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CHANGES IN COMPOSITION AND FUNCTION OF THYLAKOID MEMBRANES AS A RESULT OF PHOTOSYNTHETIC ADAPTATION OF CHLOROPLASTS FROM PEA PLANTS GROWN UNDER DIFFERENT LIGHT CONDITIONS

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The hypothesis that chloroplasts having different light-saturated rates of photosynthesis will have different proportions of the intrinsic thylakoid complexes engaged in light-harvesting and electron transport (Anderson, J.M. (1982) Mol. Cell. Biochem. 46, 161–172) has been tested. Peas were grown in light regimes which varied in light intensity, quality and time of irradiance, and ranged from sunlight through red to blue-enriched light of very low radiation. The electron-transport capacity at saturating light of Photosystem I and Photosystem II of chloroplasts isolated from light-adapted peas was 2-fold and 5–6-fold lower, respectively, in the lowest radiation compared to sunlight. There was a marked increase in the amount of total chlorophyll associated with the main chlorophyll *a*/*b*-proteins (LHCP^1, LHCP^2 and LHCP^3) and a 2-fold decrease in the core reaction centre complex of Photosystem II (CP a) as the radiation decreased; the LHCP$^{1-3}$/CP a ratio changed from 3.5 to 9.0. The amount of chlorophyll associated with Photosystem I varied from 34% in sunlight to 27% in the lowest radiation, but the antenna size of Photosystem I was not markedly different; there was a 2-fold decrease in the amount of cytochrome *f* on a chlorophyll basis, which partly accounted for the decreased electron-transport capacity of Photosystem I. Since the increases or decreases in the levels of each of the components correlated with decreasing radiation, it is clear that the light-adaptation of both light-harvesting and electron-transport components is indeed closely co-ordinated.

Introduction

Most of the polypeptides of thylakoid membranes are arranged in supramolecular, membrane-spanning complexes composed of extrinsic and intrinsic proteins [1,2]. Three complexes are involved in electron transport: the PS II complex has two proteins (each 40–50 kDa) which bind the P-680, Chl *a* and β-carotene molecules of the core PS II reaction centre, cytochrome *b*-559$_{HP}$ (HP, high-potential), a 32 kDa herbicide-binding protein and the oxygen-evolving polypeptides; the cytochrome *b*-*f* complex which includes the Rieske FeS centre; and the PS I complex which has a main 68 kDa polypeptide which binds P-700, Chl *a* and β-carotene molecules of the core PS I reaction centre and several other polypeptides. Chl *b* is present mainly in the Chl *a*/*b*-proteins of the light-harvesting complexes of PS II [3] but also in the minor Chl *a*/*b*-proteins specific to PS I [4,5]. The proton gradient generated during electron

Abbreviations: Chl, chlorophyll; PS, photosystem; CP 1a^1, CP 1a^2, CP 1, chlorophyll proteins of PS I; CP a, reaction centre complex of PS II; LHCP^1, LHCP^2, LHCP^3, Chl *a*/*b*-proteins of the main light-harvesting complex; Tricine, *N*-tris(hydroxymethyl)methylglycine; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

transport is utilized by ATP synthase (CF_1-CF_0).

Light is a dominant environmental factor in the regulation of plant growth (cf. Ref. 6). Higher plants respond to the amount of incident light available during growth with specific changes in the composition, function and structure of their chloroplasts [7–12]. Associated with the higher efficiency of photosynthetic quantum conversion of sun or high-light plants, there are higher Chl *a*/Chl *b* and lower xanthophyll/*β*-carotene ratios, and higher levels of electron carriers and ribulosebisphosphate carboxylase on a chlorophyll basis [7–13]. Chloroplasts of high-light plants have fewer thylakoid membranes with smaller grana stacks and thus fewer appressed membranes than chloroplasts of low-light plants, which have more thylakoid membranes with giant grana stacks and consequently more appressed membranes [7–13]. These light-adapted changes in the composition, structure and function of chloroplasts suggest that light is regulating the relative amounts of thylakoid complexes [6]. Because of the marked lateral asymmetry of the distribution of thylakoid complexes between appressed and non-appressed membranes [14–16], Anderson [6] postulated that chloroplasts with different extents of grana stacking will have different proportions of the thylakoid complexes, and hence exhibit different maximum light-saturated rates of photosynthesis. To test this hypothesis, we have grown peas under light regimes deliberately chosen to vary in light intensity and quality as well as duration of irradiance, to see whether different light conditions will produce coordinated changes in the amounts of chlorophyll-proteins and thylakoid complexes involved in electron transport. We demonstrate that peas do indeed adapt themselves efficiently to their environment through the interplay of the relative amounts of chlorophyll-proteins and of electron-transport complexes.

