Biochimica et Biophysica Acta 894 (1987) 69–75
Elsevier

BBA 42639

Variations in the relative content of the peripheral and inner light-harvesting chlorophyll *a*/*b*-protein complex (LHC II) subpopulations during thylakoid light adaptation and development

Ulla K. Larsson [a,*], Jan M. Anderson [a,**] and Bertil Andersson [b]

[a] *Department of Biochemistry, University of Lund, Lund and* [b] *Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, Stockholm (Sweden)*

(Received 22 May 1987)

Key words: Light-harvesting complex; Light regulation; Thylakoid development; Photosystem II; Thylakoid membrane

The light-harvesting chlorophyll *a*/*b*-protein complex of Photosystem II (LHC II) is composed of two subpopulations. One population is tightly bound to the Photosystem II core and contains predominantly a 27 kDa polypeptide. The other population, enriched in a 25 kDa polypeptide, is more peripheral bound and is able to undergo reversible detachment from the core due to protein phosphorylation or moderate heat. This pool of LHC II is therefore thought to be responsible for short-term adaptation of the Photosystem II antenna. In this study we show that the peripheral subpopulation is also responsible for long-term adaptation to variations in light regime. By two-dimensional gel electrophoresis it was found that spinach or pea grown under low irradiance contained an increased amount of total LHC II and also showed an increased proportion of the 25 kDa polypeptide relative to the 27 kDa polypeptide. The additional LHC II incorporated during the low-light adaptation was calculated to possess a 27/25 kDa polypeptide ratio around 2, a ratio close to what has previously been found for the peripheral pool of LHC II. This demonstrates a specific variation in the level of the peripheral LHC II in response to long-term light adaptation. By analyzing the LHC II polypeptide composition of developing pea etioplasts, we also demonstrate that in the early stage of membrane development the Photosystem II core and its tightly bound LHC II antenna is first inserted, while the peripheral LHC II is inserted at a later stage concomitant with grana formation and segregation of the two photosystems.

* Present address: Botanisches Institut der Universität München, Menzinger Strasse 67, D-8000 München 19, F.R.G.

** Permanent address: Division of Plant Industry, CSIRO, GPO Box 1600, Canberra A.C.T. 2601, Australia.

Abbreviations: LHCII, light-harvesting chlorophyll a/b-protein complex of Photosystem II; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; SDS, sodium dodecyl sulphate

Correspondence: B. Andersson, Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-10691 Stockholm, Sweden.

Introduction

There is much evidence that the photosynthetic apparatus of the chloroplast is able to adapt to changes in the environmental conditions [1–4]. This suggests that the composition, function and structure of the thylakoid membrane is dynamic rathe than static. Long-term adaptations allow for adjustments in the relative proportions of light-harvesting pigments, Photosystem I and II reac-

tion centres, electron carriers and ATP synthetase. In low irradiance or in shade, plants maximize light-harvesting capacity and reduce the amounts of electron-transport complexes and ATP synthetase relative to chlorophyll (cf. Ref. 4). Hence there is a marked increase in the amount of chlorophyll *a/b*-protein antenna of Photosystem II (LHC II) relative to the Photosystem II core complex [5].

In addition to long-term adaptations, the chloroplast possesses a rapid mechanism of adaptation. This short-term adaptation involves a reversible phosphorylation of LHC II and results in migration of a subpopulation of LHC II from Photosyste II in the appressed regions to the non-appressed stroma regions, thereby increasing the association of LHC II with Photosystem I [6–9]. A detachment of LHC II from the Photosystem II core, followed by lateral rearrangements, is also found in response to elevated temperatures [10–12].

The biosynthesis of LHC II is regulated by light [13,14] and by the activity of photoreceptors [15,16]. The stable assembly of LHC II in the thylakoid membranes appears to depend on the concomitant light-driven synthesis of chlorophyll [14,17]. The apopolypeptides of LHC II are encoded by small gene families as part of the nuclear genome [18] and some of these genes seem to be expressed differentially during leaf development or under varying environmental conditions [19].

