Biochimica et Biophysica Acta, 975 (1989) 277–282
Elsevier

BBABIO 43025

Effects of bicarbonate on thylakoid protein phosphorylation

Cecilia Sundby, Ulla K. Larsson and Tomas Henrysson

Department of Biochemistry, University of Lund, Lund (Sweden)

(Received 23 January 1989)

Key words: Protein phosphorylation; Bicarbonate effect; Photoinhibition; Light harvesting complex; Thylakoid membrane; (Spinach)

The addition of $NaHCO_3$ to either intact chloroplasts or isolated thylakoid membranes was found to affect thylakoid protein phosphorylation. It was found that bicarbonate highly stimulated the [^{32}P]phosphate incorporation into the 25 kDa polypeptide of LHC II, whereas phosphorylation of the 27 kDa polypeptide of LHC II was unaffected. Addition of $NaHCO_3$ had a negative influence on the phosphorylation of the 9 kDa phosphoprotein of PS II. Moreover, depletion of endogenous bicarbonate stimulated its phosphorylation. These data indicate that it is the phosphorylation of the 25-kDa-containing peripheral pool of LHC II that is under the regulation of bicarbonate. In addition, $NaHCO_3$ also seems to prevent photoinhibition.

Introduction

In the last years many studies have focused on light-induced thylakoid phosphorylation and its role in the regulation of excitation energy distribution. There are several pieces of evidence that phosphorylation of the LHC II chlorophyll–protein complex causes a decrease in the antenna size of PS II [1–4]. However, there are conflicting reports concerning the effects of phosphorylation on both the PS I antenna and the so-called state transitions [3–8]. Thylakoid phosphorylation has also been suggested to be protective against photoinhibition [9] and to be affected by variations in the metabolic state of the chloroplast [10–12].

Upon light-induced thylakoid phosphorylation, phosphate groups are incorporated mainly into the 25 and 27 kDa polypeptides of LHC II and into the 9 kDa phosphoprotein of PS II [1,13]. The LHC II contains more 27 than 25 kDa polypeptides, but the 25 kDa polypeptide has higher specific phosphate incorporation and becomes phosphorylated on a much faster timescale than the 27 kDa polypeptide [14,15]. The 25 kDa polypeptide is probably present only in the so-called peripheral pool of LHC II [15,16], which, upon phosphorylation, dissociates from the PS II core and its tightly bound pool of LHC II. The LHC II that is tightly bound to the PS II core consists merely of 27 kDa polypeptides. The 9 kDa phosphoprotein of PS II has been purified and sequenced [17,18], but its function in the thylakoid membrane is hitherto unknown. It has been suggested that its phosphorylation is involved in regulation of the PS II-dependent electron flow [19].

We started out to study the phosphate incorporation into thylakoid polypeptides when intact chloroplasts were or were not supplied with $NaHCO_3$ for CO_2 fixation during illumination. We found that $NaHCO_3$ has a pronounced effect on the light-induced thylakoid phosphorylation, that it is acting directly on the thylakoid membrane and that it affects phosphorylation of the three phosphoproteins in very different ways.

Materials and Methods

Preparation of intact chloroplasts and of isolated thylakoid membranes. Intact chloroplasts were prepared essentially according to Ref. 20 from spinach (*Spinacia oleracea* L.) and suspended in 50 mM Hepes (pH 7.6)/2 mM EDTA/0.5 mM K_2HPO_4/10 mM NaCl/0.33 M sorbitol. Stacked thylakoid membranes were isolated according to Ref. 21 and suspended in 50 mM Tricine (pH 7.2)/20 mM NaCl/5 mM $MgCl_2$/100 mM sorbitol.

Phosphorylation of chloroplasts and of thylakoids. Just before phosphorylation chloroplasts or thylakoids were

Abbreviations: PS I, Photosystem I, PS II, Photosystem II; LHC II, light-harvesting chlorophyll *a*/*b*–protein complex of PS II; PpBQ, phenyl-*p*-benzoquinone; SDS-PAGE, sodium dodecylsulphate polyacrylamide gel electrophoresis; Tricine, *N*-(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)glycine; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid, Chl, chlorophyll.

