Journal of Biochemistry and Molecular Biology, Vol. 34, No. 6, November 2001, pp. 496-501

JOURNAL OF
Biochemistry
and
Molecular Biology

Regulation of Chlorophyll-Protein Complex Formation and Assembly in Wheat Thylakoid Membrane

I. M. Guseinova*, S. Y. Suleimanov and J. A. Aliev
Institute of Botany, National Academy of Sciences, Patamdar shosse 40, Baku 370073, Azerbaijan

Received 30 March 2001, Accepted 31 July 2001

Lincomycin, an inhibitor of plastid protein synthesis, was found to block the synthesis of apoprotein P700 with a molecular mass of 72 kDa and the assembly of the Chl *a*-protein of PS I. Synthesis of the polypeptides of 48, 43.5, and 32 kDa of the PS II complex is also suppressed. This process is accompanied by the disappearance of the PS II reaction center Chl *a* at 683 nm, and of the PS I reaction center Chl *a* at 690, 696, and 705 nm on the fourth derivative of the absorption spectra at 77K. Lincomycin does not affect the synthesis of LHC subunits. It increases the content of the two main Chl forms of LHC at 648 nm (Chl *b*) and 676 nm (Chl *a*). The low-temperature fluorescence ratio F736/F685 is also increased. However, the effect of cycloheximide (an inhibitor of cytoplasmic protein synthesis) leads to the reduction of polypeptides of the light-harvesting Chl *a/b*-protein complex in the range of 29.5-22 kDa. Under these conditions, the relative amount of Chl *b* and the F736/ F685 fluorescence ratio decrease significantly. This is obviously the result of blocking the LHC I and LHC II synthesis. At the same time rifampicin and actinomycin D (inhibitors which block transcription in chloroplast and nuclear genome, respectively) inessentially affect the characteristics of these complexes.

Keywords: Biogenesis, Chlorophyll fluorescence, Chloroplast, Regulation

Introduction

The photochemical reactions of photosynthesis proceed in the thylakoid membranes of the chloroplasts with the participation of four multisubunit complexes: Photosystem II, cytochrome b_6f complex, Photosystem I, and ATP synthase complex (CF_0-CF_1) (Nelson, 1987). The light-harvesting Chl *a/b*-protein of PS II contains Chl *a*, Chl *b*, and preferentially zeaxanthin and lutein. It connects the basic mass of Chl and protein, and is the main component of the thylakoid membrane (Dreyfuss and Thornber, 1994a). The LHC II apoprotein is encoded by the multi-gene family of cab (recently renamed Lhcb) in nuclear DNA. The presence of a plastid factor that controls the expression of nuclear genes that encodes LHC II apoproteins has been inferred (Tonkyn *et al.*, 1992).

The reaction centers of the PS I and PS II complexes contain only Chl *a*, and are enriched by β-carotine. CP 47 and CP 43 are Chl *a* PS II core antennas, each consists of one polypeptide that ranges from 45-51 kDa and 40-45 kDa, respectively. Both are served as intermediate transducers of the excitation energy from the Chl *a/b* peripheral antenna (LHC II) to the PS II reaction center Chl (Horton *et al.*, 1996). The PS-I core complex consists of two heterodimeric subunits in equimolar amounts: PS I-A and PS I-B (Xu and Chitnis, 1995). The PS I-A and PS I-B polypeptides are homologous, containing the Chl *a* PS I reaction center (P700). PS I-A, PS I-B, and P700 make up the PS I reaction center complex that is designated as CP I when resolved on SDS-PAGE. LHC Ia and LHC Ib are Chl *a/b* peripheral antenna complexes of PS I (Dreyfuss and Thornber, 1994b).

The stability of Chl-binding proteins is regulated indirectly by light, because light is an absolute requirement for Chl synthesis in angiosperms (Green and Salter, 1996; Tziveleka and Argyroudi-Akoyunoglou, 1999); in the absence of Chl, the Chl-binding proteins are turned over. The stability of the other thylakoid polypeptides is dependent on their incorporation into complete macrocomplexes: in the absence of one polypeptide, part of the complex it forms is destabilized and all of the polypeptides are turned over at a faster rate.

