THERMOLUMINESCENCE CHANGES DURING INACTIVATION AND REACTIVATION OF THE OXYGEN-EVOLVING SYSTEM IN ISOLATED CHLOROPLASTS

Yorinao INOUE, Takashi YAMASHITA*, Yoshichika KOBAYASHI and Kazuo SHIBATA
Laboratory of Plant Physiology, The Institute of Physical and Chemical Research, Wako-shi, Saitama 351 and
**Institute of Biological Sciences, University of Tsukuba, Ibaraki 300-31, Japan*

Received 11 August 1977

1. Introduction

Thermoluminescence from leaves and algal cells is a sort of chemiluminescence emitted upon heating plant materials after irradiation at low temperature. The glow curves of mature leaves recorded as a function of emission temperature generally show four luminescence peaks at different temperatures of −155°C, −5°C, +25°C and +55°C, which were denoted by Arnold [1] as Z, A, B and C bands, respectively. These bands result from recombination of electrons and positive holes which were formed by chlorophyll photoreactions in PS II and stabilized as frozen state. Previous studies [2–4] on thrermoluminescence from higher plant leaves and algal cells demonstrated that the B band is closely related to the O_2-evolving activity of chloroplasts. We have measured angiosperm leaves greened under intermittent illumination with flashes at long intervals or gymnosperm leaves and green algal cells greened in darkness, which did not show the water-splitting activity in the Hill reaction whereas the pigment systems of both PS I and PS II had been developed almost completely. The glow curves of such leaves and algal cells were devoid of the B band found for mature leaves and cells. On exposure of these materials to continuous light or flashes at short intervals, the B band was developed rapidly, being accompanied by generation of the Hill activity with water as electron donor. A similar relationship was found for Mn-deficient algal cells [5]. The deficient cells showed an extremely low B band, and illumination of such cells in the presence of Mn^{2+} greatly intensified the low B band to a normal height. All these observations indicated that the O_2-evolving system stores the light energy for emission of the B band.

In the present study, glow curves were measured for isolated chloroplasts during inactivation of the O_2-evolving system by Tris-treatment and during reactivation of the inactivated chloroplasts by reduced DCIP(2,6-dichlorophenolindophenol)-treatment followed by illumination with continuous light. It was hoped that such measurements combined with the reversible inactivation and reactivation techniques will give us further insight into the nature of the B band in relation to the O_2-evolving activity.

2. Experimental

Chloroplasts were prepared from young spinach leaves with a medium containing 0.05 M Tris (pH 7.4), 0.4 M sucrose, 0.01 M NaCl and 2 mM ascorbate [6]. The isolated chloroplasts were subjected to inactivation of the O_2-evolving activity by Tris-treatment [6] and reactivation by washing the inactivated chloroplasts with reduced DCIP [7,8] as briefly described below. A chloroplast precipitate was suspended in 0.8 M Tris solution, either at pH 8.2 or pH 8.8, and kept in darkness at 4°C for 20 min or 60 min, respectively. The two types of chloroplasts treated with Tris at different pH values are denoted in this paper as Tris(pH 8.2)- and Tris(pH 8.8)-treated chloroplasts, respectively. The O_2-evolving activities of these inactivated chloroplasts were less than 2 μmol/mgChl.h. For reactivation, the inactive chloroplasts were exposed to 0.3 mM reduced DCIP solution for 20 min at 4°C. The O_2-evolving activity of the Tris(pH 8.2)-treated

chloroplasts was recovered by this treatment to be about half the initial activity, but the activity of Tris(pH 8.8)-treated chloroplasts remained completely inactive after this treatment. The inactive Tris(pH 8.8)-treated chloroplasts washed with reduced DCIP were then subjected to light reactivation [8]; the inactive chloroplasts were suspended in a medium containing 15 mM Tris(pH 7.8), 20 mM NaCl, 4 mM $MgCl_2$, 2 mM Ca-acetate, 50 μM Mn-acetate and 0.5 mM dithiothreitol and exposed to weak white light (70 $\mu W/cm^2$) at room temperature for 20 min. The illuminated chloroplasts showed about 35% of the initial activity. The activity of O_2-evolution was monitored with a Clark-type O_2-electrode with ferricyanide as electron acceptor as described previously [6].

A square piece of filter paper (2.2 × 2.2 cm^2) was moistened with 0.1 ml chloroplast suspension containing about 20 μg chlorophyll and placed on a metal heater. The sample on the heater was first cooled, illuminated for excitation at a fixed low temperature with strong red light (0.7 mW/cm^2) for 1 min, then heated at a rate of 0.5°C/s up to 80°C. Thermoluminescence emitted during heating was recorded against temperature as described previously [6].

