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STUDIES ON A BARLEY MUTANT LACKING CHLOROPHYLL *b*

II. FLUORESCENCE PROPERTIES OF ISOLATED CHLOROPLASTS

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SUMMARY

1. Chloroplasts isolated from a barley mutant which lacked chlorophyll *b* were less fluorescent than the chloroplasts of the normal strain, at both 20°C and 77°K. At 20°C, the quantum yields of fluorescence of the mutant chloroplasts were from 1/12 to 1/3 of the yields of the normal chloroplasts, depending on the wavelength and intensity of excitation.

2. A study of the time-course of the fluorescence intensity showed that the increase in fluorescence upon illumination was much smaller for the mutant chloroplasts. The fluorescence yields of the mutant chloroplasts were less dependent on redox conditions.

3. Fluorescence excitation spectra of the mutant chloroplasts at 77°K showed a pronounced shoulder at 670 mμ instead of the band at 650 mμ observed with the normal chloroplasts.

4. The mutant chloroplasts were more susceptible to disruption by digitonin, but the fluorescence properties of the small and the large particles did not indicate a fractionation of the photochemical systems.

5. The results are discussed in terms of the two pigment systems of photosynthesis.

INTRODUCTION

Photosynthesis in the green plant is a two quantum process: the two quanta are absorbed by different groups of pigments (pigment systems 1 and 2) which then cooperate in the primary photochemistry[1]. Reduction of $NADP^+$ is carried out by pigment system 1 and its associated electron carriers (collectively known as Photosystem 1), while O_2 evolution arises from pigment system 2 and its electron carriers (Photosystem 2). Evidence from action spectra[2,3] and from studies on the fractionation of the photochemical systems[4–7] show that in normal plants both pigment systems contain chlorophyll *a* (chl *a*) and chlorophyll *b* (chl *b*) and the major carotenoids, but in different proportions. Chl *b* is preferentially concentrated in pigment system 2

Abbreviations: chl *a*, chlorophyll *a*; chl *b*, chlorophyll *b*; Tricine, tris(hydroxymethyl)-methylglycine.

while system I has a higher content of β-carotene. Photosynthetic studies with a barley mutant lacking chl *b*[8,9] indicated that chl *b* is not an essential component of either pigment system. Leaf sections from the barley mutant showed photosynthetic rates which did not differ significantly from the rates obtained with normal plants[8]. Chloroplasts isolated from the barley mutant were more active per mg of total chlorophyll in the Hill reaction if assayed at high light intensities, but at lower intensities ($<$ 15000 lux) the mutant chloroplasts were less active than chloroplasts isolated from normal plants[9]. It was suggested that this lower efficiency of the mutant chloroplasts may be due either to a decreased efficiency of energy transfer to the reaction centres, or to a decreased amount of chlorophyll in Photosystem 2.

In the present study, fluorescence measurements have been made, at both 20°C and 77°K, on normal and mutant chloroplasts, and on the particles obtained by digitonin fragmentation in order to gain more insight into the pigment systems of the barley mutant. The smaller rises of fluorescence intensity with time observed with the mutant chloroplasts, together with their low quantum yields of fluorescence appear to support the view that their lower photochemical efficiency is due to a decreased amount of chlorophyll in Photosystem 2, compared with Photosystem I.

Excitation spectra of mutant chloroplasts, measured at 77°K show that a form of chl *a* absorbing at about 670 mμ (Ca-670) can perform some of the function of chl *b*.

MATERIALS AND METHODS

Preparation of chloroplasts

The normal and mutant strains of barley (*Hordeum vulgare*) used in these studies were described previously[8,10]. Chloroplasts from either mutant or normal plants were usually prepared by hand-grinding the leaves in an ice-cold mortar as described in the previous paper[9]. Two alternative methods for the preparation of chloroplasts were compared with the standard procedure of hand-grinding. In one method, the leaves were blended in a Servall Omni-mixer[5], and in the other a hand-chopping procedure was used[11].

