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COMPETITION BETWEEN THE 735 nm FLUORESCENCE AND THE PHOTOCHEMISTRY OF PHOTOSYSTEM I IN CHLOROPLASTS AT LOW TEMPERATURE

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Summary

Fluorescence emission spectra of chloroplasts, initially frozen to —196°C, were measured at various temperatures as the sample was allowed to warm. The 735 nm emission band attributed to fluorescence from Photosystem I was approx. 10-fold greater at —196° C than at —78° C. The initial rate of photooxidation of *P*-700 was also measured at —196° C and —78° C and was found to be approximately twice as large at the higher temperature. It is proposed that the 735 nm emission band is fluorescence from a long wavelength form of chlorophyll, C-705, which acts as a trap for excitation energy in the antenna chlorophyl system of Photosystem I. Furthermore, it is proposed that C-705 only forms on cooling to low temperatures and that the temperature dependence of the 735 nm emission is the temperature dependence for the formation of C-705. C-705 and *P*-700 compete to trap the excitation energy in Photosystem I. It is estimated from the data that at —78°C *P*-700 traps approx. 20 times more energy than C-705 while, at —196°C, the two traps are approximately equally effective. By analogy, the 695 nm fluorescence which also appears on cooling to —196° C is attributed to traps in Photosystem II which form only on cooling to temperatures near —196° C.

Introduction

It is generally assumed that the 735 nm fluorescence band which appears in the fluorescence emission spectrum of green plants as the temperature is lowered toward —196°C is due to fluorescence from Photosystem I. However, the relationship of that fluorescence to the photochemistry of Photosystem I has not been established and the reasons for the strong temperature dependence of the emission band have not been adequately elucidated. On cooling, another emission band at 695 nm, generally attributed to fluorescence from

Photosystem II, also appears near −196°C with an even steeper temperature dependence than the 735 nm band. The purpose of the work reported here was to determine if the fluorescence at 735 nm was in competition with the photochemistry of Photosystem I at low temperatures and to examine the temperature dependence of the 735 and 695 nm emission bands.

In Photosystem II, the yield of photochemistry at low temperature is limited by a temperature-dependent competition between a charge stabilization reaction and a direct back-reaction in the primary reaction center couple [1]. The limitation on the yield of photochemistry set by the back-reaction was also manifest by the relative ineffectiveness of single saturating flashes to accomplish stable charge separation in the Photosystem II reaction centers at −196°C. It was shown previously, however, that this type of back-reaction does not appear to exert a major limitation on the photochemistry of Photosystem I at low temperature since most of the *P*-700 could be photooxidized by a single saturating flash at −196°C [2]. Thus, we feel justified in proceeding with our analysis of a temperature-dependent competition between fluorescence and photochemistry in Photosystem I.

Materials and Methods

Spinach chloroplasts were prepared by the method of Yamashita and Butler [3]. For measurements of fluorescence, the chloroplasts were suspended at 10 μg chlorophyll/ml in 0.05 M Tris · HCl, pH 7.2, and frozen to liquid nitrogen temperature in a vertical cylindrical cuvette and Dewar system similar to that described previously [4]. In this case, however, the cuvette, 1 cm in diameter, had 2-cm thick stainless steel walls to increase the heat capacity of the cuvette. Fluorescence was measured with a triple-arm, fiber-optic, light-pipe assembly described previously [5]. A thermocouple was frozen into the sample to monitor the temperature. The frozen sample was excited on the top surface with light from a small Ne-He laser (633 nm, 2 mW/cm^2) through one of the light-pipe arms. Another light-pipe arm transmitted the fluorescence from the top surface to a monochromator (either a Bausch and Lomb High Intensity Monochromator, 5 nm passband, or a Bausch and Lomb Double Grating Monochromator, 2 nm passband). Fluorescence was measured from the sample at the F_M level, i.e. when the Photosystem II reaction centers were all closed. The measurements at −196°C were made with the sample submerged in liquid nitrogen. For measurements at −78°C, the sample was first frozen to −196°C and then was allowed to warm to −78°C with solid CO_2 around the cuvette. To obtain the temperature profile of fluorescence emissions at various wavelengths, the liquid nitrogen was allowed to boil away and emission spectra were measured at various temperatures indicated by the thermocouple as the sample warmed.

