Photosynthesis Research 10: 393–403 (1986)

ON THE STRUCTURE AND FUNCTION OF CYTOCHROME b-559

W. A. CRAMER*, S. M. THEG, and W. R. WIDGER

ABSTRACT. A summary of biochemical, biophysical, and molecular biological data is presented which led to the identification of two different polypeptides (α and β, MW = 9.16 and 4.27 kDa) in the cytochrome b-559 protein. The presence of a single His residue on each polypeptide, and the conclusion from spectroscopy that the heme coordination must be bis-histidine led to an obligatory requirement for coordination of a single heme through a heme cross-linked dimer. This structure does not have a precedent among soluble or membrane bound cytochromes. The possible participation of the cytochrome in the pathway of photoactivation is discussed.

I. *Introduction*

Cytochrome b-559 is an intrinsic polypeptide of photosystem II (1), and its M_r 10,000 polypeptide is recognized in SDS-PAGE gels of PSII particles (e.g., ref. 2). The location of the cytochrome genes (3), as well as their nucleotide sequence in the spinach plastid chromosome (4), is known. The presence of a cytochrome b-559 polypeptide in the minimum PSII complement naturally suggested a function associated with the water splitting reactions. The following such functions have been proposed: (i) A redox function for cytochrome b-559 in water splitting (5,6), as well (ii) as a function in which the reduced cytochrome might function as an H^+ acceptor of the water splitting reactions (7,8); (iii) a cycle around PSII (9-10), based initially on the discovery of b-559 photooxidation by PSII at 77°K (12); consistent with such a cycle are the proposals (iv) that the cytochrome may function as a donor to the PSII donor, Z^+, or to ADRY-like compounds interacting with Z^+, inferred from effects of ADRY reagents on cytochrome turnover (13-15) and Z^+ reduction (16), or (v) in a cycle that functions in photoheterotrophic but not autotrophic algae (17).

Regarding structure, recent advances in understanding of the amino acid composition, heme coordination, nucleotide and amino acid sequence, and polypeptide composition of cytochrome b-559 have relied on a combination of biochemical, biophysical, and molecular biological approaches (3,4,18-20). These data have led to conclusions concerning the arrangement of the cytochrome in the membrane (21, see below):

II. *Studies on the Structure of Cytochrome b-559.*

The initial step in the purification, the extraction of membranes with 2% Triton X-100 and 4 M urea, was developed by Wasserman and coworkers (22,23), who reported that the purified cytochrome was made of small electrophoretically similar subunits of M_r 5,600 with three

different NH_2-termini, in a complex oligomeric (octameric) lipoprotein of 110,000 molecular weight. However, the absence of histidine in the amino acid composition (23) was extremely unlikely for a heme protein, and the heterogeneity in the NH_2-termini was also an indication of lack of preparation purity. In retrospect, the small polypeptide thought to be a b-559 subunit was probably a heterogeneous mixture of peptides resulting from background proteolysis, since the amino acid composition was quite different from that later determined for pure cytochrome b-559 (4,18). Furthermore, the molecular weight of the lipoprotein is probably not ~100,000, and this value is probably only a mean of values for the large heterogeneous non-specific aggregates (MW = 100-300,000) of the cytochrome that are obtained in aqueous solution (24).

It was difficult to reproducibly obtain a high yield of cytochrome in the initial extraction until we systematically unstacked the thylakoids beforehand, suggesting that the extracted cytochrome b-559 arises from the appressed membrane region. Because of the small size of the cytochrome and a tendency toward proteolysis, all procedures were carried out in the presence of a cocktail of protease inhibitors (18).

Starting from 600 mg of chlorophyll, 5-10 mg of the cytochrome could be purified after three chromatography steps, as judged by a predominant M_r 10,000 band on SDS-PAGE gels (18). A similar M_r value for the cytochrome was obtained starting with PSII particles from maize (19). The gels also showed other minor bands, particularly one near M_r 8,000 and a weakly staining M_r 6,000 band. Because the M_r 8,000 band was found to be more pronounced in the absence of proteolysis inhibitors, it and the 6 kDa band were initially assumed to be proteolysis products of the dominant M_r 10,000 polypeptide. It was realized at this time, however, that the small amount of the M_r 6,000 band might be a result of a weak affinity for Coomassie stain, since (a) the amino acid composition of the M_r 6,000 component was somewhat similar to the M_r 10,000 with one His residue in each (8), and (b) it showed a significant 280 nm absorbance relative to the M_r 10,000 band in the HPLC elution profile (Fig. 1). Therein will lie a story.

