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KINETIC CHARACTERIZATION OF T-JUMP THERMOLUMINESCENCE IN ISOLATED CHLOROPLASTS

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SUMMARY

Luminescence triggered by temperature jump in lettuce chloroplasts was measured at different final temperatures in the range of 30–70 °C. The initial temperature was 20 °C. This type of luminescence is suggested to result from a reaction of recombination of precursors formed during the preillumination period. The rate constant for this second order reaction was derived and shown to be proportional to the ratio between the luminescence peak and the total luminescence integral. An Arrhenius plot for these rate constants yields the value of 0.68 ± 0.08 eV for the activation energy. This value is compared with those reported by other investigators and a mechanism in terms of the semi conductor concepts is proposed for luminescence.

INTRODUCTION

Chloroplasts emit a pulse of light if after a prior irradiation they are subjected to a sudden temperature increase (T-jump thermoluminescence)[1]. Thermoluminescence was also demonstrated previously by several workers[2–4] by preirradiation at low temperatures followed by subsequent relatively slow warming at constant rate. In this case the luminescence was recorded as a function of temperature (glow curve). In the glow curve a number of peaks which are indicative of various metastable states of different energies were observed. There is a transition at particular temperature values from these states to restore the chlorophyll excited state. The primary process leading to luminescence is presumably the acquiring of sufficient thermal energy to reach the chlorophyll excited state energy level.

The nature of the metastable states was not defined with certainty. It is unlikely that they represent long lived electronically excited states, since their life times are too long at the conditions in which they are formed. A likely assumption is that they represent a pair of oxidized and reduced moieties formed by the first act of photosynthesis. Whether these are the primary electron donor which is oxidized and the electron acceptor which is reduced, as suggested by Lavorel[5], or rather more transitory forms: an electron solvated in the pigment medium and a "hole" (possibly chlorophyll$^+$), as Arnold and Azzi[2,6] suggested, remains still to be decided. The occurrence of several peaks in the glow curve makes the latter assumption likely; the different energies are due to different electron traps formed in the heterogeneous pigment system. On the other hand, the assumption of defined chemical entities will give only

one energy and hence only one peak in the glow curve. Rubin and Venediktov[3], however, have extended the idea of Lavorel in assuming that possibly several secondary acceptors with different potentials that react back with the oxidized primary electron donor could give different peaks in the glow curve. In this case each electron carrier is associated with a different activation energy.

There is a disagreement in the literature with regard to the positions of the peaks in the glow curves[2–4]. Moreover, the conversion of peak position into activation energy is inaccurate. Arnold and Azzi[6] used the formula by Randall and Wilkins[7] which contains two parameters, the frequency factor and the activation energy, in one equation. The activation energy could be estimated only roughly, from an arbitrary plausible value given to the frequency factor. Hence, one cannot have much confidence in the glow curve data for exact determination of activation energies, except perhaps for qualitative conclusions. Shuvalov and Litvin[4] presented a very detailed analysis of activation energies by dividing the decay curve of delayed light into five components, analysing each component according to a first-order decay. However, it was shown that the decay of delayed light may be better described by a second-order decay kinetics, at least for the range between 10 ms and 1 s (refs. 8, 9). Also, no account is made for the efficiency of producing luminescence (*cf.* Theoretical) as function of temperature.

The T-jump is superior to the glow curve method to investigate the kinetics and the activation energy of the process of emission, since the temperature is constant during the emission process. The initial luminescence peak obtained by a T-jump is proportional to the rate of the decay of the luminescence precursors, and its change with temperature yields a measure of the activation energy. We have found, however, that the total emission (integrated over the time) changes in a significant manner at various temperatures and for different samples. One way to account for this is to introduce an efficiency parameter for the creation of excited states by the luminescence generating reaction. With this efficiency parameter, we obtained a consistent account of the kinetic data, and a unique value for the activation energy of 0.68 ± 0.08 eV, for different sets of experiments.

