Sequence Analysis of the D1 and D2 Reaction Center Proteins of Photosystem II

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A compilation of 38 sequences for the D1 and 15 sequences for the D2 reaction center proteins of photosystem II is presented. The sequences have been compared and a similarity index that takes into account the degree of conservation and the quality of the changes in each position has been calculated. The similarity index is used to identify and describe functionally important domains in the D1/D2 heterodimer. Comparative hydropathy plot are presented for the aminoacid sidechains that constitute the binding domain of the tyrosine radicals, Tyr_Z and Tyr_D, in photosystem II. The structure around Tyr_Z is more hydrophilic than the structure around Tyr_D. The hydrophilic residues are clustered in the part of the binding pocket for Tyr_Z that is turned towards the lumenal side of the thylakoid membrane. Most prominent is the presence of two conserved carboxylic aminoacids, D1-Asp170 and D1-Glu189. Their respective carboxyl-groups come close in space and are proposed to constitute a metal binding site together with D1-Gln165. The distance between the proposed metal binding site and the center of the ring of Tyr_Z is approximately 7 Å. The cavity that constitutes the binding site for Tyr_D is composed of residues from the D2 protein. Its character is more hydrophobic than the Tyr_Z site and the environment around Tyr_D lacks the cluster of putative metal binding sidechains.

Introduction

A major breakthrough in bioenergetic research is the determination to high resolution of the 3-dimensional structure for the photosynthetic reaction center from purple bacteria [1, 2]. This achievement is important also for the understanding of oxygenic photosynthesis since the reaction center in photosystem II (PS II) is thought to be homologous to the bacterial reaction center. The reaction center in PS II is composed of a heterodimer of two hydrophobic proteins, D1 and D2 [3], that show considerable sequence homologies with their bacterial equivalents, the L and M subunits [4–7]. The D1 and D2 proteins most probably contain five membrane spanning helices in analogy to the L and M subunits [8]. In addition, sequence alignment studies with the bacterial reaction center proteins have been used to place the start and end points of the five transmembrane helices [7, 9, 10] and to identify potentially important residues in the D1 and D2 proteins [7, 9, 11].

The D1/D2 heterodimer in PS II binds the primary electron donor, P_{680}, the acceptor complex including the intermediary pheophytin acceptor, the first and second quinone acceptors, Q_A and Q_B, and the acceptorside iron (for a recent review covering most aspects to PS II see [12]). The binding of those components is thought to be quite similar to the binding of the corresponding components in the bacterial reaction center [13]. In addition, the D1 and D2 proteins contain two redox-active tyrosine residues with functions on the donor side of PS II (Tyr_Z, the immediate electron donor to P_{680}^+, is D1-Tyr 161 and Tyr_D, an accessory electron donor, is D2-Tyr161) [14–17]. Also the Mn-cluster involved in the oxidation of water is thought to be bound to the D1/D2 heterodimer [10, 18]. Both the D1 and D2 proteins are highly conserved proteins [10, 19] which reflects the intricate and complicated photochemistry that is carried out by the

Abbreviations: P_{680}, primary donor chlorphyll(s) in PS II; PS II, photosystem II; Q_A, first quinone acceptor in PS II; Q_B, second quinone acceptor in PS II; Tyr_D, D2-Tyr161, accessory electron donor in PS II; Tyr_Z, D1-Tyr161, immediate electron donor to P_{680}.

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The amino acid numbering used in this paper is according to the sequences for the D1 and D2 proteins in spinach.

D1/D2 heterodimer. Despite this overall conservation it is highly likely that functionally important amino acids and regions in the sequences show a higher degree of conservation than less important elements. Therefore, careful analysis and comparison of the available aminoacid sequences will prove useful in attempts to study different components and partial reactions carried out by the heterodimer using site-directed mutagenesis.

In earlier computer assisted model studies we have used the homology between PS II and the bacterial reaction center to predict the three-dimensional structure around Tyr_Z and Tyr_D in the D1 and D2 proteins respectively [10]. The tyrosine residues were found in cavities close to the lumenal side of the heterodimer. The cavities were very similar in size and location but we pointed out that the environment around Tyr_D seemed to be more hydrophobic than the environment around Tyr_Z. From considerations of data in the literature we suggested that the hydrophilic loop between helices A and B in the D1 protein is putative binding region for the Mn-cluster and we proposed that Mn-ligands were to be found among the almost 10 polar amino acids (histidines and carboxylates) that are conserved in this loop on the D1 protein.

Here we take this work further. We make a more detailed sequence comparison of the D1 and the D2 proteins including statistical analysis of the sequences. In addition, we present a hydrophobicity plot of the cavities that contain the two tyrosine-radicals. A cluster of hydrophilic side-chains in the immediate vicinity of Tyr_Z that might constitute a metal-binding site is also described.

Materials and Methods

The computer manipulation of the sequences were done with the UWGCG sequence analysis software package [20] on a microVAX computer. Most of the sequences are available in the databases Genbank, EMBL, NBRF and SwissProt. When only the DNA sequence was available, it was translated to its peptide sequence using standard codon translation. The sequences were aligned pairwise by insertion of gaps with the program GAP.

The compared D1 protein sequences are from higher plants [21–39], liverwort [40], algae [41, 42], *Euglena gracilis* [43, 44], *Cyanophora paradoxa* (cyanelle) [45], *Prochlorothrix hollandica* [46] and cyanobacteria [47–54]. The sequences for the D2 protein are from higher plants [55–63], liverwort [40], algae [64] and cyanobacteria [65–67]. The sequences for the D1 proteins from *Chenopodium album* (residues number 212–280; [26]) and *Triticum aestivum* (residues number 1–70; [37]) are not complete. The sequence for the D2 protein from *Triticum aestivum* (residues number 1–10; [63]) is also incomplete. In the published sequence for the D1 protein from *Vicia faba* [33] His215 is wrongly claimed to be an glutamine residue (K. Ko personal communication). Only one [43] of the two available sequences [43, 44] for the D1 protein from *Euglena gracilis* has been used in Table IA and in our calculations. The two sequences differs in position 79 (Ile in [43] and Leu in [44]) due to different interpretations of the intron-exon boundaries. We have used the sequence [43] that is identical to all other D1 sequences in position 79. In the sequence for the D2 protein from *Hordeum vulgare* the published sequence in [68] contains two errors (correct in [60]) which lead us to treat D2-Ala171 and D2-Ser173 as non-conserved in our earlier publication (Fig. 1B in [10]) although they are entirely conserved among the available sequences.

