Plant Physiol. (1979) 63, 174–182
0032-0889/79/63/0174/09/$00.50/0

Composition and Function of Thylakoid Membranes from Grana-rich and Grana-deficient Chloroplast Mutants of Barley[1]

Received for publication February 6, 1978 and in revised form July 13, 1978

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ABSTRACT

Chlorophyll-deficient barley (*Hordeum vulgare*) mutants were studied that had chlorophyll *a*/*b* ratios either higher or lower than the wild type. Mutants with high ratios (>5.2) had a reduced proportion of their photosynthetic lamellae appressed into grana ("grana-deficient" mutants) compared with wild type (chlorophyll *a*/*b* = 3.2), while the majority of lamellae in the chloroplasts with low chlorophyll *a*/*b* ratios (2.0–2.4) were organized into grana ("grana-rich" mutants).

All mutants catalyzed photosystem I and photosystem II electron transport, were tightly coupled as evidenced by increased rates of electron transport in the presence of methylamine, and were able to generate a light-dependent transmembrane proton gradient. Differences were evident in rates of electron transport per mole of chlorophyll. The mutants having high chlorophyll *a*/*b* ratios catalyzed 15- to 50-fold higher rates of ferricyanide photoreduction than the mutants having low chlorophyll *a*/*b* ratios, and 5- to 7-fold higher than the wild type.

Low temperature absorption spectra of chloroplast fragments showed that the grana-deficient mutant with a high *a*/*b* ratio had a chlorophyll spectrum characteristic of a PSI preparation while mutants with the low ratio had a spectrum typical of a PSII preparation.

The temperature fluorescence emission spectra of thylakoid membrane fragments from the two types of mutants were also strikingly different from one another, as were the electrophoretic patterns of the thylakoid polypeptides.

Light is required for the assembly of the chloroplast lamellar systems. The ultrastructural changes occurring during chloroplast development and their relationship to the photochemical properties of chloroplast thylakoid membranes have been extensively described (*e.g.* see ref. 4). The course of chloroplast differentiation is, in some plants, altered by the environmental light intensity under which they grow. It was reported many years ago that in certain species the leaves occupying sunny habitats have a high photosynthetic capacity on a Chl basis compared to those occupying shady habitats (7). Recently, distinct differences were described in the ultrastructure, pigment composition, and photosynthetic capabilities of chloroplasts in sun and shade plants (2, 5). Sun plants have a smaller proportion of their thylakoids appressed in grana and a higher Chl *a*/*b* ratio than shade plants. To investigate the relationship between thylakoid organization and photosynthetic properties further, several barley mutants were compared that had Chl *a*/*b* ratios either significantly higher or lower than wild type barley seedlings when grown under similar environmental conditions.

MATERIALS AND METHODS

Plant Material. Wild type barley (*Hordeum vulgare* L. cv. Svalöf's Bonus) and six recessive lethal chloroplast mutants (24) were used. Stocks of these nuclear gene mutants were maintained as heterozygotes and seeds from the stocks were germinated and grown in plastic trays containing Vermiculite moistened with tap water. The seedlings homozygous for the recessive allele were distinguished by their aberrant color. Unless stated otherwise, all seedlings were grown at 21 C under a continuous illumination of 170 ft-c provided by Philips H-8 fluorescent tubes. The apical 5-cm of the primary leaves of 7-day-old seedlings were used for all experiments.

Electron Microscopy. Leaf segments were fixed for 2 hr in cold 10 mM Sørensen's phosphate buffer (pH 7.2) containing 3% (w/v) glutaraldehyde. After washing with 70 mM phosphate buffer (pH 7.2), the samples were treated for 1 hr with 70 mM phosphate buffer (pH 7.2) containing 1% osmium tetroxide. They were dehydrated using a graded series of ethanol washes and then embedded in a low viscosity epoxy-resin according to Spurr (21). Sections cut on a Reichert ultramicrotome were counterstained using uranyl acetate and lead citrate and then were analyzed using either a Siemens Elmiskop 1A or a Zeiss EM9A electron microscope.

Oxygen Evolution from Leaves. The upper surface of the apical 5-cm section of the primary leaf was abraded gently with emery cloth and then placed in contact with a platinum electrode as described by Kirk and Goodchild (14). The leaf section was equilibrated on the electrode in the dark at 23 C and then illuminated with collimated light (4.6×10^2 w cm^{-2} sec^{-1}) emitted from a 200-w Osram lamp and filtered through 20 cm of water and a red RG-1 Jena filter.

Chloroplast Isolation. The apical primary leaf segments were cut into small pieces approximately 2 mm in length and 3 to 5 g of this material were suspended in 30 ml of ice-cold 50 mM Sørensen's phosphate buffer (pH 7.5) containing 30 mM NaCl, 1 mM EDTA, and 0.5% (w/v) BSA. This suspension was blended in an Omni-Mixer operating at full line voltage for two periods of 10

[1] Financial support was given by United States Public Health Service Grant GM22051 from the National Institutes of Health, and by the Danish Natural Science Research Council. CIW-DPB Publication No. 618.

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sec each in order to release the chloroplasts. The resulting brei was pressed through two layers of 28-μm nylon mesh and then filtered through two layers of Miracloth (Calbiochem). The chloroplasts were sedimented by centrifuging 10 min at 2,445*g* in a SS-34 Sorvall rotor. The chloroplasts were washed by suspending in 5 mM Tricine-NaOH (pH 7.5) containing 30 mM NaCl and 0.05% (w/v) BSA and sedimented as before. Finally, the pelleted chloroplasts were suspended in 1 ml of the wash medium. Examination of such preparation by electron microscopy reveals predominantly naked lamellae systems and very few grana (18). The Chl was extracted using 80% (v/v) acetone containing 0.2% NH_4OH, and the *A* at 645 and 663 nm were measured in order to calculate the content of Chl *a* and *b* (3).

Photoreduction of Ferricyanide and DCIP.[5] The rate of photoreduction of ferricyanide was determined by the decrease in *A* at 420 nm. The assay mixture (0.75 ml) contained chloroplasts (10 μg Chl ml^{-1}), 5 mM Tricine-NaOH (pH 7.5), 30 mM NaCl, 0.05% (w/v) BSA, and 0.33 mM potassium ferricyanide. The rate of DCIP photoreduction was measured by the decrease in *A* at 590 nm in an assay mixture (0.75 ml) containing chloroplasts (5 μg Chl ml^{-1}), 5 mM Tricine-NaOH (pH 7.5), 30 mM NaCl, 0.05% (w/v) BSA, and 0.05 mM DCIP. Where indicated, 60 mM methylamine (pH 7.5) was included in the reaction mixtures. The reactions were carried out at 23 C in 3-ml cuvettes using a Cary 17 spectrophotometer that had an actinic light source and a scattered transmission accessory. The actinic light was from a forced air-cooled, 200-w Osram bulb positioned above the cuvette, and following collimation was passed through a RG2-630 red cut-off filter. The light intensity emitted from the bulb was controlled using a rheostat and was calibrated against a thermopile. Unless otherwise specified, the reaction mixture was illuminated with red light at 6.8×10^2 w cm^{-2} sec^{-1}.