A similar work, in principle, was outlined by Jursinic and Govindjee[10], for a more limited temperature range in 3-(3,4-dichlorophenyl)-1,1-dimethylurea-treated chlorella. Starting from the fact that the decay curve of the delayed light is second order (in the square root of the luminescence), they drew an Arrhenius plot of the decay rate constant *versus* temperature. Since the rate constant for luminescence decay is an artificial quantity related to the real rate constant of recombination by a square-root relation (*cf.* Theoretical), their calculated value for the activation energy (approx. 0.36 eV) must be multiplied by a factor of 2 (*cf.* Appendix). Also, there is no account of possible change of the excited-state formation yield (expressed by the total luminescence integral) at different temperatures, which we show here to be important.

EXPERIMENTAL

The apparatus and procedure used for performing the T-jump experiments and recording the data were described before[11,12]. The essential characteristics were as follows:

A sample of 1 ml of lettuce chloroplast suspension (50 μg chlorophyll) was irradiated for a short period (t_I=0.5 s; I approx. $150 \cdot 10^{-9}$ Einstein$\cdot$cm$^{-2}\cdot$s^{-1}). After a certain dark period (t_D approx. 0.5 s), 1 ml of hot water (max. 90 °C) was rapidly injected. The time of injection and mixing was about 0.1 s. Following this injection a pulse of emission is observed. The final temperature was calculated according to the standard thermometric calculation. An experimental calibration of the final temperature which agreed with the calculated values was also performed. This shows that there is almost no heat loss from the cuvette during the first seconds after injection when emission is observed. In one run of experiments the volumes were 0.25 ml chloroplasts and 0.75 ml hot water, which allowed a wider range of T-jump.

THEORETICAL

It was previously shown[10,12] and will be confirmed here (*cf.* Results) that the decaying part of the light emission pulse induced by a T-jump behaves like a second order reaction in $\sqrt{L}\cdot L$ is the momentary emission intensity. This result might be rationalized by assuming a second order reaction of the precursors formed during the preillumination and is symbolized by the following equations:

$$A + B + Chl \rightarrow (A + B) + Chl^* \rightarrow (A + B) + Chl + h\nu \qquad (i)$$

Chl=chlorophyll. A and B may be charged species which recombine, *e.g.* solvated electrons and "holes"*:

$$Chl^+ + e^- \rightarrow Chl^* \rightarrow Chl + h\nu \qquad (ia)$$

Reaction i is too slow at room temperature and the emission is weak. Its rate is accelerated considerably at the final temperature T_f followed by a stronger emission. There is a build up of the emission during the heating period. In the following analysis we shall assume that the build up time is negligible compared to the time of decay, and will take the zero time at the position of the luminescence peak. At t=0 the the concentration c of the precursors A or B is c_0 (assuming [A]=[B] which is determined by the preillumination conditions and, therefore, is independent of T_f). The decay of A (or B) is given by:

$$-\frac{dc}{dt} = kc^2 \qquad (1)$$

hence:

$$\frac{1}{c} = \frac{1}{c_0} + kt \qquad (2)$$

$$L = -\gamma\frac{dc}{dt} = \gamma kc^2 \qquad (3)$$

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* We do not imply any specific model of luminescence. Some authors accept Lavorel's[5] view that luminescence is generated by the back reaction of Z^+ and Q^- (*cf.* Introduction). We have reasons not to accept this hypothesis *a priori*, and make the more general assumption of the existance of electrons and "holes" in the pigment matrix (*cf.* also ref. 13). The relevancy of these species to the photosynthetic electron transport is not clear, but they may be formed in the preillumination either in a main photosynthetic reaction or in a side reaction.

where γ is a proportionality factor which includes: (a) the efficiency, α, in which the singlet excited state of chlorophyll is formed as a result of the recombination reaction; (b) the efficiency, Φ, of emission from the excited state; (c) a geometrical factor, G, of the apparatus which also takes into account the units in which L is expressed. Obviously $\gamma = \alpha \cdot \Phi \cdot L$. γ may depend on several unknown features of the system. It may be temperature dependent and also may differ from sample to sample. We assume, however, that it is time independent*.

