EMISSION SPECTRA OF THE TRIGGERED LUMINESCENCES IN ISOLATED CHLOROPLASTS OBTAINED IN A FLOW APPARATUS

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1. Introduction

Since the discovery of the ability of chloroplasts to emit a pulse of luminescence as a result of an acid - base transition (Mayne and Clayton [1]) extensive studies were carried out in several laboratories, including ours, on the subject of triggered luminescence. These led to the discoveries of a series of different treatments which can induce preilluminated chloroplasts to emit a pulse of luminescence in a similar manner. For reviews on these studies, cf. that by Mayne and Fleischman [2] and that by Lavorel [3].

The emission spectra of some luminescences were either not measured at all or measured only roughly. In the two cases where a spectrum was reported (acid –base type as shown by Mayne and Clayton [1]; acid and salt induced types as described by Miles and Jagendorf, [4]) these spectra were obtained point by point by measuring separately the emission peak at each wavelength. The wavelength setting was changed either by use of interference filters or by a monochromator. From our experience, the scattering of results obtained in this way is quite considerable.

The purpose of the present work was to construct a flow apparatus capable of measuring continuously the spectra of the triggered emissions from chloroplasts suspensions. We present below the spectra of the luminescences induced by HCl, by sodium benzoate, by methanol, by dimethyl-sulfoxide, and by T-jump. The spectrum of fluorescence was also measured on the same apparatus, and brought for comparison.

2. Materials and methods

Fresh chloroplasts from lettuce leaves were prepared according to Avron [5] except for the final resuspension which was in 0.2 M sucrose and 0.1 M KCl. The details of the apparatus are schematically shown in fig. 1. The chloroplast suspension was illuminated by light from a projector (Braun) transmitted through a Corning C.S. 4–96 filter (light wavelength is approximately between 400 and 600 nm). The chloroplasts were mixed with the appropriate reagent in a Y shaped glass tube (the mixing chamber). The flow velocities of the chloroplasts and of the reagent were 3.5 and 5.0 ml/sec respectively.

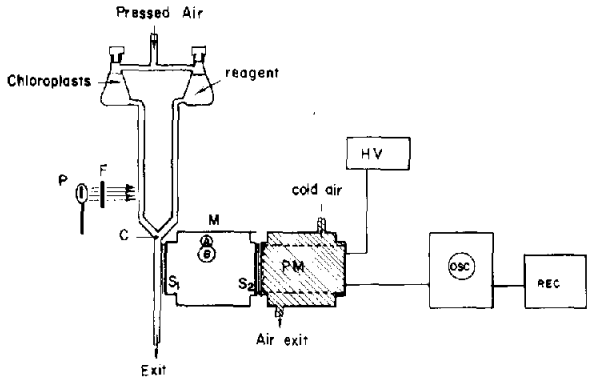

Fig. 1. Experimental arrangement of the flow apparatus. P = Projector; F = Filter; C = Mixing Chamber; M = Monochromator; PM = Cooled Photo = multiplier; OSC = Oscilloscop amplifier; REC = Recorder. HV = High Voltage Power Supply.

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The mixing chamber and the 1.5 cm glass tube following it were placed in front of a monochromator (Jarrel Ash 82–410, 0.25 m). The emission induced inside the mixing chamber passed through the monochromator to a cooled photomultiplier (EMI 9658 B). The output of the PM was amplified by a differential amplifier unit of an oscilloscope (Tektronix 3A9 unit) and traced on a chart recorder (Easterline Angus 370). The wavelength scanning was controlled using an electrical wavelength drive. The synchronization between the wavelength scanning and the tracing on the recorder was achieved by connecting both the wavelength drive motor and the chart recorder to a common power source via one switch. The linear dispersion of the monochromator was 4.95 nm/mm and the slit widths were 1.5 mm. In one experiment we used narrower slits for better spectral resolution causing however an increase in the noise/signal ratio. There was no essential difference in the spectra with wide or narrow slits.

The following reagents were used to trigger the luminescence as a result of mixing with the chloroplast suspension: (a) 0.1 1N HCl solution, (b) 1.5 M sodium benzoate solution, methanol analar grade, (d) dimethyl sulfoxide analar grade and (e) hot distilled water at approx. 90°C. The concentration of the chloroplasts was varied in preliminary experiments to find an optimal value. It was found that 45 μg chlorophyll/ml gave maximal response. This value was then used in all subsequent experiments.

