Biochimica et Biophysica Acta 810 (1985) 295–301
Elsevier

BBA 41888

Mechanism of the light-state transition in photosynthesis. V. 77 K linear dichroism of *Anacystis nidulans* in State 1 and State 2

Doug Bruce and John Biggins

Division of Biology and Medicine, Brown University, Providence, RI 02912 (U.S.A.)

(Received July 18th, 1985)

Key words: Photosynthesis; State transition; Linear dichroism; Phycobilisome; Pigment orientation; (*A. nidulans*)

Linear-dichroism spectra of *Anacystis nidulans* at 77 K were determined for whole cells chemically fixed in light State 1 and light State 2. Whole cells were oriented by the squeezed gel technique using 5% gelatin 2.2 M sucrose gels. Peaks with positive dichroism were observed at 638 nm and 688 nm with shoulders at approx. 650 nm and 700 nm. The amplitude of the 650 nm shoulder was greater for cells in State 2 than those in State 1, and the State-2-minus-State 1 difference spectrum had a single peak at 656 nm. The linear dichroism spectrum of phycobilisomes isolated from *A. nidulans* showed peaks at 635 nm (phycocyanin) and 656 nm (allophycocyanin). The spectrum for thylakoid membranes free of phycobilisomes had one peak at 685 nm with a shoulder at 698 nm. We suggest that the change in dichroism at 656 nm between cells in State 1 and State 2 results from a change in orientation of the allophycocyanin core of the phycobilisome. This result is discussed in the context of our model for the light-state transition in phycobilisome-containing organisms.

Introduction

The distribution of excitation energy between PS II and PS I is regulated in both Chl *b*- and phycobilin-containing photosynthetic organisms [1–3]. The response of these organisms to preferential excitation of one photosystem is to compensate by redistributing the absorbed excitation energy more equally between the photosystems. This process of excitation energy redistribution between PS II and PS I has been called the light state transition after the original terminology of Bonaventura and Myers [1]. There is now substantial evidence that the mechanism for the light state transition in Chl *b*-containing organisms (green plants and green algae) is different from that in phycobilisome-containing organisms (rhodophyceae and cyanobacteria) [4–7].

In green plants the transition to State 2, following the preferential excitation of PS II, is triggered by reduced plastoquinone which activates a membrane-bound protein kinase. The kinase phosphorylates the LHC II in the grana membrane regions (rich in PS II) and the phosphorylated LHC II redistributes to the unstacked stroma membrane regions (rich in PS I). This lateral migration of LHC II from grana to stroma regions increases the absorbance cross-section of PS I at the expense of PS II, directly, by changing the relative size of the antenna pigment pools of PS II and PS I. Corresponding changes in electron transport for PS I and PS II then occur. The reverse transition to State 1 occurs upon either dark adaptation or preferential excitation of PS I, (oxidized plastoquinone) and is attended by dephosphoryla-

Abbreviations: PS, Photosystem; Chl, chlorophyll; LHC II, chlorophyll-*a*/*b* light-harvesting complex of Photosystem II; $NaKPO_4$, NaH_2PO_4 titrated with K_2HPO_4 to a given pH.

tion of the phosphorylated LHC II followed by lateral migration of the LHC II back into the appressed regions of the membrane. For an extensive review, see Ref. 8.

Work in this laboratory has established that the mechanism for the state transition in phycobilisome-containing organisms is different. No reversible protein phosphorylation event accompanying the state transition has been detected in these organisms, and the kinetics of the transition are much faster than those in green plants [5,6]. We have suggested that a small reversible change in thylakoid membrane conformation is responsible for regulating the efficiency of excitation energy transfer from PS II Chl *a* to PS I Chl *a* by changing the proximity between the two photosystems.

