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Relevance of the Photosynthetic Reaction Center from Purple Bacteria to the Structure of Photosystem II†

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The conversion of the energy of light into chemical energy is one of the fundamental processes of life. Photosynthetic organisms are able to oxidize organic or inorganic compounds upon the absorption of light, and they use the extracted electron for the fixation of carbon dioxide. The most important oxidation product is oxygen due to the splitting of water. In eukaryotes these processes occur in photosystem II of chloroplasts. Among prokaryotes photosynthetic oxygen evolution is restricted to cyanobacteria and prochloron-type organisms. The phylogenetically older "purple photosynthetic bacteria" and "green photosynthetic bacteria" can oxidize sulfur compounds or organic materials in a so-called anoxygenic photosynthesis, and they are able to derive energy from a cyclic electron flow that is coupled to photophosphorylation.

How water is split in the "oxygen-evolving complex" of photosystem II belongs to the most important questions to be answered. It is clear that a direct photolysis of water does not take place. The primary charge separation occurs in the reaction center of photosystem II. This reaction center is a complex consisting of peripheral and integral membrane proteins, several chlorophyll A molecules, two pheophytin A molecules, two or three plastoquinone molecules, and one non-heme iron atom. Upon absorption of light the primary electron donor, which is a chlorophyll A molecule or a pair of chlorophyll A molecules, is photooxidized. It then oxidizes a manganese compound in the oxygen-evolving complex via an intermediate electron carrier ("Z") that may be a plastoquinol (O'Malley & Babcock, 1984). The manganese complex can accumulate four positive charges, which then extract four electrons from water, leading to the release of protons and oxygen [for recent reviews, see Dekker and van Gorkom (1987) and Brudvig (1987)]. From the primary electron donor ("P680") the electron is transferred to a pheophytin (Klevanik et al., 1977). Then the two plastoquinones ("Q_A" and "Q_B") act in series. An electron is transferred first to the more firmly bound Q_A and then to Q_B. The Q_B plastoquinone receives two electrons successively and becomes protonated, and the resulting plastoquinol is replaced by another plastoquinone. Q_B is the site of action of commercially used herbicides, which apparently act by displacing the Q_B plastoquinone.

The location of the photosystem II reaction center is still a matter of debate. Nakatani et al. (1984) concluded from fluorescence measurements that a protein of apparent molecular weight 47 000 (CP47) is the apoprotein of the photosystem II reaction center. A different view emerged from work with the photosynthetic reaction centers from the purple bacteria. Williams et al. (1983) obtained the amino acid sequence of the M(edium) subunit of the reaction center from *Rhodopseudomonas (Rps.) sphaeroides* [now called *Rhodobacter (Rb.) sphaeroides*] and discovered sequence homologies with the D1 protein from spinach (Zurawski et al., 1982), which is the *psbA* gene product and is also known as herbicide binding protein, Q_B protein, or 32-kDa protein. They suggested conservation of functionally analogous regions of the two proteins. D1 had been shown to become covalently labeled by the herbicide azidoatrazine in photoaffinity-labeling experiments (Pfister et al., 1981). Later on it became apparent that the L(ight) subunit of the bacterial reaction center possesses sequence homology with the M subunit and the D1 proteins (Youvan et al., 1984; Williams et al., 1984; Michel et al., 1986). In parallel, the sequence of another photosystem II protein, D2, became known (Rasmussen et al., 1984; Rochaix et al., 1984; Alt et al., 1984; Holschuh et al., 1984). D2 also possesses sequence homologies to the D1 proteins and the L and M subunits. It appears likely that gene duplications gave rise to the L and M subunits as well as to the D1 and D2 proteins and that all these subunits are derived from a common ancestor.