An array of similarity indices, indicative of how conserved the residues are along the sequence, was calculated for the D1 and D2 proteins in the following way: In the aligned nonidentical sequences (A-Z for the D1 protein in Table IA and A-L for the D2 protein in Table IB) all residues at the same position were compared pairwise to each other. A score value was assigned to every pair and their average was taken as the similarity index for that particular position. The similarity indices were calculated for every position in the sequence and were plotted against the residue number. The score values were taken from the Dayhoff mutational difference score matrix [69] normalized by Gribskov and Burgess [70] which takes into consideration the quality of the aminoacid changes. In the score matrix the highest value is 1.5 for identical residues, and the lowest value is −1.2 for a change from cystein to tryptophane. The same score of −1.2 was also given to a gap. Thus, the average similarity index is ranging from 1.5 (completely conserved residue) to −1.2 (a residue which is inserted into only one of the sequences, *e.g.* residue 4 in the D1 protein from *Euglena gracilis*).

The hydropathy plots were done with the program QMAGINT using the average membrane preference scale (AMP07) shown to be optimal for membrane proteins with redox functions [71]. The analyzed sequences were artificially created from the aminoacids surrounding Tyr_Z and Tyr_D (see legend to Fig. 2). Therefore, the studied residues are not neighbours in the protein backbone. For the hydropathy calculations a window size of one was used. The curves were then smoothed by a moving average of three consecutive values.

Results and Discussion

Sequences for the D1 and D2 proteins

The aligned aminoacid sequences of the D1 and D2 proteins, from 38 and 15 organisms respectively, are shown in Table I A and B. The complete spinach sequence is shown at the top of each table. To align the D1 proteins, gaps were inserted in the N-terminus and in the C-terminus, while for the D2 protein gaps only had to be inserted in the N-terminus. This is because the sequences for the D2 protein are more similar in length than the D1 sequences (Table I).

From Table I it is clear that both the D1 and the D2 proteins are very conserved. About 65% of the residues in the D1 protein and 75% in the D2 protein have not been altered during the long evolutionary time-span from cyanobacteria to higher plants. The somewhat lower value for the D1 protein is probably not due to a lesser degree of conservation in this protein but instead it might reflect the higher number of sequences available for the comparison.

With this extensive sequence information available it is possible to make a statistical comparison of the sequences to identify conserved and non-conserved stretches in the proteins. The interest in this approach is that very important functions, like the primary photochemical reactions, the oxygen evolution and the plastoquinone reduction are likely to occur in the more conserved parts of the structure while less conserved regions can be predicted to have less importance for both structure and function. To accomplish this comparison, we have calculated a similarity index for the aligned sequences (Fig. 1). Negative scores are given to positions in the sequences where there occur many changes or where the changes that occur are to aminoacids of very different type. High positive scores are given to positions with few changes between the organisms or where the changes are minor.

In both the D1 and the D2 protein the highest degree of conservation (Table I; Fig. 1) is found in the central helical core of the proteins which is considered to be the region for the primary charge separation reaction [7, 9, 10, 12]. Especially the membrane spanning D helix and the CD helix, lying parallel to the membrane plane on the lumenal side of the thylakoid membrane, are almost totally conserved. The central helical core most

