OSCILLATION OF THERMOLUMINESCENCE AT MEDIUM-LOW TEMPERATURE

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1. Introduction

The glow curves of mature green leaves usually show six luminescence peaks emitted at different temperatures. The six bands which we observed were denoted in [1,2] as Z, Z_v, A, B_1, B_2 and C bands with partial modification of Arnold's nomenclature; in [3,4] the six bands were denoted as Z, I, II, III, IV and V. The A, B_1 and B_2 bands were found to be closely related to the oxygen-evolving activity of chloroplasts [5–7]. We have demonstrated that dark-grown green leaves of gymnosperm, dark-grown green cells of algae and angiosperm leaves greened under flashing light of long dark intervals of 30 s do not emit these three bands, unless the latent oxygen-evolving system in these leaves and cells is activated by continuous light or by short interval flashes of a few seconds apart [8,9]. This view was supported by further observations that these bands are absent in the glow curves of Mn-deficient algal cells but appear rapidly on illumination of such deficient cells in the presence of Mn^{2+} [10], and that the B band (mixture of B_1 and B_2 bands) of spinach chloroplasts disappears on inactivation of the oxygen-evolving activity by Tris-treatment, at pH 8.8 and reappears on reactivation of Tris-treated chloroplasts by treatment with reduced DCIP followed by illumination in the presence of Mn^{2+} and Ca^{2+} [11].

All these observations suggested that positive charges for the emission of the B band are accumulated possibly as the oxidized S species proposed by [12,13]. According to the current concept on luminescence from chloroplasts summarized [14], the degree of oxidation of S greatly affects the luminescence intensity with stronger luminescence from more highly oxidized S states. This concept is based on the measurements of delayed fluorescence of various life times [15,16] and the luminescence triggered by acid, salts or temperature jump [17].

In the present study, the glow curves from isolated spinach chloroplasts after excitation with short saturating flashes at various temperatures ranging from +17°C to −65°C were compared as a function of the number of flashes given to see the temperature dependency of the oscillatory pattern. It was found that each photo-oxidation step of S species has a different temperature dependency.

2. Experimental

Chloroplasts were prepared from spinach leaves with 0.05 M Tris buffer (pH 7.4) containing 0.4 M sucrose and 0.01 M NaCl, and suspended in 0.05 M tricine buffer (pH 7.4) containing 0.4 M sucrose at the chlorophyll concentration of 200 μg/ml. A square piece of filter paper (4.8 cm^2) was moistened with an aliquot (0.1 ml) chloroplast suspension, cooled to a desired temperature, and illuminated with saturating flashes from a Xe strobe with a short duration less than 8 μs (4 J/flash) through a red glass filter ($\geqq$ 630 nm). The illumination was repeated at intervals of 1 s. The illuminated sample was dipped in liquid nitrogen to be rapidly cooled to 77°K. The sample was, then, heated in darkness at a rate 0.5°C/s up to +80°C, and the luminescence emitted during heating was recorded against temperature as described [1].

3. Results and discussion

Figure 1 shows the glow curves of isolated chloro-

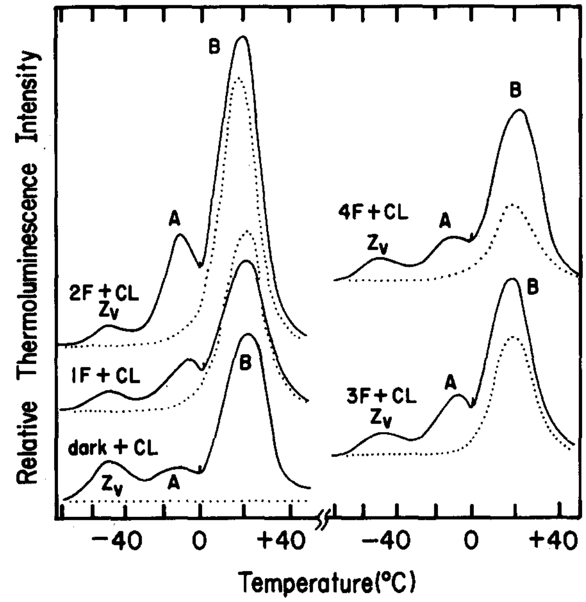

Fig.1. Glow curves of isolated spinach chloroplasts measured after different excitation conditions. Dotted glow curves were obtained after high temperature excitation at +17°C with 8 μs flashes repeated for various times indicated by F on each curve, and solid glow curves were obtained after additional low temperature excitation at −65°C with continuous light (CL) given to the chloroplasts pre-illuminated with flashes at +17°C.