Photosystem I Activity. This activity was assayed in a reaction mixture (3.0 ml) containing chloroplasts (20–25 μg Chl ml^{-1}), 50 mM Tricine-NaOH (pH 8.5), 30 mM NaCl, 3 mM $MgCl_2$, 3 mM isoascorbate, 0.2 mM N,N,N′,N′-tetramethylphenylenediamine, 0.1 mM methylviologen, and 1.0 μM DCMU. O_2 uptake was measured using a Rank electrode constructed of clear Lucite and water-jacketed at 23 C (Rank Bros., Bottisham, U.K.). Collimated actinic light as 4.6×10^5 ergs cm^{-2} sec^{-1} from a 200-w Osram lamp was first filtered through a RG-1 Jena red filter and 20 cm of water. Contamination of the preparations by catalase was routinely measured as a reappearance of O_2 following turning off the light and the cessation of PSI activity. In no case was a rate of O_2 reappearance exceeding 5% of the light-dependent rate of O_2 consumption noted. As a further control, azide was added to some assays with membranes from wild type and grana-deficient chloroplast preparations, but consistent increases in light-dependent O_2 consumption were not found.

Proton Pump Activity. The light-dependent generation of a proton gradient was measured using the O_2 electrode apparatus described above, modified so that the inner compartment accepted a conical plug holding a GK 2322C Radiometer combination electrode (Radiometer, Copenhagen, Denmark). The pH changes were monitored with a model 26 Radiometer pH meter coupled to a recorder having a 1-mv full scale sensitivity. The reaction mixture (3.0 ml) contained chloroplasts (10–16 μg Chl ml^{-1}), 30 mM NaCl, 3 mM $MgCl_2$, and 0.05 mM DCIP, or 12.5 μM pyocyanine perchlorate at an initial pH of 7.5. Each reaction mixture was calibrated by adding a known aliquot of NaOH and observing the resulting change in pH.

Spectra. Suspensions of isolated plastids were sonicated to generate small thylakoid fragments by placing a 50-ml polycarbonate tube holding the suspension in an ice bath and sonicating at maximum cavitation for 5 sec, followed by a 25-sec cooling period, using an M.S.E. sonicator equipped with an oscillating probe. This procedure was repeated for a total period of 5 min. The sonicate was centrifuged at 50,000*g* for 30 min in a Sorvall SS-34 rotor. The supernatant subsequently was diluted with the wash medium and frozen either in NMR tube cuvettes for the fluorescence measurement or in special 2-mm thick cuvettes for absorption measurements (10), using liquid N_2. The fluorescent emission spectra were obtained using a Perkin-Elmer MPF-31 spectrophotometer. Excitation was at 440 ± 5 nm. The spectra of several different sample dilutions were examined to ensure that there was not a significant distortion of fluorescence by reabsorption. The emission spectra were corrected by subtracting the baseline due to scattered light obtained using a buffer blank. After digitizing, the spectra were subjected to curve-fitting analysis (10).

Gel Electrophoresis. Gel electrophoresis of chloroplast membrane protein on 7.5% polyacrylamide gels was carried out using the phenol-acetic acid-8 M urea system described previously (15).

RESULTS

Chl Content. The mutants had either the *xantha* (yellow-green leaves) or *viridis* (pale green leaves) phenotypes, and consequently the total Chl content of all of them was lower than in the wild type (Table I). The particular mutants used in this study were selected because the ratio of Chl *a* and *b* in their leaves was significantly different from the wild type. The Chl *a*/*b* ratio for *xantha-l*[35] and *viridis-k*[23] was above 5 compared with the average wild type value of 3.2. In the four remaining mutants with higher Chl content, it was lower than the wild type ratio and varied between 2.0 and 2.4.

The leaf Chl content decreased and the ratio of Chl *a* to *b* increased in the mutants *viridis-k*[23] and *xantha-l*[35] when the seedlings were grown under successively higher light intensities (Table II). At 1,640 ft-c, the highest light intensity used, Chl *b* in the *viridis-k*[23] seedlings was barely detectable using absorption spectrophotometry. These trends were not observed with the wild type seedlings where both the total Chl content and the Chl *a*/*b* ratio were almost independent of light intensity.

The mutant *xantha-b*[12] was previously analyzed when grown at different light intensities and also temperatures (20). There was a large decrease in both the total Chl content and photochemical activity as the light intensity increased and temperature decreased. Despite these changes, and in contrast to the results with *xantha-l*[35] and *viridis-k*[23], the Chl *a*/*b* ratio remained below 2.5 at all of the light intensities studied.

Chloroplast Membrane Organization. The mutants divided into two groups in Table I on the basis of their Chl content and Chl *a*/*b* ratio can also be separated into the same two groups based on their internal chloroplast ultrastructure (2, 23). The group with high ratios of Chl *a* to *b* and low Chl content had chloroplasts with very few grana and will be referred to as grana-deficient mutants. The mutants having lower Chl *a*/*b* ratios than wild type

Table I. Chlorophyll Content and Ratio of Chlorophyll *a* to *b* in Wild Type and Mutant Barley

Genotype	Total Chlorophyll	Chlorophyll *a*/*b*
	μg chlorophyll g fresh wt^{-1}	
Wild type	1760	3.2
High Chlorophyll *a*/*b* ratio		
xantha-7[35]	106	5.2
viridis-k[23]	156	5.2
Low chlorophyll *a*/*b* ratio		
xantha-b[12]	326	2.2
xantha-c[23]	372	2.4
xantha-d[31]	376	2.4
viridis-zd[69]	497	2.0

[5] Abbreviation: DCIP: 2,6-dichloroindophenol.

had chloroplasts in which most of the lamellae were in grana and intergrana lamellae were few. These are referred to here as grana-rich mutants.

The chloroplast profile shown in Figure 1 is typical for the two grana-deficient mutants, *viridis-k*^{23} and *xantha-l*^{35}, and is characterized by parallel arrays of unpaired lamellae. Some appression between adjacent lamellae to form small grana and spheroidal grana were observed, even in the *viridis-k*^{23} seedlings grown at 1,640 ft-c.

The chloroplast shown in Figures 2 and 3 illustrate the main characteristics of the grana-rich mutants. Their chloroplasts contained large grana and there was a distinct reduction in the amount of unpaired lamellae compared with wild type barley chloroplasts. As pointed out previously (25), the grana are of two types, either they have a large diameter (giant grana, *cf.* Figs. 2 and 3), or they have a normal diameter with many appressed discs (*cf.* Fig. 3). The grana characteristically are randomly oriented with respect to one another within the plastids and profiles in the plant of the discs are frequently observed.

Chloroplast preparations isolated from both grana-deficient mutants contained mostly vesicles (Fig. 5) and some appressed swollen thylakoids (Fig. 4). The latter were apparently induced during isolation since grana with such large diameters are not evident *in vivo*.