Time-resolved fluorescence spectroscopy has supported the contention that the mechanism involves a change in the efficiency of excitation energy transfer from PS II Chl *a* to PS I Chl *a* in both the red alga *Porphyridium cruentum* and the cyanobacterium *Anacystis nidulans* [7]. The efficiency of energy transfer from PS II Chl *a* to PS I Chl *a* could be affected by changes in the distance between Chl *a* molecules (and/or) changes in their orientation. Linear dichroism measurements have shown that light-harvesting pigments associated with PS II and PS I are oriented in whole cells, chloroplasts, thylakoid membranes and pigment-protein complexes in both Chl *b* containing and phycobilin-containing organisms [9–12]. Changes in linear dichroism have been reported to occur upon phosphorylation of the thylakoid membranes of pea [13]. These changes were shown to be dependent on polarized light scattering and related to the degree of membrane stacking rather than a reorientation of pigments.

The objective of this investigation was to determine and characterize possible changes in pigment orientation which might accompany the state transition in *A. nidulans*. The linear dichroism spectra of cells in State 1 and State 2 were found to differ and we suggest that a change in the orientation of a phycobilisome core component accompanies the state transition. This result is discussed in the context of our working model for the mechanism of the light-state transition in phycobilisome-containing organisms.

Materials and Methods

A. nidulans was grown autotrophically at a light intensity of 25 $\mu E \cdot m^{-2} \cdot s^{-1}$ on medium C of Kratz and Myers [14]. Cells were harvested in the late log growth phase. Cells used for linear dichroism measurements were chemically fixed in State 1 or State 2 using glutaraldehyde as previously described [4] with the following modifications. The cells were suspended in medium C during the fixation procedure and washed three times after fixation with 100 mM sucrose, 20 mM Tricine (pH 8). State 1 was induced by illumination with blue light (Corning glass 5.60 plus a 600 nm short pass filter) at 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ for 3 min prior to the addition of glutaraldehyde. State 2 was induced by exposure to 590 nm light (590 nm interference filter, Baird-Atomic), for 3 min at 30 $\mu E \cdot m^{-2} \cdot s^{-1}$.

Phycobilisomes from *A. nidulans* were isolated by the method of Glazer [15,16]. Three grams of previously frozen cells were resuspended to 0.12 g cells/ml in 0.65 M $NaKPO_4$ (pH 8) and passed through a French press three times. Cells were then incubated for 30 min in 2% Triton at room temperature with slow stirring. The cells were centrifuged at 31 000 × *g* for 30 min at 18°C and the resulting blue supernatant was collected. The supernatant was centrifuged for 15 h at 55 000 × *g* and the phycobilisome pellet resuspended in 0.65 M $NaKPO_4$ (pH 8). To prevent dissociation during the orientation procedure for the linear dichroism measurements, the phycobilisomes were cross-linked with glutaraldehyde as follows. Phycobilisomes were suspended in 50 ml 0.65 M $NaKPO_4$ (pH 8) and treated with 0.3% glutaraldehyde for 5 min. The excess glutaraldehyde was then quenched with glycine at a final concentration of 20 mM. The cross-linked phycobilisomes were then dialysed against 20 mM Tricine (pH 7.4) and separated from free phycocyanin (as detected by fluorescence spectroscopy) by centrifugation for 2–4 h at 30 000 × *g*. The phycobilisomes remained energetically well coupled after fixation and incorporation into gels as determined by low temperature fluorescence emission (data not shown).

Thylakoid membranes devoid of phycobilisomes were isolated using a modification of the technique described by Shatz and Witt [17]. Four

grams of previously frozen cells were incubated in 15 ml of 0.5 M mannitol/10 mm $MgCl_2$/2 mM K_2HPO_4/20 mM Hepes (pH 7.8) with 0.1% lysozyme for 1.5 h at 37°C. The cells were cooled to room temperature, and passed through a French press, osmotically lysed by resuspension in 100 ml 10 mM $MgCl_2$/2 mM K_2HPO_4/20 mM Hepes (pH 7.8) and centrifuged at $4000 \times g$ for 5 min to pellet remaining whole cells. The supernatant was then centrifuged at $30000 \times g$ for 20 min to pellet membranes. The membranes were resuspended and washed once with 0.5 M mannitol/10 mM $MgCl_2$/2 mM K_2HPO_4/20 mM Hepes (pH 7.8).