The M_r 10,000 band could be purified on reverse phase HPLC (Fig. 1) as a single band on an overloaded gel, and the purity was confirmed by an NH_2-terminal amino acid sequence of twenty-seven residues obtained from it (18). Monospecific polyclonal antibody to the HPLC-purified polypeptide was used to locate the gene for this cytochrome b-559 polypeptide in the spinach plastid chromosome by hybrid selection-translation using a library of restricted DNA fragments and immunoprecipitation (3).

A reading frame on the spinach plastid chromosome could be located that corresponded exactly to that determined for the twenty-seven residues of the NH_2-terminus of the M_r 10,000 polypeptide, and whose nucleotide sequence corresponded, after post-translational processing of the NH_2-terminal residue, to an 82 amino acid polypeptide with MW = 9,162 (4). The presence of a single histidine residue is an important aspect of the sequence, since at just about this time spectroscopic

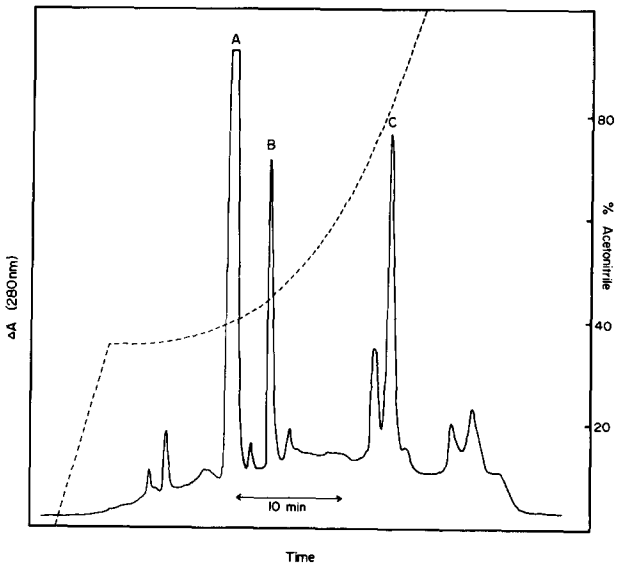
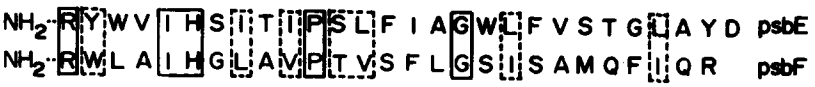


Fig. 1. Separation by reverse phase HPLC of the purified polypeptide components of cytochrome b-559 (13). The elution bands A-C contain free heme, the 4.27 kDa, and the 9.16 kDa polypeptides, respectively.

measurements carried out with the purified cytochrome showed that the heme coordination must be bis-histidine (20). The absence of methionine as the sixth ligand was proven by the lack of a 695 nm absorption band of the oxidized cytochrome (Fig. 2), the absence of lysine in the protein (4), and EPR and Raman spectral data (20).

Continuation of the nucleotide sequence in Herrmann's laboratory showed that the TAG stop codon of this reading frame overlapped a ribosome binding sequence, GGAGG, and that an initiator codon for another reading frame encoding a 4.27 kDa polypeptide was present nine nucleotides downstream from the terminal G codon (4). Proximity of the downstream reading frame suggested possible relevance to the b-559 protein. This idea was strengthened by alignment of the sequences which showed that the polypeptide products of each gene, psbE and psbF, contain a single His residue positioned five residues from an Arg residue on the NH_2-terminal side of a 25-26 residue non-polar domain. The corresponding position of the Arg residue defining the end of the hydrophobic domain and the homology between identical (solid box) and like (dashed box) residues in the hydrophobic domain is shown (Table I).

Table I. Comparison of psbE and psbF genes.