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A substantial amount of structural information could be obtained with the reaction center from *Rhodopseudomonas viridis*, which could be crystallized (Michel, 1982). The crystallographic analysis yielded a complete picture of the pigment arrangement and the structure of the protein subunits (Deisenhofer et al., 1984, 1985). The most striking feature is the symmetric arrangement of the pigments and the L and M subunits. Both subunits are needed to establish the binding sites for the primary electron donor and the electron-accepting quinone–iron complex. These characteristics and the sequence homologies led to the proposal that the D1 and D2 proteins form the core of the photosystem II reaction center. Due to specific sequence homologies and the fact that the L subunit (de Vitry & Diner, 1984) like the D1 subunit (Pfister et al., 1982) can be labeled by azidoatrazine, D1 was proposed to correspond to the L subunit and D2 to the M subunit (Michel & Deisenhofer, 1986; Deisenhofer et al., 1985). Independently, Hearst (1986) came to the same conclusion on the basis of the particularly good sequence homology between the D1 and D2 proteins and the L and M subunits from *Rhodobacter capsulatus*. This view received argumentative support (Trebst & Depka, 1985; Trebst, 1986). Strong experimental support was provided by the recent isolation of a complex of the D1 and D2 proteins together with cytochrome *b*-559 (Nanba & Satoh, 1987; Satoh et al., 1987; Barber et al., 1987). This complex shows light-driven electron transfer to a pheophytin but has lost the plastoquinones. Here we discuss the structure of the photosynthetic reaction center from the purple bacterium *Rps. viridis* and describe the role of those amino acids that are conserved between the bacterial and photosystem II reaction center.

Structure of the Reaction Center from Rps. viridis

The photosynthetic reaction center from *Rps. viridis* is found in stacked photosynthetic membranes of this Gram-negative bacterium. The photosynthetic membranes are invaginations of the inner bacterial membrane and are contiguous to it (Drews & Giesbrecht, 1965). It is therefore appropriate to use the term periplasmic side for the side of the photosynthetic membrane that is connected to the periplasm. The opposite side of the membrane faces the cytoplasm of the bacterium. The isolated reaction center consists of the H(eavy), L(ight), and M(edium) subunits and a firmly bound cytochrome subunit containing four hemes. The amino acid sequences of all four protein subunits are known (Michel et al., 1985, 1986a; Weyer et al., 1987). In addition, it contains four bacteriochlorophyll B molecules, two bacteriopheophytin B molecules, one menaquinone as Q_A, one ubiquinone as Q_B, and a ferrous non-heme iron atom. Two of the bacteriochlorophylls form the primary electron donor; the two other are called "accessory" bacteriochlorophylls. As has been shown by the X-ray crystallographic analysis (Deisenhofer et al., 1984, 1985), the core of the reaction center is made up of the L and M subunits. Both proteins possess a primarily α-helical secondary structure. Each subunit contains five long membrane-spanning α-helices and several short ones. The membrane-spanning α-helices of the L subunit are called LA–LE and those of the M subunit MA–ME. The short α-helices in the connections of transmembrane α-helices are called LCD and LDE in the L subunits and MCD and MDE in the M subunit. The backbones of the major α-helices of both subunits and the ring systems of the photosynthetic pigments are related by approximate twofold symmetry. The major α-helices and the pigments are shown in Figure 1. The symmetry axis runs vertically in the center of the figure between the special pair bacteriochlorophylls (near the periplasmic side of the membrane) and through the ferrous non-heme iron atom (near the cytoplasmic side of the membrane).

