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PHOTOSYNTHETIC ENERGY CONSERVATION INVESTIGATED BY THERMOLUMINESCENCE

ACTIVATION ENERGIES AND HALF-LIVES OF THERMOLUMINESCENCE BANDS OF CHLOROPLASTS DETERMINED BY MATHEMATICAL RESOLUTION OF GLOW CURVES

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Summary

Thermoluminescence of isolated chloroplasts was analysed by a computer-assisted multicomponent curve fitting procedure to determine the activation energies, the free energies of activation, frequency factors and half-lives of the component bands of the glow curve. Optimal fit was obtained in the temperature region from −80°C to +80°C by the resolution of the glow curve into seven bands with peak positions at −24, −12, +12, +17, +28, +44 and +69°C.

All of the activation free energies of the thermoluminescence bands were much higher than 0.59 eV, the minimum free energy of activation required for the back reaction of the primary charge separation as calculated on the basis of the theory of Ross and Calvin (Ross, R.T. and Calvin, M. (1967) Biophys. J. 7, 595—614).

The high free energies of activation and long half-lives (longer than 50 ms) of the thermoluminescence bands suggest that thermoluminescence in the temperature region from −80°C to +80°C does not reflect the charge recombination of primary products but represent the reversal of subsequent stabilization steps of

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Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; E, activation energy; ΔF, free energy of activation; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; s_0, frequency factor; $t_{1/2}$, half-life; T_m, temperature at the maximum of a thermoluminescence band.

the charge separation process which proceed along the acceptor and donor sides of Photosystem II.

Introduction

Thermoluminescence is generated by the recombination of positive and negative charges accumulated during energy conservation in the photosynthetic process [1—3]. The activation energy for thermoluminescence is the measure of the stabilization energy of the photoreaction [4,5]. The minimum amount of free energy of activation (the loss of energy during energy conservation) can be estimated on the basis of the theoretical calculation of Ross and Calvin [6]. A red photon at 680 nm represents 1.82 eV and the maximum free energy stored in the Photosystem II is 1.23 eV, (cf. Ref. 8), hence the minimum free energy of activation cannot be less than 0.59 eV. A similar value, 0.57 eV, was obtained by Malkin for the minimum activation energy calculated from delayed luminescence measurements [7].

Since more than one component of the electron transport chain is involved in the energy storage, several bands appear in the glow curve [2,4,9—11]. To determine the parameters of these bands (activation energy, frequency factor and amplitude) it is necessary to fit a theoretical equation containing these parameters to the entire glow curve [4,12,13]. This is a difficult procedure because of the overlapping of the bands. Arnold and Azzi [14] calculated the activation energy from the peak positions of the thermoluminescence bands using a formula given by Randall and Wilkins [12]. Since the frequency factor was taken from the thermoluminescence of inorganic crystals, the meaning of their activation energies (0.53, 0.60 and 0.64 eV) is uncertain. Shuvalov and Litvin [15] estimated the activation energies (0.35 and 0.90 eV) from Arrhenius plots of the rising side of the glow bands. The experimental error of the low thermoluminescence values measured at the rising side of the bands also makes the activation energies obtained by this method uncertain. Lurie and Bertsch attempted to resolve the glow curve into three overlapping bands according to rigorous theoretical considerations [4]. They calculated the activation energies (0.48, 0.57 and 0.80 eV) and frequency factors from the temperatures at the maximum and half-maximum intensities of the separated bands. However, it has been shown recently that the glow curve consists of more than three bands [2,3,11] and therefore, the conversion of peak position into activation energy is inaccurate due to the uncertainty in the number and positions of the bands. One must be also careful in accepting activation energies determined by temperature-jump thermoluminescence measurements because of the large degree of overlapping of the bands in the glow curve [5,16]. The shortcomings of the methods mentioned above result in unreliable values for the activation energies and several of these energies do not satisfy the limit of the minimum activation energy, 0.57 eV, estimated by Malkin from delayed luminescence measurements [1,3,4,14,16].

