Luminescence from Photosystem I at High Temperatures

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The changes in the fluorescence and delayed fluorescence intensity of spinach leaf as affected by temperature were studied. It was observed that the delayed fluorescence showed a maximum at about 45 °C whereas the fluorescence maximum was at about 55 °C. An examination of the emission spectra of delayed fluorescence at different temperatures showed that at higher temperatures the relative emission at 735 nm was increased. It is argued that at higher temperatures the luminescence from photosystem I contributes to delayed fluorescence.

Introduction

The phenomenon of delayed light emission or delayed fluorescence (DF) from photosynthetic membranes was first detected by Strehler and Arnold [1]. Several reports have provided valuable information on the characteristics of DF since then as has been extensively reviewed from time to time [2–6]. It is suggested that DF arises in the photosystem II reaction and probably involves regeneration of the chlorophyll singlet by recombination of charges in the photoelectric PS II dipole $^{+}Y.Chl.Q^{-}$ where ^{+}Y is the oxidisable primary donor, Chl is the reaction centre chlorophyll and Q^{-} is the reduced primary acceptor (see ref. [3]). Several approaches have been used to obtain information on the nature and origin of DF. One among them is the technique of thermoluminescence which in more recent years has proved quite useful. Basically this technique involves slow warming of pre-illuminated photosynthetic membranes, leaves from low temperature (mostly 77 K) to high temperature (about 325 K). During this slow warming in the dark the pre-illuminated photosynthetic membranes/leaves give out light at distinct temperatures (the glow curves). The thermoluminescence studies carried out by Lurie and Bertsch [7] and Ichikawa *et al.* [8] suggest that these glow peaks also originate in the PS II. Earlier studies by Shuvalov and Litvin [9] and more recent studies by us [10–12] have shown that besides the glow peaks that originate in PS II there are some that originate in PS I. The major evidence for assigning peaks IV and V (according to our nomenclature) appearing around + 25 °C and + 45 °C respectively to PS I was that they could be excited by far red light [10] and that in a PS I enriched chloroplast fraction, these two peaks constituted the major part of the total luminescence [12]. These observations suggested to us that if the glow peaks are indeed related to the phenomina of DF [9] then it should be possible for us to observe DF from PS I. Considering the fact that PS I glow peaks appear at higher temperatures than PS II glow peaks we thought that DF from PS I, if present, will be observed at higher temperatures. The data presented in this communication show that the emission spectra of delayed fluorescence at high temperature is characterized by an increased emission at 735 nm (relative to 685 nm) suggesting that at high temperatures DF also originates in PS I.

Abbreviations: ATP, adenosine triphosphate; DCPIP, 2,6-dichlorophenolindophenol; DF, delayed fluorescence; NADPH, reduced nicotinamide adenine dinucleotide phosphate; PS, photosystem.

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Methods

Leaves from field grown spinach were used in all the experiments. The leaf pieces cut to a size of about 4 mm × 10 mm were inserted in the quartz dewar of the phosphoroscope assembly and held in position by an aluminium holder. The DF was measured by using the phosphoroscope assembly of the Aminco Bowman Spectrophotofluorometer and an X-Y recorder. The DF was excited by 440 nm and the light emitted from the front surface was monitored after 10 ms at the desired wavelength using a photomultiplier with an S-20 response (R-446). The temperature of the sample was gradually and continuously increased from 10 °C to 70 °C using a circulating water bath. The exact temperature of the leaf piece was monitored by an iron con-

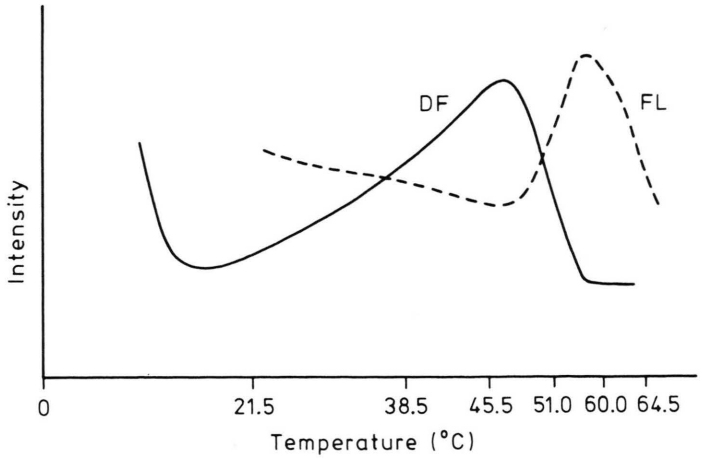

Fig. 1. The changes in the intensity of fluorescence (FL) and delayed fluorescence (DF) of spinach leaf as a function of temperature.

stantan thermocouple. During warming of the sample the DF was continuously monitored at 685 nm. In those experiments where the emission spectrum of the DF was studied, the temperature of the sample was raised or decreased by injecting an appropriate amount of hot or cold water of a desired temperature. The ratio of E 685/E 735 was calculated from the peak hights of the spectra obtained in several different experiments. For monitoring fluorescence under identical conditions the chopper of the phosphoroscope assembly was removed and the total fluorescence yield at 685 nm was monitored on the spectrophotofluorometer. The intensity of the exciting light was approximately 10^5 ergs · cm^{-1} · sec^{-1}.