Materials and Methods

Pea (*Pisum sativum* L.) seedlings were grown in vermiculite in growth cabinets at 22 ± 2°C, or inside a glasshouse. The eight different light conditions used are summarized in Fig. 1 and Table I. Emission spectra of the light sources were recorded with a Techtum Spectroradiometer, QSM 2500.

Chloroplasts were isolated from 2–3-week-old seedlings. To eliminate physiological differences in leaves, whole seedlings were used for chloroplast isolation. Chloroplasts were isolated as described previously [17], but in a homogenization buffer of 50 mM Tricine (pH 8.0), 0.4 M sucrose, 10 mM NaCl and 5 mM $MgCl_2$. Chloroplasts were resuspended in 50 mM Tricine (pH 8.0), 10 mM NaCl and 5 mM $MgCl_2$. Thylakoids were washed in glass-distilled water, then 1 mM EDTA (pH 8.0) followed by two washes in 50 mM Tricine (pH 8.0) and resuspended in 50 mM Tricine (pH 8.0) (1–4 mg Chl/ml) prior to storage in liquid N_2. Total chlorophyll and Chl *a*/Chl *b* ratios were determined in 80% acetone [18].

Discontinuous slab (140×1.5 mm cross-section) SDS-polyacrylamide gel electrophoresis was performed using a modification of the published procedure [19]. The acrylamide/*N*,*N*′-methylenebisacrylamide ratio was 29.2 : 0.8; the acrylamide was 8.0% in the separating and 4.0% in the stacking gel, with 0.1% SDS and the buffer system of Neville [20] was used. Chloroplast membranes (50 μg Chl) were solubilized without prior lipid extraction in a solution (100 μl) containing 50 mM Tris-HCl (pH 8.0), 10% (v/v) glycerol and SDS to give an SDS/Chl ratio of 7.5 (w/w). Samples (equivalent to 20 μg Chl) were applied immediately to the gel which had been pre-electrophoresed at 10 mA for at least 30 min. Gels were run at 4°C in a Bio-Rad Protean slab cell at 10 mA for 3 h. Total migration distance was about 5 cm. Immediately after electrophoresis, these 'green' gels were scanned at 675 and 650 nm on a Varian 635 spectrophotometer fitted with a gel scanning attachment. The relative distribution of chlorophyll on the gels was estimated as described previously [19].

Photoreduction of ferricyanide and DCIP was measured with a Hitachi-Perkin Elmer 557 spectrophotometer as described previously [21]. Photosystem I activity was assayed as O_2 uptake with a Hansatech electrode at 20°C as described in Ref. 21.

P-700 was assayed by the photochemical method [22] and cytochrome *f* was determined from the hydroquinone-reduced minus ferricyanide-oxidized difference spectrum [23].

Results

Characteristics of the light regimes

Peas (a sun-adapted species) were grown for the same time in eight widely varying light regimes. The spectroradiometric scan from 740 to 400 nm of each light climate is shown in Fig. 1. Their characteristics are compared in Table I where the light climates are listed in order of decreasing photon fluence rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$), measured from 740 to 400 nm. The same order of light regimes is maintained for all tables. The light climates ranged from full and screened sunlight where the spectral intensity and quality and also duration of irradiance will vary throughout the day and from day to day, to intermediate radiation of continuous tungsten-enriched fluorescent light, to the 12-h fluorescent light regime and, finally to the very low radiation of continuous red fluorescent light and the even lower irradiance of the blue-enriched fluorescent light regimes.