Normal and mutant chloroplasts were fragmented by incubation with digitonin for 30 min at 0°, as described previously for spinach chloroplasts[5]. The concentration of total chlorophyll in the suspensions varied from 0.3 to 0.4 mg/ml for both normal and mutant chloroplasts. Fractions of different size were separated by differential centrifugation[4,5].

Fluorescence measurements

Fluorescence emission and excitation spectra were recorded on a fluorescence spectrometer incorporating automatic correction for photomultiplier and monochromator responses, and variation in energy output of the light source. A schematic diagram of the spectrometer is shown in Fig. 1. Excitation was by means of a quartz-iodine lamp (625 W) and a Bausch and Lomb 500-mm diffraction grating monochromator (M_1). The monochromator had a linear wavelength dispersion of 3.3 mμ/mm and was blazed for maximum efficiency at 500 mμ. A sample of the excitation light from M_1 was reflected onto the excitation photomultiplier, PM_1. The spectral response of PM_1 was determined by reference to a thermopile (Zeiss Type V.Th.8.), and a cor-

rection applied by a potentiometer (C_1), linked to the wavelength drive (D_1) of M_1 to adjust the photomultiplier electrical gain accordingly. The electrometer amplifier (A_1) gave a signal S_1 proportional to the intensity of the excitation light.

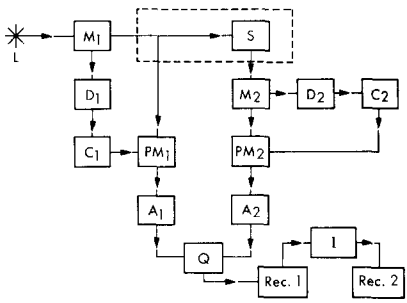

Fig. 1. Schematic diagram of fluorescence spectrometer. See text for explanation.

The sample was contained in a 1-cm optically clear rectangular cell (S) and fluorescence was viewed at right angles to the excitation beam by the monochromator, M_2, also a Bausch and Lomb 500-mm diffraction grating, but blazed at 750 mμ. The output of the fluorescence monochromator was measured by a photomultiplier, E.M.I. Type 9558 (PM_2). The spectral response of the combination of M_2 and PM_2 was determined and corrected by means of a potentiometer (C_2) linked to the wavelength drive (D_2) of M_2 to adjust the electrical gain of PM_2 with wavelength. The output of the electrometer amplifier (A_2) gave a signal S_2 proportional to the intensity of the fluorescence light.

The fluorescence signal S_2 was then automatically divided by the excitation signal S_1 in the divider Q. This process eliminated the variation of the output of the combination of light source and the efficiency of M_1 with wavelength, which would otherwise appear in the output S_2. The output signal S_2/S_1 was applied to the potentiometric recorders (Rec. 1 and Rec. 2). Correction to an accuracy of $\pm$ 2 % was possible over the fluorescence emission range of 400–750 mμ and over the exictation range 300–600 mμ. The excitation range could be extended with partial correction and less accuracy over the range 600–720 mμ.

The instrument was operated normally with an excitation band width of $\pm$ 1.5 mμ and a fluorescence emission band width of $\pm$ 1.0 mμ, at a scanning speed of 40 mμ/min, and with a recorder scan of 10 mμ/cm. The excitation intensities reaching the sample cell, at band widths of $\pm$ 1.5 mμ and with the quartz-iodine lamp operated at 80 V were (erg·cm^{-2}·sec^{-1}) $I_{436\ m\mu} = 90$, $I_{470\ m\mu} = 130$, $I_{650\ m\mu} = 170$, $I_{670\ m\mu} = 140$. Ten-fold higher intensities ($I \times 10$) were obtained with an excitation band width of approx. $\pm$ 6 mμ. For measurements at the higher intensities the emission band width was maintained at the $\pm$ 1.0-mμ value. The amplifiers A_1 and A_2 were provided with stepped gain ranges of the order of 100/1 to enable the instrument to cater for a wide range of sample concentrations and quantum efficiencies.

