CHARACTERIZATION OF SIX PUTATIVE PHOTOSYSTEM I MUTANTS IN BARLEY

by

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Six putative photosystem I mutants in five different nuclear genes of barley have been characterized with respect to their photosynthetic electron transport capabilities, *P700*, cytochromes, chlorophyll-proteins and other thylakoid polypeptides. Three of these mutants (*viridis-n*³⁴, *-zb*⁶³, and *-h*¹⁵) have normal photosystem II activities but are wholly or partly deficient in photosystem I electron transport, *P700*, chlorophyll *a*-protein I and its apoprotein. These three mutants also lack low molecular weight polypeptides which are believed to be integral photosystem I iron-sulfur centres. Of the remaining mutants, *xantha-q*⁷⁵ and *-q*⁷⁶, which are allelic, are mainly depleted in photosystem II and the other (*viridis-q*⁴²) had about 75 % of the wild-type activity in both photosystems. The data are discussed in relation to the fluorescence induction kinetics and low temperature fluorescence emission spectra of the mutants.

Abbreviations: Asc = ascorbic acid; BSA = bovine serum albumin; Chl = chlorophyll; Cyt = cytochrome; DCIP = 2,6-dichlorophenolindophenol; DCMU = 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FeCN = potassium ferricyanide; *F*_m = maximal fluorescence; *F*₀ = initial fluorescence; *F*_s = steady state fluorescence; HEPES = *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; MeV = methyl viologen; PD = *o*-phenylenediamine; PS = photosystem; SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis; Tricine = *N*-(tris-(hydroxymethyl)-methyl)-glycine; Tris = tris-(hydroxymethyl)aminomethane.

1. INTRODUCTION

A powerful tool for the correlation of thylakoid polypeptides with respect to their function is the use of mutants affecting polypeptide composition (20, 28). With the possible exception of the barley chlorophyll *b*-less mutant and the *xantha-f*, *-g* and *-h* mutants in barley blocking steps in chlorophyll *a* synthesis, relevant mutants have not been sufficiently well characterized to separate the primary lesion from pleiotropic secondary effects. Five photosystem I mutants from maize (21) selected on the basis of their fluorescence induction kinetics have been shown to lack chlorophyll *a*-protein 1. In addition to the loss of chlorophyll *a*-protein 1 with its associated *P700* activity, three of these mutants were also deficient in chlorophyll *a/b* proteins. One of these mutants, *hcf*E1481* was examined with respect to total thylakoid polypeptide composition (22) and found to be deficient in an additional low molecular weight polypeptide (18,000 mol. wt.). Whether the mutants lack other polypeptides is not known at present. Photosystem I mutants of *Chlamydomonas* have been more extensively characterized as to the relationship between *P700*-chlorophyll *a*-protein 1, a 70,000 mol. wt. apoprotein, and photosystem I activity (10). Whether there is an obligatory relationship between all these components is still uncertain as is their relationship to the bound iron-sulfur centres known to be an integral part of photosystem I (11). It has recently been shown (24) that a photosystem I mutant from barley (*viridis-n*³⁴) is deficient in chlorophyll *a*-protein 1 and three low molecular weight polypeptides two of which are probably iron-sulfur centres. In this report we have extended the observations on *viridis-n*³⁴ and examined an additional five potential photosystem I mutants of barley which were selected from a group of 42 *viridis* mutants on the basis of their fluorescence induction kinetics (25).

2. MATERIALS AND METHODS

2.1. Plant material

Seeds of wild-type barley (*Hordeum vulgare* cv. Svalöf's Bonus) and the recessive mutants *viridis-h*¹⁵, *xantha-q*⁷⁵, *viridis-n*³⁴, *viridis-q*⁴², *xantha-q*⁷⁶ and *viridis-zb*⁶³ were germinated in vermiculite moistened with tap water. Seedlings

were harvested after growing for 7 days at 20 °C in continuous white light (1700 lux). The mutant seedlings were separated from the wild-type phenotype by their paler green color. All mutants are seedling lethals and are therefore maintained in the heterozygous state (25, 26).