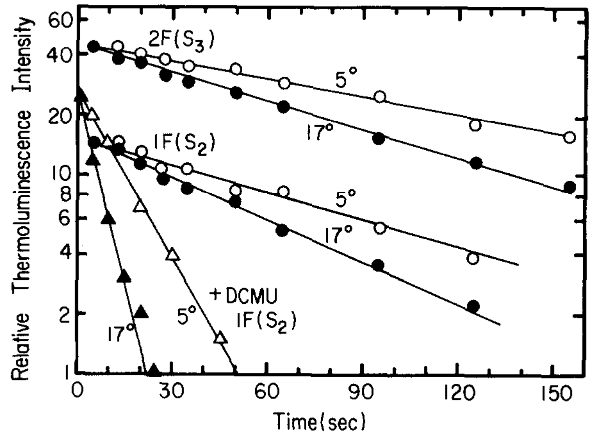

Fig.2. Decay course of the charged state for the B-band emission attained by flash excitation. Excitation and subsequent dark-incubation were made at two different temperatures of +17 and +5°C. F numbers indicate the number of flashes given, and *S* with subscripts in parentheses are the possible dominant *S* species attained by the respective number of flashes given. Concentration of DCMU was 10 μM.

plast measured after different excitation conditions. Chloroplasts were illuminated with a certain number of flashes indicated as 1F, 2F, (. . .) in the figure at a high excitation temperature of +17°C, and then illuminated at a low excitation temperature of −65°C for 10 s with continuous light (CL). The glow curves measured before and after the low temperature excitation with continuous light are shown by dotted and solid curves, respectively. As seen from these profiles, the chloroplasts excited only by flashes at +17°C emitted the B band but did not emit the Z_v and A bands. The height of the B band was dependent on the number of flashes given. The height after 2nd flash was about 2-times higher than that measured after a single flash, whereas the heights after 3rd and 4th flashes were rather lower than that after a single flash.

The decay courses of excited charged states in darkness are shown by fig.2. In the experiment, the glow curves were measured after various incubation periods in darkness at +17°C and +5°C subsequent to flash excitation. The B-band height plotted against the incubation time showed a clear first order decay. The half decay time of the state charged by a single flash was 42 s at +17°C and 78 s at +5°C(1F). The B-band height after 2nd flash (2F) was about 2 times higher than that observed after 1st flash, but the half lives measured at +17°C and +5°C were 46 s and 100 s, respectively, being only slightly longer than those observed after 1st flash. These rates of decay were so slow that we may neglect the loss of charges during the dark interval between flashes and during cooling the sample to liquid nitrogen temperature (about 3 s). The decay was markedly accelerated by a factor of 7 in the presence of 10 μM DCMU, as shown by the solid and open triangles. Considering that DCMU lowers the intensity of delayed fluorescence observed after a single flash illumination [7,18], this marked acceleration of decay by DCMU seems due to a non-fluorescent recombination of the separated charges.

The B-band height observed after flashes at +17°C is plotted against the flash number and shown by the dotted lines with open circles in trace I of fig.3, which indicate oscillation of four flash cycles with maxima at 2nd, 6th and 10th flashes and minima at 4th, 8th and 12th flashes. The B-band height after the additional low temperature excitation with continuous light showed a similar oscillatory pattern with maxima at 2nd, 6th and 10th flashes (solid circles with solid line in trace I), but the minima at 4th, 8th and 12th flashes were much less pronounced. Between these results with and without the post low temperature excitation, an appreciable difference was found in the B-band height after 3rd and 4th flashes, but a small or practically no difference after 1st and 2nd flashes (fig.1).