Development of photosynthetically competent thylakoid membranes requires the coordinated synthesis and assembly of a large number of polypeptides of both cytoplasmic and chloroplast origins (Webber and Baker, 1996). The synthesis and assembly of thylakoid membrane-protein complexes may be regulated at several levels of gene expression. These

*To whom correspondence should be addressed.
Tel: 994-12-38-11-64; Fax: 994-12-39-33-80
E-mail: dj_aliev@baku.ab.az

include transcription, mRNA stability, translation, and protein turnover (for a review, see Gruissem and Tonkyn, 1993; Gray, 1996). The biogenesis of these complexes in higher plants and green algae is a multi-step process that initiates with the transcription and translation of the various polypeptide subunits.

In this present paper, we used a systematic approach for a more detailed study of the formation and assembly of wheat Chl-protein complexes. To understand how the coordination in the thylakoid membranes, protein synthesis assembly, and turnover may be controlled during chloroplast development, we investigated the absorption and fluorescence characteristics of *in situ* forms of chloroplast pigments, photochemical activity, and polypeptide composition of isolated chloroplasts after treatment of the plants with different nucleic acid and protein synthesis inhibitors.

Materials and Methods

Wheat seeds (*Triticum durum L.*) were germinated and grown in a phytotron on wet filter paper that was moistured by water at 23 ± 2°C and a 50-60% relative humidity. Five six-day-old-etiolated seedlings without caryopsis and root were transferred in the darkness into inhibitor solutions, or pure water (control). The treatment of the seedlings by antibiotics was carried out according to Giller *et al.* (1986). For inhibition of plastid or cytosolic protein synthesis, lincomycin or cycloheximide (Sigma) were used at final concentrations of 100 or 10 mg/mL, respectively. To inhibit the transcriptional activity in chloroplasts or in nuclei, rifampicin or actinomycin D (Sigma) were used at final concentrations of 100 or 10 mg/mL.The transferred seedlings were kept for 24 h in darkness, then illuminated for 48 h by a fluorescent lamp (LB-40, Russian) with a light intensity of 50 W/m^2.

Chloroplast isolation and thylakoid membrane sedimentation were carried out by osmotic shock according to Giller *et al.* (1986). The collected membrane was resuspended in 5 mM Tris-HCl buffer, pH 8.0. The chlorophyll concentration was estimated spectrophotometrically in a 80% acetone extract according to MacKinney (1941). The photochemical activity of the chloroplasts was determined on an analyzer Type OH 102 (Radelkis, Hungary) by a Clark-type electrode. The reaction mixture contained 80 mM sucrose, 10 mM NaCl, 10 mM $MgCl_2$, 30 mM Tris-HCl, pH 7.4. The chloroplast content in the mixture was equivalent to 100 μg Chl.

Thylakoid proteins were separated with SDS-PAGE, according to Laemmli (1970), using a 6% stacking gel and a 10 to 25% (w/v) acrylamide gradient in a separating gel (30.0 : 0.8 acrylamide/bisacrylamide). The gels were 1.0 mm thick, 16 cm wide, and 18 cm long. The electrophoresis buffer was 192 mM glycine and 24 mM Tris (pH 8.3). SDS was added to 0.1% (w/v) of the upper buffer reservoir. Prior to electrophoresis, the samples were solubilized for 30 min at room temperature in a solution that contained 5 mM Tris-HCl, pH 8.0, 2% SDS, 1% 2-mercaptoethanol, and sucrose. After electrophoresis, the protein bands were visualized by staining the gel with a solution of 0.25% (w/v) Coomassie Brilliant Blue R-250 in methanol : acetic acid : H_2O [5 : 1 : 4] (by vol.). The excess dye was removed using charcoal in acetic acid : ethanol : H_2O [3 : 10 : 27] (by vol.). Molecular weight markers were purchased from Pharmacia. The gels were scanned on an ULTROSCAN-2202 Laser Densitometer (LKB, Sweden). When necessary, the gels were dried in a SLAB GEL Dryer 2003 (LKB, Sweden).

