The Topology of the Plastoquinone and Herbicide Binding Peptides of Photosystem II in the Thylakoid Membrane

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Dedicated to Professor Hans Grisebach on the occasion of his 60th birthday

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The 32 kDa herbicide and Q_B binding peptide (D-1 protein) and its homologous 34 kDa peptide (D-2 protein) are integral membrane subunits of photosystem II. A model for their folding through the thylakoid membrane in five transmembrane α-helices is proposed from the comparison of amino acid sequence and hydropathy index plot homologies with subunits of the bacterial system. Following recent data on the X-ray structure of a bacterial photosystem the binding niche for Q_B is interpreted on the basis of the amino acid changes found in the 32 kDa peptide in herbicide tolerant higher plants and algae.

Photosystem II consists of five integral peptide subunits of 47, 44, 34, 32 and 10 kDa molecular weight. They bind the functional components of photosystem II: the reaction center chlorophyll, core antenna chlorophylls, pheophytin, Fe, cytochrome b_{559}, two plastoquinones Q_A und Q_B and a primary electron donor (for review see [1–3]). The 32 kDa peptide has been given particular attention, as it was the first of the photosystem II peptides of which the specific function was recognized. It was shown to bind herbicides [4] and to be identical with the rapidly turning over peptide [5] encoded by the D-1 gene. From the known mode of action of these herbicides on the acceptor side of photosystem II it is indicated that the 32 kDa peptide is involved in Q_B binding. It was also the first integral membrane peptide of the electron transport system, whose complete amino acid sequence was determined [6] (now all of the genes for photosystem II peptides have been sequenced as well as most of the cytochrome *b*/*f*-complex and of photosystem I, for review see [7, 8]). From predictions of its secondary structure [9] the suggestion developed that integral peptides of the photosynthetic membrane may fold through the membrane several times in hydrophobic helices [8]. Although this general statement seems to be accepted, the exact folding of the 32 kDa peptide remained controversial.

Rao *et al.* [9] suggested that the 32 kDa peptide folds seven times through the membrane. However, other predictions of the folding of the 32 kDa peptide have been proposed [10, 11] because it appeared that certain sequence stretches in the Rao model were not hydrophobic enough or too short to span the membrane. On the other hand hydrophobic sequences, not considered by Rao *et al.* [9], might cross the membrane [10, 11]. A main argument against any of these folding predictions is that these models do not fit well mutant data that indicate which amino acids participate in herbicide and from there Q_B binding. The 32 kDa peptide of herbicide tolerant mutants of higher plants and algae is shown to have specific amino acid changes [12–17]. These changes: val_{219}, phe_{255}, ser_{264}, leu_{275} are not readily accomodated, according to the Rao *et al.* [9] prediction, in a herbicide or Q_B binding niche because the changed amino acids were oriented towards both sides of the membrane as well as inside [18]. This paper suggests a topology of the 32 kDa herbicide and Q_B binding protein that explains the mutant data very well.

A prediction of the folding of the cytochrome b_6 subunit of the cytochrome b_6/f-complex [8, 19] and of the heme binding sites included the comparison of several homologous (mitochondrial) cytochrome *b* sequences in the interpretation of the hydropathy index plots [8, 19, 20]. Similarly the folding of the 32 kDa peptide proposed here considers in addition to its hydropathy index plot also amino acid sequences of homologous peptides from the thylakoid – of the 32 and 34 kDa peptide – and the L and M subunit of the bacterial photosystem.

The D-2 gene encoding the 34 kDa peptide of photosystem II has been sequenced recently

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[21–23]. The amino acid sequence so derived is quite homologous both in sequence and in a hydropathy index plot as noted by Rochaix *et al.* [21]. The 34 kDa peptide appears therefore to be like the 32 kDa peptide also a Q binding peptide. And so are also two subunits of the bacterial system. As is well known the functional components of photosystem II are very similar to those of the bacterial photosystem, in particular in the functional events on the acceptor side that involves Fe, Q_A and Q_B [3, 24, 25]. Three subunits called L, M and H are the integral peptides, which bind these components in the bacterial system. Youvan *et al.* [26] pointed out that the L and M peptides have amino acid sequence homology to the 32 kDa peptide of photosystem II.

