THERMOLUMINESCENCE CHARACTERISTICS OF GRANAL AND AGRANAL CHLOROPLASTS OF MAIZE

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1. Introduction

Materials illuminated at temperatures below 0°C emit light upon heating. This phenomenon is called thermoluminescence and was first observed with chloroplasts by Arnold and Sherwood [1]. The emission bands of thermoluminescence are a result of charge recombination between the various positively charged electron donors and negatively charged electron acceptors of the photosystems [2–4]. According to most authors thermoluminescence originates only from photosystem II [5–7] but Sane et al. [2] as well as Shuvalov and Litvin [8] attributed some of the glow peaks to photosystem I.

Inoue and Shibata [3,9] demonstrated that the emission of the bands at +25°C and +40°C is linked to the positively charged *S* states of the water-splitting enzyme. Few data, however, are available on the location of negative charges participating in the charge recombination [2–4]. The aim of the present study was to obtain further information about the involvement of photosystem I in thermoluminescence and to correlate the main bands of the glow curve with the different components of the electron transport chain.

Abbreviations: Q, the primary electron acceptor of photosystem II; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; Adry, acceleration of the deactivation reactions in the water-splitting enzyme Y; DCMU, 3-(3,4-dichlorophenyl)-1,1 dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone

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The results presented indicate that thermoluminescence is generated only in photosystem II and the thermoluminescence bands appearing at +10°C and +20°C originate from charge recombination between positively charged *S* states and negatively charged Q and plastoquinone, respectively.

2. Materials and methods

Intact chloroplasts were obtained from enzymatically isolated mesophyll protoplasts and bundle sheath cells using the method in [10]. The chloroplasts were suspended in a medium containing 0.4 M D-sorbitol, 10 mM NaCl, 1 mM $MnCl_2$, 5 mM $MgCl_2$, 2 mM EDTA and 50 mM Hepes (pH 7.5) at 170 μg/chlorophyll ml. The measurement of thermoluminescence was carried out in the temperature region from –80°C to +90°C using equipment similar to that in [11]. Samples were illuminated with white light at 10 $W.m^{-2}$ for 5 min during continuous cooling from +20°C to –80°C, then heated at a constant rate of 10°C/min to measure the thermoluminescence. Chemicals were added before the illumination was started and the samples were frozen and thawed twice in the dark to ensure the penetration of compounds into the chloroplasts. This treatment did not change the glow curve of the control chloroplasts.

3. Results and discussion

In maize leaves the mesophyll chloroplasts have a high granum content and exhibit both photosystem I

and photosystem II activities; the bundle sheath chloroplasts do not contain grana and are deficient in photosystem II activity [10]. Figure 1 shows the glow curves of granal and agranal chloroplasts and the effect of NH_2OH on the thermoluminescence of granal chloroplasts. In the temperature range from −80°C to +90°C, the glow curve of granal chloroplasts exhibited 7 bands: at −20, −10, 0, +10, +20, +45 and +70°C (fig.1A). The glow curve of agranal chloroplasts, however, was smooth and the overall intensity of thermoluminescence was very low (fig.1B).

After inhibition of the donor side of photosystem II with a low concentration of NH_2OH the glow curve of granal chloroplasts became similar to that of agranal chloroplasts (fig.1C). These observations

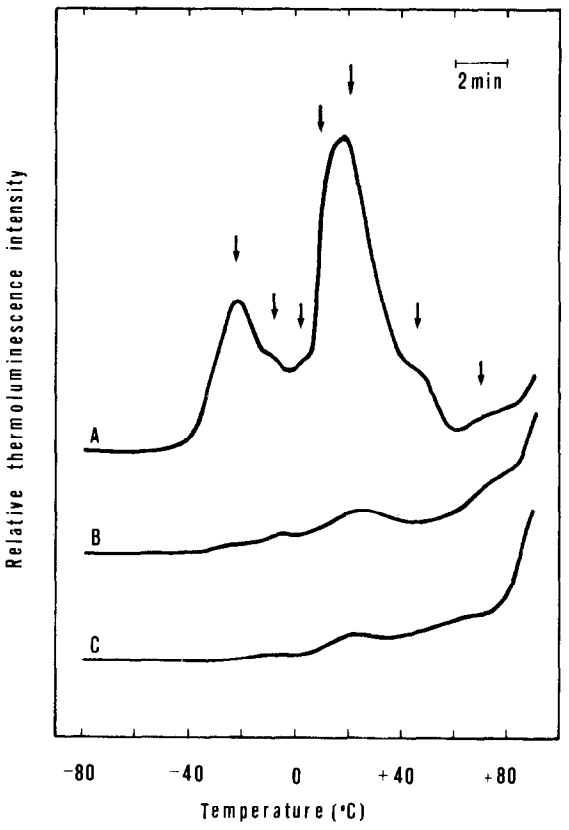

Fig.1. Glow curves of isolated granal and agranal chloroplasts of maize: (A) granal chloroplasts (positions of bands are indicated by arrows); (B) agranal chloroplasts; (C) granal chloroplasts treated with 0.5 mM NH_2OH. All curves start out at zero intensity.