2.2. Chloroplast and thylakoid isolation

Chloroplasts were prepared by homogenizing precooled leaves in a medium composed of 0.4 M-sucrose, 200 mM-NaCl, 50 mM-Tricine/NaOH, pH 7.9. The homogenate was filtered through two layers of 31 µm nylon gauze and centrifuged 5 min at 4,000 g. Chloroplast pellets to be used for the analysis of thylakoid polypeptide content by SDS-PAGE were resuspended and osmotically shocked in 10 mM-EDTA, 25 mM-HEPES/NaOH, pH 7.5. The thylakoid pellet obtained after centrifugation for 5 min at 10,000 g was resuspended in a small volume of 1.90 M-sucrose, 5 mM-EDTA, and 25 mM-HEPES, pH 7.5, and the thylakoids purified by the flotation technique described earlier (20). Thylakoids used for the analysis of chlorophyll-proteins were subjected to two additional washes with 100 mM-Tris/acetate, pH 9.2. When chloroplasts were prepared for measurements of photosynthetic electron transfer reactions, 0.5 % BSA was included in the homogenization medium and the chloroplast pellet was resuspended in 50 mM-Tricine, pH 8.0, 50 mM-NaCl, and 5 mM-MgCl₂ together with 0.5 % BSA.

2.3. Chlorophyll determination

Chlorophyll content of leaves and chloroplasts was determined spectrophotometrically in 80 % acetone (5). For analysis of leaves, only the top 5 cm was used and this was cut into small pieces and ground in a mortar and pestle in 85 % acetone. Calcium carbonate was present during the grinding to avoid the formation of pheophytin.

2.4. Photochemical measurements

2.4.1. Photosystem I and II measurements

Spectrophotocatalytic measurements of photosystem I and II were carried out as described earlier (24) except that methylamine was used as

an uncoupler instead of gramicidin D. Electron transport was also assayed by use of a Rank Bros. oxygen electrode. Photosystem I plus photosystem II was determined with H_2O as electron donor and methyl viologen as electron acceptor. Photosystem I electron transport was measured in the presence of DCMU using ascorbate/DCIP as donor and methyl viologen as acceptor. Total electron transport from water to CO_2 was estimated from the amount of oxygen evolved upon illumination of small leaf pieces (approx. 20 mg) in a medium of 100 mM- NaHCO_3 .

2.4.2. Cytochromes

Cytochromes were measured at room temperature as described previously (24). Low temperature spectra of the cytochromes were obtained as described by BOARDMAN and ANDERSON (9).

2.4.3. *P700*

P700 was measured from the ferricyanide oxidized minus ascorbate reduced difference spectrum of chloroplasts dispersed in a Triton X-100 contained buffer (18). The duplicate cuvettes containing the following assay mixture (2.2 ml): chloroplasts (150 μg chlorophyll), Triton X-100 (11.0 mg) and 42 mM-Tricine, pH 8.0. The spectrophotometer was operated in the split beam mode and the two cuvettes were measured against each other from 660 to 730 nm to establish a baseline. Ferricyanide was then added to the reference cuvette to a final concentration of 1 mM and sodium ascorbate to the measuring cuvette to produce a 1 mM-solution. The two cuvettes were equilibrated for 4 min before the difference spectrum was recorded. A millimolar extinction coefficient of 64 mequivalents $^{-1}$ cm $^{-1}$ was used to convert the observed change in absorbance at 698 minus that at 725 nm into concentration of *P700* (18). Light induced *P700* content was assayed as described by ANDERSON et al. (3).

2.5. Difference spectra of wild-type minus mutants

Cuvettes containing wild-type and mutant chloroplasts were adjusted to give equal absor-

bance ($A = 1.0$) at the red chlorophyll absorption maximum. The difference spectrum was then recorded from 600 to 750 nm at both room and liquid nitrogen temperatures using an Aminco DW-2a spectrophotometer. For measurements at 77 K the chloroplasts were suspended in buffer containing 66 % (v/v) glycerol.

2.6. Fluorescence emission spectra

The fluorescence emission spectra were obtained from whole leaves at liquid nitrogen temperature (77 K) using an Applied Photophysics fibre optics spectrophotometer (25).

2.7. Sodium dodecyl sulfate polyacrylamide gel electrophoresis

The proportions of chlorophyll in the different chlorophyll protein complexes and the free chlorophyll band were estimated by scanning cylindrical 8 % SDS-PAGE gels at 670 nm as previously described (16) except that all gels were run at 4 °C and at pH 9.2. An SDS/chlorophyll ratio of 10:1 (w/w) was used to solubilize the thylakoid membranes prior to electrophoresis. Increased ratios (up to 20:1 (w/w)) were necessary for proper solubilization of thylakoids from mutants with a low chlorophyll content.

