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EFFECT OF LINCOMYCIN ON THE CHLOROPHYLL PROTEIN COMPLEX I CONTENT AND PHOTOSYSTEM I ACTIVITY OF GREENING LEAVES

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SUMMARY

1. Greening barley and pea leaves treated with lincomycin have a reduced chlorophyll content. Lincomycin does not alter the proportion of chlorophyll in chlorophyll-protein complex II (CPII) but greatly reduces that in chlorophyll-protein complex I (CPI).

2. Difference spectra show that chloroplasts from lincomycin-treated leaves are deficient in at least two long wavelength forms of chlorophyll *a*. These have maxima at 77 K of 683 and 690 nm.

3. The chemically determined *P*-700/chlorophyll ratio of chloroplasts is unaffected by lincomycin but the photochemical *P*-700/chlorophyll ratio is less than half of that of the control. It is less affected than the chlorophyll-protein complex I content.

4. Photosystem I activity expressed on a chlorophyll basis is unaffected by lincomycin but the light intensity for half saturation is increased 8-fold.

5. Chlorophyll-protein complex I apoprotein content is reduced by lincomycin. No evidence was found for an accumulation of its precursor(s). The relative abundance of major peptides of 18 000, 15 000 and 12 000 daltons in lincomycin-treated chloroplasts is attributed to a general inhibition of greening and associated membrane formation.

INTRODUCTION

There is evidence that up to 70 % of the chlorophyll in green plants is associated with two chlorophyll-protein complexes [1]. Approx. 10–20 % of the total chlorophyll is associated with CPI which contains only chlorophyll *a* together with *P*-700, the reaction centre of Photosystem I. It has been suggested that there is an obligate association between the occurrence of CPI and Photosystem I electron transport activity as nuclear [2] and plastome mutants [3] lacking both CPI and

Abbrevitions: DCIP, 2,6-dichlorophenolindophenol; CPI, chlorophyll-protein complex I; CPII, chlorophyll-protein complex II; SDS, sodium dodecyl sulphate.

Photosystem I activity are known. During greening of bush bean leaves the appearance of CPI correlated with Photosystem I activity as measured by photo-oxidation of *P*-700 [4]. CPI formation is reduced by inhibitors of protein synthesis on 70-S ribosomes [5, 6] and both the extent of cytochrome *f* photo-oxidation and the Photosystem I electron transport via methyl viologen to oxygen is reportedly inhibited by chloramphenicol [7]. It has, however, also been reported [8] that Photosystem I activity is more resistant to inhibition by chloramphenicol treatment during greening of leaves than is that of Photosystem II. In addition many Photosystem I activities are substantial at the earliest stages of greening before CPI can be detected [9, 10].

In this paper we have examined the CPI content and Photosystem I activity of greening barley and pea leaves treated with lincomycin, an inhibitor of protein synthesis on 70-S ribosomes. Our results show that Photosystem I activity is not affected under conditions which greatly inhibit addition of antennae chlorophylls and their associated peptides to CPI.

MATERIALS AND METHODS

Barley (*Hordeum vulgare* "Abyssinia") and pea (*Pisum sativum* "Greenfeast") was grown in vermiculite at 25 °C in darkness and watered with (1 g/l) Aquasol (Hortico LTD, Laverton North, Vic. 3028). Barley leaves were harvested after 7.5 days and pea shoots after 9 days. The excised leaves or shoots were pretreated for 24 h with either water or lincomycin before exposure for a further 24 h to light from a bank of Philips 40 W white fluorescent lamps. The light intensity at the leaf surface was 0.3 $mW \cdot cm^{-2}$. After illumination the top 1 cm of barley leaf was discarded and chloroplasts were prepared by blending the next 4 cm of leaf for twice 15 s at full line voltage in a Sorvall "omnimix" in a medium containing 0.3 M sucrose, 0.01 M KCl and 0.05 M potassium phosphate, pH 7.2. After filtering the homogenate through two layers of miracloth, it was centrifuged at 2500 × *g* for 15 min. The chloroplasts were washed once by resuspending the pellet in the above medium and recentrifuging.

Photosystem II was assayed by the spectrophotometric measurement of DCIP reduction. Red actinic light (40 $mW \cdot cm^{-2}$) was provided by a tungsten projection lamp together with Corning glass filters numbers 2-60 and 1-75. Photosystem I was assayed either by the disproportionation of 1,5-diphenyl semicarbazone (actinic light as above) or by the measurement of oxygen uptake in a Rank oxygen electrode in presence of the couple ascorbate-DCIP with methyl viologen as autoxidisable electron acceptor. Actinic light (240 $mW \cdot cm^{-2}$) was provided by a tungsten projection lamp and filtered through 10 cm of water. Reduced light intensities were obtained by the use of wire mesh screens. The reaction mixture contained in 2 ml, 50 mM phosphate buffer, pH 7.2, 0.01 M KCl, 10^{-5} M methyl viologen, $5 \cdot 10^{-5}$ M DCIP, 10^{-2} M sodium ascorbate and chloroplasts to give 3–20 μg chlorophyll.