In this paper, we have considered all of the experimental points of the glow curve using a mathematical curve resolution technique in the determination of the possible number of thermoluminescence bands, the related activation ener-

gies, free energies of activation, frequency factors and half-lives. The high free energies of activation and the long half-lives of the bands indicate that thermoluminescence in the temperature region from —80° C to +80° C does not reflect the charge recombination of primary products but represents the reversal of subsequent stabilization steps of the charge separation process which proceed along the donor and acceptor sides of Photosystem II (for definition of stabilization steps see Ref. 17).

Theory

Determination of activation energy, free energy of activation, frequency factor and half-life

The first theoretical treatment of thermoluminescence was given by Randall and Wilkins for inorganic crystals [12]. In this theory the equation for an isolated thermoluminescence band had a form:

$$I = -c\frac{dn}{dt} = cnk_A \tag{1}$$

where I is the instantaneous thermoluminescence intensity, n is the number of electrons in the traps, c is a proportionality factor and k_A is the rate constant of electron detrapping. The temperature dependence of the rate constant was considered by a relation similar to that found empirically by Arrhenius for the temperature dependence of the rate constant of chemical reactions:

$$k_A = s\exp(-E/\boldsymbol{k}T) \tag{2}$$

where s is the frequency (or pre-exponential) factor, E is the trap depth (namely the Arrhenius activation energy, which is temperature independent), T is absolute temperature and $\boldsymbol{k}$ is Boltzmann's constant.

The modern theory of reaction kinetics makes it possible to give a thermodynamic interpretation of the rate constant [18].

$$k_A = \frac{\boldsymbol{k}}{h}T\exp(-\Delta F/\boldsymbol{k}T) \tag{3}$$

where h is Planck's constant, ΔF is the free energy of activation. In this equation, which was given by Eyring [18] the exponential term now involves a free energy of activation rather than a temperature independent activation energy. By definition

$$\Delta F = E - T\,\Delta S \tag{4}$$

where E is the activation energy, ΔS is the entropy of activation. From Eqns. 3 and 4

$$k_A = s_0 T\exp(-E/\boldsymbol{k}T) \tag{5}$$

where

$$s_0 = \frac{\boldsymbol{k}}{h}\exp(\Delta S/\boldsymbol{k})$$

is the frequency factor.

The substitution of Eqn. 5 into Eqn. 1 results in an equation which can be applied for the characterization of thermoluminescence bands originating from the back reactions of photosynthesis.

$$I = -c\frac{dn}{dt} = cns_0T\exp(-E/kT) \tag{6}$$

Introducing the temperature, T, as variable, instead of the time, t, into Eqn. 6:

$$I = -cB\frac{dn}{dT} = cns_0T\exp(-E/kT) \tag{7}$$

Holding the heating rate, B, constant the solution of Eqn. 7 gives:

$$I = cn_0s_0T\exp(-E/kT)\exp\left[-\int_{T_0}^{T}\frac{s_0}{B}T'\exp(-E/kT')\,dT'\right] \tag{8}$$

where n_0 is the initial number of trapped electrons, T_0 is the initial temperature. After approximating the integral in Eqn. 8 according to the method of Grossweiner [13] the thermoluminescence intensity is

$$I = AT\exp\left[(-E/kT) + \frac{s_0k}{BE}T^3\exp(-E/kT)\right] \tag{9}$$

where

$$A = cn_0s_0\exp\left[\frac{s_0k}{BE}T_0^3\exp(-E/kT_0)\right]$$

In the case of $E/kT > 25$ (e.g. $E > 0.7$ eV, $T = 300$ K) the error in Eqn. 9 (due to the approximation) is less than 5% on average.

