A Contact Site between the Two Reaction Center Polypeptides of Photosystem II Is Involved in Photoinhibition

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Dedicated to Professor Wolfgang Haupt on the occasion of his 70th birthday

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A new contact site between the two reaction center polypeptides D1 and D2 of photosystem II close to arg238 and arg234 respectively is proposed. The amino acid sequences involved are between the 4th transmembrane and a connecting parallel helix. The sequence includes a trypsin sensitive site in both polypeptides, the likely cleavage site in the rapid turnover of the D1 polypeptide and part of the herbicide binding site. The contact site is oriented towards both quinone binding sites Q_A and Q_B. A folding of the backbone of the amino acid sequences involved is proposed.

Introduction

The reaction center P_{680} and the two plastoquinone acceptor sites of photosystem II are bound to two protein subunits, the D1 and D2 polypeptides (see review in [1]). A folding model [2] of their amino acid sequences was based on their homology to the L and M polypeptides of the purple bacteria reaction center of *Rps. viridis,* for which a X-ray structure is available [3]. Amino acid substitutions in inhibitor tolerant mutants (see [4]) were essential for support of the folding prediction of the quinone (and herbicide) binding sites [2, 5]. Accordingly the niche for quinone binding (Q_A and Q_B) on these polypeptides is formed by the ends of the 4th and 5th transmembrane helices and a connecting parallel helix between these two. The two polypeptides of the reaction center and their two quinone binding sites are interconnected by an Fe-atom, that is bound by four histidines, two from each subunit on their 4th and 5th transmembrane helix [3]. There are further contact sites between the two reaction center polypeptides in various places in the reaction center of purple bacteria [3], and these are likely very similar in photosystem II. But in addition to these there seems to exist another contact site specific for photosystem II between the D1 and the D2 polypeptide and the Q_A and Q_B site. This additional contact site in PS II is in amino acid sequences lacking in the L and M subunit of the purple bacteria. It appears to provide a communication between the two quinone binding sites in regulatory phenomena like in photoinhibition.

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Results

The trypsin sensitivity of photosystem II is well known [6, 7]. In thylakoids only the acceptor side of PS II is accessible to trypsin; the donor side and the watersplitting system are protected because of the closed vesicle structure. Under these conditions trypsin treatment of the thylakoids results in a ferricyanide reduction by PS II alone, which is insensitive to inhibitors in the Q_B site [6, 7]. This was explained by a loss of Q_B and herbicide binding and an acquired accessibility of Q_A to the hydrophilic ferricyanide [8]. It was shown that indeed trypsin cleaves the D1 protein, that carries the Q_B and herbicide binding site [9, 10]. The cleavage site was identified at arg237 [11]. Later we showed [12] that trypsin under the same conditions also cleaves the D2 polypeptide, even preferentially over the D1 protein. This explained the acquired Q_A accessibility. The cleavage site in the D2 protein was identified at arg234 [12].

It was shown early that DCMU interferes with the trypsination of the D1 protein [6, 7, 13–15]. This was extended by showing that protection from trypsin cleavage is obtained by DCMU and other compounds of its inhibitor family but not by inhibitors of the phenol type [13–16]. Such data of experiments with B. Depka are shown and extend-

Table I. Effect of the two types of PS II inhibitors on the trypsin cleavage (5 min) of the two reaction center polypeptides in isolated spinach thylakoids.

Inhibitor [μM]	Remaining protein [%] D1 protein	D2 protein
None	11	12
10 DCMU	100	42
10 Atrazine	74	48
10 Metribuzine	36	50
300 *o*-Phenanthroline	45	17
10 Bromonitrothymol	0	11
10 Hydroxypyridine	21	0
1 Hydroxyquinoline	20	10
10 Ioxynil	50	
20 Pyridate	70	75
Control without trypsin	100	100

Hydroxyquinoline: 3-bromo-2-trifluoromethyl-5,8-dichloro-4-hydroxy-quinoline. Hydroxypyridine: 2-fluoro-tribromo-4-hydroxy-pyridine. Pyridate: 6-chloro-3-phenyl-4-hydroxy-pyridazine (*i.e.* the hydrolyzed commercial product).

ed in Table I. They show that DCMU and bromonitrothymol are on the extremes in full or no protection. Other inhibitors are in between, reflecting the individual orientation of the inhibitors in the binding niche. This is explored somewhere else [5, 17]. Ioxynil and pyridate (and other phenyl substituted phenols, reported on elsewhere) are called "intermediate" phenols as against "pure" phenol types, like bromonitrothymol. The effect of pyridate, already studied [18], is explored in more detail by M. Jansen and M. Edelman, who suggested the use of pyridate to us.