The sample cell (S) was located in a gas-tight sealed compartment. For low temperature measurements, a separate access to this compartment was made from above. A polyurethane-insulated copper pot of capacity 1 l was mounted above the compartment. Inside the compartment, the sample cell was placed in a lagged copper

container which was clipped on to a 2.5-cm diameter solid copper finger attached to the pot above. The copper container was fitted with air-gapped quartz windows located for right-angle viewing. Liquid N_2 in the copper pot served to reduce the cell temperature to 77° K, the temperatures being monitored by a copper–constantan thermocouple and millivoltmeter amplifier. The external windows of the cell container were heated to avoid frosting. The whole compartment was sealed and could be purged with dry N_2 gas. This arrangement allowed the sample to be maintained at 77° K for periods of up to several hours.

Quantum efficiency (ϕ) was determined by an extension of the relative method[12]. Fluorescein in 0.1 M NaOH at 10^{-6} M was taken as a convenient standard with an absolute value of quantum efficiency of 0.92 at 20° C (ref. 13). Quantum efficiencies were determined from the equation,

$$\phi_2 = \phi_1[(1 - 10^{-A_1})/(1 - 10^{-A_2})] \quad (\int F_2 d\lambda / \int F_1 d\lambda)$$

where ϕ_1 and A_1 are the quantum efficiency and the absorbance of the standard at its excitation wavelength, and ϕ_2 and A_2 are similar quantities for the sample. $\int F_1 d\lambda$ and $\int F_2 d\lambda$ are the integrals of the fluorescence emission spectrum of the standard and sample, respectively. A simple linear wavelength correction enables energy to quanta conversion so that the integral ratio is expressed as relative quanta. When A_1 and A_2 are < 0.1 as is necessary for linearity, $(1 - 10^{-A_1}) \simeq A_1(1 - 1.15 A_1)$. A_1 and A_2 were measured at the appropriate wavelengths by means of a Cary Model 14 recording spectrophotometer fitted with a scatter transmission accessory. The integrals were recorded on the second channel of a two-channel recorder by means of an operational amplifier, I, connected as an integrator.

For fluorescence measurements, the chloroplasts were diluted to an absorbance of 0.2 at the particular excitation wavelength. Fluorescence yields at room temperature were determined in 0.05 M phosphate buffer (pH 7.2), containing 0.3 M sucrose and 0.01 M KCl. Low-temperature measurements were made in 0.05 M phosphate buffer (pH 7.2), containing 60 % glycerol.

Measurements of the time-course of fluorescence intensity were made at 683 mμ, the maximum of the fluorescence emission spectrum. Fluorescence intensity was recorded as a function of time on a recorder (Rikadenki, Japan — Model B-34, full scale response time < 0.5 sec), operated normally at a speed of 80 mm/min.

RESULTS

Steady-state quantum yields of fluorescence of normal and mutant chloroplasts excited at a number of wavelengths and at two levels of light intensity are shown in Table I. The fluorescence yields of the mutant chloroplasts were considerably lower than the corresponding values from normal chloroplasts, the ratio ϕ_{normal} to ϕ_{mutant} varying with the wavelength of excitation. As might have been expected, the mutant chloroplasts were least fluorescent relative to the normal ones when excited at 470 or 650 mμ where chl *b in vivo* shows absorption maxima. Increasing the light intensity 10-fold led to large increases in the quantum yields of fluorescence of the normal chloroplasts. In contrast, the quantum yields of the mutant chloroplasts were only slightly dependent on light intensity. Chloroplasts prepared by either the

blending or chopping procedures gave fluorescence yields which did not differ significantly from the yields reported in Table I.