The spectral measurements were performed using a double-beam Hitachi-557 (Japan) spectrophotometer and a Hitachi-850 (Japan) fluorescence spectrophotometer, as described previously (Asadov *et al.*, 1986). The fluorescence emission spectra were corrected for the spectral sensitivity of the spectrofluorometer using rhodamine B. The samples were immersed in liquid N_2 during measurements. The relative content of the *in situ* chlorophyll forms in the control and inhibitor-treated chloroplasts was determinated from the fourth derivatives of their absorption spectra at 77K by the two different methods. In the first case, the areas of peaks of each of the individual chlorophyll forms were measured. For this purpose, the left and right minima of the fourth derivative of the absorption spectrum of the individual chlorophyll form were connected by a straight line (Asadov *et al.*, 1986). The area of the corresponding peak was measured by weighing. The areas of all of the peaks were summed and taken as 100%. In the second case, the amplitudes of the peaks of each individual chlorophyll form were estimated. For this purpose, a line was drawn between the maximum of the corresponding peak and the central point of the segment that connected the left and right maxima of the fourth derivative of the absorption spectrum of the chlorophyll form.

Results and Discussion

Treatment of seedlings by protein synthesis inhibitors in chloroplasts (lincomycin) and in cytoplasm (cycloheximide) resulted in significant changes of composition and content of the thylakoid membrane proteins. Lincomycin blocks and cycloheximide partially blocks the synthesis of the P700 apoprotein (PS I-A/B) with a molecular mass of approximately 72 kDa, and the assembly of the Chl *a*-protein of PS I (CP I), i.e. the main structural element of PS I (Fig. 1, A and B). The sensitivity of the synthesis of the chlorophyll a-protein of CP I to the inhibitors of the protein synthesis on 70S ribosomes, and the existence of the plastid CP I-less mutants, indicate the participation of chloroplast DNA in the synthesis of the CP I polypeptides. The decrease of Chl *a*-protein content in the CHI-treated chloroplasts is somewhat surprising (Fig. 1, A and B). However, nuclear mutants of *C.reinhardtii* that affect the translation of the two reaction center subunits of PS I , the psa A and psa B (Stampacchia *et al.*, 1997), have been described. These results demonstrate that the translational activity in the nuclei is necessary for normal accumulation of the PS I protein complex. According to our densitometric data, synthesis of the 48, 43.5, and 32 kDa proteins that belong to the PS II complex is also suppressed by LM. Hence, the genome of plastids, and their protein-synthesizing system, connect directly with the encoding and translation of these polypeptides. However, the CHI treatment also decreases the amount of these polypeptides in thylakoid membranes. Photosynthesis-deficient nuclear mutants that are