Total thylakoid polypeptides were analyzed as described earlier (24).

3. RESULTS

3.1. Partial electron transport reactions

The mutants used in this study were selected as potential photosystem I mutants from *viridis* mutants, i.e. mutants originally identified by a reduction in chlorophyll content (25). The mutants contain 10 % to 79 % of the wild type chlorophyll levels as determined by extraction of chlorophyll from leaf pieces (Table I). Lower values obtained for the same mutants by this method compared to the estimates obtained previously from measuring the absorbance of intact leaves are probably due to the relatively large contribution of light scattering in leaves of low chlorophyll content. Exclusive and complete loss of chlorophyll *a*-protein 1 from chloroplasts in leaves having a chlorophyll *a* to chlorophyll *b* ratio of 3.0 would result in a chlorophyll *a* to

Table I

Chlorophyll content and chlorophyll *a/b* ratios of wild-type and mutant barley seedlings. Chlorophyll content is expressed as mg · g⁻¹ fresh weight, and as its percentage relative to wild-type. Data previously obtained (25) for chlorophyll content estimated by absorbance measurements of mutant leaves are also included (% *).

	wild-type	<i>vir-h¹⁵</i>	<i>vir-zb⁶³</i>	<i>vir-n³⁴</i>	<i>xan-q⁷⁵</i>	<i>xan-q⁷⁶</i>	<i>vir-q⁴²</i>
chl content	1.63	0.45	0.75	1.28	0.22	0.16	0.46
%	100	28	46	79	13	10	28
% *	100	45	64	76	29	26	25
chl <i>a</i> /chl <i>b</i> ratio	3.1	5.2	5.2	3.0	3.0	3.0	3.1

Table II

Photosynthetic electron transport in chloroplasts of wild-type and mutant barley.

	wild-type	<i>vir-h¹⁵</i>	<i>vir-zb⁶³</i>	<i>vir-n³⁴</i>	<i>xan-q⁷⁵</i>	<i>xan-q⁷⁶</i>	<i>vir-q⁴²</i>
Oxygen electrode measurements	μmoles O ₂ consumed · hr ⁻¹ · mg ⁻¹ chlorophyll						
PS I + PS II (H ₂ O → MeV)	105	9	0	21	0	0	32
PS I (Asc/DCIP → MeV)	185	44	0	64	110	165	134
Spectrophotometric measurements	μmoles of reduced acceptor · hr ⁻¹ · mg ⁻¹ chlorophyll						
PS I + PS II (H ₂ O → FeCN)	359	66	56	207	—	—	205
PS II (H ₂ O → Pd/FeCN)	541	454	590	736	31	36	402

Table III

***P700* content of wild-type and mutant barley thylakoids expressed as molar ratios of total chlorophyll to *P700*.**

	wild-type	<i>vir-h¹⁵</i>	<i>vir-zb⁶³</i>	<i>vir-n³⁴</i>	<i>xan-q⁷⁵</i>	<i>xan-q⁷⁶</i>	<i>vir-q⁴²</i>
Chemical chl/ <i>P700</i>	323	>10,000	>10,000	2,130	4,150	6,070	406
Photochemical chl/ <i>P700</i>	324	>15,000	>15,000	3,520	—	—	—
	416	—	—	—	—	—	840

Table IV

Cytochrome content of wild-type and mutant barley thylakoids. Results are expressed as the molar ratio of total chlorophyll to cytochrome.

	wild-type	<i>vir-h¹⁵</i>	<i>vir-zb⁶³</i>	<i>vir-n³⁴</i>	<i>xan-q⁷⁵</i>	<i>xan-q⁷⁶</i>	<i>vir-q⁴²</i>
chl/cyt <i>f</i>	1,220	3,750	6,280	960	8,400	8,790	1,260
chl/cyt <i>b₅₅₉</i> HP.	496	1,300	350	320	14,000	∞	862
chl/cyt <i>b₆</i>	275	283	203 ¹	358	442	650	360

¹This value is predominantly due to cytochrome *b₅₅₉* low potential — see text.

Table V

Chlorophyll-proteins of wild-type and mutant barley. Results are expressed as a percentage of the total chlorophyll content in the different chlorophyll containing bands in the gel.