Oxygen Evolution by Barley Leaf Sections. Figure 6 compares light-dependent O_2 evolution of leaf sections from wild type and *viridis-k*^{23} seedlings grown at high and low light intensities. Application of this technique and interpretation of the data generated have been considered in detail by Kirk and Goodchild (14), as well as by Kahn and Carlsen (12). The data revealed that *viridis-k*^{23}, even when grown at 1,640 ft-c where it becomes very deficient in Chl *b*, can evolve substantial amounts of O_2. The studies of Kahn and Carlsen (12) showed that it was the maximum initial rate at which the electrical potential changed upon illumination that was related to the photosynthetic rate. In the case of this parameter, the value for *viridis-k*^{23}, grown at either high or low light intensity, was only 20 to 30% lower than the corresponding value for wild type. This result agrees well with that obtained by direct measurements of photosynthetic CO_2 fixation made by Carlsen (9). A reduction in the Chl content of *viridis-k*^{23} to less than 10% of the wild type content was not paralleled by a corresponding large reduction in O_2-evolving capacity per area of leaf.

Photochemical Activities. The photoreductive activity with ferricyanide as the oxidant and PSI activity of chloroplast preparations from the wild type and mutant plants are given in Table III. Ferricyanide reduction was measured in the presence of the uncoupler methylamine.

Both ferricyanide photoreduction and PSI activity were higher on a Chl basis in the two mutants deficient in grana than in wild type. The ferricyanide photoreductive activity showed the largest difference; the ratio of this activity to PSI activity in chloroplasts from these two mutants was about four times that obtained with wild type chloroplasts (Table III). The values for *viridis-k*^{23} shown in the table are for plants grown at 1,640 ft-c, and in this particular experiment Chl *b* content was so low it could not be detected in

Table II. Effect of Light Intensity on Chlorophyll Content of Two Mutants and Wild Type Barley

Plants were grown for 7 days at 21 C in continuous white light at the intensities indicated. Chlorophyll was extracted from the apical 5-cm of the primary leaf and measured as described in Materials and Methods.

Light intensity	Genotype					
	Wild type		*viridis-k*^{23}		*xantha-l*^{35}	
	Total chlorophyll	Chlorophyll *a/b*	Total chlorophyll	Chlorophyll *a/b*	Total chlorophyll	Chlorophyll *a/b*
ft-c	μg chlorophyll g fresh wt^{-1}		μg chlorophyll g fresh wt^{-1}		μg chlorophyll g fresh wt^{-1}	
1,640	1,890	2.9	160	13	51	24
640	1,870	3.2	190	7.2	93	5.5
260	1,790	3.6	250	6.4	100	5.5
78	1,800	3.3	320	4.9	160	5.3
40	1,490	3.8	360	4.8	150	5.6

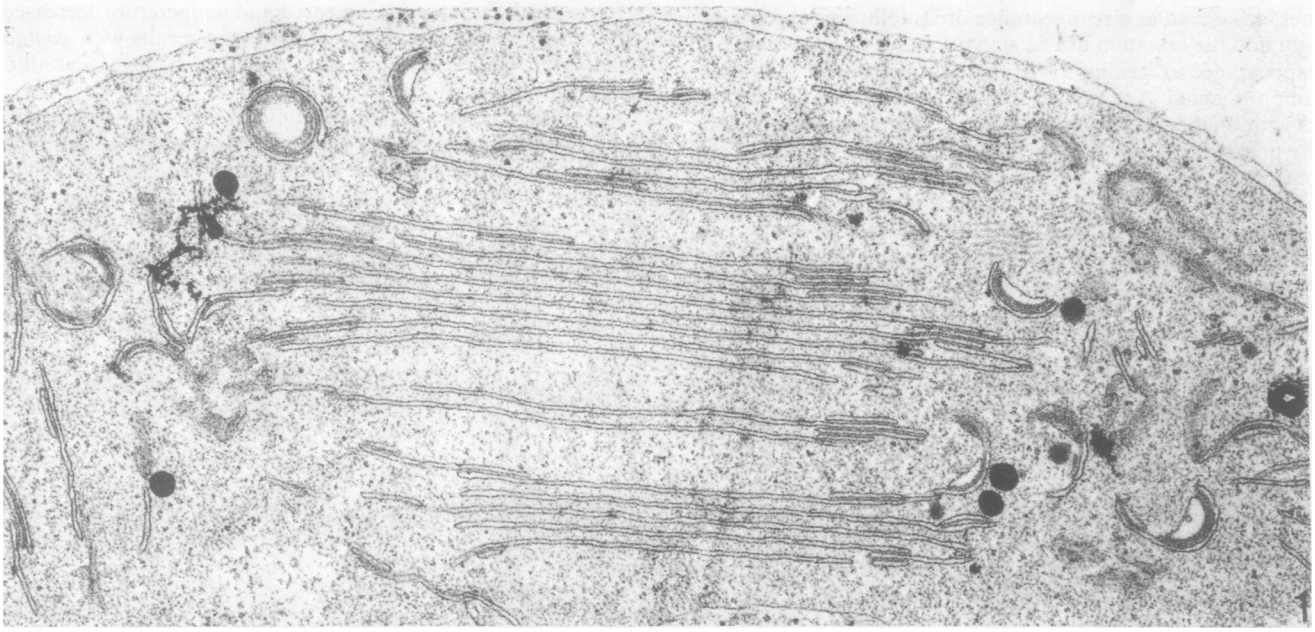

FIG. 1. Chloroplast section from *viridis-k*^{23} containing long parallel unpaired thylakoids, a few grana-like structures, and spheroidal grana. Mutant *xantha-l*^{35} is phenotypically similar to this mutant. Seven days under 320 ft-c, 21 C. (× 31,000).