Correspondence: C. Sundby, Department of Biochemistry, University of Lund, P.O. Box 124, S-221 00 Lund, Sweden.

diluted to 200 μg Chl/ml with the media above. Phosphorylation was carried out in presence or absence of added bicarbonate. Where indicated, 20 mM $NaHCO_3$ was added to dark-adapted samples from a freshly prepared 0.5 M stock solution 2 min before phosphorylation was started. To all samples 10 mM NaF was supplied to inhibit phosphatase activity. Intact chloroplasts were then supplied with carrier-free [^{32}P]orthophosphate (120 μCi/ml) and isolated thylakoids were given 0.4 mM [γ-^{32}P]ATP (300000 cpm/nmol ATP). Phosphorylation was performed at 20°C by 10 min illumination with red light (greater than 600 nm) at 200–15000 $\mu E \cdot m^{-2} \cdot s^{-1}$.

Bicarbonate depletion of thylakoids. In case of bicarbonate depletion prior to phosphorylation, the thylakoids were suspended at 600 μg Chl/ml in 20 mM sodium formate, 15 mM Tricine (pH 5.8), 20 mM NaCl, 5 mM $MgCl_2$, 100 mM sorbitol and bubbled with N_2 gas for 30–60 min. Control thylakoids were treated likewise but with no addition of formate. After incubation, samples were diluted 3-fold with 15 mM Tricine (pH 7.8)/20 mM NaCl/5 mM $MgCl_2$/100 mM sorbitol to yield pH 7.2. Thereafter, phosphorylation was immediately performed as described above. To check that bicarbonate depletion indeed was obtained, a 100 μl sample was withdrawn before phosphorylation, added to 0.9 ml PS II activity assay medium (see below) and the PS II activity was directly determined oxygraphically with or without the addition of 2 mM $NaHCO_3$.

Analysis of ^{32}P incorporation into thylakoid polypeptides. Immediately after the phosphorylating illumination, samples were spun down and the thylakoids were solubilized for electrophoresis. In the case of intact chloroplasts, these were osmotically broken and their thylakoid membranes were washed twice to remove stromal proteins prior to solubilization. SDS-PAGE was performed in the gel system of Ref. 22. For quantification of [^{32}P]phosphate incorporation, the polypeptide bands corresponding to the 25 and 27 kDa apopolypeptides of LHC II and the 9 kDa phosphoprotein of PS II were excised from the gels, solubilized in H_2O_2/perchloric acid and counted for radioactivity.

PS II activity measurements. To estimate the PS II activity after phosphorylation, samples were spun down from the media in which they were phosphorylated and the PS-II-dependent electron flow from H_2O to phenyl-*p*-benzoquinone (PpBQ) was measured in a Clark type of oxygen electrode with thylakoids corresponding to 20 μg Chl/ml in 10 mM sodium phosphate buffer (pH 7.4)/5 mM NaCl/5 mM $MgCl_2$, 10 mM NaF, 100 mM sucrose and 0.3 mM PpBQ. In the cases where intact chloroplasts had been phosphorylated these were osmotically broken and their thylakoid membranes were washed twice before measurement. Excitation light was passed through a red light filter (> 600 nm) and was of saturating light intensity.

Determination of PS II antenna size from activity measurements. PS II activity light-saturation curves were recorded by varying the light intensity of the excitation light. The light-saturation curves were transformed into Eadie-Hofstee plots where V/I (= PS II activity in μmol O_2/mg Chl per h divided by light intensity) is plotted versus V (see Ref. 3).