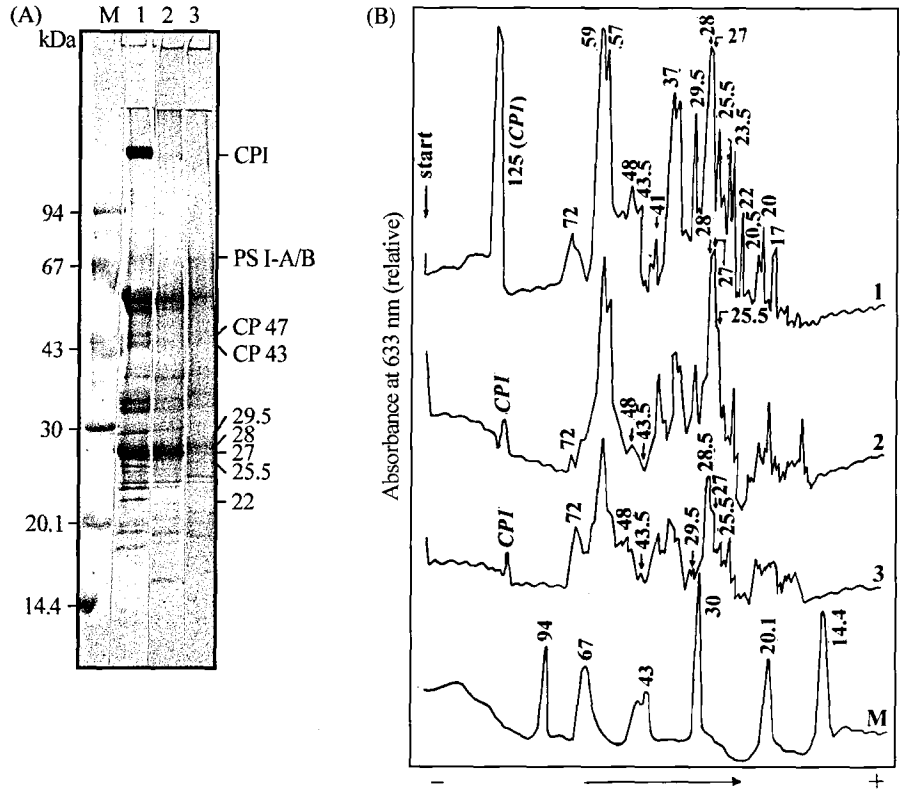

Fig. 1. (A) Coomassie blue staining of thylakoid membrane proteins that are isolated from different samples of wheat seedlings after SDS-PAGE. 1-control; 2-after treatment by lincomycin; 3-after treatment by cycloheximide. (B) Density patterns of SDS-PAGE; Standard proteins are shown in lane M (kDa): 94 (phosphorylase B), 67 (bovine serum albumin), 43 (ovalbumin), 30 (carbonic anhydrase), 20.1 (trypsin inhibitor), 14.4 (lactalbumin). Electrophoresis was carried out in a Tris-glycine buffer pH 8.3 at 4°C for 16 h.

unable to accumulate transcripts from the psbA (Girard-Bascou *et al.*, 1992), psbB (Hird *et al.*, 1991; Monod *et al.*, 1992), and psbC (Rochaix, 1989) genes have also been isolated. In each case, the mutation appears to specifically block the translation of a single chloroplast message. In these plants, the steady-state level of these polypeptides was much lower in the presence of this inhibitor than in the untreated control seedlings. It has been suggested that the polypeptides of the reaction centers of PSs are synthesized in chloroplasts, but some minor components that are synthesized on cytoplasmic ribosomes are necessary for their targeting in the formed membrane and assembly into the functional units (Gray, 1987; Shen and Bryant,1995). It is interesting to note that LM does not reduce the content of the LHC II polypeptides (Fig.1, A and B, 29.5, 28, 27 and 25.5 kDa). In some experiments, it even increases. These data agree with the results that were obtained by using another inhibitor of the protein synthesis in chloroplasts-chloramphenicol, which stimulated LHC II assembly and accumulation of its apoproteins (Duysen *et al.*, 1985; Tziveleka and Argyroudi-Akoyunoglou, 1999). These results suggest that the LHC apoproteins can be stabilized under conditions where the synthesis of PS I and PS II RCs is inhibited. Therefore, there is competition between the PS I and PS II RCs polypeptides, and the LHC apoproteins, for newly synthesized Chl molecules during thylakoid membrane assembly.

Inhibition of cytoplasmic protein synthesis by cycloheximide, however, had a significant effect on the accumulation of polypeptides in the 29.5-22 kDa region (Fig.1, A and B). The data confirm the point of view that subunits of LHC II are encoded in a nucleus and synthesized on 80S ribosomes of cytoplasm.

In the low-temperature (77 K) absorption spectra of the treated samples, the main band, which is located at 676 nm in the red region, is shifted after treatment by 1-2 nm to a shorter wavelength region (Fig. 2, A and B). Simultaneously, its intensity decreases in comparison with that of the control. The fourth derivatives of the absorption spectra (A^{IV}) of the investigated preparations show that the LM effect results in the disappearance of the three longest wavelength Chl forms (Chl *a* 690, 696 and 705 nm) due to the reaction center of PS I. The Chl 683 form that belongs to the reaction center of PS II also disappears after this treatment. At the same time, the relative content of the two main Chl forms of LHC II increased-Chl *b* 648 (14.8% versus 12.4% in control) and Chl *a* 676 (40.7% versus 34.3% in control) are seen after treatment. Similar changes were observed by Sarvari and Nyitrai (1985) in kidney beans. However, under the CHI