The initial (at $t=0$) value of L, L_0, is given from Eqn 3 by

$$L_0 = \gamma k c_0^2 \tag{4}$$

From Eqns 2 and 3 we obtain that L decays in a second-order kinetics with respect to $\sqrt{L}$:

$$\frac{1}{\sqrt{L}} = \frac{1}{\sqrt{L_0}} + \sqrt{\frac{k}{\gamma}}\, t \tag{5}$$

We define k_{exp} as the experimental second-order constant for the decay of $\sqrt{L}$

$$k_{exp} = \sqrt{\frac{k}{\gamma}} \tag{6}$$

The total integrated emission is given by

$$\mathscr{L} = \int_0^\infty L\mathrm{d}t = \int_0^\infty -\gamma \frac{\mathrm{d}c}{\mathrm{d}t} = \int_{c_0}^0 -\gamma \mathrm{d}c = \gamma c_0 \tag{7}$$

The following simple analysis follows when we wish to evaluate k and γ from a given experiment. We solve k and γ from Eqns 4 and 7. The result is:

$$\gamma = \frac{\mathscr{L}}{c_0} \tag{8}$$

$$k = \frac{L_0}{\mathscr{L} c_0} \tag{9}$$

Both γ and k are expressed on a relative basis only, because of the unknown c_0. From Eqns 6, 8 and 9 it follows that:

$$k_{exp} = \sqrt{\frac{L_0}{\mathscr{L}}} \tag{10}$$

It is sufficient to use any two of the Eqns 8–10 in order to obtain the parameters γ and k. The third equation must be fulfilled automatically if Eqn 5 is obeyed. Relation 10 is in fact obtained directly from Eqn 5 by integration. It may be used for cross checking the measurements of k_{exp}, or for evaluating L from the linear plot of Eqn 5 without the necessity of numerical integration, or area measurements.

For evaluating the activation energy of Process i one ought to plot k, as obtained from Eqn 9, as a function of temperature, according to the Arrhenius equation. The plot of L_0 alone may give rise to erroneous results if γ changes considerably with T.

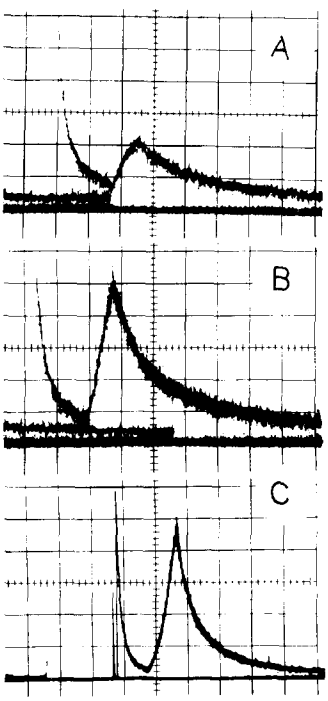
* We did not check the quantum yield of fluorescence emission and its possible change during the T-jump procedure (*cf.* ref. 14).

The plot of k_{exp} *vs* T has no direct meaning. For constant γ it will give an apparent activation energy which is half of the true value, since k_{exp} (for constant γ) is proportional to $\sqrt{k}$ and $\sqrt{k}=\sqrt{k_0}\exp(-{}^1/_2\, E/RT)$.

RESULTS

Fig. 1 represents a few oscilloscopic pictures of the T-jump emission. Usually, as the temperature increases the peak value, L_0, increases too and the decay kinetics becomes faster. This is due to a combined effect of k and γ.

Fig. 2 shows that the total integrated luminescence, $\mathscr{L}=\int_0^\infty L\ dt$, is not constant as a function of T. In many samples there is a tendency toward a saturation at high temperatures, but this is not observed in all samples. From Eqn 8 the variation of $\mathscr{L}$ reflects directly the variation of γ. $\mathscr{L}$ was measured by graphical integration, and also computed in relative units by use of Eqn 9. Both methods gave proportional answers, as required by the second-order behaviour of $\sqrt{L}$ (*cf.* below).