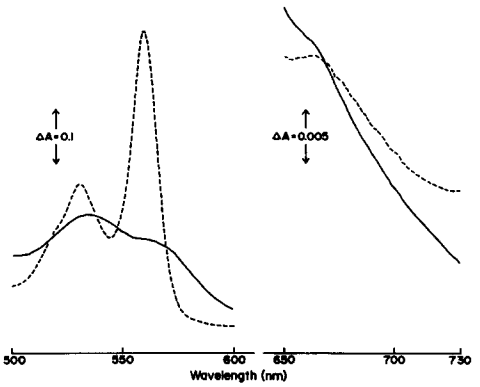


Fig. 2. Comparison of the visible light absorbance properties of purified cyt b-559 in the α-band (left) and 695 nm (right) region, the latter diagnostic of a methionine-ferric heme interaction that generates an absorption band with ϵ_{mM} = 800 M^{-1} cm^{-1} (20). Note the 20-fold scale expansion for the right-hand panel; (——) oxidized with ferricyanide; (---) reduced by dithionite (ref. 20). From the α-band spectra the ϵ_{mM} values are: peak (559.5nm) minus trough (577nm) of difference spectrum, 21.5; reduced minus oxidized at 559.5 and 552 nm, 17.5 and 5.0, respectively. The isobestic points are at 570, 548, and 538 nm.

Given the DNA sequence data, we were led to ask whether the small polypeptide was present in the SDS-PAGE gels. As stated above, the band running near M_r 6,000 had originally been thought to be a proteolysis product of the M_r 10,000 band. The initial amino acid composition data were consistent with this designation since only a single histidine residue was found (18). An amount of the small polypeptide sufficient for NH_2-terminal sequencing was separated and isolated by the reverse phase HPLC. Comparison of the sequences showed that the M_r 6,000 polypeptide was the product of the downstream gene (21):

Measured
NH_2 ... Thr-Ile-(Asp)-X-(Thr)-Tyr-Pro-Ile-Phe- ... COOH
Predicted from Nucleotide Sequence
NH_2 ... -Thr-Ile-Asp-Arg-Thr-Tyr-Pro-Ile-Phe- ... COOH

The predicted amino acids were also present in positions 3 and 5, but one other residue was found in these cycles. The residue in position 4 could not be determined. The smaller b-559 polypeptide, like the larger one, appears to have lost the N-terminal amino acid by post-translational processing, so that the molecular weights are 9,162 and 4,268, respectively. Establishment of the identity of the b-559 M_r 6,000 polypeptide with the protein product of the downstream gene allowed the stoichiometry of the 9.16 and 4.27 kDa polypeptides to be

determined through the respective areas under the peaks in the HPLC elution profile normalized to tryptophan content. The stoichiometry is close to 1:1, implying that the polypeptides are present in equal molar ratio in the b-559 protein (21). The 1:1 stoichiometry of the 9.16 kDa (α): 4.27 kDa (β) polypeptides and the bis-histidine coordination resulting from polypeptides containing only a single histidine imply that the simplest model for coordination of a single b-559 heme is a heme cross-linked ($\alpha\beta$) dimer. Assuming that the 25-26 residue non-polar domains on each polypeptide span the membrane in an α-helical conformation, the two histidine residues on α and β would be positioned five residues into the hydrophobic phase from Arg residues positioned at the polar interface (Fig. 3). This heme cross-linked dimeric structure does not have a precedent among soluble or membrane-bound cytochromes.

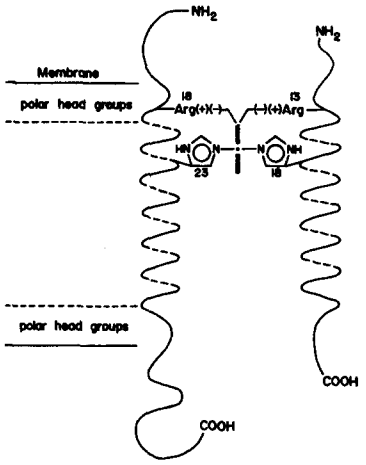


Fig. 3. Model for folding of a monoheme unit of cytochrome b-559 in the hydrophobic core of the thylakoid membrane (16).

Without any information on the arrangement of the cytochrome b-559 protein relative to other PSII proteins, the nature of the packing of the heme is not known. The well-known lability of this cytochrome *in situ* (1) may be a consequence of this structure. Recent studies of the orientation of the heme plane of ferric-cytochrome b-559 show it to be perpendicular to the plane of the membrane (25), consistent with the model shown in Fig. 3. Upon formation of a high-spin cytochrome after aging of the membranes, however, the heme plane tilts by 45° (25). Evidently, coordination of at least one of the His residues is labile with respect to this treatment, and may also be correlated with the shift from high to low midpoint potential of the cytochrome (1,26).

