Biochimica et Biophysica Acta, 462 (1977) 307–313

BBA 47380

FLUORESCENCE EMISSION SPECTRA OF PHOTOSYSTEM I, PHOTOSYSTEM II AND THE LIGHT-HARVESTING CHLOROPHYLL *a*/*b* COMPLEX OF HIGHER PLANTS

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(Received March 14th, 1977)

SUMMARY

Fluorescence emission spectra excited at 514 and 633 nm were measured at −196 °C on dark-grown bean leaves which had been partially greened by a repetitive series of brief xenon flashes. Excitation at 514 nm resulted in a greater relative enrichment of the 730 nm emission band of Photosystem I than was obtained with 633 nm excitation. The difference spectrum between the 514 nm excited fluorescence and the 633 nm excited fluorescence was taken to be representative of a pure Photosystem I emission spectrum at −196 °C. It was estimated from an extrapolation of low temperature emission spectra taken from a series of flashed leaves of different chlorophyll content that the emission from Photosystem II at 730 nm was 12 % of the peak emission at 694 nm. Using this estimate, the pure Photosystem I emission spectrum was subtracted from the measured emission spectrum of a flashed leaf to give an emission spectrum representative of pure Photosystem II fluorescence at −196 °C. Emission spectra were also measured on flashed leaves which had been illuminated for several hours in continuous light. Appreciable amounts of the light-harvesting chlorophyll *a*/*b* protein, which has a low temperature fluorescence emission maximum at 682 nm, accumulate during greening in continuous light. The emission spectra of Photosystem I and Photosystem II were subtracted from the measured emission spectrum of such a leaf to obtain the emission spectrum of the light-harvesting chlorophyll *a*/*b* protein at −196 °C.

INTRODUCTION

It is apparent from the accompanying paper [1] that a greater fraction of the excitation energy is distributed to Photosystem I with excitation at 514 nm compared to excitation at 633 nm. Thus, we would expect that the shape of emission spectra from flashed leaves at −196 °C should depend to some extent on the wavelength of excitation and, specifically, that 514 nm light should excite more of the 730 nm emission band of Photosystem I relative to the 694 nm band of Photosystem II than 633 nm light. The purpose of the present paper is to explore such differences in the emission

spectra from flashed leaves and greened leaves in an attempt to deduce the spectral quality of the fluorescence from Photosystem I, Photosystem II and the light-harvesting chlorophyll *a*/*b* protein.

MATERIALS AND METHODS

The flashed leaves were grown as described in the accompanying paper [1]. Fluorescence was measured from the front surface of 1.4 cm discs of the leaves frozen to −196 °C in our vertical cuvette and Dewar system with the triple-arm, fiber-optic light-pipe assembly described previously [2, 3]. Excitation at 514 nm was from an argon laser through a Bausch and Lomb High Intensity Monochromator set at 514 nm. Excitation at 633 nm was from a helium-neon laser through a 633 nm interference filter. The intensities of both excitations were adjusted to be 1.5 mW/cm^2 at the surface of the sample. Emission spectra were measured with a Bausch and Lomb Double Grating Monochromator with a 2 nm bandwidth on line with a small computer. The spectra presented were corrected for the spectral response of the monochromator and phototube and were plotted directly by the computer.

RESULTS AND DISCUSSION

Fluorescence emission spectra from a flashed leaf at −196 °C are shown in Fig. 1 for excitation at 514 and 633 nm with the two spectra being normalized at 694 nm. As noted in the Introduction, we would expect that the ratio of Photosystem I to Photosystem II emission should be greater with 514 nm excitation than with 633 nm excitation. We assume that Photosystem I emission is negligible at 694 nm so that we take the difference spectrum, Δ, in Fig. 1 to be a pure Photosystem I emission spec-

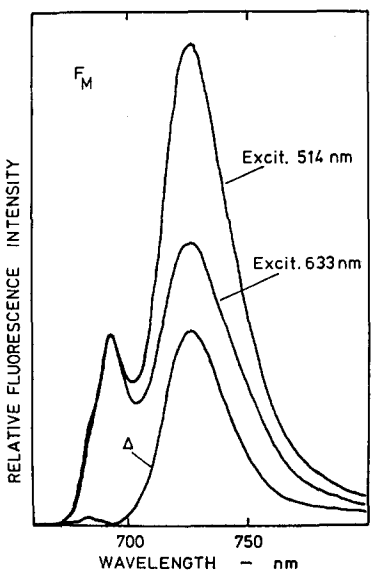

Fig. 1. Emission spectra of a flashed leaf at −196 °C excited at 514 nm and at 633 nm. Δ is the difference spectrum.