In spinach and pea, the predominant gene products of LHC II are a 27 kDa and a 25 kDa polypeptide. Previously we have shown that there are two subpopulations of LHC II with different relative proportions of the 27 and 25 kDa polypeptides and different structural association with Photosystem II [20,31]. The 27 kDa polypeptide is dominant in the LHC II pool tightly bound to the core of Photosystem II, while the peripheral pool of LHC II has a relatively high abundance of the 25 kDa polypeptide. Due to the capability of the peripheral subpopulation to dissociate rapidly from Photosystem II in response to light variations [7,20,22] or elevated temperatures [11], this pool of LHC II appears to be the one involved in short-term adaptation of the light-harvesting apparatus. In this study, by analyzing the polypeptide composition of LHC II in plants adapted to different irradiances, we demonstrate that the peripheral pool of LHC II is not only responsible for short-term, but also for long-term adaptation. We also show a heterogeneity in the insertion and assembly of the two different subpopulations of LHC II in developing thylakoid membranes.

Materials and Methods

Spinach (*Spinacia oleracea* L.) and pea (*Pisum sativum* L.) leaves were grown under different white light intensities of 50, 180 and 480 $\mu E \cdot m^{-2} \cdot s^{-1}$. After 6 weeks thylakoids were isolated essentially as in Ref. 23.

Thylakoids from 7-days-old pea etioplasts were isolated after 6, 12 and 24 h of illumination. Light intensity was approx. 70 $\mu E \cdot m^{-2} \cdot s^{-1}$. For phosphorylation, thylakoids from light-grown or greening etioplasts were suspended in 15 mM Tricine (pH 7.8), 20 mM NaCl, 5 mM $MgCl_2$, 10 mM NaF, 100 mM sucrose to a concentration of 400 μg chlorophyll/ml. Thylakoids were phosphorylated in the dark for 10 min at room temperature in the presence of 1 mM NADPH, 10 μM ferredoxin and 0.4 mM [γ-^{32}P]ATP (500 000 cpm/nmol ATP).

The chlorophyll *a/b* ratios were determined according to Arnon [24].

Chlorophyll-protein complexes from the thylakoid membrane were resolved by mild SDS-polyacrylamide gel electrophoresis [25] with an SDS/chlorophyll ratio of 7.5. The gels were scanned at 675 and 650 nm to quantify the chlorophyll-protein complexes [26]. Denaturing SDS-polyacrylamide gel electrophoresis was performed in the gel system of Laemmli [27]. The gels were stained with Coomassie brilliant blue R-250, destained and scanned using an LKB 2202 laser densitometer.

Quantification of the apopolypeptides of LHC II was done using two-dimensional gel electrophoresis as described in Ref. 20. The amounts of the LHC II polypeptides were quantified from their peak areas.

In experiments using [γ-^{32}P]ATP, the apo-polypeptide bands were excised from the SDS-polyacrylamide gels, solubilized in H_2O_2/perchloric acid and counted for radioactivity in Aquasol.

Results

Polypeptide composition of LHC II in high- and low-light-adapted spinach and pea thylakoids

The chlorophyll *a/b* ratios of the thylakoids from spinach and pea grown under different irradiances varied between 3.1 and 2.7 (Table I), the lower ratio determined from low light-adapted leaves, which is consistent with previous results [4,5]. These variations in chlorophyll *a* relative to chlorophyll *b* were also reflected in variations in the relative amounts of chlorophyll-protein complexes (Table I). The most striking difference is the incrased proportion of total chlorophyll associated with LHC II (LHCP1 + LHCP3) in low-light-adapted thylakoids. Concomitantly, there is a decrease in the relative amount of the Photosystem II core (CPa). Hence, the (LHCP1 + LHCP3)/CPa ratio increases from 3.5 to 5.1 (spinach) or from 3.1 to 4.8 (pea) when the thylakoids adapt to low irradiance. In contrast, the relative proportion of total chlorophyll in the bands corresponding to Photosystem I (CP1a + CP1) is not as much affected.