The differential effect of light intensity on fluorescence yields of normal and mutant chloroplasts also was apparent from studies of the time-course of fluorescence intensity. Figs. 2 and 3 show some typical curves. The fluorescence–time curves obtained with the normal chloroplasts without additions (Figs. 2a and b) were distinctly biphasic in agreement with previous observations with spinach chloroplasts[14–16]. At the higher light intensity, the fluorescence intensity increased from an initial value (F_0) to a steady-state value (F_∞) which was 3–4-fold higher. The fluorescence–time curves were completely reproducible, provided the chloroplasts

TABLE I

STEADY-STATE QUANTUM YIELDS OF FLUORESCENCE OF NORMAL AND MUTANT CHLOROPLASTS AT 20° C

Chloroplasts were isolated from normal or mutant leaves by hand-grinding. Quantum yields of fluorescence were determined in 0.05M phosphate buffer (pH 7.2) containing 0.3M sucrose and 0.01M KCl. The chloroplasts were diluted to an absorbance of 0.2 at the particular excitation wavelength. Light intensities were either $I \times 1$ or $I \times 10$ where (erg·cm^{-2}·sec^{-1}) $I_{436\ m\mu} = 90$, $I_{470\ m\mu} = 130$, $I_{650\ m\mu} = 170$, $I_{670\ m\mu} = 140$.

Excitation wavelength (mμ)	*Quantum yield (φ in %)*					
	Light intensity I × 1			*Light intensity I × 10*		
	Normal	*Mutant*	*φ normal / φ mutant*	*Normal*	*Mutant*	*φ normal / φ mutant*
436	0.67	0.26	2.6	1.3	0.28	4.7
470	0.71	0.13	5.5	1.5	0.13	12.0
650	0.75	0.15	5.0	1.6	0.18	8.8
670	0.93	0.25	3.7	1.45	0.35	4.1

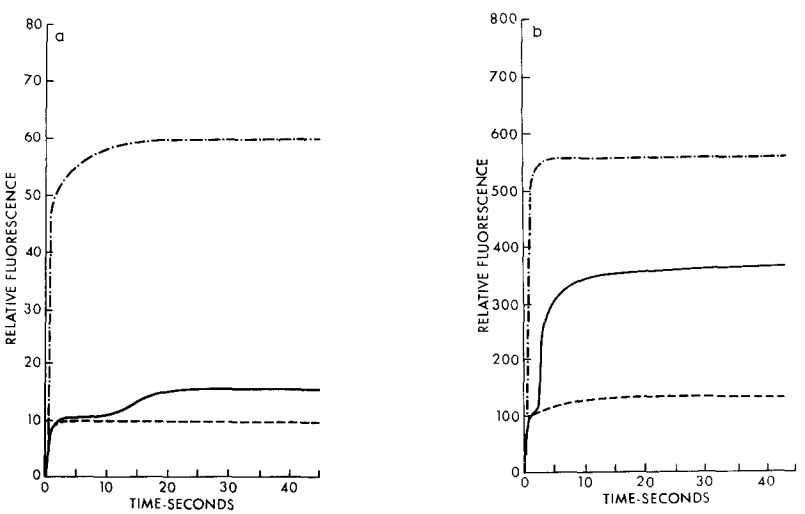

Fig. 2. Time-course of fluorescence intensity at 683 mμ of normal chloroplasts. Temperature 20° C. Excitation wavelength 650 mμ. Concentration of chloroplasts 0.20 $A_{650\ m\mu}$ unit. Light intensity (a) 170 erg·cm^{-2}·sec^{-1}; (b) 1700 erg·cm^{-2}·sec^{-1}; ———, no additions; – – – –, + ferricyanide; –·–·–, + dithionite.

remained in the dark for at least 2 min between illuminations. At the lower light intensities the increase in fluorescence intensity ($F_\infty - F_0$) was smaller and its magnitude varied with the wavelength of excitation (Table II).

A comparison of the fluorescence–time curves for mutant and normal chloroplasts and the corresponding values of F_∞/F_0 (Table II) shows clearly that the increases in the fluorescence intensity upon illumination ($F_\infty - F_0$) were smaller for the mutant chloroplasts. Excitation with the higher intensity light at 670 mμ gave the highest value of F_∞/F_0 for mutant chloroplasts, but it was still low compared with the corresponding value for normal chloroplasts.

