Biochimica et Biophysica Acta, 408 (1975) 228–239

BBA 47000

CHARACTERISTICS OF THERMOLUMINESCENCE BANDS OF INTACT LEAVES AND ISOLATED CHLOROPLASTS IN RELATION TO THE WATER-SPLITTING ACTIVITY IN PHOTOSYNTHESIS

TETSUO ICHIKAWA, YORINAO INOUE and KAZUO SHIBATA

Laboratory of Plant Physiology, The Institute of Physical and Chemical Research (Rikagaku Kenkyusho) Wako-shi, Saitama (Japan)

(Received May 12th, 1975)

SUMMARY

Plant materials (intact leaves, chloroplasts or subchloroplast particles) preilluminated at a low temperature (e.g. −60 °C) were rapidly cooled to −196 °C and then the luminescence emitted from the sample on raising the temperature was measured as a function of temperature, by means of a sensitive photo-electron counting technique. Mature spinach leaves showed five luminescence bands at different temperatures which were denoted as Z_v, A, B_1, B_2 and C bands. The A, B_1, B_2 and C bands appeared at constant temperatures, −10, +25, +40 and +55 °C, respectively, being independent of the illumination temperature, but the Z_v band appeared at a variable temperature slightly higher than the illumination temperature. The B_1 and B_2 bands were absent in the thermoluminescence profiles of samples devoid of the oxygen-evolving activity, such as heat-treated spinach leaves, wheat leaves greened under intermittent illumination and photosystem-II particles prepared with Triton X-100. It was deduced that these luminescence bands arise from the energy stored by the electron flow in photosystem II to evolve oxygen, and other bands were ascribed to charge-separation in some other sites not related to the oxygen evolving system.

INTRODUCTION

After the discovery of delayed fluorescence from green plant materials by Strehler and Arnold [1], Arnold and Sherwood [2] found that algae and leaves can store some of the light energy absorbed by chlorophylls at temperatures below 0 °C. The stored energy is re-emitted upon heating the sample in darkness. Such luminescence has been interpreted as the result of the recombination of electrons and holes which were trapped in a frozen state during illumination followed by rapid cooling.

Arnold was the first who measured the thermoluminescence of dried chloroplasts [2, 3] and intact algal cells [4], and found four luminescence bands emitted at

---

Abbreviations: Ind Cl_2, 2,6-dichloroindophenol; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

different temperatures. Rubin and Venediktov [5] made a similar measurement and found that the glow profile is greatly dependent on the system of illumination; continuous illumination of leaves during cooling yielded a profile with four peaks, whereas illumination of leaves at a low temperature around −50 °C yielded a profile with two peaks. In recent studies, Lurie and Bertsch [6, 7] applied the continuous illumination method to isolated chloroplasts and obtained three bands in the profile. They discussed several mechanisms to store energy for thermoluminescence. Arnold and Azzi [8] attempted to calculate the activation energy, and Shuvalov and Litvin [9] correlated some of the glow peaks to delayed fluorescence components with different decay constants. In order to explain the presence of more than three peaks in a glow profile, Rubin and Venediktov [5] considered various cations and anions in the electron transport chain as energy carriers. Recombination between such cations and anions results in the emission of several bands at different temperatures. More recently, Sane et al. [10] observed five thermoluminescence bands emitted from intact leaves. They investigated in particular the emission spectrum of the 118 °K band (Arnold's Z band) and found that the spectrum shows a single maximum at 740 nm. On the basis of this emission spectrum, they arrived at the conclusion that the band is an emission from the triplet states of chlorophylls in vivo.

In the present study, we have attempted firstly, to measure the thermoluminescence bands precisely by means of a sensitive photon-counting technique and secondly, to characterize the bands from the data obtained for various samples such as mature spinach and wheat leaves, intermittently illuminated wheat leaves, heat-treated spinach leaves and subchloroplast particles of photosystems I and II prepared from spinach leaves. These data are presented in this paper together with some deductions drawn from them.

