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The far red induced slow component of delayed light from chloroplasts is emitted from Photosystem II

Evidence from emission spectroscopy

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Abstract

In spinach chloroplasts illuminated with far red light, the relative intensity maximum during the decay of delayed light is emitted at 680–690 nm. This finding supports previous models predicting emission from Photosystem II, and contradicts earlier attributions to Photosystem I.

Due to self absorption, the emission spectrum of the relative maximum is shifted to longer wavelengths and displays apparent Photosystem I characteristics in chloroplast samples of higher concentration or in leaves. This may have caused earlier investigators to ascribe the emission to Photosystem I.

A differences between the spectral width of the emission spectra of delayed fluorescence and the relative maximum indicates that these two phenomena represent emission from different sub-populations of Photosystem II centers.

Abbreviations: PS I – Photosystem I; PS II – Photosystem II; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea

Introduction

After illumination with far red light, the decay of delayed light emission from algae (Bertsch and Azzi 1965, Björn 1971a, Bonaventura and Kindergan 1971, Krause et al. 1987, Schmidt and Senger 1987a,b), protoplasts (Nakamoto et al. 1988) and leaves of higher plants (Björn 1971b, Desai et al. 1983, Sundblad et al. 1989) passes through a relative maximum between 1–4 min after the end of the illumination. The appearance of this maximum depends on several factors, such as the growth condition of the algae (Bertsch and Azzi 1965) or the prehistory of the leaves (Desai and Ross 1985, Sundblad et al. 1989), the intensity and duration of the excitation (Schmidt and Senger 1987b, Schmidt 1988), the presence of oxygen (Björn 1971a, Nakamoto et al. 1988) and the temperature during the measurement (Bertsch et al. 1967, Desai et al. 1983).

Since the relative maximum only develops upon illumination with light above 700 nm, all studies agree that the excitation of PS I is criti-

cal, but there is a lack of agreement on the energy storage system leading to the temporal increase in light intensity and on the question of the emitters.

Reporting on the first observation of the relative maximum, Bertsch and Azzi assumed the involvement of an enzymatic process and emission from unspecified chlorophyll molecules (Bertsch and Azzi 1965, Bertsch et al. 1967).

Later, Björn (1971a,b) demonstrated that the relative maximum in *Elodea* leaves and *Chlorella* is associated with the cyclic electron transport around PS I and the transthylakoid pH, and presented a model featuring emission from PS II stimulated by reactions of PS I. Similarly, Krause et al. (1987) suggested that the relative maximum in *Chlorella* is produced in PS II from electrons activated at PS I.

Recent investigations of Sundblad and coworkers showed that in barley leaves and protoplasts, the relative maximum was due to recombination in PS II, between positive charges stored in a special, far red induced redox state of the oxygen evolving complex and electrons of a reverse flow from PS I (Nakamoto et al. 1988, Sundblad 1989, Sundblad et al. 1989).

All studies committed to the mechanism of the process conclude that, although the excitation of PS I is critical, the relative maximum is emitted from PS II. Surprisingly, the available two reports on the spectral distribution of the emission at the relative maximum seem to contradict this. Based on the observation of a peak around 720 nm in the emission spectrum of *Pothos* leaves, Desai and Ross (1985) suggested that the relative maximum is emitted from PS I. A similar spectrum was reported in *Scenedesmus* by Schmidt and Senger (1987a).

Apart from the disagreement with the PS II characteristics of the emission (like the sensitivity to DCMU, a blocking agent between the two photosystems (Björn 1971, Nakamoto et al. 1988)), it is hard to accommodate this conclusion with the extremely low fluorescence yield of PS I at room temperature (Bertsch et al. 1967, Krause et al. 1987). Also, the above spectra were not corrected for the self absorption of the samples, which may, particularly in leaves, distort the original emission spectrum.

