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CHARACTERIZATION OF THE LOW TEMPERATURE THERMOLUMINESCENCE BAND Z_V IN LEAF

AN EXPLANATION FOR ITS VARIABLE NATURE

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Summary

Out of the six thermoluminescence bands reported for a mature leaf, one band (Z_v) appearing at the lowest temperatures is dependent on the temperature of illumination. The characteristics of this band in fresh leaf are compared with those in a leaf heated to 60°C for 5 min. It is concluded here that this band, following illumination at temperatures lower than 173 K, is part of Arnold and Azzi's Z band (Arnold, W. and Azzi, J.R. (1971) Photochem. Photobiol. 14, 233—240). However, it is a part of peak I when observed subsequent to illumination beyond 173 K. An explanation for the appearance of this band at different temperatures is proposed.

The Z_v band reported recently by Shibata and his associates [1—3] is peculiar in comparison with several other thermoluminescence bands observed in leaves [1—9] as it appears at different temperatures, T_{max}, depending upon the temperature during illumination (T_i) whereas the temperature maxima for the other peaks do not change even if T_i is changed. In view of this, the above authors designated this band as Z_v. Ichikawa et al. [1] proposed that the Z_v may either be a component of the Z band of Arnold and Azzi [4] or of a partially stabilized fraction of component I of Shuvalov and Litvin [5] which seems to be identical to peak I reported by Desai et al. [6] and Sane et al. [7]. These proposals were based on the observation that some of the characteristics of Z_v were similar to those of the Z band of Arnold and Azzi [4] and others to those of component I of Shuvalov and Litvin [5]. In this communication we provide an explanation for the behav-

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Abbreviation: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea.

iour of the Z_V band and demonstrate that when excited at lower temperatures (less than 173 K) Z_V is a part of the Z band but when excited at higher temperatures it is a part of peak I of Desai et al. [6].

The glow curves were recorded according to the methods described earlier [6]. The illumination of the spinach leaf disc at different temperatures was carried out on the stage of a cryostat [10] using white light and the disc was subsequently quickly frozen to 77 K before the recording of the glow curves. The total period of illumination was 2 min and the intensity of illumination was 10 $W \cdot m^{-2}$.

The integrated yield of the Z_V band as the temperature during illumination (T_i) is changed is shown in Fig. 1, curve A. As T_i increases from 125 K the yield decreases giving a minimum yield at about 173 K after which it rises. If the temperature during illumination is less than 125 K the Z_V band becomes indistinguishable from the Z peak in respect of its shape, emission and sensitivity to heating. (The leaf disc used in these experiments gave all the other peaks previously described by Desai et al. [6].) Fig. 1 curve B shows part of the glow curve of the Z band in the temperature range 125 to 175 K obtained on illumination of the leaf at 77 K. It shows that the tail of the Z band extends right upto 173 K.

The yield of the Z_V band as a function of T_i in the leaf disc previously heated to 60°C for 5 min is shown in Fig. 2. Such a leaf shows only the Z band and the other bands (peaks I to V) associated with electron transport are absent (data not shown). With increase in T_i the yield of Z_V decreases, the rise in the yield at T_i higher than 173 K is not observed and the Z_V yield approaches a zero value. A similar result is observed if extracted chlorophylls are used in place of a leaf disc. The extracted chlorophylls also show only the Z band, the other peaks associated with electron transport are absent