Results

When intact chloroplasts were illuminated, the [^{32}P]phosphate incorporation into the 25 kDa polypeptide of LHC II was highly stimulated by the presence of 20 mM $NaHCO_3$ (Table I). The bicarbonate stimulation was 30–40% and fairly independent of the light intensity used during phosphorylation. Phosphorylation as well as its bicarbonate stimulation saturated already at 200 $\mu E \cdot m^{-2} \cdot s^{-1}$. In contrast to the 25 kDa polypeptide, phosphorylation of the 27 kDa polypeptide of LHC II was not affected by the presence of $NaHCO_3$ during phosphorylation, at least not at lower light intensities. At higher intensities a small stimulation was seen, but this was rather due to a decrease in the [^{32}P]phosphate incorporation in control chloroplasts than to a positive effect by $NaHCO_3$. By contrast, for the 9 kDa polypeptide of PS II the phosphorylation was negatively influenced by bicarbonate. The [^{32}P]phosphate incorporation into this polypeptide markedly decreased (−25%) when phosphorylation was performed in the presence of $NaHCO_3$.

To test whether the bicarbonate stimulation is an effect of its functioning as a final electron acceptor in ongoing CO_2 fixation in the intact chloroplasts or if it even affects protein phosphorylation directly on the thylakoid membrane level, phosphorylation was also performed with isolated thylakoid membranes. In Table II is seen that addition of $NaHCO_3$ to the thylakoid membranes before phosphorylation caused a stimulation of the [^{32}P]phosphate incorporation into the 25 kDa polypeptide (+44%). Phosphorylation of the 27 kDa polypeptide was not, or very weakly, affected by $NaHCO_3$ (+4%). Finally, phosphorylation of the 9 kDa polypeptide is negatively affected by the addition of $NaHCO_3$ (−10%). The kinase responsible for phosphorylation is thought to be regulated by the redox state of the plastoquinone pool [23] or the cytochrome *b/f* complex [24–26] and differences in electron transfer may therefore activate the kinase to different degrees. We therefore measured the actual electron flow at the beginning of phosphorylation and found that under these conditions (200 μg Chl/ml, no PpBQ present) the activity, a pseudocyclic electron flow with oxygen as final electron acceptor, detected as an oxygen consumption [27], was the same with and without added bicarbonate (data not shown).

TABLE I

Effect of bicarbonate on light-induced phosphorylation of intact chloroplasts

Intact chloroplasts were supplied with [^{32}P]phosphate and illuminated with red light of the indicated intensity for 10 min, in the absence (Cl I control) or presence (Cl I $NaHCO_3$) of 20 mM $NaHCO_3$. After phosphorylation the chloroplasts were osmotically broken and spun down. Their thylakoid membranes were washed and thereafter solubilized for SDS-PAGE. After electrophoresis the individual polypeptide bands were excised from the gel and counted for radioactivity. The total incorporation of [^{32}P]phosphate into the 25 and 27 kDa apopolypeptides of LHC II and of the 9 kDa PS II protein is expressed in cpm.

Light intensity		Total incorporation of [^{32}P]phosphate (cpm)		
		25 kDa	27 kDa	9 kDa
200 $\mu E \cdot m^{-2} \cdot s^{-1}$	Cl I control	12276	16791	6373
	Cl I $NaHCO_3$	17239	16881	4808
	effect of $NaHCO_3$	+40%	0%	−24%
1000 $\mu E \cdot m^{-2} \cdot s^{-1}$	Cl I control	13281	17058	7852
	Cl I $NaHCO_3$	18008	17007	5866
	effect of $NaHCO_3$	+36%	0%	−25%
400 $\mu E \cdot m^{-2} \cdot s^{-1}$	Cl I control	12008	15548	6870
	Cl I $NaHCO_3$	15400	17194	5578
	effect of $NaHCO_3$	+28%	+12%	−19%
15000 $\mu E \cdot m^{-2} \cdot s^{-1}$	Cl I control	12488	15142	8266
	Cl I $NaHCO_3$	16326	17548	6197
	effect of $NaHCO_3$	+31%	+16%	−25%