In an attempt to resolve the contradiction between kinetic models and emission spectra, Sundblad (1988) suggested that the long wavelength characteristics of the spectrum reflect emission from a sub-population of PS II, PS II_β (Melis and Homann 1975, 1976), located in the stroma exposed, PS I enriched segments of the thylakoid membrane (Anderson and Melis 1983) rather than emission from PS I itself.

In this report, the relative maximum is studied in isolated spinach chloroplasts for the first time. Using samples with low chlorophyll concentration (with low self absorption) we examined the emission spectrum of far red induced delayed light emission during the relative maximum and present a direct evidence for its PS II origin. A comparison of the emission spectra of delayed fluorescence and the relative maximum supports the above idea of emission from different types of PS II centers. Studies of leaves and chloroplasts at high concentration suggest that, in these samples, the same emission appears with apparent PS I features due to self absorption.

Materials and methods

Leaf disks and chloroplasts of market spinach (prepared as described by Takahashi and Asada (1982)) were illuminated with a microscope lamp through a 720 nm interference filter (Toshiba, Japan) for 2 min, and transferred to the measuring apparatus within 10 s. All measurements were carried out at 22 °C.

Light emission was measured with a high sensitivity photon counting system (Inaba et al. 1982) using an R1333 photomultiplier tube (Hamamatsu Photonics, Japan), in a cooled housing, protected by a motor driven shutter. Data were collected with a gated two channel photon counter (Stanford Research Systems, USA) connected to a personal computer (NEC, Japan).

Emission spectra were detected with an R1333 photomultiplier based spectral analyzer utilizing colored glass filters (Toshiba, Japan) on a computer controlled, rotating filter wheel (Inaba 1988). Leaf disks and isolated chloroplasts were measured in a 1.6 cm diameter glass tube at 22 °C, as described earlier (Hideg et al. 1990a). Photon counting data were collected in the same

way as for the kinetic measurements. The spectral analysis was carried out by calculating the count rate for each spectral region defined by the subtraction of the two transmission curves of the corresponding colored glass filters, as described earlier (Inaba 1988). Spectra were corrected for the absorption of the sample determined with a double beam spectrophotometer (Hitachi U-3400, Japan), and for the spectral sensitivity of the photomultiplier tube.

Results and discussion

Figure 1 shows the decay of delayed light emission arising from chloroplasts (5 μg ml^{-1}) between 10 s and 5 min following far red (720 nm) irradiation. Similarly to previous studies on algae, protoplasts and leaves (Bertsch and Azzi 1965, Bertsch et al. 1967, Björn 1971a,b, Bonaventura and Kindergan 1971, Desai and Ross 1985, Krause et al. 1987, Schmidt and Senger 1987a,b, Nakamoto et al. 1988, Sundblad et al. 1989), we found that 2–4 min after illumination the monotonic decrease of the intensity is interrupted by a relative maximum.

As it is shown in Fig. 1, the spectral distribution of the emission in the course of the monotonous decay displays a maximum around 680–690 nm (Fig. 1, inset A). In samples of low chlorophyll concentration, the effect of self absorption is not significant (compare corrected and uncorrected spectra in Fig. 1A).

In an earlier study we showed that, in chloroplasts illuminated with white (400–700 nm) light, delayed fluorescence emerges from PS II as a spectral feature at 685 nm during the first 50 s of the decay (Hideg et al. 1989, 1991). The 680–690 nm emission peak, reported here in the same time region but in far red illuminated samples, also demonstrates PS II emission. It is probably due the slight excitation of PS II which also occurs at 720–730 nm (Björn 1971a, Bonaventura and Kindergan 1971). Supporting this, we observed the same emission spectrum during the first minute following far red excitation in DCMU treated samples (where the decay in monotonic (Björn 1971a, Nakamoto et al. 1988)) as in untreated ones (data not shown).

The spectrum of delayed light during the emission of the relative maximum (2–4 min after excitation) exhibits a peak at 680–690 nm (Fig.