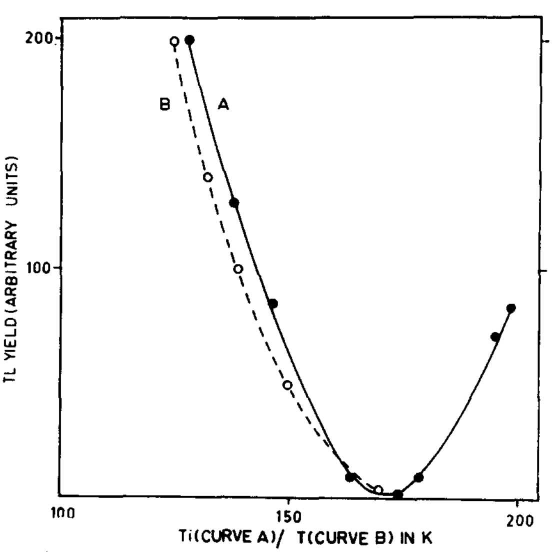

Fig. 1 (A) Integrated thermoluminescence yield of Z_V band in a leaf as a function of temperature during illumination (T_i) with white light. (B) Part of the glow curve of the Z band (T_i=77 K) in the temperature (T) range 125 to 175 K.

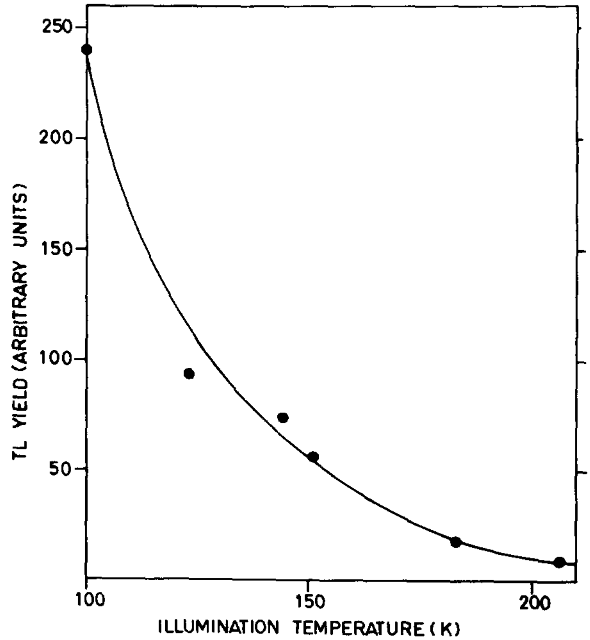

Fig. 2. Integrated thermoluminescence yield of the Z_v band in a leaf preheated to 60° C for 5 min.

[8]. This suggests that the Z_v band appearing at higher temperatures may be a part of peak I with a temperature maxima of 236 K, whereas the Z_v appeai ing at T_i lower than 173 K may be due only to the Z band. In this case, it should be possible to show that the Z_v appearing at higher T_i values has a different emission than Z_v appearing at a lower T_i since the Z band has an emission maximum at 740 nm [5,8] whereas the peak I has the emission characteristics identical to delayed light emission i.e. with a maximum of $<$ 700 nm (data not shown).

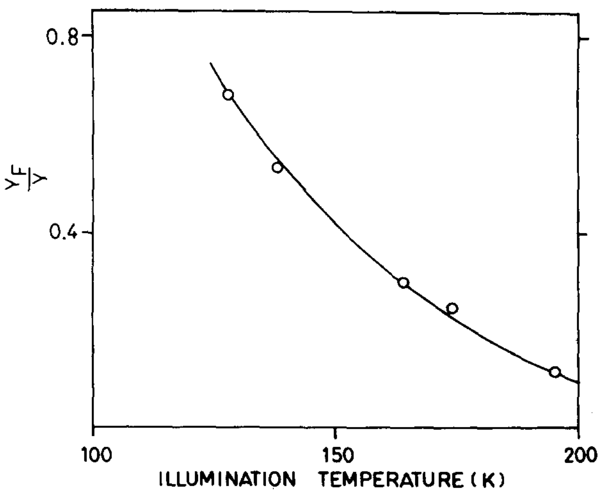

Fig. 3. The ratio Y_F/Y as a function of T_i. Y_F, integrated yield in the presence of the filter (Corning No. CS 7-69); Y, yield in the absence of the filter.