As seen in Tables I and II, bicarbonate affects phosphorylation very similarly in intact chloroplasts and thylakoid membranes. Therefore bicarbonate stimulation of phosphorylation must be a direct effect on the thylakoid membrane. One such direct bicarbonate effect on the thylakoid membranes has been described previously [28–30], namely a stimulatory effect of $NaHCO_3$ on the Hill reaction, especially after bicarbonate depletion by formate treatment. In order to optimize the bicarbonate effect seen on protein phosphorylation, we incubated isolated thylakoid membranes with sodium formate at low pH to obtain bicarbonate depletion. We obtained a partial depletion as judged from withdrawn samples, that were stimulated 2–3 -fold by the addition of 2 mM $NaHCO_3$ to the assay medium, whereas control thylakoids were not (not shown). After depletion treatment the thylakoids were phosphorylated in the absence or presence of $NaHCO_3$. We then found that the negative influence of bicarbonate on the phosphorylation of the 9 kDa polypeptide was seen even more clearly with this approach (Table III). While previous data (Table I and II) already indicated a negative influence of added $NaHCO_3$ on its phosphorylation, removal of endogenous $NaHCO_3$ markedly stimulated the [^{32}P]phosphate incorporation, by 58%. The phosphorylation of the two LHC II polypeptides was not much affected by the $NaHCO_3$ depletion. Readdition of $NaHCO_3$ to the depleted thylakoid membranes again showed a negative effect on the phosphorylation of the 9 kDa polypeptide (data not shown).

To test if the changed phosphorylation of the 9 kDa PS II protein leads to any change in PS II activity, we

TABLE II

Effect of bicarbonate on light-induced phosphorylation of isolated thylakoid membranes

Isolated thylakoid membranes were supplied with [γ-^{32}P]ATP and illuminated with red light (200 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 10 min in the absence (Cl II control) or presence (Cl II $NaHCO_3$) of 20 mM $NaHCO_3$. The total incorporation of [^{32}P]phosphate into the 25, 27 and 9 kDa polypeptides was quantified as described in Table I.

	Total incorporation of [^{32}P]phosphate (cpm)		
	25 kDa	27 kDa	9 kDa
Cl II control	21903	49681	15259
Cl II $NaHCO_3$	31620	51934	13674
Effect of $NaHCO_3$	+44%	+4%	−10%

TABLE III

Effect of bicarbonate depletion by sodium formate on light-induced phosphorylation of isolated thylakoid membranes

Isolated thylakoid membranes were depleted of bicarbonate in 20 mM sodium formate pH 5.8 at 20 °C for 60 min (Cl II formate). Control thylakoids were likewise treated without the addition of formate (Cl II control). Thereafter the thylakoids were supplied with [γ-^{32}P]ATP and illuminated with red light (200 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 10 min. The total incorporation into the 25, 27 and 9 kDa polypeptides was quantified as described in Table I.

	Total incorporation of [^{32}P]phosphate (cpm)		
	25 kDa	27 kDa	9 kDa
Cl II control	1299	1778	231
Cl II formate	1469	1765	364
Effect of formate	+13%	−7%	+58%

measured the light-saturated PpBQ-dependent oxygen evolution after phosphorylation (Table IV). To distinguish an effect of phosphorylation from direct effects of formate/bicarbonate on the PS II activity [28–30], the different samples, all supplied with NaF to prevent dephosphorylation, were spun down and resuspended in an assay medium containing neither formate nor bicarbonate. We found that, although there is a 24% difference in phosphorylation of the 9 kDa protein between intact chloroplasts phosphorylated in the absence and presence of bicarbonate (Table I), their thylakoid membranes show the same PS II activity (Table IV, Cl I control and Cl I $NaHCO_3$). The 58% higher phosphorylation of the 9 kDa protein yielded for thylakoid membranes that were bicarbonate depleted by formate before phosphorylation (Table III) did not differ much in PS II activity compared to the control either (Table IV, Cl II control and Cl II formate). However, in this case the comparison of the PS II activities is less certain, since the illumination used to induce phosphorylation in itself caused a decrease in PS II activity of the control thylakoid membranes (Table IV, Cl II control), and presumably also of the formate treated thylakoid membranes (Cl II formate). Notably, the addition of $NaHCO_3$ before phosphorylation seemed to prevent this photoinhibition (Cl II $NaHCO_3$ and Cl II formate/$NaHCO_3$). That bicarbonate protects the thylakoid membrane against photoinhibition is an interesting and new observation. Partly it may be explained by a smaller LHC II antenna (see below).