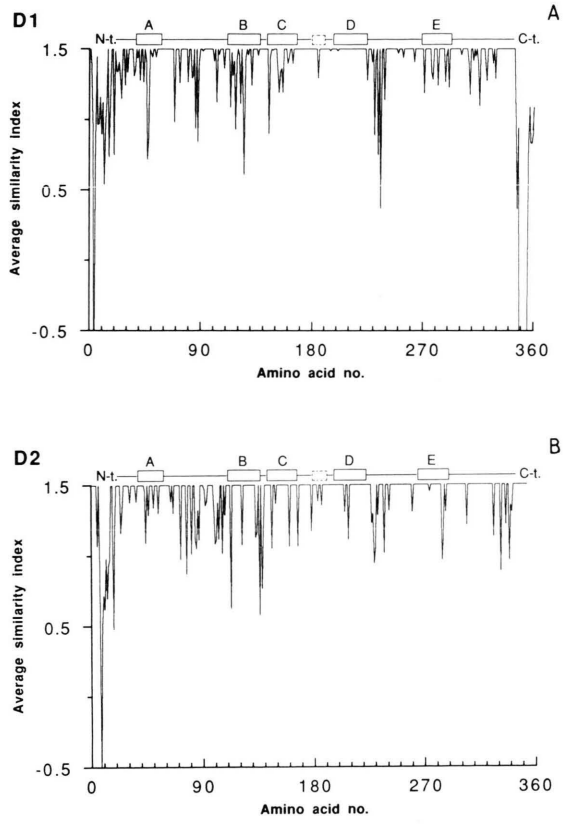
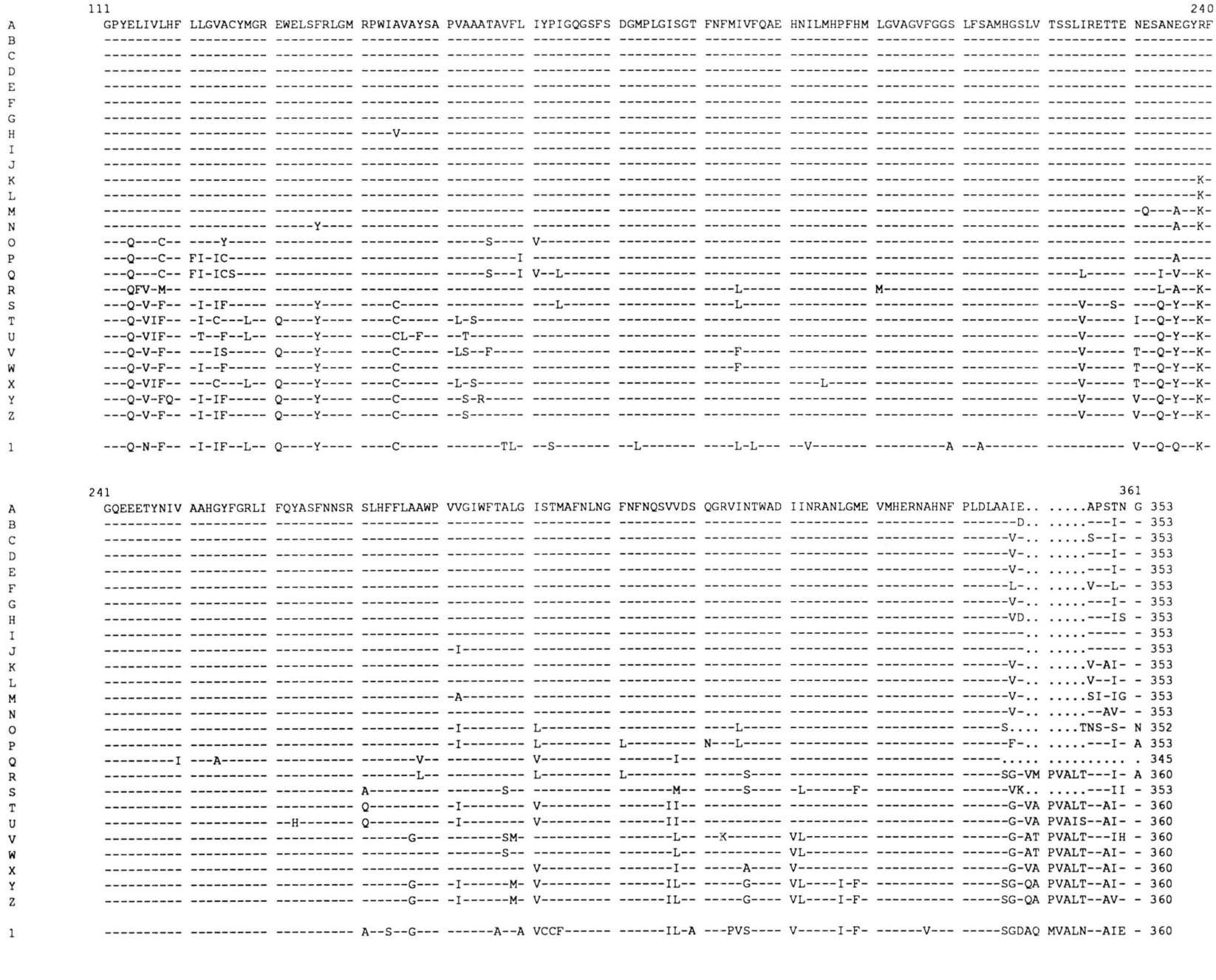


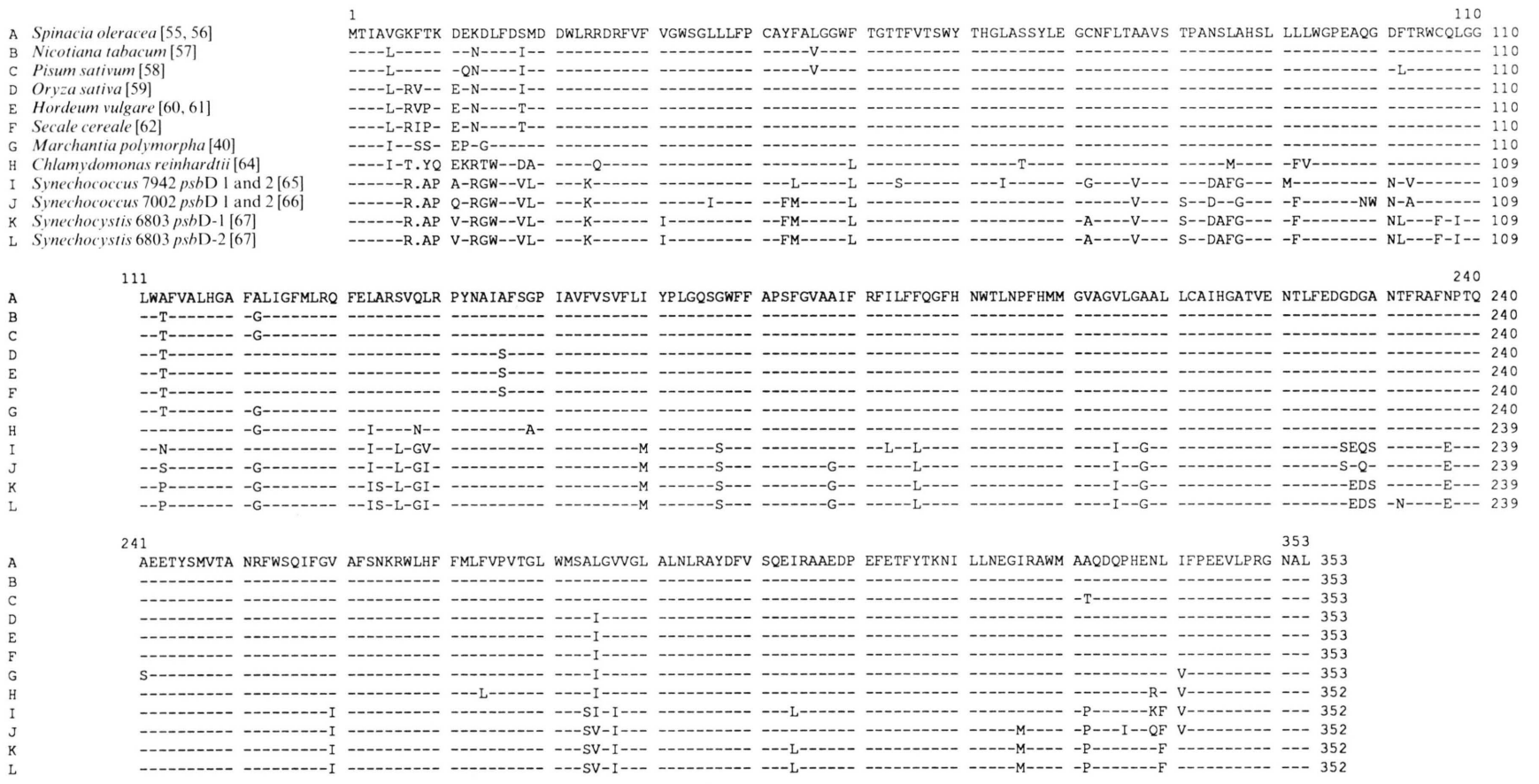
Fig. 1. A. Similarity index for the D1 protein. The numbering is taken from the aligned sequences in Table I B. B. Similarity index for the D2 protein. The numbering comes from the aligned sequences in Table I B. Only complete sequences have been used in the calculations. The sequence for *Synechocystis* 6803 *psb*A-1 has not been used because this gene has never been shown to be expressed *in vivo* [72]. In the top of the figure the approximate positions of the membrane spanning helices and the CD helix (dashed box) are indicated.