Actinic light intensities were measured by means of a Hewlett-Packard 8330 Radiant Flux meter. *P*-700 was determined chemically from ascorbate-reduced, ferricyanide-oxidised difference spectra as described by Anderson et al. [11]. Photochemically active *P*-700 was determined in the presence of 1 % Triton X-100 and 10^{-3} M sodium ascorbate using an Aminco-Chance dual wavelength spectrophotometer from the light-driven decrease in absorbance at 700 nm using 730 nm as reference wavelength. Actinic light (50 $mW \cdot cm^{-2}$) was provided by a tungsten projection

lamp together with Corning glass filters 4-96 and 1-75. The photomultiplier was shielded from the actinic light by means of a Schott RG 695 glass filter. An extinction coefficient for *P*-700 of $80 \cdot 10^3$ l · mol^{-1} · cm^{-1} was assumed.

Coupling factor (CF1) was removed from the chloroplasts by means of NaBr [13]. The chloroplasts were lysed in 0.01 M Tricine buffer, pH 7.8, and then treated three times with 2 M NaBr. Supernatants were removed each time by a twice dilution with distilled water and centrifuging. The residual pellet which contained the lamellae was analysed electrophoretically as described below. Combined supernatants containing the coupling factor were made to 5 % with trichloroacetic acid, the resulting precipitate dissolved in 1 % SDS and its constituent peptides separated by polyacrylamide gel electrophoresis.

D144 particles were prepared by differential centrifugation of chloroplasts incubated in 0.5 %, (w/v) digitonin as described by Anderson and Boardman [12].

Chlorophyll-protein complexes were determined by washing the chloroplasts twice in 0.1 M Tris/acetate, pH 8.4, and solubilising the lamellae in 1 % SDS/Tris/acetate (SDS : chlorophyll > 40 : 1, w/w). After centrifuging at 20 000 × *g* for 20 min to remove insoluble matter, the green supernatant was made to 4 % with sucrose and 25 µl applied to polyacrylamide gels (8.0 % w/v polyacrylamide; 0.2 % w/v methylene bisacrylamide). After electrophoresis for 15 min (top and bottom buffers 0.1 M Tris/acetate, pH 8.4, with 0.15 % SDS) the gels were removed and scanned at 670 and 650 nm in a Gilford gel scanner. The proportion of the total chlorophyll in CPI and CPII was determined by averaging at the two wavelengths the integrated areas under the chlorophyll containing peaks [14]. For analysis of lamellar peptides the procedure above was followed but the electrophoresis was continued until the front had migrated approx. 10 cm. Gels were then fixed in methanol/acetic acid/water (5 : 1 : 5, v/v) and stained for 3 h with 0.025 % (w/v) Coomassie Blue in the above mixture [15]. Destaining was done with alternate washes of 7 % (w/v) acetic acid and methanol/acetic acid/water. Gels were calibrated for molecular weights by comparison of the samples with the mobility of the following proteins of known molecular weight, bovine serum albumin 68 000, ovalbumin 46 000, chymotrypsinogen 26 000, myoglobin 17 200, and cytochrome *c* 12 600.

Chlorophyll was determined in 80 % acetone extracts of both leaves and chloroplasts using the equations of Arnon [16].

RESULTS

The effect of different concentrations of lincomycin on the chlorophyll content and proportions of chlorophyll in CPI and CPII in greening barley leaves is shown in Table I. Chlorophyll biosynthesis is inhibited approx. 75 % at a concentration of 100 µg/ml lincomycin. At these inhibited levels of chlorophyll formation CPII has the same proportion of chlorophyll as in the control but the proportion in CPI is greatly reduced. This represents a 6-fold preferential inhibition of CPI formation at 100 µg/ml lincomycin but the proportion of CPI in all inhibitor treatments is significantly lower than that in the control. The effect of lincomycin at concentrations of 100–1000 µg/ml was investigated in a single experiment (results not shown). Although there was a small increase in the extent of CPI inhibition at these higher concentrations of lincomycin, the amounts of CPI and of chlorophyll were within the

TABLE I

THE EFFECT OF LINCOMYCIN ON TOTAL CHLOROPHYLL IN BARLEY LEAVES GREENED FOR 24 h AND ON THE PROPORTION OF CHLOROPHYLL ASSOCIATED WITH CHLOROPHYLL-PROTEIN COMPLEXES I AND II IN THE CHLOROPLASTS

The number of experimental observations contributing to each value is given in parentheses.

