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Chloramphenicol-induced stabilization of light-harvesting complexes in thylakoids during development

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Chloramphenicol treatment allows light-harvesting apoprotein accumulation in thylakoids of intermittent light plants and prevents its digestion in plants transferred to darkness after brief pre-exposure to continuous light. This suggests that under conditions where reaction center-core protein synthesis is inhibited, the light-harvesting apoproteins can be stabilized. The results support the competition hypothesis proposed to occur between reaction center polypeptides and light-harvesting apoproteins for chlorophyll, whenever the rate of chlorophyll formation relative to that of the polypeptide components is limited.

Light-harvesting complex; Synthesis control; Protein stabilization; Chloramphenicol effect

1. INTRODUCTION

The reaction center (RC) and light-harvesting (LHC) apoproteins of photosynthetic units (PS) are stabilized in thylakoids through chlorophyll binding and Chl-protein complex formation. Upon exposure of etiolated plants to continuous light, complete PS units are formed which contain, in addition to the RC core complexes, LHCs. The rate of Chl accumulation in this case is high and parallels that of the other thylakoid components. In plants exposed to intermittent light, however, Chl is bound selectively on RC proteins and only RC core complexes are stabilized in thylakoids [1,2]. Since no LHCs can be detected in the membrane, in spite of the fact that the mRNA for the LHC-II apoprotein is present and can be translated [3], it has been proposed that LHC stabilization is controlled at the post-translational level [4,5]: whenever the rate of Chl accumulation is reduced, while polypeptide synthesis remains unaffected, there is a competition between polypeptides for the small amount of Chl available. The RC polypeptides, having higher 'affinity' for Chl than those of LHC, bind Chl and become stabilized, while the LHC apoproteins, unstable in the absence of Chl binding, are digested.

Such competition between thylakoid polypeptides was also evident in plants transferred to the dark after brief pre-exposure to CL. Under these conditions, the LHC apoprotein formed in CL was drastically reduced in the dark [6,7], while new small-sized PS units were formed [7–9]. Since this was found to occur only in plants which are still in the process of development and have the need/capacity to form new PS units to attain the

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Abbreviations: PS, photosystem; LHC, light-harvesting complex; Chl, chlorophyll; CPI, P700-Chl *a* protein complex of PS I; CPIa, PS I Chl-protein complex containing CPI and LHC-I; CAP, chloramphenicol; CL, continuous light; LDC, light-dark cycles; ImL, intermittent light; D, dark; cyt., cytochrome

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number of mature chloroplasts, the effect was attributed to a reorganization process [7–9] rather than to increased LHC apoprotein turnover in the dark [6]: in the absence of Chl synthesis in darkness the RC apoproteins formed remove the Chl already bound in LHCs to form new PS units, while the LHC polypeptides are digested.

To check this hypothesis, we have attacked the problem in the past and found that, by increasing the relative rate of Chl accumulation, i.e. by decreasing the duration of the dark interval in ImL (vis 28 min vs 98 min), LHC apoproteins can be stabilized [10,11]; this suggests that some Chl becomes available for binding on LHC apoproteins as well, resulting in their stabilization.

Here, we attempted to reduce the formation of the RC apoproteins relative to that of Chl or LHC apoproteins. To prevent chloroplast coded RC polypeptide synthesis, we treated plants with chloramphenicol. Our results show that CAP treatment allows the stabilization of LHC apoproteins in ImL thylakoids and prevents their dissociation in plants transferred to darkness.

2. MATERIALS AND METHODS

6-day etiolated *Phaseolus vulgaris* leaves were used, grown and handled as in [1]. The leaves with one cotyledon removed were either exposed to ImL [2 min white light alternating with 98 min dark in cycles], or to CL for 18 h and then transferred to the dark. In the first case, leaves were immersed for 3 min in CAP solution (200 μg/ml), 7 h before and immediately prior to ImL exposure, according to [12]. In the latter, leaves were immersed in CAP once just before transfer to the dark and once more after 7 h in darkness; the leaves were dried on filter paper and kept in darkness until the second CAP treatment. Leaves handled in the same manner but immersed in H_2O were used as controls. Chl was extracted from leaves as in [1] and determined according to [13]. PS I and PS II activities were monitored as in [11]. Low-temperature fluorescence spectra were recorded in a fluorometer set-up according to [11]. SDS-PAGE of thylakoid polypeptides was performed as described [11], after thorough washing of thylakoids with 0.05 M Tricine, pH 7.2. All determinations in 18 h CL leaves were made after immersing the leaves in H_2O and drying as above.