III. *Structural Models for a Two Heme Cytochrome b-559.*

The choice of models for the arrangement of the b-559 polypeptide in the membrane becomes less obvious if the protein contains two hemes. There is a substantial amount of evidence indicating the presence of two b-559 hemes per P680 reaction center positioned at two different environments in the membrane. The stoichiometry of one or two hemes

per P680 depends, however, upon the chlorophyll/P680 stoichiometry (27,28). The b-559 content in different PSII particle preparations can be one or two per P680 (29-32). Two different heme environments are indicated by the biphasic oxidation by ferricyanide of two high potential hemes (33). This provides an explanation for the observations that one b-559 heme can be photo-oxidized at 77°K (34), and that one heme can be photoreduced by PSII in 100 msec at room temperature (35), although the question of whether these hemes are distinct has not been answered. The simplest models for coordination of two hemes in one cytochrome b-559 protein would involve a tetramer of polypeptides to coordinate the two hemes (Fig. 4).

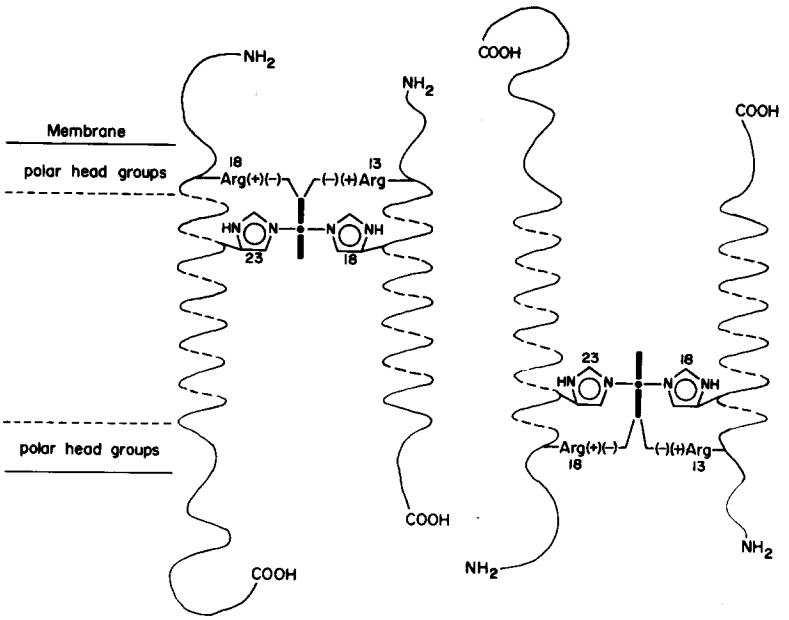


Fig. 4. Tetrameric polypeptide model for arrangement of cytochrome b-559 containing two hemes in the hydrophobic core of the thylakoid membrane, assuming two heme cross-linked heterodimers.

The two models that can be constructed for a cytochrome b-559 polypeptide tetramer would consist of two heme cross-linked dimers, two homodimers $(\alpha)_2$ $(\beta)_2$, or heterodimers $(\alpha\beta)_2$ [Fig. 4], with the hemes on opposite sides of the hydrophobic phase. Both of these models for cytochrome b-559 invoke the precedent of the model proposed for the trans-membrane arrangement of the hemes in cytochrome b of the mitochondrial and chromatophore b-c_1 complexes and cytochrome b_6 of the chloroplast b_6-f complex (36-38).

An argument against a possible $(\alpha)_2(\beta)_2$ homodimer model is that the two dimers differ greatly in charge, since the α and β subunits have a net charge at neutral pH of +2 and -3, respectively. The two homodimers would then differ by 10 charge units, as well as by about 10 kDa in molecular weight, and should have been separable on the

detergent-containing DEAE columns used for purification of the protein. An argument against the heterodimer model is that there is no a priori basis for two identical ($\alpha\beta$) units to orient oppositely in the membrane during its synthesis. An answer to the latter point is that the b-559 polypeptide could be assembled in the membrane as a tetrameric unit. With either model, the predicted heme content, one heme per 14,000 molecular weight, is close to the measured value of ~1/17,000 (18). The two different protein and lipid environments proposed for the two cytochrome hemes, one close to the stromal, and the other close to the lumenal interface, might also explain the greater lability toward conversion to lower potential (not reducible by hydroquinone) of approximately half the cytochrome b-559 population (1). No indication has been obtained thus far in the purification work for the presence of a distinct low potential b-559 protein (discussed in ref. 18).