By comparing the hydropathy index plots and amino acid sequences of these four peptides: L and M from the bacterial system and D-1 and D-2 from photosystem II, five hydrophobic segments can be detected to be common to all four peptides (Fig. 1). These sequences are very hydrophobic and are long enough to span the 35 Å of the membrane in an α helix. Accordingly five hydrophobic helical spans are predicted for the D-1 and D-2 (32 and 34 kDa) peptide of photosystem II (Figs 2 and 3) (rather than seven as in the Rao *et al.* model [9]). The bacterial photosystem of *Rhodopseudomonas viridis* has been crystallized and the X-ray structure become available [27–29]. The data show that of the three subunits of the reaction center the L and M subunit fold through the membrane five times. This then shows directly that the predicted five helices of the hydropathy plot exist indeed. From this it has been suggested already [8, 10, 27, 28] that the two D-1 and D-2 peptides of photosystem II are homologous to the L and M subunit of the bacterial system, both in structure and function.

The amino acids involved in Fe and Q_B binding in the bacterial system are indicated by the X-ray structure of Deisenhofer, Michel *et al.* [28, 29]. It had already been proposed that histidines are involved in Fe and quinone binding from EXAF studies [30]. Four histidines involved in Fe binding are indicated in the X-ray structure [27–29]. These histidines are conserved in the homologous helical spans IV and V in the D-1 and D-2 subunits of photosystem II (see