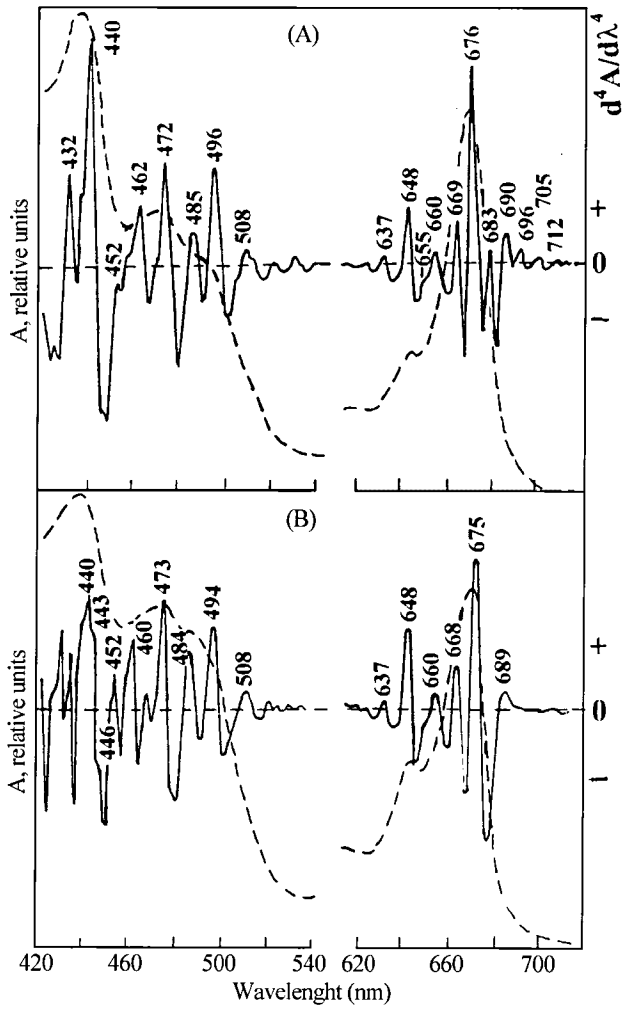

Fig. 2. Absorption spectra (dashed curves) and fourth derivatives of the absorption spectra (solid curves) at 77K of chloroplasts that were isolated from wheat seedlings in the absence (A) or presence of lincomycin (B). Interval of differentiation: $\Delta\lambda = 7$ (A) and 6 (B) respectively. Data are from four or five separate chloroplast preparations for each set of plants.

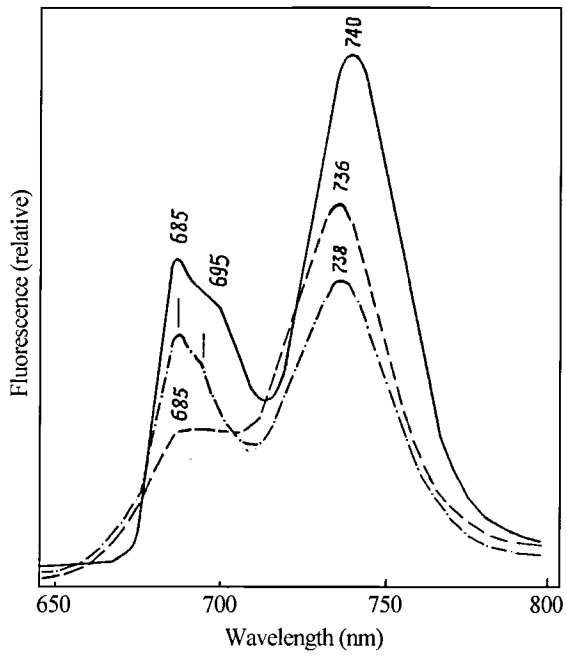

Fig. 3. Fluorescence emission spectra at 77 K that was obtained from wheat chloroplasts: control (solid curves), after treatment by lincomycin (dashed curves), after treatment by cycloheximide (dashed dot curves). Spectra were normalized to the equivalent emission at 687 nm. Each curve is from four or five separate chloroplast preparations for each set of plants. The excitation wavelength was 440 nm.

effect, the content of the 648 and 676 nm peaks decreased significantly in comparison with the control-Chl *b* 648 (3.8% versus 12.4% in control) and Chl *a* 676 (10.2% versus 34.3% in control) (figure not shown). Thus, it seems that these Chl forms bind with LHC II proteins, which may be reduced significantly under CHI application. This is good data on the spectral investigations of LHCs that were isolated from the thylakoid membranes of chloroplasts (Asadov *et al.*, 1986).

A comparative analysis of the fourth derivatives of the absorption spectra at 77 K of chloroplasts in the blue region showed that the LM treatment decreased, in comparison with control, the ratio of peak amplitudes of Chl *a* (at 440 nm) and Chl *b* (at 472 nm) in correspondence with the data that was obtained in the red region of the spectrum (Fig. 2, A and B). At the same time, the LM treatment of chloroplasts only had a minor effect on the absorption bands of carotenoids (peaks at 452, 460, 484, 494 and 508 nm).

