DETERMINATION OF ACTIVATION ENERGIES AND HALF-LIVES OF THERMOLUMINESCENCE BANDS OF CHLOROPLASTS APPLYING THE METHOD OF MULTICOMPONENT CURVE RESOLUTION

I. VASS, G. HORVÁTH+, T. HERCZEG† and S. DEMETER+,*

Department of Theoretical Physics, József Attila University, H-6722 Szeged, +Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged and *†Department of Biophysics, József Attila University, H-6720 Szeged, Hungary*

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1. Introduction

Thermoluminescence probably results from a thermal recombination of positive and negative charges accumulated during photosynthetic quantum conversion [1–4]. The intensity of the emitted light as a function of temperature reflects the amount of energy stored in the early steps of photosynthesis. Because more than one component of the electron transport chain is involved in the energy storage, several bands appear in the glow curve [2–5].

The free energies of activation calculated from the bands of the glow curve represent the energy deficit between the free energy of the absorbed photon and the conserved energy. A photon having a wavelength of 680 nm represents an energy quantum of 1.82 eV. The maximum amount of free energy stored is 1.23 eV/photon absorbed by photosystem II [6]. This means that the minimum free energy of activation calculated from TL cannot be <0.59 eV. A similar value (0.57 eV) was obtained for the minimum activation energy calculated from delayed luminescence measurements [7]. Surveying the activation energies of TL bands published earlier we note that only a few fulfill this requirement [2,8,9] while most of them are too low and thus energetically unacceptable [1–3,9–11]. On the other hand, activation energies for certain bands are not available in the literature.

Here, we have determined the possible number of TL bands, the related activation energies, free energies of activation, frequency factors and half-lives using a mathematical curve resolution technique. The high free energies of activation and the long half-lives of the bands indicate that TL at –80 – +80°C does not reflect the reversal of the primary charge separation but represents the reversal of subsequent stabilization steps of the charge separation process which proceed along the acceptor and donor sides of photosystem II.

2. Materials and methods

Intact chloroplasts were obtained from enzymatically isolated mesophyll protoplasts of maize using the method in [12]. Chloroplasts were resuspended in a medium containing 0.4 M d-sorbitol, 10 mM NaCl, 1 mM.$MnCl_2$, 5 mM $MgCl_2$, 10 mM EDTA, 0.4% bovine serum albumin and 50 mM Hepes (pH 7.5) and were diluted to 170 μg chl/ml.

Glow curves were measured at –80 – +80°C using an apparatus similar to that in [13]. Samples were illuminated with white light at 10 W/m^2 for 5 min during continuous cooling from +20 – –80°C. For best resolution of the peaks the rate of heating was 10°C/min as in [4].

In the mathematical curve resolution of the overlapping bands of glow curves the method of damped least squares estimation was applied [14]. The equation for an individual band was taken from the method

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Abbreviations: TL, thermoluminescence; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid; Q, the primary acceptor of photosystem II; T_m, temperature at the maximum of the TL band; *E*, activation energy; ΔF, free energy of activation; s_0, frequency factor; $t_{1/2}$, half-life; chl, chlorophyll

* To whom reprint requests should be addressed

in [15] based on the model in [16] taking into account the linear temperature dependence of the pre-exponential factor, $s = s_0 . T$, where s_0 was the frequency factor and T was absolute temperature [17]. The program modified the input parameters of the bands (activation energy, frequency factor and amplitude) to obtain the optimal fit of their sum to the glow curve. The input parameters were calculated from the measured glow curves either by Arrhenius's or by Grossweiner's method [2]. The number of the components was taken from [5]. The activation energies and frequency factors of the component bands were obtained using the mathematical fitting procedure [14]. After the activation energy and frequency factor had been determined, the entropy of activation was estimated from the frequency factor, and the free energy of activation, ΔF, was calculated from $\Delta F = E - T\,\Delta S$ where E was the activation energy, ΔS was the entropy of activation and T was absolute temperature [17].

3. Results and discussion

The mathematical resolution of the glow curve of maize mesophyll chloroplasts is shown in fig.1. The glow curve was decomposed into 7 bands, with peak positions at −24, −12, +12, +17, +28, +44 and +69°C. An extra band was applied above 70°C in the curve resolution to consider the effect of high temperature on the photomultiplier. Curve resolutions obtained with various input parameters showed that the glow curve could not be fitted well with the 6 bands experimentally observed [5]. Consideration of a new band at ~+28°C was required to get a fairly good fit. The in vivo existence of this band was also proven by the method of thermal cleaning in [18] as follows. The excited samples were heated up to −30°C, −10°C, +15°C and +25°C and cooled down again to −80°C without further excitation. The glow curves represented a continuous shift in the position of the main band suggestion the presence of two bands at +15 – +35°C (fig.2A). Subtraction of any two members of the series of glow curves obtained by this thermal cleaning process indeed confirms the presence of two TL bands at +15 – +35°C (fig.2B). 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. On the basis of these results we conclude that the glow curve of chloroplasts consists of 7 bands at −80 – +80°C.