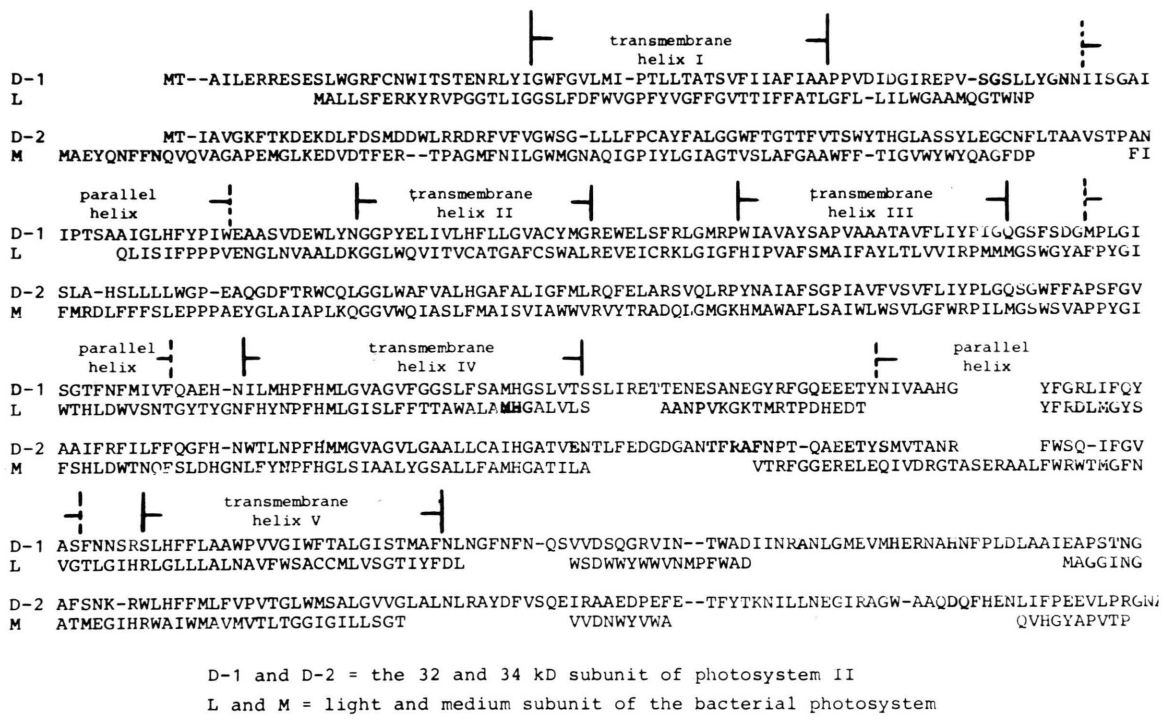

Fig. 1. Alignment of the amino acid sequences of the D-1 and D-2 subunits of photosystem II with the L and M subunits of the photosystem of *Rh. capsulata* indicating five hydrophobic segments common to each.

Fig. 1). This determines which hydrophilic sequences of D-1 and D-2 are extending into the matrix space of the thylakoid because Q_A and Q_B are reduced in photosystem II on that side.

Accordingly in a model for photosystem II the binding niche for the acceptor quinones – and from there also for the inhibitors and herbicides – is composed of two subunits, one each of the D-1 and D-2 protein that both span the membrane five times in a highly symmetrical manner. The four histidines on helices IV and V of both the D-1 and D-2 subunits: his_{215} and his_{275} (in D-1) are, by comparison with the bacterial system, involved in Fe binding. The menaquinone Q_A in the crystallized bacterial center is close to a his, to a peptide bond and to a $tryptophan_{250}$ in a parallel helix of the M subunit [29]. Again this tryptophan is conserved in D-2 indicating that this is the Q_A binding site of photosystem II. It is equivalent to phe_{255} in the D-1 protein. From the homologous folding of the D-1 (and L) subunit it follows that his_{215} and phe_{255} are involved in Q_B binding (Fig. 4). This predicted folding of the 32 kDa/D-1 protein seems not to be in agreement with trypsin digestion experiments [31], nor with the contention that the carboxylend of D-1 is on the matrix side, where a precursor is processed [31]. Instead it would be inside the lumen space according to Fig. 2.

The predicted folding of the 32 kDa peptide, however, allows quite reasonable explanations for those amino acid changes, indicated above, that are changed in herbicide tolerant mutants of higher plants and algae [12–17]. According to the folding of

