SHORT COMMUNICATIONS

BBA 43243

Fluorescence properties of fragments from sonicated spinach chloroplasts

The fluorescence emission spectrum of spinach chloroplasts at 20° C shows a sharp band at 683 nm and a minor broad band with a maximum at about 735 nm. The spectrum is similar in shape to that of chlorophyll in organic solvents, although the fluorescence yield is much smaller for chloroplasts. On cooling chloroplasts to 77°K, a three banded fluorescence emission spectrum is obtained with maxima at 683, 693 and 735 nm[1,2]. The most intense band is now at 735 nm, with 75% of the total emission appearing in this band. BRODY AND BRODY[3] observed that concentrated solutions of chlorophyll *a* in organic solvents gave, at 77°K, an intense fluorescence emission at about 720 nm, which was ascribed to the chlorophyll dimer. They suggested further that the 735-nm emission from chlorophyll *in vivo* at 77°K was derived from a chlorophyll dimer, and the emission at about 690 nm from a chlorophyll monomer.

BRODY *et al.*[4] examined the fluorescence properties of fragments prepared from spinach chloroplasts by a sonic treatment. The relative intensities of the 683- and 735-nm bands at 77°K were a function of particle size of the fragments, the larger particles showing an increased relative emission at 735 nm compared with the smaller fragments. BRODY *et al.*[4] suggested that sonication of chloroplasts may produce fragments which contain few, if any, aggregates of chlorophyll, and that the intensity of fluorescence at 735 nm from the aggregate may approach zero as the size of the fragment decreases.

In contrast to sonication, digitonin incubation of spinach chloroplasts which effects a fractionation of the photochemical systems produced small fragments which showed a greatly enhanced relative fluorescence emission at 735 nm at 77°K (ref. 2).

It seemed desirable to establish to what extent, if any, the fluorescence properties of chloroplast fragments are influenced by particle size. In this work, we have reinvestigated the fluorescence emission spectra at 77°K of fragments produced by sonication of chloroplasts. We conclude that the spectra are independent of fragment size over a wide range, but dependent on the redox state of a quencher.

Spinach leaves (16 g) were blended in two lots, each in 60 ml of 0.05 M phosphate buffer (pH 7.2) containing 0.3 M sucrose and 0.01 M KCl in a Servall Omnimixer as described previously[5]. The chloroplasts were purified by sedimentation at 1000 × *g* for 10 min and washing once with sucrose–phosphate buffer. The final chloroplast pellets were resuspended in distilled water (27.5 ml) and allowed to stand for 10 min at 0° C before fragmentation with a sonic oscillator (Raytheon 250 W, 10 kcycles) for 2 min. The chamber of the oscillator was maintained at about 4° C by circulation of cold water. The chloroplast fragments were separated by differential centrifugation at 1000 × *g* for 10 min, followed by 10 000 × *g* for 30 min, 50 000 × *g* for 30 min, 144 000 × *g* (40 000 rev./min Rotor No. 40 Spinco Model L centrifuge) for 1 h and finally a further spin at 144 000 × *g* for 3 h. The pellets from each centrifugation were resuspended in 0.05 M phosphate buffer (pH 7.2) containing 0.01 M KCl.

Absorption spectra of the fractions were recorded on a Cary Model 14 R spectrophotometer, fitted with a scattered-transmission accessory. For fluorescence measurements, the fractions were diluted to an absorbance of 0.2 at 470 nm in order to minimize reabsorption of the emitted light. Absorption spectra at 77°K were recorded in 60% glycerol, as described previously[6].

Fluorescence emission spectra were determined on a fluorescence spectrometer, which incorporated automatic correction for photomultiplier and monochromator responses, and variation in energy output of the light source[7]. The instrument was operated with an excitation band width of ± 1.5 nm and a fluorescence band width of ± 1.0 nm. Fluorescence kinetics were recorded on a Rikadenki B-34 recorder at a chart speed of 80 mm/min. Quantum yields of fluorescence were calculated as described previously[7]. For measurements at 77°K, the sample was diluted into 0.05 M phosphate buffer (pH 7.2), containing 60% glycerol, and placed in a cylindrical glass cell which had an effective path length of 0.3 cm. The method of cooling the sample was described previously[7].