Table 1

Chl content, Chl *a*/Chl *b*, PS I, PS II and PS II/PS I ratios in 6-day etiolated bean leaves exposed either to intermittent light in the presence or absence of CAP, or transferred to darkness in the presence or absence of CAP after brief pre-exposure to CL

(1) Sample	(2) Chl *a* (μg/g fresh wt)	(3) Chl *b* (μg/g fresh wt)	(4) Chl *a*/Chl *b*	(5) PS I/mg Chl per h	(6) PS I/g fresh wt per h	(7) PS II/mg Chl per h	(8) PS II/g fresh wt per h	(9) PS II/PS I
14 LDC								
+ CAP	103.0	4.9	21.1					
− CAP	164.7	0	high					
35 LDC								
+ CAP	226.7	33.4	6.8	263	68.4	280	73.0	1.24
− CAP	401.8	0	high	454	190.0	1150	481.0	2.74
55 LDC								
+ CAP	269.9	55.4	4.9					
− CAP	487.1	27.8	17.5					
86 LDC								
+ CAP	479.4	65.4	7.3					
− CAP	502.1	32.5	15.5					
	(μg/leaf)				(PS I/leaf per h)		(PS II/leaf per h)	
18 h CL	22.90	6.41	3.57	238	6.97	333	9.76	1.60
+ CAP								
+ 24 h D	21.75	5.80	3.75					
+ 48 h D	22.60	4.59	4.92	285	7.75	370	10.06	1.43
− CAP								
+ 24 h D	24.91	5.30	4.70					
+ 48 h D	21.33	2.70	7.90	434	10.43	1111	26.70	2.88

The PS I and PS II represent V_{max} activities calculated from the $1/V$ vs $1/I$ plots. The PS II/PS I ratio was estimated on the assumption that in mature chloroplasts it is equal to unity

3. RESULTS

Table 1 shows that CAP treatment inhibits Chl synthesis in leaves exposed to (2 min light + 98 min dark) LDC. The inhibition is overcome, however, after exposure to ImL for 6 days (86 LDC), suggesting that the action of CAP does not last for long periods. As expected, Chl *b* is absent from untreated leaves, appearing only after 55 LDC (table 1) [1]. In contrast, CAP-treated leaves accumulate appreciable amounts of Chl *b*, as shown in table 1. The Chl *a*/Chl *b* ratio, therefore, is lower in CAP-treated than in control leaves (table 1). The presence of Chl *b* suggests the presence of LHC apoprotein in the thylakoids of CAP-treated leaves. Indeed, the SDS-PAGE resolution pattern shows the presence of the 25 and 21 kDa polypeptides, the apoproteins of LHC-II and LHC-I, respectively, in CAP-treated leaves, in contrast to control samples (fig.1a). The 21 kDa polypeptide is present, however, in smaller amounts. On the contrary, the chloroplast coded polypeptides, as expected, are missing from CAP-treated plants (68 and 48 kDa of PS I and PS II RC, 33 kDa of cyt. *f*, 32 kDa of the Q_B-binding protein and 12 kDa of cyt. *b*-559). However, the 43 kDa PS II RC peripheral polypeptide is detected, suggesting that the CAP treatment applied may not be fully inhibitory. Moreover, an