EXPERIMENTAL

The leaf sample was a square segment ($2.2\ \mathrm{cm} \times 4.0\ \mathrm{cm}^2$) of spinach leaf or 10 segments of wheat leaves, which were placed or arranged close together on two layers ($2.2\ \mathrm{cm} \times 4.0\ \mathrm{cm}^2$) of moist filter paper (Toyo No. 2). The sample of chloroplasts or subchloroplast particles in the form of suspension was put on the two layers of filter paper to be adsorbed on the paper. The absorbance of the sample leaves at 678 nm was about 1.5. The sample leaf or particles on the filter paper were sandwiched between a heater and a glass plate, both being the same dimension as that of the filter paper. The heater was a 100-ohm Nicrom wire wrapped with thin mica films and was fixed near the middle of a long holder ($2.2 \times 22\ \mathrm{cm}^2$, aluminium plate), so that the sample on the heater could be put into a Dewar bottle with a nob at an end of the long holder, to be cooled to a desired temperature with cold nitrogen gas and/or liquid nitrogen. The temperature of the sample was monitored with a Cu-constantan thermocouple (0.2 mm in diameter) inserted between the sample and the glass plate.

The sample was illuminated at a fixed temperature (T_i) for 1 min with the light at an intensity of $600\ \mu\mathrm{W/cm}^2$ from a tungsten lamp in a projector through a red glass filter (VR-63, Toshiba Kasei Co.) and a heat-absorbing water layer (depth 11 cm). Immediately after illumination, the sample was cooled rapidly to −196 °C by dipping it into liquid nitrogen in a Dewar bottle. The sample was then transferred with the Dewar bottle to a housing of a photo-electron counting device of the digital lock-in type (Jasco, model KC-200) equipped with a 30-Hz mechanical chopper and a red-sensitive photomultiplier (EMI 9659QB). The sample was heated slowly at a rate of 30 °C/min and the photons emitted from the leaf during the heating were counted through a red glass filter (VR-63, Toshiba Kasei Co.) which transmits light at a wavelength higher than 630 nm. The heating rate was controlled by varying the voltage supplied to the heater. The digital photon count in every 32 Hz was converted to an analogue signal with a rate meter, and recorded against time on a strip chart recorder or against temperature on an X-Y recorder.

Two different samples of wheat leaves were prepared as described in previous papers [11, 13] by illuminating 7-day old etiolated seedlings continuously, or intermittently (1-ms light+5-min dark) for 24 h at 24±1 °C. Chloroplasts were isolated from spinach leaves with 0.04 M Tris buffer (pH 7.5) containing 0.4 M sucrose and 0.01 M NaCl. The particles of photosystems I and II were prepared by treating the chloroplasts with 4 % Triton X-100 according to the method of Vernon et al. [12].

The Hill activities of chloroplasts or subchloroplast particles were measured as the rate of Ind Cl_2 (2,6-dichlorophenolindophenol) photoreduction with water or diphenylcarbazide as electron donor [13]. The chloroplasts prepared from the intermittently illuminated leaves and the photosystem-II particles prepared from spinach chloroplasts with Triton X-100 showed no activity with water as electron donor but appreciable activities of 20±4 and 30±5 (μmol Ind Cl_2/mg chlorophylls/h), respectively, with diphenylcarbazide as the donor. The chloroplasts prepared from wheat leaves greened by continuous illumination showed an activity of 80±10 (μmol Ind Cl_2/mg chlorophylls/h) either with water or with diphenylcarbazide as electron donor.

RESULTS

Effects of heating rate and illumination temperature

Fig. 1 shows the effect of the heating rate on the thermoluminescence band of mature spinach leaves. The rate affected not only the height of each luminescence band but also the relative heights of various bands. Curves A and B obtained at slow rates (11 and 19 °C/min) showed flat obscure bands on a low level. At higher rates of 24 and 31 °C/min (curves C and D), the luminescence bands above 0 °C were remarkably intensified, and neighboring bands were clearly distinguished from each other. Even higher rates (63 and 85 °C/min) did not however, improve the distinction but rather diffused the bands (curves E and F). Despite these great changes of relative heights and total area of bands recorded as a function of temperature, the total photon count integrated against time over the several bands was much less affected by the change of heating rate; the relative total photon count for profiles A, B, C, D, E and F were 62, 79, 84, 100, 102 and 92 respectively. The relative count increased slightly when the rate increased from 11 to 19 °C/min, but did not change at all above 31 °C/min. The medium rate of 30 °C/min, which was obtained by an energy supply of 1.3 W/cm^2 was thus found to be optimal to observe luminescence bands sensitively and distinctly. This rate was used in the further experiments presented in this paper.