Since the glow curve of chloroplasts is the superposition of several thermoluminescence bands, in order to determine the parameters of these bands (namely A proportionality factor, E activation energy and s_0 frequency factor) it is necessary to simultaneously fit the whole glow curve with the sum of thermoluminescence bands described by Eqn. 9. It is a great advantage of the multicomponent curve fitting method that the activation energies and frequency factors of the component bands can be obtained directly as a result of the fitting procedure. Once E and s_0 are determined the entropy of activation can be estimated from the frequency factor:

$$\Delta S = k\ln\frac{h}{k}s_0 \tag{10}$$

and the free energy of activation is given by Eqn. 4. The half-life of the state ($t_{1/2}$) can also be calculated for the appropriate temperature:

$$t_{1/2} = \frac{\ln 2}{s_0T}\exp(E/kT) \tag{11}$$

The method applied in our paper for the mathematical analysis of thermoluminescence possesses two advantages in comparison with previous methods.

(a) In determining the parameters of thermoluminescence bands the multi-

component curve fitting procedure was applied, taking into consideration all of the experimental points in the calculations. We can assume, therefore, that this method gives a more reliable estimation of the parameters than those previously published.

(b) As a result of the calculation not only the activation energies but the free energies of activation could also be determined for each of the thermoluminescence bands.

However, even this method considers only one route of decay of luminescence precursors; non-radiative recombination and decay to other products are neglected.

Consideration of the phase transition of water in correction of the experimental glow curve

The influence of the solid-liquid phase transition of water at about 0°C on the shape of an individual thermoluminescence band was examined in a model experiment. A single thermoluminescence band was calculated from Eqn. 9 holding the heating rate, B, constant. In this case, phase transition was not considered (Fig. 1A). The effect of phase transition on the glow curve can be simulated if the temperature dependence of B, determined experimentally, is considered in Eqn. 7. In this case, the differential equation could be only solved by numerical integration [19]. As shown in Fig. 1A, the phase transition creates an artificial peak in the glow curve at about 0°C. Curves similar to those calculated mathematically were measured experimentally with chloroplasts suspended in glycerol or water. To obtain a single band in the region of phase transition the chloroplasts were treated with DCMU (Fig. 1B). On the basis of these results it can be concluded that the solid-liquid phase transition of water creates an artificial peak in the glow curve and this fact has to be taken into account in the multicomponent curve fitting.

Materials and Methods

Intact chloroplasts were isolated from mesophyll protoplasts of maize (*Zea mays* KSC 360) prepared by enzymatic digestion [20]. The chloroplasts were resuspended in a medium containing 0.4 M (+)-sorbitol, 10 mM NaCl, 1 mM $MnCl_2$, 5 mM $MgCl_2$, 2 mM EDTA, 0.4% bovine serum albumin and 50 mM Hepes at pH 7.5 [21], and were diluted to a chlorophyll concentration of 170 μg/ml. 0.6 ml aliquots of the suspension were used for the measurement of thermoluminescence. The measurement of glow curves was carried out in the temperature region from −80°C to +80°C using an apparatus similar to that described by Tatake et al. [22]. The light emission of the samples was measured by a red-sensitive photomultiplier (EMI 9558B) and the signal was amplified through a home-made differential amplifier and fed to a Philips PM 8120 X-Y recorder. The temperature of the sample holder was monitored using a platinum resistor thermometer placed below the samples. Samples were illuminated with white light from a NARVA halogen lamp of 650 W for 5 min during continuous cooling from +20°C to −80°C. The exciting light was passed through a heat-absorbing water filter (thickness 10 cm) and a Balzers neutral density filter giving an illumination intensity of 10 $W \cdot m^{-2}$. For the best resolu-

tion of peaks the rate of heating was 10°C per min, as used by Sane et al. [2].

In the mathematical curve resolution the method of damped least squares was applied to resolve the overlapping bands [23]. The individual bands were described by Eqn. 9 (see Theory) and the initial values of activation energies and frequency factors were calculated from the experimental glow curves either by the method of Arrhenius or of Grossweiner [4]. The number of the components was taken from earlier experimental results [11]. The program modified the input parameters of the bands to obtain the optimal fit of their sum to the glow curve and the fit was characterized by the sum of the squares of deviations of the data points from the calculated fit. In our calculation this sum was less than 10^{-2} for 50 points of the glow curve. This means that the maximum error of the fit was less in average than 1% in each data point.