Measurements of the rate of photooxidation of *P*-700 were made with chloroplasts suspended at 150 μg chlorophyll/ml in 0.05 M Tris · HCl, pH 7.2, in the same vertical cylindrical cuvette. The sample thickness was about 2 mm. The chloroplasts were frozen at −196°C to give an optically dense, highly scattering sample. The *P*-700 measurements were similar to those described previously [5,6]. The *P*-700 was transformed by a repetitive series of non-saturating

3-μs xenon flashes (1 flash/s) delivered to the bottom of the sample through a light-pipe arm terminating at the glass bottom of the Dewar. The 703 nm measuring beam from a monochromator and an interference filter, both at 703 nm, was also incident at the bottom of the Dewar through another light-pipe arm. The 703 nm light transmitted by the sample was taken to a phototube with appropriate cut off filters by a light pipe which rested directly on top of the frozen sample. The transmittance at 703 nm as a function of time (or flash number) was monitored by a Hewlett-Packard Model 7047A X-Y Recorder with a 0.1 s time response. At each flash the recorder trace shows a rapid upward transient but it recovers between flashes to indicate the value of the transmittance. The lower envelope of the flash profile indicates the kinetics of the phototransformation of *P*-700. The weak 703 nm measuring beam had no appreciable actinic effect. For measurements at −78°C the sample was first frozen to −196°C and then allowed to warm to −78°C.

Results

Fluorescence emission spectra of chloroplasts at −196°C and at −78°C are shown in Fig. 1A. The three emission bands near 685, 695 and 735 nm in the emission spectrum at −196°C have been attributed to the light-harvesting chlorophyll *a/b* complex, the antenna chlorophyll *a* associated directly with Photosystem II units and the antenna chlorophyll *a* of Photosystem I units, respectively. The temperature-dependence of the fluorescence at 682, 693 and 733 nm is shown in Fig. 1B. It is apparent from both Figs. 1A and 1B that the emission bands at 735 and 695 nm are much more sensitive to temperature than the band at 685 nm. The data in Fig. 1B were taken from emission spectra measured with a 5 nm passband (rather than the 2 nm passband used for the spectra in Fig. 1A) so that there may be some overlap of the emission bands in these data. For instance, the steeper increase of F_{682} at temperatures below −160°C may be due to overlap from F_{693} which shows a major increase in this

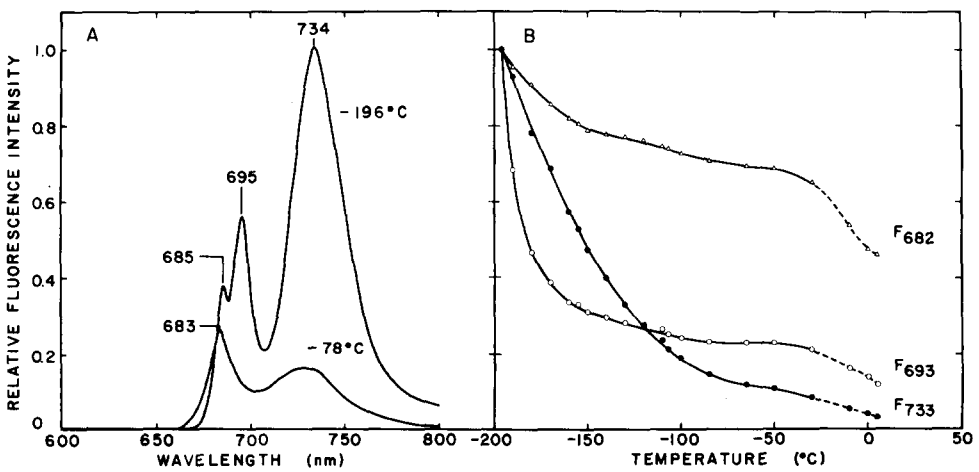

Fig. 1. The temperature dependence of fluorescence emission from spinach chloroplasts. (A) Fluorescence emission spectrum at −196°C and at −78°C. (B) Fluorescence emission at 682 nm (F_{682}), at 693 nm (F_{693}) and at 733 nm (F_{733}) vs. temperature. Conditions otherwise as in Materials and Methods.