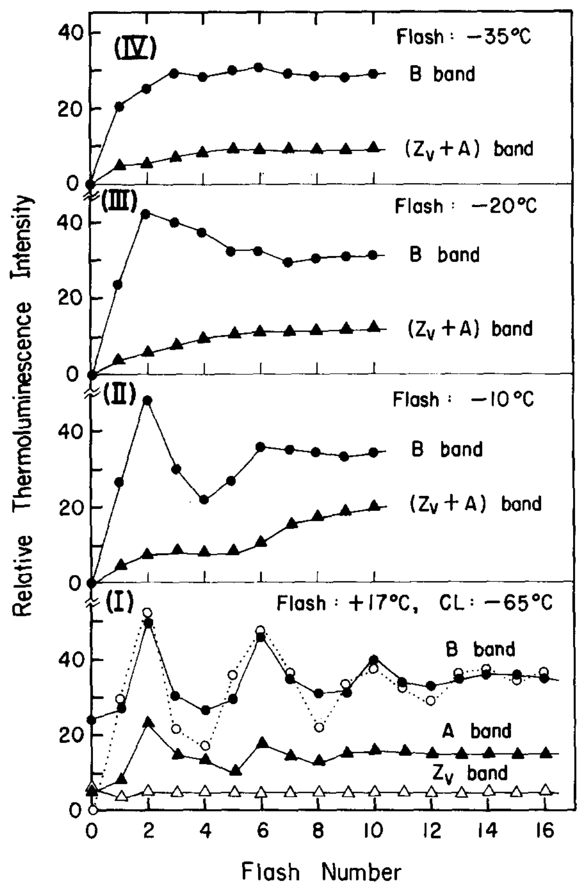

Fig.3. Oscillation of thermoluminescence as effected by flash excitation at four different temperatures. In trace I, open circles with dotted line show the B-band heights observed after high temperature excitation at +17°C with varied number of flashes, and solid circles with solid line show the B-band heights observed after additional low temperature excitation at −65°C by continuous light (CL) given to the chloroplasts pre-illuminated at +17°C with varied number of flashes. Solid and open triangles in trace I show the heights of A and Z_V band, respectively, observed after the additional low temperature excitation at −65°C. Solid circles and triangles in the Traces II, III and IV show the heights of the B and (Z_V + A) bands, respectively, observed after excitation by varied number of flashes given at respective temperatures of −10°C, −20°C and −35°C as indicated.

The oscillation of the B-band height can be correlated to the conversion of the S species in the oxygen-evolving system. If we assume that the most dominant species in dark-adapted chloroplasts is S_1 according to [13], the first maximum found after 2nd flash may be due to the luminescence from S_3, and the weaker luminescence after 1st flash is ascribed to S_2. This view is consistent with the conclusion drawn from the studies on triggered luminescence and delayed fluorescence with a long life time [15,17]. The fact that the low temperature excitation with continuous light after flashes did not affect the B-band height after 1st and 2nd flashes may indicate that the conversions from S_2 to S_3 and from S_3 to S_4 and then to S_0 do not proceed at the low temperature of −65°C. The B band charged by the low temperature excitation without any preceding flash illumination was as high as that observed after 1st flash. This indicates that S_0 and S_1 which exist in darkness are converted to S_2 by the low temperature excitation. This may also explain the fact that the B band after the 3rd or 4th flash was enhanced by the low temperature excitation, since the dominant species after these flashes are expected to be S_0 and S_1, respectively.

Similar experiments were carried out with flashes at lower temperatures but with no additional excitation by continuous light. When the flash excitation was made at −10°C, the B-band height showed an oscillation similar to that observed in the experiment at +17°C up to 6th flash, but decreased gradually thereafter with no further oscillation (solid circles in trace II in fig.3). This indicates that the first cycle of S conversion was completed but the second cycle proceeded only partially to the stage, probably, of S_3 after 6th flash. Possibly, one molecule of oxygen was evolved at each oxygen-evolving center from two molecules of water which had been bound to S species before cooled to −10°C, but the second molecular oxygen was not evolved because additional water molecules could no longer be incorporated into the open S sites at −10°C.

Trace III in fig.3 shows the results of a similar experiment at –20°C. The B-band height increased steeply on 2nd flash but decreased gradually thereafter without showing any oscillation. The gradual decrease after 3rd and 4th flashes implies that the conversion of S_3 to S_4 was blocked at this low temperature, whereas the conversions from S_1 to S_2 and from S_2 to S_3 can proceed normally. At still lower excitation temperature of –35°C, the B-band height increased steeply on 1st flash, but gradually increased thereafter without any oscillation (Trace IV in fig.3). This indicates that the conversion from S_2 to S_3 is partially blocked at –35°C.

These results are summarized in table 1, in which the temperature dependencies of the reactions involving the S species estimated above are listed. Among the reactions, binding of free water molecules to open S species was most sensitive to low temperature. This critical temperature of –10°C may, probably, be the freezing point of free water in the thylakoid space. At lower temperatures, oxidations of the S species are blocked step by step, and the oxidation of S to higher oxidized species was more sensitive to low temperature. We have recently shown that thylakoid proteins undergo conformational changes accompanying the oxidation of S species [19]. This suggested that the PS-II reaction center is more exposed to chemical modification when the S species are oxidized progressively. It seems, therefore, possible to suggest a specific conformational change occurring on each step of S oxidation which is blocked at a different temperature, leading to the inhibition of further S oxidation.