It has previously been shown that phosphorylation causes a 15% decrease in the size of the LHC II antenna [3]. Is the bicarbonate stimulated increase in LHC II phosphate incorporation reflected in a further decreased antenna size compared to phosphorylation without $NaHCO_3$? To test this we phosphorylated intact chloroplasts in the presence or absence of $NaHCO_3$. After phosphorylation the chloroplasts were osmotically broken and the PS-II-dependent electron flow was measured as a function of light intensity (Fig. 1). As a measure of the antenna size we used $(-1/K_M$, where K_M is the required light intensity for half-maximal activity ($\frac{1}{2} V_{max}$). $-1/K_M$ is obtained if the data of Fig. 1 are transferred into an Eadie-Hofstee plot (inserted in Fig. 1). We found that the 40% higher phosphate incorporation into the 25 kDa polypeptide that was obtained in the presence of $NaHCO_3$ (Table I) was indeed reflected in a 31% smaller PS II antenna, comparing the antenna resulting from phosphorylation of intact chloroplasts in the presence and absence of $NaHCO_3$ (Fig. 1). Essentially the same results were obtained when thylakoid membranes were phosphorylated with and without $NaHCO_3$ (not shown).

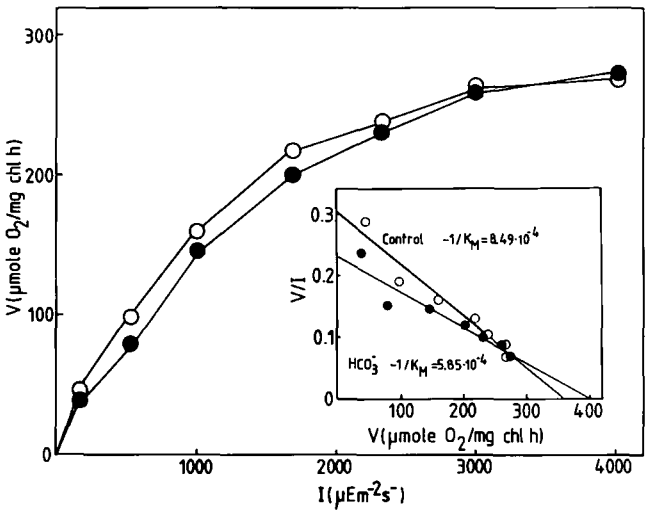

Fig. 1. Intact chloroplasts was phosphorylated in the presence (●) or absence (○) of $NaHCO_3$ and then osmotically broken. PS-II-dependent electron flow was measured as a function of light intensity. As a measure of the antenna size we used $-1/K_M$, which was obtained as the slope when the data of Fig. 1 were transformed into an Eadie-Hofstee plot (inset).

TABLE IV

Light-induced phosphorylation of intact chloroplasts and of isolated thylakoid membranes. Effects on PS II dependent electron flow

Intact chloroplasts (Cl I) or isolated thylakoid membranes (Cl II, treated as described in Table III) were phosphorylated at 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ in the absence and presence of $NaHCO_3$. After phosphorylation all samples were spun down and resuspended in assay medium. In case of intact chloroplasts these were osmotically broken and their thylakoid membranes washed twice before resuspension. The oxygen evolution was measured with a Clark-type oxygen electrode in an assay medium containing 10 mM sodium phosphate buffer (pH 7.4)/5 mM NaCl/5 mM $MgCl_2$/10 mM NaF/100 mM sucrose and thylakoid membranes corresponding to 20 μg Chl/ml. Phenyl-*p*-benzoquinone (0.3 mM) was added to the oxygraph.