Whole cells, membranes and phycobilisomes were oriented for linear dichroism measurements using the squeezed gel technique [18]. The use of polyacrylamide and glycerol for gels, previously used for this technique [19], led to large changes in the low-temperature fluorescence emission spectra of the cells indicative of dissociation of phycobiliproteins from phycobilisomes and the thylakoid membrane. Consequently, a procedure was developed using gelatin and sucrose as a gel medium which was optimized to give excellent orientation and could also be cooled to 77 K without frosting or cracking. 5 ml hot stock gel solution (5% gelatin, 2.2 M sucrose) were allowed to cool slowly at room temperature and, just prior to solidification, 200 µl of the specimen of interest was mixed into the solution and poured into 8 mm by 5 cm casting blocks to a depth of 8 mm. The gels were allowed to solidify completely at 4°C for 20 min before use. Orientation was achieved by squeezing 8 mm by 8 mm by 2 cm gel blocks in one dimension in a specially designed cuvette to a final 8 mm by 4 mm by 4 cm. The cuvette consisted of an aluminum cold finger 8 mm thick, 2 cm wide and 10 cm long with a central aperture 8 mm wide and 4 cm long. One side of the aperture was covered with a 2 mm thick lucite window, and gel squeezing was accomplished with a lucite plunger 3.9 cm long, 7.5 mm wide and 6 mm thick which also formed the other window. Linear dichroism spectra were then determined at 77 K using the apparatus described previously [11]. Fluorescence emission spectra were measured with a Spex spectrofluorometer as described previously [4].

Results

Fig. 1 compares the 77 K fluorescence emission spectra of whole cells frozen in State 1 and State 2 in growth media to those of cells fixed in State 1 and State 2 with glutaraldehyde and incorporated

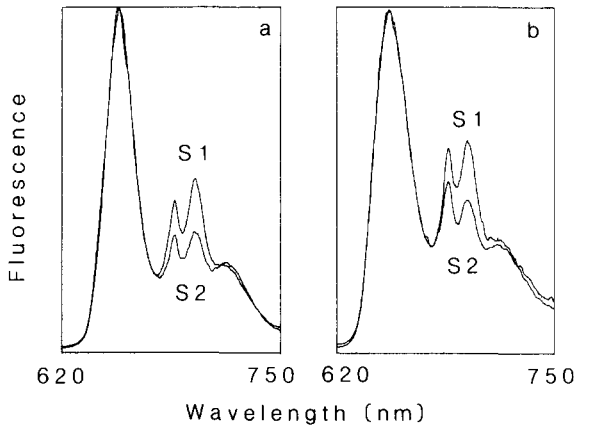

Fig. 1. 77 K fluorescence emission spectra of *A. nidulans* in State 1 and State 2. Excitation was at 590 nm and spectra were normalized at 650 nm. (a) Cells were suspended in growth media and brought to State 1 or State 2 by a 2 min exposure to blue (Corning glass 5.60 plus a 600 nm short pass filter) or green (590 nm interference filter) light before freezing. (b) Cells were chemically fixed in State 1 or State 2 with glutaraldehyde and incorporated into 5% gelatin, 2.2 M sucrose gels before freezing as described in Materials and Methods.