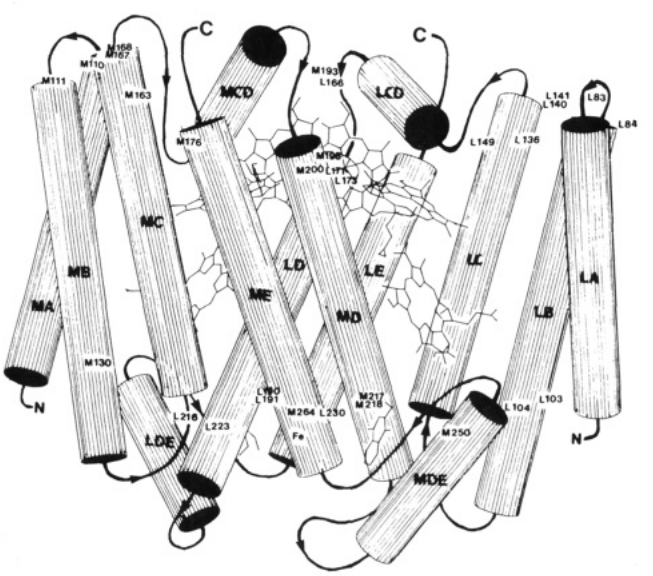

FIGURE 1: Representation of the major α-helices and the pigments in the reaction center from *Rps. viridis*. The α-helices are represented as columns. The transmembrane α-helices of the L (M) subunit are labeled by LA–LE (MA–ME) and the major α-helices in the connections by LCD (MCD) and LDE (MDE). The α-helix connections are shown schematically. The view is parallel to the plane of the membrane. The special pair bacteriochlorophylls are at the interface of the L and M subunits between the D and E α-helices, the accessory bacteriochlorophylls below α-helices LCD and MCD, and the bacteriopheophytins near the C α-helices. The binding site for Q_A (menaquinone in *Rps. viridis*) is between the MDE and MD α-helices and that for Q_B between the LDE and LD α-helices. The location of amino acids conserved between all L and M subunits and all D1 and D2 proteins (see text), as well as specifically in the quinone binding sites, is shown.

The structural differences in protein folding of the L and M subunits are mainly at the amino terminus on the cytoplasmic side of the photosynthetic membrane (M has a longer amino terminus), in the connection of the transmembrane α-helices A and B (M shows an insertion of seven amino acids), in the connection of the D and E transmembrane α-helices (M possesses an additional loop providing the side chain of glutamic acid M232 as a ligand to the iron atom), and at the carboxy terminus, where the M subunit is considerably longer. These differences can also be seen in the alignment of the sequences of the L and M subunits (see Figure 2, lines LV and MV). At the interface of both subunits the primary electron donor (the "special pair") is found near the periplasmic side, whereas the iron atom connects the L and M subunits near the cytoplasmic side via histidine ligands of the D and E α-helices.

The accessory bacteriochlorophylls, the bacteriopheophytins, and the quinones form two structurally equivalent, symmetry-related branches. One branch is more closely associated with the L subunit and the other with the M subunit. The bacteriopheophytin absorbing at longer wavelength is involved in light-driven electron transfer across the photosynthetic membrane in *Rps. viridis* (Vermeglio & Paillotin, 1982) as well as in *Rb. sphaeroides* [see, e.g., Kirmaier et al. (1985)]. Comparison of the linear dichroism spectra of crystals (Zinth et al., 1983) and the X-ray data shows that this is the bacteriopheophytin more closely associated with the L subunit (Zinth et al., 1985; Knapp et al., 1985). Therefore, it is the pigment branch more closely associated with the L subunit that is used for light-driven electron transfer across the photosynthetic membrane. At the end of this branch the primary