The addition of an oxidizing agent such as $K_3Fe(CN)_6$ to mutant or normal chloroplasts almost completely eliminated the time-dependent rise in fluorescence intensity, so that F_∞ approximated to F_0 (Figs. 2 and 3). In the presence of a strong reductant, such as dithionite, the chloroplasts gave levels of fluorescence (F_{reduced})

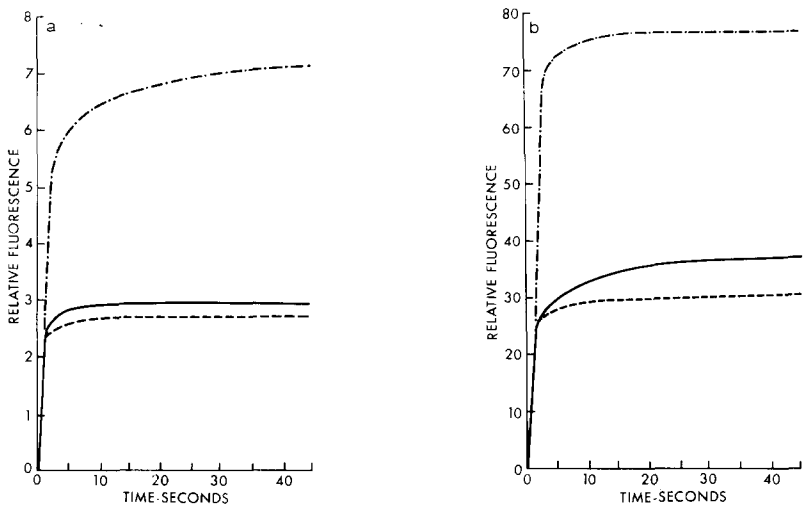

Fig. 3. Time-course of fluorescence intensity at 683 mμ of mutant chloroplasts. Conditions as for Fig. 2; ———, no additions; – – – –, + ferricyanide; –·–·–, + dithionite.

TABLE II

RATIOS OF THE STEADY-STATE FLUORESCENCE INTENSITY TO THE INITIAL FLUORESCENCE INTENSITY

Fluorescence intensity was measured at 683 mμ. F_0 was the initial fluorescence intensity upon opening the slit between the light source and the excitation monochromator and F_∞ was the final steady-state value. Other conditions as for Table I.

Excitation wavelength (mμ)	$\frac{F_\infty}{F_0}$			
	Light intensity I × 1		*Light intensity I × 10*	
	Normal	*Mutant*	*Normal*	*Mutant*
436	1.10	1.11	3.03	1.39
470	1.28	1.16	3.23	1.12
650	1.56	1.20	3.57	1.37
670	1.85	1.17	3.33	1.64

which were always higher than normal steady-state values (F_∞) (*cf.* ref. 17). A striking difference between the normal and mutant chloroplasts was in the ratio $\phi_{reduced}/\phi_{oxidized}$ (Table III); the values obtained with the mutant chloroplasts were considerably below those obtained with normal chloroplasts. Table IV shows a

TABLE III

RATIOS OF STEADY-STATE QUANTUM YIELDS OF FLUORESCENCE IN DITHIONITE AND FERRICYANIDE

Conditions as for Table I. Ferricyanide was added to one sample of normal or mutant chloroplasts to give a final concentration of $5 \cdot 10^{-5}$ M. Dithionite (1–2 mg) was added to an identical sample. Quantum yields were determined on each sample.

Excitation wavelength ($m\mu$)	*Light intensity*	$\phi_{reduced}/\phi_{oxidized}$	
		Normal chloroplasts	*Mutant chloroplasts*
436	$I \times 1$	5.9	2.2
	$I \times 10$	5.3	3.3
470	$I \times 1$	5.0	2.5
	$I \times 10$	4.6	2.2
650	$I \times 1$	5.6	2.6
	$I \times 10$	4.2	2.6
670	$I \times 1$	4.2	2.8
	$I \times 10$	3.0	2.0

TABLE IV

RATIOS OF STEADY-STATE QUANTUM YIELDS OF FLUORESCENCE OF NORMAL AND MUTANT CHLOROPLASTS IN THE PRESENCE OF DITHIONITE

Conditions as for Table I. Dithionite (1–2 mg) was added to each sample.