Chlorophyll bands	wild-type	<i>vir-h15</i>	<i>vir-zb63</i>	<i>vir-n34</i>	<i>xan-q75</i>	<i>xan-q76</i>	<i>vir-q42</i>
Chl _a -P1	24.1	0	0	9.9	2.7	2.3	17.8
Chl _{a/b} -P1 + Chl _{a/b} -P2 + Chl _a -P2 + Chl _a -P3	63.8	77.2	86.0	82.6	68.2	71.1	66.4
Free chl	12.1	22.8	14.0	7.5	29.1	26.4	15.8

Table VI

Chlorophyll-proteins of wild-type and mutant barley thylakoids after extended electrophoresis. Results are expressed as a percentage of the total chlorophyll content in the different chlorophyll containing bands in the gel.

Chlorophyll bands	wild-type	<i>vir-h15</i>	<i>vir-zb63</i>
Chl _a -P1	22.1	0	0
Chl _{a/b} -P2*	12.6	11.1	14.5
Chl _a -P3 + Chl _a -P2	11.0	24.2	28.9
Chl _{a/b} -P2	36.7	27.8	30.5
Free chl	17.6	36.9	26.0

chlorophyll *b* ratio of 1.8 and a chlorophyll content per leaf of approx. 70 % of that of the wild-type. With the exception of the mutant *viridis-n34*, exclusive loss of chlorophyll *a*-protein I can not explain the observed reduction in chlorophyll content nor the observed chlorophyll *a* to chlorophyll *b* ratios.

The mutants studied are all known to be lethal at the seedling stage (25, 26) when grown under field conditions. Localization of the defect may be deduced from measurements of partial electron transport reactions in chloroplasts obtained from seedlings. Results of such studies are presented in Table II. All the mutants show a substantial decrease in the rate of electron transport from water to methyl viologen when this is expressed on a chlorophyll basis. Only in *viridis-zb63* does this result reflect a complete absence of electron transport through photosys-

tem I. However, in mutants *viridis-n34* and *-h15* there is a significant reduction in the rate of photosystem I electron transport. The complete absence of total photosynthetic electron transport in the mutants *xantha-q75* and *-q76* is surprisingly due to an almost complete absence of photosystem II whereas PSI is only slightly reduced. The mutant *viridis-q42* has a substantial electron transport capacity in both photosystem I and II whereas measurement of oxygen evolution by leaf pieces using CO₂ as electron acceptor showed an inhibition of 85 % compared to the value in leaves of wild-type. When this is expressed on a chlorophyll basis, however, the degree of inhibition is only 50 %.

3.2. P700 content

The P700 content of wild-type and mutants was determined to see if there is a correlation between the presence of the reaction centre and the photosystem I driven electron transport (Table III). Only two of the mutants appear to be completely deficient in P700, namely *viridis-h15* and *-zb63*. The mutants *viridis-n34*, *xantha-q75* and *-q76* have approx. 10 % of the wild-type level of P700 whereas their photosystem I activities (Table II) vary between 30 and 90 % of the wild-type level. The mutant *viridis-q42* seems to have close to normal levels of P700 and about 70 % of wild-type photosystem I activity. As usually observed, the photochemically determined chlorophyll/P700 values are slightly greater than those determined chemically but follow the same general pattern.

3.3. Cytochromes

The cytochrome content of the photosystem I mutant *viridis-n*³⁴ was previously shown to be similar to that of wild-type (24). It was therefore of interest to examine whether this was also true of the photosystem I mutants *viridis-zb*⁶³ and *-h*¹⁵. As shown in Table IV both mutants have a decreased content of cytochrome *f* but apparently normal content of cytochrome *b*₆, the other cytochrome associated with photosystem I. However, examination of the dithionite minus hydroquinone spectrum of mutant *viridis-zb*⁶³ indicated an absorption maximum of 559 nm rather than the usual value of 562 nm for cytochrome *b*₆. Calculation of the content of low potential cytochrome *b*₅₅₉ relative to that of cytochrome *b*₅₆₃ showed that the latter was only 30 % of that expected. (Ratio of cytochrome *b*₅₆₃ to cytochrome *b*₅₅₉ of 0.6 versus 2.0 for wild-type.) A difference spectrum of dithionite treated chloroplasts of wild-type minus *viridis-zb*⁶³ at 77 K confirmed this deficiency of cyt *b*₅₆₃ in addition to the relative absence of cytochrome *f* (Table IV). Mutants *xantha-q*⁷⁵ and *-q*⁷⁶ which are more properly described as photosystem II deficient mutants (Table II) lack cytochrome *b*₅₅₉ high potential which is associated with photosystem II. They also, however, have only trace amounts of cytochrome *f*.