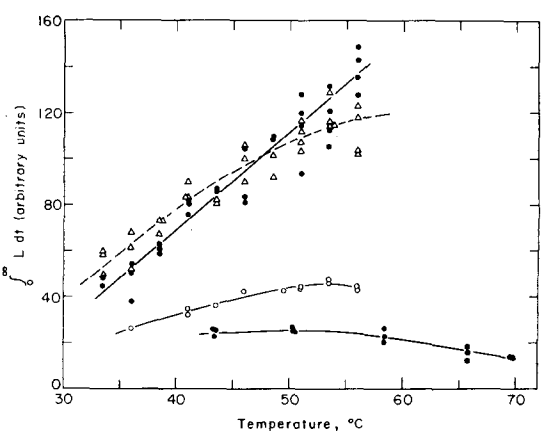


Fig. 1. Time course of T-jump induced luminescence at three different temperatures. The chloroplasts suspension at 22 °C was injected manually with 1 ml hot water. Final temperature: (A) 33.5 °C; (B) 43.5 °C; (C) 56 °C. Time scale 0.5 s/division. Sensitivity in C is smaller by a factor of 2 than in A and B. Preceding the luminescence signal in each picture, the delayed light emission is also observed.

Fig. 2. Total integrated luminescence *vs* final temperature. The different curves are for experiments in different chloroplast preparations on different days. In the experiment represented by the points (○) 1st curve from bottom, the chloroplasts volume was 0.25 ml and that of the hot water 0.75 ml. Other details as in Experimental.

Fig. 3 shows that the decay of L is second order in $\sqrt{L}$ at different temperatures, at least up to one second after the peak, in accordance with Eqn 4. We thus confirmed our earlier results[12] and also the results of Jursinic and Govindjee[10].

Fig. 4 shows an Arrhenius plot of k, calculated from Eqn 9. Since c_0 is assumed to be constant it is sufficient to take the parameter $L_0/\mathscr{L}$ (*cf.* Eqn 9) which is proportional to k. In this figure we actually plotted lg $(L_0/\mathscr{L})$ *vs* $1/T$. For different samples we obtained similar values for the activation energy in spite of the differences of behaviour between the samples (as shown, *e.g.* by the behaviour of $\mathscr{L}$ *vs* T). The activation energy value is about 14 ± 1 kcal/mole $=0.6\pm0.04$ eV. The error estimation is for unsystematic sources and sample variations. When we consider also systematic sources of error (*cf.* below) the value of the activation energy is increased and is estimated to be 16 ± 2 cal/mole (0.68 ± 0.08 eV).

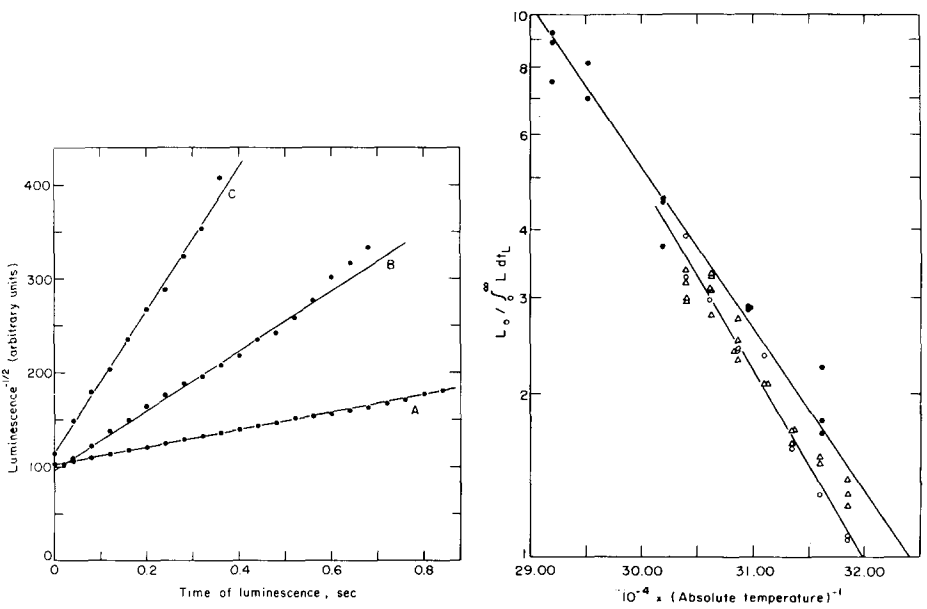


Fig. 3. Time course of T-jump luminescence, expressed as $1/\sqrt{L}$, at three different temperatures. Final temperature: (A) 36 °C; (B) 46 °C; (C) 56 °C. Other details, as in Experimental.