Table I. A. The aligned D1 protein sequences from different organisms. B. The aligned D2 protein sequences from different organisms. The first sequence in each table is the spinach sequence. Identical residues are represented by – and where the sequences differ the aminoacids are designed by their one letter codes. The gaps from the alignment analysis are marked as dots. Only one of each identical sequence is shown in the table. Identical to the D1 protein from *Spinacia oleracea* [21] are the sequences from *Nicotiana debneyi* [21], *Nicotiana tabacum* [22], *Nicotiana plumbaginifolia* [23], *Gossypium hirsutum* [24], *Solanum nigrum* [25] and *Chenopodium album* (residues 212–280) [26]. Identical to the D1 protein from *Hordeum vulgare* [36] is the sequence from *Triticum aestivum* (residues 1–70) [37]. Identical to the D2 protein from *Secale cereale* [62] is the sequence from *Triticum aestivum* (residues number 1–10) [63].

D1 sequences

		1									110	
A	*Spinacia oleracea* [21]	MTA.ILERRE	SESLWGRFCN	WITSTENRLY	IGWFGVLMIP	TLLTATSVFI	IAFIAAPPVD	IDGIREPVSG	SLLYGNNIIS	GAIIPTSAAI	GLHFYPIWEA	ASVDEWLYNG 109
B	*Glycine max* [27]	---.------	----------	----------	----------	----------	----------	----------	----------	----------	----------	---------- 109
C	*Sinapis alba* [28]	---.------	----------	----------	----------	----------	----------	----------	----------	----------	----------	---------- 109
D	*Medicago sativa* [29]	---.-----D	--T-------	----------	----------	----------	----------	----------	---------C	----------	----------	---------- 109
E	*Brassica napus* [30]	---.------	----------	----------	----------	----------	----------	----------	----------	----------	----------	---------- 109
F	*Oryza sativa* [31]	---.------	-T--------	----------	----------	----------	----------	----------	----------	----------	----------	---------- 109
G	*Pisum sativum* [32]	---.-----D	--N-------	----------	----------	----------	----------	----------	----------	----------	----------	---------- 109
H	*Vicia faba* [33]	---.-----D	-HN-------	---T------	----------	----------	----------	----------	----------	--------V-	----------	----D---I- 109
I	*Petunia hybrida* [34]	---.------	----------	----S-----	----------	----------	----------	----------	----------	----------	----------	---------- 109
J	*Amaranthus hybridus* [35]	---.------	----------	----------	----------	----------	----------	----------	----------	----------	----------	---------- 109
K	*Hordeum vulgare* [36]	---.------	-T--------	----------	----------	----------	----------	----------	----------	----------	----------	---------- 109
L	*Secale cereale* [38]	---.------	-T--------	----------	----------	----------	----------	----------	----------	----------	----------	---------- 109
M	*Pinus contorta* [39]	---.-I----	-AN--S---D	----------	----------	----------	----------	----------	----------	----------	----------	---------- 109
N	*Marchantia polymorpha* [40]	---.T-----	-A-I-----D	-V--------	----------	----------	----------	----------	----------	----------	----------	---------- 109
O	*Chlamydomonas reinhardtii* [41]	---.------	NS---A---E	----------	------I---	C---------	----------	----------	---------T	--V----N--	----------	--L------- 109
P	*Chlamydomonas moewusii* [42]	---.------	-T---A---E	--------I-	------I---	----------	----------	----------	----------	--V----N--	----------	--L------- 109
Q	*Euglena gracilis* [43]	-ISPV-KKYA	RP---Y---A	-VA-KK----	V---------	-----AT---	----------	----------	--F------T	--VV---N--	----------	T-L------- 110
R	*Cyanophora paradoxa* [45]	---.T---NA	-V---EQ--G	F---------	--------F-	L-----TL--	---V------	--------A-	--F-------	--V--S----	-M--------	--L------- 109
S	*Prochlorothrix hollandica* [46]	--T.A-RQ--	-ANA-EQ--Q	--A-------	V-----I---	------IC--	----------	--------A-	--M-------	--VV-S-N--	----------	--M------- 109
T	*Anabaena* 7120 *psb*A-1 [47]	--T.T-QQ-S	-ANV-E---T	--------I-	V---------	---A--VC--	---V------	--------A-	--I-------	--VV-S-N--	----------	--L------- 109
U	*Anabaena* 7120 *psb*A 2, 3 and 4 [47, 48]	---.T-QQ-K	-ANV-EQ--E	-----N----	----------	---A--TC--	----------	--------A-	--I-------	--VV-S-N--	----------	--L------- 109
V	*Synechococcus* 7942 *psb*A-1 [49]	--S.--REQR	RDNV-D---E	-V---D--I-	V---------	------IC--	V---------	--------A-	--M-------	--VV-S-N--	----------	--L------- 109
W	*Synechococcus* 7942 *psb*A 2 and 3 [49]	--T.A-Q---	-A---QQ--E	-V---D----	V---------	------IC--	V---------	--------A-	--M-------	--VV-S-N--	----------	--L------- 109
X	*Fremyella diplosiphon* [50]	--T.T-QQ-S	RA-V-D---E	--------I-	----------	---A--AC-V	----------	--------A-	--I-------	--VV-S-N--	----------	--L------- 109
Y	*Synechocystis* 6714 *psb*A-1 [51]	--T.T-QQ--	-A---EQ--Q	-V---N--I-	V----T----	------TC--	----------	--------A-	----------	--VV-S-N--	----------	--L------- 109
Z	*Synechocystis* 6803 *psb*A 2 and 3 [52, 53]	--T.T-QQ--	-A---EQ--Q	-V---N--I-	V----T----	------TC--	----------	--------A-	----------	--VV-S-N--	----------	--L------- 109
1	*Synechocystis* 6803 *psb*A-1 [54]	--T.TQLGLQ	EQ---S---C	-----S----	----------	------TC--	----------	-------IA-	---------T	A-VV-S-N--	A---------	H-L------- 109