LHC II consists of two different subpopulations; one inner or tightly bound to the Photosystem II core and containing mainly the 27 kDa polypeptide, and another more peripherally associated with the Photosystem II core showing a relatively high abundance of the 25 kDa polypeptide [20,21]. The ratio between the 27 and 25 kDa polypeptides in the peripheral pool of LHC II has been shown to be close to 2. In light of this heterogeneity it is of interest to see whether the increase of LHC II under low light conditions (Table I) is attributed to an incrase of both LHC II subpopulations or mainly to one of them. We have therefore, by two-dimensional gel electrophoresis [20], investigated the relative amounts of the 27 and 25 kDa LHC II polypeptides in the high- and low-light-adapted spinach and pea plants. When the relative proportion of the two apopolypeptides of LHC II was determined, pronounced differences could be seen (Table II). The ratio between the 27 and 25 kDa polypeptides of high-light-adapted thylakoids is 4.1–4.3. This value decreases significantly whenthe plants were grown at lower irradiance, reaching a 27/25 kDa polypeptide ratio of 3.3. This indicates an increased amount of the 25 kDa polypeptides in the LHC II from low-light-grown plants both for spinach and pea.

Assuming a relative constant Photosystem II core content in high- and low-light-adapted plants, the polypeptide composition of the additional LHC II in low-light thylakoids can be estimated. This was done by relating the observed changes in the (LHCP1 + LHCP3)/CPa ratio (Table I) to the changes seen in the ratio between the 27 and 25 kDa polypeptides (Table II). These calculated val-

TABLE I

CHLOROPHYLL *a/b* RATIOS AND RELATIVE DISTRIBUTION OF CHLOROPHYLL (%) BETWEEN THE CHLOROPHYLL-PROTEIN COMPLEX IN THYLAKOIDS OF SPINACH AND PEA GROWN AT DIFFERENT LIGHT CONDITIONS

The chlorophyll-protein complexes of thylakoids isolated from high- (HL), medium- (ML) and low- (LL) light-adapted plants were resolved by mild SDS-polyacrylamide gel electrophoresis [25]. CP1a + CP1, Photosystem I complexes; CPa, Photosystem II complex; LHCP, light-harvesting complex of Photosystem II; FC, free chlorophyll. Chlorophyll *a/b* ratios were determined according to Ref. 24. Light irradiances (measured in $\mu E \cdot m^{-2} \cdot s^{-1}$): HL, 480; ML, 180; LL, 50.

Chlorophyll-protein complex	Spinach			Pea		
	HL	ML	LL	HL	ML	LL
Chl *a/b*	2.9	2.8	2.7	3.1	3.0	2.8
CP1a + CP1	25	25	24	32	30	30
CPa	14	12	11	13	12	10
LHCP1 + LHCP3	49	51	56	40	44	48
FC	12	12	9	15	14	12
(LHCP1 + LHCP3)/CPa	3.5	4.2	5.1	3.1	3.7	4.8

TABLE II

RATIO BETWEEN THE 27 AND 25 kDa APOPOLYPEPTIDES OF LHC II IN THYLAKOIDS OF SPINACH AND PEA GROWN AT DIFFERENT LIGHT CONDITIONS

Thylakoids adapted to high- (HL), medium- (ML) and low- (LL) light intensities were isolated. The amounts of the two apopolypeptides of LHC II were quantified from their peak areas after two-dimensional gel electrophoresis [20]. Light irradiances (measured in $\mu E \cdot m^{-2} \cdot s^{-1}$): HL, 480; ML, 180; LL, 50.

	Spinach			Pea		
	HL	ML	LL	HL	ML	LL
27/25 kDa ratio	4.3	3.5	3.3	4.1	3.7	3.3

ues suggest 27/25 kDa polypeptide ratios of 2.0 and 2.3 for the additional LHC II of low-light spinach and pea thylakoids, respectively. Since these values are very close to the one demonstrated for the peripheral subpopulation of LHC II [20,21], this result indicates a specific increase of the peripheral LHC II in plants grown under low irradiance.