IV. *The Question of Function.*

Independent of the question of homo- vs. hetero-dimers, the model of Fig. 4 suggests that the two hemes would span most of the membrane dielectric, implying that this cytochrome could participate in a trans-membrane pathway. The obvious possibility for such a pathway would be a cycle around photosystem II. The lack of evidence for such a cycle in chloroplasts with unimpaired water splitting (1) is consistent with rapid reduction of P680 by other intermediates in the water splitting pathway (e.g., ref. 39). Furthermore, competent electron transfer in PSII with water as the donor can occur in the absence of high potential (hydroquinone-reducible) cytochrome b-559 (1,40-42), thereby arguing against hypotheses for b-559 function that would obligatorily involve a high potential b-559 in water splitting, the most recent such proposal concerning a function in proton-linked PSII electron transport (7). Thus, we know of no viable hypotheses linking cytochrome b-559 to electron transport in PSII while the water splitting enzyme is operational. Because of these data, and the fact that the amplitude of the photooxidation of cytochrome b-559 by PSII is increased when water splitting is blocked or impaired (e.g., 12) one is led to look for the function of the cytochrome in chloroplasts with impaired PSII activity.

V. *A Proposal for the Function of Cytochrome b-559 in Chloroplast Development and in Response to Stress.*

In this section we would like to propose a role for cytochrome b-559 as a mediator in the (re)assembly of the water splitting enzyme, either in developing chloroplasts or in thylakoids recovering from stress-induced damage.

It has been known for some time that light is required for the assembly of the components of the oxygen evolving complex into a functional water splitting enzyme (for a review, see 43). This process, known as photoactivation, has been demonstrated in both algae (green and blue-green) and in higher plants, and occurs when the oxygen evolving complex is in a non-functional, but "ready to assemble" state. Such a situation is found, for instance, in algae and chloroplasts of plants grown without Mn^{2+} (44), or washed with NH_2OH (45) or TRIS (46) and then supplied with externally added Mn^{2+},

and in chloroplasts of seedlings grown in darkness (47,48) or intermittent light (49,50).

It is clear that photoactivation is a PSII-dependent process. Both its action spectrum and absorption cross-section are the same as those of PSII, it is sensitive to DCMU, and it has been observed in a PSI-less mutant (43). The rate of photoactivation is also proportional to the number of inactive PSII units (43), suggesting that the oxidants produced in reaction centers not connected to functional oxygen evolving complexes play a critical role in the process.

As discussed above, while cytochrome b-559 has never been shown to compete effectively with the oxygen evolving complex for oxidants produced at the PSII reaction center, it is readily photooxidized by PSII when the water splitting enzyme is inhibited (e.g.,12). In developing chloroplasts, its photooxidation by PSII can be observed at the onset of assembly of the photochemical apparatus (51). It was, in fact, noted in ref. 43 that "the terminal step in formation of the capacity for O_2 evolution may be the photoactivation of Mn." It therefore seems plausible that the cytochrome could play a role in funneling the oxidizing potential of P680 in inhibited chains to the thylakoid component responsible for the final assembly of the oxygen evolving complex, and specifically to the oxidation of Mn^{2+} (Fig. 5). Consideration of the mid-point potentials of Mn-containing superoxide dismutases that range between +180 and +320 mV (52) indicates that the middle-high potential forms (Em > 200 mV) of cytochrome b-559 could be capable of oxidizing complexed Mn. In addition, the requirement for low light intensities for photoactivation (4% of saturating intensity for photosynthesis (43)) may be a result of the relatively slow redox reaction times (100 ms, 36) of cytochrome b559. Finally, although a number of seemingly unrelated compounds (uncouplers, DCCD) act through unknown mechanisms to inhibit photoactivation in certain cases (54), one can understand the inhibition by hydrazine and hydroquinone (43) as being due to their competition with cytochrome b-559 for the PSII oxidant. Indeed, our hypothesis predicts that competent PSII electron donors should be able to inhibit photoactivation. [The idea that cytochrome b-559 might be involved in the pathway of photoactivation was also independently put forward by Prof. G. Cheniae (personal communication)].

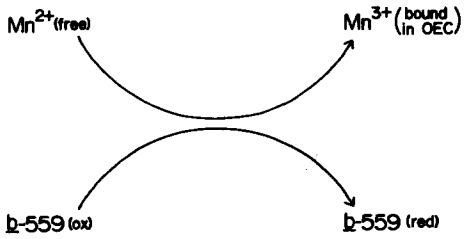


Fig. 5. Proposed role for cytochrome b-559 in the oxidation of Mn^{+2} to Mn^{+3} during the process of photoactivation or repair of stress damage to PSII.

