F-695 EMISSION FROM THE PURIFIED PHOTOSYSTEM II CHLOROPHYLL *a*–PROTEIN COMPLEX

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1. Introduction

Early fractionation experiments [1–4] as well as the analysis of fluorescence excitation spectra [5,6] and of fluorescence inductions [7,8] supported the conclusion that F-685 and F-695 belong to photosystem II (PSII) while F-735 belongs to photosystem I (PSI) in photosynthesis. However, the F-695 band, which has been postulated as fluorescence originating from the antenna of the PSII reaction center [9], appeared neither in the purified preparations of light-harvesting ([13], but see [23]) nor in the purified reaction center complex [10–15] of PSII. This discrepancy was first explained by the hypothesis [13] that F-685 and F-695 are due to the light-harvesting and the reaction center complex of PSII, but that the peak positions are blue-shifted in the detergent-solubilized preparations to 681 and 685 nm, respectively. The hypothesis, however, is challenged by the facts that a prominent emission band near 685 nm is observed on the spectrum of chloroplasts from mutant barley lacking the light-harvesting chlorophyll *a*/*b*–protein [16] and that the F-685 emission of the purified PSII chlorophyll *a*–protein complex (the chlorophyll *a*–protein comprising the reaction center of PSII [11,13,17]) no longer shows the characteristic temperature dependence of chloroplast F-695 emission.

In order to gain more information about the fate of F-695 in isolation of pigment–protein complexes from the thylakoid membranes, an analysis was carried out to detect F-695 band by a sensitive method which uses 1,10-phenanthroline in the presence of chaotropic reagents [18]. The results clearly indicated that the purified PSII chlorophyll *a*–protein complex contains two fluorescent components, F-685 and F-695, at 77 K, although the molecular environment for the latter component is modified to some extent.

2. Materials and methods

Chloroplasts were prepared from spinach (*Spinacia oleracea*) leaves as in [13]. Light-harvesting chlorophyll *a*/*b*–protein, PSII chlorophyll *a*–protein complex, and PSI chlorophyll *a*–protein complex were isolated by digitonin-treatment of spinach chloroplasts and purified using isoelectrofocussing as in [13].

Fluorescence emission spectra were measured either with a computer-controlled fluorescence spectrometer of Drs W. L. Butler and R. J. Strasser (fig.1,2) or with the instrument in (fig.3,4) [19].

3. Results and discussion

Figure 1 shows a comparison between the fluorescence emission spectrum at 77 K from spinach chloroplasts with those of the 3 major chlorophyll–protein complexes purified therefrom. It is evident that the characteristic emission peak at 695 nm observed on the chloroplast emission spectrum is hardly seen in any of the 3 purified complexes. On the contrary, a new emission band, which is not seen in the chloroplast emission spectrum at 77 K and which is 10–20-times more intense, on a chlorophyll basis, than that of the chloroplast emission bands or of the F-685 and F-734 emission bands of the purified chorophyll protein complexes, appears in the purified light-harvesting chlorophyll *a*/*b*–protein at 681 nm. Closer inspection of the emission spectrum of the PSII