Polypeptide composition of LHC II in greening pea etioplasts

In order to see if there are any differences in the appearance of the 27 and 25 kDa polypeptides of LHC II in developing thylakoid membranes, we investigated the ratio between the two polypeptides in greening pea etioplasts. The thylakoids were isolated after 6, 12 and 24 h of illumination of dark-grown pea plants and the apopolypeptides were resolved by denaturing SDS-polyacrylamide gel electrophoresis. Control thylakoids were isolated from 6-week light-grown plants. As seen in Fig. 1, correlating with the times of greening there is an increasing amount of polypeptides in the 25–27 kDa range. However, the appearance of

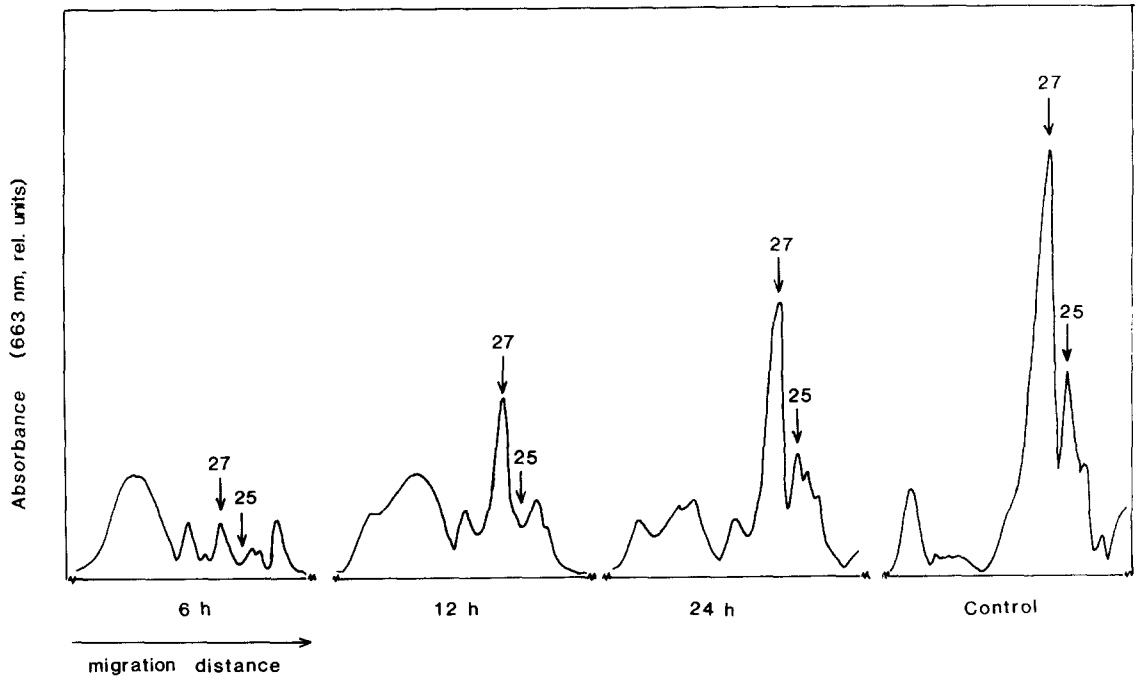

Fig. 1. SDS-polyacrylamide gel analysis of the apopolypeptides resolved from thylakoids of control and greening pea etioplasts. Only the apopolypeptides in the range of 20–35 kDa are shown. Approximately the same amount of chlorophyll was loaded on the gels. The gels were stained with Coomassie brilliant blue, destained and scanned using a laser densitometer.