Lincomycin (μg/ml)	Chlorophyll (μg/g fresh weight)	Chlorophyll in CPI (%)	Chlorophyll in CPII (%)
0	566±87 (10)	13.0±2.2 (15)	21.0±4.9 (15)
10	330±41 (5)*	8.2±1.8 (5)*	24.5±6.5 (5)
25	250±16 (5)*	6.4±1.4 (5)*	22.9±2.5 (5)
50	193±17 (5)*	3.5±1.8 (5)*	23.7±4.8 (5)
100	201±28 (10)*	2.3±1.4 (10)*	18.2±4.7 (10)

* Significantly different from corresponding control, $P < 0.01$.

standard error for these values at 100 μg/ml lincomycin reported in Table I. There appears to be a residual level of chlorophyll and CPI which is not susceptible to inhibition by lincomycin under the conditions of our experiments.

In contrast to the preferential inhibition of complex I formation, the activity of Photosystem I whether measured as a methyl viologen-catalysed oxygen uptake, or the disproportionation of diphenyl carbazone, is not inhibited by lincomycin (Table II). Additionally there is no significant inhibition of the chemically determined *P*-700 content although a shift in its absorbance maximum from 699 to 696–697 nm was sometimes observed. Photochemically determined *P*-700 is reduced more than 2-fold in lincomycin-treated chloroplasts although the reduction is not as great as that in complex I. Omission of Triton X-100, or greatly increasing the actinic light intensity, did not affect the above results but their real significance is uncertain. As noted by other workers Photosystem II is strongly inhibited [17, 18].

The above results were obtained with chloroplasts isolated from a short segment beginning 1 cm below the apex of the barley leaf. As the developmental

TABLE II

THE EFFECT OF LINCOMYCIN ON *P*-700 CONTENT, PHOTOSYSTEM I AND PHOTOSYSTEM II ACTIVITY IN CHLOROPLASTS FROM BARLEY LEAVES GREENED FOR 24 h IN THE PRESENCE OF LINCOMYCIN

For the disproportionation of 1,5-diphenyl carbazone, control and 100 μg/ml lincomycin values were 812 and 874 μmol · mg chlorophyll^{-1} · h^{-1}, respectively. The number of experimental observations contributing to each value is given in parentheses.

Lincomycin (μg/ml)	Photosystem I (μmol O_2 · mg chlorophyll^{-1} · h^{-1})	Chlorophyll/*P*-700 (chemical)	Chlorophyll/*P*-700 (photochemical)	Photosystem II (μmol DCIP · mg chlorophyll^{-1} · h^{-1})
0	445± 75 (5)	316± 97 (12)	582± 39 (12)	74±21 (5)
50	425± 65 (5)	377±154 (5)	–	–
100	385±110 (5)	408± 73 (7)	1305±169 (12)*	7± 6 (5)*

* Significantly different from corresponding control, $P < 0.01$.

TABLE III

EFFECT OF LINCOMYCIN (100 μg/ml) ON CHLOROPHYLL CONTENT OF THE LEAVES AND CPI CONTENT AND ACTIVITY OF PHOTOSYSTEM I IN CHLOROPLASTS ISOLATED FROM DIFFERENT PARTS OF THE BARLEY LEAF GREENED FOR 24 h

Photosystem II was more than 90 % inhibited in all segments by the lincomycin treatment.

Distance from tip (cm)	Total chlorophyll (μg/g fresh weight)		CPI content (percent total chlorophyll)		Photosystem I activity (μmol O_2 · mg chlorophyll^{-1} · h^{-1})	
	Control	Lincomycin	Control	Lincomycin	Control	Lincomycin
0–2	317	122	9.0	trace	840	743
2–4	556	243	12.6	2.2	519	538
4–6	477	226	13.1	2.6	316	495
6–8	314	143	14.5	2.7	208	425
8–10	211	41	13.3	trace	140	221

stage of the chloroplasts changes from tip to base of the cereal leaf [19] we examined the effect of lincomycin on chlorophyll content, CPI content and Photosystem I activity in chloroplasts isolated from a series of 2-cm segments down the barley leaf. The results are presented in Table III. In all parts of the lamina, lincomycin has inhibited both the synthesis of chlorophyll and the formation of CPI but Photosystem I activity on a chlorophyll basis is unchanged, or increased, except in the upper 2 cm.

Treatment of greening pea shoots with lincomycin gave similar results to those reported above for barley (Table IV). Complex I is greatly reduced but Photosystem I activity persists even at the highest concentration of lincomycin where complex I is not detectable. Photosystem II is again much more sensitive than is Photosystem I to lincomycin treatment.

The large reduction in the proportion of chlorophyll in CPI unaccompanied by a change in the Photosystem I activity was unexpected. We therefore examined the dependence of Photosystem I activity on the actinic light in chloroplasts from control and lincomycin-treated leaves. As shown in Fig. 1 the lincomycin-treated chloroplasts require much higher light intensities for saturation and about five times higher light intensity to reach half maximal rate.