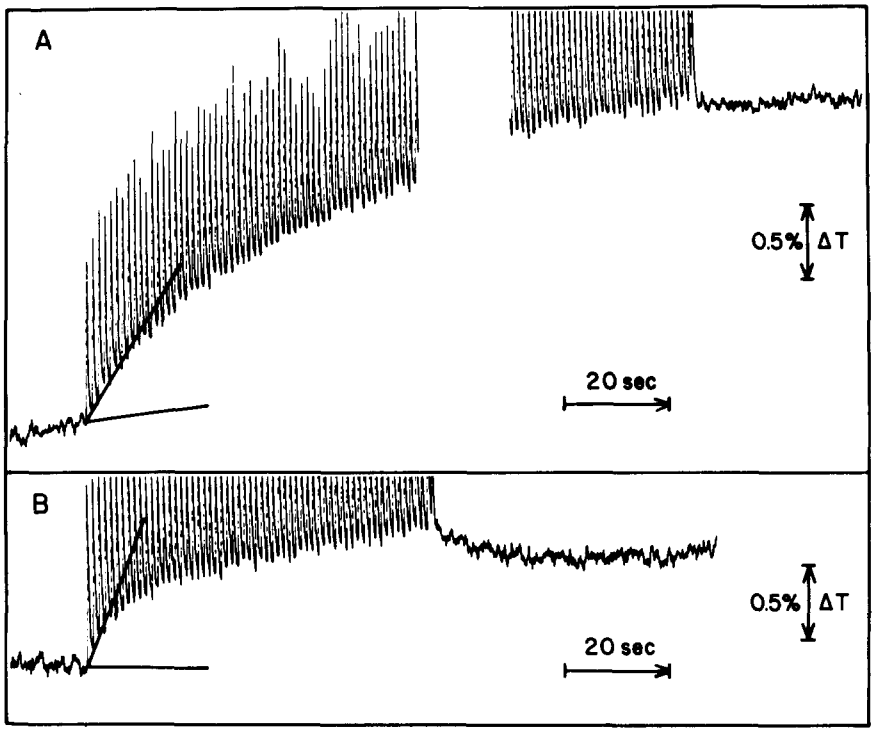

Fig. 2. The photooxidation of *P*-700 in spinach chloroplasts at −196°C (A) and at −78°C (B). Where curve A is interrupted, 68 flashes were applied to the sample. The indicated angles represent the estimated initial rates of photooxidation of *P*-700.

temperature region. On the other hand, the rather flat temperature-dependence of F_{693} at temperatures above −160°C may be due to F_{682} which shows a similar flat temperature-dependence. The intensity of the fluorescence at 735 nm increases about 7-fold on cooling from −78 to −196°C. Since the long wavelength tail of the 685 nm emission band contributes to some extent to the 735 nm fluorescence, the 735 nm emission band of Photosystem I must increase at least by a factor of 10 or more over this temperature range. The results in Fig. 1B agree reasonably well with the earlier report of Govindjee and Yang [7] on the temperature dependence of fluorescence at 685, 696 and 735 nm over the range from −196°C to 0°C.

The kinetic curves for the photooxidation of *P*-700 at −196°C and at −78°C are shown in Fig. 2. The rate of the photoreaction, estimated from the initial slope of the absorbance change at the onset of irradiation, is approximately twice as fast at −78°C as it is at −196°C. In an average of nine such comparisons the initial slope at −78°C was 0.109 ± 0.006 (S.D.) $\%\Delta T/s$ while that at −196°C was 0.053 ± 0.003 $\%\Delta T/s$ for an average ratio of 2.1.