Table I also shows the effect of the inhibitors on the trypsin sensitivity of the D2 protein. When first observed [12] it seemed as a surprise that the DCMU type inhibitors in the Q_B site also protect the cleavage of the D2 protein. As shown in Table I the effect of the inhibitors on the protection of the D2 protein is very similar to that on the D1 protein, although there are fine differences.

It is exactly this that I want to point out in this paper: an inhibitor in the Q_B site not only effects the D1 polypeptide, but also the Q_A binding polypeptide D2.

We have discussed [16] that the properties of trypsin cleavage and the effect of inhibitors resemble in certain aspects that of the cleavage of the two proteins in photoinhibition.

In photoinhibition photosystem II is quickly inactivated and then its protein subunits photodegraded (for a recent review see [19]). The degradation starts at the D1 polypeptide, followed by that of the D2 protein [19, 20]. Likely the phenomenon of rapid turnover of the D1 protein, established earlier and in great detail (for a recent review see [21]), is part of the photoinhibitory process and therefore its properties are very relevant. The cleavage site in rapid turnover is likely close to glu 240 of the D1 polypeptide [22]. Particularly relevant for this paper is that the cleavage is blocked by inhibitors of the DCMU type [21], but not necessarily by inhibitors of the phenol type [23]. In *Chlamydomonas rh.* [19, 20], but also in isolated thylakoids photoinhibition results in the degradation of the D1 and of the D2 polypeptide, as reported by several groups [20, 24–27]. In UV-triggered photoinactivation both types of inhibitors protect the degradation of both protein subunits [27].

Photoinactivation of photosystem II without any photodegradation occurs in illuminated thylakoids in the absence of an electron acceptor and under strict anaerobic conditions [20, 26, 28]. This fast photoinactivation is prevented by inhibitors of the DCMU type, but not of the phenol type [20]. As Table II is to show a preillumination of thylakoids under anaerobic conditions leads to a loss of about 80% of their oxygen evolving capacity. If

Table II. Effect of the two type of photosystem II inhibitors on the photoinactivation of PS II under anaerobic conditions in isolated spinach thylakoids. For testing activity of the thylakoids that had been preilluminated in the presence of an inhibitor enriched PS II (BBY) particles were prepared. The Triton washings were done in the presence of phenanthroline, that competes and therefore removes the other inhibitors and is itself washed off the membrane easily in the final washing.

Pretreatment	Activity of oxygen evolution after 5 min pretreatment [μmol/mg chlorophyll]
Dark	420
Light	90
Light + inhibitor	
10 μM DCMU	408
10 μM Atrazine	240
10 μM Metribuzine	360
100 μM *o*-Phenanthroline	420
1 μM Bromonitrothymol	72
1 μM Ioxynil	72
10 μM Pyridate	48

Pyridate: 6-chloro-3-phenyl-4-hydroxy-pyridazine.

the preillumination is done in the presence of DCMU, atrazine, metribuzine or *o*-phenanthroline, all or most of the activity is preserved. If, however, the preillumination is done in the presence of any phenol type inhibitors no or even a further loss of activity is observed (Table II).

Discussion

It is, of course, well established that the classical PS II herbicides of the DCMU/triazine family block photosynthetic electron flow by displacing plastoquinone Q_B from the D1 polypeptide [29] and by shifting the redox equilibrium between Q_A and Q_B [30]. Also the phenol type inhibitors bind at the Q_B site. But as there are a number of differences in their inhibitor pattern (reviewed in [31]) they are grouped as a separate PS II inhibitor family [5, 17]. Cross resistance studies have recently supported the concept of two different types of PS II inhibitors that are oriented differently in the Q_B binding niche.

Both types of inhibitors bind then to the Q_B site on the D1 polypeptide. However, it became clear recently that Q_B site inhibitors affect properties of the D2 polypeptide as well [12, 16]. The effect of the two type of inhibitors on the D2 polypeptide is again different. Data given and quoted under "Results" show effects of inhibitors on the D1 and the D2 polypeptide:

1. Not only electron flow but also other properties of the D1 polypeptide are affected by inhibitors in the Q_B site. Trypsin cleavage of the D1 polypeptide at arg238 is prevented by inhibitors of the DCMU/triazine family. "Pure" phenol type inhibitors do not. Rapid turnover and the degradation of the D1 polypeptide in photoinhibition are prevented or slowed down. There are again differences of the two types of inhibitors on this.

2. Trypsin cleavage of the D2 polypeptide at arg234 is slowed down by certain inhibitors in the Q_B site. The same is true for its degradation in photoinhibition both *in vivo* and *in vitro*.

3. The fast photoinactivation of PS II under anaerobic conditions preceding photodegradation of the two subunits is prevented by inhibitors of the DCMU, but not of the phenol type.