Relative distribution of chlorophyll between the chlorophyll-proteins

The Chl *a*/Chl *b* ratios of the light-adapted pea thylakoids ranged from 2.96 to 1.91 (Tables II and III). This is consistent with published results on adaptation, since plants grown in sunlight or high-intensity light have higher Chl *a*/Chl *b* ratios

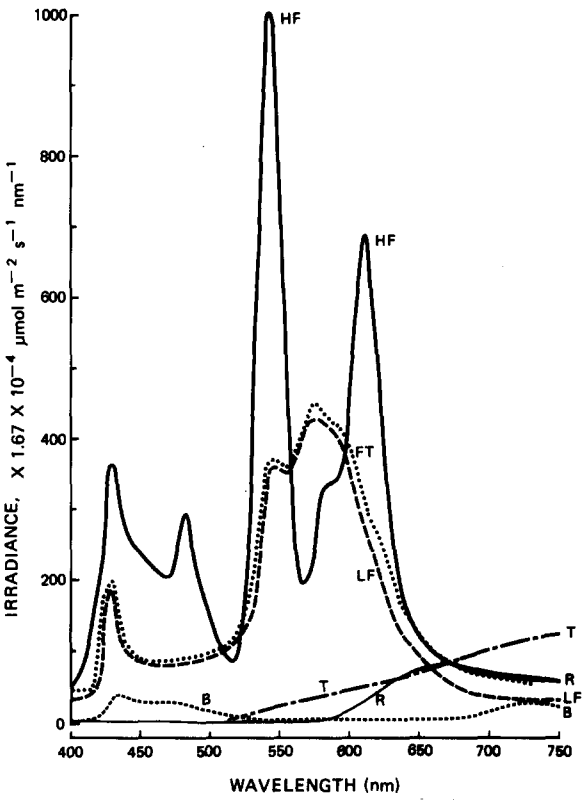

Fig. 1. Spectroradiometric scans of the light climates used for growth of pea plants (see Table I for abbreviations).

TABLE I

LIGHT REGIMES USED FOR THE GROWTH OF PEA SEEDLINGS

Light condition	Abbreviations	Light/dark regime	Emission maximum (nm)	Photon-fluence rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$) (400–740 nm)
Full sunlight	FS	–	740	1080 [a]
Screened sunlight	SS	–	740	540 [a]
High white fluorescent light	HF	Continuous	545	140
White fluorescent light + tungsten light	FT	Continuous	585	100
Low white fluorescent light	LF	12 h light/dark	585	100
Tungsten light	T	Continuous	740	24
Red fluorescent light	R	Continuous	665	15
Blue-enriched fluorescent light	B	Continuous	435	6

[a] A typical noon measurement. Approximate day length was 10 h.

TABLE II

THE PERCENTAGE DISTRIBUTION OF CHLOROPHYLL IN THE CHLOROPHYLL-PROTEINS OF THYLAKOIDS OF LIGHT-ADAPTED PEA PLANTS

The values are the average of three experiments. The light regimes are defined in Table I and Fig. 1. FC, free chlorophyll.

Light-adapted thylakoids	Percentage chlorophyll in chlorophyll-proteins							
	CP $1a^1$	CP $1a^2$	CP 1	$LHCP^1$	$LHCP^2$	CP a	$LHCP^3$	FC
FS	9.0	17.9	7.9	23.7	1.3	12.0	16.6	10.1
SS	8.0	16.3	8.4	29.3	1.5	11.8	15.0	9.5
HF	8.0	17.3	9.0	28.6	2.5	11.6	14.1	8.9
FT	7.2	16.7	9.8	26.9	2.7	9.9	16.1	9.8
LF	7.4	16.5	8.6	27.3	3.6	8.5	16.8	10.3
T	6.8	13.4	8.4	31.7	2.3	8.1	19.3	8.5
R	6.4	12.7	8.8	29.8	4.0	6.0	21.6	9.7
B	5.6	12.2	9.6	34.3	3.9	6.5	20.6	6.3

than plants grown in shade or low-intensity light [7–13]. Melis and Harvey [10] have shown that the Chl *a*/Chl *b* ratios are influenced by light intensity rather than quality. Given these differences in Chl *a*/Chl *b* ratios one would expect to find variations in the relative amounts of the chlorophyll-protein complexes which may be resolved by mild polyacrylamide gel electrophoresis [19].