Figure 3 shows the low-temperature fluorescence spectra of chloroplasts from the control and inhibitor-treated plants. The main fluorescence band that is localized at 740-742 nm shifted after treatment by 4-6 nm to the short wavelength region. Possibly, the inhibitor of synthesis of some of the PS I polypeptides resulted in the suppression of the formation of the longest wavelength chlorophyll forms. This is apparently connected with the decrease in the amount of chlorophyll in the PS I antenna (LHC I). A similar spectral shift was attributed to the removal of chlorophylls from the peripheral antenna (Guseinova *et al.*, 2000). The shoulder at 695 nm on the fluorescence spectrum disappeared after the treatment. The intensity of the band at 685 nm was also remarkably decreased, which is connected with the suppression of the formation of polypeptides in the reaction center of PS II (Mc Cormac *et al.*, 1996). At the same time, the ratio of fluorescence bands F736/F685 increased in comparison with the control. This could be connected with the stimulation of LHC I synthesis during the inhibition of RC by LM. The F736/F685 ratio in the control and LM treated chloroplasts is equal to 1.6 and 2.4 respectively. Under the CHI application, the F736/F685 bands ratio in the low-temperature fluorescence spectra of chloroplasts decreases to 1.2. Possibly this reflects the blockage of LHCs I and II synthesis after the CHI treatment. Therefore, the $(F736/F685)_{LM}$ ratio was greater

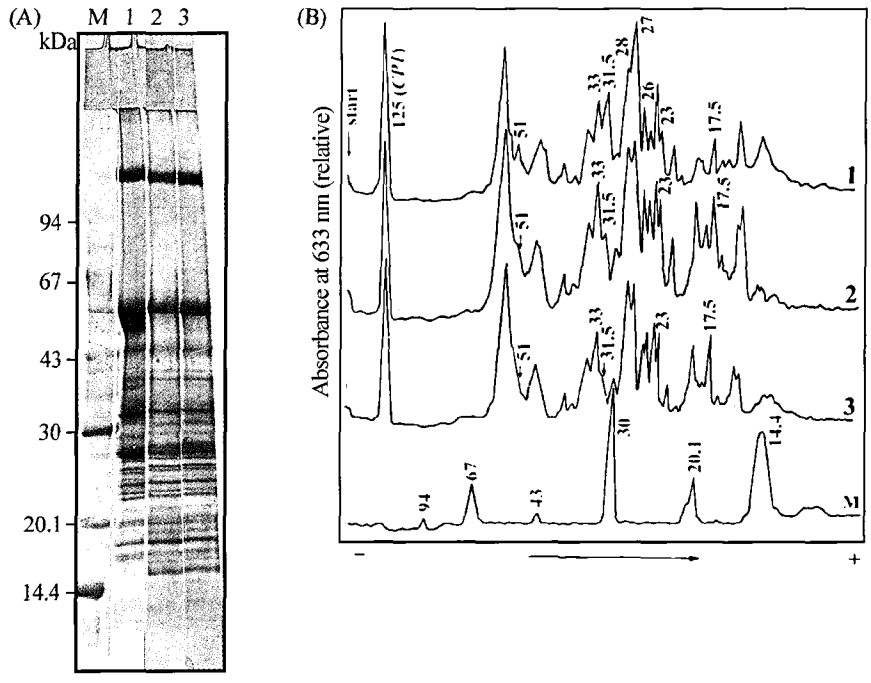

Fig. 4. (A) SDS-PAGE and (B) Density patterns of proteins from wheat thylakoid membranes: control (1), and after treatment by rifampicin (2) or by actinomycin D (3); M-protein markers (as in Fig. 1).

than the $(F736/F685)_{CHI}$ ratio.