The data obtained from the experiments in which the thermoluminescence yield of Z_v of the leaf as a function of T_i was measured in the presence (Y_F) and absence (Y) of a Corning No. CS 7-69 filter is shown in Fig. 3. This filter transmits less than 1% at 720 nm, 40% at 740 nm and 75% at 780 nm. If there was only one type of emission from the Z_v band at T_i values lower and higher than 173 K the ratio Y_F/Y should have remained constant irrespective of T_i. If, however, there were two species involved, the low temperature one (Z) emitting with a maximum at 740 nm and the high temperature one (peak I) emitting with a maximum around 685 nm; then as the contribution of Z decreases and that of peak I increases the ratio Y_F/Y should decrease monotonously. As is evident from Fig. 3 the ratio decreased with increase in T_i showing that the emission characteristics of Z_v at lower temperatures are different from those at higher temperatures. Thus it seems that the origin of Z_v is different at lower and higher temperatures.

We have previously shown [7] that peak I is completely lost on the addition of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU). If peak I was contributing to Z_v at T_i values higher than 173 K then the Z_v appearing at higher T_i values should be lost in a DCMU-treated leaf whereas the Z_v appearing at lower T_i values will not be affected by DCMU addition since the Z band is insensitive to DCMU. This in fact was observed by us. In a DCMU-treated leaf the Z_v appearing as a result of illumination at higher T_i values was absent and the plot of Z_v yield as a function of T_i was similar to the one obtained in Fig. 2. This result further supports the conclusion that Z_v appearing at T_i values higher than 173 K is due to peak I whereas at lower T_i values it is due to the Z band.

On the basis of the above data we propose that Z_v as observed by Ichikawa et al. [1] is due to the partial charging of Z band and peak I of Desai et al. [6] or component I of Shuvalov and Litvin [5] depending upon T_i. At T_i lower than 173 K it is mainly due to the Z band and at T_i higher than this temperature it is due to peak I. The Z_v cannot be observed at much higher temperatures because of the appearance of peak I at these temperatures. We, therefore, propose the following mechanism for the appearance of Z_v.

It was reported earlier that the peak temperature of the Z band is 118 K [6—8]. The high temperature tail of the Z band is found to extend upto about 173 K. Thus if the leaf disc is illuminated at any intermediate temperatures between 120 and 173 K then those traps situated above T_i will be filled whereas those below the T_i will not be filled. As a result, on warming, the thermoluminescence yield will be due only to those traps that are situated between T_i and 173 K. As the T_i is shifted towards higher temperatures, fewer traps will be filled and therefore, the thermoluminescence yield will decrease. This is indeed experimentally observed by us. The curves obtained by plotting the yield of Z_v against T_i (Fig. 1, curve A) follows the curve of the Z band following illumination at 77 K (Fig. 1, curve B) in this temperature range. Since only those traps that are situated above T_i are filled, it is expected that the resulting glow curve will have a peak temperature higher than T_i. A shift in peak temperature as a result of a shift in T_i is hence predicted. Such a shift is also expected in the case of Z peak annealed to different temperatures, since annealing empties traps situated below the temperature of annealing, leaving those situated at higher temperatures relatively undisturbed. This indeed was observed by us (data not shown). A shift in the temperature of the glow curve dependent on T_i is not unique to Z band. In fact, such a behaviour is also observed with other biomolecules. As an example, glow curves of adenine obtained by illuminating with ultraviolet light (Philips TUV 6, germicidal lamp) at different T_i are shown in Fig. 4. A shift in glow peak temperature to temperatures slightly higher than T_i and decrease in the thermoluminescence yield with higher T_i are observed as expected.

On the basis of the above mechanism one cannot explain a gradual rise in Z_v yield at T_i above 173 K. As stated earlier this increase is mostly due to peak I. Since the yield of peak I increases as the T_i increases [3] the yield of Z_v which now is due to peak I also rises for T_i above 173 K. That the Z_v band is a part of Z band at lower temperature is further supported by lower yields of Z_v at T_i values higher than the peak temperature of Z band until 173 K. The light curves of various glow peaks reported earlier [7] show that the Z band appears only when the rest of the bands are almost saturated This agrees with the finding of Ichikawa et al. [1] who showed that Z_v bears a complementary relationship with their B_1 and B_2 bands.