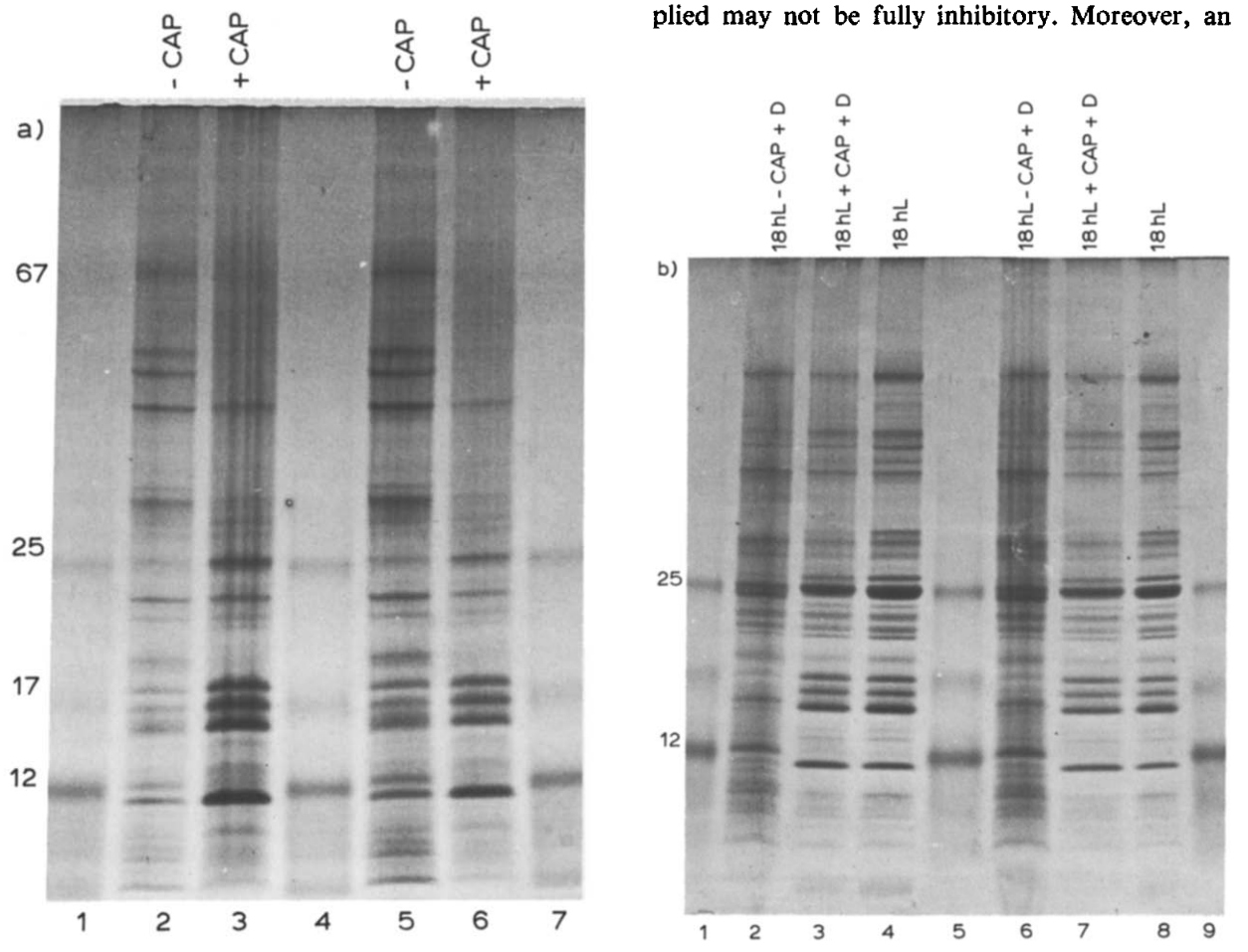

Fig.1. SDS-PAGE of thylakoid polypeptides obtained from leaves exposed (a) to intermittent light (35 LDC) in the presence or absence of CAP (4 μg Chl loaded on slots 2,3; 140 μg protein on slots 5,6); (b) to CL for 18 h and then transferred to darkness for 48 h in the presence or absence of CAP (8 μg Chl loaded on slots 6–8; 140 μg protein on slots 2–4). Slots 1,4,7 in (a) and 1,5,9 in (b) contain molecular mass marker proteins (molecular mass in kDa indicated on the left).