It can be argued that the effect of Q_B site inhibitors on the proteolytic cleavage of the D2 polypeptide is due to a covering up of the D2 polypeptide by the D1 polypeptide and therefore the protective effect is indirect. Or the communication of the inhibitors in the D1 towards the D2 polypeptide occurs *via* the central Fe, bound to both polypeptides. Here it is proposed that 1. there is a direct interaction between those amino acids in the D1 and D2 polypeptide that are cleaved in trypsin treatment or in photodegradation and that 2. these amino acids are part of both the Q_A and Q_B site.

The results show that interaction between the D1 and D2 polypeptides occurs in a part of their amino acid sequence that has no homologous sequence in either the L or M subunit of the purple bacteria and is therefore sometimes called the "extra loop". The interaction of the D1 and D2 polypeptide in their respective arginines (238 and 234 respectively) gives an important clue for an attempt to model the new contact site and the "extra loop". A further parameter given is that the arginines are part of the Q_A and Q_B site respectively. There is good evidence for that for Q_B but direct evidence for an interaction of Q_A with arg234 of the D2 protein is scare, except of the acquired Q_A accessibility after cleavage at arg234.

Any folding model for the reaction center II will start with identifying conserved functional amino acids [2, 3, 32], in particular histidines for Fe binding, two of which also provide hydrogen bridges to the quinones, as shown in the X-ray structure of the purple bacterial reaction center [3]. The central Fe interconnects 4 transmembrane helices [3]. Table III shows the position of these histidines in the amino acid sequences and the number of amino acids between them. Between those histidines the D1 polypeptide is 17 amino acids longer than the homologous L subunit and the D2 polypeptide is 7 amino acids longer than the M subunit. One can break down these additional amino acids, by counting from the first Fe binding histidine on helix IV (or helix D) to the phe (or trp in the D2 and M polypeptide) that is in the connecting parallel helix and is oriented below the quinone towards pheophytin [3]. Then count from these amino acids to the serine in the D1 and L polypeptide that provides another hydrogen bridge to Q_B and is substituted in triazine tolerance both in PS II (see [4]) and in purple bacteria [33–35]. It is an ala in the D2 and M polypeptide, whose backbone nitrogen appears to be involved in Q_A binding [2, 3]. And finally count from the serine to the histidine

Table III. Number of amino acids between equivalent functional sites in quinone and Fe binding in the protein subunits of the reaction center of photosystem II (*i.e.* D1 and D2) *vs.* that of *Rps. viridis* (*i.e.* L and M).

Protein subunit	Amino acid at position:		Distance in number of AS		Amino acid at position:
	The Q_B binding proteins				
D1	His_{215}		57		His_{272}
L	His_{190}		40		His_{230}
D1	His_{215}	Phe_{255}	9	Ser_{264}	His_{272}
	40			8	
L	His_{190}	Phe_{216}	7	Ser_{223}	His_{230}
	26			7	
	The Q_A binding proteins				
D2	His_{215}		54		His_{269}
M	His_{217}		47		His_{264}
D2	His_{215}	Trp_{254}	9	Ser_{263}	His_{269}
	39			6	
M	His_{217}	Trp_{250}	8	Ala_{258}	His_{264}
	33			6	

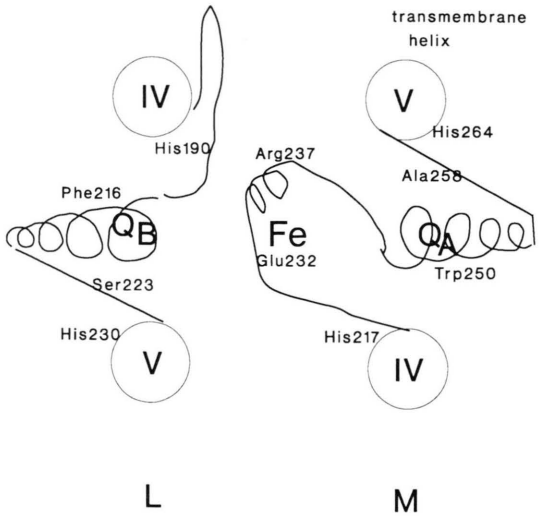

Fig. 1. Schematic drawing of the backbone folding of the amino acid sequence of the L and M subunit of the reaction center of *Rps. viridis* that are involved in quinone binding. Redrawn from the folding model according to the X-ray data in [3]. Viewed from the top.

on helix V (or helix E) liganding the central Fe. From Table III it is clear that the extra amino acids in the PS II polypeptides are mainly between helix IV and the parallel helix – additional 14 in the D1 and 6 in the D2 polypeptide, when compared with the L and M subunit. Whereas the L and M subunits are different from each other in length in this area, the D1 and D2 polypeptides are almost identical in length. The proteolytic cleavage sites in trypsin treatment, rapid turnover and photoinhibition discussed above are in these extra amino acids.