The fragments obtained by the sonication of chloroplasts under our conditions of very low ionic strength had the same chl *a*/chl *b* ratio as the chloroplasts and the same absorption spectrum, both at 20° C and 77°K. These observations are in agreement with previous work[8,9], although more recently it was shown that sonication of chloroplasts for short times under conditions of higher ionic strength yields fragments with different chl *a*/chl *b* ratios[10,11]. The continuous lines in Figs. 1a and 1b show a comparison of the fluorescence emission spectra at 77°K of the large fragments contained in the 10 000 × *g* fraction (SF-10) and the very small fragments of the 144 000 × *g* fraction from the final centrifugation (SF-144_2). Both spectra have bands with maxima at 683, 693 and about 735 nm, but it is apparent that there is a difference in the fraction of the fluorescence emitted at the 735-nm band. The emission at 735 nm remains reasonably constant, but the emissions at 683 and 693 nm are smaller for SF-144_2. Ratios of quantum yields of fluorescence are given in Table I. For SF-10, 73% of the fluorescence was emitted at the 735-nm band, compared with 83% for SF-144_2. The fragments obtained from the first centrifugation at 144 000 × *g* (SF-144_1) were similar in their fluorescence properties to SF-144_2. The 50 000 × *g* fraction which emitted 76% of its fluorescence at the 735-nm band was intermediate between the SF-144_1 and SF-10. The fluorescence spectrum of the supernatant from the final cen-

TABLE I

QUANTUM YIELDS OF FLUORESCENCE OF CHLOROPLAST FRAGMENTS

The quantum yield of fluorescence at the 735-nm band is expressed as a percentage of the total quantum yield of fluorescence. Conditions as for Fig. 1.

Fraction	$\phi_{735\ nm}/\phi_{total}$ (%)	
	−ferricyanide	*+ferricyanide*
Chloroplasts	71	81
SF-10	73	82
SF-50	76	80
SF-144_1	84	85
SF-144_2	83	84
Supernatant	47	44

trifugation differed markedly from the sedimented fractions, and it resembled in fact, the fluorescence spectrum of chlorophyll *a* in ethanol at 77°K.

These results are not in agreement with the earlier work of BRODY *et al.*[4], where the sonication time was 15 min. We therefore tested the influence of sonication time on the fluorescence properties of the fragments. Fragments produced by sonication of chloroplasts under our conditions for 15 min gave similar fluorescence spectra to the fragments produced by sonication for 2 min, but the amount of chlorophyll in the supernatant was greater after the longer time of sonication.

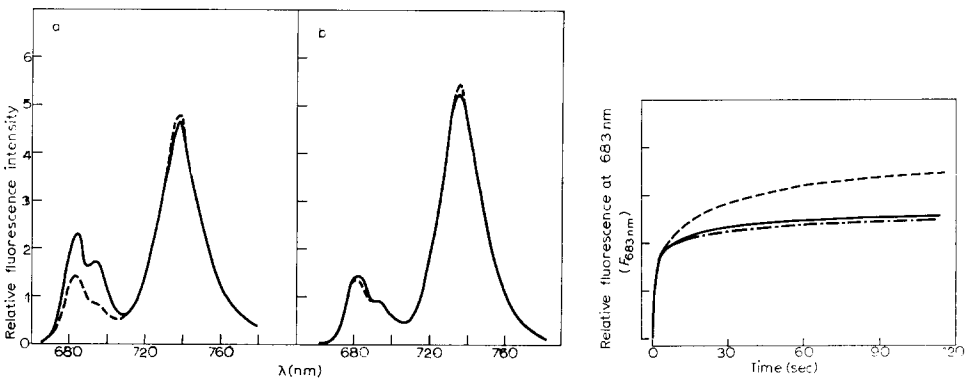

Fig. 1. (a) Fluorescence emission spectra of SF-10 at 77°K; ———, without addition of ferricyanide; — — —, with addition of ferricyanide. (b) Fluorescence emission spectra of SF-144_2 at 77°K; ———, without addition of ferricyanide; — — —, with addition of ferricyanide. Excitation wavelength, 470 nm. Light intensity 130 ergs·cm^{-2}·sec^{-1}. Ferricyanide concn., 50 μM.