	PS II activity (μmol O_2/mg Chl per h)	
	nonphosphorylated	phosphorylated
Cl I control	266	270
Cl I $NaHCO_3$	275	273
Cl II control	102	68
Cl II $NaHCO_3$	114	112
Cl II formate	118	76
Cl II formate/$NaHCO_3$	114	119

Discussion

The results presented in this paper taken together demonstrate that thylakoid protein phosphorylation is markedly influenced by bicarbonate. Interestingly, there are reports claiming that under in vivo conditions phosphorylation depends on variations in the demand for NADPH and ATP by the carbon reductive cycle [10–12]. Thus, the bicarbonate stimulation of LHC II phosphorylation we have found could be the actual mechanism by which the carbon reductive cycle regulates phosphorylation.

Upon phosphorylation both the 25 and 27 kDa polypeptides of LHC II becomes phosphorylated. However, it is only the 25 kDa containing peripheral pool of LHC II that migrates away from the PS II core and not the tightly bound pool of LHC II from which the 25 kDa polypeptide is absent [15,16]. It has furthermore been demonstrated that the amount of 25 kDa polypeptide in LHC II varies as an adaption to different light conditions during growth [31]. Our present data show that although both the 27 and 25 kDa LHC II polypeptides become phosphorylated it is only the phosphorylation of the 25 kDa polypeptide that is bicarbonate stimulated. An important conclusion is therefore that it is only the phosphorylation of the 25-kDa-containing pool peripheral pool of LHC II that is under the regulation of bicarbonate. This further strengthens the notion that it is the 25-kDa-containing peripheral pool of LHC II that is the variable part of the light-absorbing system [15,31]. In addition, the fact that the phosphorylation of the 27 kDa polypeptide of LHC II is not affected by bicarbonate may also hint that the phosphorylation of the 27 kDa polypeptide may serve a purpose other than phosphorylation of the 25-kDa-containing variable part of LHC II. This would obscure any discussion concerning the role of LHC II phosphorylation.

For the 9 kDa polypeptide of PS II it has been claimed that there is a correlation between its phosphorylation and a decreased electron flow from H_2O to ferricyanide/benzoquinone [19]. However, our own set of PS II-activity measurements in Table IV do not support this. Nevertheless, formate treatment of thylakoid membranes to increase phosphorylation of the 9 kDa protein should be a fruitful future approach to investigate the role of the phosphorylation of the 9 kDa PS II protein.

The existence of two different types of bicarbonate site in the thylakoid membrane has been reported [32]. The high affinity bicarbonate site is suggested to be located within or in a close proximity to the so-called Q_B protein [33–35]. The other type of bicarbonate site reported is a low-affinity site whose location is unknown [32]. One may speculate that, since the phosphorylation of the 9 kDa polypeptide is affected pronouncedly by the bicarbonate depletion treatment, its phosphorylation may be connected to the high-affinity site. This site may even be located on the 9 kDa polypeptide, which thus should be in close proximity to the Q_B protein. If the bicarbonate high-affinity binding site is on the 9 kDa protein, its function in the membrane would obviously involve regulation of the PS-II-dependent electron flow. Even more speculative, as the phosphorylation of the 25 kDa LHC II polypeptide is affected mainly when the $NaHCO_3$ concentration is raised from the endogenous level up to 20 mM, its phosphorylation may rather be connected with the low-affinity site. This site could then be on the 25 kDa polypeptide of LHC II. Thus, our data would imply a hitherto unknown way of regulating phosphorylation, where a prerequisite for kinase activity should still be the reduction of the plastoquinone pool [23] or the cytochrome *b/f* complex [24–26]; but if this criterion is fulfilled, the degree of phosphorylation would depend on the amount of bicarbonate bound to the 25 kDa polypeptide of LHC II.

Acknowledgements

We thank Ms. Maria Mattsson for excellent technical assistance. This work was supported by the Swedish Natural Research Council and the Carl Trygger Foundation.