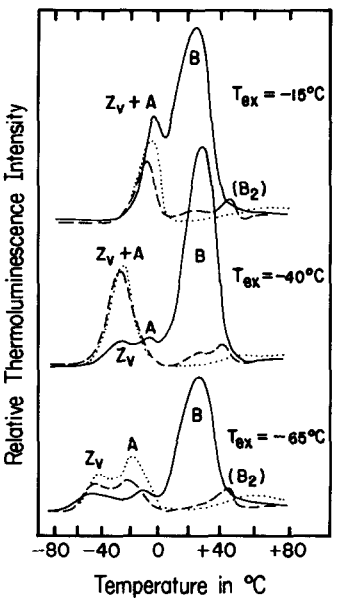

Fig.1. Effects of Tris treatment on the glow curves of isolated chloroplasts as observed by excitation at three different temperatures (T_{ex}). Solid, broken and dotted curves are the glow curves obtained for intact, Tris(pH 8.2)-treated and Tris(pH 8.8)-treated chloroplasts, respectively. The O_2-evolving activities of these chloroplasts are listed in table 1.

3. Results and discussion

Figure 1 shows the effect of Tris treatment on the glow curves measured after excitation at three different temperatures (T_{ex}). All the solid curves of intact chloroplasts show a distinct B band at +25°C. The B band of intact leaves sometimes splits into two, B_1 and B_2 bands, but the B band of isolated chloroplasts in the figure looks single at neutral pH. At acidic pH values, around pH 5, however, the B band of isolated chloroplasts splits into two bands emitted at +25°C and +40°C. As reported previously, the emission temperature of the Z_v band shifts toward higher temperatures as the excitation temperature (T_{ex}) increases. This shift made the Z_v band observed at T_{ex} −15°C overlap over the A band at −5°C.

The broken and dotted curves in the figure are the glow curves obtained for the Tris(pH 8.2)- and Tris(pH 8.8)-treated chloroplasts, respectively. The glow curves of these inactive chloroplasts were almost completely deprived of the B band. Only a very weak band was found around +45°C in some of the glow curves. The Z_v and A bands behaved quite differently on the Tris treatment. The Z_v and A bands measured by excitation at T_{ex} −65°C were intensified by a factor of 2 to 3 by the Tris treatment. The intensification was even greater at T_{ex} −45°C, but less distinct at T_{ex} −15°C. It is evident from these curves that the Tris treatment destroys the mechanism by which light energy is stored for emission of the B band but not the mechanism for emission of the Z_v and A bands. This accords with the previous conclusion that the B band is closely correlated to the O_2-evolving activity, since the Tris treatment specifically inhibits the O_2-evolving activity.

Curves in fig.2A show the result obtained after reactivation of the Tris-(pH 8.2)-treated chloroplasts with reduced DCIP. The Z_v and A bands, which had been intensified by the Tris treatment, were flattened by the DCIP treatment to the low levels for intact chloroplasts and, instead, the missing B band was

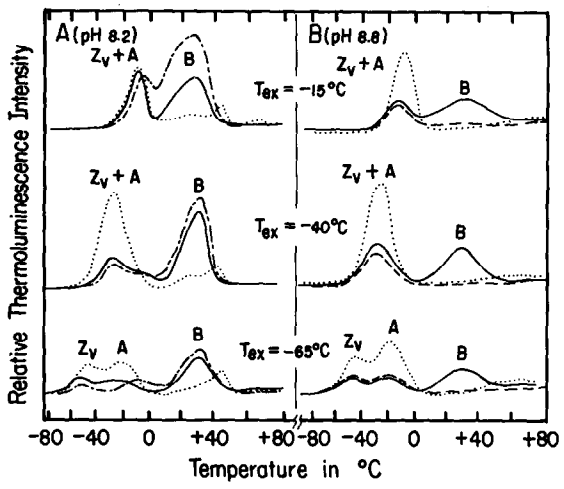

Fig.2. Changes of thermoluminescence bands on reactivation of Tris-treated chloroplasts. Dotted and solid curves in fig.2A are the glow curves before and after the DCIP-treatment of the Tris(pH 8.8)-treated chloroplasts, respectively, and the broken chain curves is the glow curve of intact chloroplasts treated with DCIP. The curves in fig.2B are the glow curves of the Tris(pH 8.8)-treated chloroplasts. The Tris(pH 8.8)-treated chloroplasts (dotted curve) were washed with DCIP and incubated in a medium containing $MnCl_2$ in the dark (broken curve) and in the light (solid curve). The O_2-evolving activities of these chloroplasts are listed in table 1.