Results

The changes in the peak intensity of delayed fluorescence (DF) and total fluorescence (FL) as a function of temperature are shown in Fig. 1. The intensity of DF is minimum around room temperature but increases steadily as the temperature rises up to 45 °C. A further rise in temperature results in a sharp decrease in DF intensity reaching a minimum at 55 °C. There is also an increase in the intensity of DF as the temperature is lowered from 20 °C. The changes in fluorescence intensity (F 685) did not parallel the changes observed in DF. The fluorescence intensity continues to decrease gradually as the temperature is raised until about 45 °C. Beyond this the fluorescence increases, giving a peak around 55 °C. It comes down gradually thereafter. There are two important features brought out by the Fig. 1 that at temperatures higher than 20 °C the DF and fluorescence intensity bear an inverse relationship with each other, and the DF shows a sharp decline beyond 45 °C whereas decline in fluorescence occurs beyond 55 °C. The significance and implication of these results are discussed later.

In order to find out if the DF at higher temperatures is qualitatively different than at room temperature or lower temperatures the emission characteristics of the DF were studied. Fig. 2 shows the typical spectra observed at 3 different temperatures. At all the temperatures, the DF shows a maximum at about 685 nm and a shoulder at around 735 nm.

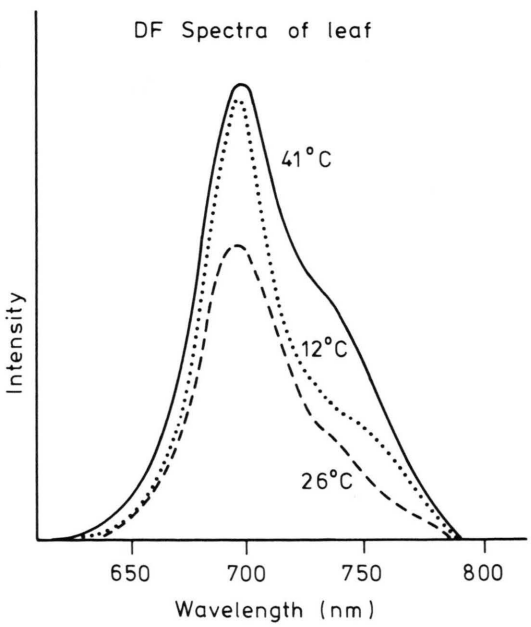

Fig. 2. Emission spectra of delayed fluorescence of spinach leaf at three different temperatures.

However, if one calculates the ratio of E 685 to E 735 it is seen from a number of experiments that this ratio is greater at lower or room temperatures but it is considerably smaller at higher temperatures *i. e.* beyond 45 °C. This change in the ratio shows that the 735 nm emission is relatively increased at higher temperatures. Since it is known that 735 nm emission primarily originates in PS I [13] these observations indicate that PS I contributes more to DF at higher temperatures.

Discussion

The data presented in this communication show that at higher temperatures not only the intensity of DF is increased but that the relative emission at 735 nm is higher. This indicates that PS I contributes to the DF at high temperatures. The observed increase in 735 nm emission cannot be attributed to the effect of temperature, as such, on the relative intensity of different bands because 735 nm band becomes stronger at lower rather than at higher temperatures.

We have earlier shown [10, 12] that of the several thermoluminescence peaks, the peak V, that appears at high temperature (48 °C) originates exclusively in PS I. Additionally the peak IV appearing at 25 °C was shown to arise at least partly in PS I. In view of this and the data reported by Shuvalov and Litvin [9] showing the relationship between the glow peaks and DF we would like to suggest that the light emission associated with these two peaks contributes to the 10 ms component of the DF at high temperatures.

Several workers have previously studied the effect of temperature on DF and fluorescence [14, 15], see also [3–5]. But most of the studies reported were conducted on isolated, broken chloroplasts. The present studies were carried out on leaf as such and the DF component studied by us was the one emitted after 10 ms. Of direct relevence to our work are the reports of Murata and Fork [16], Schreiber and Berry [17], Ono and Murata [18] and Itoh [19] and these will be considered in the subsequent discussion.