TABLE IV

THE EFFECT OF LINCOMYCIN ON CHLOROPHYLL CONTENT, CHLOROPHYLL PROTEIN COMPLEXES. AND PHOTOSYNTHETIC ACTIVITIES OF CHLOROPLASTS FROM PEA LEAVES GREENED FOR 24 h

Photosystem I and Photosystem II activities are expressed as μmol substrate transformed · mg chlorophyll^{-1} · h^{-1}.

Lincomycin (μg/ml)	0	10	25	50	100
Chlorophyll (μg/g fresh weight)	175	147	136	121	118
Chlorophyll *a*/*b*	2.87	2.46	2.32	2.46	2.38
Photosystem I activity	324	364	337	376	192
Photosystem II activity	65	43	15	7	0
Percent of chlorophyll in complex I	9.0	1.3	1.6	trace	0
Percent of chlorophyll in complex II	22.6	32.0	23.0	28.7	23.0

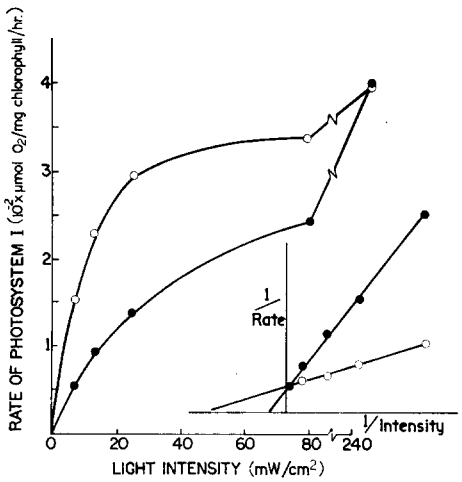

Fig. 1. Photosystem I activity at different actinic light intensities in chloroplasts from control (○ — ○) and lincomycin-treated (100 µg/ml) (● — ●) barley leaves. Inset: the same experimental data plotted as 1/rate against 1/light intensity.

The pooled results of four separate experiments gave values of 11 ± 5 and 90 ± 30 mW · cm^{-2} for the light intensities required for half saturation for control and lincomycin-treated chloroplasts, respectively. A dependence of Photosystem I activity on light intensity of type shown in Fig. 1 can occur when the products of the photochemical reaction are subsequently processed by a dark enzymic reaction which becomes rate limiting at high light intensities. In the plot of 1/rate against 1/light intensity, the greater slope for the lincomycin-treated chloroplasts is consistent with a decreased efficiency of light transfer to the reaction centres. The same maximum rates for control and lincomycin-treated chloroplasts implies a similar concentration (expressed on a chlorophyll basis) of a rate-limiting component.

The results shown in Fig. 1 are for oxygen uptake in the Mehler reaction but similar results were also obtained from the spectroscopically determined disproportionation of 1,5-diphenyl carbazone.

The Photosystem I activities reported in Tables II, III and IV, while substantial, are lower than those reported from greening barley when tetramethylenephenylene-

TABLE V

PHOTOSYSTEM I ACTIVITY OF DIGITONIN SUBCHLOROPLAST PARTICLES (D144), AND CHLOROPLASTS TREATED WITH PLASTOCYANIN (5 µM) OR TRITON X-100 (0.5 %), ISOLATED FROM BARLEY LEAVES GREENED FOR 24 h IN THE PRESENCE OF LINCOMYCIN

Photosystem I activities are expressed as µmol O_2 uptake · mg chlorophyll^{-1} · h^{-1}.

	No addition	+Plastocyanin	No addition	0.5 % Triton X-100	Original chloroplasts	D144 particles
Control	425	674	407	6947	469	875
Lincomycin	470	693	428	7411	413	1400

diamine rather than DCIP was the electron carrier [9]. It is also well known that treatment of chloroplasts with detergents and/or plastocyanin may lead to a large stimulation of the rate of Photosystem I. To exclude the possibility that the lack of Photosystem I inhibition is due to restricted entry of electrons to the reaction centre, we examined a number of situations in which the rate of Photosystem I was stimulated. The results are presented in Table V. Despite the variation in absolute rates brought about by the additions shown in Table V, there is no evidence that the lincomycin-treated chloroplasts are different from those of the control. The addition of Triton X-100 to a final concentration of 0.5 % gave a large stimulation of Photosystem I immediately and this was not dependent on added plastocyanin. The D144 particles were analysed for CPI content and a small enrichment of CPI in control particles was noted compared to that of the starting chloroplasts. The lincomycin D144 particles contained an identical low CPI content to that of the starting material. Chemically determined chlorophyll/*P*-700 ratios were 192 for the control but 777 for the lincomycin D144 particles, suggesting little correlation between *P*-700 content and Photosystem I activity.