regenerated to some extent. This is most clearly shown in the middle profiles measured at T_{ex} −40°C. The extent of regeneration of the B band was approx. 25% of the initial height, although the recovery of the O_2-evolving activity was as high as 48% of the initial activity as listed in table 1. This was found to be due to the effect of DCIP on the glow curves. The treatment of intact chloroplasts with reduced DCIP lowered the B band considerably at the three excitation temperatures, as shown by broken curves in the same figure. This lowering of the B band was not accompanied by any change of the O_2-evolving activity (table 1). DCIP may induce a cyclic electron flow around the PS II reaction center which will neutralize the charges separated by the photoreaction. Such an ADRY characteristic of this reagent may be responsible for the lowering of the B band [9].

Figure 2B shows the changes of glow curves observed on reactivation of the Tris(pH 8.8)-treated chloroplasts. The chloroplasts treated with Tris at a higher pH of 8.8 were first washed with reduced DCIP, then incubated in a Mn-containing medium in the light or in the dark at room temperature. The dotted curves on the Tris(pH 8.8)-treated chloroplasts (reproduced form fig.1 for reference) were changed to the solid curves by washing the chloroplasts with DCIP followed by incubation with Mn^{2+} in the light and to broken curves by the same washing and incuba-

Table 1
Relative heights of thermoluminescence bands and oxygen-evolving activities of intact and Tris-inactivated and DCIP light- (or dark-) reactivated chloroplasts

Treatment	O_2-Evolution (μmol/mgChl.h)	Relative band height[a] (emission temperature)		
		Z_v (−45°C)	A(−20°C)	B(+25°C)
Intact	106	0.6	0.8	51
Intact DCIP	105	0.3	0.5	17
Tris(pH 8.2)	2	10	11	8[b]
Tris(pH 8.2)-DCIP	51	0.6	0.5	14
Tris(pH 8.8)	0	14	20	0
Tris(pH 8.8)-DCIP-dark incubation	8	0.7	0.8	0
Tris(pH 8.8)-DCIP-light incubation	36	0.7	0.8	10

[a] Excitation temperature was T_{ex} −65°C
[b] Emitted at +45°C (see fig.1)

tion in the dark. The DCIP treatment followed by the dark incubation brought the Z_v and A bands back to the original low heights, but did not develop the missing B band. The B band was developed by the same period of incubation in the light to be as high as 20% of the original height(solid curve). The activity was recovered to 36% of the original activity by the incubation in the light, but the activity recovered after the dark incubation was only 7% (table 1). It may be concluded from this close parallelism in recovery between the B band and the O_2-evolving activity that the B band results from some reaction involved in the water-splitting process whereas the other Z_v and A bands are not directly concerned with oxygen evolution.

The emission of the B band from the O_2-evolving system, which had been suggested by previous studies [2–4] with intact leaves under development, was thus confirmed in the present study with isolated chloroplasts. The development of the B band in Mn-deficient algal cells required a multi-quantum photoreaction in the presence of Mn^{2+} [5,10]. Multi-quantum photoreactivation of Tris(pH 8.8)-treated chloroplasts with flashes will be reported elsewhere.

Acknowledgements

This study was supported by a research grant on 'Photosynthetic oxygen evolution' given by the Ministry of Education and by a grant for 'Life sciences' at The Institute of Physical and Chemical Research (Rikagaku Kenkyusho), Japan.

References

[1] Arnold, W. and Sherwood, H. (1957) Proc. Natl. Acad. Sci. USA 43, 105–114.
[2] Inoue, Y., Ichikawa, T. and Shibata, K. (1976) Photochem. Photobiol. 23, 125–130.
[3] Ichikawa, T., Inoue, Y. and Shibata, K. (1975) Biochim. Biophys. Acta 408, 228–239.
[4] Inoue, Y., Furuta, S., Oku, T. and Shibata, K. (1976) Biochim. Biophys. Acta 449, 357–367.
[5] Inoue, Y. (1976) FEBS Lett. 72, 279–282.
[6] Yamashita, T. and Butler, W. (1969) Plant Physiol. 44, 1342–1346.
[7] Yamashita, T., Tsuji, J. and Tomita, G. (1971) Plant Cell Physiol. 12, 117–126.
[8] Yamashita, T. and Tomita, G. (1976) Plant Cell Physiol. 17, 571–582.
[9] Renger, G. (1973) Biochim. Biophys. Acta 314, 390–402.
[10] Radmer, R. and Cheniae, G. M. (1971) Biochim. Biophys. Acta 253, 182–186.