case, the yield of TL emission is again determined by the advancement of charge accumulation at the donor side of PS II. TL intensity exhibits period-4 oscillation (fig.2D), corresponding to the four successive redox states of the water-splitting system.

The oscillation patterns of TL of orthophenanthroline-, atrazine- and DNOC-treated chloroplasts are similar to those obtained with DCMU treated chloroplasts. Therefore, in fig.3 we present only the binary TL oscillations of dark-adapted chloroplasts after treatment with orthophenanthroline, atrazine or DNOC.

The inactivation of the oxygen-evolving system during the long-term dark adaptation of chloroplasts was checked by the measurement of the rate of electron transport from water to *p*-benzoquinone. It was found that after 6 h dark adaptation at +6°C the chloroplasts lost ~40% of their oxygen-evolving capacity. This residual inactivation is reflected in the decreased amplitude of the TL yield after restoring period-4 oscillation by pre-illumination (fig.2D).

These results document that oscillation of the TL yield in a sequence of pre-illuminating flashes fol-

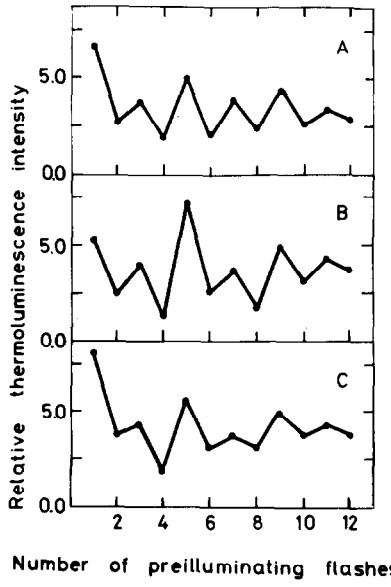

Fig.3. Oscillation of TL after flash pre-illumination of chloroplasts followed by inhibitor addition. Chloroplasts stored for 6 h in the dark at +6°C were excited by flashes at −5°C prior to the addition of (A) 2.5 mM orthophenanthroline and (B) 25 μM atrazine. Treatment by 180 μM DNOC (C) was done at −10°C following flash pre-illumination.

lowed by the addition of inhibitors is determined by the redox states of the oxygen-evolving system and that of the B pool prior to inhibitor addition. I propose that following a long-term dark adaptation of chloroplasts the binary oscillation of the TL yield reflects the binary oscillation of the redox state of B.

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