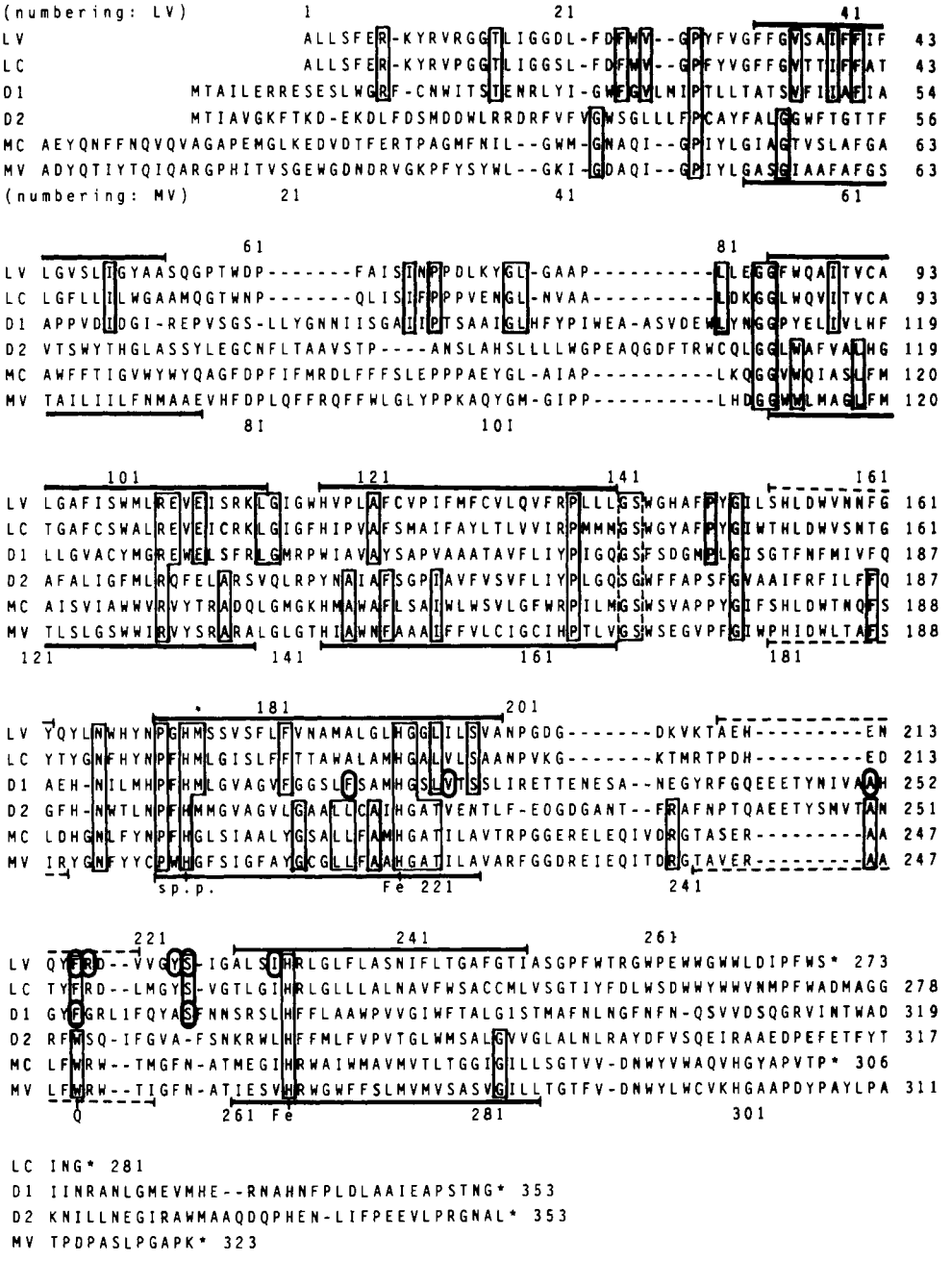

FIGURE 2: The amino acid sequences of the L and M subunits from the purple bacteria *Rps. viridis* (LV, MV; Michel et al., 1986a) and *Rb. capsulatus* (LC, MC; Youvan et al., 1984) compared with those of the D1 and D2 proteins from spinach chloroplasts (Zurawski et al., 1982; Alt et al., 1984; Holschuh et al., 1984). Amino acids common to all six subunits, the L subunits and D1, or the M subunits and D2 are boxed. The position of the transmembrane α-helices in the *Rps. viridis* reaction center is indicated by bars above the sequences of the L subunits and below the sequences of the M subunits. The positions of the short α-helices in the connections of transmembrane α-helices C and D, as well as D and E, are indicated by dashed lines. The histidine ligands of the special pair bacteriochlorophylls and of the non-heme iron atom are marked by sp.p. or Fe. Circles show amino acids known to be mutated in herbicide-resistant reaction centers from the purple bacteria or from photosystem II (for references, see text).

quinone Q_A is found. The binding site for the quinone ring is made up exclusively by the connections of the transmembrane α-helices D and E of the M subunit.

Most of the secondary quinone Q_B is lost during isolation of the reaction center and crystallization (Shopes & Wraight, 1985; Gast et al., 1985). It was therefore not visible in the original electron density map. Its binding site was deduced by soaking quinones and competitive inhibitors like *o*-phenanthroline and the herbicide terbutryn [2-(methylthio)-4-(ethylamino)-6-(*tert*-butylamino)-*s*-triazine] into the crystals and subsequent difference Fourier analysis (Deisenhofer et al., 1985; Michel et al., 1986). The binding site is found in a pocket formed by the α-helix connections of the transmembrane α-helices D and E of the L subunit in a position related to the Q_A binding site by the twofold rotation axis. In the crystal structure of the reaction center from *Rb. sphaeroides*, which became available recently, electron density for Q_B is seen in the equivalent position (Allen et al., 1986, 1987). Since the twofold symmetry axis runs perpendicular to the membrane, the electron transfer from Q_A to Q_B occurs parallel to the membrane plane.