References

1 Steinback, K.E., Bose, S. and Kyle, D.J. (1982) Arch. Biochem. Biophys. 216, 356–361.
2 Horton, P. and Lee, P. (1984) Biochim. Biophys. Acta 767, 563–567.
3 Larsson, U.K., Ögren, E., Öquist, G. and Andersson, B. (1986) Photobiochem. Photobiophys. 13, 29–39.
4 Deng, X. and Melis, A. (1986) Photobiochem. Photobiophys. 13, 41–52.
5 Farchaus, J.W., Widger, W.R., Cramer, W.A. and Dilley, R.A. (1982) Arch. Biochem. Biophys. 217, 362–367.
6 Haworth, P., Kyle, D.J. and Arntzen, C.J. (1982) Biochim. Biophys. Acta 680, 343–351.
7 Bonaventura, C. and Meyers, J. (1969) Biochim. Biophys. Acta 189, 366–383.
8 Williams, W.P. and Allen, J.F. (1987) Photosynth. Res. 13, 19–45.
9 Horton, P. and Lee, P. (1985) Planta 165, 37–42.
10 Baker, N.R., Markwell, J.P. and Thornber, J.P. (1983) Photobiochem. Photobiophys. 5, 181–190.
11 Horton, P. (1983) FEBS Lett. 152, 47–52.
12 Fernyhough, P., Horton, P. and Foyer, C. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. III, pp. 299–302, Martinus Nijhoff/Dr. W. Junk, Dordrecht.
13 Bennett, J. (1984) Physiol. Plant. 60, 583–590.
14 Jennings, R.C., Islam, K. and Zucchelli, G. (1986) Biochim. Biophys. Acta 850, 483–489.
15 Larsson, U.K., Sundby, C. and Andersson, B. (1987) Biochim. Biophys. Acta 894, 59–68.
16 Spangfort, M., Larsson, U.K., Anderson, J.M. and Andersson, B. (1987) FEBS Lett. 224, 343–347.
17 Widger, W.R., Farchaus, J.W., Cramer, W.A. and Dilley, R.A. (1984) Arch. Biochem. Biophys. 233, 72–79.
18 Allen, J.F. and Findlay, J.B.C. (1986) Biochem. Biophys. Res. Commun. 138, 146–152.
19 Packham, N.K. (1987) Biochim. Biophys. Acta 893, 259–266.
20 Jensen, R.G. and Bassham, J.A. (1966) Proc. Natl. Acad. Sci. USA 56, 1095–1101.
21 Andersson, B., Åkerlund, H.-E. and Albertsson, P.-Å. (1976) Biochim. Biophys. Acta 423, 122–132.
22 Laemmli, U.K. (1970) Nature 227, 680–685.
23 Horton, P., Allen, J.F., Black, M.T. and Bennett, J. (1981) FEBS Lett. 125, 193–196.
24 Bennet, J., Shaw, E.K. and Baker, S. (1987) FEBS Lett. 210, 22–26.
25 Gal, J.N., Shahak, Y., Shuster, G. and Ohad, I. (1987) FEBS Lett. 221, 205–210.

26 Wollman, F.A. and Lemaire, C. (1988) Biochim. Biophys. Acta 933, 85–94.
27 Mehler, A.H. (1951) Arch. Biochem. Biophys. 34, 339–351.
28 Warburg, O. and Krippahl, G. (1960) Z. Naturforsch. 156, 367–369.
29 Govindjee and Van Rensen, J.J.S. (1978) Biochim. Biophys. Acta 505, 183–213.
30 Vermaas, W.F.J. and Van Rensen, J.J.S. (1981) Biochim. Biophys. Acta 636, 168–174.
31 Larsson, U.K., Anderson, J.M. and Andersson, B. (1987) Biochim. Biophys. Acta 894, 69–75.
32 Stemler, A. (1977) Biochim. Biophys. Acta 460, 511–522.
33 Khanna, R., Govindjee and Wydrzynski, T. (1977) Biochim. Biophys. Acta 462, 208–214.
34 Snel, J.F.H. and Van Rensen, J.J.S. (1983) Physiol. Plant. 57, 422–427.
35 Nugent, J.H.A., Corrie, A.R., Demetriou, C., Evans, M.C.W. and Lockett, C.J. (1988) FEBS Lett. 235, 71–75.