3.4. Thylakoid polypeptides

The chlorophyll contents and chlorophyll *a/b* ratios (Table I) indicate that each mutation may have multiple effects on the chlorophyll-protein content. This was investigated by determining the proportion of the amount of chlorophyll associated with each of the principal chlorophyll-proteins after solubilization with SDS. Initially, a brief electrophoresis was used to obtain clear separation of chlorophyll *a*-protein 1 from other chlorophyll-proteins together with a minimum

of free chlorophyll formation (Table V). There appears to be a complete absence of chlorophyll *a*-protein 1 in both *viridis-zb*⁶³ and *-h*¹⁵ and only about 10 % of the wild-type level in mutants *xantha-q*⁷⁵ and *-q*⁷⁶. These amounts of chlorophyll *a*-protein 1 correlate well with the *P*700 content of the mutants. This also applies to mutant *viridis-q*⁴² (Table III). The proportion of chlorophyll *a*-protein 1 in *viridis-n*³⁴ is somewhat greater than would be expected from a direct correlation with its reaction centre content but is consistent with the measured photosystem I electron transport rate (Table II).

The chlorophyll content of *viridis-n*³⁴ is so high that visual selection of the mutant leaves from those of the heterozygote and the wild-type is difficult. This may result in some contamination of the mutant chloroplasts with those from normal leaves. To check this possibility, chloroplasts were isolated from single leaves which were indisputably mutant and wild-type, respectively. It was found that the mean chlorophyll *a*-protein 1 content of four mutant leaves was 7.3 % and that of the wild-type 21.1 % expressed as a percentage of the total chlorophyll in the gel. We therefore conclude that the results reported for the large scale chloroplast isolations from *viridis-n*³⁴ are representative of the mutant alone.

The apparent loss of chlorophyll *a*-protein 1 from mutants *viridis-h*¹⁵ and *-zb*⁶³ is accompanied by an increased overall chlorophyll *a/b* ratio. This can only be accommodated by an increase in the proportion of some other chlorophyll *a*-protein and/or a decrease in chlorophyll *a/b*-protein 2. To investigate this matter, the electrophoretic separation was extended until adequate resolution of chlorophyll *a*-proteins 2 and 3 from chlorophyll *a/b*-protein 2 was achieved (Table VI). Although this extended electrophoretic separation was accompanied by a substantial increase in the proportion of free

Figure 1. Thylakoid polypeptide pattern of wild-type and mutant barley.

The same amount of chlorophyll was applied to each slot and the separation was carried out at 19 °C on an 11–15 % SDS-polyacrylamide gradient gel. The position of the green bands observed corresponding to chl_{a/b}-P1 and chl_{a/b}-P2 were marked by the use of metal wires before the gel was fixed (right track). The polypeptide composition of photosystem I particles was analyzed for reference purposes. The molecular weights assigned to the polypeptides in the low molecular weight range are inaccurate and only given as a guide. The nomenclature system used for the chlorophyll-proteins is defined in (20).

Figure 2. Absorbance and difference spectra of wild-type and *viridis-zb63*.

a) Absorbance spectra of wild-type (—) and *viridis-zb63* (-----). b) Difference spectrum recorded at room temperature. c) Difference spectrum recorded at liquid nitrogen temperature.

Figure 3. Absorbance and difference spectra of wild-type and *viridis-n34*.

a) Absorbance spectra of wild-type (—) and *viridis-n34* (-----). b) Difference spectrum recorded at room temperature. c) Difference spectrum recorded at liquid nitrogen temperature.

Figure 4. Absorbance and difference spectra of wild-type and *viridis-q42*.

a) Absorbance spectra of wild-type (—) and *viridis-q42* (-----). b) Difference spectrum recorded at room temperature. c) Difference spectrum recorded at liquid nitrogen temperature.

chlorophyll, a remarkable increase of the proportion of the total chlorophyll in chlorophyll *a*-proteins 2 and 3 compared to that in the wild-type was observed.