Role of Conserved Amino Acids

Figure 2 shows the aligned amino acid sequences of the L and M subunits from *Rps. viridis*, *Rb. capsulatus*, and the D1 and D2 proteins from spinach chloroplasts. The sequence homology seems not to be significant during the first quarter of the sequences. In this region the D1 and D2 proteins are

considerably longer, and the existence in this sequence region of two transmembrane α-helices in D1 and D2 instead of only one in L and M is possible on the basis of hydropathy plots. Even models with a total of seven or eight transmembrane α-helices have been proposed for the D1 protein [see, e.g., Rao et al. (1983) and Kyle (1984)]. However, these models can be excluded from the results of topological experiments using site-specific antibodies directed against peptides of the D1 protein (Sayre et al., 1986). The immunological studies are only compatible with a total of five transmembrane α-helices. The sequence homology starts to become significant with a glycine–glycine pair at positions L83, L84, M110, and M111, corresponding to D1-109, D1-110, D2-109, and D2-110, which is at the start of the transmembrane α-helices B. This is in contrast to the folding model for D1 by Kleier et al. (1987), where transmembrane helix B was predicted to end previously at D1-100 by a combination of various prediction methods. Within the B α-helices an arginine residue (L103, M130, D1-129, D2-129) is conserved. In the bacterial reaction center the arginine side chains are located at the carboxy termini of the short α-helices (LDE, MDE) in the connections of the D and E transmembrane α-helices. Their major function may be to balance the permanent partial negative charge at the carboxy termini of these short α-helices. The existence of such arginines in the B α-helices of D1 and D2 can be taken as an argument in favor of the occurrence of such short α-helices in the connections of the D and E α-helices in photosystem II. Prolines L136 and M163 give rise to a kink of 30–40° in the C α-helices. We therefore expect to find a similar kink in the C α-helices of the D1 and D2 proteins of photosystem II due to prolines D1-162 and D2-162. At the ends of the transmembrane α-helices C the amino acid pair glycine–serine is found in the L and M subunits and D1. In D1 it is exchanged for serine–glycine, which may lead to an equivalent protein folding. Glycines L149 and M176, corresponding to D1-175 and D2-175, are close to the start of α-helices LCD and MCD in a tightly packed region of the protein. The conserved asparagines L166 and M193 are located in turns of the peptide chains and stabilize the turns by hydrogen bonds. This may be similar in the D1 and D2 proteins. The occurrence of proline L171 (M198) at the start of transmembrane α-helix LD (MD) is in line with the observation that prolines are frequently found at the starts of α-helical segments. Its conservation may indicate that D α-helices in the D1 and D2 proteins start with prolines D1-196 and D2-196, respectively. The histidines L173 and M200, corresponding to D1-198 and D2-198, are the protein ligands to the magnesium atoms of the special pair bacteriochlorophylls, and histidines L190 and M217 are ligands to the ferrous non-heme iron atom. Their conservation must be taken as a hint that a special pair of chlorophylls and a ferrous non-heme iron atom exist also in the photosystem II reaction center. Of special interest is the conservation of the following glycines, L191 and M218. A side chain there would interfere with the close approach of the α-helices LD and ME (or MD and LE, respectively). The conservation of these glycines therefore can be considered as diagnostic for a close approach of the LD and ME α-helices as well as the MD and LE α-helices in the D1 and D2 proteins near the ferrous non-heme iron atom. Beyond L191 and M218 other sequence alignments than those shown in Figure 2 are possible. These alignments [see, e.g., Hearst (1986) and Michel et al. (1986a)] show a slightly higher sequence identity for the E α-helices, but the histidines L220, M264, D1-272, and D2-272 would not be lined up.

In summary, primarily glycines and prolines of structural importance at or close to ends of α-helices are conserved, as well as the histidine ligands to the special pair bacteriochlorophylls and the non-heme iron atom. It is remarkable that the amino acids conserved between the L, M, D1, and D2 subunits are found at, or close to, the ends of the α-helices at the outer or the inner surfaces of the photosynthetic membranes. This can be seen nicely in Figure 1.

In the L subunits and D1 proteins phenylalanine L216 (corresponding to D1-255) and serine L223 (D1-264), which form part of the binding site of Q_B (see Figure 3), and the herbicide terbutryn (Michel et al., 1986b) are found in identical positions in Figure 2. In D2 and the M subunits phenylalanine L216 is replaced by a tryptophan (M250, D2-254), which forms a major part of the Q_A binding site and may be involved in electron transfer from the bacteriopheophytin to Q_A. Another critical amino acid seems to be glutamic acid L104 corresponding to D1-130. Apparently it is protonated and hydrogen-bonded to the bacteriopheophytin in the L branch (Michel et al., 1986b). It is not found in the M subunit or D2.

