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On the origin of the thermoluminescence band at around +50 °C in isolated subchloroplast particles

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The glow band appearing at around +50 °C was studied in various subchloroplast preparations. The band was intensified by addition of detergent to the chloroplast suspension and it was present in all preparations obtained by detergent treatment as well as in artificially prepared protein-pigment–detergent micelles. The band could only be generated by continuous light, and not with flash excitation, and it disappeared when the detergent concentration was decreased in the sample. Our results indicate that the band appearing in subchloroplast preparations at around +50 °C is an artifact generated by detergent–protein-pigment interaction and it has to be distinguished from the +50 °C band observable in leaves and isolated chloroplasts.

Thermoluminescence is a burst of light emission observed when irradiated organic or inorganic materials are subsequently warmed in darkness. The phenomenon, characteristic of photosensitive semiconductors, is generally accepted as due to thermally activated recombination of electrons and positive holes produced by irradiation and stabilized in frozen states at low temperature [1].

In the photosynthetic membrane at room temperature, some of the negative and positive charges in the reaction centers are metastable and recombine spontaneously to release energy. When chloroplasts are cooled rapidly during or after irradiation, some of the metastable charges are stabilized. On warming such frozen samples, the stabilized positive and negative charges recombine, being thermally activated over the barrier of activation energy, and light is emitted. The recorded emission intensity against the temperature, the so-called glow curve, can be resolved into several bands [2]. Since the late 1960s a number of studies have focussed on the origin of the various glow bands [3–11]. It is now quite clear that most of the glow peaks are related to the photosynthetic electron transport but in some cases the exact location of the charges participating in charge recombination is still uncertain. For example, there are conflicting data in the literature about the origin of the band appearing at around +50 °C. This band is also known as C band or peak *V* [12], can be observed in both intact leaves [7,13] and isolated chloroplast, and is enhanced by DCMU treatment [10,14].

On the one hand, a relationship between peak *V* and PS I photochemistry has been proposed, since this band is charged by 740 nm light [13,15], and it is present in Tris-washed chloroplasts [16] and when the O_2-evolving enzyme was not developed [17]. In addition, PS I subchloroplast particles were relatively enriched in this band [14].

On the other hand, there is evidence to indicate that peak *V* originates from PS II. $HgCl_2$ which inhibits the electron flow between plastocyanin and P-700 had little or no effect on peak *V* [10,18]. Furthermore, in flash experiments the band at +50 °C was found to exhibit a binary oscillation with flash number. From this result, it was concluded that S_0Q_A and S_1Q_A redox couples were involved in the charge recombination responsible for the peak [19]. Peak *V* was found to be present in PS II particles (Rutherford's unpublished result; for a reference, see Ref. 12).

The above-mentioned data are completely contradictory with respect to the location of the charges generating the thermoluminescence band at +50 °C. It is especially confusing that this peak has been found in both PS I and PS II preparations. In the present work, therefore, we have reinvestigated the presence of peak V in different subchloroplast preparations obtained by

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Abbreviations: LHC II, light-harvesting complex of Photosystem II; Chl, chlorophyll; PS, Photosystem; Q_A, 'primary' acceptor of PS II; BSA, bovine serum albumin; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

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various detergent treatments. Comparing the thermoluminescence characteristics of these preparations to those of artificially prepared protein-pigment-detergent micelles indicates that in subchloroplast preparations, the band appearing at + 50 °C is an artifact which is a result of the pigment-detergent interaction.

Peas (*Pisum sativum* L.) were germinated and grown under greenhouse conditions for 14 days, and chloroplasts were isolated as described previously [20]. Digitonin fractionation of chloroplasts was carried out according to Sane et al. [14]. Functional PS II particles were isolated as described by Bertold et al. [21]. Isolation of LHC II complex followed the procedure of Ryrie et al. [22]. Pigments were extracted with a mixture of acetone/ethyl ether (3 : 1) and washed acetone free with distillated water. To obtain the protein-detergent-pigment solution, dried pigments (solvent was evaporated by N_2 gas) were dissolved in a 10% Triton and 0.4% BSA containing suspension buffer and diluted to a final concentration of 0.5% Triton and 0.4% BSA with the original suspension buffer.