trum. The accompanying paper [1] shows that the excitation spectra for Photosystem I and Photosystem II of a flashed leaf at −196 °C are appreciably different, especially in the spectral regions from 500 to 520 nm and from 600 to 650 nm. If there were an appreciable Photosystem I emission at 694 nm the excitation spectra for F_0 and F_V at 694 nm should be different since F_0 would consist of fluorescence from both Photosystem I and Photosystem II while F_V is due solely to emission from Photosystem II. The excitation spectra of F_0 and F_V for the 694 nm fluorescence at −196 °C shown in the accompanying paper are virtually identical which supports our contention that Photosystem I shows no appreciable fluorescence at 694 nm.

Given the emission spectrum of Photosystem I fluorescence, we should be able to subtract out the Photosystem I emission from the overall spectrum and obtain a pure Photosystem II emission spectrum if we knew how much of the fluorescence at 730 nm was due to the tail of the Photosystem II emission. In the accompanying paper we concluded from the wavelength dependence of the rate of photooxidation of *P*-700 in the flashed leaves that most of the emission at 730 nm is due to Photosystem I but we cannot conclude, on the basis of these measurements, that all of the emission at 730 nm is due to Photosystem I. In fact, we estimated from the emission spectrum of Photosystem II particles that the emission from Photosystem II at 730 nm was 10–15 % of the emission at the 694 nm peak. In the present work we have used emission spectra from flashed leaves at different stages of development to estimate the amount of 730 nm emission due to Photosystem II.

The primary leaves from different dark-grown bean plants which have been subjected to the repetitive flash regime vary widely in their chlorophyll content. The light flashes set off a pattern of developmental changes which include the opening of the plumular hook, the extension of the primary leaves beyond the cotyledons, the

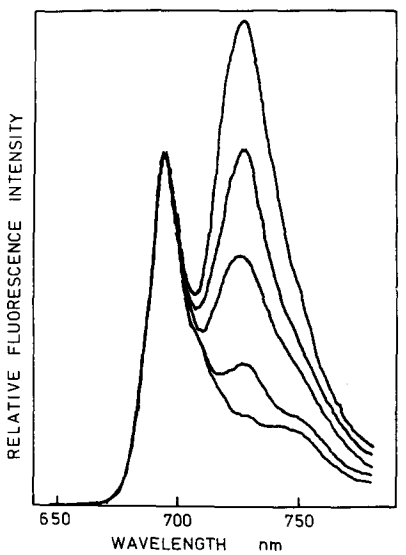

Fig. 2. Emission spectra of different flashed leaves at −196 °C excited at 633 nm. The leaves were the same age and had been subjected to the same number of flashes but had different chlorophyll content. The spectra were normalized at 694 nm.

unfolding and expansion of those leaves and the accumulation of chlorophyll in the leaves. Some plants show a more rapid development in the light flashes than others and the primary leaves of those plants accumulate more chlorophyll. Emission spectra at −196 °C from a series of flashed bean leaves, all taken from plants of the same age and subjected to the same number of flashes, are shown in Fig. 2 with the spectra being normalized at the 694 nm emission peak. The extent of the 730 nm emission band is correlated with the chlorophyll content of the leaves, with the greener leaves showing more of the 730 nm emission. We assume that the shape of the Photosystem II emission is the same in the various fluorescence spectra and that the spectra differ in the relative contribution of the 730 nm emission band of Photosystem I. The wide diversity in the extent of the 730 nm emission band will be used to extrapolate out the 730 nm emission due to Photosystem I in order to determine how much of the 730 nm emission is due to Photosystem II.