18 kDa polypeptide is absent from CAP-treated ImL plants.

The presence of LHCs in CAP-treated ImL plants is also reflected by the lower light intensity requirement for saturation of PS I and PS II and the lower PS I/Chl and PS II/Chl values (fig.2, table 1). These facts suggest that the PS units formed in the presence of CAP are larger than those in control leaves, even though Chl accumulation in CAP-treated leaves is lower than that in untreated leaves.

The stabilization of LHCs in the presence of CAP is also obvious from experiments with plants transferred to darkness after brief pre-exposure to CL. As already pointed out, the pre-existing LHCs in CL are digested in darkness as long as new PS units are formed. CAP treatment, therefore, was expected to allow LHC stabilization by preventing new RC protein synthesis in the dark. Indeed, SDS-PAGE of thylakoid polypeptides obtained from plants transferred to the dark in the presence of CAP shows that the amount of the 25 and 21 kDa polypeptides is not significantly reduced (fig.1b). In contrast, the LHC apoprotein is considerably reduced in thylakoids of untreated plants transferred to darkness in the absence of CAP. The dissociation of the LHCs in darkness, occurring in control leaves, is reflected by the increase in Chl *a*/Chl *b* ratio (table 1). The increase in this ratio is lower in CAP-treated plants, suggesting that Chl *b* degradation in this case is lower than for untreated controls. The Chl *a* level in both cases remains constant during the dark incubation. Stabilization of the LHCs in darkness in CAP-treated leaves is also indicated by the lower light intensity requirement for saturation of PS I and PS II in plastids of CAP-treated plants, and the lower PS I/Chl and PS II/Chl ratios found (fig.3, table 1). These results, therefore, show that transfer to darkness, in the presence of CAP, of plants pre-exposed to CL does not affect the size of the PS units already formed in CL.

The organization of LHC-I into the PS I unit of CAP-treated ImL plants is also suggested by the 77 K fluorescence emission spectra of leaves (fig.4a). The 730 nm emission peak of CAP-treated leaves suggests the organization of LHC-I with CPI into CPIa [14]; on the contrary, the 714 nm emission of untreated leaves indicates the presence of the CPI core [14,15]. Similarly, the PS I unit formed after transfer to darkness of leaves pre-exposed to CL is in a more organized state in CAP-treated leaves (fig.4b). The emission peak of 18 h CL leaves is shifted to 720 nm after 48 h dark incubation and the $F_{730-720}/F_{690}$ ratio is reduced, suggesting the degradation of LHC-I

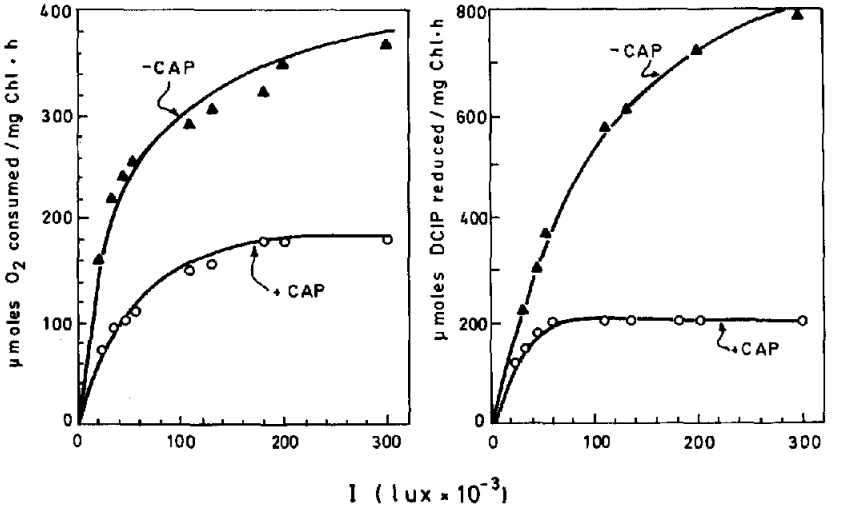

Fig.2. PS I (left) and PS II (right) activities of leaves exposed to 35 (2 min light + 98 min dark) LDC in the presence or absence of CAP.