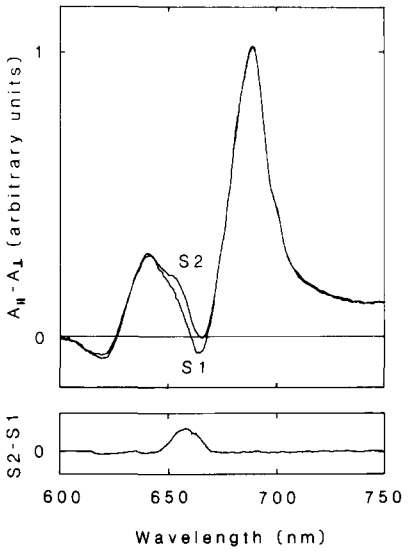

Fig. 2. 77 K linear dichroism spectra of the cells in Fig. 1b. The lower box shows the State 2-minus-State 1 difference spectrum. The dichroic ratio ($A_{\parallel}/A_{\perp}$) of the 688 nm peak was typically 1.1–1.3 for whole cells.

into 5% gelatin, 2.2 M sucrose gels. It can be seen that the fixation and orientation procedures do not detrimentally affect the characteristic fluorescence emission spectra of cells in State 1 and State 2.

Fig. 2 shows 77 K linear dichroism spectra of the glutaraldehyde fixed whole cells whose fluorescence emission spectra are shown in Fig. 1b. Positive peaks in linear dichroism are seen at 638 nm and 688 nm, and shoulders are consistently present at 650 nm and 700 nm. The 638 nm and 650 nm contributions are most likely due to phycocyanin and allophycocyanin, and the 688 nm and 700 nm bands from PS I Chl a antenna and P-700, respectively. PS II Chl a is expected to contribute to the short wavelength side of the 688 nm peak. The difference spectrum for cells in State 2 minus cells in State 1 shows one positive peak at 656 nm which has a width at half height of 15 nm. The change in linear dichroism observed between cells in State 1 and State 2 could be due to a change in orientation of a chromophore or could result from a wavelength-dependent change in polarized light scattering. Control experiments were, therefore, performed by measuring the polarized light scattering directly as previously described [13]. No differences were detected in the scattering spectra of the samples whose linear dichroism spectra are shown in Fig. 2 (data not shown).

The presence of allophycocyanin-containing complexes in the phycobilisome core of *A. nidulans* with absorbance maxima at 650 nm, 653 nm and 655 nm [20] suggested the possibility that one or more of these complexes may undergo a change in orientation which results in the linear dichroism difference spectrum shown in Fig. 2. To test this hypothesis and also determine if there were any contributions to the linear dichroism spectrum at 656 nm by components in the thylakoid membrane, linear dichroism spectra were measured for both isolated phycobilisomes and thylakoid membrane preparations (Fig. 3). The measurements in Fig. 3 were also much less sensitive to polarized scattering contributions due to the smaller particle size of phycobilisomes and thylakoid membrane fragments as compared to whole cells. The linear dichroism spectrum for phycobilisomes shows positive peaks at 635 nm and 656 nm with a prominent shoulder at 625 nm. The linear dichroism spectrum of thylakoids is characterized by a positive peak at 685 nm and a shoulder at 697 nm. The linear dichroism spectrum for the membrane preparation correlates well with that of whole cells in the region 660 nm to 750 nm although it is blue-shifted by approx. 3 nm. The phycobilisome linear dichroism spectrum shows an exaggerated 656 nm peak and an additional shoulder at 625 nm when compared to that for whole cells. This suggests that the orientation of isolated phycobilisomes in the gel is not identical to their orientation when attached to the thylakoid membrane. The larger amplitude of the 656 nm peak in the linear dichroism of the phycobilisome suggests that the phycobilisome core contributes to the anisotropy of the phycobilisome resulting in at least a partial alignment of the absorption dipoles of allophycocyanin with the stretch axis of the gel. Our results indicate that the change in linear dichroism at 656 nm, which is the only detectable change in linear dichroism between cells in State 1 and State 2 (Fig. 2), originates from a component in the phycobilisome rather than from the membrane.