It was discovered that inhibitors that block the transcription process in chloroplasts (rifampicin), and in a nucleus (actinomycin D), affect the synthesis of polypeptide with M_r 51 kDa (Fig.4, A and B). The synthesis of 31.5 kDa polypeptide is also blocked. This is a minor component of the thylakoid membranes and by virtue of the peculiarities of the amino acid structure (protein does not contain lysine) is weakly stained by Coomassie Brilliant Blue R-250. Since our experiments used this stain, it is impossible to confirm that the bands that were observed on the electrophoregramme with M_r 31.5 kDa is the D1-protein, which is the carrier of P680. No observations were made on the inhibitors effects on the special changes in the composition and contents of the LHC II apoproteins in the 28-26 kDa region. The same effect at the RNA level is not observed, in spite of the fact that translation inhibitors on plastids stimulate accumulation of LHC II apoproteins. The content of 33, 23, and 17.5 kDa polypeptides that participate in water oxidation is appreciably increased under both inhibitors' effect.

The content of the Chl form at 683 nm in the fourth derivative of the absorption spectra at 77 K of the treated preparations almost coincides with the level of the control. In the fluorescence spectra of chloroplasts that were treated by AcD and Rf, there is a small shift of the basic maximum at 740 nm (figures not shown).

LM and CHI significantly suppress the PS II photochemical activity of the PS II reaction center. Contrary to the translation inhibitors, the oxygen evolution rate in preparations that were treated by RNA synthesis inhibitors was nearly on the level of the control, or even slightly higher (data not shown). It is possible that the considerable difference between the effects of inhibitors that suppress the translation and transcription process on the PS II that function in cell organelles are connected with the action of rifampicin and actinomycin D, not only on the transcription processes of chloroplast and nuclear DNA, but also on cell metabolism.

From these results, we conclude that the LHC I and LHC II content stimulate, while RC_s of PS I and II synthesis are blocked. In these experiments, the hypothesis of Tzinas and Argyroudi-Akoyunoglou (1988 and 1999) was confirmed. They suggested a competition between the apoproteins of LHC and RC for newly synthesized Chl molecules during chloroplast development. The regulation of biogenesis and assembly of chlorophyll-protein complexes is controlled by a post-transcriptional event. In the leaves of higher plants *in vivo,* there is some cooperation between protein-synthesizing systems of the cytoplasm and chloroplasts in the formation and function of these complexes. This cooperation is expressed not only in a joint assembly of its individual parts (RC, their minor components, and Chl *a/b* LHC_s), but also in the regulation of the level of their synthesis.

References

Asadov, A. A., Zulfugarov, I. S., Suleimanov, S. Yu. and Aliev, J. A. (1986) Pigment organization in light-harvesting complex and pigment-protein complex of photosystem I of spinach chloroplasts. *Dokl. Akad. Nauk SSSR* **287**, 444-447.

Dreyfuss, B. W. and Thornber, J. P. (1994a) Assembly of light-

harvesting complexes (LHCs) of Photosystem II: Monomeric LHC IIb complexes are intermediates in the formation of oligomeric LHC IIb complexes. *Plant Physiol.* **106**, 829-839.

Dreyfuss, B. W. and Thornber, J. P. (1994b) Organization of the light-harvesting complex of Photosystem I and its assembly during plastid development. *Plant Physiol.* **106**, 841-848.

Duysen, M. E., Freeman, T. P., Williams, N. D. and Huckle, L. L. (1985) Chloramphenicol stimulation of light-harvesting chlorophyll protein complex accumulation in a chlorophyll b deficient wheat mutant. *Plant Physiol.* **78**, 531-536.

Girard-Bascou, J., Choquet, Y., Schneider, M., Delosme, M. and Dron, M. (1987) Characterization of a chloroplast mutation in the psa A2 gene of Chlamydomonas reinhardtii. *Curr. Genet.* **12**, 489.

Girard-Bascou, J., Pierre, Y. and Drapier, D. (1992) A nuclear mutation affects synthesis of the chloroplast psbA gene production Chlamydomonas reinhardtii. *Curr. Genet.* **22**, 47.

Giller, Yu. E., Asoeva, L. M., Kolyago, V. M. and Fradkin, L. I. (1986) Cooperation of protein synthesizing systems of cytoplasm and chloroplasts in the formation of pigment system of the photosynthetic apparatus. *Dokl. Akad. Nauk SSSR* **287**, 502-506.

Gray, J. C. (1987) Genetics and synthesis of chloroplast membrane proteins; in *Photosynthesis, Amesz, J.* (ed.), pp. 319-342, Elsevier, Amsterdam, New York, Oxford.