An important aspect of this hypothesis is that cytochrome b-559 would be important not only for chloroplast development, but also as part of a stress-response system for PSII, mediating repair to damaged oxygen evolving complexes. These enzymatic complexes are among the most labile in the electron transport chain, and have been shown to be damaged by heat (55), chilling (56,57), and water stress (O. Canaani, personal communication), all conditions potentially encountered by plants in their natural habitats. Thus, in spite of the lack of a demonstrated role of cytochrome b-559 in chloroplasts with a normally functioning water splitting complex, it would be understandable that this cytochrome is maintained throughout the life of the chloroplast and not degraded after the completion of development.

A number of experiments can be suggested to test this working hypothesis. First, one could examine the process of photoactivation in plants or algae in which the gene for cytochrome b-559 is altered or deleted. Such a deletion has been accomplished in the Synechocystis 6803 cyanobacterium (H. Pakrasi, personal communication). Alternatively, one could investigate the effects of an antibody to the cytochrome on photoactivation of Mn-extracted PSII membrane preparations. The use of PSII preparations may be essential in this case, as there appears to be an accessibility problem with the antibody added to typical thylakoid preparations (unpublished data). In either experiment, the model predicts that photoactivation should be severely hampered. Second, cytochrome b-559 could be monitored during a train of photoactivating flashes; the model predicts that the cytochrome should undergo continuous oxidation-reduction cycles, the reduction should be faster in the presence of added Mn^{2+}, and the rate and/or extent of oxidation by PSII should decrease as the recovery proceeds. Experiments such as these could determine whether the model proposed above brings us closer to uncovering the elusive function of cytochrome b-559 in chloroplasts.

ACKNOWLEDGMENT

W. A. C. is indebted to Warren for a great deal, including an introduction to the problem discussed in this article. Research supported by the National Science Foundation and the Indiana Corporation for Science and Technology.

REFERENCES

1. Cramer WA and Whitmarsh J (1977) Ann Rev Plant Physiol 28:133-172
2. Tang X-S and Katoh S (1985) FEBS Lett 179:60-64
3. Westhoff P, Alt J, Widger WR, Cramer WA, and Herrmann RG (1985) Plant Molec Biol 4:103-110
4. Herrmann RG, Alt J, Schiller B, Widger WR and Cramer WA (1984) FEBS Lett 176:239-244
5. Lundegärdh H (1961) Proc Natl Acad Sci USA 53:703-710
6. Cox RP and Bendall DS (1972) Biochim Biophys Acta 283:124-135
7. Butler WL (1978) FEBS Lett 95:19-25
8. Matsuda H and Butler WL (1983) Biochim Biophys Acta 724:123-127
9. Boardman NK, Anderson JM and Hiller RG (1971) Biochim Biophys Acta 234:126-136