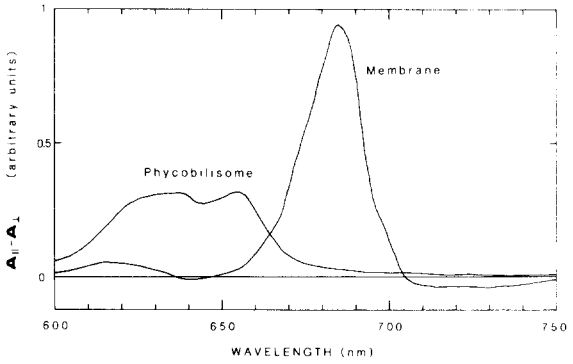

Fig. 3. 77 K linear dichroism spectra of isolated phycobilisomes and thylakoid membranes free of phycobilisomes. The two spectra have been normalized at 688 nm and 638 nm to the same relative heights of these two peaks in Fig. 2. The dichroic ratio ($A_{\parallel}/A_{\perp}$) was typically 1.3–1.5 for the 685 nm peak of membranes and 1.05–1.07 for the 656 nm peak of phycobilisomes.

Discussion

The data presented show that the transition from State 1 to State 2 in *A. nidulans* is accompanied by an increase in the amplitude of a positive peak in the low-temperature linear dichroism spectrum at 656 nm. We attribute the 656 nm linear dichroism signal to a change in orientation of the

phycobilisome core. This conclusion is supported by the presence of a 656 nm peak in the linear dichroism spectrum of isolated phycobilisomes of *A. nidulans*. A positive peak at 655 nm in the linear dichroism spectrum of isolated phycobilisomes from *P. cruentum* has been reported by Hoarau et al. [10], who showed that allophycocyanin was the most highly oriented chromophore in the phycobilisome. As can be seen directly from structural models for the phycobilisome of *A. nidulans* [20], core components have a much greater potential for orientation than rod components. The possibilities for orientation of the core which would lead to a positive dichroism peak are complicated by the in vivo organization of the thylakoid membranes in *A. nidulans*. *A. nidulans* is a rod-like cell whose thylakoid membranes are most easily modeled as concentric cylinders inside the cell. Orientation of whole cells by the gel-squeezing technique is expected to align the long axis of the cell with the stretch axis of the supporting gel. However, it is apparent that the thylakoid membranes will not be oriented planar to this stretch axis unless the cell is compressed by the gel squeezing technique which is unlikely due to the rigidity of the cell wall. Therefore, orientation of thylakoid membranes in situ is most likely confined to orientation of concentric cylinders along the stretch axis (Fig. 4b). Under these conditions, linear dichroism measurements will not be as sensitive to absorption dipole orientation with respect to the plane of the thylakoid membrane as they are for samples which have oriented planar membranes (Fig. 4a). This idea is supported by the fact that the dichroic ratio, $A_{\parallel}/A_{\perp}$, of the 688 nm peak in *A. nidulans* was lower in whole cells than in isolated thylakoid membrane preparations. The linear dichroism measurement is, however, sensitive to absorption dipoles which are oriented with respect to the long axis of the cell.

The observed change in dichroism of a core component between cells in State 1 and State 2 could result from a reorientation with respect to either the plane of the thylakoid membrane or to

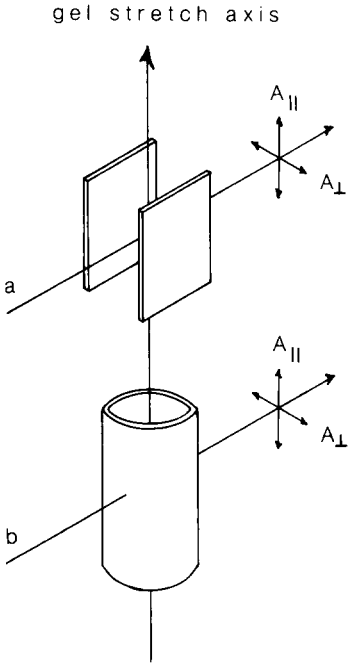

Fig. 4. Model for the presumed orientation of isolated thylakoid membrane fragments (a) and in vivo thylakoid membranes (b) of *A. nidulans* by the squeezed gel technique showing the orientation of $A_{\parallel}$ and $A_{\perp}$ with respect to the stretch axis of the gel.