these proteins do not follow the same kinetics. After 6 h of illumination, only a 27 kDa polypeptide is present. There are no or very small amounts of a 25 kDa polypeptide. After 12 h of greening, proteins of this molecular weight become more visible and after 24 h, the peak height ratio between the polypeptides at 27 and 25 kDa is almost the same as in the control. This suggests that the 27 kDa polypeptide of LHC II is inserted in the developing thylakoid at an earlier stage compared to the 25 kDa polypeptide. However, a true quantification of the 27 and 25 kDa polypeptides of LHC II from the gel scans of Fig. 1 is difficult to make, since comigrating polypeptides may obscure the quantification. Unfortunately, the thylakoids from the greening pea etioplasts were very sticky, probably due to nuclear contamination and hence, it was not possible to run two-dimensional gel electrophoresis for a careful analysis of the relative amounts of the apopolypeptides of LHC II, as was performed above. As an additional analysis we therefore estimated the content of the 27 and 25 kDa polypeptides according to their relative incorporation of [^{32}P]phosphate. To allow for maximal incorporation independent of the photochemical activities of the membranes, the isolated thylakoids were phosphorylated in the dark in the presence of NADPH and ferredoxin. Fig. 2 shows histograms of the total incorporation of [^{32}P]phosphate (cpm) into the 27 and 25 kDa polypeptides of control thylakoids and thylakoids from etioplasts illuminated for 12 or 24 h. In the thylakoids isolated after 6 h of greening, we could not find any labelling of the apopolypeptides of LHC II. As seen in the control thylakoids, the total incorporation in the 27 kDa polypeptides was 1.5-times higher than in the 25 kDa polypeptides, consistent with previous data [20]. After 12 h of greening a small amount of [^{32}P]phosphate was found in the 27 kDa polypeptides. Not until thylakoids from etioplasts illuminated for 24 h were phosphorylated, we first found incorporation of [^{32}P]phosphate into the 25 kDa polypeptides. However, the proportion of phosphate labelling in the LHC II apopolypeptides of the developing thylakoids was quite different from what could be expected from control thylakoids. The total phosphorylation of

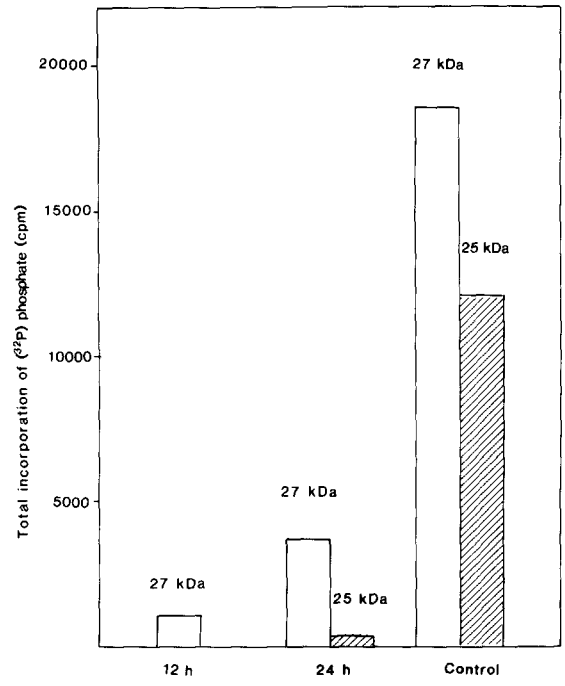

Fig. 2. Histograms of total [^{32}P]phosphate incorporation into the 27 and 25 kDa polypeptides of LHC II found in greening etioplasts. The apopolypeptides were resolved by SDS-polyacrylamide gel electrophoresis and the total incorporation of [^{32}P]phosphate, expressed in cpm, was analyzed after cutting out the individual apopolyppetide bands from the gels.

the 27 kDa polypeptides of etioplasts illuminated for 24 h was almost 10-times higher than that of the 25 kDa polypeptides compared to an incorporation ratio of 1.5 for control thylakoids. This observation suggests that the very young developing membranes have a low content of the 25 kDa polypeptides in accordance with the SDS-polyacrylamide gel electrophoresis (Fig. 1). Hence, we suggest that the Photosystem II antenna of developing membranes consists of predominantly inner LHC II, while peripheral LHC II is inserted later during the maturation.

Discussion

Several studies suggest the involvement of a specific subpopulation of LHC II in various short-term adaptations. These include dissociation of a part of the LHC II antenna from the core of Photosystem II as a result of light variations

(phosphorylation [6–9]) or elevated temperatures [10–12], and is followed by rapid changes in the excitation energy distribution to the reaction centres of Photosystem I and II. In recent studies, this subpopulation of LHC II has been referred to as the peripheral subpopulation of LHC II with a high relative abundance of the 25 kDa polypeptide [20–22]. In this study, we demonstrate that the level of the peripheral pool of LHC II is also responsible for long-term light adaptation. Analyses of the polypeptide composition of LHC II in thylakoids isolated from spinach or pea plants grown under various light irradiances show that low light-adapted chloroplasts contain an increased amount of the peripheral subpopulation of LHC II per Photosystem II reaction centre compared to high-light adapted chloroplasts. In contrast to the peripheral pool of LHC II, the inner pool is obviously not as much affected by different light quantities. It may even be so that the ratio between the inner LHC II pool and the Photosystem II core remains constnat during long-term light adaptation. The peripheral subpopulation of LHC II may therefore act as the 'regulatory unit' for both short-term and long-term adaptation within the light-harvesting antenna of Photosystem II, as previously proposed [21].