Fig. 2. Time-course of fluorescence emission at 683 nm at 77°K. — — —, SF-10; ———, SF-144_1; –·—·–, SF-144_2. Conditions as for Fig. 1.

Figs. 1a and 1b also show the effect of the addition of ferricyanide, prior to cooling, on the fluorescence spectra of SF-10 and SF-144, at 77°K. The small fragments are not affected to any significant extent by the ferricyanide, but the 10 000 × *g* fraction shows a reduction in the fluorescence bands at 685 and 693 nm. A similar decrease was observed with unsonicated chloroplasts, and to a lesser extent, with the SF-50. Table I shows the percentage of fluorescence emitted at the 735-nm band after addition of ferricyanide. With the exception of the supernatant, there is now little difference between the various fractions. The conclusion is reached, therefore, that the small differences in the fluorescence properties of the fragments from sonicated chloroplasts are not due to a difference in particle size. The effect of ferricyanide suggests that the spectral differences are due to differences in the redox state of the quencher Q (ref. 12), and this suggestion was supported by measurements of the time-course of the fluorescence emission. From their studies DUYSENS AND SWEERS[12] concluded that fluorescence is quenched when Q is in the oxidized state.

KOK[13] and MURATA[14] studied the effect of time of excitation on the course of the fluorescence intensity at 683, 693 and 735 nm ($F_{683\,nm}$, $F_{693\,nm}$, $F_{735\,nm}$) of chloroplasts cooled to 77°K. $F_{693\,nm}$ showed a considerable increase from its initial instantaneous value to a final steady-state level. A similar time-course was observed for $F_{683\,nm}$, but $F_{735\,nm}$ showed only a small increase with time of illumination. It was

concluded that the amplitudes of $F_{683\,nm}$ and $F_{693\,nm}$ (which arise mainly from Photosystem 2, ref. 2) are influenced by the redox state of the quencher Q (ref. 12), and that Q can be photoreduced even at 77°K. $F_{735\,nm}$ which belongs mainly to Photosystem 1 (ref. 2) is influenced to a much smaller extent by the redox state of a quencher. Our experiments with spinach chloroplasts confirmed the observations of KOK[13] and MURATA[14] and showed further that the induction of $F_{683\,nm}$ and $F_{693\,nm}$ is much smaller in SF-144_1 and SF-144_2 than in SF-10. Fig. 2 shows the time-course of fluorescence for $F_{683\,nm}$. Similar results were obtained with $F_{693\,nm}$.

It appears that the small fragments from sonicated chloroplasts (SF-144_1 and SF-144_2) are less active in the photoreduction of Q at 77°K than the larger fragments (SF-10). The fluorescence emitted at 683 and 693 nm by SF-144_1 and SF-144_2 is more highly quenched than the corresponding fluorescence from SF-10. The level of $F_{735\,nm}$ remains fairly constant with particle size. Therefore, in the absence of ferricyanide the small particles emit a greater percentage of their fluorescence at the 735-nm band, and we obtain an apparent dependence of the fluorescence spectrum on particle size. A somewhat surprising result is that ferricyanide is able to interact with Q at a temperature as low as 77°K and minimize its photoreduction by light. The independence of fluorescence spectrum on particle size implies that the scattering of fluorescence followed by reabsorption is not a significant factor in our experiments.

Particles enriched in photosystem 1 would show an increased relative emission at 735 nm and be less dependent on ferricyanide than chloroplasts[2], but we have rejected this as an explanation for our present results in view of the constancy of the chl *a*/chl *b* ratios and the absorption spectra.

The spectrum obtained with the supernatant suggests at least a partial disruption of the photosynthetic units with either loss of energy transfer to the far-red form of chlorophyll (Chl-705) (ref. 15) or a disruption of chlorophyll dimers as suggested by BRODY *et al.*[4].

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C.S.I.R.O., Division of Plant Industry,
Canberra, 2601 (Australia)

N. K. BOARDMAN
S. W. THORNE

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