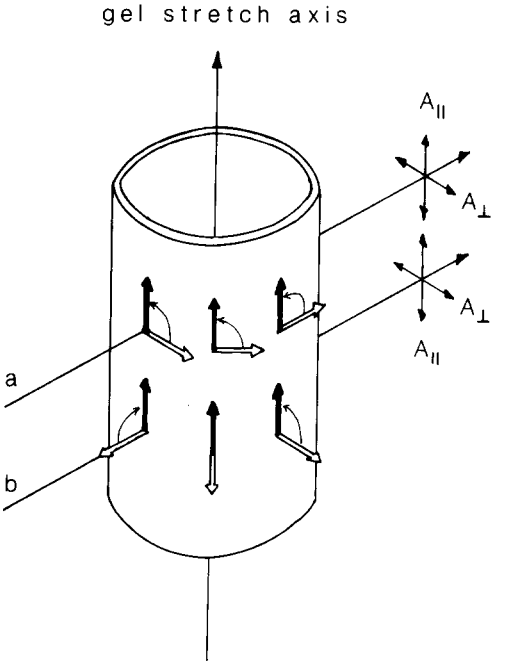

Fig. 5. Model for the in vivo orientation of the thylakoid membranes of *A. nidulans* by the squeezed gel technique showing possible orientation changes of an absorption dipole which would result in an increase in dichroism ($A_{\parallel} - A_{\perp}$). (a) Rotation of the dipole about an axis normal to the membrane surface which results in a net orientation along the stretch axis of the gel. (b) Tilt of the dipole into the membrane plane.

tilt of the phycobilisome core in the plane of the membrane (Fig. 5b) and the latter to a rotation of the core about an axis normal to the membrane surface (Fig. 5a). Due to the approximate radial symmetry of the phycocyanin rods, a rotation of the entire phycobilisome about an axis normal to the plane of the membrane would not influence their contribution to the linear dichroism spectrum, whereas a tilt of the whole phycobilisome with respect to the membrane plane would. Additionally, structural considerations such as the large size of the phycobilisome and its close association with the thylakoid membrane limit the degree of tilt possible. We suggest, therefore, that the specific change in linear dichroism we observed at 656 nm is either due to a change in orientation of a component of the allophycocyanin core itself or is a reflection of a rotational movement of the entire phycobilisome. The latter would have to result in an orientation of the core with respect to the long axis of the cell.

Our previous results with *A. nidulans* have shown no large differences in the fluorescence lifetimes of either phycocyanin or allophycocyanin between cells in State 1 and State 2 [7]. Therefore, if allophycocyanin undergoes re-orientation with respect to the phycobilisome, then it does so without affecting the efficiency of energy transfer from phycocyanin to allophycocyanin to the terminal phycobilin emitter. It seems more likely that the observed change in dichroism at 656 nm is a reflection of total phycobilisome reorientation. Furthermore, as the phycobilisome core is relatively tightly bound to PS II [17], and the fluorescence lifetime of the terminal emitter of the phycobilisome core is not affected as much as the lifetime of PS II Chl *a* during the state transition [7] it becomes apparent that the orientation change observed for the phycobilisome core component may be an indicator of a concerted movement of both phycobilisome and PS II together. A reorientation of the phycobilisome/PS II complex in the thylakoid could be responsible for regulating the efficiency of energy transfer between PS II Chl *a* and PS I Chl *a* by changing the proximity between PS II and PS I. This mechanism is consistent with both our previous fluorescence lifetime determinations and the present linear dichroism data.