111
240
241
361


D2 sequences
1
110
A Spinacia oleracea [55, 56] MTIAVGKFTK DEKDLFDSMD DWLRRDRFVF VGWSGLLLFP CAYFALGGWF TGTTFVTSWY THGLASSYLE GCNFLTAAVS TPANSLAHSL LLLWGPEAQG DFTRWCQLGG 110
B Nicotiana tabacum [57] ----L----- --N----I-- ---------- ---------- -----V---- ---------- ---------- ---------- ---------- ---------- ---------- 110
C Pisum sativum [58] ----L----- -QN----I-- ---------- ---------- -----V---- ---------- ---------- ---------- ---------- ---------- -L-------- 110
D Oryza sativa [59] ----L-RV-- E-N----I-- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 110
E Hordeum vulgare [60, 61] ----L-RVP- E-N----T-- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 110
F Secale cereale [62] ----L-RIP- E-N----T-- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 110
G Marchantia polymorpha [40] ----I--SS- EP-G------ ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 110
H Chlamydomonas reinhardtii [64] ----I-T.YQ EKRTW--DA- ----Q----- ---------- ---------L ---------- -----T---- ---------- -----M---- -FV------- ---------- 109
I Synechococcus 7942 psbD 1 and 2 [65] ------R.AP A-RGW--VL- ---K------ ---------- ---L-----L ---S------ ---I------ -G----V--- ---DAFG--- M--------- N-V------- 109
J Synechococcus 7002 psbD 1 and 2 [66] ------R.AP Q-RGW--VL- ---K------ -----I---- --FM-----L ---------- ---------- ------V--- S--D--G--- -F------NW N-A------- 109
K Synechocystis 6803 psbD-1 [67] ------R.AP V-RGW--VL- ---K------ I--------- --FM-----L ---------- ---------- -A----V--- S--DAFG--- -F-------- NL---F-I-- 109
L Synechocystis 6803 psbD-2 [67] ------R.AP V-RGW--VL- ---K------ I--------- --FM-----L ---------- ---------- -A----V--- S--DAFG--- -F-------- NL---F-I-- 109
111
240
A LWAFVALHGA FALIGFMLRQ FELARSVQLR PYNAIAFSGP IAVFVSVFLI YPLGQSGWFF APSFGVAAIF RFILFFQGFH NWTLNPFHMM GVAGVLGAAL LCAIHGATVE NTLFEDGDGA NTFRAFNPTQ 240
B --T------- -G-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 240
C --T------- -G-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 240
D --T------- ---------- ---------- -----S---- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 240
E --T------- ---------- ---------- -----S---- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 240
F --T------- ---------- ---------- -----S---- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 240
G --T------- -G-------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 240
H ---------- -G-------- --I----N-- --------A- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 239
I --N------- ---------- --I--L-GV- ---------- ---------M ------S--- ---------- --L--L---- ---------- ----I--G-- ---------- ------SEQS ------E--- 239
J --S------- -G-------- --I--L-GI- ---------- ---------M ------S--- -------G-- -----L---- ---------- ----I--G-- ---------- ------S-Q- ------E--- 239
K --P------- -G-------- --IS-L-GI- ---------- ---------M ------S--- -------G-- -----L---- ---------- ----I--G-- ---------- -------EDS ------E--- 239
L --P------- -G-------- --IS-L-GI- ---------- ---------M ------S--- -------G-- -----L---- ---------- ----I--G-- ---------- -------EDS -N----E--- 239
241
353
A AEETYSMVTA NRFWSQIFGV AFSNKRWLHF FMLFVPVTGL WMSALGVVGL ALNLRAYDFV SQEIRAAEDP EFETFYTKNI LLNEGIRAWM AAQDQPHENL IFPEEVLPRG NAL 353
B ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --- 353
C ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- -T-------- ---------- --- 353
D ---------- ---------- ---------- ---------- ----I----- ---------- ---------- ---------- ---------- ---------- ---------- --- 353
E ---------- ---------- ---------- ---------- ----I----- ---------- ---------- ---------- ---------- ---------- ---------- --- 353
F ---------- ---------- ---------- ---------- ----I----- ---------- ---------- ---------- ---------- ---------- ---------- --- 353
G S--------- ---------- ---------- ---------- ----I----- ---------- ---------- ---------- ---------- ---------- V--------- --- 353
H ---------- ---------- ---------- ---L------ ----I----- ---------- ---------- ---------- ---------- --------R- V--------- --- 352
I ---------- ---------I ---------- ---------- ---SI-I--- ---------- ---L------ ---------- ---------- -P------KF V--------- --- 352
J ---------- ---------I ---------- ---------- ---SV-I--- ---------- ---------- ---------- -----M---- -P---I--QF V--------- --- 352
K ---------- ---------I ---------- ---------- ---SV-I--- ---------- ---L------ ---------- -----M---- -P-------F ---------- --- 352
L ---------- ---------I ---------- ---------- ---SV-I--- ---------- ---L------ ---------- -----M---- -P-------F ---------- --- 352