Results

The mathematical resolution of the glow curve of maize mesophyll chloroplasts is shown in Fig. 2. The glow curve was decomposed into seven bands, with peak positions at −24, −12, +12, +17, +28, +44 and +69°C. These bands are designated in this paper as bands at −20, −10, +10, +20, +30, +45 and +70°C. The effect of high temperature on the photomultiplier had to be corrected for, due to a noticeable elevation of the background signal above 70°C. In the mathematical resolution of the glow curve, this was accomplished by applying an extra band (unrelated to the photosynthetic back reactions referred to in this paper) in the high temperature region of the glow curve. Curve resolutions obtained with various input parameters showed that the glow curve could not be fitted well with the six bands observed in earlier experiments

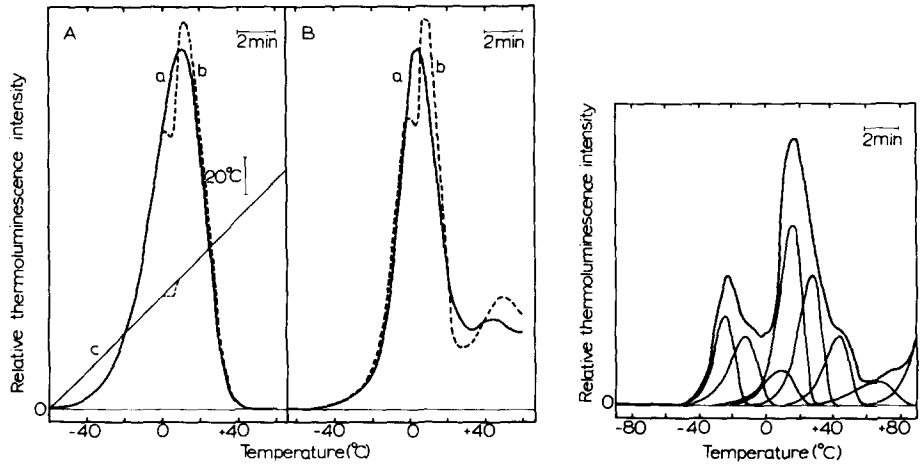

Fig. 1. The influence of the solid-liquid phase transition of water on the shape of an individual thermoluminescence band. (A) Mathematical simulation of a single thermoluminescence band. (a) no phase transition (solid line); (b) phase transition between 0 and 5°C (dashed line); (c) the temperature of the sample in case (a) (solid line), and (b) (dashed line). (B) Experimentally measured glow curve of DCMU-treated chloroplasts measured: (a) in 65% glycerol; (b) in 50 mM Hepes buffer, pH 7.5.

Fig. 2. Mathematical curve resolution of the glow curve of maize mesophyll chloroplasts. The mathematical methods applied in the curve resolution and the measuring conditions are described in Materials and Methods.