Unfortunately, measurements of the half-time for the photoreaction, which would be more precise than estimates of the initial slope, would be misleading. In order to have enough *P*-700 to measure accurately, the absorbance of the frozen sample at the actinic wavelengths of the xenon flash was quite high. Thus, the actinic intensity at the rear surface of the sample was much less than

it was at the front surface. The effect of this variation of actinic intensity with sample depth is to average together a range of rate constants from relatively rapid ones at the front surface to much slower ones at the rear surface which makes the first-order photoreaction appear more second order in character, especially at −196°C where the dark reduction of $P\text{-}700^+$ is negligible. At higher temperatures where the dark reaction is more appreciable, the dark reduction of $P\text{-}700^+$ from the front region of the sample counteracts the photooxidation at the rear regions so that the kinetics of the overall reaction appear more first order. The effect of this change of kinetics with temperature is to prolong the half-time of the photoreaction at the lower temperatures. The initial slope of the photoreaction is not affected by the dark reduction of $P\text{-}700^+$ so that so long as the samples have comparable optical properties at the different temperatures the initial slopes are considered to be valid for comparisons of relative rates. Measurements were made of the transmittance at 633 nm as the sample warmed up from −196°C. No detectable changes were noted up to −50°C where phase transitions in the medium began to occur.

Discussion

We take the results showing that the increased yield of fluorescence at 735 nm which occurs on lowering the temperature is accompanied by a decreased yield for the photooxidation of *P*-700 to indicate that fluorescence and photochemistry in Photosystem I are in competition with one another at low temperature. However, the nature of that competition and the reasons for the strong temperature dependence of the 735 nm emission still remain to be elucidated. Even the nature of the chlorophyll responsible for the 735 nm emission band must be specified since we cannot assume a priori that that fluorescence emanates from the antenna chlorophyll of Photosystem I.

It was found previously [8] that the excitation spectrum for the 735 nm fluorescence from chloroplasts at 196°C has a long wavelength excitation band at 705 nm. A small absorption band was also found at 705 nm in the absorption spectrum of chloroplasts at −196°C and was attributed to the presence of small amounts of a long wavelength form of chlorophyll denoted C-705. It was proposed on the basis of those spectra that light energy absorbed by the antenna chlorophyll is transferred to C-705 and that C-705 is source of the 735 nm emission band at low temperatures [8]. The question was raised as to whether C-705 is present but non-fluorescent at room temperature or whether C-705 only forms on cooling to low temperatures [8]. We now tend to favor the latter hypothesis and to attribute the temperature dependence of the 735 nm emission band to the temperature dependence for the formation of C-705. The proposed formation of C-705 on cooling is consistent with experimental observations that the absorption band of C-705 is observed only at temperatures approaching −196°C. However, these observations do not prove the proposal since the appearance of the 705 nm absorption band could be due to the increased resolution of the absorption spectra which occurs at low temperatures.

According to our hypothesis the 735 nm fluorescing C-705 molecules (or aggregates) are energy traps in the antenna chlorophyll system of Photosystem

I which trap energy in competition with the photochemically active *P*-700 reaction center traps. We have also proposed [9,10], to account for the absence of light-induced fluorescence yield changes in Photosystem I, that *P*-700$^+$ traps excitation energy as effectively as *P*-700: energy trapped by *P*-700 is used for photochemistry, energy trapped by *P*-700$^+$ is dissipated as heat. As the temperature is lowered more C-705 traps form (C-705 could be an aggregated state of chlorophyll which is stable only at low temperatures), the emission at 735 nm increases and the rate of photooxidation of *P*-700 decreases. Comparison of the absorption and fluorescence excitation spectra of chloroplasts [11] and bean leaves [12] at −196°C indicates that the yield of the 735 nm fluorescence is at least twice as great at 705 nm where the excitation of C-705 is mainly by direct absorption than it is at shorter wavelengths where the energy is absorbed by antenna chlorophyll and transferred to C-705. In the latter case competition for the energy by *P*-700 would decrease the fluorescence from C-705.

An alternative but operationally similar proposal would be to assume that C-705 is present at normal temperatures but has a shorter wavelength absorption maximum which permits energy transfer to *P*-700. According to this hypothesis lowering the temperature would cause the absorption maximum of C-705 to shift to longer wavelength where it would become a terminal energy trap. With either of these hypothesis, lowering the temperature causes the accumulation of 705 nm absorbing, 735 nm fluorescing, energy trapping centers which complete with *P*-700 for the excitation energy in Photosystem I.