Fig. 2 summarizes the effect of illumination temperature (denoted as T_i), which varied in a wide range between −10 and −196 °C. The sample leaf of spinach was illuminated for 1 min at a fixed temperature and then rapidly cooled down to −196 °C from which the measurement of luminescence with heating was started. The curves in

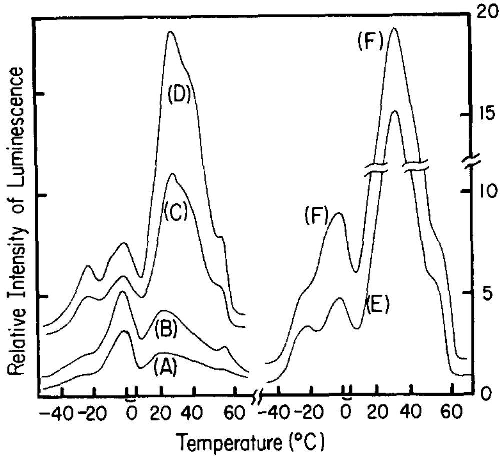

Fig. 1. Effects of heating rate on the thermoluminescence profile of mature spinach leaves. Sample leaves were illuminated at −40 °C, cooled down to −196 °C and then heated at rates of 11, 19, 24, 31, 63 and 85 °C/min for curves A, B, C, D, E and F, respectively. The luminescence intensity was recorded against time and then replotted against the leaf temperature recorded separately.

the figure show the remarkable effects of illumination temperature on the thermoluminescence profile. The profile (curve A) obtained at the highest illumination temperature of $T_i = -10$ °C shows a major luminescence band (denoted as A band) around −7 °C and weak bands above 0 °C. A variety of changes occurred as the illumination temperature decreased. Between −10 and −55 °C, the A band greatly decreased in height, and a strong band (B_1 band) appeared above 0 °C with two shoulders (B_2 and C bands), so that the total photon count below 0 °C decreased by 90 % and the count above 0 °C increased approximately 4-fold. The thermoluminescence bands in this paper are denoted as above according to the nomenclature of Arnold with partial modification; the assignments for designation of the five bands found in the present study are discussed in the Discussion with Table I. The band designated as x in the figure is an artifact due to the melting of the ice. As seen from curve T, the leaf temperature stopped rising until the ice formed in the sample melted. This caused an abrupt drop of emission on the left side of the B_1 band which is shown by an arrow on curve B. We may, therefore, expect a stronger artifact band for a stronger B_1 band such as shown on curve B, and a weaker artifact effect for the weakest B_1 band on curve G.

Qualitatively different changes were found when the illumination temperature was lowered below −55 °C. The B_1 and B_2 bands decreased in height, but the height of the C band does not seem to be much affected, so that a sharp C band appeared on curve G obtained at $T_i = -196$ °C. Furthermore, the Z_v band on curve C observed at −55 °C behaved differently from others. This band shifted progressively to lower temperatures with decreasing illumination temperature, being located slightly higher (10–30°) than the illumination temperature. This band was called Z_v band after Arnold's Z band observed at −155 °C, but subscript "v" was added in order to distinguish this band emitted at variable low temperature from other bands emitted at fixed temperatures. Curve H is the profile obtained by illuminating the sample leaf

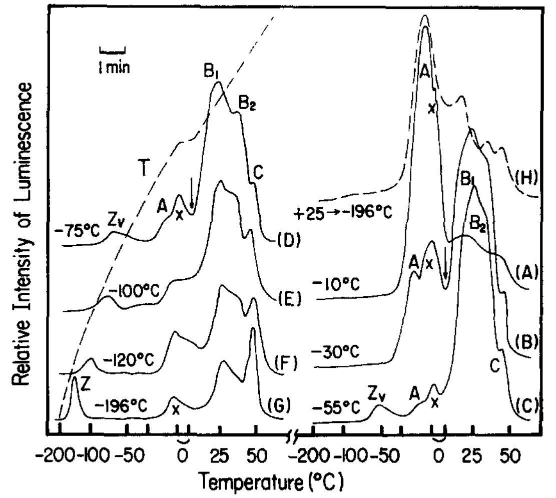

Fig. 2. Thermoluminescence profiles of intact spinach leaves illuminated at different temperatures. Leaves were cooled down to a temperature (T_1 indicated on each curve), illuminated for 1 min with red light (above 630 nm, 600 μW/cm^2), rapidly cooled to −196 °C and then heated at a rate of 30 °C/min to measure the luminescence. The broken curve (T) indicates the leaf temperature recorded against time. Curve H is the profile obtained by continuous illumination of the sample during cooling from room temperature to −196 °C. The five observed bands were denoted as Z_v, A, B_1, B_2 and C bands as indicated on the curves in the figure. The x band is an artifact due to melting of ice.

continuously during cooling from room temperature to −196 °C. The highest band around −15 °C seems to be a composite of the A and x bands, and the three peaks above this band may be assigned as the B_1, B_2 and C bands, respectively. Curve H is similar to curve A obtained at $T_i = -10$ °C. This suggests that the profile obtained after continuous illumination over the wide temperature range from +25 to −196 °C is determined by the storage of light energy between +25 and −10 °C. The storage in this case may be completed around −10 °C.