By using the data for the three-dimensional folding of the reaction center from *Rps. viridis* derived from the X-ray structure [3] and keeping the principal features, one can attempt to model the "extra loops" in PS II. The two additional amino acids between the parallel helix and transmembrane helix 5 in the D1 polypeptide and one in the D2 polypeptide, when compared with the L and M subunit, although likely of profound importance for the differences in quinone and inhibitor function between PS II and purple bacteria, are ignored here and therefore not changed in Fig. 1 *vs.* Fig. 2.

Fig. 1 shows schematically the folding of the backbone of the amino acids between helix IV (or

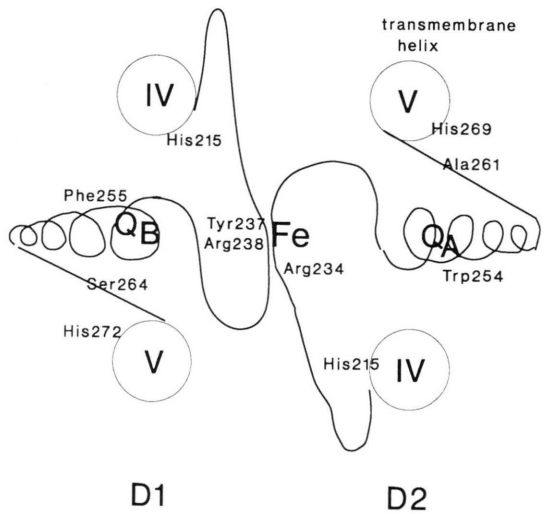

Fig. 2. Schematic drawing of the backbone folding of the amino acid sequence of the D1 and D2 polypeptide of photosystem II, that are involved in quinone binding. A contact site between the two subunits is proposed close to the respective arginines 237 and 234. Viewed from the matrix side towards the top of the four transmembrane and the two parallel helices.

helix D) of the bacterial reaction center of *Rps. viridis*, as taken from the three-dimensional folding derived from the X-ray structure [3]. One can then model the backbone of the additional amino acids in PS II like in Fig. 2. For the folding of the D1 polypeptide start at the N side from the parallel helix like that of the M subunit; but then extend the sequence towards the transmembrane helix 4 like in the L subunit. For the folding of the D2 polypeptide add the 6 additional amino acids onto the folding of the M subunit like the turn in the L subunit at the C side of the 4th helix. This is drawn schematically in Fig. 2. Accordingly the "extra loops" in the sequences of both the D1 and D2 polypeptide fold very symmetrically, unlike the asymmetric folding of the L and M subunits, which are different in length in this part of the sequence. After transmembrane helix IV the amino acid sequences extend somewhat towards the outside, but then fold back inwards. As both subunits of PS II do so, they touch each other above the Fe and then continue to the parallel helix. The sequence of the D2 subunit has to be pushed sideways in comparison with the M subunit in order to accommodate the D1 sequence. This is another reason why there is no homology in the D2 subunit to glu 232 of the M subunit. The contact site is assumed to be close to the arginines from the results discussed above. This way the gap above the central Fe is closed in photosystem II and no polypeptide equivalent to the H subunit in the bacterial system is required in PS II. This is not to indicate at all that they get close to the Fe. Actually the distance to it is large. The trypsination experiments actually suggest the immediate accessibility of those arginines from the matrix space. The heme groups of (two) cytochrome(s) b_{559} might be arranged in the space outside helices IV and V of the D1 and D2 polypeptide respectively and symmetrically on the opposite side. This way cytochrome b_{559} might contribute to the folding (and stability) of the reaction center polypeptides by preventing that the "extra loops" discussed here extend to far outside and then would not touch each other. Indeed also the large subunit of Cyt b_{559} is easily cut by trypsin [36].

Fig. 2 is to indicate schematically how the occupancy and redox state of the Q_B site is communicated to the D2 polypeptide (like in the trypsination of the D2 polypeptide) and how *vice versa* that of the Q_A site may induce cleavage of the D1 polypeptide in photoinhibition. This is, if "overreduction" of Q_A after or in photoinactivation of PS II is indeed the trigger for photodegradation [26, 37].

That the amino acid sequence around arg 238 is part of the Q_B and DCMU binding site is directly observed in the photoaffinity labelling experiment, where tyr 237 is tagged by azidomonuron [38]. The "pure" phenol type inhibitors will orient in the Q_B binding site such, that they do not "see" that tyrosine 237 and therefore do not protect from proteolytic cleavage. (Recent evidence for phenyl substituted phenols, including pyridate, shows that they do "see" arg 238, as already indicated in Table I.) When only displacement of plastoquinone Q_B is required (like in inhibition of e-flow or in UV-triggered degradation [27]) the phenol type inhibitors behave like DCMU, but otherwise they don't.

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