The roles of all these residues are in excellent agreement with the proposal that D1 and D2 constitute the core of the photosystem II reaction center. Between the starts of the B α-helices and the ends of the D α-helices, the L and M subunits, and the D1 and D2 proteins, differ in lengths by only one amino acid residue. This fact indicates a very similar protein folding of these regions in the D1 and D2 proteins and the L and M subunits. There are also two interesting differences. From the sequence homologies there is no evidence for the presence of binding sites for the accessory chlorophylls in D1 and D2. One possible explanation would be that accessory chlorophylls in photosystem II do not exist. Nanba and Satoh (1987), however, reported the presence of about five chlorophyll molecules per complex of D1/D2/cytochrome *b*-559. Therefore, the absence of accessory chlorophylls in the photosystem II reaction center seems unlikely. Another possibility is that the accessory chlorophylls are bound to the D1 and D2 proteins in a different position, or in a different way.

The D1 and D2 proteins possess a higher degree of sequence identity than the L and M subunits, and the length of the connections of transmembrane α-helices D and E is nearly equal when D1 and D2 are compared, but not when the L and M subunits are compared. The M subunit possesses an insertion of seven amino acids in the connection of the transmembrane α-helices D and E, which contains glutamic acid M232 as fifth protein ligand to the ferrous non-heme atom. Since the connections of the transmembrane α-helices D and E in the D1 and D2 proteins are equally long, a deviation from the symmetric protein folding allowing either D1 or D2 to donate a fifth protein ligand to the ferrous non-heme iron atom is unlikely. Having in mind the well-known effects of bicarbonate at the electron-accepting site of photosystem II [for review, see Vermaas and Govindjee (1982)], we consider bicarbonate as a likely candidate to be the fifth iron ligand in D1 and D2.

Another interesting difference is the presence of two histidines in D1 and D2 but not in L and M subunits: histidines D1-118 and D2-118 may be involved in binding other cofactors (manganese, accessory chlorophylls, or even the hemes of cytochrome *b*-559). If they were ligands to chlorophylls, the planes of the chlorophyll ring system must be expected to be perpendicular to the membrane and not nearly parallel as found for the accessory bacteriochlorophylls in the bacterial system. Histidines 190 in both the D1 and D2 proteins are rather close to the special pair binding site. They might be

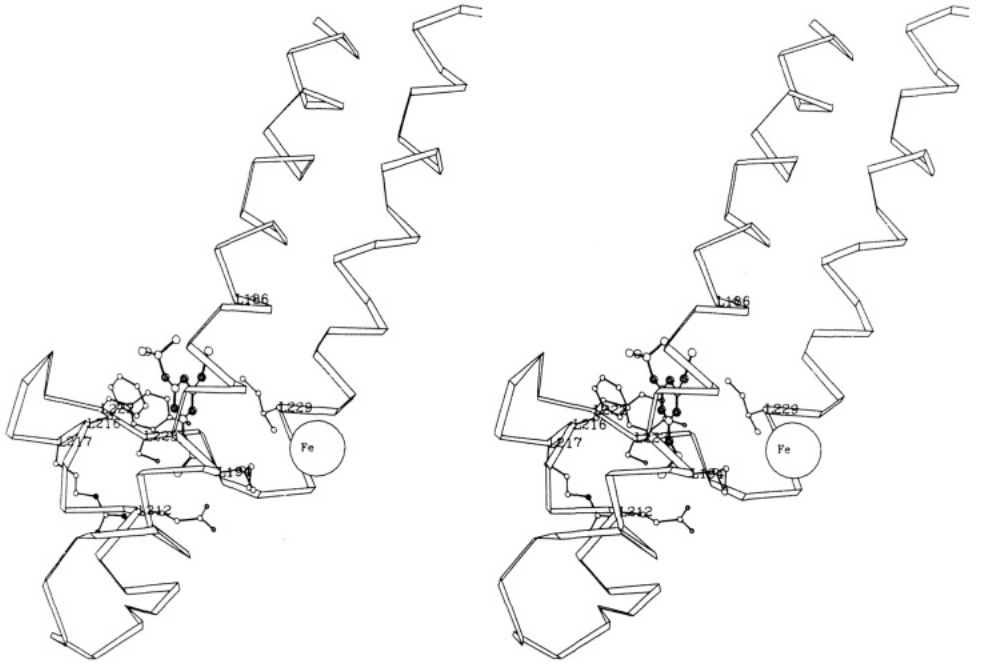

FIGURE 3: Stereo drawing of the terbutryn binding site in the photosynthetic reaction center from *Rps. viridis*. The peptide chain is represented as a ribbon drawing. Amino acids that are mutated in herbicide-resistant reaction centers from purple bacteria, as well as the amino acids corresponding to mutated amino acids in photosystem II (see Figure 2), are shown as atomic models.