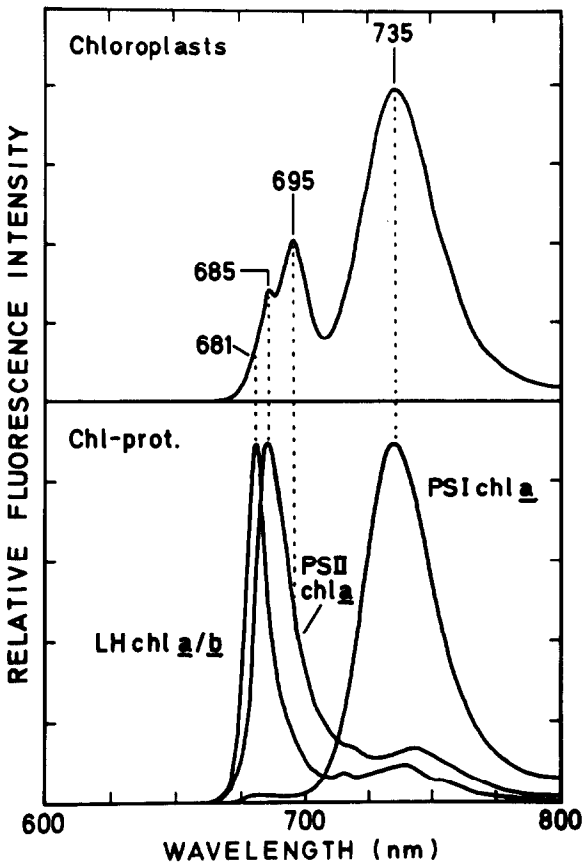

Fig.1. Comparison between the fluorescence emission spectrum at 77 K of spinach chloroplasts (upper) with those of chlorophyll–protein complexes (lower). Fluorescence was excited at 633 nm (He–Ne laser), analyzed by a Bousch and Lomb double grating monochromator (band pass, 2 nm), and corrected for the sensitivity of photomultiplier and the transmission efficiency of the monochromator. The intensities of the emission from different chlorophyll–protein complexes were normalized at the highest peak values. Chlorophyll was 10 μg/ml in 50 mM Tris–HCl (pH 7.2). *Abbreviations:* LH chl *a*/*b*, light-harvesting chlorophyll *a*/*b*–protein; PSII chl *a*, PSII chlorophyll *a*–protein complex; PSI chl *a*, PSI chlorophyll *a*–protein complex

chlorophyll *a*–protein complex, however, indicates that there is a weak shoulder around 690–695 nm; thus the half-band width (15–17 nm) of the emission (F-685) of this complex is much larger than that of the F-681 band (10 nm) of light-harvesting chlorophyll *a*/*b*–protein (see also [19]). The temperature profile of this 695 nm shoulder was very different from that of the main band (F-685); it showed a much higher temperature coefficient than that of the F-685 band over 150–77 K, suggesting that there are some contributions of a component similar to that involved in the F-695 emission of chloroplasts.

The F-695 emission band is not always a distinct band on the spectrum at 77 K in the case of chloroplasts from all higher plants and algae. For example, it is often very difficult to detect a distinct F-695 band on the emission spectrum at 77 K in the case of chloroplasts from wheat or maize [15,20]. However, this component still seems to be ubiquitous in all of the higher plants and algae at 77 K, because there always exists a temperature-sensitive shoulder in the 695 nm region of the emission spectrum, although in some materials its intensity is very low. This conclusion is also supported by the observation that the addition of 1,10-phenanthroline together with chaotropic reagents to the chloroplasts from such materials results in activation of a prominent emission band at 695–700 nm with similar characteristics to the F-695 band of typical chloroplasts; e.g., the emission has a variable part, increases at <150 K, and has the same excitation spectrum as those of the F-685 and F-695 emission bands [18]. The mechanism of this phenanthroline effect is not clearly understood yet. However, it probably reflects some changes in the interaction between chlorophyll and the protein within the PSII chlorophyll–protein complexes. One benefit on this phenomenon is that it can be used as a sensitive method to detect F-695 in the preparations with low F-695 emission. Figure 2 shows the effect of 1,10-phenanthroline in the presence of 0.1 M guanidine–HCl on the fluorescence emission spectrum of spinach chloroplasts at 77 K. Guanidine–HCl, at 0.1 M, causes a minor, but definite effect on the emission spectrum at 77 K (compare chloroplast emission spectrum in fig.1 with that without 1,10-phenanthroline in fig.2) [18]. This effect is not discussed further here. The fluorescence difference spectrum shown in fig.2 and the 4th derivative analysis of the emission spectra indicate that the effect of 1,10-phenanthroline is to create a new emission band at 700 nm at the expense of F-695 emission. The new band is intensified, possibly because of the inhibition of uphill transfer due to the difference in energy between the molecules involved.