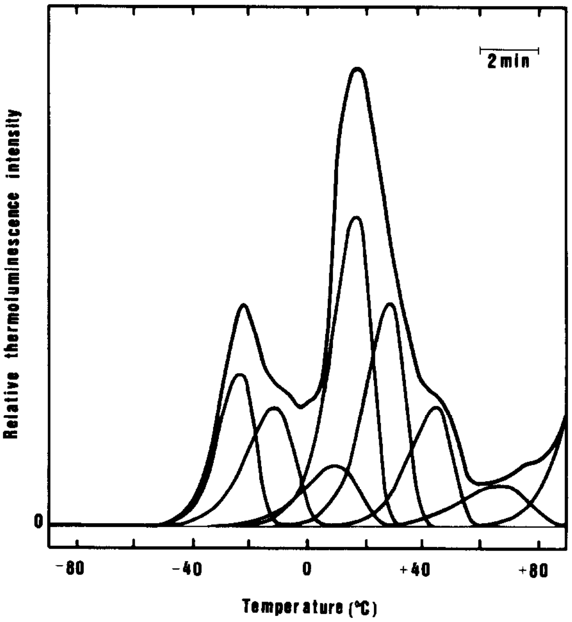

Fig.1. Mathematical curve resolution of the experimentally measured glow curve of maize mesophyll chloroplasts. The maximum error of the mathematical fit was <5% in each data point.

The characteristics of the bands are given in table 1. The half-lives of the individual bands were calculated from the activation energies and frequency factors according to [2] with the exception that in our calculations the temperature dependence of the pre-exponential factors was also considered.

The activation energies are much higher than the activation energy estimated [7] for the reversal of the primary charge transfer act (0.57 eV). The activation energies of the TL bands cannot be less than this value. Most of the activation energies in [1–3, 9–11] did not fulfill this requirement. These activation energies were calculated from the peak positions and geometrical factors of the bands or from the Arrhenius plot of the rising side of the bands. Moreover, the conversion of peak position into activation energy is inaccurate due to the uncertainty of the number and positions of the bands. The Arrhenius method is not satisfactory because only the initial part of each glow band is used in the calculations. In contrast to these methods we considered the whole glow curve in the calculation of activation energies.