3. Results

The positions of the emission peaks and their coincidence with the fluorescence spectra of chloroplasts as shown in fig. 2 leave no doubt in identifying the chlorophyll *a* as the species which emits these luminescences upon triggering. The wavelengths of the peaks shown in fig. 2 fall in the range of 682 to 682.5 nm. These results were characterized to all (around 30) spectra measured in repeated experiments, the peaks of which never fall out of the range 681–683 nm. Fig. 2 was obtained after smoothing out the

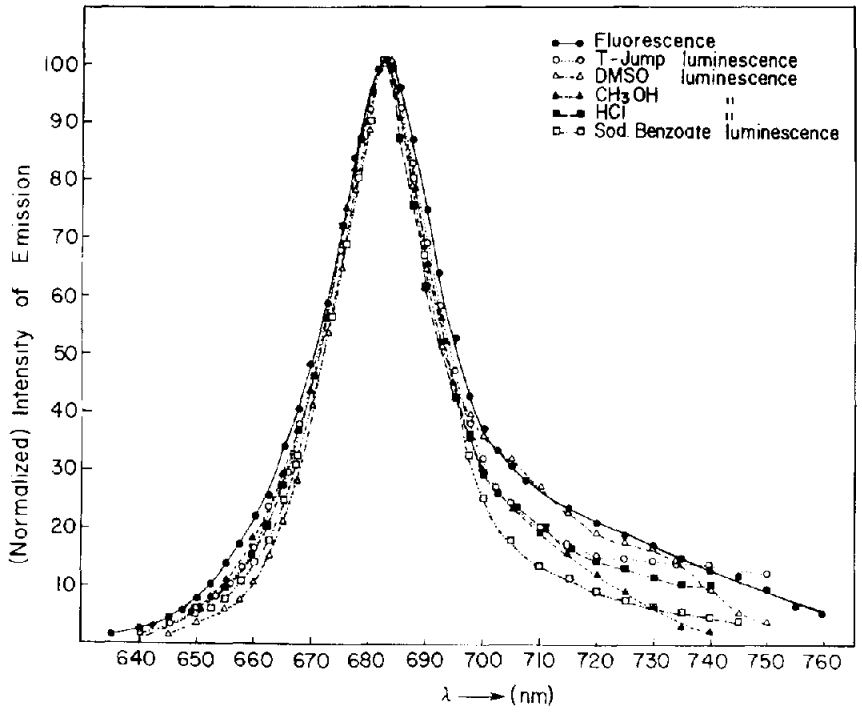

Fig. 2. Emission spectrum of the specified triggered luminescences. The points were obtained from the original curves by normalizing the peak to 100.

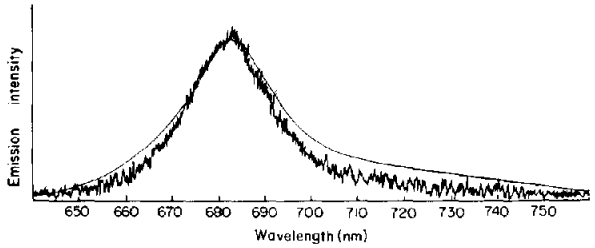

Fig. 3. Original recording of the HCl induced luminescence, and also the fluorescence.

noise, obtained in the original recording. Fig. 3 shows the original spectrum as recorded by the instrument in the case of HCl induced luminescence. In comparison, a fluorescence spectrum is also shown measured with the same batch of chloroplasts. There is much less noise in the trace of fluorescence, since the fluorescence is much brighter than luminescence and the photomultiplier operates under a much lower voltage. Because of the relatively high noise level we do not place significance at the moment to the small differences in the tail part of the spectra (> 700 nm).

4. Control experiments

We were careful to check that the emission obtained was indeed the triggered luminescence and not a fluorescence that somehow tunneled the irradiation section by scatterin and light guide effects. To show this we switched off the flow of reagent or of the chloroplasts while the exciting light was on. In this case the luminescence response disappeared (the scattered fluorescence should remain). It also disappeared when the exciting light was turned off, as one would naturally expect (fig. 4).

5. Discussion

The observation of the coincidence in the traces of the different types of luminescence and that of the fluorescence implies some important conclusions regarding the origin of these luminescences. It was suggested that Photosystem II is the source for the acid–base luminescence from the typical action spectrum [6] and also the emission spectrum [2]. Our ob-

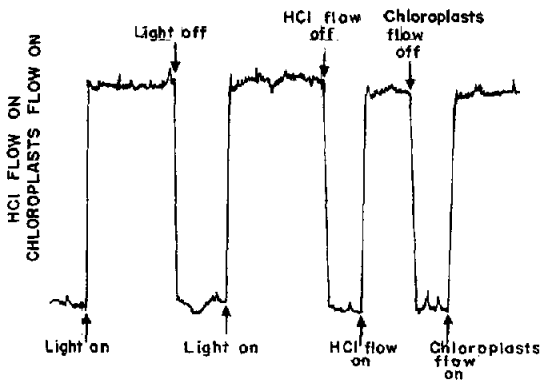

Fig. 4. Luminescence signal for control experiment.

servations generalize these conclusions: The coincidence of the spectra given in fig. 2 suggests that all the triggered luminescences are emitted from the same Photosystem II chlorophyll *a*, which emits also the fluorescence.

Although the natures of the different triggering effects are different, we were able to show that they possess common properties, as for example the typical 'oscillations' when preilluminated by flashes [7] and the enhancement in the presence of electron transport inhibitors, parallel to the fluorescence [8]. In the present study we have shown that they also possess very similar emission spectra. These kinds of observations leave very small doubt that photosystem II is involved in all the triggered luminescences, and that Chlorophyll a_{11} is the emitting species.

References

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