As the chlorophyll-protein complexes might be artifacts of the SDS solubilisation of the chloroplast lamellae, experiments were performed to see if any long wavelength forms of chlorophyll *a* were missing from chloroplasts isolated from lincomycin-treated leaves. Evidence for the probable in vivo existence of CPI was obtained from the difference spectrum of control and lincomycin-treated chloroplasts which had not been treated with SDS. Two cuvettes containing an equal concentration of chlorophyll were scanned at both room and liquid nitrogen temperatures (Fig. 2). The lincomycin chloroplasts are relatively deficient in long wavelength forms of chlorophyll *a* having an absorbance maximum at 685 nm at room temperature, additional peaks at 633 and 443 nm were also seen. At liquid nitrogen temperatures, a peak at 683 nm together with a shoulder at 690 nm was obtained. Both room and liquid nitrogen temperature spectra show lincomycin-treated chloroplasts to be relatively enriched in chlorophyll species absorbing in the 650–660 nm region. Although part of this is due to chlorophyll *b* much must be due to a chlorophyll *a* species absorbing at 660 nm and presumably not part of complex II. In a barley mutant lacking chlorophyll *b* and complex II, the lincomycin-treated chloroplasts again show relatively increased absorbance at 660 nm (Hiller, R. G., unpublished).

When difference spectra were run at room temperature on control—lincomycin-treated chloroplasts solubilized in 1 % Triton X-100 the lincomycin chloroplasts were deficient in a chlorophyll *a* species having an absorbance maximum at 677 nm, the maximum described for CPI purified from Triton-solubilised chloroplasts [21].

In a *Scenedesmus* mutant which lacks CPI the chlorophyll *a*/chlorophyll *b* ratio of the whole cell does not differ from that of the wild type [20]. In barley (Table VI) but not in pea (Table IV) we have also noticed that the chlorophyll *a*/chlorophyll *b* ratio of the entire leaves is not affected by lincomycin, although it is significantly reduced in the isolated chloroplasts (Table VI). The reduction in chlorophyll *a*/chlorophyll *b* ratio in the chloroplasts is almost identical with the reduction expected from the inhibition of CPI formation. The discrepancy between the whole leaf ratios and those of the chloroplasts might be explained in two ways. Firstly a fractionation occurs during the isolation of the lincomycin-treated chloroplasts such that particles

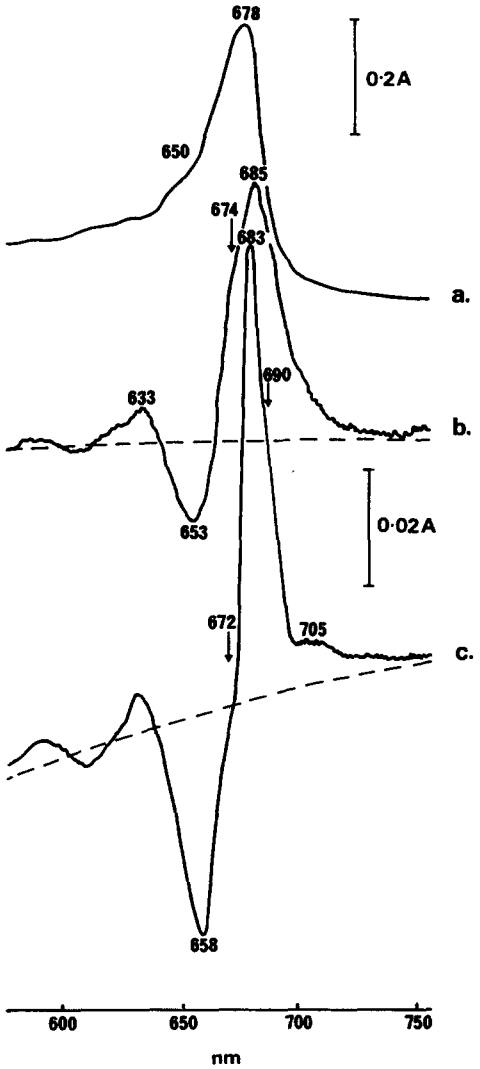

Fig. 2. Difference spectra of control−lincomycin-treated (100 μg/ml) chloroplasts. Spectrum b is at 297 K and 10 μg chlorophyll/ml chlorophyll. Spectrum c is at 77 K and 15 μg chlorophyll/ml. Spectrum a is of control chloroplasts alone at 297 K. The curved baselines are an estimate based on the difference in light scattering between control and lincomycin-treated chloroplasts. In the blue region of the difference spectra a clear peak was seen at 445 nm at both 297 and 77 K and troughs at 428 and 406 nm.

enriched in chlorophyll *a* (and perhaps in CPI) remain in the supernatant of the 2500 × *g* centrifugation or secondly that chloroplasts enriched in chlorophyll *a* remain attached to cell and tissue debris and that there are more of these in lincomycin-treated leaves. Examination of the pellets centrifuged for 15 min at 10 000 × *g* and for 20 min at 40 000 × *g* showed that their CPI content was always very low in chloroplasts from lincomycin-treated leaves so the first possibility may be excluded.