Green, B. R. and Salter, A. H. (1996) Light regulation of nuclear-encoded thylakoid proteins; in *Molecular Genetics of Photosynthesis*, Anderson, B., Salter, A. H. and Barber, J. (eds.), pp. 75-104, Oxford University Press, New York.

Gruissem, W. and Tonkyn, J. C. (1993) Control mechanisms in plastid gene expression. *Crit. Rev. Plant Sci.* **12**, 19-55.

Guseinova, I. M., Suleimanov, S. Y., Zulfugarov, I. S. and Aliev, J. A. (2000) Assembly of the light-harvesting complexes during plastid development. *J. Fluorescence* **10**, 255-259.

Hiller, R. G., Pilger, T. B. G. and Genge, S. (1977) Effect of lincomycin on the chlorophyll protein complex I content and Photosystem I activity of greening leaves. *Biochim. Biophys. Acta* **460**, 431-444.

Hird, S. M., Webber, A. N., Wilson, R., Dyer, T. A. and Gray, J. C. (1991) Differential expression of the chloroplast genes for the 47 kDa chlorophyll a-protein and the 10 kDa phosophoprotein during chloroplast development in wheat. *Curr. Genet.* **19**, 199-206.

Horton, P., Ruban, A. V. and Walters, R. G. (1996) Regulation of light-harvesting in green plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 655-684.

Krugh, B. W. and Miles, D. (1995) Energy transfer for low temperature fluorescence in PS II mutant thylakoids. *Photosynth. Res.* **44**, 117-125.

Laemmli, V. K. (1970) Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature* **227**, 680-685.

MacKinney, G. (1941) Absorption of light by chlorophyll solutions. *J. Biol. Chem.* **140**, 315-322.

Monod, C., Goldschmidht-Clemont, M. and Rochaix, J-D. (1992) Accumulation of chloroplast psbB RNA requires a nuclear factor in Chlamydomanas reinhardtii. *Mol. Gen. Genet.* **231**, 449-459.

Nelson, N. (1987) Structure and function of protein complexes in the photosynthetic membrane; in *Photosynthesis, Amesz*, J. (ed.), pp. 213-232, Elsevier, Amsterdam-New York-Oxford.

Rochaix, J.-D., Kuchka, M., Mayfield, S., Schirmer-Rahire, M., Girard-Bascou, J. and Bennoun, P. (1989) Nuclear and chloroplast mutations affect the synthesis or stability of the chloroplast psbC gene product in Chlamydomonas reinhardtii. *EMBO J.* **8**, 1013-1021.

Sarvari, E. and Nyitai, P. (1985) Comparison of different methods for isolating photosystem 1 on lincomycin treated and untreated bean seedlings. *Photosynthetica* **19**, 504-513.

Shen, G. and Bryant, D. A. (1995) Characterization of a Synechococcus sp. strain PCC 7002 mutant lacking Photosystem I. Protein assembly and energy distribution in the absence of the Photosystem I reaction center core complex. *Photosynth. Res.* **44**, 41-53.

Stampacchia, O., Girard-Bascou, J., Zanasco, J.-L., Zerges, W., Bennoun, P. and Rochaix, J.-D. (1997) A nuclear-encoded function essential for translation of the chloroplast psaB mRNA in Chlamydomonas. *Plant Cell* **9**, 773-782.

Tzinas, G. and Argyroudi-Akoyunoglou, J. H. (1988) Chloramphenicol-induced stabilization of light-harvesting complexes in thylakoids during development. *FEBS Lett.* **229**, 135-141.

Tziveleka, L. A. and Argyroudi-Akoyunoglou, J. H. (1999) Proteolytic mechanism in LHC II stabilization; in *The Chloroplast: from Molecular Biology to Biotechnology*, Argyroudi-Akoyunoglou, J. H. and Senger, H. (eds.), pp. 277-282, Kluwer Academic Publishers, Dordrecht, The Netherlands.

Webber, A. N. and Baker, N. R. (1996) Control of thylakoid membrane development and assembly; in *Oxygenic Photosynthesis: The Light Reactions*, Ort, D. R. and Yocum, C. F. (eds.), pp. 41-58, Kluwer Academic Publishers, Dordrecht, The Netherlands.

Xu, Q. and Chitnis, P. R. (1995) Organization of Photosystem I polypeptides: identification of PsaB Domains that may interact with Psa D. *Plant Physiol.* **108**, 1067-1075.