The light-adapted pea thylakoids were solubilized at 4°C with an SDS/chlorophyll weight ratio of 7.5 : 1 and subjected to discontinuous electrophoresis at 4°C. Eight chlorophyll-containing bands were resolved and identified by their previously characterised spectral properties [19] (Fig. 2). They are listed in order of increasing mobility: CP $1a^1$, CP $1a^2$, CP 1, $LHCP^1$, $LHCP^2$, CP a, $LHCP^3$ and free chlorophyll. Three of these chlorophyll-proteins are associated with PS I: CP $1a^1$ and CP $1a^2$ are undissociated PS I complexes which include CP 1 and colourless polypeptides, and CP 1 is the β-carotene-P-700-Chl *a*-protein. CP a is the presumed reaction centre complex of PS II and the three Chl *a*/*b*-proteins ($LHCP^1$, $LHCP^2$ and $LHCP^3$) with Chl *a*/*b* ratios of 1.3–1.1 belong to the main light-harvesting complex of PS II [19,24].

Comparison of the profiles of the chlorophyll-protein bands resolved from each of the light-

TABLE III

COMPARISON OF THE CHLOROPHYLL DISTRIBUTION IN THE CHLOROPHYLL-PROTEINS OF PS I AND PS II IN THYLAKOIDS OF LIGHT-ADAPTED PEAS

For light regimes see Table I and Fig. 1.

Light-adapted thylakoids	Chl *a* / Chl *b*	$LHCP^{1-3}$	CP a	PS I *	PS II **	$\frac{LHCP^{1-3}}{CP\ a}$	$\frac{PS\ II}{PS\ I}$	$\frac{PS\ I}{CP\ a}$	$\frac{PS\ I + CP\ a}{LHCP^{1-3}}$
FS	2.96	41.6	12.0	34.8	53.6	3.5	1.54	2.9	1.13
SS	2.68	45.8	11.8	32.7	57.6	3.9	1.76	2.8	0.97
HF	2.73	45.2	11.6	34.3	56.8	3.9	1.66	3.0	1.00
FT	2.54	45.7	9.9	33.7	55.6	4.6	1.65	3.4	0.95
LF	2.67	47.7	8.5	32.5	56.2	5.6	1.73	3.8	0.86
T	2.40	53.3	8.1	28.6	61.4	6.6	2.14	3.5	0.69
R	2.13	55.4	6.0	27.9	61.4	9.2	2.20	4.7	0.61
B	1.91	58.8	6.5	27.4	65.3	9.0	2.38	4.2	0.58

* PS I = CP $1a^1$ + CP $1a^2$ + CP 1.