TABLE VI

EFFECT OF 100 μg/ml LINCOMYCIN ON CHLOROPHYLL *a*/CHLOROPHYLL *b* RATIOS IN LEAVES AND ISOLATED BARLEY CHLOROPLASTS

The number of experimental observations contributing to each value is given in parentheses.

	Leaves Chlorophyll *a*/Chlorophyll *b*	Chloroplasts Chlorophyll *a*/Chlorophyll *b*
Control	3.23±0.32 (10)	3.06±0.24 (10)
Lincomycin	3.25±0.44 (10)	2.68±0.19 (10)*

* Significantly different from corresponding control, $P < 0.01$.

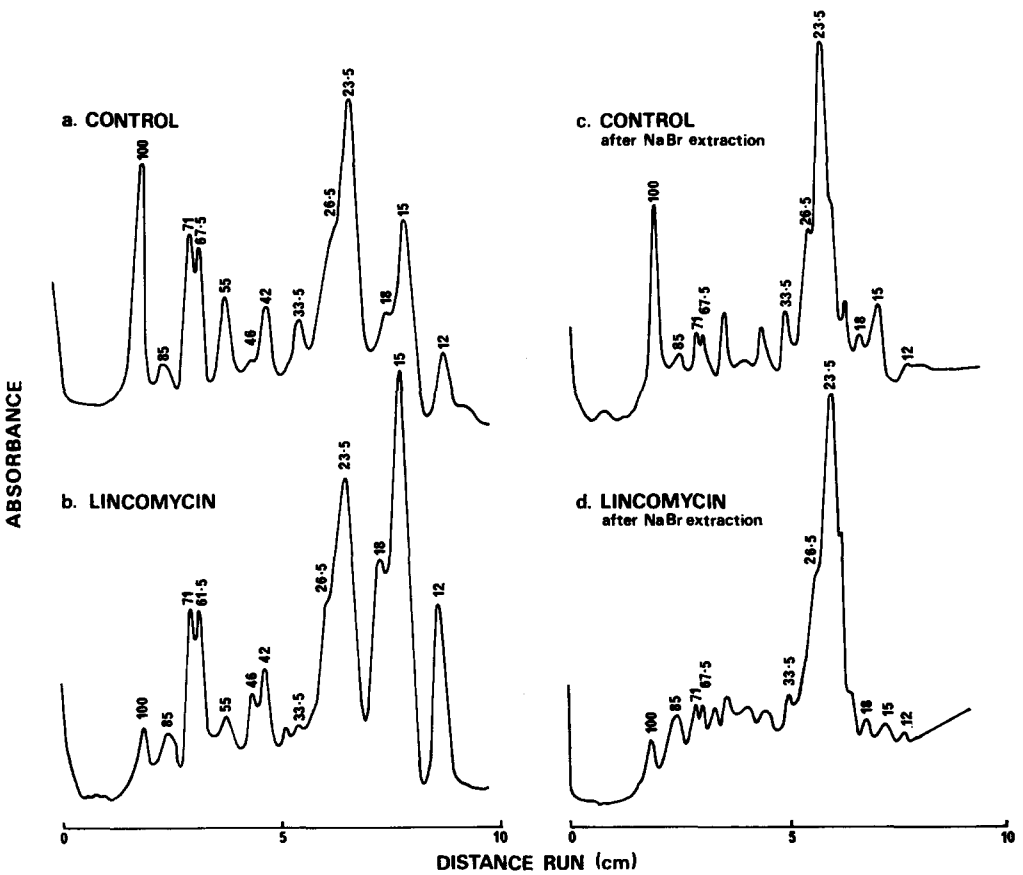

Fig. 3. SDS chloroplast lamellar peptides separated by polyacrylamide gel electrophoresis and stained with Coomassie Blue. (a) Control chloroplasts. (b) Lincomycin-treated (100 μg/ml) chloroplasts. (c) Control chloroplasts after NaBr extraction. (d) Lincomycin-treated chloroplasts after NaBr extraction. The peaks are identified by their average molecular weight in daltons. Chlorophyll remains attached to CPI and CPII under the conditions of this electrophoretic separation. CPI is associated with the peptide at 100 000 daltons and CPII with the peptides at 26 500 and 23 500 daltons. The two largest subunits of coupling factor are associated with the bands at 71 000 and 67 500 daltons.