The maximum amount of free energy stored in the

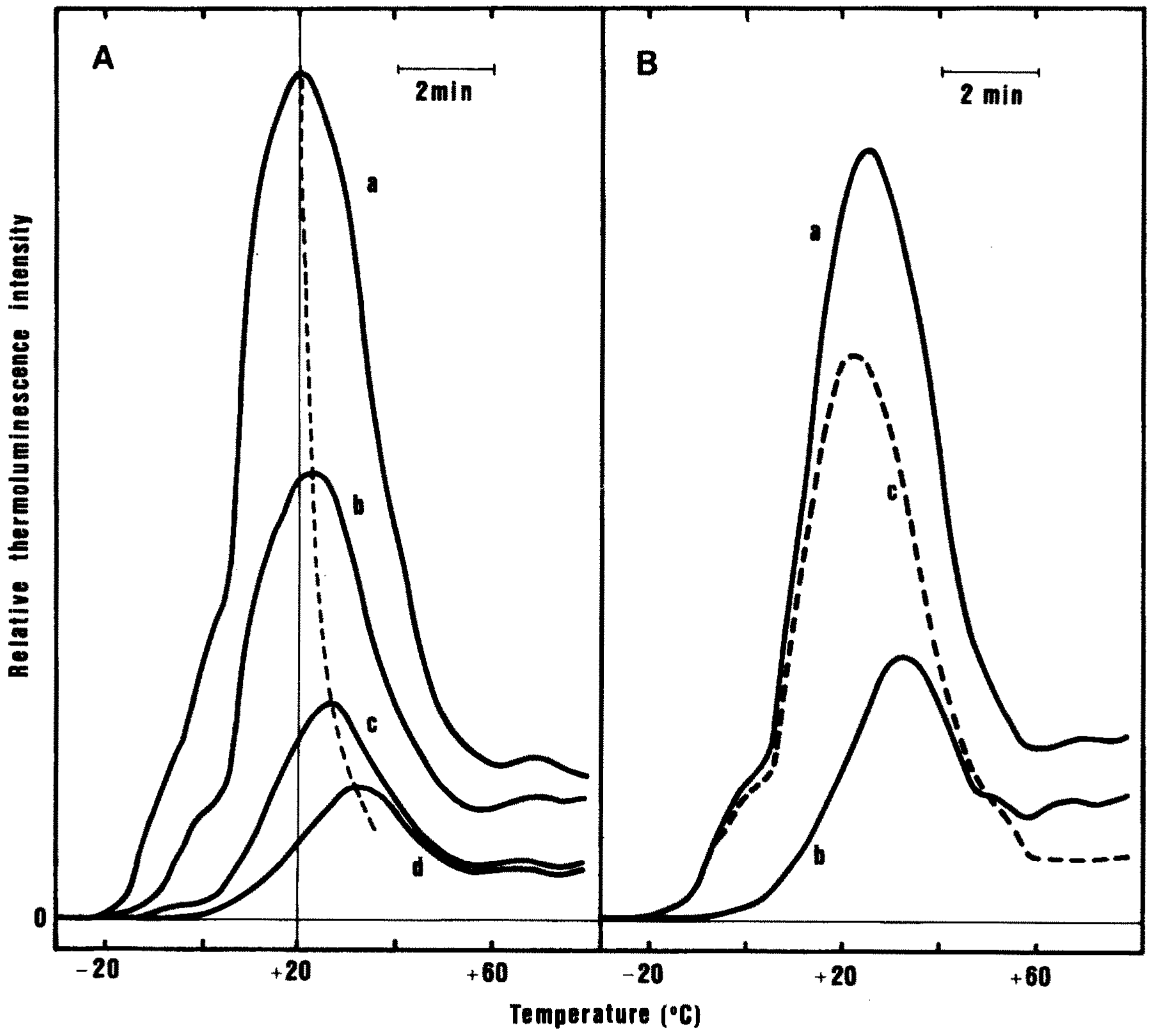

Fig.2. The experimental demonstration of the in vivo existence of two TL bands at +15 – +35°C (A) Shift in the peak position of the main band induced by thermal cleaning. Excited samples were raised to −30°C (a); −10°C (b); +15°C (c) and +25°C (d). After recooling the samples to −80°C without further excitation, the glow curves were recorded. (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) (a) minus (b).

primary charge separation is 1.23 eV according to theoretical calculations [6] and it is 1.30 eV as determined from delayed luminescence measurements [19]. The free energies of activation calculated from both values (0.59 eV, 0.52 eV) are lower than our lowest free energy of activation (0.69 eV). Thus we conclude 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 stabilization steps follow each other increasing the activation energy along the recombination path [7,20]. Thus the long $t_{1/2}$ of the TL bands calculated for +25°C (table 1) support the conclusion that TL at −80 – +80°C does not reflect the back reaction of the primary charge separation but represents the reversal of the subsequent stabilization steps at the donor and acceptor sides of photosystem II (for the definition of stabilization

Table 1
Characteristics of the individual thermoluminescence bands obtained from the mathematical resolution of the glow curve

Designation of the bands	T_m (°C)	E (eV)	ΔF (25°C) (eV)	s_0 (s^{-1})	Half-life, $t_{1/2}$ (s) 0°C	25°C	T_m °C
−20	−24	0.81	0.69	2.5×10^{12}	1.1	0.05	33
−10	−12	0.67	0.77	6.9×10^{8}	1.1×10	0.9	44
+10	+12	0.73	0.82	3.7×10^{8}	1.7×10^{2}	11.0	44
+20	+17	1.04	0.83	8.5×10^{13}	4.7×10^{2}	11.0	33
+30	+28	1.12	0.87	4.1×10^{14}	2.9×10^{3}	50.0	32
+45	+44	1.16	0.93	1.6×10^{14}	4.1×10^{4}	605.0	38
+70	+69	0.89	0.98	5.1×10^{8}	1.2×10^{5}	4770.0	49

The meaning of the symbols is given in *Abbreviations*

steps see [20]). At +25°C all the processes related to electron transport are much shorter than the $t_{1/2}$ of the bands at +45°C (605 s) and +70°C (4770 s). The very long-lived delayed luminescence (from several minutes to hours) may reflect oxygen dependent chemiluminescence [7]. On the basis of the long $t_{1/2}$, we can assume that the TL bands appearing at +45°C and +70°C might originate from a process similar to that proposed for long-lived delayed luminescence.