The changes in DF and fluorescence observed by us in the spinach leaf are probably related to a number of processes such as electron transport, phosphorylation, rates of CO_2 fixation etc. affected by temperature. Since earlier workers have not reported the changes in DF and fluorescence from the leaves covering a wide range of temperatures as studied in the present work an explanation based on the known effects of temperature on the photochemical reactions, membrane potential and carboxylation reactions, for the changes observed in the intensity of DF and fluorescence is provided below.

Changes in delayed fluorescence

(A) DF at temperatures below 18 °C: The high DF at lower temperatures is due to slower rate of electron transport between Q and the subsequent acceptors and or due to a slower rate of water splitting at these temperatures. This explanation is supported by the observations of Nolan and Smillie [20] who have shown that the Hill activity of the isolated barley chloroplasts when monitored as DCPIP reduction with water as the donor is slow at low temperature but increases as the temperature rises until 38 °C beyond which it declines. The lower rate of either water splitting *i. e.* a block between H_2O to Y (the primary electron donor to PS II) electron transport or a block between Q^- to subsequent acceptors (due to slow enzymatic reactions) would result in the accumulation of $Y^+ \cdot Chl \cdot Q^-$ species. If we accept the proposal that DF results from recombination of charges in the photoelectric dipole of PS II, then accumulation of $Y^+ \cdot Chl \cdot Q^-$ would result in high DF from PS II. The emission spectra of DF at low temperature is in aggrement with the proposal that at low temperature, the DF primarily originates in PS II. (B) DF at room temperature (around 20 °C): The lowest intensity of DF is seen around room temperature. This is due to the fact that at room temperature the enzymatic reactions associated with the CO_2 fixation are most active resulting in efficient utilization of both reducing power *i. e.* NADPH and high energy compound ATP. This prevents accumulation of the $Y^+ \cdot Chl \cdot Q^-$ species and hence the luminescence is lower. (C) DF at high temperature (from 35 °C to 45 °C): The high intensity of DF at temperatures well above room temperature results from the loss of membrane function resulting in the disconnection of PS I from PS II. This prevents efficient electron flow between Q^- and $P\,700^+$. As a consequence the only way by which Q^- can be oxidized is the recombination pathway resulting in increased DF from PS II centres.

At higher temperatures in addition to DF from PS II centres there is also DF originating in PS I.

Bjorn [21] had previously shown that emission from PS I decays slowly at room temperature. At higher temperatures this decay could be faster and is detected as a 10 ms component. Shuvalov [22] has demonstrated that in an isolated PS I fraction DF from PS I arises as a result of a back reaction between $P\,700^+$ and $P\,430^-$ – the primary electron acceptor of PS I. He has also shown that the luminescence intensity increases with a rise in temperature and has a peak at 710 nm. Considering Shuvalov's observations and the results presented in this paper we suggest that contribution by PS I DF at higher temperatures is increased and is reflected in the emission spectra of DF at high temperature in addition to total DF intensity. Two possibilities for increased contribution by PS I could be considered: a. If the PS II centres are damaged at high temperatures the DF from PS II will be reduced, and b. At high temperatures PS I reaction which is less sensitive to heating proceeds at a faster rate. However, since non-cyclic electron flow is prevented due to damaged PS II centres, the recombination between $P\,700^+$ and $P\,430^-$ is increased. This results in increased emission from PS I. (D) Decrease in DF beyond 45 °C: This decrease is associated with the rise in fluorescence intensity (Fig. 1) suggesting that beyond 45 °C the chloroplast membrane may be damaged due to phase transition of membrane components. Earlier workers [16, 18, 19] have shown that changes in phase transition are associated with the changes in DF and fluorescence.

Changes in fluorescence

The changes in fluorescence intensity observed by us are similar to those observed by earlier workers [17, 18]. The sudden increase in fluorescence intensity from 45 °C to 55 °C is due to the disorganization of the thylakoid membrane as seems evident from a sudden decrease in DF in this temperature range. The decrease in fluorescence beyond 55 °C is due to the introduction of a variety of quenchers as a result of the total destruction of the photosynthetic membrane.

The temperatures at which maximum DF and fluorescence is observed are different at high temperature. The observation that the 10 ms DF component shows a peak at high temperature is a new observation and that at this temperature the contribution by PS I DF is higher is also new. These data together with our earlier data on thermoluminescence bands [10, 12] suggest that DF from PS I can be best observed at higher temperatures. The possibility that PS I could contribute to DF was evident from some of the earlier studies (see refs. [4, 5]).

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