Total SDS-PAGE thylakoid polypeptide patterns of four of the mutants discussed above are shown in Figure 1. The levels of chlorophyll *a*-protein 1 observed on the gels correspond with the chlorophyll *a*-protein 1 amounts detected on the cylindrical gels. A very faint Coomassie blue stained band at the position of chlorophyll *a*-protein 1 in *viridis-zb63* and *-h15* may represent a comigrating polypeptide or a residual amount of

Figure 5. Difference spectrum of mutants *viridis-q42* minus *viridis-zb63*.

a) Difference spectrum recorded at room temperature. b) Difference spectrum recorded at liquid nitrogen temperature. c) Control: wild-type minus wild-type, difference spectrum recorded at room temperature.

chlorophyll *a*-protein 1. It should be noted that none of the mutants showed an increase in polypeptide content of the 70,000 mw region of the gels. Such an increase might have been expected if the lesion resulted in a decreased stability of chlorophyll *a*-protein 1 to SDS. The mutant *viridis-n*³⁴ has a reduced amount of three low molecular weight polypeptides (24) two of which are believed to be polypeptides of the iron-sulfur centres known to be an integral part of photosystem I. The two photosystem I mutants *viridis-zb*⁶³ and *-h*¹⁵ also lack those same low molecular weight polypeptides. In other respects, the polypeptide composition of all six mutants appears normal with the exception of mutants *xantha-q*⁷⁵ and *-q*⁷⁶ which are possibly deficient in chlorophyll *a*-proteins 2 and/or 3 (data not shown). Two additional low molecular weight polypeptides (mol. wt. 13,100 and 10,200) in these two mutants and in *viridis-h*¹⁵ are believed to be of nuclear origin (19).

In the mutants *viridis-h*¹⁵, and *-zb*⁶³, substantial changes in the content of chlorophyll-proteins migrating between chlorophyll *a/b*-protein 2 and its oligomer chlorophyll *a/b*-protein 2* were observed after a brief electrophoretic separation. The apoproteins of these components have not yet been localized with certainty in the total thylakoid polypeptide pattern. Thus it is not known if changes in chlorophyll *a*-protein content reflect changes in total polypeptide content.

As noted above, in all the mutants the intensity of protein staining in the region of chlorophyll *a*-protein 1 or of its apoprotein, correlates well with the proportion of the total chlorophyll found in chlorophyll *a*-protein 1 after a brief electrophoresis. The possibility of this chlorophyll-protein existing in vivo but being especially unstable in detergent under our conditions cannot, however, be entirely dismissed. Absence of chlorophyll *a*-protein 1, which is enriched in long wave-length species of chlorophyll, should be reflected in the absorbance spectrum of thylakoids prior to their solubilization with detergent. This situation can be investigated by measuring the difference spectrum of wild-type minus mutant chloroplasts after normalization to a particular wave-length. In our study we normalized the spectra of both wild-type and mutant chloroplasts to an absor-

bance of 1 at the wave-length of maximum absorbance and the resulting difference spectra are shown in Figures 2 to 4. When compared to wild-type thylakoids, those of mutants *viridis-zb*⁶³, *-n*³⁴ and *-h*¹⁵ (data not shown) as well as *viridis-q*⁴² are clearly deficient in forms of chlorophyll *a* absorbing at wave-lengths of 692 nm and beyond. Low temperature difference spectra show a peak at 690 nm with a pronounced shoulder around 707 nm. Mutant *viridis-zb*⁶³ is, as previously noted, also deficient in chlorophyll *b* and chlorophyll *a/b*-protein 2 and this is clearly shown in the difference spectrum (Figure 2) by the peak at 649 nm. Because of arbitrary normalization, differences in light scattering between mutants and overlapping absorbance spectra of all their chlorophyll proteins, it is not possible to use the difference spectra to make quantitative observations as to the proportion of chlorophyll *a*-protein 1 missing from the mutants in vivo. Despite the above uncertainties, we estimate that *viridis-zb*⁶³ has only 80% of the absorbance at 692 nm possessed by the wild-type, while *viridis-n*³⁴ has 90%. This result is consistent with the partial loss of chlorophyll *a*-protein 1 from *viridis-n*³⁴ thylakoids compared to the complete loss from *viridis-zb*⁶³. We conclude from the difference spectra that the absence or reduction of chlorophyll *a*-protein 1 from detergent solubilized thylakoids does not result from an altered stability but reflects the in vivo loss of a major chlorophyll *a* species absorbing preferentially at wave-lengths greater than 692 nm.