probably binds several of the redox-components that are involved in the primary photochemistry in PS II. The primary donor in PS II, P_{680}, is thought to be a chlorophyll dimer with its central Mg-ions being coordinated to the entirely conserved His 198 in both proteins. The location of these histidines on the lumenal end of the D helix is analogous to the location of the corresponding histidines in the bacterial reaction center [7, 9]. In addition the central helical core contains Tyr_Z and Tyr_D, the two redox active tyrosine residues in PS II. Tyr_Z (D1-Tyr 161) is situated close to the lumenal end of the C helix (Fig. 2A). Tyr_D (D2-Tyr 161) is found at the corresponding location in the D2 protein (Fig. 2A). The tyrosines are situated in two hydrophobic cavities built up of helices C, D, E and CD on both proteins [10]. The proteic structures around the tyrosine radicals are discussed in detail below.

There is strong experimental support for the assumption that the photoactive pheophytin is bound similarly in PS II and purple bacteria [73, 74]. In the reaction center from purple bacteria L-Glu 104 forms a hydrogen bond to the 9-keto group in pheophytin [75]. In PS II, D1-Glu 130 was recently suggested to form a similar hydrogen-bond to the pheophytin [73, 74]. This residue is located towards the C-terminal end of the B helix [10, 11] where it is surrounded by a quite conserved stretch of aminoacids (Fig. 1A). However, D1-Glu 130 is not conserved in the low-light forms of the D1 protein from some cyanobacteria (Table I) where it is exchanged to a glutamine residue which also might participate in formation of a hydrogen bond. Turned towards the pheophytin on the neighbouring turn of the B helix purple bacteria have L-Trp 100 which forms a hydrogen bond to the pheophytin [75]. In a similar position on the D1 protein we find D1-Tyr 126 which is conserved in all species. The C helix from the L subunit provides two residues, L-Pro 124 and L-Phe 121 that form contacts with the ringplane of pheophytin [75]. The proposed configuration of the C helix from the D1 protein (Fig. 1A; [10, 11]) places in exactly the same positions the conserved residue D1-Pro 150 and D1-Tyr 147 which is a tyrosine in all D1 proteins except *psb*A 2, 3 and 4 from *Anabaena* 7120 where it is a phenylalanine. Considering these similarities between the binding site for the pheophytin in PS II and purple bacteria we believe that the earlier prediction [10, 11] of the organisation of helices B, C and D is correct. Thus, these parts of the protein can be used as a starting point for more elaborate model building studies of less conserved domains in the D1/D2 heterodimer like the Q_A site and to investigate the possible existence of a chlorophyll monomer in PS II by molecular modelling.

The first half of the C-terminus in the D2 protein is totally conserved while the corresponding part in the D1 protein contains some minor alterations (Fig. 1). The high conservation in the C-terminii suggests that these parts of the proteins are very important for the function of the reaction center. This is supported by the fact that the LF 1 mutant in *Scenedesmus* [76] which has an unprocessed (*i.e.* too long) C-terminus on the D1 protein lacks a functional Mn-cluster [77, 78]. The C-terminii are thought to protrude from the thylakoid membrane on the lumenal side of the membrane which is also the vectorial location of the oxygen evolving complex. The C-terminii also contain several entirely conserved, putative metal-binding (Mn or Ca) side-chains (carboxylates and histidines) and together these observations have led to suggestions that either parts of the catalytically active Mn-cluster or the catalytically necessary Ca-ions are bound to the C-terminus of either or both proteins (see for example [79, 80]). There is also experimental evidence that indicates that Mn binds to one of the histidine residues in the C-terminus of the D1 protein [81, 82]. The degree of conservation is lower in the outer parts of the C-terminii and in the D1 protein the analysis breaks down towards the end of the C-terminal stretch. The reason for this is that the primary transcripts for the D1 proteins have different lengths. The D1 proteins in higher plants have 353, in *Euglena* 345, in *Chlamydomonas reinhardtii* 352 and in cyanobacteria 360 residues (Table I).