The fluorescence at 730 nm at the F_M level, $F_{730(M)}$, will be considered to have two sources of emission, one due to Photosystem I, $F_{I(M)(730)}$ and one due to Photosystem II, $F_{II(M)(730)}$. These values will be normalized against the F_M level of fluorescence at 694 nm, $F_{694(M)}$, which is assumed to be due solely to Photosystem II.

$$\frac{F_{730(M)}}{F_{694(M)}} = \frac{F_{I(M)(730)}}{F_{694(M)}} + \frac{F_{II(M)(730)}}{F_{694(M)}} \tag{1}$$

The last term in Eqn. 1 is the ratio of the Photosystem II emission at 730 nm to that at 694 nm which we seek to determine by the extrapolation. In order to effect that extrapolation we need an experimentally determined value of Photosystem I fluorescence at 730 nm which is not contaminated by any Photosystem II fluorescence, in order to extrapolate the second term in Eqn. 1 to zero. To obtain such values, the flashed leaves were frozen to −196 °C in complete darkness and F_{730} was measured as a function of F_{694} on an X-Y recorder as the 633 nm excitation beam converted the fluorescence yield from the F_o level to the F_M level. These X-Y plots at −196 °C always give straight lines which can be extrapolated back to the F_{730} axis to give the value of $F_{I(\alpha)}$ [3, 4]. These values of $F_{I(\alpha)}$ are due only to Photosystem I emission; any Photosystem II emission at 730 nm will extrapolate out (as F_{694} is extrapolated to zero all Photosystem II emission should go to zero). These determinations of $F_{I(\alpha)}$ were made on each of the leaves indicated in Fig. 2; the emission spectra of the leaves

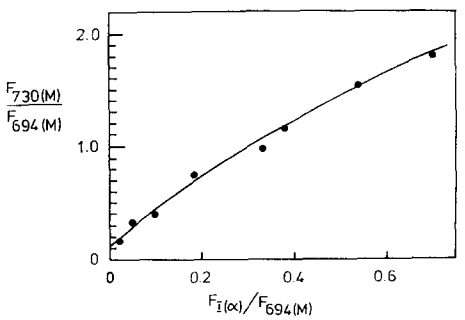

Fig. 3. Extrapolation of $F_{730(M)}/F_{694(M)}$ vs. $F_{I(\alpha)}/F_{694(M)}$. $F_{I(\alpha)}$, $F_{694(M)}$ and $F_{730(M)}$ were measured on each of the leaves indicated in Fig. 2. The extrapolation indicates that Photosystem II has emission at 730 nm which is 12 % of the peak emission at 694 mn.

at the F_M level being measured after each X-Y plot was completed. To achieve the extrapolation indicated by Eqn. 1, $F_{730(M)}/F_{694(M)}$ was plotted as a function of $F_{I(\alpha)}/F_{694(M)}$ with the assumption that $F_{I(M)730}$ goes to zero as $F_{I(\alpha)}$ goes to zero. That extrapolation shown in Fig. 3 indicates that the Photosystem II emission at 730 nm is 12 % of the peak value at 694 nm. This value obtained from the intact leaves is in good agreement with our previous estimate of 10–15 % taken from the emission spectrum of Photosystem II particles.

There could be some question as to whether $F_{I(M)730}$ extrapolates to zero as $F_{I(\alpha)}$ goes to zero. From the accompanying paper;

$$F_{I(M)} = F_{I(\alpha)} + F_{I(\beta)}$$

If $F_{I(\beta)}$ should not extrapolate to zero along with $F_{I(\alpha)}$, then we have included some Photosystem I fluorescence in our extrapolated value and we have overestimated the amount of Photosystem II fluorescence at 730 nm. Since our estimate of 12 % is already fairly low we don't consider this to be a significant source of error.

We have used this value of 12 % to construct a pure Photosystem II emission spectrum by subtracting the Photosystem I emission spectrum, calculated as the difference spectrum in Fig. 1, from the measured emission spectrum leaving 12 % of the 694 nm emission remaining at 730 nm. Such a calculated spectrum (Fig. 4) indicates that the Photosystem II emission spectrum has a small broad long wavelength maximum at about 750 nm in addition to the sharp major peak at 694 nm. The broad 750 nm band in the Photosystem II emission spectrum was also observed in a calculated Photosystem II emission spectrum from *Porphiridium cruentum* [5].

If the flashed leaves are placed in continuous light they accumulate the light-harvesting chlorophyll *a*/*b* protein and the low temperature emission spectrum develops a well-defined shoulder at about 685 nm. The emission spectrum of such a leaf at −196 °C excited with 633 nm light is shown in Fig. 5. The spectrum of the fluorescence excited at 514 nm was also measured (data not shown) and the spectral