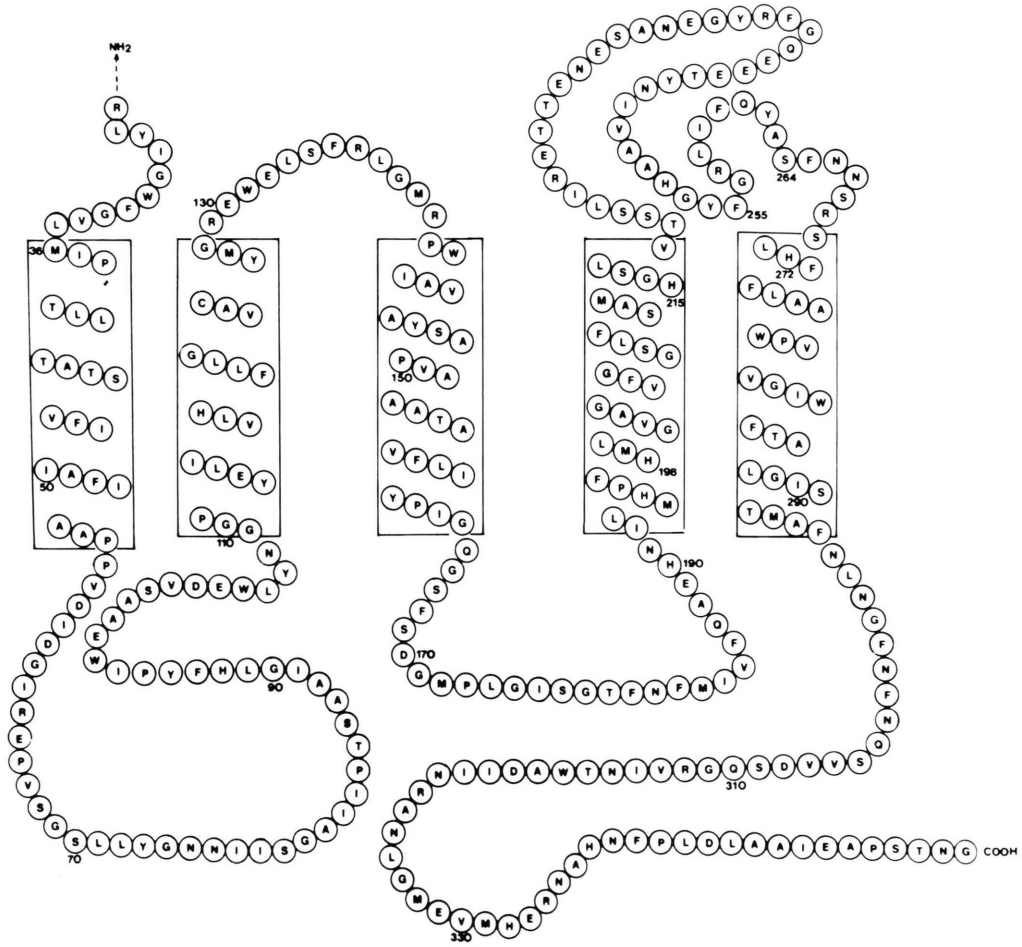

Fig. 2. Predicted folding of the amino acid sequence of the 32 kDa subunit of photosystem II in five hydrophobic helices spanning the membrane.