Previous workers [5, 6] have shown that the protein (100 000 dalton band in Figs. 3a and 3b) coincident with CPI is reduced in chloroplasts obtained from leaves treated with lincomycin or chloramphenicol. This was confirmed in the present work (Figs. 3a and 3b), as was the marked reduction of the peptide at 33 500 [5, 6], relative to complex II apoprotein and the two largest subunits of coupling factor. Lincomycin treatment also caused large relative increases in peptides of 46 000, 18 000, 15 000, and 12 000 daltons. Since there are prominent peptides in these positions in extracts of etioplasts and chloroplasts during the earliest stages of greening, we tentatively attribute their relative abundance to a general inhibition of the greening processes by lincomycin. (The molecular weights of peptides on acrylamide gels are used as a

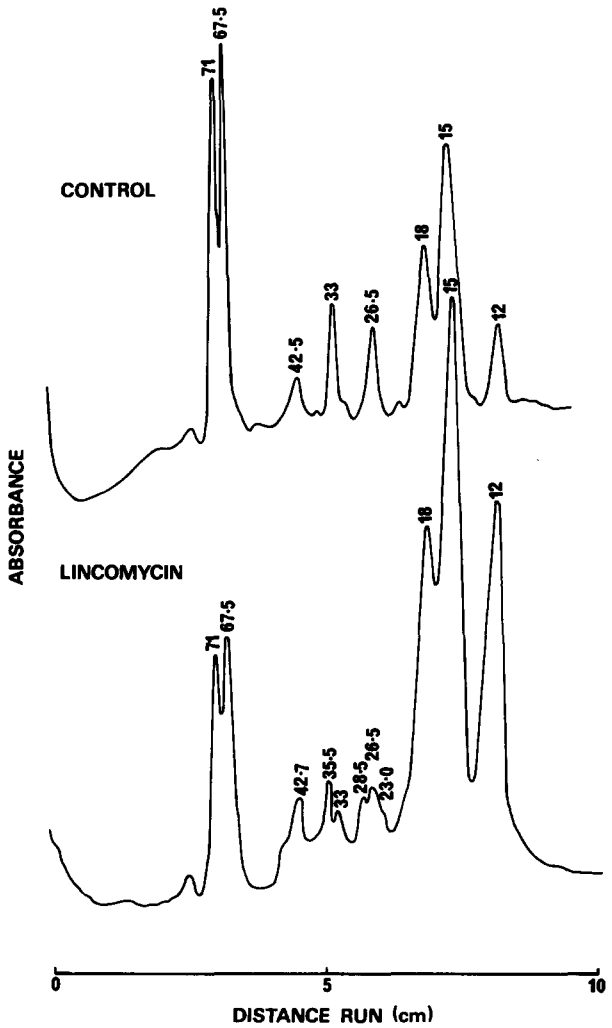

Fig. 4. Peptides extracted by 2 M NaBr from chloroplasts and separated by SDS-polyacrylamide gel electrophoresis. (a) Control chloroplasts. (b) Lincomycin-treated chloroplasts. The two largest subunits of coupling factor are associated with the bands at 71 000 and 67 500 daltons.

means of identification only. As lipids were not removed prior to electrophoresis anomalous migration of peptides is very likely).

When chlorophyll is removed from CPI, its constituent peptide(s) may migrate more rapidly and then lie in the same region of the gels as the two large subunits of coupling factor [2, 32]. There is thus the possibility that the apoprotein(s) of CPI is still made in the presence of lincomycin but its assembly inhibited [6]. We removed coupling factor from chloroplasts by treatment with NaBr [13] as the usual EDTA washing removed only a small part of the coupling factor.

The lamellar peptides remaining after NaBr treatment were separated by polyacrylamide gel electrophoresis and are shown in Figs. 3c and 3d. The peptides of 100 000 (belonging to CPI) and 26 500 and 23 500 daltons (belonging to CPII) are the most prominent feature. No substantial peptide remains in the region of the two largest subunits of coupling factor in either the control or lincomycin-treated material. The major peptides of 18 000, 15 000 and 12 000 daltons have also been removed by the NaBr treatment.

Proteins (including coupling factor) removed from the chloroplasts by washing with NaBr were also examined (Fig. 4). There is a considerable number of peptides besides those attributable to coupling factor. In the lincomycin treatment, peptides of 18 000, 15 000 and 12 000 daltons are again considerably more abundant compared to those of the two largest subunits of coupling factor. These same peptides are present in NaBr extracts of the etioplast and their relative abundance is probably of no significance, although it is possible one or more contributes to the apoproteins of the complexes. The removal of these peptides of 18 000, 15 000 and 12 000 daltons by NaBr without use of detergent may be a first step in elucidating their role.

DISCUSSION

Inhibitors of protein synthesis on 70-S ribosomes reduce chlorophyll synthesis in greening leaves although inhibition is rarely if ever complete. The inhibition is usually of the order of 60–80 % at the highest concentrations of inhibitor. Part of this (maximum 20 %) can be attributed to the preferential effect on chlorophyll *a* in CPI. The remainder is probably accounted for by a general inhibition of chloroplast membrane synthesis [23, 24] and in the present work would include CPII whose apoprotein(s) is coded for by nuclear DNA [25], since CPII contains a constant proportion of a reduced chlorophyll content. Although the Photosystem I electron transport activities expressed on a chlorophyll basis are not reduced by lincomycin it may be concluded that they are inhibited if expressed on a per leaf basis as is the formation of many electron transport components, including cytochromes [25].