Fig. 4. Log $(L_0/\mathscr{L})$ *vs* $1/T$. $L_0/\mathscr{L}$ is proportional to k, the recombination rate constant as shown in Eqn 9. The different symbols represent the results of three different experiments. The full circles represent the experiment in which 0.25 ml chloroplast suspension was injected with 0.75 ml hot water. Other details as in Experimental.

DISCUSSION

A basic fact arising from this work is that the kinetic form of the luminescence decay is pure second order (in $\sqrt{L}$) overall the temperature range investigated. This means that we deal essentially with a unified process in the temperature range investigated (34–70 °C). In considering the variation in the total integrated emission we could perhaps account for increased emission by operation of an additional mecha-

nism at higher temperatures. This is ruled out on the basis of the above statement and also because of the fact that sometimes the total emission decreases rather than increases with temperature.

The emission observed by us must correspond, at the range of temperature where it is emitted, to the glow curve peak at about 50 °C observed by Rubin and Venediktov[3], or to the peak at 36 °C observed by Arnold and Azzi[2,6] (these differences may be due to different rates of heating). All the results are probably confined to this one type of emission.

In the framework of molecular solid state concepts[6] we could think of three essential steps: (a) Untrapping the electrons into the conjuction level or to levels where they can diffuse easily. One has to consider also the back reaction of electron retrapping. Step a is essentially an equilibrium reaction. (b) Diffusion of the recombining species. (c) Mutual approach of the recombining species and recombination. This may be summarized by the following equations:

$$e^-_{\text{trapped}} \rightleftharpoons e^-_{\text{untrapped}} \text{ (Step a)} \tag{ii}$$

$$e^-_{\text{untrapped}} + \text{Chl}^+ \rightarrow \text{Chl}^* \text{ (Steps b and c)} \tag{iii}$$

The formation of Chl* becomes second order if Reaction iii is slow enough compared to Reaction ii. Only in this case the perturbation caused by Reaction iii to Reaction ii is small and Reaction iii limits the overall rate. In such a case one may write for Reaction ii the Boltzmann expression:

$$[e_{\text{untrapped}}] = [e^-_{\text{trapped}}] \exp(-E/RT) \approx e^-_{\text{total}} \exp(-E/RT) \tag{11}$$

The recombination rate would be proportional to:

$$[e^-_{\text{untrapped}}] \cdot [\text{Chl}^+] \approx [e^-_{\text{total}}] \exp(-E/RT) \cdot [\text{Chl}^+] \approx [e^-_{\text{total}}]^2 \exp(-E/RT) \tag{12}$$

since $[e^-_{\text{total}}] = [\text{Chl}^+]$.

If one accepts the hypothesis originated by Lavorel[5] that the recombining species are Z^+ and Q^-, the oxidized electron donor and the reduced electron acceptor of Photosystem II, it is necessary to add the assumption that at least one of the recombining species must be diffusable. Otherwise the recombination must be within a pair Z^+Q^- (geminate recombination) which will give rise to first order kinetics[12]. Therefore we prefer the more general idea of electrons and "holes". Our analysis is nevertheless applicable to any type of mobile precursors, which react according to a second order law.

It is seen from the results (Fig. 2) that the function γ *vs* T as reflected by $\mathscr{L}$ *vs* T does not behave in a reproducible manner. This reflects the fact that quantum yields of photochemical and photophysical processes are sensitive function of the molecular arrangements, and of presence of impurities, which in the complex system of chloroplasts are difficult to control reproducibly. However, the energetics of the process which is much less sensitive to the above factors, is indeed reproducible.

The value of about 0.68 eV obtained reflects probably the stabilization energy of the recombining species compared to the energy of excited chlorophyll of 1.8 eV. The difference of about 1.12 eV is sufficient to drive the primary reactions of electron

transport. Hence this value is also consistent with the assumption that the recombining species may be the primary products of photosynthesis.

We cannot compare directly this value of the activation energy with the reported values from glow curves, because, as we stated in the introduction, these values are only rough estimates, and are based on a guess for the frequency factors. Still some values in the literature do come close to ours. Arnold and Azzi[6] give the values: 0.54, 0.60 and 0.64 eV for all the glow curve peaks at -6, $+30$, $+52$ °C correspondingly. The data of Jursinic and Govindjee[10] is complex and indicates the occurrence of several processes. Nevertheless, one of their numbers for activation energy, which is in the relevant range, 20–35 °C, if multiplied by 2, as we have shown is necessary, comes close (within 6%) to our value (0.72 and 0.67 eV, *cf.* Table in the Appendix; This does not take into account the factor γ). Therefore, the numerical value of the activation energy may be significant and universal for all photosynthetic systems.