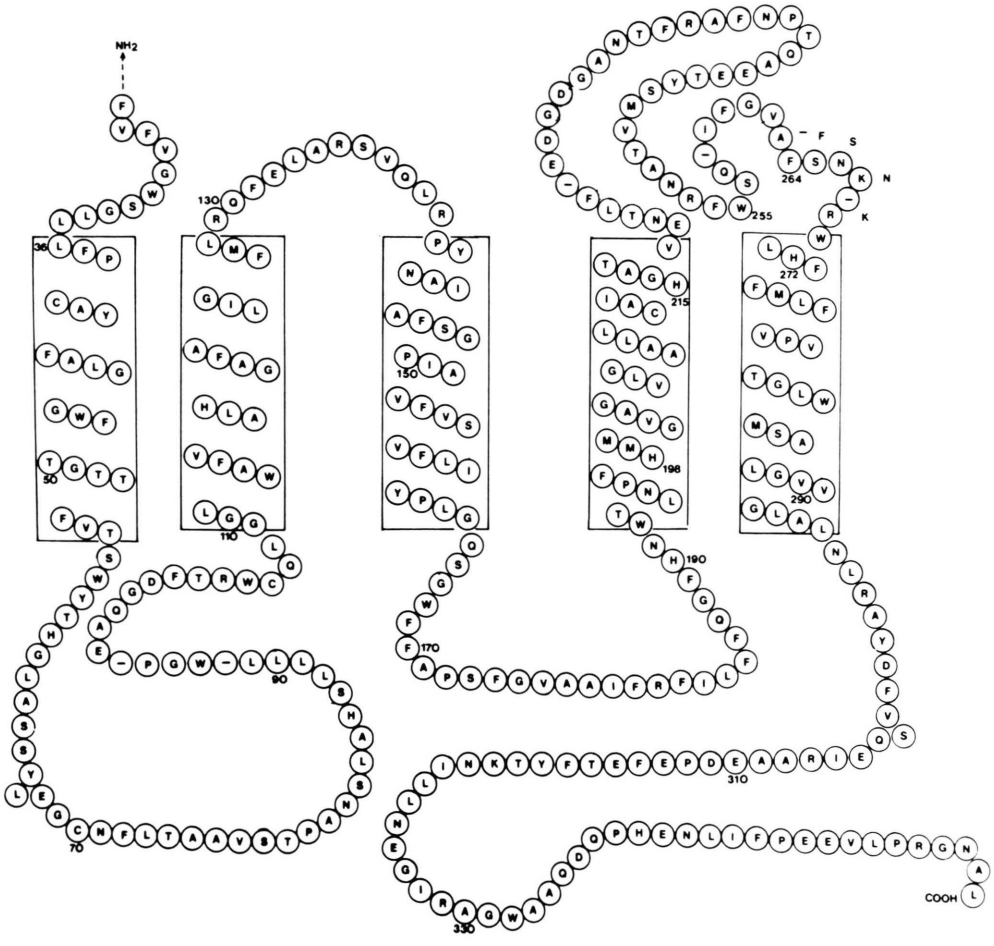

Fig. 3. Predicted folding of the amino acid sequence of the 34 kDa subunit of photosystem II in five hydrophobic helices spanning the membrane.

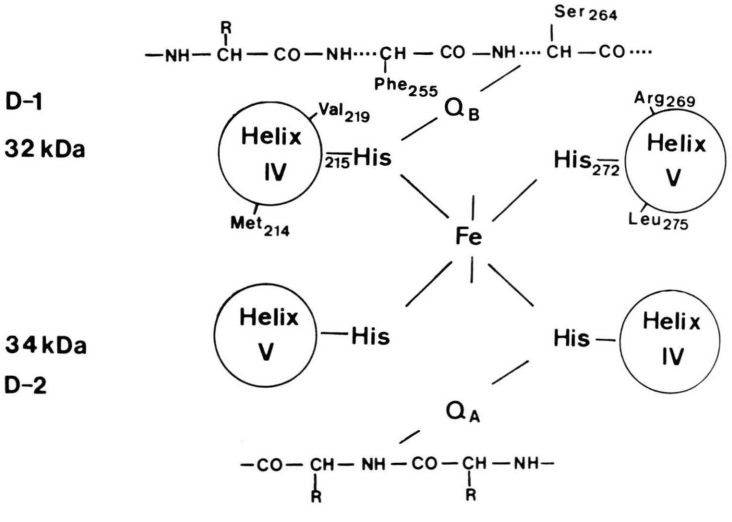

Fig. 4. Proposed binding of Q_B (and herbicides) and Fe via four histidines of the D-1 and D-2 subunits of photosystem II, following the homology to and the X-ray structure of a bacterial photosystem [28, 29]. The amino acid changes in herbicide tolerant mutants are indicated (val_{219}, phe_{255}, ser_{264}, leu_{275}).

Fig. 2 of the 32 kDa subunit all changes are on that part of the peptide that is oriented towards the matrix side, as is to be expected for inhibitors of Q_B function on the matrix side. As indicated in Fig. 4, the val_{219}, changed in diuron tolerance [15], is above that his_{215} that is implied directly in Q_B binding. As val_{219} is in the membrane spanning hydrophobic helix, its precise orientation in respect to the his_{215} can be taken from a helical wheel (see [8, 9]), as in an α helix 3.6 amino acids are in one turn. Possibly, increasing the bulky sidechain from valine to isoleucine in the mutation leads to steric hindrance of binding of an inhibitor towards the histidine below. Particularly supporting the model of the D-1/D-2 folding is the mutation of phe_{255} [15]. Because the X-ray structure of the bacterial system shows [29] that this phe is on a helix parallel to the membrane and extends into the hydrophobic space below the quinone binding. This phe might also interact with those herbicides with an aromatic ring (see, however, the