Excitation wavelength ($m\mu$)	*Light intensity*	$\frac{\phi_{reduced,\ normal}}{\phi_{reduced,\ mutant}}$
436	$I \times 1$	6.7
	$I \times 10$	3.9
470	$I \times 1$	11.1
	$I \times 10$	10.0
650	$I \times 1$	8.1
	$I \times 10$	6.5
670	$I \times 1$	3.6
	$I \times 10$	3.8

comparison of the fluorescence yields of normal and mutant chloroplasts under conditions of maximum fluorescence, *i.e.* in the presence of a strong reductant. Ratios of $\phi_{reduced,\ normal}/\phi_{reduced,\ mutant}$ varied from 3.6 at 670 mμ to 11.1 at 470 mμ. Light intensity had only a slight effect on the ratios.

Fluorescence spectra at 77° K

Fig. 4 shows the fluorescence emission spectra at 77° K of normal and mutant chloroplasts, excited at 436 mμ. The emission spectrum of chloroplasts from normal barley resembles that obtained previously with spinach chloroplasts[6], but the band at

695 mμ is less pronounced. Although the fluorescence yield of the mutant chloroplasts at 77° K was considerably lower than that of the normal chloroplasts, the fraction of the fluorescence emitted at the 735-mμ band did not differ significantly between the normal and mutant chloroplasts (Fig. 4), Excitation spectra for the fluorescence bands at 735 mμ are shown in Fig. 5. In the spectrum of the normal chloroplasts, bands at 650 and 475 mμ due to chl *b* are clearly visible. Quanta absorbed at 470 and 490 mμ relative to those absorbed at 436 mμ are less effective in exciting fluorescence in the mutant, than in the normal chloroplasts. This result again reflects the lack of chlorophyll *b* in the mutant. The bands at 495 and 465 mμ seen in the excitation spectrum of the mutant chloroplasts are thought to be due to carotenoids. Bands at these wavelengths were also visible in low-temperature absorption spectra of mutant chloroplasts[9]. The excitation spectrum of the mutant chloroplasts shows a

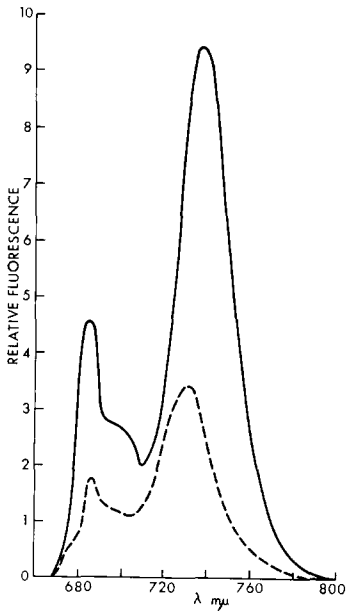

Fig. 4. Fluorescence emission spectra of normal (———) and mutant (– – – –) chloroplasts at 77°K. Excitation wavelength 436 mμ. Light intensity 90 erg·cm^{-2}·sec^{-1}. Concentration of chloroplasts 0.1 $A_{436\ m\mu}$ unit.

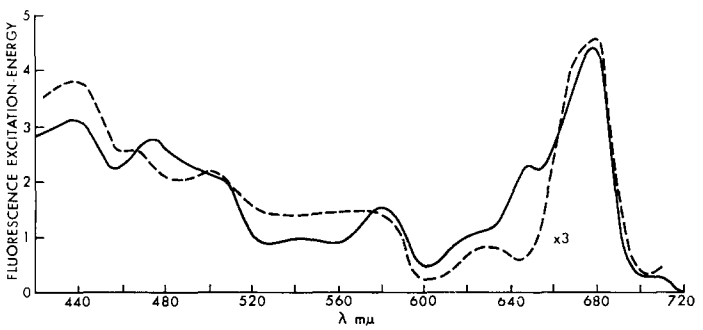

Fig. 5. Fluorescence excitation spectra of normal (———) and mutant (– – – –) chloroplasts at 77°K. Emission wavelength 735 mμ. Concentration of chloroplasts 0.1 $A_{436\ m\mu}$ unit.

pronounced shoulder at 670 mμ, instead of the band at 650 mμ, seen with normal chloroplasts. A similar result was obtained when the fluorescence excitation spectrum of a mutant barley leaf was measured at 77° K, using front surface illumination. It appears that a form of chl *a* absorbing at 670 mμ (Ca-670) can perform some of the function of chl *b*. The presence of Ca-670 in the mutant chloroplasts was deduced previously from low-temperature absorption spectra[9].