Although mutant *viridis-q*⁴² was selected as a lethal mutant with a possible lesion in photosystem I, the partial rates of electron transport measured do not substantiate this and neither is there any major deficiency in chlorophyll *a*-protein 1. Difference spectra of wild-type minus *viridis-q*⁴² (Figure 4) indicate that this mutant is also deficient in long wave-length forms of chlorophyll *a* although the spectra are not identical (690 nm peak vs 692 nm peak at room temperature) with those of wild-type minus mutants *viridis-h*¹⁵, *-zb*⁶³ and *-n*³⁴. This was confirmed by recording the difference spectrum of mutant *viridis-q*⁴² minus mutant *viridis-zb*⁶³ (Figure 5). *Viridis-q*⁴² contains more of a chlorophyll species absorbing at 696 nm than does *viridis-zb*⁶³ but is deficient in a species

absorbing at 685 nm. A difference spectrum between wild-type and mutant *viridis-q*⁴² of unresolved chlorophyll-proteins exclusive of chlorophyll *a*-protein 1, showed a maximum at 685 nm (data not shown). This preliminary result indicates that *viridis-q*⁴² may either be partly deficient in chlorophyll *a*-protein 2 and/or 3 or have an altered chlorophyll *a/b*-protein.

4. DISCUSSION

The selection of putative photosystem I mutants by means of their fluorescence induction kinetics is based (25) on the presence of an initial fluorescence (F_0), followed by a rise of the fluorescence to a maximum (F_m) mainly due to photosystem II and the lack of a subsequent decrease which, if present, is due to electron transport through photosystem I (12). If the decrease from the maximum to steady state fluorescence (F_s) is substantially less than in the wild-type such mutants have also been designated as potentially deficient in photosystem I (25). Of the six putative photosystem I mutants studied here, only one, *viridis-zb*⁶³, showed a complete absence of photosystem I activity as indicated both by fluorescence induction kinetics (25) and in vitro electron transport measurements. The other five mutants showed photosystem I activities which ranged from 25 % to 90 % of that of the wild-type (*xan-q*⁷⁶ > *vir-q*⁴² > *xan-q*⁷⁵ > *vir-n*³⁴ > *vir-h*¹⁵) and decreases in F_m to F_s which were 5 % to 65 % of that of the wild-type (*vir-n*³⁴ > *xan-q*⁷⁶ > *vir-q*⁴² > *vir-h*¹⁵ > *xan-q*⁷⁵) (25). There is therefore no correlation in the ranking of mutants based on activities of photosystem I electron transport partial reactions and those based on the absolute values of the fluorescence decrease. Furthermore, two of the mutants, *xantha-q*⁷⁵ and *-q*⁷⁶ must, on the basis of the results reported here, be more properly described as photosystem II mutants since this was more strongly inhibited than was photosystem I. The two allelic mutants *xantha-q*⁷⁵ and *-q*⁷⁶ had the lowest variable fluorescence of the six mutants but still 50 % of that in the wild-type. This compares with a photosystem II electron transport activity of less than 10 % of that in the wild-type. Mutants can therefore not be designated as photosystem I mutants solely from the extent of the decrease from F_m to F_s or

from the absence of biphasic variable fluorescence. The small decrease in F_m to F_s observed in mutants *xantha-q*⁷⁵ and *-q*⁷⁶ may be ascribed to the deficiency found in their cytochrome *f* content in addition to that found in *P700* and chlorophyll *a*-protein 1.

The mutants *viridis-h*¹⁵ and *-zb*⁶³ had extremely low *P700* contents and the lowest photosystem I rates together with the complete absence of chlorophyll *a*-protein 1. However, there was no such clear correlation in any of the other mutants. This situation may be similar to that in *Chlamydomonas* during re-greening following treatment with chloramphenicol (6) and in etiolated barley greened in the presence of lincomycin (17) where chlorophyll *a*-protein 1 was reduced more than was the photosystem I activity expressed on a chlorophyll basis (18). It has also been demonstrated during greening of etiolated seedlings that photosystem I electron transport develops more rapidly than does measurable *P700* activity or formation of stable chlorophyll *a*-protein 1 (1, 14). This situation might be further explored with the isolation of chlorophyll *a*-protein 1 or photosystem I particles from the mutant *viridis-n*³⁴ in which the *P700* content is apparently more reduced than is either photosystem I activity or chlorophyll *a*-protein 1 content. The values for *P700* content and photosystem I activity in *viridis-n*³⁴ here reported are relatively higher than previously observed (24). The variability of the results may be explained by the use of seed material from a different harvest in the present study.