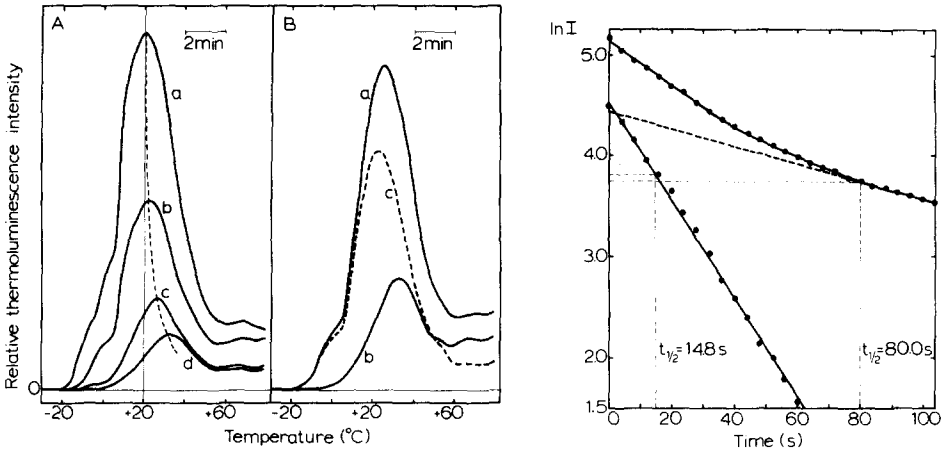

Fig. 3. The experimental demonstration of the in vivo existence of two thermoluminescence bands in the temperature region from +15 to +35°C. (A) Shift in the peak position of the main band induced by thermal cleaning. Excited samples were heated up to −30°C (a); −10°C (b); +15°C (c) and +25°C (d). After recooling the samples to −80°C the glow curves were recorded without further excitation. (B) Resolution of the main band at +20°C into two components, (a) glow curve after thermal cleaning at +10°C; (b) glow curve after thermal cleaning at +25°C and (c) Δ (a − b).

Fig. 4. Resolution of the decay of thermoluminescence intensity measured at +25°C into two exponentials. Thermoluminescence was measured as described in Materials and Methods, however the heating was stopped at +25°C and the decay of thermoluminescence intensity was recorded vs. time.

[11]. Consideration of a new band at about +30°C was required to obtain a fairly good fit. The in vivo existence of this band was established essentially as described in Ref. 24. The excited samples were heated up to different temperatures (−30, −10, +15 and +25°C) and cooled down again to −80°C. The glow curves recorded without further excitation showed a continuous shift in the position of the main band suggesting the presence of two bands in the temperature region from +15 to +35°C (Fig. 3A). Substraction of any two members of the series of glow curves obtained by this thermal cleaning process indeed confirms the presence of two thermoluminescence bands in the temperature region from +15 to +35°C (Fig. 3B). The exact peak positions of these two hidden bands cannot be determined experimentally by the method of thermal cleaning because of the overlapping of these bands with the band appearing at +45°C.

Our conclusion as to the existence of two bands in the temperature region from +15 to +35°C was also supported by the measurement of decay of thermoluminescence intensity at +25°C. Fig. 4 shows that the decay can be resolved into two exponential components with half-lives of 14.8 s and 80 s. On the basis of these results we assume that the glow curve of chloroplasts consists of seven bands in the temperature region from −80°C to +80°C.

The mathematical resolution of the glow curve of chloroplasts inhibited by DCMU and DBMIB is shown in Fig. 5. Since the glow curve measured after DCMU treatment (Fig. 5A) is strongly distorted around 0°C due to the solid-liquid phase transition of water, the experimental points in the temperature region between −2°C and +15°C were not used in the curve fitting procedure. The parameters of the main band (peak position at +7°C) were, therefore, eva-

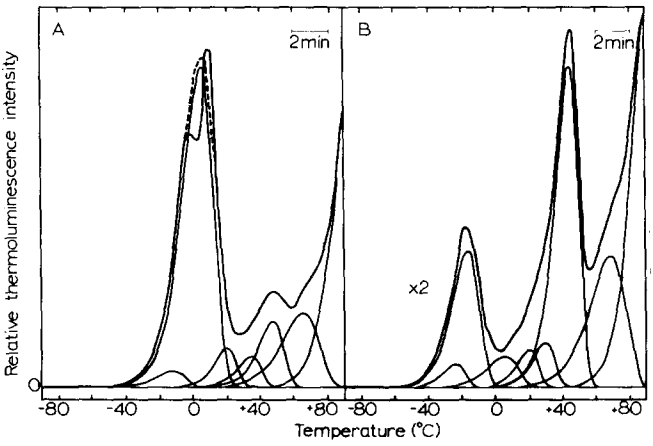

Fig. 5. Mathematical curve resolution of the glow curves of DCMU- and DBMIB-treated chloroplasts. (A) 10 μM DCMU. Solid lines represent the experimentally measured glow curve and the thermoluminescence bands obtained from the mathematical curve resolution. In the temperature region from −2°C to +15°C the dashed line illustrates the sum of the component bands. (B) 5 μM DBMIB. Other measuring conditions are as in Materials and Methods.