Fig. 3 shows the effects of illumination temperature on the emission temperatures of these five bands observed in the case of mature spinach leaves. The emission temperatures of the four bands except the Z_v band were independent of the illumination temperature, but the emission temperature of the Z_v band changed almost linearly with the illumination temperature, being higher by 10–30° than the illumination temperature. The emission temperatures of the A, B_1, B_2 and C bands were estimated from these data to be −10, +25, +40 and +55 °C, respectively. It was previously reported by Arnold [3] that the luminescence below −140 °C is charged by blue light but not by red light. However, Sane et al. [10] showed recently that his 118 °K band, which is equivalent to Arnold's Z band, can be charged by red light as well as by blue light. The above experiment for our Z_v band was, therefore, repeated with 650-nm red light from a monochromator or from a He-Ne laser. These red light beams were found to be as effective as the red light through a red glass filter used in most of the present experiments.

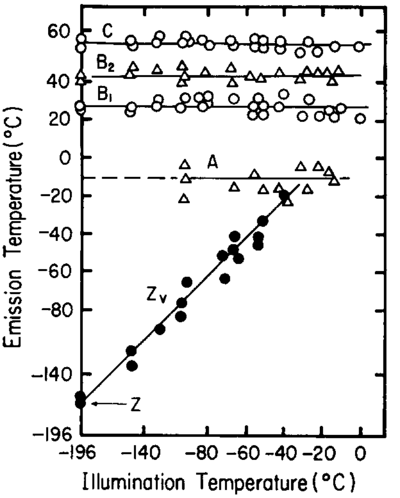

Fig. 3. Relationship between illumination and emission temperatures. Sample spinach leaves were illuminated at a varied temperature and the emission temperatures for the five luminescence bands Z_v, A, B_1, B_2 and C were estimated from the profile and plotted against the illumination temperature (T_i). The experimental conditions were similar to those for Fig. 2.

Thermoluminescence from various leaves and chloroplast preparations

The thermoluminescence profiles of various samples were measured at $T_i = -60\pm5$ °C. Curves A to E in Fig. 4 show the effect of heat treatment on the profile of spinach leaves. The sample leaf dipped in warm water at a desired temperature for 5 min under dim green safety light was kept in darkness for 30 min and then subjected to measurement. The A, B_1, B_2 and C bands were markedly lowered by the treatment at 40 °C and disappeared completely after the treatment at 45 °C (curves B and C). Considering that the heat treatment at 40 °C for 5 min inactivates the oxygen-evolving system specifically [14], some of these bands may be emitted from the system or from its related structures. The Z_v band, on the other hand, was much more resistant to the heat treatment and remained unchanged even after the treatment at 45 °C. When the temperature was raised to 55 °C, all the bands including the Z_v band disappeared completely (curve D). Above 65 °C, however, a weak broad band appeared around 50 °C. This band may result from denatured pigments or proteins.

Broken curves in the same figure show the effects of some inhibitors infiltrated into spinach leaves under vacuum. CCCP (carbonyl cyanide m-chlorophenylhydrazone) at 10 μM and sodium pentachlorophenolate at 50 μM lowered the bands uniformly (curves G and H). 3-(3',4'-Dichlorophenyl)-1,1-dimethylurea, (DCMU), on the other hand, affected the bands in a different manner. With increasing DCMU concentration, the Z_v band first disappeared and then the B_1 and B_2 bands decreased in height. At higher concentrations (e.g. 50 μM, curve F), these bands disappeared almost completely, a new peak appeared at +10 °C and the C band was intensified. This effect of DCMU is similar to that obtained by Rubin and Venediktov [5], who found a shift of their major luminescence peak corresponding to our B_1 band towards lower temperature on treating *Chlorella* cells with DCMU. However, Arnold and Azzi [8] reported that their B band is resistant to DCMU treatment while their A and