While this paper was in preparation Sarvari et al. [26] reported on the effects of lincomycin on chlorophyll accumulation, and fluorescence and absorbance spectra of greening *Vicia faba* leaves. They also found that long wavelength forms of chlorophyll *a* are preferentially inhibited although it is not possible to assign any maxima to these from the data presented. Curve fitting analysis of chloroplast spectra has revealed the existence of up to six forms of chlorophyll *a* including species having absorbance maxima at 684 and 691 nm [27]. Partially purified CPI is preferentially enriched in chlorophyll *a* forms having maxima at 684 and 691 nm [28]. Our difference spectra indicate that it is these components which are inhibited by lincomycin and provide some evidence for the in vivo existence of CPI.

The changes in chlorophyll *a*/chlorophyll *b* ratio due to lincomycin reported both in this paper and by Sarvari et al. [26] can be accounted for by the preferential inhibition of CPI. In greening peas the relative suppression of chlorophyll *a* by chloramphenicol [30] is much greater than the total chlorophyll in CPI. This might indicate that chlorophyll *a* associated with some component besides CPI or CPII is also inhibited by lincomycin. However, the barley mutant which lacks chlorophyll *b* and CPII is no more sensitive to lincomycin than is the wild type, but does show the same reduction in long wavelength forms of chlorophyll *a* (Hiller, R. G., unpublished).

This paper provides clear evidence that CPI can be greatly reduced by lincomycin without interfering with Photosystem I activities as measured by the Mehler reaction. The higher light intensities required for saturating this reaction in lincomycin-treated chloroplasts would be consistent with a reduction or alteration in the antennae chlorophylls around Photosystem I, especially if long wavelength forms of chlorophyll *a* such as *P*-685 and *P*-690 are not distributed randomly through the antenna but are arranged in blocks close to the reaction centre [35]. As CPI contains *P*-700 the reaction centre for Photosystem I, the determination of reaction centre content of chloroplasts from lincomycin-treated leaves is of importance. Our data for chemically determined *P*-700 indicate that on a chlorophyll basis, the reaction centre content is not affected by the inhibitor. On the other hand the photochemically determined *P*-700 values determined in 1 % Triton solution suggest that lincomycin-treated chloroplasts may be relatively deficient in functional reaction centres, even though this deficiency is much less than that of CPI. It is possible that the chemically determined *P*-700 values are erroneous due to increased oxidation of bulk chlorophyll in lincomycin-treated chloroplasts and the shift to lower wavelengths in the maximum in the difference spectrum may be an indication of this. Even if this explanation is correct, the paradox of identical Photosystem I rates accompanying very different reaction centre contents, remains.

This paradox may be due to (i) instability of *P*-700 in Triton; this seems unlikely in view of the exceptionally high Photosystem I activity revealed by Triton X-100 addition. (ii) The turnover rate of *P*-700 is potentially high but the maximum rate of electron transfer to oxygen is restricted by subsequent dark reactions. In this case, when results are expressed on a unit chlorophyll basis, the reaction centre concentration might be reduced without affecting the maximum rate of oxygen uptake. (iii) The chemical and photochemically determined *P*-700 are artifacts bearing no relation to the content of the real reaction centre of Photosystem I. (iv) During development *P*-700 is not the only reaction centre.

At present we would favour the second explanation as, firstly, Triton X-100 addition produces such a large stimulation of Photosystem I activity showing that this is not usually limited by *P*-700 content. Secondly, the second-order rate constant for the reduction of *P*-700 by plastocyanin is $1 \cdot 10^8$ $l \cdot mol^{-1} \cdot s^{-1}$ [30]. For a Photosystem I activity of 0.3 μmol electrons transferred to $O_2 \cdot mg$ $chlorophyll^{-1} \cdot s^{-1}$, ($\cong$ 1000 μmol $e^- \cdot mg$ $chlorophyll^{-1} \cdot h^{-1}$) the concentration of *P*-700 need be only $1/10^3$ that of plastocyanin instead of the 1/1 ratio usually observed. During greening photochemically active *P*-700 has been found to be absent or lag behind the development of Photosystem I activity [9, 32, 33]. If two types of reaction centre exist during the earliest stages of greening then by delaying the general devel-

opment of the chloroplast, lincomycin might favour the persistance of the non-*P*-700 reaction centre. In this case chloroplasts from control and lincomycin-treated leaves might fortuitously show similar maximum Photosystem I activity.

There is now good evidence that CPII has only a light harvesting role in photosynthesis. It has been suggested [34] that much of CPI must have only a similar role and the results presented here support this view. It would clearly be of interest to isolate the CPI from lincomycin-treated leaves and examine it for *P*-700 content and its peptide composition. We have attempted this isolation by many methods but without success, perhaps because these methods depend on the properties of the apoproteins of the light harvesting chlorophylls in Photosystem I. It is these apoproteins whose formation or assembly is so sensitive to inhibitors of protein synthesis on 70-S ribosomes.

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