It must be stressed, however, that the present analysis is applicable only to the main T-jump pulse. We did not analyse the tail of the emission, which probably behaves in a different way[15]. It is our view[12], that many mechanisms and probably different precursors may exist, which give rise to the various luminescences; this may apply possibly to the main T-jump pulse (in the range of a few seconds) and the tail (in the range of many seconds or even minutes[15]).

The possibility that γ may be time dependent, especially through the variation of Φ represents some difficulty. In future work we intend to measure the fluorescence emission during the T-jump procedure, in order to ascertain our assumptions. Another objection is the possible influence of the various oxidation states of the primary election donor (which causes oscillations of luminescence after excitation by flashes[16], which may decay in an heterogeneous way during the T-jump. Because of the above objections our conclusions in the present paper cannot be regarded as final.

A possible source of systematic error

In principle the method of T-jump should give a consistent and accurate answer for the activation energy. However, there is one experimental drawback which is the finite time period to reach the final temperature. In our case it was not negligible, especially not at the higher temperatures where the decay was quite fast. We estimated that up to about 25% of the total emission was emitted during the rise period to the peak, which introduces a similar uncertainty in c_0, at the time where the luminescence peak is achieved ($t=0$). Since we assumed the constancy of c_0 for a given sample at all temperatures this is the main factor causing a systematic error in the result. The following consideration gives an estimate for the error in the activation energy. From this consideration it seems that our value is too low by less than 2.4 kcal/mole (about 17% of our value). We cannot estimate the actual error, but only its maximal limit.

To see this we use the formula which gives the activation energy E, using data obtained at two different temperatures:

$$E = R \ln(k_1/k_2)/(1/T_2 - 1/T_1)$$

From Eqn 9 k is inversely proportional to c_0. We took c_0 as constant and omitted it from Arrhenius equation. If c_0 is not constant, but less within say 25%, as estimated above, k should be bigger by 33% or less. If both k_1 and k_2 in the above equation have

the same relative error the ratio k_1/k_2 would not be affected and hence also not the activation energy. A maximal error in E would be obtained if we allow an error in one of the k's and assume that the other has got the correct value. Since the error is large at higher temperatures where the emission decay is faster, the correct procedure is to assume an error Δk in k_1, at the higher temperature T_1. The corresponding error in E, ΔE is given by:

$$\Delta E = R \ln(1+\Delta k/k_1)/(1/T_2 - 1/T_1)$$

Substituting for T_2 and T_1, the edges of our temperature range ($1/T_2 = 31.7 \cdot 10^{-4} K^{-1}$, $1/T_1 = 29.2 \cdot 10^{-4} K^{-1}$) and the above estimated value for $\Delta\ k/k_1$ (0.33), one gets approximately $\Delta E = 2.4$ kcal which gives a systematic error of about 17%. On this basis the true value may be higher than cited before and reach value of about $16.5 \pm$ 1 kcal/mole. To allow for both systematic and unsystematic errors we may give a round figure for the activation energy as 16+2 kcalmole (0.68+0.08 eV).

APPENDIX (Received February 8th, 1973)

TABLE

CORRECTED ACTIVATION ENERGIES FROM DATA OF JURSINIC AND GOVINDJEE[10]

After Govindjee, personal communication.

(*A*) *From their Fig. 12*

Temperature range (°C)	*Corrected values* (*eV*)
5–10	0.87
10–20	0.08
20–35	0.72

(*B*) *From T-jump (their Table 2)*

Initial temperature (°C)	*Final temperature* (°C)	*Corrected activation energy* (*eV*)
2	5	1.12
2	10	0.92
2	15	0.57
2	20	0.46
12	20	0.09
24	30	0.47
24	35	0.67

It is important to point out that measurements presented in this paper are in the higher temperature range (34–70 °C), and all the initial temperatures were room temperature.

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