It is generally considered that the long wavelength fluorescence emission at low temperature arises from chlorophyll species associated with photosystem I (11, 12) and more specifically with chlorophyll *a*-protein 1 (2). The evidence for this is the fluorescence emission of digitonin and Triton photosystem I particles and of isolated chlorophyll *a*-protein 1 obtained from gels (2). The results with the mutants studied here do not, however, support such a simple assignment since leaves of *viridis-zb*⁶³ and *-h*¹⁵, which do not have any chlorophyll *a*-protein 1, retain a high ratio of long wavelength fluorescence which differs from the wild-type only in a 6 nm blue shift of the emission maximum (Figure 6). It is conceivable that the long wavelength fluorescence emission at 77 K arises from

Figure 6. Low temperature (77 K) fluorescence emission spectra of leaves from wild-type (—) and *viridis-zb63* (-----).

an outer part of chlorophyll *a*-protein 1 whose activity is retained *in vivo* but lost on solubilization by SDS. An alternative explanation is that the long wave-length fluorescence has multiple origins and that a minor component of shorter emission wave-length in the wild-type becomes predominant when chlorophyll *a*-protein 1 is lost due to mutation.

Although absence of chlorophyll *a*-protein 1 on SDS-PAGE might be the result of decreased stability to SDS-treatment, the difference spectra of wild-type minus mutants *viridis-h15*, *-zb63* and *-n34*, show a marked reduction in long wave-length forms of chlorophyll *a*. A similar loss of chlorophyll *a*₆₉₀ and *a*₇₀₀ has been reported for *Chlamydomonas* mutants which lack chlorophyll *a*-protein 1 and photosystem I activity (8). From a study of photosystem I particles containing variable chlorophyll/*P700* ratios, MULLET et al. (23) concluded that the outer antennae of photosystem I absorbed preferentially at 683 and 701 nm and the inner at 685 to 690 nm and 696 nm. If this is correct our results

would suggest that only the core antennae of photosystem I together with the reaction centre are lost from the mutants *viridis-h15*, *-zb63* and *-n34*. This may be contrasted with the restriction of chlorophyll *a*-protein 1 formation by greening leaves in the presence of lincomycin where the difference spectra indicated a removal of chlorophyll species absorbing predominantly at 683, 692 and 705 nm although in this case photosystem I activity was unimpaired (17).

The loss of chlorophyll *a*-protein 1 and its apoprotein is accompanied in all mutants with a similar reduction in the content of three polypeptides with molecular weights of 18,300, 15,200 and 13,500. Two of these polypeptides incorporate ⁵⁹Fe (unpublished data) and have consequently been assigned to the iron-sulfur centres shown by low temperature ESR to be an integral part of photosystem I (13). The chloroplast photosystem I reaction centre (*P700*) appears to form an integral complex with several polypeptides of low molecular weight (7). The extent to which this complex can be assembled in the absence of any one of its components is uncertain. If all are required for the proper synthesis and assembly, then there are likely to be several non-allelic *viridis* mutants which lack a functional photosystem I. In addition, mutants lacking polypeptides necessary for the correct assembly of the complex are expected. These may well have pleiotropic effects which result in the partial loss of components somewhat removed from the photosystem I reaction centre.

Among the mutations studied here, only that in *viridis-n34* produces a lesion which is confined to the integral components of photosystem I, that is, chlorophyll *a*-protein 1, *P-700*, and associated iron-sulfur proteins. Mutants *viridis-h15* and *-zb63* are also partly deficient in chlorophyll *a/b*-protein 2 and cytochrome *f* and may represent assembly mutants. Mutant *viridis-zb63* should be the most useful for further studies on photosystem II electron transport. This mutant is not only completely lacking photosystem I activity but the deficiency in chlorophyll *a*-protein 1 and additionally in chlorophyll *a/b*-protein 2 results in an enrichment of photosystem II components which is comparable with that in photosystem II particles prepared by fractionation with digitonin.

Specific deficiency in chlorophyll *a*-protein 1

could be caused by mutation in three nuclear genes of barley. It may also be noted that mutations in chloroplast DNA can lead to loss of chlorophyll *a*-protein 1 (8, 15). It is evident that this type of photosystem I mutants deficient in chlorophyll *a*-protein 1 can only be useful in further resolving these complex structural inter-relationships if in vitro protein synthesis from RNA is investigated. An alternative approach is the search for higher plant mutants which are compositionally indistinguishable from the wild-type but which have a functionally incompetent photosystem I.

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