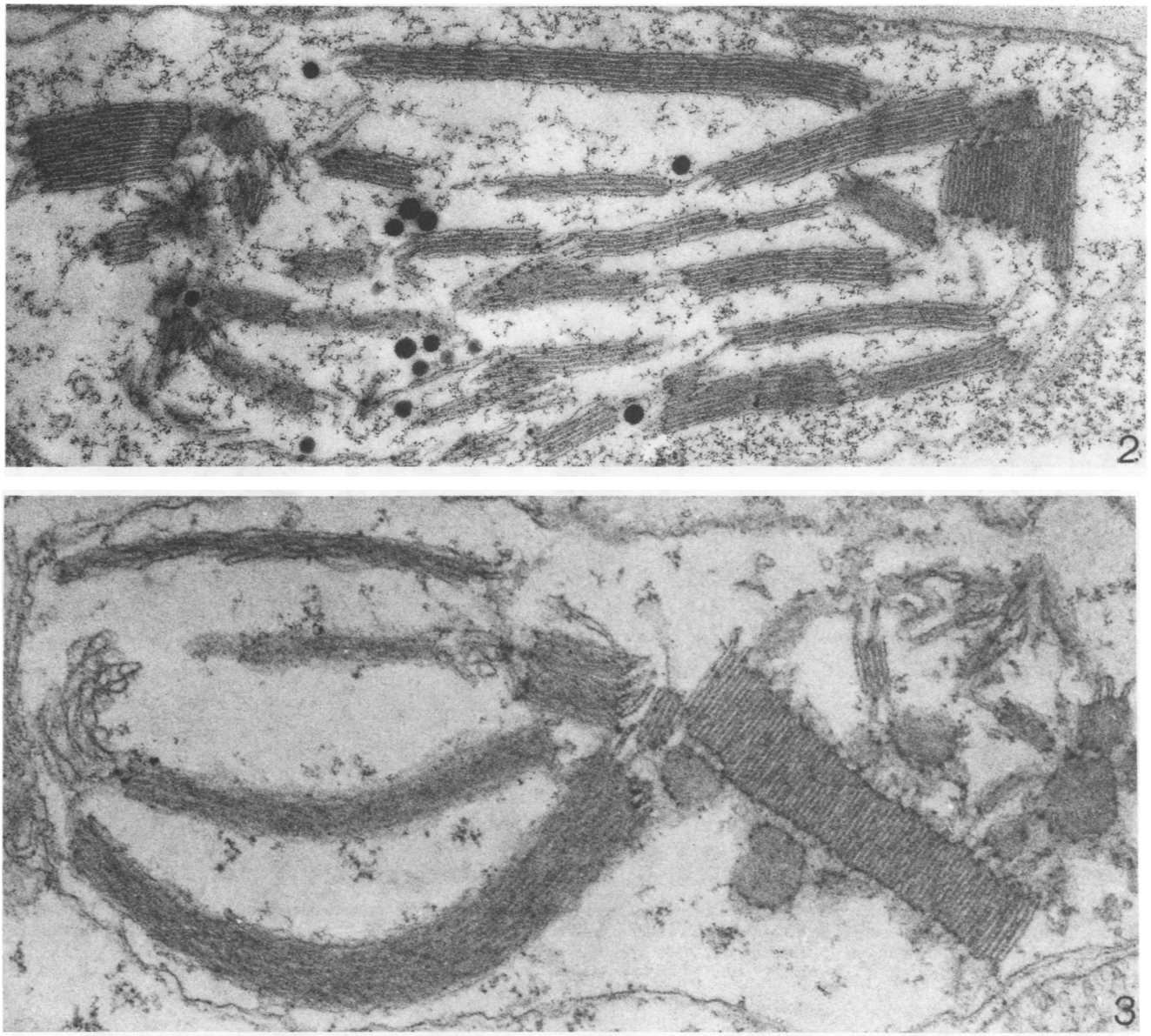

FIG. 2 and 3. Mutant *xantha-c*[23] forms giant grana in addition to grana with normal diameter and width. Intrathylakoid membranes are poorly developed. Seven days under 170 ft-c, 14 C and 21 C, respectively. (× 36,000).

the isolated chloroplasts. Plants grown at lower light intensities gave the same rates of ferricyanide photoreduction but slightly lower PSI activities.

In contrast, the rate of ferricyanide photoreduction was lower in the chloroplasts from grana-rich mutants compared with the value for wild type chloroplasts. The low values obtained for the grana-rich mutants are likely to be an overestimation as the activity was not completely sensitive to DCMU. Photoreduction of ferricyanide by chloroplasts from *xantha-b*[12], *xantha-c*[23], *xantha-d*[31], and *viridis-zd*[69] was inhibited 52, 88, 89, and 74%, respectively, by 6–7 μM DCMU. Most of the increase in activity in *xantha-b*[12] and all of that in the other three mutants obtained by including methylamine in the reaction mixture was DCMU-sensitive. The activities of wild-type and the two grana-deficient mutants for ferricyanide photoreduction were inhibited 98% by the same concentration of DCMU. The rates of photoreduction of DCIP for the grana-rich mutants were 30 to 50% of the values shown for ferricyanide reduction. They were completely inhibited by DCMU and were either not stimulated or only marginally so by 0.5 mM 1,5-diphenylcarbazide, a PSII donor. PSI activity in the grana-rich mutants was comparable with the wild type activity and showed a reasonable correlation with the total Chl content of the preparations but not with Chl *b* content or the relative proportions of granal and stromal lamellae. Assuming that ferricyanide photoreduction in the presence of methylamine expresses maximal rates of PSII electron transport then PSII activity correlated with neither total Chl content, Chl *b* content, nor the amount of grana present.

In a number of other chloroplast systems deficient in both Chl *b* and grana lamellae, as occurs naturally in the bundle sheath cells of maize and in pea grown under an appropriate light/dark cycle, electron transport via PSII is not saturated even by very high intensities of red light (19). Also, the normal coupling of phosphorylation to noncyclic electron flow appears to be impaired since Hill activity is not increased by the addition of an uncoupler such as methylamine. This was not the situation with the grana-deficient barley mutants *xantha-l*[35] and *viridis-k*[23]. Figure 7 shows photoreductive activities of chloroplasts from *xantha-l*[35], *viridis-k*[23] (grown at 40 or 1,640 ft-c) and wild type plants as a function of the actinic light intensity used for the assay. Light saturation