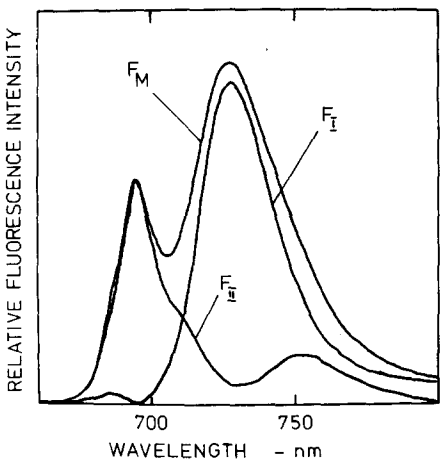

Fig. 4. Emission spectrum of a flashed leaf at −196 °C excited at 633 nm, F_M, taken from Fig. 1. Emission spectrum of Photosystem I, F_I, (see Fig. 1) adjusted so that the difference $F_M - F_I$ at 730 nm is 12 % of the peak at 694 nm. F_{II} is the difference spectrum, $F_M - F_I$.

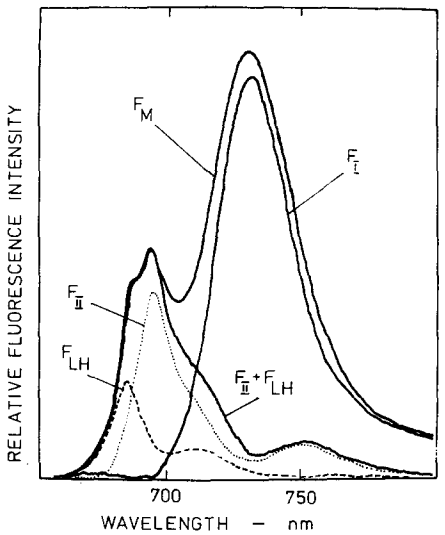

Fig. 5. Emission spectrum at −196 °C of a flashed leaf which had been illuminated 4 h with continuous white light, F_M. $F_{II}+F_{LH}$ is the difference spectrum F_M-F_I, as in Fig. 4. F_{II} was taken from Fig. 3 and adjusted in magnitude according to the text. F_{LH} is the difference spectrum between $F_{II}+F_{LH}$ and F_{II}.

shape of the Photosystem I emission was determined from the difference spectrum by the procedures indicated in Fig. 1. The wavelength maximum of the Photosystem I emission shifts from about 728 nm for the flashed leaf to about 735 nm as the leaf greens. The wavelength maximum of the Photosystem II emission remains at 694 nm during the greening process. The Photosystem I emission spectrum, F_I in Fig. 5, was subtracted from the measured spectrum of the leaf to give the emission spectrum due to Photosystem II chlorophyll plus the light-harvesting chlorophyll *a*/*b* complex, $F_{(II+LH)}$. The Photosystem II emission spectrum, F_{II} from the flashed leaf (Fig. 4), was then subtracted from $F_{(II+LH)}$ to give the emission spectrum of the light-harvesting chlorophyll *a*/*b* complex, F_{LH}.

The amount of F_{II} used in Fig. 5 was not arbitrary. The fourth derivative of the emission spectrum of a green leaf shows a maximum at 682 nm for the chlorophyll *a*/*b* complex even though the shoulder on the emission spectrum is closer to 685 nm. If we consider the sum of the emissions from Photosystem II and the chlorophyll *a*/*b* complex;

$$F_{(II+LH)} = F_{II}+F_{LH}$$

and take the first derivative of that sum;

$$\frac{dF_{(II+LH)}}{d\lambda} = \frac{dF_{II}}{d\lambda}+\frac{dF_{LH}}{d\lambda}$$

we can set the last term equal to zero at 682 nm. The emission spectra for $F_{(II+LH)}$ (Fig. 5) and for F_{II} (Fig. 4) are available as digitized spectral data in the computer so that the first derivatives of those spectra are also readily available. Thus, the magnitude of F_{II} used in Fig. 5 was selected so that $dF_{II}/d\lambda$ was equal to $dF_{(II+LH)}/d\lambda$ at 682 nm.

The leaf used for the spectra shown in Fig. 5 was sufficiently green that the shorter wavelengths of fluorescence may have been subjected to some reabsorption by the leaf even with the front surface measurements. Thus, the spectrum of F_{LH} may be suppressed somewhat at the peak relative to the longer wavelengths.

We conclude that the emission spectra shown in Fig. 5 for F_I, F_{II} and F_{LH} are representative of fluorescence from the three types of antenna chlorophyll postulated in the tripartite model for greenplant photosynthesis [6, 7].

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Science Foundation, PCM76-07111. R. S. is the recipient of a grant from the Swiss National Science Foundation, NR. No. 831.190.73.

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