luated from the correct points of the glow curve. The sum of the bands obtained from the curve resolution resulted in an envelope which was not distorted by phase transition (Fig. 5A, dashed line). It can be seen in Fig. 5A, that DCMU abolished the band at −20°C and considerably increased the peak at +10°C. The glow curve of DBMIB-treated chloroplasts contained all of the bands present in the thermoluminescence of untreated chloroplasts and the bands at −10°C and +45°C were enhanced (Fig. 5B). Both DCMU and DBMIB treatment considerably decreased the bands at +20°C and +30°C and thus their contribution to the total thermoluminescence intensity was negligible. These very small bands may have originated from chloroplasts unaffected by the inhibitors and we may assume that these two bands would not be present after complete inhibition. In the presence of inhibitors (DCMU and DBMIB) the elevation of the signal at high temperatures was more pronounced than in the case of untreated chloroplasts. We may assume that, besides the effect of high temperature on the photomultiplier (black body radiation), the light emission appearing at higher temperatures may have been related to the rising side of the glow band found above 100°C by Arnold and Sherwood [25] and by Tumerman et al. [26]. In our curve resolutions the band at 70°C is distorted by this high temperature glow, however, the other component bands are not influenced (Fig. 5A and B).

The characteristics of the thermoluminescence bands obtained from the resolution of glow curves are given in Table I. The parameters of the individual bands were not significantly influenced by DCMU and DBMIB treatment. From this fact we conclude that the bands which are present in the glow curves of DCMU- and DBMIB-treated chloroplasts are the same, as in that of the untreated ones.

The activation energies given in Table I are higher than the activation energy calculated by Malkin for the back reaction of primary separated charges (0.57

TABLE I

CHARACTERISTICS OF THE INDIVIDUAL THERMOLUMINESCENCE BANDS OBTAINED FROM THE MATHEMATICAL RESOLUTION OF THE GLOW CURVES

The meaning of symbols is given in Abbreviations on p. 140.

Designation of peaks	T_m (°C)	E (eV)	ΔF (25°C) (eV)	s_0 (s^{-1})	$t_{1/2}$ (T_m) (s)	$t_{1/2}$ (25°C) (s)
Control chloroplasts						
−20	−24	0.81	0.69	$2.5 \cdot 10^{12}$	33	0.05
−10	−12	0.68	0.77	$6.9 \cdot 10^{8}$	44	0.9
+10	+12	0.72	0.82	$3.7 \cdot 10^{8}$	44	11.0
+20	+17	1.04	0.83	$8.5 \cdot 10^{13}$	33	11.0
+30	+28	1.12	0.87	$4.1 \cdot 10^{14}$	32	50.0
+45	+44	1.16	0.93	$1.6 \cdot 10^{14}$	38	605.0
+70	+69	0.89	0.98	$5.1 \cdot 10^{8}$	39	4770.0
DCMU-treated chloroplasts						
−20	—	—	—	—	—	—
−10	−12	0.67	0.76	$6.8 \cdot 10^{8}$	34	0.81
+10	+7	0.72	0.81	$7.3 \cdot 10^{8}$	35	5.0
+20	+21	1.11	0.85	$5.7 \cdot 10^{14}$	30	16.0
+30	+34	1.15	0.89	$4.8 \cdot 10^{14}$	36	137.0
+45	+48	1.17	0.94	$1.7 \cdot 10^{14}$	30	841.0
+70	+66	0.88	0.98	$4.5 \cdot 10^{8}$	52	3660.0
DBMIB-treated chloroplasts						
−20	−24	0.81	0.69	$2.3 \cdot 10^{12}$	32	0.05
−10	−15	0.66	0.75	$7.2 \cdot 10^{8}$	38	0.5
+10	+7	0.73	0.81	$8.7 \cdot 10^{8}$	36	6.0
+20	+20	1.10	0.83	$9.0 \cdot 10^{14}$	22	10.0
+30	+30	1.14	0.87	$7.9 \cdot 10^{14}$	27	56.0
+45	+45	1.17	0.93	$2.7 \cdot 10^{14}$	28	530.0
+70	+67	0.88	0.98	$4.5 \cdot 10^{8}$	60	4810.0