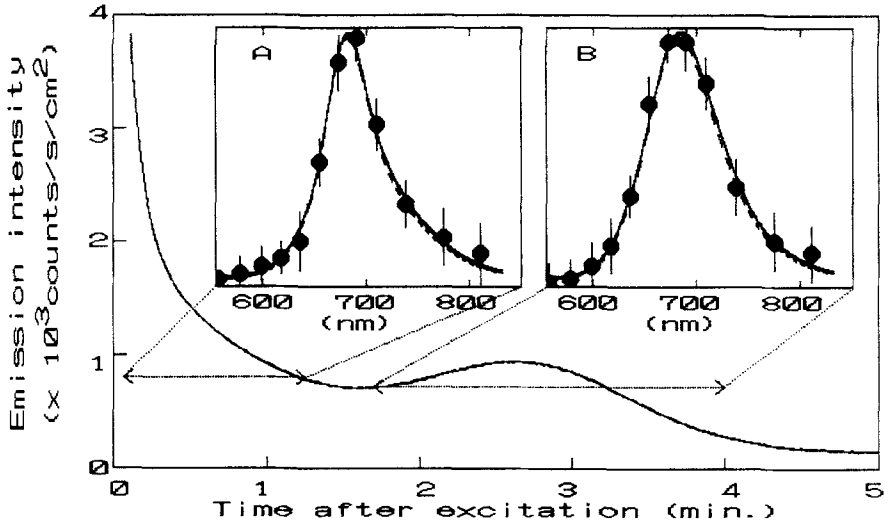

Fig. 1. Decay of delayed light emission from far red (720 nm, 2 min) irradiated spinach chloroplasts (22 °C, chlorophyll concentration 5 μg ml^{-1}). Background counts (measured while having suspending buffer in the sample holder) were less than 3% of the delayed fluorescence intensity after 5 min (data not shown). *Insets*: Normalized spectra of delayed light emission before (A) and during (B) the relative maximum. Full circles indicate data points. Error bars were omitted when they were smaller than the symbols. Solid lines indicate an approximation of the spectra, dashed lines show spectra corrected for the self absorption of the sample.

1B), showing that the emitters of the relative maximum belong to PS II, as suggested previously (Björn 1971a,b, Bonaventura and Kindergan 1971, Krause et al. 1987, Nakamoto et al. 1988, Sundblad et al. 1989).

Further comparison of the emission spectra before and during the relative maximum, reveals a difference in the width of the emission features. As it is shown in the insets of Fig. 1, the emission peaks are the same, but their spectral width is different: The spectrum at the relative maximum is broader than that of delayed fluorescence. This difference indicates that the PS II centers involved in the emission of the relative maximum are different from the emitters of delayed fluorescence, in accordance with a previous suggestion of Sundblad (1988).

In a preceding publication, based on a mathematical analysis of the emission spectra of one second delayed fluorescence, we suggested that the main peak could be the sum of two components with similar maxima (around 685 nm) but with different spectral widths (one is approximately twice the other), representing emission from different populations of chlorophyll molecules in PS II (Hideg et al. 1990b). Since the emission spectrum of fluorescence does not change with the delay time during the first minute of the decay (Hideg et al. 1991), we assume that this spectral heterogeneity is present in the delayed fluorescence spectrum presented here, in Fig. 1A, although the resolution of these latter data does not permit mathematical analysis. That the emission spectrum at the relative maximum appears broader than the spectrum of delayed fluorescence, could be due to a change in the amplitude ratios of the above two components, in favor of the broader one.

In order to compare our findings with previous reports on leaves, where the self absorption of the sample remarkably modifies the spectrum of any emission process, the emission spectrum of the relative maximum was examined in high concentration chloroplast samples and in leaves.

Figure 2 shows the emission spectrum of delayed light from far red excited chloroplasts (Fig. 2A) and spinach leaves (Fig. 2B) around the relative intensity maximum. Both spectra, when uncorrected for self absorption, show an emission maximum around 710–720 nm, similar to the spectrum reported by Desai and Ross (1985) using *Pothos* leaves.