The accuracy of the data presented in table 1 was experimentally confirmed. As an example, the decay of TL intensity measured at +17°C resulted in $t_{1/2}$ ~35 s (fig.3A) in accord with the calculated value of 33 s (table 1). Samples excited and kept in the dark at 0°C for 1 min did not exhibit bands below 0°C (fig.3B) and after 40 min dark incubation only the bands over +20°C could be seen (fig.3B). These results are in agreement with the $t_{1/2}$ calculated for 0°C (table 1).

Comparison of free energies of activation obtained

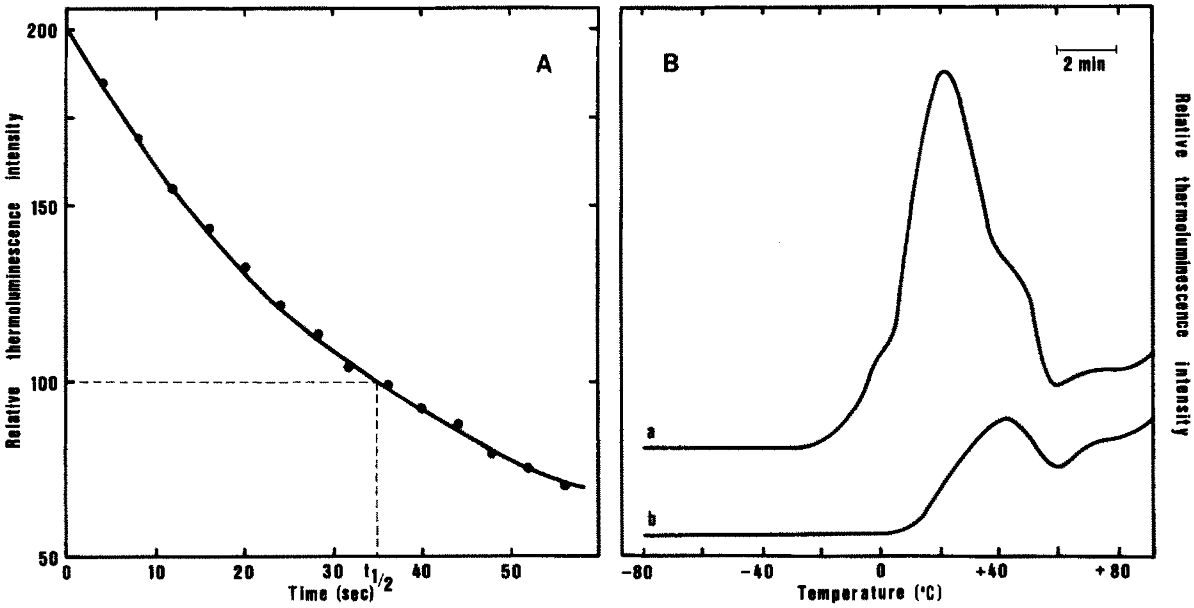

Fig.3. Experimental test of the theoretically calculated half-lives. (A) Decay of thermoluminescence intensity measured at +17°C; (B) Glow curves of samples excited at 0°C for 1 min. After excitation samples were kept in dark (a) 1 min; (b) 40 min, cooled down to −80°C and measured as in section 2.

from TL with the free energies of activation calculated from the midpoint potentials of the different redox couples of the electron transport chain provides an opportunity to relate the TL 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 $\gtrsim$+0.82 V, the midpoint potential of the H_2O/O_2 couple, whereas that of Q/Q^- is ~–0.25 V [21]. Thus the redox span between these couples corresponds to $\geqslant$1.07 eV of the free energy conserved during the photosynthetic process. The loss during energy conservation, namely the free energy of activation for TL calculated from this redox span, is 0.75 eV. This value is approximately equal with our lowest free energies of activation (0.69 eV,0.77 eV). Therefore we can conclude that one of the TL bands appearing below 0°C might be related to the charge recombination between the positively charged S_4 state and Q^-. This conclusion is in agreement with the suggestion [3] that the cation pool for the band with peak positions at –10°C related to the S_4 state. The different values for the oxidation–reduction potential of P680 available in [19,22] do not allow an unambigous determination of the midpoint potentials of the lower S states [23]. For this reason, correlation of the other bands of TL with the different redox couples of the electron transport chain requires further investigation.

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