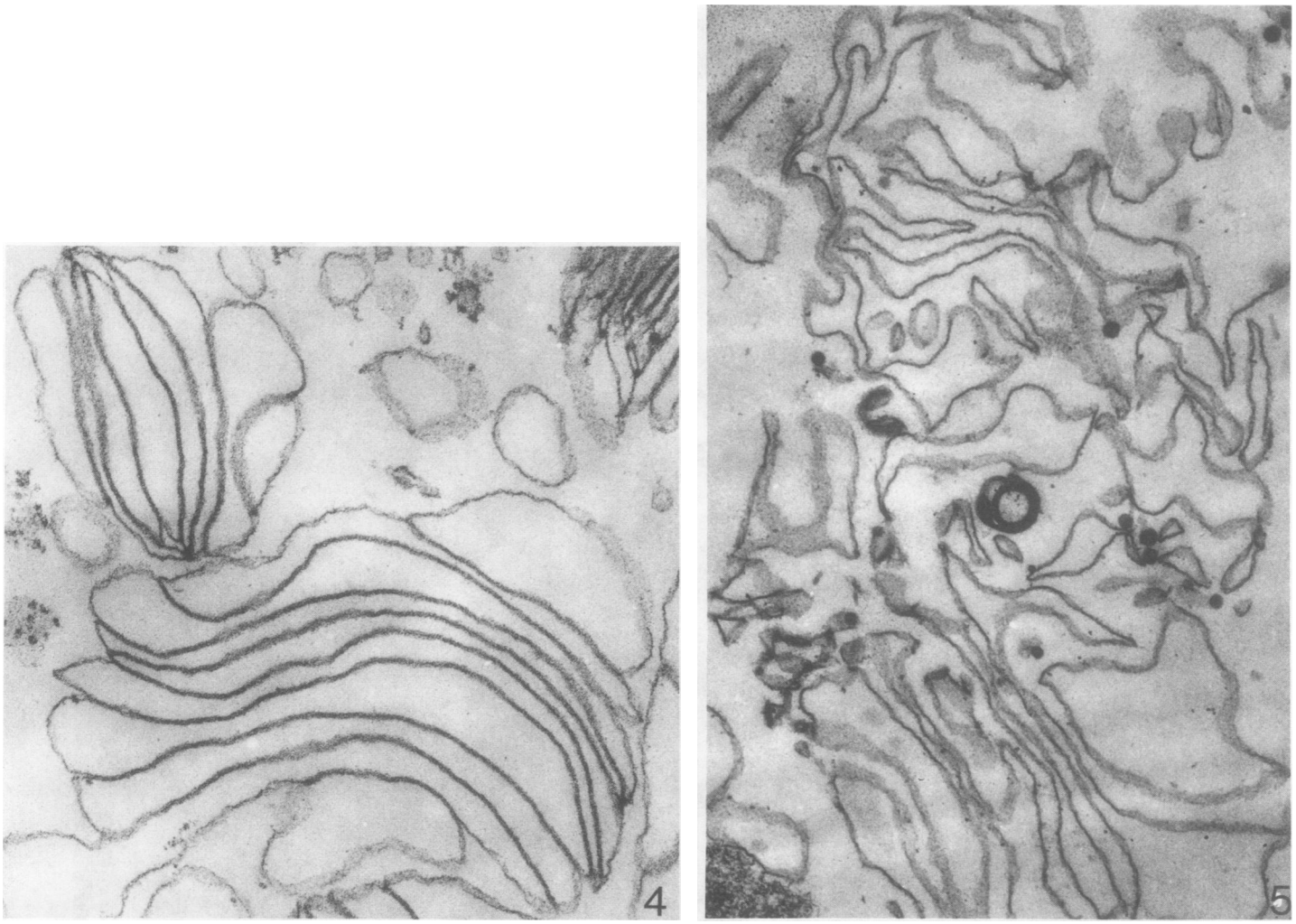

FIG. 4 and 5. Portion of a preparation of isolated lamellar systems from *viridis-k*^{23} (Fig. 4) which show swollen grana. A few of the numerous swollen thylakoids present in the preparation are evident. In Figure 5 swollen membranes isolated from *xantha-f*^{35} are shown. A spheroidal granum can be seen in the center. Standard growth conditions. (× 29,000 and × 30,000, respectively).

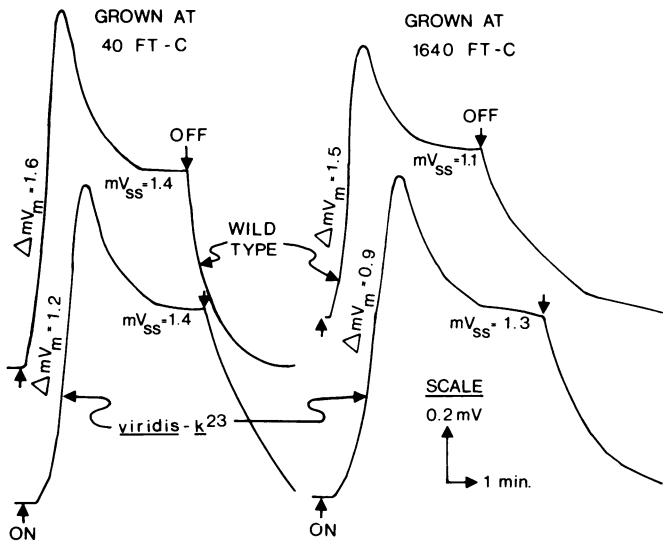

FIG. 6. O_2 evolution by leaf sections of wild type and *viridis-k*^{23} grown under low (40 ft-c) and high (1,640 ft-c) intensities of white light. ΔmV indicates slope of trace or maximum rate of change in signal (mV min^{-1} cm^{-2}); mV_{ss} is the steady-state change in potential (mV cm^{-2}).

Table III. Activities of Photosystems in Mutant and Wild Type Barley Chloroplasts

The plants were raised under standard growth conditions, except for *viridis-k*^{23}, which was grown at 1,640 ft-c. Results for *xantha-b*^{12} are from (20). Ferricyanide photoreduction was measured as described in Materials and Methods in the presence of 60 mM methylamine.

Genotype	Photoreduction of Ferricyanide	Photosystem I Activity (O_2 uptake)	Ratio of Ferricyanide Reduction to Photosystem I
	μmoles min^{-1} mg chlorophyll^{-1}	μequiv min^{-1} mg chlorophyll^{-1}	
Wild type	6.9	19.5	0.35
Deficient in granal lamellae			
xantha-7^{35}	31.4	25.6	1.2
viridis-k^{23}	43.2	31.0	1.4
Deficient in stromal lamellae			
xantha-b^{12}	0.88	18.6	0.05
xantha-c^{23}	1.92	16.2	0.12
xantha-d^{31}	2.06	20.4	0.10
viridis-zd^{69}	1.35	17.7	0.07

for ferricyanide reduction was achieved with all four chloroplast preparations, although slightly higher intensities were required to saturate the mutant preparations compared with wild type. Figure 7 also shows that the rate of ferricyanide reduction was stimulated by the addition of methylamine for both the mutants and wild

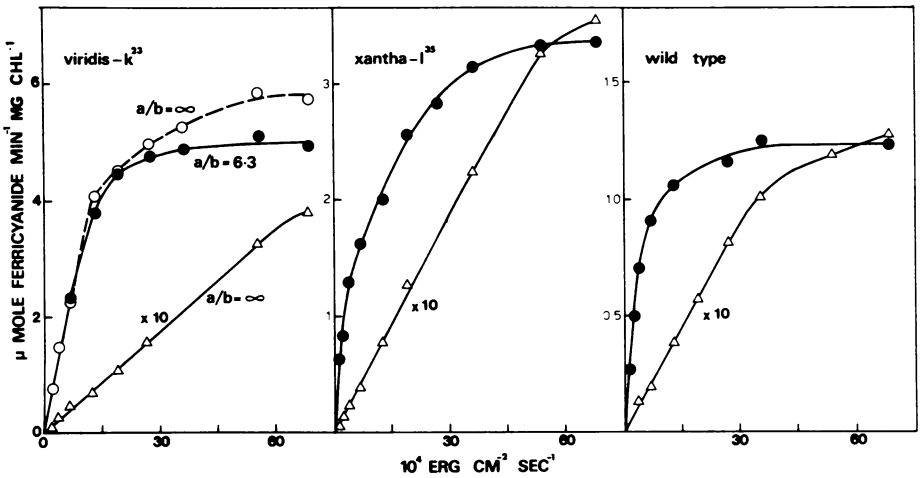

FIG. 7. Photoreduction of ferricyanide at different intensities of actinic red light. The mutant *viridis-k*^{23} was grown under 1,640 ft-c (Chl *b* not detected), or 40 ft-c (Chl *a* to *b* = 6.3). The wild type and *xantha-l*^{35} were grown under 170 ft-c of white light; (○) and (●): no methylamine; (△): 60 mM methylamine present in reaction mixture. All values for activities measured in the presence of methylamine are to be multiplied by 10.

type. In each case the shape of the curve became more linear and higher light intensities became necessary to saturate the reaction.