Fluorescence properties of particles obtained by digitonin fragmentation of chloroplasts

The fluorescence behaviour, both at 20° C and 77° K, of the particles obtained by digitonin fragmentation of normal barley chloroplasts was similar to that reported for spinach[6] indicating a partial fractionation of the photochemical systems. The distribution of chlorophyll among the various centrifugal fractions was also similar. For example, in one experiment, incubation of normal barley chloroplasts with 0.5 % digitonin for 30 min at 0° gave 55 % of the chlorophyll in the 10000 × *g* fraction and 8 % in the 144000 × *g* fraction. In contrast, the mutant chloroplasts were more susceptible to disruption with digitonin. Incubation with 0.5 % digitonin fragmented the chloroplasts to such an extent that only 19 % of the chlorophyll was recovered in the 10000 × *g* fraction. Incubation with 0.25 % digitonin for 10 min at 0°, and dilution of the incubation mixture with 9 vol. of 0.05 M phosphate buffer before differential centrifugation resulted in less fragmentation of the mutant chloroplasts; 46 % of the chlorophyll was recovered in the 10000 × *g* fraction and 8.5 % in the 144000 × *g* fraction.

Examination of the fluorescence properties of the 10000 × *g* and 144000 × *g* fractions from the mutant chloroplasts showed no significant differences between the fractions, in distinct contrast to the fractions from the normal barley. The 10000 × *g* and 144000 × *g* fractions from mutant chloroplasts each gave quantum yields of fluorescence of 0.24 %, whereas the 10000 × *g* fraction from normal chloroplasts gave a quantum yield of 0.8 % and the 144000 × *g* fraction a yield of 0.16 %. At 77° K the spectrum of each fraction from the mutant chloroplasts resembled that of whole chloroplasts.

DISCUSSION

In Part I of this series[9], two alternative suggestions were put forward to explain the lower photochemical efficiency of the mutant chloroplasts at low light intensities: (1) a structural defect in the light-harvesting pigment assemblies results in a decrease in the efficiency of resonance transfer of energy to the photochemical reaction centres. Electron microscopy of normal and mutant leaf sections indicated that the mutant chloroplasts were more disorganized than the normal ones[18]. There were fewer grana in the mutant chloroplasts and fewer thylakoids per granum, but more single lamellae; (2) the mutant chloroplasts have a decreased amount of chlorophyll in pigment system 2, compared with pigment system 1. This results in a greater fraction of incident light being absorbed by the light-harvesting assemblies in Photosystem 1.

The room-temperature fluorescence measurements reported in the present paper favour the second hypothesis. The fluorescence emission of chloroplasts at 20° emanates mainly from pigment system 2 (refs. 6, 17), so that a decrease in the fraction of light absorbed by pigment system 2 would result in a lower quantum yield of

fluorescence. On the other hand, a decrease in the efficiency of resonance transfer of energy to the reaction centres, as postulated in the first hypothesis might be expected to increase the probability that an absorbed quantum would be re-emitted as fluorescence from the light-harvesting chlorophylls.

The fraction of light absorbed by pigment system 2 of the normal chloroplast varies with the wavelength, since pigment system 2 contains a higher proportion of chl *b*. Compared with the normal, the mutant chloroplasts which lack chl *b* should be at a particular disadvantage at 470 and 650 mμ, where chl *b* absorbs maximally. The wavelength dependence of the relative quantum yield (Table I) is thus consistent with the lack of chl *b*.