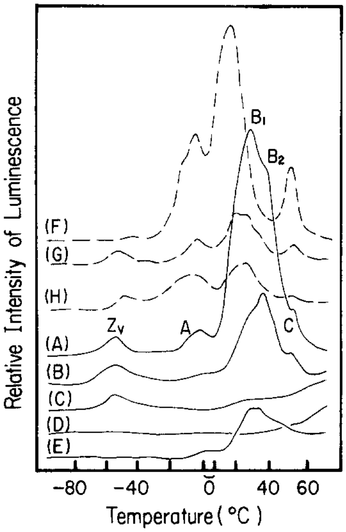

Fig. 4. Effects of treatment with chemicals and heat on the thermoluminescence profiles of intact spinach leaves. Curve A is the profile of a control leaf without treatment and curves B, C, D and E are the profiles obtained after heat-treatment at 40, 45, 55 and 65 °C respectively. Sample leaves were heated in warm water for 5 min and incubated in darkness for 30 min before measurement. Broken curves of F, G and H are the profiles obtained after treatment with DCMU (50 μM), CCCP (10 μM) and sodium pentachlorophenolate (50 μM), respectively. The chemicals were infiltrated into the leaves under vacuum for 10 min at room temperature in darkness.

C bands are sensitive to the treatment, and Shuvalov and Litvin [9] reported that only their component IV (Arnold's B band) is resistant to DCMU treatment.

Fig. 5 shows the profiles of whole chloroplasts isolated from spinach leaves and subchloroplast particles of photosystems I and II. Whole chloroplasts (curve C), possessing the photosystem II water-splitting activity as well as the photosystem I activity, showed the five bands found for intact leaves; but the photosystem II particles (curve A) prepared with Triton X-100, which undergoes the photosystem-II reaction only in the presence of diphenylcarbazide showed distinct Z_v and A bands and a small but distinct C band superimposed on the strong emission above 60 °C due to solubilized chlorophylls; they did not show the two other bands. Addition of diphenylcarbazide (1 mM) to the photosystem-II particles in suspension did not alter the profile of curve A. The profile (curve B) of photosystem-I particles showed strong emission above 60 °C but no bands below 40 °C. A separate experiment on solubilized chlorophylls suggested that the strong emission at the higher temperatures is from free chlorophylls solubilized with Triton X-100 from chloroplasts. The shoulder near 50 °C on the steeply rising curve may be the C band masked with the strong emission. Shuvalov and Litvin [9] stated that the emission band at +20 °C (their component IV) observed for intact leaves originates from photosystem I, based on their observation that the far-red light was more effective in exciting this band. However, the present data show that photosystem-I particles emit no bands except the C band, and that intact wheat leaves greened under intermittent illumination, whose chloroplasts have both photosystems I and II but are specifically lacking in oxygen-evolv-

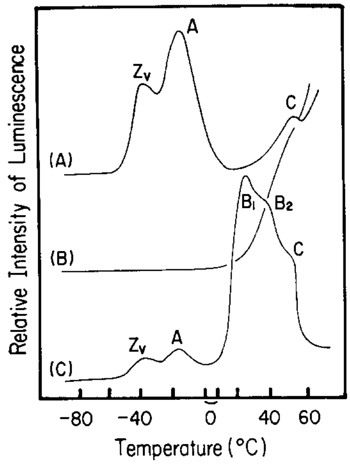

Fig. 5. Thermoluminescence profiles of isolated chloroplasts (curve C) and subchloroplast particles of photosystem I (curve B) and photosystem II (curve A). Spinach chloroplasts were isolated with Tris buffer and the subchloroplast particles were prepared by the treatment of chloroplasts with Triton X-100 according to the method of Vernon et al. [12].

ing activity, show only two bands of Z_v and C. These Z_v and C bands emitted at −50 and +55 °C are considered to be different from component IV of Shuvalov and Litvin [9].

Fig. 6 shows the profile of etiolated wheat leaves and its changes during greening by intermittent or continuous illumination. The profile (curve A) of etiolated

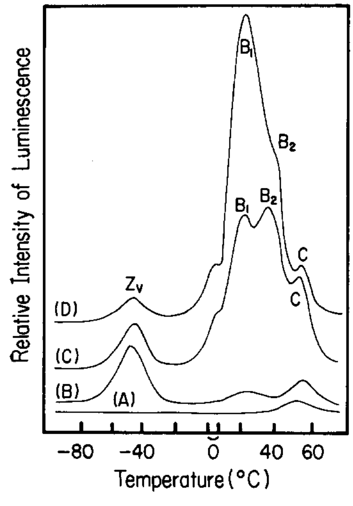

Fig. 6. Thermoluminescence profiles of etiolated wheat leaves before (curve A) and after 24-h greening by continuous illumination with strong white light (750 μW/cm^2, curve D) and by intermittent illumination (1-ms light+5-min dark) with xenon flash light (1.5 · 10^4 ergs/cm^2 per flash, curve B). Curve C is the profile obtained after continuous illumination of the intermittently illuminated leaves with 680-nm red light (2 μW/cm^2) for 5 min. The activities of Ind Cl_2 photoreduction with water as electron donor of the chloroplasts prepared from the leaves for curves B, C and D were 0, 19, 80 μmol Ind Cl_2/mg chlorophylls/h, respectively.