As fig.3 shows, basically the same results can be obtained if 1,10-phenanthroline together with guanidine–HCl is added to the purified PSII chlorophyll *a*–protein complex, except that the peak position of the new band is shifted (695 nm). However, 1,10-phenanthroline plus guanidine–HCl are entirely

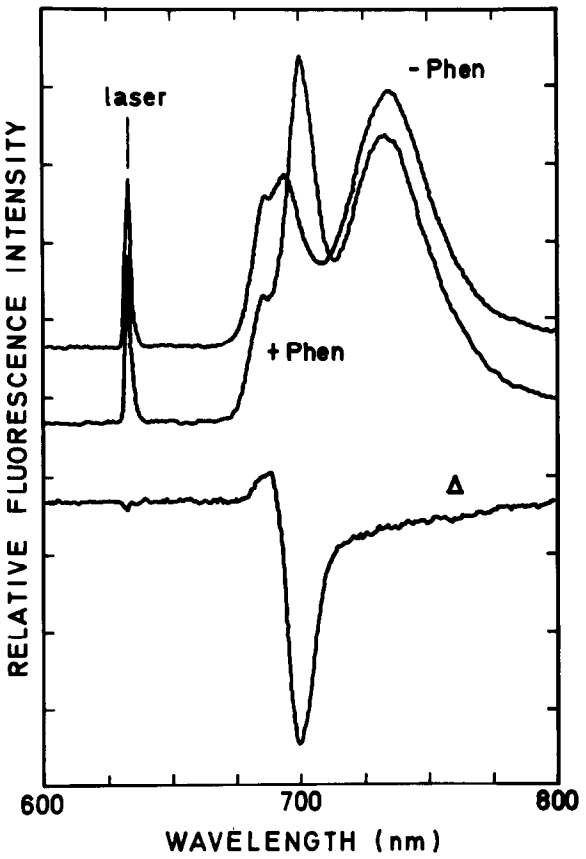

Fig.2. Fluorescence emission spectra at 77 K of spinach chloroplasts in the presence (+Phen) and in the absence (−Phen) of 1 mM 1,10-phenanthroline. Chloroplasts were suspended at 5 μg chl/ml in 50 mM Tris–HCl (pH 7.2) and 50 mM guanidine–HCl. Curve at the bottom (Δ) represents fluorescence difference spectrum, (−Phen) minus (+Phen). See text and fig.1 for further explanations.

inactive in creating a new band in the light-harvesting chlorophyll *a*/*b*–protein (fig.3) or in the PSI chlorophyll *a*–protein complex (data not shown). The new band is highly temperature dependent over 150–77 K as shown in fig.4, just as in the typical F-695 emission of chloroplasts, while the F-685 (fig.4) and F-681 bands were only slightly dependent on temperature.

On the basis of the increase in the emission band at 695–700 nm which takes place to compensate for the decrease of F-695 emission shown in fig.2 and of the characteristic temperature-dependence which, together with the inductions and the excitation spectra [18], are common to both emission bands, it is concluded that the emission band created by the addition of 1,10-phenanthroline plus guanidine–HCl is a transformed species of the F-695 band. The F-695 emission band in the purified PSII chlorophyll *a*–protein complex probably is blue-shifted and markedly quenched, because the new band formed by the addition of 1,10-phenanthroline plus guanidine–HCl is 5 nm blue-shifted as compared with the corresponding band in chloroplasts (fig.2). This implies that there are some changes in structural integrity around the F-695 molecules in the detergent-treated preparations. For PSII preparations, the extent of the F-695 emission appears to decrease with increasing degrees of purification. None of the highly purified PSII preparations hitherto reported [10–15] showed a prominent emission peak at 695 nm which has been postulated as a component of the PSII reaction center complex [9]. However, from the analysis reported here it is concluded that the F-695 is a component localized in the PSII chlorophyll *a*–protein complex, and that this complex contains two fluorescent components, F-685 and F-695, at 77 K, although the latter is modified to some extent (see also [15]).