In the D1 protein two interesting agglomerations of conserved side-chains are found close to the ends of helices A and B in the lumenal loop between the A and B helices (Table IA, Fig. 1A). These two stretches (D1 54–67 and D1 92–113) contain many conserved residues that are putative metal-binding sites and it has been proposed that the AB-loop on the D1 protein constitutes part of the Mn cluster [10]. The overall conservation is much lower in the middle part of the AB loop on

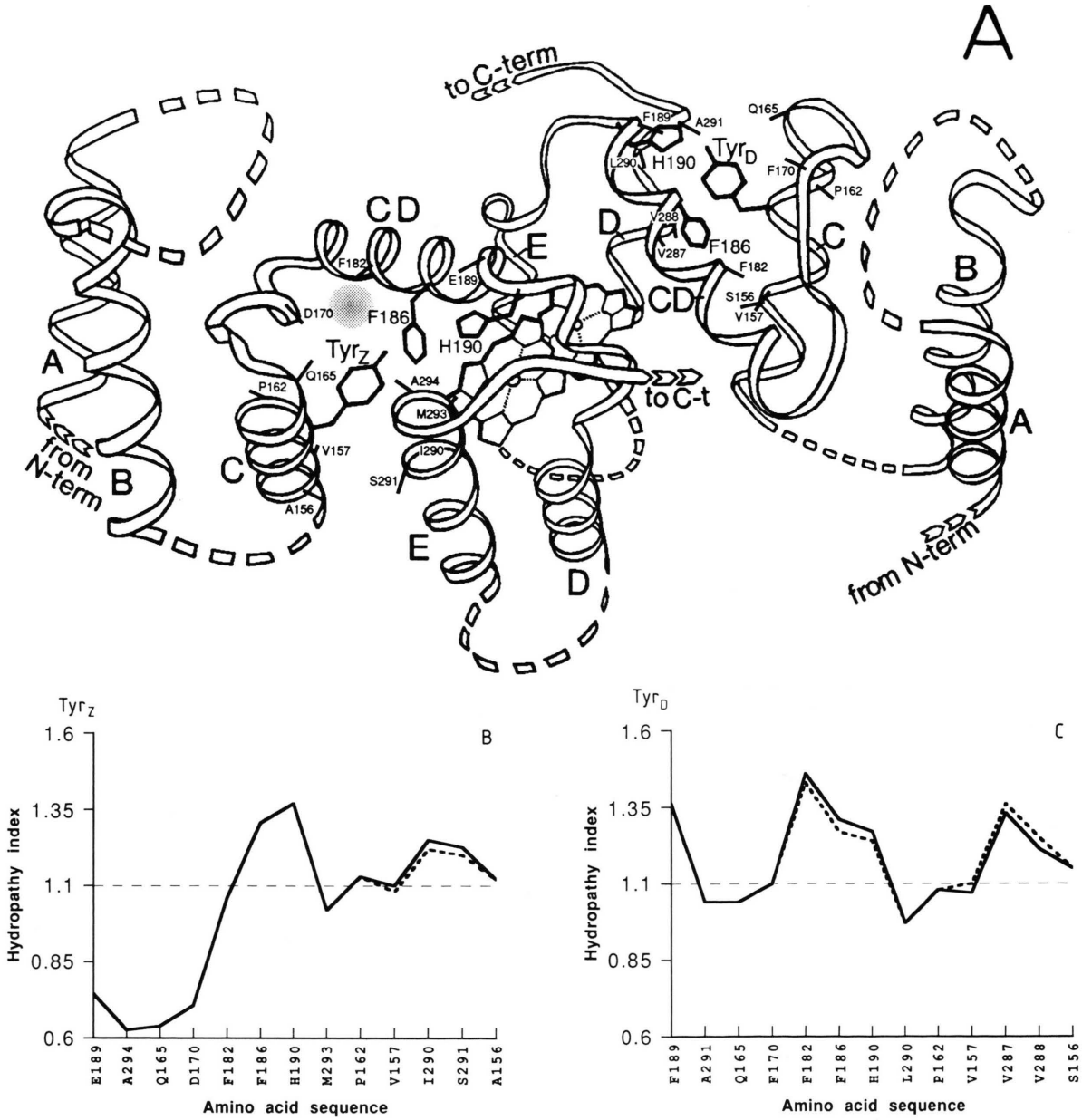


Fig. 2. A. The donor side of PS II. The D1 and D2 proteins are seen obliquely above from the lumenal side of the thylakoid membrane. The side-chains of Tyr 161, Phe 186 and His 190 have been inserted in the structure. The dotted area indicates the approximate position of the proposed metal-binding site composed of the side-chains of D1-Gln 165, D1-Asp 170 and D1-Glu 189. In the protein domains around the tyrosines, the positions of the Cα and Cβ carbons from the neighbouring residues are indicated to facilitate the prediction of the approximate side-chain position of these residues. The peptide backbone and the Cα and Cβ carbons are placed identically to the corresponding atoms in the three dimensional structure from the bacterial reaction center in *Rhodopseudomonas viridis* [1]. The numbering of the side-chains is according to the sequences from spinach. B, C. Hydropathy plot for the aminoacids that constitute the immediate surroundings to Tyr_Z on the D1 protein and Tyr_D on the D2 protein respectively. To analyze whether there exist clusters of hydrophobic or hydrophilic side-chains the residues in the neighbourhood of each tyrosine are plotted in the order they appear when one looks down into the membrane from the lumenal side along the membrane normal. To group aminoacids that come close in space we have started with residue number 189 (which is closest to the membrane surface) and proceeded down inwards the membrane along a clockwise spiral. The full lines show the hydropathy plots for spinach. The dotted lines show the plots for those sequences that are nonidentical to spinach. For the D1 protein the differences are at position 290, where the Ile is changed to either a Val or a Leu (see Table I A). Both changes result in practically identical plots. For the D2 proteins the differences are at positions 186 and 287, where in all cyanobacteria the Phe is changed to a Leu and the Val to a Ile, respectively.

the D1 protein. The AB loop in the D2 protein is also much less conserved (Fig. 1 B).