**PS II = CP a + $LHCP^1$ + $LHCP^2$ + $LHCP^3$.

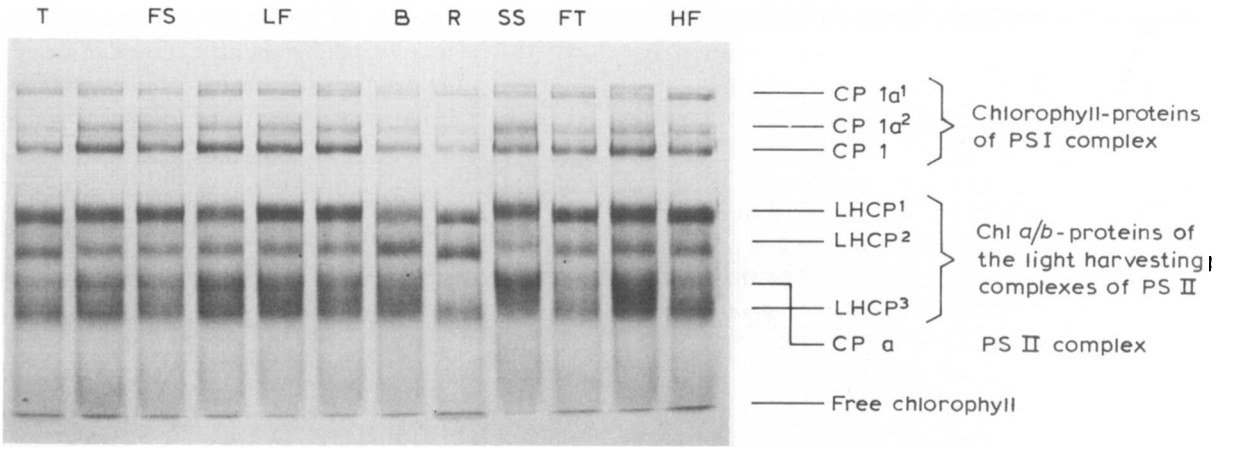

Fig. 2. Unstained SDS-polyacrylamide gels after electrophoresis of chloroplast thylakoids from peas grown under eight different light conditions (see Table I and Fig. 1). Letters on top of each slot indicate light conditions during growth (see Table I). The designation of the chlorophyll-proteins resolved is shown on the right-hand side of the gel.

adapted pea thylakoids (Fig. 2) shows that they all have the same chlorophyll-protein bands as judged by electrophoretic mobilities. The identity of these chlorophyll-protein bands was confirmed by showing that the absorption spectra and low-temperature fluorescence spectra of the chlorophyll-proteins resolved from pea plants grown in sunlight were identical to those of spinach [19,24]. The same seven chlorophyll-proteins were resolved in each case, since there were no differences in the spectral properties of the individual chlorophyll-protein bands resolved from the eight light-adapted thylakoids.

The relative amounts of chlorophyll associated with each of the seven chlorophyll-protein bands were determined (Table II). Since the amounts of free chlorophyll were low and roughly comparable (6–10% of the total chlorophyll), a valid comparison can be made of the relative chlorophyll distribution between the various chlorophyll-protein complexes. As shown in Table III, as the Chl *a*/Chl *b* ratios decrease with decreasing radiation, the amount of chlorophyll associated with $LHCP^1$, $LHCP^2$ and $LHCP^3$ increases from 42 to 59%. In contrast, the relative amounts of chlorophyll associated with CP a decrease markedly, with thylakoids from plants grown in blue-enriched light having only half the amount of chlorophyll in CP a compared to thylakoids from peas grown in sunlight (Table III). Thus, the changes associated with $LHCP^{1-3}$ are in inverse direction to the larger changes of CP a. The ratios of light-harvesting chlorophyll-proteins of PS II to the core reaction centre of PS II complex ($LHCP^{1-3}$/CP a ratio) vary markedly (Table III). Thylakoids from sun-grown peas have an $LHCP^{1-3}$/CP a ratio of 3.5 in agreement with those found in other sun plant species [6]. At the other end of the scale, the thylakoids from blue-enriched light-grown peas have a ratio of 9.0. The total amount of chlorophyll associated with the chlorophyll-proteins of PS I (CP $1a^1$, CP $1a^2$ and CP 1) ranges from 35% with peas grown in full sunlight to 27% when grown in the blue-enriched light. Hence, the relative proportion of total chlorophyll associated with PS I compared to that associated with PS II (CP a, $LHCP^1$, $LHCP^2$ and $LHCP^3$) is not greatly different. The PS II/PS I chlorophyll content ratios fall into three groups: 1.5 in full sunlight, 1.7 for the intermediate radiation regimes and 2.1–2.4 for the very low radiation regimes. Finally, the ratio of the amounts of chlorophyll associated with the core reaction centre complexes of PS II and PS I compared to that associated with the light-harvesting complex (CP a plus PS I/$LHCP^{1-3}$ ratio) decreases from 1.13 at the top to 0.58 at the bottom of the radiation scale (Table III).