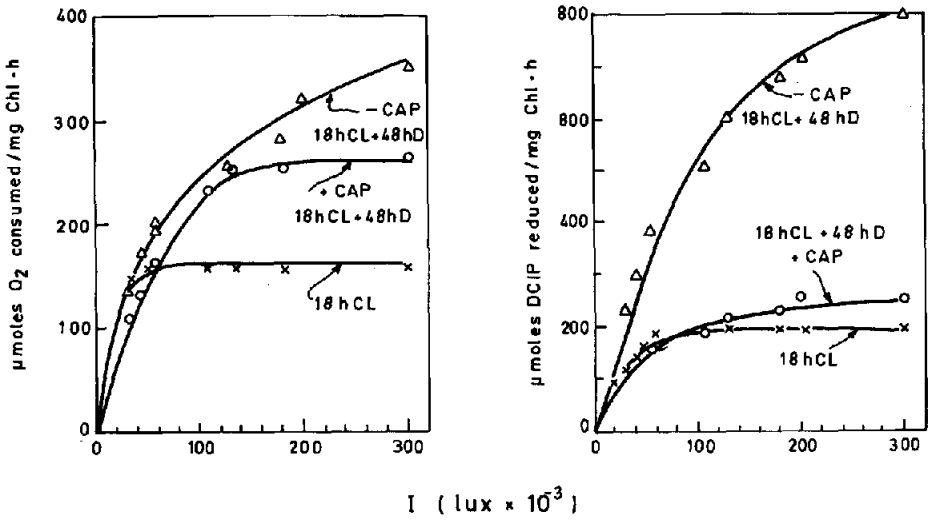

Fig.3. PS I (left) and PS II (right) activities of leaves exposed to 18 h CL and then transferred to darkness for 48 h in the presence or absence of CAP.

[9,14,15]; in leaves transferred to the dark in the presence of CAP, however, the long-wavelength peak is shifted to 725 nm and the F_{725}/F_{690} ratio remains considerably higher.

The number of PS units formed after CAP treatment in ImL is lower than in controls (see columns 6,8; table 1). This was expected, since CAP treatment prevents RC protein synthesis. However, since CAP treatment also inhibits Chl synthesis in this case, it is not possible to state whether the lower PS unit number is due to the lower amount of Chl, or to the reduced RC polypeptide synthesis. However, in the case of plants transferred to darkness in the presence of CAP, the Chl *a* content remains constant in the dark irrespective of CAP treatment. The reduced number of PS units