The ability of methylamine to stimulate Hill reaction activity for the mutants is compared in Table IV. The stimulation obtained with chloroplasts from *xantha-l*^{35} and *viridis-k*^{23} was large and comparable with the wild type. The stimulation of ferricyanide or DCIP photoreduction by the four grana-rich mutants was approximately half of that found for the wild type. These results suggested that the mutants were capable of generating a proton gradient in the light and this was verified directly by measuring the proton pump activity (Table IV).

Absorption Spectra. The low temperature absorption spectrum of *xantha-b*^{12} in Figure 8 is essentially typical for a PSII (8) preparation in that it is high in the Chl *b* region and low in the content of the long wavelength Chl forms C_a684 and C_a691. By contrast the *viridis-k*^{23} spectrum looks like that for a PSI preparation, having high *A* in the long wavelength forms and in C_a670 (8). The characteristics and proportions of the different forms of Chl of both preparations are given in Table V. The *xantha-b*^{12} mutant contains a significant amount of the 640 form of Chl *b* while the other does not. The mutant *viridis-k*^{23} provides an interesting example of a naturally occurring PSI Chl *a* absorption spectrum with less complication of residual Chl *b* than in the usual fractions of particles from green plants enriched in PSI.

The low broad bands near 588 and 625 nm in both spectra are unresolved complex mixtures of the short wavelength secondary bands correlated with the identifiable longer wavelength components. They are included in the curve analysis only because the overlap of their long wavelength sides with the absorption of the Chl *b* region requires their inclusion to avoid exaggeration of the actual Chl components.

Fluorescence Emission Spectra. Low temperature fluorescence emission spectra of chloroplast membrane fragments from the wild type, one grana-deficient mutant and one grana-rich mutant, are shown in Figure 9. The spectra of wild type membranes had the expected two major emission bands, one with a maximum at 686 nm and a shoulder at 696 nm and a second more prominent band with a maximum at 738 nm. In the grana-deficient mutant *viridis-k*^{23}, the emission band at 686 nm was the most conspicuous. In the grana-rich mutant, *xantha-b*^{12}, the two bands were about the same in fluorescence intensity. In both mutants the peak for the long wavelength emission was shifted toward the blue, by 5 nm for *viridis-k*^{23} and by 10 nm for *xantha-b*^{12}. Mutant *xantha-l*^{35} gave a spectrum very similar to the one given by *viridis-k*^{23}.

As shown in Figure 9, the three spectra can be fitted to curves

Table IV. Stimulation of Photosynthetic Electron Transport by Methylamine and Proton Pump Activity

Values for methylamine stimulation of the photoreduction of ferricyanide given are the rate of photoreduction in the presence of 60 mM methylamine divided by the rate in the absence of methylamine. Assay procedures are given in Materials and Methods. The light intensities used to grow *viridis-k*^{23} are shown in the table; otherwise standard growth conditions apply. Data for *xantha-b*^{12} are from ref. (20).

Genotype	Stimulation of Photoreduction by Methylamine		Proton Pump
	Ferricyanide	DCIP	
			μmole H^+ pumped mg chlorophyll^{-1}
Wild type	7.9	4.1	0.30
Deficient in granal lamellae			
xantha-l^{35}	10.5	4.0	0.28
viridis-k^{23}			
(40 ft-c)	8.6	4.6	0.06
(320 ft-c)	7.6	4.4	0.15
(1,640 ft-c)	7.3	3.8	0.26
Deficient in stromal lamellae			
xantha-b^{12}	2.8	2.3	0.17
xantha-c^{23}	3.1	2.5	0.15[1]
xantha-d^{31}	4.1	2.5	0.18[1]
viridis-zd^{69}	3.1	2.7	0.34

[1]The electron acceptor was pyocyanine instead of DCIP

composed of eight Gaussian components shown in Table VI. The above mentioned shift toward the blue in the peaks for the long wavelength emission bands of the mutant membranes can be largely accounted for by a reduction in the 740 nm Gaussian component relative to the 725 nm component.

Chloroplast Membrane Proteins. The proteins in the chloroplast thylakoids of the wild type, *viridis-k*^{23} and *xantha-b*^{12} were solubilized in phenol-acetic acid-urea and yielded upon electrophoresis the patterns shown in Figure 10. The protein patterns of the mutants differed from the wild type. In the grana-deficient mutant *viridis-k*^{23}, the protein band with an apparent mol wt of 39,000 was strongly reduced compared with the wild type. This protein band increases strongly during the development of etioplasts into chloroplasts in wild type leaves, as do the bands of mol wt 34,000, 41,000 and 100,000 (15).

On the other hand, in the grana-rich mutant *xantha-b*^{12}, the protein band of apparent mol wt 100,000 was reduced compared with the other chloroplast specific bands in the 34,000 to 41,000 mol wt range. The band of mol wt 70,000, which was present in both etioplasts and chloroplasts, was somewhat reduced in this mutant.

DISCUSSION

Results of studies we reported earlier showed that wild type barley chloroplasts prepared as described in this report consist of naked lamellar systems with almost no grana (18). Salt concentrations higher than those used in the isolation media, or those used in the assay mixtures, would be required if grana structure were to have been maintained, or if appression between adjacent thylakoids were to have been re-established (18). On this basis, differences in electron transport activity due to varying proportions of "stacked" *versus* "unstacked" thylakoids among the mutants seen *in situ* would not be expected *in vitro*.