The observed differences in the proportion of

chlorophyll associated with the individual chlorophyll-proteins show that a marked variation in chlorophyll distribution occurs within pea thylakoids, which is dependent on the light conditions during plant growth. These results extend previous findings that shade plants have more $LHCP^{1-3}$ and less PS I complex than found in sun plants [25] and low-light-adapted radish seedlings have a higher proportion of $LHCP^{1-3}$ than high-light-adapted seedlings which have a greater proportion of PS I and PS II core reaction centre complexes [13].

Effect of available radiation during growth of peas on electron-transport capacity

The rates of PS I and PS II electron-transport capacity under saturating light conditions were compared in freshly isolated thylakoids from the light-adapted peas. The rate of O_2 uptake in the presence of methyl viologen was assayed to determine PS I photochemical activity. The light-saturated rates of the PS I activity of thylakoids from light-adapted plants fall into three groups, with a 2.3-fold decrease in photochemical activity of chloroplasts from peas grown in the red and blue-enriched light compared to sunlight (Table IV). Potassium ferricyanide or DCIP were used as electron acceptors for PS II. In both cases photoreduction of the electron acceptors was completely inhibited by 3 μM DCMU, and is therefore a measure of PS II activity only [26]. The light-saturated rates of PS II reduction in the presence of the uncoupler methylamine hydrochloride are rather variable in the 'intermediate' radiation range (Table IV); however, there is a 5–6-fold decrease in the uncoupled PS II electron-transport capacity of the thylakoids from plants grown in low radiation compared to those grown in sunlight (Table IV). Overall, the light-saturated rates of PS I are decreased in thylakoids from plants grown in low radiation, and those of PS II are even further diminished, compared to thylakoids from sun plants. Shade plants have even lower saturated rates of electron-transport capacity, particularly those of PS II [7,8,11].

Concentrations of cytochrome f and P-700 in light-saturated thylakoids

The amounts of the cytochrome *b-f* complex can be measured by determining the cytochrome *f* concentration [23], since this complex contains one molecule of cytochrome *f*, two molecules of cytochrome *b*-563 and one molecule of the Rieske FeS centre [27]. The amount of cytochrome *f* in the light-adapted chloroplasts (on a chlorophyll basis) decreased as the radiation available during growth was lowered, with a 2.2-fold difference existing between plants grown in sunlight and blue-enriched light (Table V). The amount of P-700 per unit chlorophyll also decreased with a 1.6-fold difference between thylakoids from peas grown in the highest and lowest radiation regimes (Table V).

TABLE IV

EFFECT OF VARYING THE LIGHT REGIMES DURING GROWTH OF PEAS ON THE LIGHT-SATURATED RATES OF PS I AND PS II BY ISOLATED CHLOROPLASTS

MA, methylamine (60 mM when present).

Light-adapted chloroplasts	Radiation ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	O_2 uptake ($\mu mol \cdot h^{-1} \cdot mg^{-1}$ Chl)	$K_3Fe(CN)_6$ reduction		DCIP reduction	
			−MA	+MA	−MA	+MA
			($\mu mol \cdot h^{-1} \cdot mg^{-1}$ Chl)		($\mu mol \cdot h^{-1} \cdot mg^{-1}$ Chl)	
FS	1080	462	21	336	162	366
SS	540	450	18	294	150	342
HF	140	324	23	222	108	258
FT	100	336	15	264	96	240
LF	110	354	16	384	114	300
T	24	336	12	150	96	330
R	15	204	14	132	72	132
B	6	198	12	60	60	96

TABLE V

AMOUNTS OF CYTOCHROME *f* AND P-700 IN TYHYLAKOIDS OF LIGHT-ADAPTED PEAS

Light regime during growth	Cytochrome *f* (μM)	Chl / Cytochrome *f*	P-700 (μM)	Chl / P-700	PS I Chl * / P-700	P-700 / Cytochrome *f*
FS	2.75	404	2.43	457	151	0.97
SS	2.29	484	2.30	482	159	1.00
HF	2.06	538	2.29	484	170	1.11
FT	1.93	575	2.55	444	144	1.32
LF	1.59	698	2.50	440	142	1.57
T	1.86	596	1.63	680	204	0.87
R	1.50	740	1.55	716	208	1.03
B	1.26	881	1.53	725	174	1.21

* PS I Chl = CP $1a^1$ + CP $1a^2$ + CP 1.