eV [7]) and the free energies of activation considerably exceed the minimum free energy of activation, 0.59 eV, estimated on the basis of the theory of Ross and Calvin [6]. This suggests that the photosynthetic processes represented by our activation energies cannot be related to the charge recombination of primary products.

It is known from delayed luminescence measurements that the charge recombination of the primary products of photosynthesis begins immediately after the primary photochemical act and that, within the μs time region, several electron transport steps follow each other so increasing the activation energy along the recombination path [7,17]. Thus the long half-lives of the bands calculated for +25°C (Table I) support the conclusion that thermoluminescence in the temperature region from −80°C to +80°C does not reflect the charge recombination of primary products but represents the reversal of subsequent stabilization steps at the donor and acceptor sides of Photosystem II.

It has been suggested recently by Sane et al. that the light emission associated with their thermoluminescence bands IV and V appearing around +25°C and +45°C, respectively, contributed to the 10 ms component of delayed luminescence of Photosystem I at high temperatures [27]. The thermoluminescence bands found by our curve resolution procedure at +28°C and +44°C may corre-

spond to the bands IV and V of Sane et al. [27]. Since the half-lives of these bands calculated at +45°C (at which temperature the maximum intensity of the 10 ms component of delayed luminescence was observed by Sane et al.) are 3.0 s and 33.0 s, respectively, we can conclude that in contrast to the suggestion of Sane et al. [27] the thermoluminescence bands appearing at +28 and +45°C do not contribute to the 10-ms component of delayed luminescence of Photosystem I.

At +25°C all the processes related to electron transport are much shorter than the half-lives of bands at +45°C (605 s) and +70°C (4770 s). It has been reported that the very long-lived delayed luminescence (from several minutes to hours) might be considered as oxygen-dependent chemiluminescence [7,28]. On the basis of the long half-lives of the bands at +45°C and +70°C we can assume that these two thermoluminescence bands might be generated by a similar process but it is not actually a true reversal of the photosynthetic events.

The accuracy of the data presented in Table I was experimentally confirmed. The values of the half-lives calculated from the decay of thermoluminescence intensity measured at the peak positions of the bands are in good agreement with the half-lives obtained from curve resolution (Table II). The results shown in Fig. 4 provide further evidence as to the accuracy of the data of Table I. The measured values of half-lives of two overlapping bands at +25°C (14.8 s and 80 s) are approximately equal with the calculated half-lives of the bands appearing at +17 and +28°C (11 s and 50 s).

Fleischmann [29], Crofts et al. [30] and others have suggested a mechanism for millisecond-delayed luminescence of chloroplasts. According to this theory, photosynthetic electron transport establishes a proton motive force across the membrane and this electrochemical potential gradient lowers the activation energy for charge recombination resulting in delayed fluorescence.

The response of millisecond-delayed luminescence to ionophores and uncouplers was in good qualitative agreement with the proposed model [31,32].

To find out whether our activation energies are decreased by the proton motive force (0.28—0.38 eV [30]) we investigated the effect of ionophores on thermoluminescence. As can be seen in Fig. 6 neither valinomycin nor gramicidin which are known to abolish the transmembrane potential, or nigericin, which diminishes the pH gradient, influenced the shape of the glow curve to

TABLE II

COMPARISON OF EXPERIMENTALLY MEASURED AND THEORETICALLY CALCULATED HALF-LIVES OF CERTAIN BANDS OF THE GLOW CURVE

Individual bands for the measurement of half-lives were obtained by the addition of inhibitors.

