BBA 43223

Enhancement of emission from chloroplasts at 698 nm by a naturally-occurring factor

The existence of an emission band with maximum at 698 nm (F698) has been reported for many algae and higher plants at —196° (refs. 1–7). It was proposed that the pigment giving rise to this emission is the reactive site for the photochemistry of System II in photosynthesis[3–5]. Some of the evidence which supports this assignment has been obtained from fluorescence excitation spectra determined with whole algae and chloroplasts[8,9] and from emission spectra of chloroplasts fractionated in the presence of detergents[10–12]. With the latter, it was observed that emission at 685 and 698 nm is associated with one type of particle, while longer wavelength emission is associated with a different particle whose photochemical properties relate it to System I. These fractionation procedures yield a better separation and isolation of the pigments of System I than those of System II, and even selective extraction[13] has not resulted in the isolation or enrichment of System II, or pigment F698, to the degree needed for the biochemical and spectroscopic studies which will elucidate the functional aspects of System II.

Work in our laboratory has revealed the existence of a naturally-occurring factor(s) which enhances emission at 698 nm. Chloroplasts from higher plants were isolated by a modification of the method of JAGENDORF AND AVRON[14]. Those from *Ricinus communis* (castor oil plant) were suspended in isolation medium (0.35 M NaCl–0.04 M Tris–HCl, pH 7.8), while those from other sources, *e.g. Spinacia oleracea, Zea mays,* Raphanus sp., Nicotiana sp. or *Chlamydomonas reinhardtii* strain 6270C, were suspended either in isolation medium (control) or in an aqueous cell-free extract of Ricinus leaf (prepared by a slightly modified method of MCCARTY AND JAGENDORF[15]).

In Fig. 1 may be seen the time-dependent changes in fluorescence emission which occur during incubation of Ricinus chloroplasts at 37°. (For spectrofluorimeter characteristics, see ref. 16.) At 37°, F685 seems to disappear in about 5 min; after approx. 30 min, long-wavelength fluorescence (with maximum at 710–720 nm in algae and 730–740 nm in higher plants) has diminished to such an extent, that only emission from F698 is apparent. Similar changes may be seen in Fig. 2, in which the ratio of intensities at 735 nm and 698 nm is plotted (Curve B) as a function of incubation (25°) time for Spinacia chloroplasts suspended in Ricinus extract; in the absence of extract (Curve A) these changes do not occur. That the increase in F698 and the decrease in F735 are absolute, has been determined by monitoring fluorescence yield (relative), at room temperature and the temperature of liquid nitrogen, as a function of incubation time.

Associated with changes in emission spectra are simultaneous changes in fluorescence action spectra; see Fig. 3, in which are shown low-temperature spectra for exciting long-wavelength emission as a function of incubation time. The spectrum given by Curve A in Fig. 3 may be considered to represent the control situation in Ricinus, on the basis of similarity to control chloroplasts (*i.e.* chloroplasts from other plants, suspended in the absence of the active factor).

The fluorescence changes observed in the present work are temperature-dependent, the rate of change increasing with increasing temperature of incubation (measured range 0–45°). Determination of chlorophyll ($a+b$) according to the method of

ARNON[17], reveals no significant decrease in concentration even after 2 h of incubation at 37°.

That the active factor(s) in the extract is a protein, or is associated with a protein, is indicated by the following experiments: (a) the above-described fluores-