The P-700/cytochrome *f* molar ratios varied from 0.9 to 1.6 in a random fashion. The decrease in the relative amounts of cytochrome *f* and P700 on a chlorophyll basis in shade plants is greater than that observed here [7].

Discussion

As the photosynthetically active radiation available for the growth of peas is decreased there is a marked decrease in the light-saturated rates of both PS II and PS I activities (Table IV). There are four factors limiting the light-saturated rates of photosynthesis on a chlorophyll basis: (i) the relative concentrations of PS II and PS I; (ii) the antenna size of PS II and PS I (i.e., the number of light-harvesting molecules per reaction centre); (iii) the relative effectiveness of pigment absorption by PS II and PS I which depends partly on the chlorophyll and carotenoid composition; and (iv) the relative electron-transport capacity of PS II and PS I which corresponds to the proportions of electron carriers (plastoquinone, cytochromes, plastocyanin, and ferredoxin) compared to P-680 and P-700.

The relative concentration of PS II and PS I. Clearly, there are differences in the amounts of chlorophyll-proteins in pea plants grown in varying light regimes (Tables II and III). As the radiation is decreased, more chlorophyll is found associated with the main Chl *a*/*b*-proteins of PS II than with the reaction centre complexes of PS II and PS I; the (CP $1a^1$ + CP $1a^2$ + CP 1 + CP a)/$LHCP^{1-3}$ ratio is 1.13 in plants grown in sunlight compared to 0.52 in those grown in blue-enriched light.

The antenna sizes of PS II and PS I. Despite the slight decrease in the total amount of chlorophyll associated with PS I, from 34% in thylakoids from sun-grown plants to 27% in those grown in the blue-enriched light, the antenna sizes of PS I in the various thylakoids are roughly comparable (175 ± 25) (Table V). We cannot determine the antenna size of PS II as we cannot measure P-680 or Q [10,28], but it may be that the antenna sizes of PS II are not greatly altered in the various pea thylakoids, since no differences were found in the antenna size of PS II in plants grown under different light intensities or in some shade plants [10].

The relative effectiveness of pigment absorption by PS II and PS I. The relative effectiveness of pigment absorption by PS II is likely to be very different in the various pea thylakoids, since there is a dramatic difference in the amounts of chlorophyll associated with the main Chl *a*/*b*-proteins and the core reaction centre complex (which has little or no Chl *b*). As the radiation decreased from sunlight to the blue-enriched light, the $LHCP^{1-3}$/CP a ratio changed from 3.5 to 9, hence the amount of Chl *b* in PS II is greatly increased. This may partly account for the greater decrease in electron-transport capacity of PS II compared to that of PS I (a 5–6-fold decrease in PS II and a 2-fold decrease in PS I from sunlight to blue-enriched light). The P-680/P-700 ratios are likely to vary also in the various pea thylakoids, since Melis

and co-workers [10,28] have shown that this ratio is about 2 in sun plants and 4 in shade plants.

The relative electron-transport capacity of PS II and PS I. These factors which limit quantum capture and conversion in the various pea thylakoids are not the only cause of the decrease in photochemical activities of PS I and PS II. There was a 2-fold decrease in the amount of cytochrome *f* on a chlorophyll basis between thylakoids from sunlight- and blue-enriched light-grown plants (Table V). The increase in the Chl/cytochrome *f* ratio from 404 to 881 is more marked than that of the Chl/P-700 ratio (457 to 725). Hence, by restricting the relative amount of the intermediate cytochrome *b-f* complex, the electron-transport capacities of both PS II and PS I are lowered.