Glow curves were measured at − 80– + 80 °C using an apparatus similar to that of Tatake et al. [23]. Samples were illuminated with white light at 10 W/m^2 for 2 min during continuous cooling from + 20 °C to − 80 °C, then heated at a constant rate of 20 °C/min.

Measuring in the thermoluminescence characteristics of digitonin fractionated spinach chloroplasts Sane and his coworkers [14] described a glow band at + 50 °C in the 40 000 × *g* fraction. Since this band appeared at a similar temperature as peak *V* observed in intact leaves and was present at a relatively high level in the D-40 fraction, they concluded that peak *V* originated from PS I. However, they neglected the fact that the band at + 50 °C became intensive during digitonin treatment and was dominant in both D-0 and D-10 fraction as well.

Therefore, as the first step of our experimentation, we re-investigated the effect of digitonin treatment on the band at + 50 °C. Fig. 1 shows that control chloroplasts exhibit a main band at + 20 °C and there is only a very weak band at around + 50 °C (Fig. 1A). After 30 min incubation with 0.5% digitonin, the band at + 50 °C became very intensive (D-0) and was also found to be intensive in both D-10 and D-40 fractions, similarly to the results obtained by Sane et al. (c.f. Fig. 1B–D and Table 4 in Ref. 14). Because the band at + 50 °C was intensified by digitonin and was not exclusively found in either PS I or PS II preparations, we assumed that the increased intensity of the band might be the result of the detergent–membrane interaction rather than a specific charge recombination within the photosynthetic electron-transport chain. If our assumption is correct, one might expect that the band would disappear after the removal of the detergent. Since digitonin strongly associates the membrane fractions and is hard to remove from the samples [24], in further experiments we applied Triton-X 100 to isolated subchloroplast particles. Furthermore, using Triton-X 100 as detergent, functionally active PS II particles can be isolated [21]. This offers the possibility to check Rutherford's observation that the band at + 50 °C originates from PS II [12]. Fig. 2B shows that Triton treatment of chloroplasts (30 min; 0.5% Triton) increased the intensity of the glow peak at + 50 °C to a similar level to that observed after digitonin treatment.

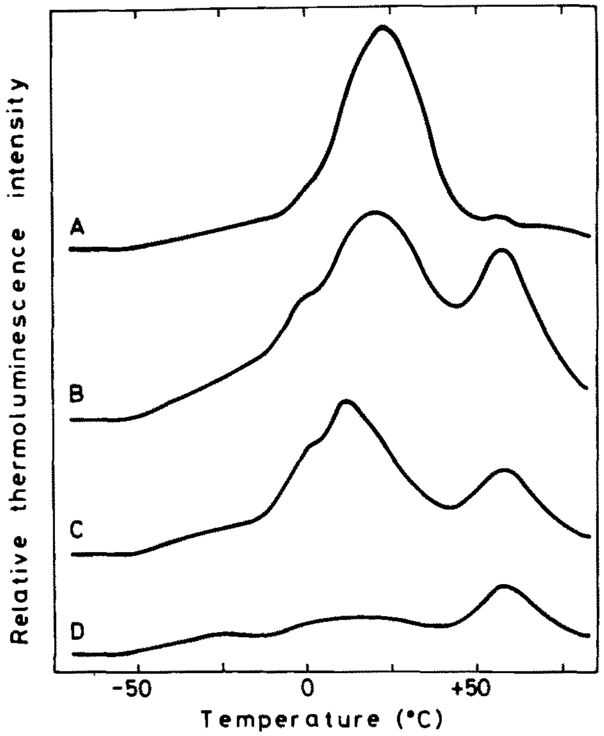

Fig. 1. The glow curves of chloroplast and subchloroplast particles obtained by digitonin treatment. (A) Control chloroplasts; (B) chloroplasts after 30 min incubation with digitonin; (C) 10 000 × *g* fraction; (D) 40 000 × *g* fraction.