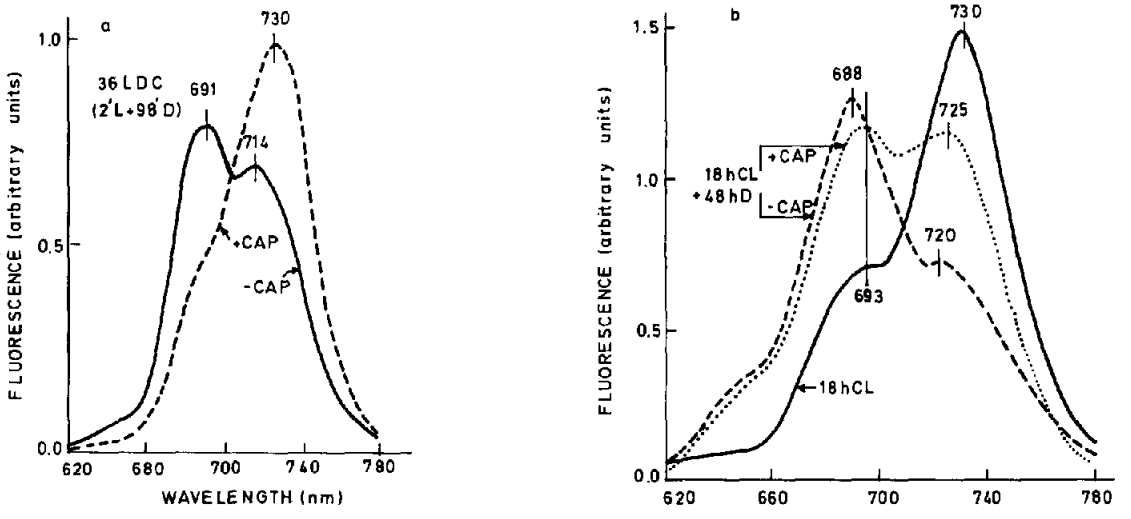

Fig.4. Low-temperature (77 K) fluorescence spectra of leaves (a) exposed to intermittent light in the presence or absence of CAP and (b) exposed to 18 h CL and then transferred to the dark in the presence or absence of CAP.

determined in this case, therefore (table 1) does reflect the reduced synthesis of the RC polypeptides. It is interesting to note that the PS II/PS I ratio in CAP-treated ImL leaves is lower than for untreated controls. It has been reported [4,11] that during development the PS II/PS I ratio is correlated with the PS II unit size, viz. the larger the size the lower the ratio. Since in CAP-treated plants the PS II unit size is larger, a lower PS II/PS I ratio was expected than in controls, and this is what we found. However, the possibility that the PS II RC polypeptides are more drastically affected by CAP treatment cannot be excluded. The PS II/PS I ratio in plants transferred to the dark after pre-exposure to CL is also lower in CAP-treated leaves than in controls; this again correlates with the finding that the PS II unit size is larger in CAP-treated plants.

4. DISCUSSION

Our results show that the stabilization of LHC polypeptides in thylakoids, ImL plants or plants transferred to darkness after brief pre-exposure to CL can be achieved when RC polypeptide synthesis is inhibited. CAP treatment of ImL plants permits stabilization of the LHC apoproteins and formation of large-sized PS units; obviously, the PS units formed in this case are few in number, since CAP inhibits RC protein synthesis. Similarly, CAP treatment of plants upon transfer to darkness after brief pre-exposure to CL prevents degradation of LHC apoproteins and the PS unit size remains unaffected in the dark. Long pre-exposure to CL has also been shown to prevent degradation of LHC polypeptides after transfer to darkness [7,9]; in this case the number of PS units formed in CL has already attained that of the mature chloroplast and no RC synthesis occurs in the dark. In all these cases, therefore, in the absence of RC protein synthesis the LHC apoproteins are stabilized. This supports the competition hypothesis proposed previously [4,5], according to which, when the rate of Chl synthesis relative to that of the other thylakoid components is low, there is a competition between RC and LHC polypeptides (LHCP) for the small amount of Chl available. The RC polypeptides having higher affinity for Chl than those of LHC bind on Chl (in ImL) or remove it from pre-existing LHC (in plants transferred to the dark after brief pre-exposure to CL); the LHC apoproteins in the absence of Chl binding are digested. In view of these findings the stimulation of LHCP accumulation by CAP treatment in the CD3 Chl deficient wheat mutant, normally deficient in LHCP [12], may be attributed to the inhibition of RC polypeptide synthesis by CAP.

The higher affinity of RC polypeptides for Chl may be due to a greater number of Chl-binding sites on RC complexes than on LHC complexes. This appears to be the case for isolated CPI, LHC-I and LHC-II of mature leaves [16–18] or leaves during development [19]. Moreover, since RC polypeptides are synthesized on thylakoid-bound ribosomes and cotranslationally incorporated into the membrane [20], while the LHC apoproteins are synthesized in the cytoplasm and post-translationally imported in the chloroplast, the former are expected to bind on Chl as soon as they are synthesized.

Our results show that the distribution of Chl among pigment-protein complexes and the formation of the PS units depend on competition between the RC and LHC apoproteins for the Chl synthesized.

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