Fluorescence excitation spectra obtained with the purified PSII chlorophyll *a*–protein complex [13] suggested that F-685 is identical with the chlorophyll absorbing at 683 nm. This indicates that F-695 is a

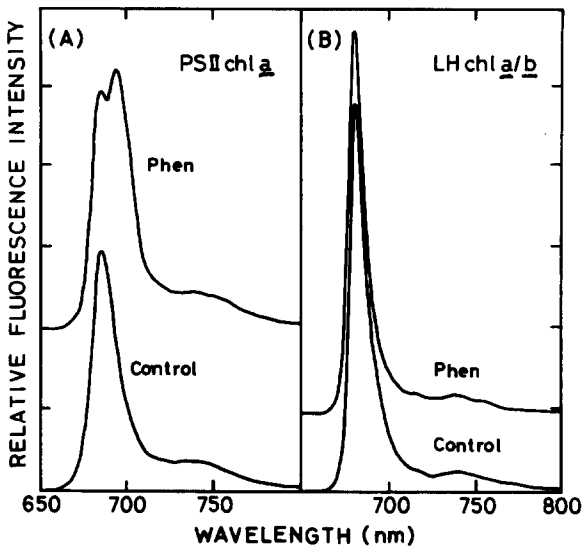

Fig.3. Fluorescence emission spectra at 77 K of PSII chlorophyll *a*–protein complex (A) and light-harvesting chlorophyll *a*/*b*–protein, purified from spinach chloroplasts, in the presence (Phen) and in the absence (control) of 1 mM 1,10-phenanthroline. Chlorophyll–protein complexes were suspended at 5 μg PSII chl *a*/ml or at 1.1 μg LH chl *a*/*b*/ml in 50 mM Tris–HCl (pH 7.2) and 50 mM guanidine–HCl. See text for further explanations.

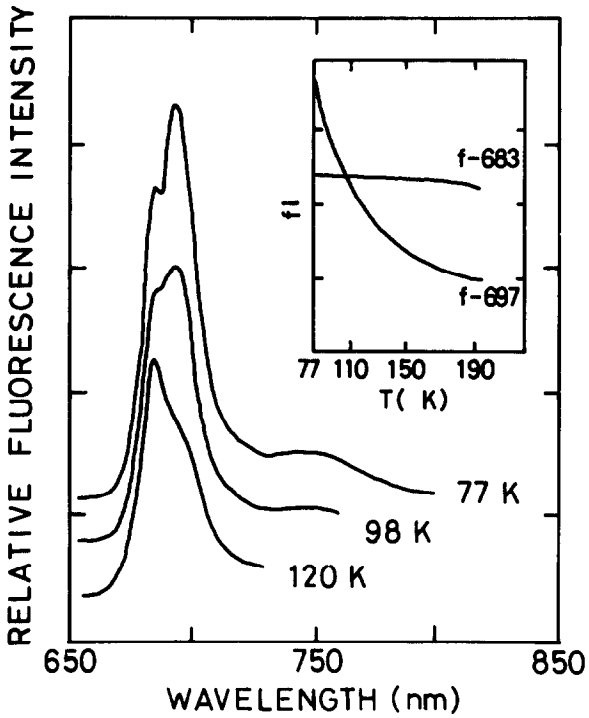

Fig.4. Temperature dependence of fluorescence emission from PSII chlorophyll *a*–protein complex in the presence of 1 mM 1,10-phenanthroline. Fluorescence emission spectra were measured at 120, 98 and 77 K. Insertion: Fluorescence emission at 683 nm (f-683) and at 697 nm (f-697) versus temperature. To obtain the temperature profile the liquid nitrogen was allowed to boil away and both the temperatures measured by a thermocouple and the fluorescence intensities were plotted on a *X*–*Y* recorder. Chlorophyll was 5 μg/ml in 50 mM Tris–HCl (pH 7.2) and 50 mM guanidine–HCl.

chlorophyll that absorbs at a longer wavelength and which is present in smaller amounts than the major antenna chlorophylls. We proposed ([21], see also [22]) that the F-695 emission band is due to a form of chlorophyll in PSII which forms on cooling to a low temperature and which acts as a trap for the excitation energy in the antenna chlorophylls of PSII. This component probably absorbs at a relatively longer wavelength than F-685 (Ca683) at room temperatures and normally transfers its excitation energy to the reaction center of PSII. It is unable to do so at low temperatures but acts as an energy trap emitting fluorescence.

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