No state-transition-induced changes in linear dichroism were detected for any Chl *a* contribution to the spectrum; this was also true in the Soret region (data not shown). Typically 80–90% of the Chl *a* in *A. nidulans* is associated with PS I (Wang et al., 1977) and the 688 nm peak and 700 nm shoulder may be tentatively attributed to the PS I Chl *a* antenna and to P-700, respectively. Our results indicate that there is no detectable change in the orientation of PS I Chl *a* with respect to the plane of the thylakoid membrane (tilt) accompanying the state transition. However, as the in vivo orientation of PS I Chl *a* with respect to the long axis of the cell may or may not be random, we are unable to determine whether a rotation of PS I Chl *a* about an axis perpendicular to the membrane plane occurs.

In summary, our data support a mechanism for the state transition which involves a conformational change of the photosynthetic apparatus in *A. nidulans*. We propose that the change in linear dichroism in the State 2-minus-State 1 linear dichroism difference spectrum is a reflection of a change in orientation of the phycobilisome/PS II complex. This structural change may be responsible in part for the change in energy-transfer efficiency from PS II Chl *a* to PS I Chl *a* previously proposed to be the mechanism of energy redistribution in *A. nidulans*.

Acknowledgements

The authors wish to thank Dr. Jacques Breton for helpful suggestions and discussions during the completion of this work. The research was supported by the Science and Educational Administration of the United States Department of Agriculture under Grant Number 81-CRCR-1-0767 from the Competitive Research Grants Office and PCM-8302983 from the National Science Foundation. In addition, D.B. gratefully acknowledges the support of a Natural Sciences and Research Council of Canada postdoctoral fellowship.

References

1 Bonaventura, C. and Myers, J. (1969) Biochim. Biophys. Acta 189, 366–383

2 Murata, N. (1969) Biochim. Biophys. Acta 172, 242–251

3 Allen, J.F., Bennett, J., Steinback, K.E. and Arntzen, C.J. (1981) Nature (London) 291, 25–29
4 Biggins, J. (1983) Biochim. Biophys. Acta 724, 111–117
5 Biggins, J., Campbell, C. and Bruce, D. (1984) Biochim. Biophys. Acta 767, 138–144
6 Biggins, J. and Bruce, D. (1985) Biochim. Biophys. Acta 806, 230–236
7 Bruce, D., Steiner, T., Biggins, J. and Thewalt, M. (1985) Biochim. Biophys. Acta 806, 237–246
8 Staehelin, L.A. and Arntzen, C.J. (1983) J. Cell Biol. 97, 1327–1337
9 Breton, J., Michel-Villaz, M. and Paillotin, G. (1973) Biochim. Biophys. Acta 314, 42–56
10 Hoarau, J., Gagliano, A.G., Geacintov, N.E. and Breton, J. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 705–708, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands
11 Biggins, J. and Svejkovsky, J. (1980) Biochim. Biophys. Acta 592, 565–576
12 Kramer, H. and Amesz, J. (1982) Biochim. Biophys. Acta 682, 201–207
13 Biggins, J. (1982) Biochim. Biophys. Acta 679, 479–482
14 Kratz, W.A. and Myers, J. (1955) Am. J. Bot. 43, 282–287
15 Yamanaka, G., Glazer, A. and Williams, R. (1978) J. Biol. Chem. 253, 8303–8310
16 Lundell, D.J., Williams, R. and Glazer, A. (1981) J. Biol. Chem. 256, 3580–3592
17 Shatz, G.H. and Witt, H.T. (1984) Photobiochem. Photobiophys. 7, 1–14
18 Adbourakhmanov, I.A., Ganago, A.O., Erokhim, Yu.E., Solovev, A.A. and Chugunov, V.A. (1979) Biochim. Biophys. Acta 546, 183–186
19 Haworth, P., Arntzen, C.J., Tapie, P. and Breton, J. (1982) Biochim. Biophys. Acta 679, 428–435
20 Glazer, A. (1984) Biochim. Biophys. Acta 768, 29–51
21 Wang, R.T., Stevens, C.L.R. and Myers, J. (1977) Photochem. Photobiol. 25, 103–108