Designation of bands	Peak positions (°C)	Half-lives at the peak positions of the bands (s)		Treatment
		Measured	Calculated	
−10	−15	42	38	5 μM DBMIB
+10	+7	33	35	10 μM DCMU
+45	+45	37	28	5 μM DBMIB

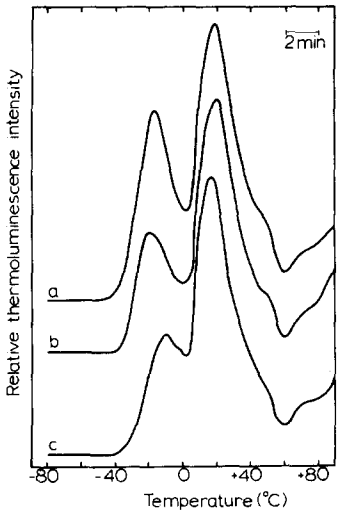

Fig. 6. The effect of ionophores on the thermoluminescence of isolated maize mesophyll chloroplasts. (a) 1 μM valinomycin; (b) 0.17 μM gramicidin; (c) 1 μM nigericin. Each sample contained 10 mM KCl.

any significant degree. This disagreement with earlier experimental results obtained by delayed luminescence can be explained by the experimental conditions used for thermoluminescence measurements. In our experiments, the heating rate was relatively low, namely 10 K/min. Therefore, the high energy state of the membrane had already decayed at the beginning of the slow heating process and thus our activation energies are real because they are not decreased by the proton motive force.

The thermoluminescence band at −20°C was enhanced relative to the control (Fig. 2), by the addition of valinomycin and decreased in the presence of nigericin (Fig. 6). These effects of ionophores on the amplitude of the thermoluminescence band at −20°C (half-life 50 ms) cannot be explained at present.

Discussion

It has been shown previously that thermoluminescence may be usefully applied for the investigation of photosynthetic energy conservation [3–5,14, 15,29]. As regards energy conservation, the conclusion obtained from thermoluminescence measurements was that the bands of glow curve represent the light-emitting back reactions of the primary photochemical processes [4,5,14, 15]. However, the relatively high activation energies and long half-lives related to the thermoluminescence bands suggest that thermoluminescence in the temperature region from −80 to +80°C does not reflect the charge recombination of primary products but represents the back reactions of subsequent stabilization steps of the charge separation process proceeding along the acceptor and donor sides of Photosystem II.

Comparison of the free energies of activation obtained from thermoluminescence with the free energies of activation calculated from the midpoint potentials of the different redox couples of the electron transport chain pro-

vides an opportunity to relate the thermoluminescence bands to electron transport components. It can be assumed that the oxidation-reduction potential of the S_4 state of the water-splitting enzyme is approximately equal to (or higher than) the midpoint potential of the H_2O/O_2 couple, +0.82 V [30], whereas that of Q/Q^- is about −0.25 V [33,34]. Thus the redox span between these couples corresponds to at least 1.07 eV of the free energy conserved during the photosynthetic process. The loss during energy conservation, namely the free energy of activation for thermoluminescence calculated from this redox span, is 0.75 eV. This value is approximately equal to our lowest activation free energies (0.69 and 0.77 eV). Therefore, we can conclude that one of the thermoluminescence bands appearing below 0°C may be related to the charge recombination between the positively charged S_4 state and Q^-. This conclusion is in agreement with the suggestion of Inoue and Shibata that the cation pool for the band with a peak position at −10°C is related to the S_4 state [3].

The different values for the oxidation-reduction potential of *P*-680 available in the literature [8,35] prevent unambiguous determination of the midpoint potentials of the lower S states [36]. For this reason the correlation of the other bands of TL with the different redox couples of the electron transport chain requires further investigation.

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