Ichikawa et al. [1] have further shown that the Z_v band is sensitive to DCMU and heating. These observations were made in experiments at T_i of 213 K. As shown in Fig. 1, and stated earlier, the Z_v band observed at T_i higher than 173 K is due to peak I of Desai et al. [6]. This peak is sensitive to heating and is very sensitive to DCMU addition [7]. In view of this the observation of Ichikawa et al. [1] regarding the sensitivity of Z_v band to

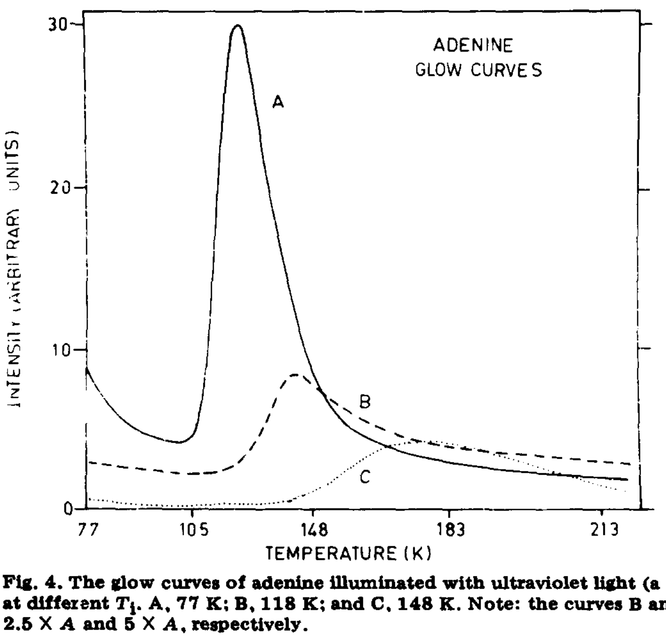

Fig. 4. The glow curves of adenine illuminated with ultraviolet light (a Philips TUV 6 germicidal lamp) at different T_i. A, 77 K; B, 118 K; and C, 148 K. Note: the curves B and C were read out at gains 2.5 × A and 5 × A, respectively.

these treatments is consistent with our proposal. If the sensitivity of the Z_v band to these treatments was studied using any T_i lower than 173 K the Z_v would be found to be resistant to DCMU and heating, since the Z band is in sensitive to these treatments [6—8].

The appearance of the Z_v band as reported by Shibata and his colleagues [1—3] can thus be explained on the basis of partial filling of the traps associated with the Z band or peak I depending upon the temperature of illumination.

References

1 Ichikawa, T., Inoue, Y. and Shibata, K. (1975) Biochim. Biophys. Acta 408, 228—239
2 Inoue, Y., Ichikawa, T. and Shibata, K. (1976) Photochem. Photobiol. 23, 125—130
3 Inoue, Y. Furuta, S., Oku, T. and Shibata, K. (1976) Biochim. Biophys. Acta 449, 357—367
4 Arnold, W. and Azzi, J.R. (1971) Photochem. Photobiol. 14, 233—240
5 Shuvalov, V.A. and Litvin, F.F. (1969) Mol. Biol. U.S.S.R. 3, 59—73
6 Desai, T.S. Sane, P.V. and Tatake, V.G. (1975) Photochem. Photobiol. 21, 345—350
7 Sane, P.V., Desai, T.S., Tatake, V.G. and Govindjee (1977) Photochem. Photobiol. 26, 33—39
8 Sane, P.V., Tatake, V.G. and Desai, T.S. (1974) FEBS Lett. 45, 290—294
9 Lurie, S. and Bertsch, W. (1974) Biochim. Biophys. Acta 357, 420—428
10 Tatake, V.G., Desai, T.S. and Bhattacharjee, S.K. (1971) J. Phys. E.: Scientific Instruments 4, 755—757