Our conclusion that plants adapted to low irradiance have relativel more peripheral LHC II compared to high light-adapted plants is suported by structural studies. Comparison of wild type and mutant chloroplasts have allowed correlations between different freeze-fracture particles and functional thylakoid complexes. It is suggested [12] that large EF_s particles represent the Photosystem II core and tightly bound LHC II, and small PF_s particles represent peripheral LHC II. In the sun plant species *Atriplex patula* grown under different irradiance, a pronounced incrase in the ratio of PF_s to EF_s particles in low-light-adapted chloroplasts was demonstrated [28]. Moreover, an increased amount of PF_s particles was also found in *Alocasia*, a shade plant species, when compared with sun plant species [29]. Thus, these ultrastructural results are consistent with our biochemical findings that it is the level of the peripheral LHC II that is modulated in response to different irradiance.

It is established that there is a rough correlation between the proportion of LHC II in the membranes and the relative extent of membrane appression (cf. Ref. 4). Shade plants and plants adapted to low irradiance have an incrased proportion of LHC II relative to the Photosystem II core and also have more and broader grana stacks than sun plants and high-light-adapted plants. As demonstrated in this study (and as indicated from the freeze-fracture analyses of Refs. 28 and 29), the increase of total LHC II in low-light-adapted chloroplasts and shade plants is due to an increased level of peripheral LHC II. Hence, we propose that the peripheral subpopulation of LHC II is the major protein component involved in membrane stacking. This is consistent with the study of Sundby and Andersson [11], showing that after moderate heating of thylakoids there is a segregation of LHC II into peripheral LHC II located into tight appressions, while Photosystem II and its inner LHC II antenna are located in non-appressed areas.

In this study we also demonstrate a heterogeneity in the appearance of the 27 and 25 kDa polypeptides of LHC II in the developing thylakoid membranes after illumination of pea etioplasts. SDS-polyacrylamide gel electrophoresis supported by [^{32}P]phosphate incorporation suggest that the 27 kDa polypeptides are inserted in the membrane of the greening etioplasts before the 25 kDa polypeptides. This indicates that developing thylakoids have a low level of the peripheral subpopulation of LHC II compared to control thylakoids. It could be argued that the lower phosphorylation of the 25 kDa polypeptide would be a result of a specified decreased kinase activity on this polypeptide. However, this explanation is very unlikely, since it has recently been shown that the phosphate incorporation kinetics for the 25 kDa polypeptide is much faster than that of the 27 kDa polypeptide [22,31]. Thus, our results suggest that the assembly of the light-harvesting complex of Photosystem II is a two-step process. During the first hours of greening, there is an insertion of the inner pool of LHC II. Later on in the development, the peripheral subpopulation of LHC II is inserted into the membranes and, concomitantly, there is a formation of grana stacks

and a segregation of Photosystem I and II. Such a two-step developmental process in the assembly of LHC II with the Photosystem-II core have been suggested by Ghirardi and Melis [30]. Studies on LHC II-deficient mutants propose that PS II_β, a Photosystem II with a smaller chlorophyll *a/b* antenna can be a precursor form of the fully developed Photosystem II (PS II_α). Ultimate experiments to prove the involvement of the peripheral subpopulation of LHC II in light adaptation, chloroplast development and membrane adhesion require the isolation of this pool from the tightly bound subpopulation of LHC II.

Although the effects of light on the regulation of gene expression of the thylakoid membrane components are very complex, there must be a selective regulation to allow plants to respond to environmental changes. As suggested by Dunsmuir [19], some of the gens coding for the apopolypeptides of LHC II may be expressed differentially during development or under varying environmental conditions. Since a heterogeneity in the polypeptide composition of LHC II in response to light adaptation has been demonstrated in this study, it would be very interesting to examine the expression of the polypeptides of the peripheral and inner subpopulations of LHC II in plants grown under different light conditions.