However, when the self absorption of the sample was considered in chloroplasts, the corrected emission spectrum (Fig. 2A, dashed line) was maximal around 690 nm and similar to low concentration samples (Fig. 1B). This finding suggests that in chloroplasts with high concentration, the relative maximum is also emitted from PS II, but due to the self absorption of the sample, the emission peak is shifted to longer wavelengths usually associated with PS I. Supporting this, we found that the relative maximum did not develop in these samples either, if DCMU was present (data not shown).

In leaves, due to difficulties of the absorption

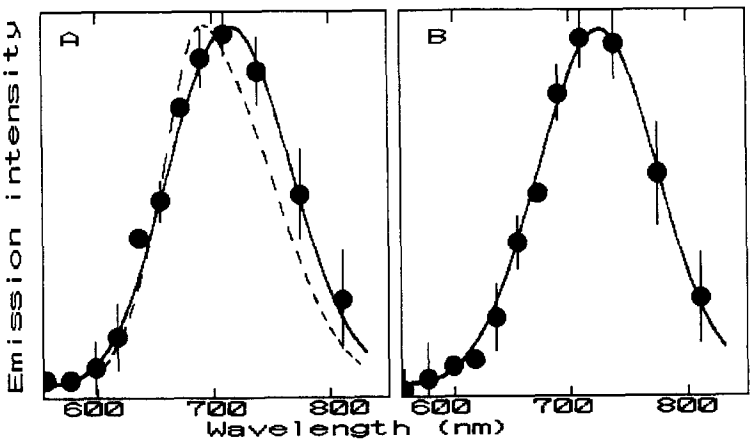

Fig. 2. Normalized spectra of delayed light during the emission of the relative maximum in far red illuminated spinach chloroplasts (100 $\mu g\,ml^{-1}$) (A) and leaves (B). Symbols and all other conditions were the same as in Fig. 1B.

measurements, corrections are more complicated and were not applied in this study. Still, the similarity between the emission spectra of leaves and high concentration chloroplast samples, combined with the likeness of the correcting functions (both absorption spectra being dominated by the absorption of chlorophylls) suggests that the relative maximum in leaves may also be from PS II, with apparent PS I like features due to self absorption.

In a hypothesis interpreting the long wavelength emission of far red induced relative maximum in leaves (Desai and Ross 1985) and in algae (Schmidt and Senger 1987a), Sundblad (1988) suggested that the PS I characteristics were only apparent and in fact reflected emission from PS II_β centers. The basis of this proposal is a preceding report on the higher contribution of PS II_β to the longer wavelength part of prompt fluorescence than that of PS II_α (Brearley and Horton 1984).

The data reported here support the assumption of emission from PS II, and we suggest that the apparent PS I characteristics could rather be due to the self absorption of the sample. Our findings are also in agreement with the suggestion that the emitters of the slow component of delayed light (which are responsible for the relative maximum) are different from the emitters of faster components. However, we found marked differences between these two phenomena not in their emission maxima but rather in their spectral width.

Following are two possible explanations accommodating our findings, the original hypothesis of Sundblad and the reported spectral features of PS II_α and PS II_β centers:

i) The relative maximum is not from PS II_β but from some other unknown sub-population of PS II centers distinguished by its broader emission spectrum.

ii) The assignment of the relative maximum to PS II_β is correct, however, the difference between the fluorescence emission spectra of PS II_α and PS II_β is not in the emission maxima (both being around 680–690 nm) but in their spectral width.

We find the second possibility more feasible, because PS II_β centers, located in the PS I enriched part of the thylakoid membrane (Anderson and Melis 1983), are very likely candidates for the site of a PS I stimulated emission from PS II (Sundblad 1988). In this picture, (which also implies that our previous attempt to associate the spectral heterogeneity with some inner structure of PS II (Hideg et al. 1990b) was incorrect) a broader emission feature from PS II_β than from PS II_α could account for a relatively bigger contribution of PS II_β to the overall emission intensity of prompt fluorescence at 720 nm (Brearley and Horton 1984).

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