10. Cramer WA and Böhme H (1972) Biochim Biophys Acta 256:358-369
11. Heber U, Kirk MR and Boardman NK (1979) Biochim Biophys Acta 546:292-306
12. Knaff DB and Arnon, DI (1969) Proc Natl Acad Sci USA 63:956-962
13. Cramer WA, and Butler WL (1967) Biochim Biophys Acta 143:332-339
14. Ben-Hayyim G (1974) Eur J Biochem 41:191-196
15. Velthuys BR (1981) FEBS Lett 126:272-276
16. Yerkes CJ and Crofts AR (1984) In Sybesma C, ed. Adv Photosyn Res I, pp. 489-492. Martinus Nijhoff/Dr. W. Junk, The Hague.
17. Mende D (1980) Plant Sci Lett 17:215-220
18. Widger WR, Cramer WA, Hermodson M, Meyer D, and Gullifor M (1984) J Biol Chem 259:3870-3876
19. Metz JG, Ulmer G, Bricker TM and Miles D (1983) Biochim Biophys Acta 725:203-209
20. Babcock GT, Widger WR, Cramer WA, Oertling WT and Metz JG (1985) Biochemistry 24:3638-3644
21. Widger WR, Cramer WA, Hermodson M, and Herrmann RG (1985) FEBS Lett. 191:186-190.
22. Garewal HS and Wasserman AR (1974) Biochemistry 13:4063-4071
23. Garewal HS and Wasserman AR (1974) Biochemistry 13:4072-4079
24. Widger WR and Cramer WA (1985) Unpublished data
25. Rutherford AW (1985) Biochim Biophys Acta 807:189-201
26. Wada K and Arnon DI (1971) Proc Nat Acad Sci USA 68:3064-3068
27. Anderson JM and Melis A (1983) Proc Natl Acad Sci USA 80:745-749
28. Whitmarsh J and Ort DR (1984) Arch Biochem Biophys 231:378-389
29. Sandusky PO, Selvius De Roo CL, Hicks DB, Yocum CF, Ghanotakis DF and Babcock GT (1983) In Inoue Y, Crofts AR, Govindjee, Murata N, Renger G and Satoh K, eds. The Oxygen Evolving System of Photosynthesis, pp 189-199. Academic Press, Tokyo.
30. Bricker TM, Metz JG, Miles D and Sherman LA (1983) Biochim Biophys Acta 724:447-455
31. Yamamoto Y, Tabata K, Isogai Y, Nishimura M, Okayama S, Matsuura K, and Itoh S (1984) Biochim Biophys Acta 767:493-500
32. Murata N, Miyao M, Omata T, Matsunami H and Kuwabara T (1984) Biochim Biophys Acta 765:363-369
33. Selak ME, Koch-Whitmarsh BE and Whitmarsh J (1984) In Sybesma C, ed. Adv Photosyn Res, pp 493-496. Martinus Nijhoff/Dr. W. Junk, The Hague.
34. Vermeglio A and Mathis P (1975) Avron M, ed. Proc 3rd Int Cong Photosyn, pp 323-334. Elsevier, Amsterdam.
35. Whitmarsh J and Cramer WA (1977) Biochim Biophys Acta 460:280-289
36. Widger WR, Cramer WA, Herrmann RG and Trebst A (1984) Proc Natl Acad Sci USA 81:674-678
37. Cramer WA, Widger WR, Herrmann RG and Trebst A (1985) Trends Biochem Sci 10:125-129
38. Cramer WA, Widger WR, Black MT and Girvin ME (1986) Topics in Photosynthesis (J Barber, ed), vol. 8, in press
39. Van Best JA and Mathis P (1978) Biochim Biophys Acta 503:178-188
40. Cramer WA, Whitmarsh J and Widger WR (1981) in Photosynthesis: Electron Transport and Phosphorylation (Akoyunoglou, G, ed) pp 509-522. Balban Int Sci Serv, Philadelphia
41. Briantais J-M, Vernotte C, Miyao M, Murata N, and Picaud M (1985) Biochim Biophys Acta 808:348-351

42. Ghanotakis DF, Yocum CF and Babcock GT (1985) Photosyn Res. In Press
43. Radmer R and Cheniae GM (1977) In Barber J, ed. Primary Processes of Photosynthesis, pp 303-348. Amsterdam:Elsevier/North Holland Biomedical Press
44. Cheniae GM and Martin IF (1971) Biochim Biophys Acta 253:167-181
45. Cheniae GM and Martin IF (1972) Plant Physiol 50:87-94
46. Yamashita T, Inoue Y, Kobayashi Y and Shibata K (1978) Plant and Cell Physiol 19:895-900
47. Oku T and Tomita G (1976) Physiol Plant 38:181-185
48. Oku T and Tomita G (1980) Physiol Plant 48:99-103
49. Ono T and Inoue Y (1982) Plant Physiol 69:1418-1422
50. Ono T and Inoue Y (1983) Biochim Biophys Acta 723:191-201
51. Baker NR and Butler WL (1976) Plant Physiol 58:526-529.
52. Lawrence GD and Sawyer DT (1978) Coord Chem Rev 27:173-193
53. Cheniae GM and Martin IF (1969) Plant Physiol 44:351-360
54. Yamashita, T and Tomita C (1975) Plant Cell Physiol 16:283-296.
55. Diner BA and Joliot P (1977) In Trebst A and Avron M, eds. Encyclopedia of Plant Physiology, Vol 5, pp 187-205. Berlin:Springer-Verlag
56. Margulies MM (1972) Biochim Biophys Acta 267:96-103
57. Smillie RM and Nott R (1979) Plant Physiol 63:796-801

Authors' address:
Department of Biological Sciences
Lilly Hall of Life Sciences, Purdue University,
West Lafayette, Indiana 47907 (U.S.A.)