Acknowledgements

This study was supported by the Swedish Natural Science Research Council (NFR). J.M.A. was a visiting Professor at the University of Lund under the auspices of the NFR.

References

1 Björkman, O. (1981) in Encyclopedia of Plant Physiology (Lange, O.K., Nobel, P.S., Osmond, C.B. and Ziegler, H., eds.), Vol. 12A, pp. 57–107, Springer-Verlag, Berlin
2 Rühle, W. and Wild, A. (1985) Naturwissenschaften 72, 10–16
3 Glick, R.E., McCauley, S.W. and Melis, A. (1985) Planta 164, 487–494
4 Anderson, J.M. (1986) Annu. Rev. Plant Physiol. 37, 93–136
5 Leong, T.-Y. and Anderson, J.M. (1984) Photosynth. Res. 5, 105–115
6 Larsson, U.K., Jergil, B. and Andersson, B. (1983) Eur. J. Biochem. 136, 25–29
7 Staehelin, L.A. and Arntzen, C.J. (1983) J. Cell Biol. 97, 1327–1337
8 Bennett, J. (1984) Physiol. Plant 60, 583–590
9 Larsson, U.K., Ögren, E., Öquist, G. and Andersson, B. (1986) Photobiochem. Photobiophys. 13, 29–39
10 Gounaris, K., Brain, A.P.R., Quin, P.J. and Williams, W.P. (1984) Biochim. Biophys. Acta 766, 198–208
11 Sundby, C. and Andersson, B. (1985) FEBS Lett. 191, 24–28
12 Staehelin, L.A. (1986) in Encyclopedia of Plant Physiology (Staehelin, L.A. and Arntzen, C.J., eds.), pp. 1–84, Springer-Verlag, Berlin
13 Apel, K. and Kloppstech, K. (1978) Eur. J. Biochem. 85, 581–588
14 Cuming, A.C. and Bennett, J. (1981) Eur. J. Biochem. 118, 71–80
15 Apel, K. (1979) Eur. J. Biochem. 97, 183–188
16 Stiekema, W.J., Wimpee, C.F., Silverthorn, J. and Tobin, E.M. (1983) Plant Physiol. 72, 717–724
17 Apel, K. and Kloppstech, K. (1980) Planta 150, 426–430
18 Dunsmuir, P., Smith, S.M. and Bedbrook, J. (1983) J. Mol. Appl. Genet. 2, 285–300
19 Dunsmuir, P. (1985) Nucleic Acids Res. 13, 2503–2518
20 Larsson, U.K. and Andersson, B. (1985) Biochim. Biophys. Acta 809, 396–402
21 Larsson, U.K., Sundby, C. and Andersson, B. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. II, pp. 677–680, Martinus Nijhoff, Dordrecht
22 Jennings, R.C., Islam, K. and Zucchelli, G. (1986) Biochim. Biophys. Acta 850, 483–489
23 Andersson, B., Åkerlund, H.-E. and Albertsson, P.-Å. (1976) Biochim. Biophys. Acta 423, 122–132
24 Arnon, D.J. (1949) Plant Physiol. 24, 1–15
25 Andersson, B. and Anderson, J.M. (1980) Biochim. Biophys. Acta 593, 427–440
26 Anderson, J.M., Waldron, J.C. and Thorne, S.W. (1978) FEBS Lett. 92, 227–233
27 Laemmli, U.K. (1970) Nature 227, 680–685
28 Björkman, O., Boardman, N.K., Anderson, J.M., Thorne, S.W., Goodchild, D.J. and Pyliotis, N.A. (1972) Carnegie Inst. Washington Yearb. 72, 115–135
29 Goodchild, D.J., Björkman, O. and Pyliotis, N.A. (1972) Carnegie Inst. Washington Yearb. 72, 102–107
30 Ghirardi, M.L. and Melis, A. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. II, pp. 261–264, Martinus Nijhoff, Dordrecht
31 Larsson, U.K., Sundby, C. and Andersson, B. (1987) Biochim. Biophys. Acta 894, 59–68