Further support for the second hypothesis is provided by the fluorescence induction experiments. The rise in fluorescence intensity of isolated chloroplasts on illumination has been attributed to the photoreduction of a primary electron acceptor, Q, in Photosystem 2 (ref. 17). Reduced Q is reoxidized in darkness, or by far-red light absorbed predominantly by pigment system 1. A preponderance of pigments in pigment system 1, compared with pigment system 2, should lead to higher steady-state oxidation levels of Q in the illuminated chloroplast, not only in far-red light, but at all wavelengths. The smaller rises of fluorescence intensity observed with the mutant chloroplasts support this view. Moreover the fluorescence yields of the mutant chloroplasts were decreased only slightly by $Fe(CN)_6^{3-}$ particularly at the lower light intensities, a result which further indicates that the quenching substance, Q, is kept mainly oxidized in these chloroplasts. The lower ratios of $\phi_{\text{reduced}}/\phi_{\text{oxidized}}$ observed with the mutant chloroplasts would appear to be consistent with a decreased amount of chlorophyll in Photosystem 2. It is known that the fluorescence yield of the chlorophyll in Photosystem 2, but not of the chlorophyll in Photosystem 1 is dependent on redox conditions[6]. A decrease in the relative amount of chlorophyll in Photosystem 2, compared with that in Photosystem 1 would give, therefore, a smaller dependence of the fluorescence of the chloroplast on redox conditions. An examination of the quantitative aspects, however, indicates that both the quantum yields of the mutant chloroplasts and the ratios of $\phi_{\text{reduced}}/\phi_{\text{oxidized}}$ appear to be too low to be accounted for solely by a decrease in the amount of pigment in Photosystem 2 due to the lack of chl *b*. Recent measurements, however, indicated that the mutant chloroplasts have a molar ratio of total chlorophyll to total cytochrome *b* which is only one-half that of the normal barley chloroplasts. This result suggests that the mutant chloroplasts have less chl *a*, as well as lacking chl *b*. A decreased amount of chl *a* in Photosystem 2, compared with Photosystem 1 would explain the fluorescence observations.

A difficulty arises when we examine the low-temperature fluorescence emission spectra of the mutant and normal chloroplast in terms of the second hypothesis. From our previous studies[6] of the low-temperature fluorescence properties of spinach chloroplasts and the particles obtained by fragmentation with digitonin, it was concluded that the fluorescence emitted at 735 mμ by chloroplasts cooled to 77° K originates mainly from pigment system 1 and that emitted at 683 and 693 mμ arises primarily from pigment system 2. A similar suggestion was made by KOK AND RURAINSKI[19]. The low-temperature fluorescence spectrum of the mutant chloroplasts does not show an abnormally large enhancement of the band at 735 mμ, as might be predicted if the mutant chloroplasts contained a higher content of pigment system 1. The inverse situation arises with a Scenedesmus mutant (No. 8) which appears to

be low in pigment system I, but shows a strong fluorescence band at 740 mμ at low temperature[20].

There is no evidence from the low-temperature fluorescence spectra of the fractions that digitonin incubation of the mutant chloroplasts gives a partial separation of the photochemical systems. The similarities between the spectra of the 10000 × *g* and 144000 × *g* fractions with the spectrum of mutant chloroplasts suggests that digitonin splits the chloroplast lamellae into smaller fragments, rather than separating the photosystems. Similar fluorescence results were obtained previously[6] with the particles obtained from sonicated spinach chloroplasts. Sonication is known to produce fragments which resemble the chloroplast in composition and photochemical activity[21–24].

Recently, ANDERSON AND VERNON[25] reported that digitonin treatment of spinach chloroplasts which had been resuspended in tris(hydroxymethyl)methylglycine (Tricine) buffer of low ionic strength did not result in a fractionation of the photochemical systems. Chloroplasts isolated in the Tricine low-salt medium lose their grana[25,26], and give rise to swollen structures containing almost continuous sheets of single lamellae. Thus in the electron microscope, the Tricine low-salt chloroplasts bear some resemblance to the mutant barley chloroplasts, which also contain a large number of single lamellae[18]. This raises the question whether a close packing of lamellae, as found in the grana are a prerequisite to a selective penetration of digitonin, which in turn causes a release of Photosystem I.

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