The decrease in photosynthetic capacity (measured as the photochemical activities of PS II and PS I) as the radiation available during the growth was lowered is attributable to changes in the levels of both the chlorophyll-proteins and electron-transport complexes. As the radiation decreases, there is a marked increase in the proportion of the light-harvesting complex, a marked decrease in the core reaction centre complex of PS II, a slight decrease in PS I complex and a more marked decrease in the cytochrome *b-f* complex. Thus, light is clearly regulating the relative proportions of the light-harvesting and electron-transport complexes. The fact that the proportions of each of these thylakoid complexes measured here (Tables II–V) generally vary in the same direction with decreasing radiation during growth suggest that the adaptation of light-harvesting and electron-transport components to the light regimes is closely co-ordinated.

The adaptation of pea plants to very low radiation did not produce chloroplasts which were equivalent to those isolated from shade plants. Sun plants do not adapt themselves to the low end of the range in the same way as in shade plants [7,8,11]. Shade plants have a 2-fold decrease in the chlorophyll associated with PS I complex (approx. 30 to 14%) and an even higher amount of light-harvesting Chl *a/b*-proteins than the pea thylakoids grown under low radiation. Also, the cytochrome *f* content on a chlorophyll basis of shade plants was 3-fold lower in contrast to 2.1-fold lower in thylakoids from blue-enriched light-grown plants. Hence, shade plants have even lower rates of PS I and PS II activities than those of the light-adapted pea thylakoids.

These results are important in view of the marked lateral heterogeneity in the distribution of the thylakoid complexes between the appressed membranes of grana partitions and the non-appressed thylakoids [14–16]. ATP synthase is located only in stroma-exposed thylakoids [29]. PS II reaction centre complex and the Chl *a/b*-proteins of the main light-harvesting complex are found mainly in appressed membranes, with 10–20% in stroma-exposed membranes, whereas most of PS I complex is found in stroma-exposed regions [14]. In contrast, some studies indicate that the cytochrome *b-f* complex is uniformly distributed between the appressed and non-appressed regions [23,30]. It was proposed that lateral asymmetry in the distribution of thylakoid complexes requires that chloroplasts with different extents of grana stacking should have different proportions of thylakoid complexes, and hence different maximal photosynthetic yields [2,6]. It is known that a surface-exposed fragment of the polypeptides of the main light-harvesting complex is required for thylakoid membrane stacking [31–33]. Therefore, the greater the amount of chlorophyll associated with the light-harvesting complex of PS II, the greater the proportion of PS II-LHCP complexes, and hence the greater the proportion of stacked relative to unstacked thylakoids. More PS II-LHCP complexes would mean less PS I complexes and ATP synthase per unit chlorophyll, and hence that both the rates of $NADP^+$ reduction and ATP synthesis would be lower; in turn, the rates of CO_2 fixation would be lower [2,6]. Our studies here of thylakoid membranes with very different amounts of thylakoid complexes and electron-transport capacities induced by growth in varying light climates confirm the proposal that photosynthetic rates are indeed influenced by the relative proportions of the intrinsic complexes present. Although detailed analysis of electron micrographs of the various pea thylakoids was not carried out, the height and width of the grana stacks increased as the radiation available during growth decreased.

Concluding remarks. The present study demonstrates that in a variety of light conditions during

growth, plants adapt themselves efficiently to their environment by regulating the synthesis and assembly of intrinsic thylakoid complexes. This adaptation presumably also involves regulation of the mobile electron carriers, plastoquinone, plastocyanin and ferredoxin, which link the transmembrane complexes. To make best use of the light energy available under conditions of low radiation, the plants have much more Chl *b* in PS II, since more of their total chlorophyll is present in the Chl *a*/*b*-proteins and relatively less in PS II reaction centre complex. In turn, this results in more stacked relative to unstacked thylakoids, and, as we have shown, slightly less PS I complex and less cytochrome *b*-*f* complex is produced to maintain lower rates of photosynthesis. In contrast, under high radiation the relative proportion of chlorophyll associated with the reaction centre complexes increases, and that of the light-harvesting complex decreases, since light for PS II is no longer a limiting factor. Such plants produce relatively more of the reaction centre complexes of PS II and PS I, cytochrome *b*-*f* complexes and thereby support greater photosynthetic rates.

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