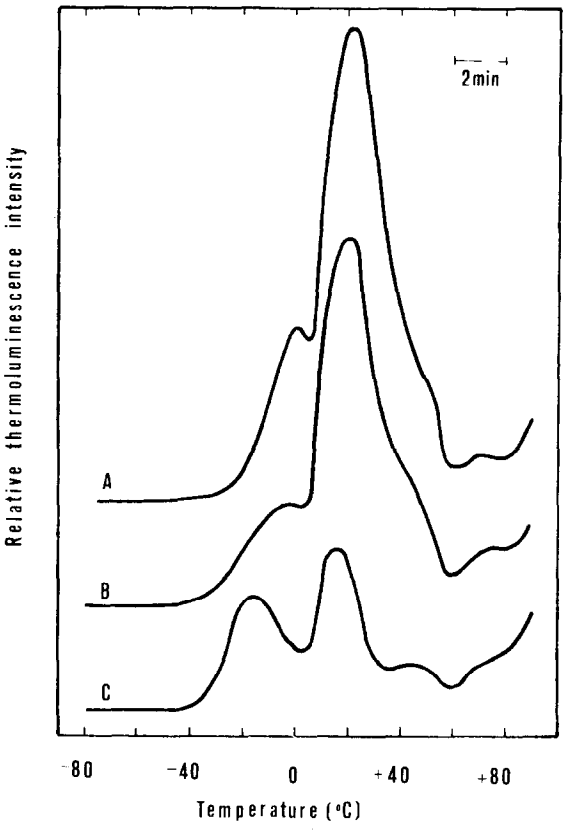

Fig.2. The effect of uncouplers on the thermoluminescence of isolated granal chloroplasts: (A) 6 mM NH_4Cl; (B) 30 μM methylamine; (C) 10 μM CCCP.

suggest that thermoluminescence originates from a functional photosystem II and that the contribution of photosystem I to the thermoluminescence of granal chloroplasts should be very small.

As shown in fig.2, NH_4Cl and methylamine considerably increased the main bands at +10°C and +20°C, CCCP, however, preferentially decreased them (fig.2A–C). It is known that NH_4Cl and methylamine can inhibit the transition from the S_4 to the S_0 state of the oxygen-evolving enzyme and cause an accumulation of high S states [12]. On the other hand, CCCP is a typical Adry reagent and specifically enhances the decay of higher S states [13]. Therefore, we can conclude that, in agreement with the suggestion of Inoue et al. [3,9], the positive charges responsible for the emission of the bands at +10°C and +20°C are accumulated in the form of oxidized S species.

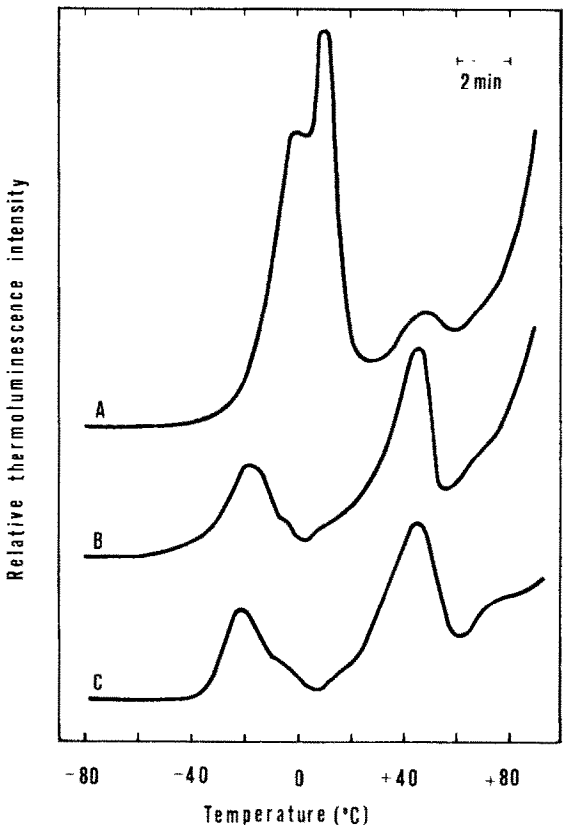

Fig.3. The effect of electron transport inhibitors and low pH on the thermoluminescence of isolated granal chloroplasts: (A) 10 μM DCMU; (B) 5 μM DBMIB; (C) pH 6.0. Measuring conditions of curve C are as in section 2 except that in the medium the 50 mM Hepes was replaced by 50 mM phosphate buffer.

The locations of the negative charges participating in the emission of thermoluminescence were investigated using electron transport inhibitors and low external pH. Conflicting evidence has been reported concerning the effect of DCMU [2,6,14]. In our experiments, using functionally highly intact chloroplasts in contrast to [2], the band at +10°C was present and the effect of DCMU (fig.3A) was similar to that obtained by Ichikawa et al. [6] and Rubin and Venediktov [14] with intact leaves and *Chlorella* cells, respectively, DCMU, which blocks the electron transport after Q and allows only one turnover of photosystem II [2], increased the peak at +10°C, suggesting that this peak is due to charge recombination of Q^- with one of the lower *S* states of the oxygen-evolving system.

The plastoquinone antagonist DBMIB cut off the main band at +20°C, with a concomitant increase in the peak at +45°C (fig.3B). It has been shown by Gould and Izawa [15] that the oxidized form of DBMIB can function as an electron acceptor for photosystem II. Kouchkovsky and Kouchkovsky [16] have concluded that high concentrations of DBMIB can even 'circuit out' the plastoquinone pool from the electron transport chain. This 'circuiting out' effect of DBMIB at high concentrations (5 μm in our case) could be the reason for negative charges not accumulating on the plastoquinone, while the absence of the peak at +20°C suggest that this thermoluminescence band belongs to platoquinone. This conclusion is supported by the effect of low pH, which results in a glow curve similar to that obtained upon addition of DBMIB (fig.3C). The disappearance of the peak at +20°C can be explained as follows:

(i) Lowering the pH from 7.5–6.0 results in a shift of the midpoint potential of plastoquinone by 90 mV in the positive direction [17].

(ii) At pH 6.0 the protonated form of plastoquinone (PQH_2) is relatively more stable than PQ, therefore, it cannot give back electrons to reduce Q [18].

Together these alterations may prevent the participation of plastoquinone in a reverse electron flow at low pH hence the absence of the +20°C peak.

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