candidates for being ligands to manganese atoms of the oxygen-evolving complex or Z, the secondary electron donor. These histidines are therefore interesting amino acids for future site-directed mutagenesis.

Q_B AND HERBICIDE BINDING SITE

The reaction centers from purple bacteria and photosystem II are both sensitive to herbicides of the *s*-triazine type like atrazine or terbutryn. Knowledge of the herbicide and Q_B binding site of the photosystem II reaction center is important for the design of new herbicides and for the generation of herbicide-resistant useful plants by molecular biologists. We therefore discuss the structure of the herbicide and Q_B binding site in detail and try to relate it to the mutations leading to herbicide resistance. The binding site of terbutryn and its mode of binding to the *Rps. viridis* reaction center have been established by X-ray crystallography (Michel et al., 1986b): apart from numerous van der Waals interactions terbutryn forms two hydrogen bonds with the protein matrix. One is found between a ring nitrogen as acceptor and the backbone N–H group of L224 as donor and the other between the serine side chain of L223 as acceptor and the aminoethyl side chain of terbutryn as donor.

The mutations leading to herbicide resistance are indicated in the sequences of Figure 2. In *Rps. viridis* three different mutants were obtained: (i) Phenylalanine L216 mutates to serine (Sinning & Michel, 1987). This phenylalanine forms a major part of the terbutryn binding site. This mutation may change the overall polarity of the binding site, or the hydrogen-bond capability of the side chain causes structural rearrangements. In the D1 protein from *Chlamydomonas* the corresponding phenylalanine 255 mutates to tyrosine (Erickson et al., 1985), which is also likely to cause structural changes. (ii) Tyrosine L222 mutates to phenylalanine in terbutryn-resistant *Rps. viridis* (Sinning and Michel, unpublished results) and to glycine in *Rb. sphaeroides* (Paddock et al., 1987). Since tyrosine L222 does not participate in terbutryn binding, a structural rearrangement is likely. (iii) Serine L223 mutates to alanine in *Rps. viridis* (Sinning & Michel, 1987) and to proline in *Rb. sphaeroides* (Paddock et al., 1987). This mutation can be expected from the mode of terbutryn binding. The absence of one out of two hydrogen bonds between terbutryn and the protein has to decrease the binding by several orders of magnitude. In *Rps. viridis* this mutation is always accompanied by a second mutation: arginine L217 is replaced by a histidine. Since arginine L217 is not involved in terbutryn binding, we consider this mutation as a secondary one somehow compensating a detrimental effect of the first one. The corresponding serine 264 in D1 mutates to alanine (Erickson et al., 1984) or glycine (Hirschberg et al., 1984) in different species. These mutations are in line with the observed mode of terbutryn binding in *Rps. viridis* and suggest the same mode of terbutryn binding in the photosystem II reaction center. The formation of a hydrogen bond between serine 264 and the herbicide had actually been proposed by Hirschberg et al. (1984).

Mutations so far not observed in *Rps. viridis* are the following: In *Rb. sphaeroides* isoleucine L229 mutates to methionine (Paddock et al., 1987). In these mutants the space available for terbutryn may be decreased due to the larger side chain. In *Rb. capsulatus* the same isoleucine (L229) has been changed to 17 amino acids by site-directed mutagenesis in order to engineer herbicide resistance (Bylina & Youvan, 1987). Leucine also led to considerable herbicide resistance. Three other mutations have been described in D1 proteins: (i) Valine 219 mutates to isoleucine (Erickson et al., 1984). The mutation influences the binding of atrazine only slightly but causes resistance to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). DCMU, however, has no effect on photosynthetic bacteria. Isoleucine L194, corresponding to valine 219 in the alignment of Figure 2, is spatially rather close to Q_B (see Figure 3). (ii) Alanine 251 from *Chlamydomonas* mutates to valine (Johanningmeier et al., 1987). The corresponding glutamate L212 (see Figure 2) is part of the binding site of Q_B. Again it is hard to understand the detailed molecular mechanism of herbicide resistance due to this mutation. (iii)