According to such a photochemical model, excitation energy in the antenna chlorophyll of Photosystem I can be trapped by *P*-700 (or by *P*-700$^+$), dissipated by non-radioactive decay or trapped by C-705. Let us assume for the sake of an illustrative argument that the rates of deexcitation by trapping by *P*-700, non-radioactive decay in the antenna chlorophyll and trapping by C-705 at −78°C are in the ratio of 18 : 1 : 1. In that case the probabilities for trapping by *P*-700 and C-705 would be 0.90 and 0.05, respectively. Now assume that on cooling further to −196°C the concentration of C-705 increases such that the rate of energy trapping by C-705 increase 19-fold to give ratios of 18 : 1 : 19. In that case the probability for trapping by *P*-700 would decrease to 0.47 while that for trapping by C-705 would increase to 0.50. Assuming that the probabilities of fluorescence from excited C-705 molecules and of photochemistry by excited *P*-700 molecules are independent of temperature, lowering the temperature from −78 to −196°C would cause the fluorescence band at 735 nm to increase 10-fold while rate of photooxidation of *P*-700 would decrease by a factor of 1.8, in reasonably good agreement with the results in Figs. 1 and 2.

It should be pointed out that we do not propose any temperature-dependent changes in the fundamental rate constants for deexcitation by any of the deexcitation pathways including non-radiative decay. The sole effect of temperature is to alter the concentration of C-705 trapping centers and, thereby, the probability for deexcitation via C-705. It would also be possible to account for the data by assuming that the rate constants for deexcitation by fluorescence, k_F, photochemistry, k_P, and non-radiative decay, k_D, in the antenna chlorophyll changed as a function of temperature. We could assume simply that k_F increased 19-fold on lowering the temperature from −78 to −196°C but the physical basis for such a change is not apparent. If we assume that k_F were constant

with temperature then we would have to assume that both k_P and k_D decreased on cooling. It is apparent that the data cannot be explained solely on the basis of a freezing-out of non-radiative decay since in that case fluorescence and photochemistry would both increase on cooling and k_D would have to be excessively large at room temperature to allow for a 10-fold increase of fluorescence at −196°C. The alternative of a temperature-dependent formation of C-705 trapping centers appears to us to provide a more plausible and specific basis to account for the experimental results. Also, we would prefere to assume that strictly photoexcitation and deexcitation processes are independent of temperature until forced to assume otherwise.

By analogy with the above analysis of the temperature dependence of the 735 nm emission band we propose that the 695 nm emission band is due to a form of chlorophyll in Photosystem II which forms on cooling to temperatures below −160°C and which acts as a trap for the excitation energy in the antenna chlorophyll of Photosystem II. This proposal is different from previous ones, suggesting that the 695 nm fluorescence band was due to the reaction center chlorophyll of Photosystem II.

In recent photochemical models of the photosynthetic apparatus by Butler and Kitajima [9] and Butler and Strasser [13] it was assumed that the fluorescence at the 695 and 735 nm at −196°C were representative of the fluorescence from the antenna chlorophyll of Photosystem II and Photosystem I, respectively. Those assumptions are still valid even if the fluorescence is actually emitted from trap molecules within the antenna system since those fluorescence bands will still be representative of the excitation energy in Photosystem II and Photosystem I. We would estimate that at −196°C the amount of energy lost through the 695 nm fluorescence is a small part of the total energy available to Photosystem II so that the 695 nm fluorescence does not represent a significant energy drain which functions in competition with other Photosystem II processes such a photochemistry and energy transfer to Photosystem I. At temperature below −196°C, however, fluorescence in the 690 nm region increases dramatically [14] which we suggest is due to the further accumulation of fluorescent trapping centers in the antenna chlorophyll system of Photosystem II. At such temperatures deexcitation through these trapping centers could represent a significant dissipating pathway for the excitation energy in Photosystem II.

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