leaves showed a very weak band around 50 °C in the temperature range of the C band. This band was observed often for etiolated and intermittently illuminated wheat leaves without actinic illumination. Intermittent illumination (1-msec light+5-min dark) of etiolated leaves for 24 h changed this profile to curve B, which shows a strong Z_v band and a very weak B_1 band. The C band was slightly enhanced by this intermittent illumination. Continuous illumination of the previously-intermittently illuminated leaves with red light (2 $\mu W/cm^2$) for 5 min intensified the B_1 and B_2 bands (curve C), and prolonged continuous illumination for 1 h brought about further intensification of these bands to yield a profile (curve D) similar to that of mature leaves. As opposed to spinach leaves used in the experiments of Figs 1–4, the A band could not be observed distinctly for wheat leaves, when illuminated at −55 °C (illumination temperature used in the present experiments). However, this A band was observed when wheat leaves were illuminated at a higher temperature of −20 °C. Under these illumination conditions, the Z_v band could hardly be observed.

DISCUSSION

Thermoluminescence from intact leaves, isolated chloroplasts and subchloroplast particles was greatly dependent on the conditions of actinic illumination and on the heating rate. Illumination of the sample at about −60 °C followed by heating at a rate of about 30 °C/min was found to be suitable for distinct separation and identification of various luminescence bands. The measurement of intact mature leaves under these conditions by a sensitive photon-counting technique clearly showed five luminescence bands at −50, −10, +25, +40 and +55 °C, which were denoted as Z_v, A, B_1, B_2 and C bands, respectively. The nomenclature of Arnold [2] was partially modified in this designation to include our new findings.

Arnold and Sherwood [2] found four luminescence bands at −155, −6, +30 and +55 °C for dried chloroplasts and denoted them as Z, A, B and C bands, respectively. These bands, except for the Z band, were interpreted by Arnold [2, 8, 15] as deriving from the recombination of trapped electrons and positive holes produced by illumination in the pigment system. The activation energies required for the recombination based on this interpretation have been calculated by several investigators [3, 7–9]. Shuvalov and Litvin [9] made a similar experiment and found four luminescence components and denoted them as components I, II, III and IV. Component I is a delayed emission with a life time of about 5 ms observed at a wide temperature range between room temperature and −196 °C, and was attributed to the triplet state of chlorophyll *a*. The others are the thermoluminescence from trapped electrons emitted at −160, −15 and +20 °C, respectively. Recently, Lurie and Bertsch [6] observed the thermoluminescence from isolated chloroplasts and found three bands at −10, +25 and +40 °C, which were denoted as peaks 1, 2 and 3, respectively. More recently, Sane et al. [10] measured the thermoluminescence of intact leaves and found five bands, two below and three above 0 °C. They investigated in particular the excitation and emission spectra of their 118 °K peak emitted at the lowest temperature (Arnold's Z band or Shuvalov's component II), and concluded that the band is an emission from the triplet state of chlorophyll *a* in vivo. They also showed that the 118 °K band is excited by red light as well as by blue light. The detailed analysis of these luminescence components was recently made by Lavorel [21]. Table I in the present paper shows the

TABLE I

CORRESPONDENCE OF THERMOLUMINESCENCE BANDS IN THE PRESENT STUDY TO THOSE REPORTED PREVIOUSLY BY VARIOUS INVESTIGATORS

Arnold and Azzi (ref. 8)	Shuvalov and Litvin (ref. 9)	Lurie and Bertsch (ref. 6)	Sane et al. (ref. 10)	Ichikawa et al. (present study)
Z(−155 °C)	Component I (D.E.)* Component II (−160 °C)	–	Peak (118 °K)	Z_v (variable) Z (−160 °C)
A (−6 °C)	Component III (−15 °C)	Peak 1 (−10 °C)	Peak (254 °K)**	A (−10 °C)
B (+30 °C)	Component IV (+20 °C)	Peak 2 (+30 °C) Peak 3 (+40 °C)	Peak (275 °K)** Peak (290 °K)**	B_1 (+25 °C) B_2 (+40 °C)
C (+52 °C)		–	Peak (320 °K)**	C (+55 °C)

* A component of delayed emission with a life time of about 5 ms.