The functionally active PS II particle (110 μmol O_2 per mg Chl per h, no detectable amount of P-700) also exhibited the band at + 50 °C (Fig. 2C, solid line), similarly to that of the D-10 fraction obtained by digitonin treatment. Washing the preparation once with detergent-free buffer resulted in the elimination of the band at + 50 °C but did not change the photosynthetic activity (103 μmol O_2 per mg Chl per h). This was also indicated by the unaltered intensity of the main band at + 10 °C (Fig. 2C, broken line). In contrast to the main band, which was excitable with single turnover flashes, the band at + 50 °C could only be observed after illuminating the samples with continuous light. These results seem to confirm our hypothesis that the band appearing in subchloroplast preparations at + 50 °C may not be related to the photosynthetic electron-transport processes.

Further evidence was obtained to verify our assumption by measuring the glow curve of LHC II complex prepared by Triton-X 100 fractionation. It can be seen in Fig. 3A that the LHC II exhibits a large band at +45 °C with an additional strong band at +70 °C. In the absence of light the samples did not show any thermoluminescence bands. The +70 °C band is known to originate from photooxidative chemiluminescence of the 'free' and/or loosely bound chlorophylls [25,26] (see also Fig. 3C). During the isolation of LHC II a large proportion of the chlorophyll is released from the membrane and part of the free pigment might be re-adsorbed by the isolated complex. The band at +45 °C could not be generated by the charge recombination of photosynthetic electron transport, since the preparation did not exhibit any electron transport with various donor and acceptor combinations and it was found electrophoretically pure as in Ref. 22 (data not shown).

From the above experiments it seems more likely that the band at around +50 °C originates from the pigment-protein–detergent interaction rather than from charge recombination occurring within the electron-transport components. If this is so, the question arises whether the generators of the band requires a specific chloroplast pigment-protein or it can be observed in any kind of artificially prepared unspecific pigment-protein–detergent micelles. Fig. 3B shows that the glow curve of the BSA-pigment-Triton micelles is practically identical to that of the LHC II. Similar curves were obtained if the BSA was substituted with β-lactalbumin, carbonic anhydrase or chicken egg albumin, indicating that no specific protein is required for the interactions. Increasing the Triton concentration in the samples from 0.2% to 0.5%, resulted in a shift of the peak position from +38 °C to +55 °C. This is in agreement with those results which demonstrated that the fluorescence properties of Chl *a* strongly depends on the detergent concentration in the solution [27]. The physical nature of the peak at around +50 °C is not yet known. There is a growing awareness of the possibility of artifact formation due to detergent–membrane interaction [28–32]. On the one hand, detergents cause structural modifications in the polypeptides which led to changes in the biological properties of these proteins [28,29]. It is assumed that the substitution of functionally essential lipids by detergent molecules is responsible for the observed phenomena [29]. On the other hand, it was found that Triton X-100 increased the chlorophyllase activity and, as a consequence, Chl *a*-trityl ester was formed in the presence of the detergent [30–32]. Both effects mentioned above may result in changes in the