Phenylalanine 211 mutates to serine in D1 from the cyanobacterium *Synechococcus* PCC 7002 (J. C. Gingrich, V. L. Stirewalt, and D. A. Bryant, unpublished observation). A serine there could cause a change in the polarity of the binding site or a structural rearrangement due to the hydrogen-bonding capability of the serine side chain, as discussed above for the mutation of the bacterial phenylalanine L216 to serine. Since alanine L186 (corresponding to phenylalanine 211) is located opposite to phenylalanine L216 (corresponding to D1-254) in the terbutryn binding site, the herbicide terbutryn may be "sandwiched" between the aromatic rings of the phenylalanines 211 and 255 in the D1 proteins.

All the mutations are indicated in Figure 2 and their spatial location is given in Figure 3. For mutations in the D1 proteins the location of corresponding amino acids in the L subunit is given according to the alignment in Figure 2. It can be seen that all these residues are rather close to the herbicide terbutryn.

In the recently published crystal structure of the reaction center from *Rb. sphaeroides* (Chang et al., 1986; Allen et al., 1986, 1987), electron density for the Q_B was found. The oxygen atoms of Q_B have been proposed to be hydrogen bonded to the side chain of serine L223 and histidine L190 (Allen et al., 1986). In the highly refined electron density at 2.3-Å resolution of the *Rps. viridis* reaction center (Deisenhofer et al., unpublished results), weak electron density is also seen, which we attribute to Q_B; 30% of Q_B was still present in the crystals used for data collection (Sinning and Michel, unpublished results). The electron density suggests possible hydrogen bonds of Q_B to histidine L190 and to the side chain of serine L223. However, the latter hydrogen bond may be shared with the backbone N–H of L225. If the hydrogen-bond pattern of Q_B is the same in D1, then it is somewhat different from the models proposed by Trebst (1986, 1987), where Q_B is hydrogen-bonded to histidine D1-215 and the N–H backbone of serine D1-264 or alanine D1-263. The sharing of one hydrogen bond of Q_B between a backbone N–H and the side chain of serine L223 also might explain why the serine L223 to alanine mutants still bind Q_B but show resistance toward terbutryn, which does not share its hydrogen bond to serine L223.

A problem in this region is the low sequence homology between L and D1, especially the fact that the connection between the α-helices D and E is considerably longer in D1. Most likely, these amino acids give rise to an additional loop of the peptide chain in the intrathylakoidal space of chloroplasts at the beginning of the small α-helix in the DE connection.

Conclusions

The arguments presented above strongly favor the proposal that the core of the photosystem II reaction center and the core of the reaction center from purple bacteria possess a very similar structure. The model presented in Figure 1 is therefore a model not only for the arrangement of the major α-helices in the reaction center from purple bacteria but also for their arrangement in the reaction center core of photosystem II. Possible differences are the mode of binding or the location of the accessory chlorophylls and the nature of the fifth ligand to the non-heme iron, which may be bicarbonate in photosystem II in contrast to a glutamyl residue in the reaction center of the purple bacteria. Similarly, Figure 3 probably presents the best available model for the herbicide binding site in the photosystem II reaction center. Sequence comparisons alone would not be sufficient to draw these conclusions since the overall sequence identity between all L and M subunits and the D1 and D2 proteins is only about 5%. However, the crystallographically based knowledge of the role of the conserved amino acids puts these conclusions on a rather safe basis. In addition, the proposal that the D1 and D2 proteins form the core of the reaction center of photosystem II immediately led to the successful isolation of a D1/D2/cytochrome *b*-559 complex from photosystem II.

Added in Proof

By a combination of site-directed mutagenesis and EPR spectroscopy, evidence has been obtained recently (R. Debus, G. T. Babcock, and L. McIntosh, unpublished results; W. Vermaas, unpublished results) that D, a slow electron donor to P680 acting parallel to Z, is identical with tyrosine D2-160. It seems possible now that Z is identical with tyrosine D1-160 and is not a plastoquinol as postulated earlier.

Acknowledgments

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