The height of the A band charged by the low temperature excitation with continuous light showed less pronounced oscillation with maxima at the 2nd and 6th flashes (solid triangles in trace I in fig.3). This may also be attributed to the conversion of S species brougt about by the preceding flashes given at +17°C. The presence of maxima at the 2nd and 6th flashes may indicate that the A band is charged much more efficiently when the additional low temperature excitation was given to the S_3 species which had been formed by the preceding flashes, and less efficiently when given to other S species. At higher excitation temperatures than –65°C, the A band and the Z_v band overlapped mutually, and the oscillation of the A band could not be observed. At –10°C, the height of the (Z_v + A) band increased steeply after 6th flash (solid triangles in trace II in fig.3), when the S_3 species had been formed and frozen by the preceding flashes. This supports the above view that the A band is charged more efficiently when the additional low temperature excitation was given to the S_3 species. At higher excitation temperature than –10°C, neither the A nor the Z_v band was emitted, as shown by dotted glow curves in fig.1.

It may be deduced from these results that the A band is indirectly dependent on the oxidation state of S species. Oxidized species of S may not be the carriers of the positive charges for the A-band emission, since this band is emitted much stronger in Tris-treated chloroplasts or in isolated PS-II particles which are no more equipped with the S apparatus [11]. Considering the fact that this band is more efficiently charged on low temperature excitation after the S_3 state has been completed, some substance(s) such as carotenoids [20] oxidized by PS-II photoreaction as a side reaction may be the carriers of positive charges for this band.

Table 1
Temperature dependency of each step of S oxidation as estimated from the oscillation of thermoluminescence

Reaction	Temperature dependency
S_0 or $S_1 \longrightarrow S_2$	Proceeds below –65°C
$S_2 \longrightarrow S_3$	Blocked below –35°C
$S_3 \longrightarrow S_4$	Blocked below –20°C
Binding of H_2O to open S sites	Blocked below –10°C

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References

[1] Ichikawa, T., Inoue, Y. and Shibata, K. (1975) Biochim. Biophys. Acta 408, 228–239.

[2] Inoue, Y., Ichikawa, T. and Shibata, K. (1976) Photochem. Photobiol. 23, 125–130.
[3] Desai, T. S., Sane, P. V. and Tatake, V. G. (1975) Photochem. Photobiol. 21, 345–350.
[4] Sane, P. V., Desai, T. S., Tatake, V. G. and Govindjee (1977) Photochem. Photobiol. 26, 33–39.
[5] Inoue, Y., Furuta, S., Oku, T. and Shibata, K. (1976) Biochim. Biophys. Acta 449, 357–367.
[6] Lurie, S. and Bertsch, W. (1974) Biochim. Biophys. Acta 357, 420–428.
[7] Jursinic, P. and Govindjee (1972) Photochem. Photobiol. 15, 331–348.
[8] Inoue, Y., Oku, T., Furuta, S. and Shibata, K. (1976) Biochim. Biophys. Acta 440, 772–776.
[9] Inoue, Y. and Shibata, K. (1976) in: Biological Solar Energy Conversion (Mitsui, A., Miyachi, S., San Pietro, A. and Tamura, S. eds) pp. 109–128, Academic Press, New York.
[10] Inoue, Y. (1976) FEBS Lett. 72, 279–282.
[11] Inoue, Y., Yamashita, T., Kobayashi, Y. and Shibata, K. (1977) FEBS Lett. 82, 303–306.
[12] Joliot, P., Barbieri, G. and Chabaud, R. (1969) Photochem. Photobiol. 10, 309–329.
[13] Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475.
[14] Lavorel, J. (1975) in: Bioenergetics of Photosynthesis (Govindjee ed) pp. 223–317, Academic Press, New York.
[15] Barbieri, G., Delosme, R. and Joliot, P. (1970) Photochem. Photobiol. 12, 197–206.
[16] Zankel, K. L. (1971) Biochim. Biophys. Acta 245, 373–385.
[17] Hardt, H. and Malkin, S. (1973) Photochem. Photobiol. 17, 433–440.
[18] Itoh, S. and Murata, N. (1973) Photochem. Photobiol. 18, 209–218.
[19] Kobayashi, Y., Inoue, Y. and Shibata, K. (1977) in: Proc. Int. Sem. Photosynthetic Oxygen Evolution, Tübingen (Metzner, H. ed) in press.
[20] Yamashita, K., Konishi, K., Itoh, M. and Shibata, K. (1969) Biochim. Biophys. Acta 171, 511–524.