recent report [17] that in a DCMU resistant blue green algae $serine_{264}$ has been mutated as in triazine resistance of higher plants and green algae). The recently reported leu_{272} change [16] is on the fifth helix. Leu_{272} is below the his_{275} that is involved in Fe binding and is also facing into the binding niche (Fig. 4). Possibly this leu has no particular function, but its change to a phenylalanine [16] might disturb energy and charge distribution in the his-Fe-his-Q_B (herbicide) interaction. The ser_{264} changed in triazine resistance in both higher plants [12] and algae [13] and in DCMU resistant Anacystis [17] is on the same subsequence as is the phe_{255} change but possibly just beyond the membrane parallel helical part of this sequence. Its peptide bond – or one close to it – might be involved in an interaction with Q_B, because the peptide bond of amino acid 258 in the equivalent area on the M subunit stabilizes Q_A in the bacterial system [29]. The mutations reported in terbutryn resistance of the bacterial system on an isoleucine next to his_{275} [32] also fits the model. The met_{214}, tagged by azidoatrazine [33] (but not by azidourea [34]) is somewhat pointing away from the binding niche – thus perhaps indicating some leeway in the exact binding during photoaffinity labeling experiments. An arginine, at the end of a helix conferring a particular dipolmoment, has been suggested to be of importance in herbicide binding by theoretical considerations [35]. An arginine with that property might be arg_{269} at the end of helix V above this his_{275} (Fig. 4).