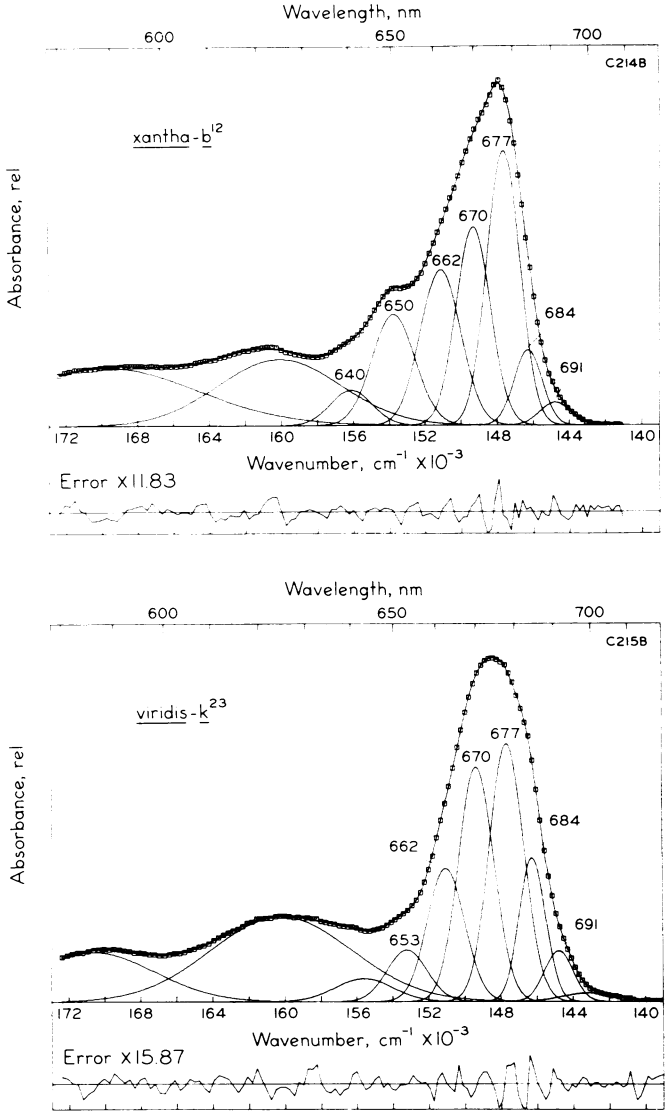

FIG. 8. Absorption spectra of two mutants at −196 C. Component bands believed to be the major peaks of particular forms of Chl are indicated. Table V gives characteristics of component Gaussian curves.

Despite the lack of grana, the chloroplast preparations catalyzed high rates of electron transport from water to both ferricyanide and DCIP, and were able to establish proton concentration gradients across membranes in the light. These activities had the expected sensitivities to various uncouplers, phenylenediamine, DCMU, and dibromothymoquinone (16). The addition of the uncoupler methylamine increased the rate of electron transport to ferricyanide up to 10-fold (16). Relatively high concentrations of methylamine (50–60 mM) are required for maximal activities of the barley (and also maize mesophyll) chloroplasts. The same activity was obtained if the 60 mM methylamine was replaced by 4 μg ml^{-1} of gramicidin D.

A stimulation of ferricyanide-Hill activity could also be elicited using phenylenediamine, and was identical in magnitude to the one caused by methylamine (16). The light saturation curves for ferricyanide photoreduction were the same in the presence of both compounds. In the former case, phenylenediamine acted by diverting electrons prior to the rate-limiting step between the two photosystems. When uncoupled according to the conditions employed in this study, ferricyanide-Hill activity was a valid indication of the capacity for PSII-linked electron transport.

The barley mutants we studied can be placed into two categories on the basis of their relative granal development and ratio of Chl *a/b*. Both mutant types showed PSII and PSI activities, but increased granal content was not correlated with enhanced PSII-linked electron transport. Instead, the grana-deficient mutants showed a higher ratio of PSII-linked activities to PSI-linked activity compared with the wild type while the grana-rich mutants showed a lower ratio than the wild type (Table III).

Thylakoids prepared from the grana-deficient mutants photoreduced ferricyanide 15 to 50 times more rapidly than those from the grana-rich mutants and five times more rapidly than those from the wild type seedlings. These differences could result either from altered photosynthetic unit sizes since these rates are expressed on a mg Chl basis, or else from faster limiting steps in electron transport within photosynthetic units of the same size. Results from the present work do not permit distinction between these two possibilities in the case of the barley mutants. Evidence favoring the latter interpretation has been presented in the case of the "*b*-less" mutant of barley (5) and Chl-deficient mutants of soybean (13), pea (5), and tobacco (5).

Significant differences in the protein composition of the thylakoids were evident between these two groups of barley mutants. The grana-deficient mutants contained small amounts of a 39,000 mol wt thylakoid polypeptide, while the grana-rich mutants contained relatively low amounts of a 100,000 mol wt protein. These bands visualized in phenol-acidic acid-urea gels appear to correspond to the apoproteins of the Chl-protein complex II and Chl-protein complex I, respectively (11). They likely reflect relative concentrations of polypeptides to which Chl is bound *in vivo* and which perform light-harvesting functions, as reviewed by Thornber (22).

Table V

Chlorophyll Components in Absorption Spectra of Chloroplast Fragments from Barley Mutants

xantha-b^{12} C214B								
Wavelength, nm:	640.6	650.4	661.7	669.7	677.3	683.6	690.7	
Half width, nm:	11.2	11.9	11.5	10.1	9.8	8.6	9.7	
Percent of area:	4.3	14.6	19.8	22.1	29.6	7.1	2.5	
Percent of long wavelength forms:							9.6	
viridis-k^{23} C215B								
Wavelength, nm:	642.6	652.7	662.1	669.6	677.3	683.8	690.7	698.9
Half width, nm:	15.7	11.2	10.6	10.3	10.4	8.0	8.8	16.9
Percent of area:	(Note 1)	6.5	15.9	27.4	30.3	13.0	5.1	1.7
Percent of long wavelength forms:							19.8	

Note 1: The 640 band is believed to represent a real form of chlorophyll *b* in the *xantha-b*^{12} but not in *viridis* so its area is omitted here.