** These temperatures were read on the glow curve in Sane's paper [10].

correspondence between these luminescence bands named differently by various authors [2, 6, 8–10] and by us.

The recent observations by Lurie and Bertsch [6, 7] and by Sane et al. [10] demonstrated the presence of two bands around 30 °C, which seem to be the B_1 and B_2 bands in our experiment. Arnold's B band or Shuvalov's component IV may be a composite of these two bands which could not be resolved at that time. Lurie and Bertsch [6] observed these bands, but did not find the Z and C bands, which is probably due to the difference in illuminating and heating conditions; they illuminated chloroplasts continuously during cooling from 0 to −196 °C and heated them at a rate of 10 °C/s.

The previous data on Arnold's Z band emitted at −155 °C (Shuvalov's component II) seem somewhat confusing. Arnold and Sherwood [2] and Shuvalov and Litvin [9] reported that the band is charged only by blue light. However, the recent observation by Sane et al. [10] showed that the band (118 °K peak) is charged not only by blue light but also by red light. Our experiment supported Sane's observation. The red light through a red filter as well as from an He-Ne laser effectively excited this low-temperature band at liquid nitrogen temperature.

When the sample leaves were excited at temperatures higher than −196 °C, a band was observed at a temperature slightly higher than the excitation temperature. This band was designated as Z_v band in this paper, and was also charged by red light as well as by blue light. Its emission temperature varied between −150 and −20 °C, depending on the excitation temperature as shown by Fig. 3. The emission intensity of our Z_v band was very low as compared with Arnold's Z band; the relative intensities of Arnold's Z band measured after excitation with red and blue light were about 3 and 10 times higher, respectively, than the intensity of our Z_v band excited by red light. Two interpretations may be given to the emission of this Z_v band; (a) considering the result by Sane et al. [10] that the red light is effective in charging Arnold's Z band, together with our result of the low quantum yield for charging our Z_v band at low temperatures above −196 °C, a fraction of Arnold's Z component may be charged at the lower temperatures to emit the Z_v band; (b) judging from the similarity in the variable characteristics of emission temperature, the Z_v band is likely to be a partially stabilized fraction of Shuvalov's component I. The emission spectrum of the Z_v band will determine which of these mechanisms is more likely and also the indentity between the Z and Z_v bands. It must be noted however that some of the characteristics of Arnold's Z band are different from those of our Z_v band; the Z band was resistant to the treatment with heat (100 °C) and to the treatment with DCMU, whereas the Z_v band is sensitive to heat (60 °C, 5 min) and DCMU treatments. Shuvalov's component I was reported to be sensitive to DCMU, and this agrees with the sensitive nature of the Z_v band to DCMU.

The thermoluminescence bands of various samples with and without the water-splitting activity are summarized in Table II. On inactivation of the water-splitting system of spinach leaves by heat treatment at 45 °C for 5 min, the A, B_1, B_2 and C bands disappear. Of these four bands, the B_1 and B_2 bands are absent in the profile of photosystem-II particles prepared with Triton X-100; they are not able to photoreduce Ind Cl_2 unless an artificial electron donor, diphenylcarbazide, is added to the preparation. These two bands are also absent in wheat leaves greened under intermittent illumination; the leaves are devoid of the water-splitting activity [16–18] whereas the reaction centers of the two photosystems have been developed almost completely in these leaves. It may be deduced from these comparative data that the B_1 and B_2 bands originate from the water-splitting site. This deduction is consistent with the previous observation by Lurie and Bertsch [6] that the photosystem-II particles (D-10) with water-splitting activity, prepared by treatment of chloroplasts with digitonin [19], showed three bands corresponding to our A, B_1 and B_2 bands .The process of development of these bands during activation of the latent water-splitting system by continuous illumination of the intermittently illuminated leaves is published elsewhere [20].

The C band seems to be less related to the photosynthetic activity, since the profile of etiolated leaves showed a weak band in the same temperature range. The C band was found for photosystem-II particles and for intermittently illuminated wheat

TABLE II

THERMOLUMINESCENCE BANDS OF VARIOUS SAMPLES OF LEAVES, CHLOROPLASTS AND SUBCHLOROPLASTS PARTICLES

Sample	Luminescence band (emission temperature in °C)				
	Z_v (Variable)	A (−10)	B_1 (+25)	B_2 (+40)	C (+55)
Intact spinach leaves	+	+	+	+	+
Heat-treated spinach leaves (45° C, 5 min)	+	—	—	—	—
Whole chloroplasts	+	+	+	+	+
Photosystem-II particles with Triton X-100	+	+	—	—	+
Photosystem-I particles	—	—	—	—	(+)**
Intact wheat leaves	+	(+)*	+	+	+
Etiolated wheat leaves	—	—	—	—	(?)***
Intermittently illuminated wheat leaves	+	—	—	—	+

* Observed often when the illumination temperature was around −10 °C.