The corresponding amino acids in the D-2 subunit as part of the binding niche are not far away from the Q_B binding site on D-1. Therefore one might expect an influence of the D-2 conformation or even direct participation in herbicide binding. Mutations might perhaps be less easily found because the conformation of D-2 protein, being the Q_A binding peptide, is of much higher importance than that of the D-1 peptide.

The model of the predicted folding of the 32 and 34 kDa peptide of photosystem II presented here follows very much the homology to the bacterial system. It is consequent therefore to suggest that the 32 and 34 kDa peptides form also the reaction center of photosystem II [27, 28]. However, so far the 47 kDa peptide is thought to be the reaction center peptide of photosystem II (see [1, 36]). It remains to further experimentation to indicate the exact role of the 47 kDa peptide in the reaction center of photosystem II, as discussed recently [36].

[1] Govindjee, T. Kambara, and W. Coleman, Photochem. Photobiol. **42,** 187–210 (1985).
[2] B. A. Diner, in: Encyclopedia of Plant Physiol. (L. A. Staehelin and C. J. Arntzen, eds.), Springer-Verlag, Berlin, Heidelberg, in press.
[3] J. L. Zimmermann and A. W. Rutherford, Physiol. Veg. **23,** in press (1985).
[4] K. Pfister, K. E. Steinback, G. Gardner, and C. J. Arntzen, Proc. Natl. Acad. Sci. USA **78,** 981–985 (1981).
[5] A. K. Mattoo, U. Pick, H. Hoffmann-Falk, and M. Edelman, Proc. Natl. Acad. Sci. USA **78,** 1572–1576 (1981).
[6] G. Zurawski, H. J. Bohnert, P. R. Whitfeld, and W. Bottomley, Proc. Natl. Acad. Sci. USA **79,** 7699–7703 (1982).
[7] R. G. Herrmann, P. Westhoff, J. Alt, J. Tittgen, and N. Nelson, in: Molecular Form and Function of the Plant Genome (L. van Vloten-Doting, G. S. P. Groot, and T. C. Hall, eds.), pp. 233–256, Plenum Publishing Corporation.
[8] W. A. Cramer, W. R. Widger, R. G. Herrmann, and A. Trebst, Trends Biochem. Sci. **10,** 125–129 (1985).
[9] J. K. M. Rao, P. A. Hargrave, and P. Argos, FEBS Lett. **156,** 165–169 (1983).
[10] D. J. Kyle, Photochem. Photobiol. **41,** 107–116 (1985).
[11] A. Trebst, in: Biochemical and Physiological Mechanisms of Herbicide Action (S. O. Duke, ed.), pp. 45–57, Tallahassee, Florida 1984.
[12] J. Hirschberg and L. McIntosh, Science **222,** 1346–1348 (1983).
[13] J. M. Erickson, M. Rahire, P. Bennoun, P. Delepelaire, P. Diner, and J. D. Rochaix, Proc. Natl. Acad. Sci. USA **81,** 3617–3621 (1984).
[14] P. Goloubinoff, M. Edelman, and R. B. Hallick, Nuc. Acids Res. **12,** 9489–9496 (1984).
[15] J. M. Erickson, M. Rahire, J. D. Rochaix, and L. Mets, Science **228,** 204–207 (1985).
[16] J. M. Erickson and J. D. Rochaix, in: Abstracts. First International Congress of Plant Molecular Biology (G. A. Galau, ed.), p. 54/OR–25–02, The Univ. of Georgia Center for Continuing Education for the Int. Soc. for Plant Mol. Biol., Athens 1985.
[17] S. S. Golden and R. Haselkorn, Science **229,** 1104–1107 (1985).
[18] J. M. Erickson, J. D. Rochaix, and P. Delepelaire, in: Molecular Biology of the Photosynthetic Apparatus (C. J. Arntzen, L. Bogorad, S. Bonitz, and K. Steinback, eds.), Cold Spring Harbour Meeting, in press (1985).
[19] W. R. Widger, W. A. Cramer, R. G. Herrmann, and A. Trebst, Proc. Natl. Acad. Sci. USA **81,** 674–678 (1984).
[20] M. Saraste, FEBS Lett. **166,** 367–372 (1984).
[21] J. D. Rochaix, M. Dron, M. Rahire, and P. Malnoe, Plant Molec. Biol. **3,** 363–370 (1984).
[22] J. Alt, J. Morris, P. Westhoff, and R. G. Herrmann, Current Genetics **8,** 597–606 (1984).
[23] K. Holschuh, W. Bottomley, and P. R. Whitfeld, Nuc. Acids Res. **12,** 8819–8834 (1984).
[24] M. Y. Okamura, G. Feher, and N. Nelson, in: Photosynthesis, **Vol. 1.** Energy Conversion by Plants and Bacteria (Govindjee, ed.), pp. 195–272, Academic Press, New York 1982.
[25] R. R. Stein, A. L. Castellvi, J. P. Bogacz, and C. A. Wraight, J. Cell. Biochem. **24,** 243–259 (1984).
[26] D. C. Youvan, E. J. Bylina, M. Alberti, H. Begusch, and J. E. Hearst, Cell **37,** 949–957 (1984).
[27] J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, J. Mol. Biol. **180,** 385–398 (1984).
[28] J. Deisenhofer, H. Michel, and R. Huber, Trends Biochem. Sci. **1985,** 243–248.
[29] J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, Nature, in press (1985).
[30] G. Bunker, E. A. Stern, R. E. Blankenship, and W. W. Parson, Biophys. J. **37,** 539–551 (1982).
[31] J. B. Marder, P. Goloubinoff, and M. Edelman, J. Biol. Chem. **259,** 3900–3908 (1984).
[32] C. J. Arntzen, A. E. Brown, C. W. Gilbert, and R. Guy, in: Molecular Biology of the Photosynthetic Apparatus (C. J. Arntzen, L. Bogorad, S. Bonitz, and K. E. Steinback, eds.), Cold Spring Harbour Meeting, in press (1985).
[33] K. E. Steinback and P. K. Wolber, in: Molecular Biology of the Photosynthetic Apparatus (C. J. Arntzen, L. Bogorad, S. Bonitz, and K. E. Steinback, eds.), Cold Spring Harbour Meeting, in press (1985).
[34] A. Boschetti, M. Tellenbach, and A. Gerber, Biochim. Biophys. Acta **810,** 12–19 (1985).
[35] L. L. Shipman, J. Theor. Biol. **90,** 123–148 (1981).
[36] A. Trebst and B. Depka, in: Chemical Physics Series **Vol. 42:** Antennas and Reaction Centers of Photosynthetic Bacteria – Structure, Interactions and Dynamics (M. E. Michel-Beyerle, ed.), pp. 215–223, Springer-Verlag, Heidelberg 1985.

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