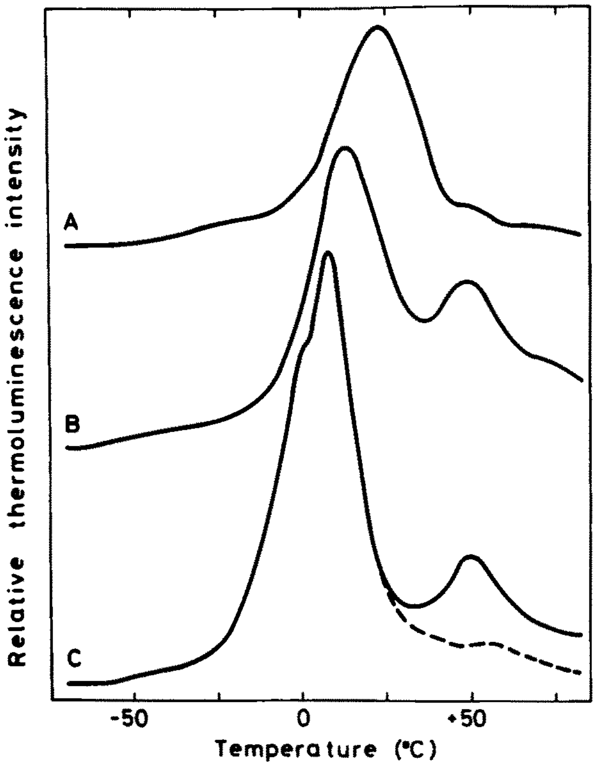

Fig. 2. The effect of Triton-X 100 on the intensity of the band at +50 °C. (A) Control chloroplasts; (B) chloroplasts after 30 min incubation with Triton-X 100; (C) functionally active PS II particle (solid line) and the same after washing with Triton-free buffer (broken line).

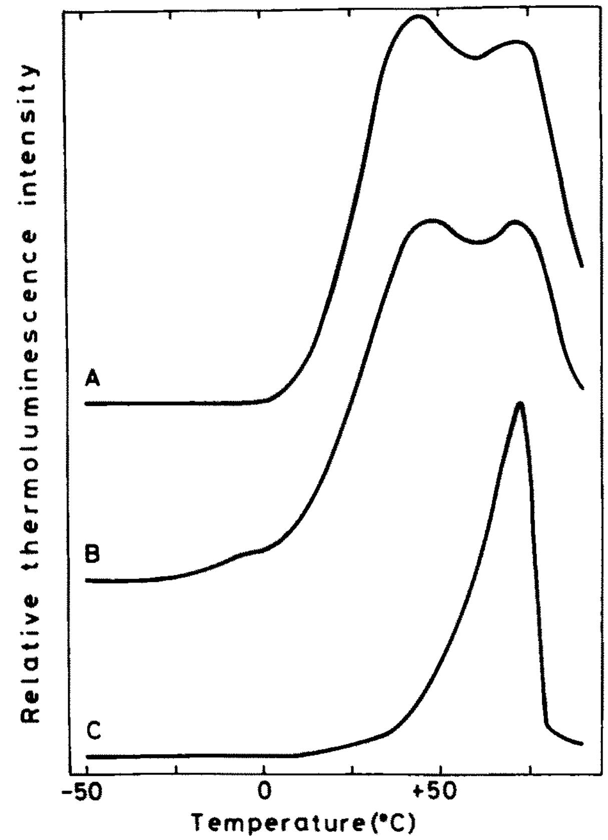

Fig. 3. The glow curves of isolated LHC II complex (A) and the artificially prepared protein-pigment-Triton micelles (B). As a reference, (C) shows the thermoluminescence curve of a pigment solution dissolved in petroleum ether 120.

fluorescence characteristics of the isolated complexes [30]. It is interesting to note that detergents were found to contain variable amounts of powerful oxidizing impurities representing a range of 0.04–0.2% H_2O_2 equivalents [33]. Desai et al. [25] showed that the thermoluminescence band appearing at +70 °C was due to chemiluminescence of chlorophylls. It might be assumed that in the presence of detergent and/or the oxidizing contamination the micelles formed are favourable for a photoinduced chemiluminescence and the reaction can even occur at temperatures lower than +70 °C. To verify this possibility further detailed investigations are required.

Summarizing our results, we can conclude that the thermoluminescence band appearing in subchloroplast preparations at around +50 °C is not generated by charge recombination occurring within the electron-transport chain. It is more likely that the band at +50 °C is an artifact resulting from the protein-pigment–detergent interaction. To avoid the possible misinterpretation of the thermoluminescence characteristics of subchloroplast particles, we propose that this peak be distinguished from peak *V* observable in intact leaves and isolated chloroplasts.

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