** Observed as a shoulder on a steeply rising emission curve.

*** Observed weakly for the sample leaves without actinic illumination.

leaves, and its height first increased and then decreased during the activation of the latent water-splitting system [20]. The A band is more difficult to characterize. However, there seems to exist a complementary relationship between this A band and the group of B_1 and B_2 bands. The A band was much more marked in the profiles of spinach leaves observed at $T_i = -10$ °C (curve A in Fig. 2) and photosystem-II particles with Triton X-100 (curve A in Fig. 5) in which the B_1 and B_2 bands were very weak or absent whereas the A band was weak in the profiles of spinach leaves observed at $T_i = -55$ °C (curve C in Fig. 2) and whole chloroplasts (curve C in Fig. 5) which show strong B_1 and B_2 bands. A similar complementary relationship was observed between the Z_v band and the group of B_1 and B_2 bands. The Z_v band was marked in the profile of intermittently illuminated wheat leaves lacking the B_1 and B_2 bands, but became progressively weaker as the B_1 and B_2 bands were developed by continuous illumination of the intermittently illuminated leaves. Spectral analysis of the luminescence of these various bands will be fruitful for further characterization of the luminescence bands in relation to the mechanism of energy storage in the pigment systems in photosynthesis.

ACKNOWLEDGEMENTS

The present study was supported by a research grant on Photosynthetic Reaction Centers given by the Ministry of Education, and by a grant for the study of "Life Sciences" at The Institute of Physical and Chemical Research. The authors thank Mrs. T. Hirata, Miss Y. Sugaya and A. Suzuki for their assistance in growing the plants and in preparing the manuscript.

REFERENCES

1 Strehler, B. L. and Arnold, W. (1951) J. Gen. Physiol. 34, 809–820
2 Arnold, W. and Sherwood, H. (1957) Proc. Natl. Acad. Sci. U.S. 43, 105–114
3 Arnold, W. and Sherwood, H. (1959) J. Phys. Chem. 63, 2–4
4 Arnold, W. (1966) Science 154, 1046–1049
5 Rubin, A. B. and Venediktov, P. S. (1969) Biofisika, 14, 105–109
6 Lurie, S. and Bertsch, W. (1974) Biochim. Biophys. Acta 357, 420–428
7 Lurie, S. and Bertsch, W. (1974) Biochim. Biophys. Acta 357, 429–438
8 Arnold, W. and Azzi, J. R. (1968) Proc. Natl. Acad. Sci. U.S. 61, 29–35
9 Shuvalov, V. A. and Litvin, F. F. (1969) Molek. Biol. USSR 3, 59–73
10 Sane, P. V., Tatake, V. G. and Desai, T. S. (1974) FEBS Lett. 45, 290–294
11 Inoue, Y., Kobayashi, Y., Sakamoto, E. and Shibata, K. (1974) Physiol. Plant., 37, 228–232
12 Vernon, L. P., Shaw, E. R. and Ke, B. (1966) J. Biol. Chem., 17, 4104–4109
13 Inoue, Y., Kobayashi, Y., Sakamoto, E. and Shibata, K. (1975) Plant Cell Physiol. 16, 687–695
14 Katoh, S. and San-Pietro, A. (1967) Arch. Biochem. Biophys. 122, 144–152
15 Arnold, W. (1965) J. Phys. Chem. 69, 788–791
16 Michel, J. M. and Sironval, C. (1972) FEBS Lett. 27, 231–234
17 Remy, R. (1973) Photochem. Photobiol. 18, 409–416
18 Ichikawa, T., Inoue, Y. and Shibata, K. (1975) Plant Sci. Lett. 4, 369–376
19 Anderson, J. M. and Boardman, N. K, (1956) Biochim. Biophys. Acta 112, 403–421
20 Inoue, Y., Ichikawa, T., Kobayashi, Y. and Shibata, K. (1975) in Proceedings 3rd International Congress on Photosynthesis. (Avron, M. ed.), pp. 1833–1840, Elsevier Scientific Publishing Company, Amsterdam
21 Lavorel, J. (1975) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 223–317, Acad. Press, New York