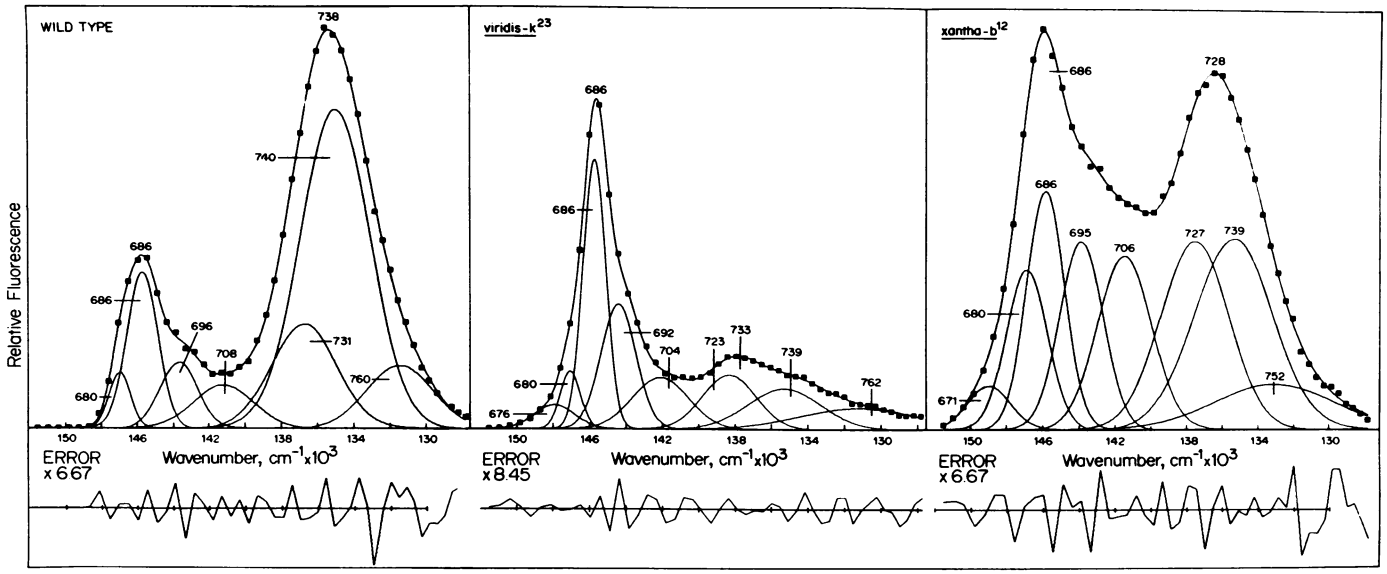

FIG. 9. Curve analysis (9) of fluorescence spectra of thylakoid fragments from wild type, *viridis-k*[23], and *xantha-b*[12] chloroplasts measured at −196 C. Uppermost curve in each panel is the sum of the Gaussian components under the spectra while the points in this curve are data from the original spectra corrected for the base line and normalized to give zero emission in the 600 to 650 nm region. The numbers identifying each Gaussian component give the wavelength assigned for maximum emission, but the ones identifying peaks in the uppermost curve identify the emission maximum in the corrected spectra.

Table VI

Curve Analyses of Fluorescence Spectra of Fragments from Barley Mutants

xantha-b[12] F21B								
Wavelength, nm:	671.3	680.9	685.7	695.1	706.9	727.0	738.6	751.9
Half width, nm:	12.3	12.3	11.6	13.7	17.7	23.7	28.3	44.5
Percent of area:	2.4	8.6	12.1	11.3	13.5	19.5	23.7	8.9
viridis-k[23] F20C								
Wavelength, nm:	676.0	680.1	686.2	692.5	703.8	723.0	739.1	761.9
Half width, nm:	11.9	6.0	6.9	10.9	18.0	18.0	25.7	44.4
Percent of area:	3.6	4.5	24.1	17.6	11.9	12.6	13.5	12.2
Wild Type Barley F22B								
Wavelength, nm:	--	680.3	686.2	695.8	708.3	731.1	740.4	760.5
Half width, nm:	--	6.5	9.5	11.9	19.5	22.6	24.8	24.7
Percent of area:	--	2.4	9.7	5.1	5.2	15.4	51.7	10.2

Examination of the low temperature fluorescence spectra of thylakoid fragments from the grana-rich and grana-deficient mutants and wild type revealed additional differences. The relative fluorescence emission at short wavelength (686 nm) compared to that at long wavelength (~730 nm) was high in the grana-deficient mutants *viridis-k*[23] and *xantha-l*[35], about equal in the grana-rich mutants *xantha-b*[12], *-c*[23], and *-d*[31], and low in wild type (Fig. 9). Curve analysis on examples of each mutant class indicated additional differences, especially in the long wavelength region of the spectrum. While the reasons for the asymmetry in the fluorescence bands in the spectra are unclear (17), the differences between the mutants could reflect changes in the environment of the chromatophores in the thylakoid membrane. The differences in the spectra could also result from different organizations or concentrations of the pigments within the membranes of the two mutant classes, and this may in turn be associated with the relative amounts of the polypeptides contributing to the 100,000 and 39,000 mol wt electrophoretic bands.

It has been proposed that PSII is responsible for the shorter wavelength emission and PSI for the longer (6). On that basis, one might expect the grana-deficient mutants, and to a lesser extent the grana-rich mutants to have relatively more PSII than PSI activity compared to wild type. Although this was the case for *viridis-k*[23], the results for *xantha-b*[12] did not meet this expectation.

The low temperature absorption spectra of *viridis-k*[23] and *xantha-b*[12] bear resemblance to PSI- and PSII-enriched particles purified from green plants, respectively (8). The poorly developed granal system, high Chl *a/b* ratio, and higher PSI activity of *viridis-k*[23] compared to *xantha-b*[12] correlate well with the accen-

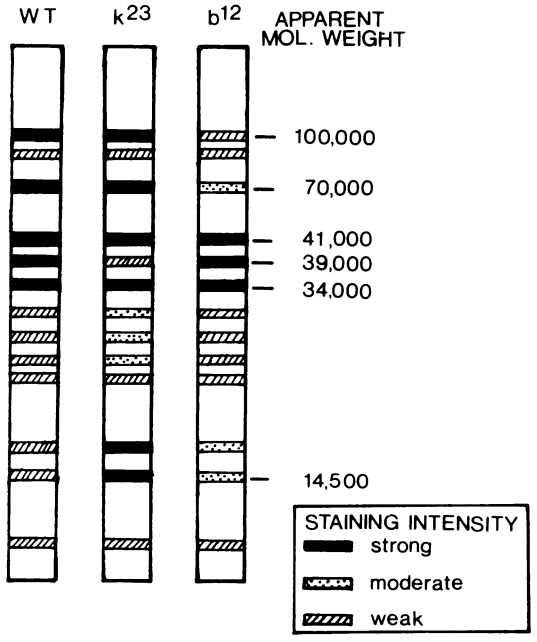

FIG. 10. Representation of electrophoretic gels obtained using chloroplast membranes isolated from wild type, a grana-deficient mutant *viridis-k*[23], and a grana-rich mutant *xantha-b*[12]. Major differences were detected in prominent bands of apparent mol wt of 100,000, 70,000, and 39,000 daltons.

tuated long wavelength *A* indicative of a PSI pigment system. By contrast, *xantha-b*[12] had far lower uncoupled rates of ferricyanide reduction than *viridis-k*[23], where one would anticipate the opposite result based on its PSII-like absorption spectrum.

The extremes in chloroplast structure and function exhibited by the grana-rich and grana-deficient barley mutants are reminiscent of the chloroplasts found in plants adapted to growth in either extremely high or low light environments. Like the chloroplasts in the grana-rich barley mutants, the chloroplasts in shade species of plants like *Alocasia, Cordyline,* and *Tomandra* have large stacks of grana, low Chl *a/b* ratios, and low rates of electron transport on a Chl basis (2, 5). At the other extreme, species such as *Atriplex patula,* like the grana-deficient barley mutants, have reduced grana content, high Chl *a/b* ratios, and high rates of electron transport/mol Chl. One might speculate that genetic lesions which affect chloroplasts frequently shift to extremes the operation of a light-sensitive adaptive mechanism common to all plants. Such a mechanism would permit different chloroplasts in the same plant or leaf to maximize net photosynthetic energy yield while minimizing that expended in the synthesis and maintenance of the photosynthetic apparatus. The mechanism could involve both the elaboration of the light-harvesting system as described by Thornber (22), and a change in the pool sizes of the various electron carriers at critical steps in the electron transport chain as discussed by Boardman (5).

Acknowledgments—We are grateful to S. Placing and B. Anderson for technical assistance, to C. Barr for help with instrumentation, and to J. Brown for her help in obtaining the fluorescence emission spectra.

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