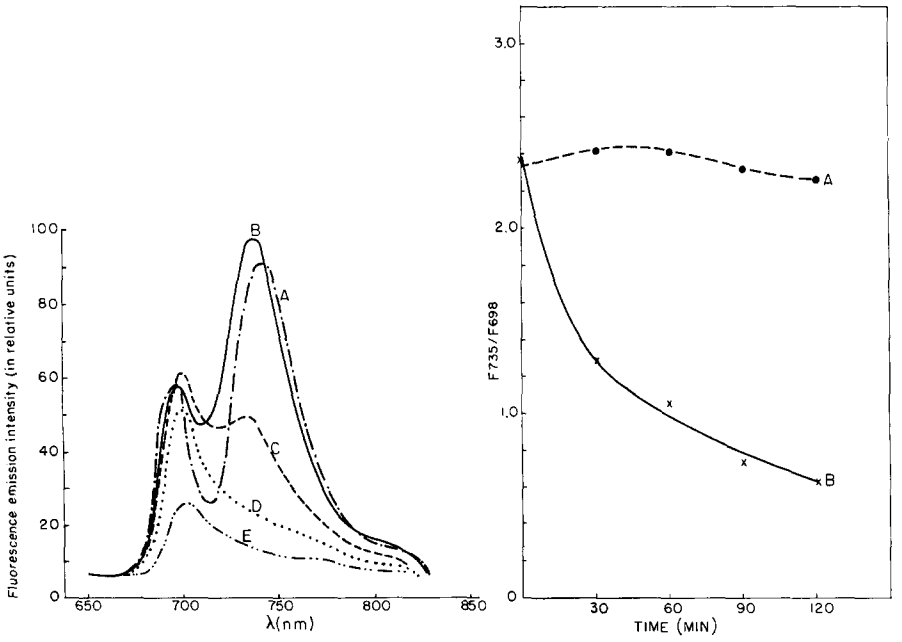

Fig. 1. Fluorescence emission spectra, excited at 435 nm, from Ricinus chloroplasts (at −196°), as a function of time of incubation at 37°. Curves A, B, C, D and E are for 0, 5, 10, 20 and 60 min, respectively.

Fig. 2. The ratio of fluorescence intensities at 735 nm and 698 nm (F735/F698) as a function of time of incubation at 25°. Curve A, spinach chloroplasts resuspended in 0.35 M NaCl–0.04 M Tris–HCl (pH 7.8); Curve B, spinach chloroplasts resuspended in Ricinus leaf extract. Fluorescence excited at 435 nm and measured at −196°.

cence changes do not occur if the extract is heated prior to incubation, or if chloroplasts are incubated in extract at 0°; (b) the active fraction of the extract can be precipitated by ammonium sulfate; and (c) the removal of dialyzable material from the precipitated extract does not result in loss of activity.

Preliminary studies with the electron microscope (in conjunction with Mr. James E. White) and experiments on oxygen evolution suggest that the observed steady-state fluorescence changes are associated with disruption of both normal lamellar structure and electron transport.

The enrichment of System II emission achieved in the present work permits more direct and quantitative investigation of the spectral and photochemical properties of the System II sensitizer than previously possible; such work, as well as studies elucidating the mechanism of this enrichment will be published in detail elsewhere.

This research was partially supported by a Faculty Research Grant to M.B.

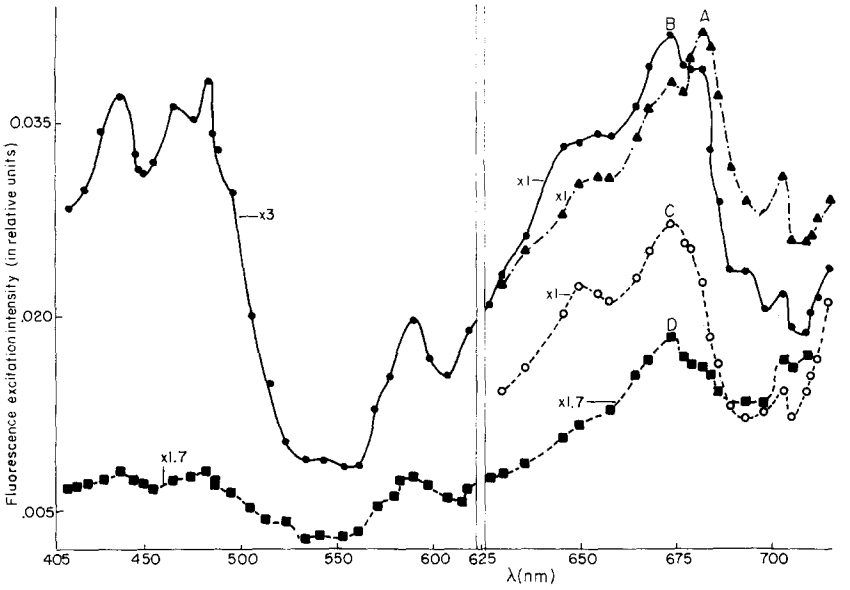

Fig. 3. Action spectra for exciting fluorescence at 735 nm from Ricinus chloroplasts (at −196°), as a function of time of incubation at 37°. Curves A, B, C, and D are for 0, 5, 10, and 20 min, respectively. Spectra have been corrected for equal number of incident quanta. Curves have been displaced upwards by indicated factors, for ease of comparison. Note that scale of abscissa differs at wavelengths shorter and longer than 623 nm.

from the City University of New York. We thank Dr. Ian Stevenson for the algal chloroplast fragments used in some of these experiments.

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Received October 7th, 1968