The extrinsic loops between helices D and E and the outer parts of helix E bind the quinone acceptors Q_A (on the D2 protein) and Q_B (on the D1 protein). This part of the sequence is more conserved on the D2 protein (Fig. 1) which might reflect a lower flexibility in the binding site of the firmly bound Q_A. The highly conserved stretch of the DE-loop (238–264) contains D2-Trp254 which has been suggested to be a ligand to Q_A [83]. The importance of D2-Trp254 in the binding of Q_A is also demonstrated from site directed mutagenesis studies in the cyanobacterium *Synechocystis* 6803 in which an alteration of D2-Trp253 (corresponds to D2-Trp254 in higher plants) to a Leu resulted in destabilization of Q_A which in the mutant might leave its site [84]. The site for Q_B is probably located to the DE-loop in the D1 protein [9]. The Q_B site is known to allow more flexibility since there exists a large number of herbicide resistant organisms with point mutations in this site that nevertheless show appreciable photosynthetic electron transport. It is likely that a similar situation exists also in photosynthetic purple bacteria where many point mutations in the Q_B site (on the L protein) exist but very few mutants have been described in the Q_A site on the M protein [85].

The extra-membraneous N-terminal is the least conserved part in both proteins (Fig. 1). The D1 protein is one of the proteins in the PS II reaction center that may become phosphorylated. The phosphorylation occurs at the threonine residue (D1-Thr2) [12] that exists in all sequences except the D1 sequence from *Euglena* (Table I A). In addition to the N-terminal the first and second membrane spanning helices (helices A and B) also show considerably flexibility in both proteins.

The environment around the tyrosine radicals

The two redox-active tyrosine residues in PS II, Tyr_Z (D1-Tyr161) and Tyr_D (D2-Tyr161) are located close to the lumenal end of the C-helices in each protein (Fig. 2A) and were earlier [10] suggested to occupy cavities (on the D1 and D2 protein respectively) between the C, D, and E membrane spanning helices close to the lumenal surface of the thylakoid membrane. The basis for this hypothesis was spectroscopic data concerning the orientation in the membrane of the tyrosine radicals [10].

Both Tyr_Z^+ and Tyr_D^+ are very oxidizing species [86]. However, while Tyr_Z^+ is reduced in the μs-ms timescale with electrons from the Mn-cluster, Tyr_D^+ is 9–10 orders of magnitude more stable in the dark ($t_{1/2}$ for the reduction of Tyr_D^+ in the S_1-state is ~ 10 h at room temperature) [86, 87]. This reason for this difference is the lack of a fast electron donor to Tyr_D^+ which has been proposed to be very isolated from the surrounding medium [87].

Here we have analyzed the protein environment in the cavities around Tyr_Z and Tyr_D with respect to conservation and hydrophobicity. We have used our hypothetical model [10] (Fig. 2A) to predict which aminoacid side-chains come close to respective tyrosine side-chain. These residues have then been analyzed using the program QMAGINT which takes into account the degree of hydropathy in each position [71]. The aminoacids used in the plots are for the spinach D1 protein: Ala156, Val157, Pro162, Gln165, Asp170, Phe182, Phe186, Glu189, His190, Ile290, Ser291, Met293 and Ala294 and for the spinach D2 protein: Ser156, Val157, Pro162, Gln165, Phe170, Phe182, Phe186, Phe189, His190, Val287, Val288, Leu290 and Ala291. The hydropathy plots are presented in Fig. 2B and C.

The results from these calculations reveal that the environment around Tyr_D is more hydrophobic than around Tyr_Z. The difference is not evenly spread in the cavity but the major difference between the two sites is found in one end of the cavity. The most important difference in hydrophobicity between the two domains is in the part of the structure that is turned away from the primary donor and, thus, turned towards the lumenal side of the membrane (Fig. 2A). The hydrophilic part of the cavity structure is on the D1 protein composed of Glu189, Ala294, Gln165 and Asp170 (lefthand part of the plot in Fig. 2B). The corresponding part of the cavity around Tyr_D is composed of Phe189, Ala291, Gln165 and Phe170 from the D2 protein (lefthand part of Fig. 2C). Thus, the difference in hydrophobicity comes from the positions 170 and 189, which on the D1 protein (close to Tyr_Z) are an aspartic acid and a glutamic acid while they on the D2 protein (close to Tyr_D) are phenylalanines. These two conserved residues (Ta-

ble I) are located close to the lumenal surface of the membrane (Fig. 2A) in the ends of the cavities that are furthest away from the primary donor (Fig. 2A). The remaining part of the cavities around the tyrosines are very similar on the D1 and D2 proteins. It is thus reasonable to suggest that the different stabilities of the two radicals depend on the different polarity in their environment due to the existence of the two carboxylic residues in the vicinity of Tyr_Z and the phenylalanines in the corresponding positions close to Tyr_D.

A putative metal binding site close to Tyr_Z on the D1 protein

The Mn-cluster, which is involved in the oxygen evolution, is proposed to be partially bound to the extrinsic A-B loop in the D1 protein [10]. Asp 170 and Glu 189 on the D1 protein (see above) have an interesting location approximately midways between Tyr_Z and the proposed binding region for the Mn-cluster. It is difficult to predict the exact location of the side-chains of Asp 170 and Glu 189 since they lack identical equivalents in the bacterial reaction center. However, if one makes the very conservative assumption that the peptide backbone and β-carbons of the corresponding side-chains have the same orientation in the bacterial reaction center and PS II (see Fig. 2A) it is found that D1-Asp 170 and D1-Glu 189 (the corresponding side-chains in Rps. viridis are L-His 144 and L-Gln 163) may come close together in space at a position approximately 7 Å from the center of the phenolic ring in Tyr_Z (Fig. 2A). The side-chain of D1-Gln 165 will also be located in the close vicinity of the carboxylic side-chains. Together these three residues might constitute part of a metal-site. The approximate location of this site is marked as a dotted area in Fig. 2A. This is very interesting since Asp 170 has been pointed out as a putative Mn-ligand from experiments with site-directed mutagenesis in *Synechocystis* 6803 [88].

It is tempting to speculate that the putative metal-binding site is part of the Mn-cluster and that the region in the A-B loop earlier proposed by us to bind Mn constitutes the remaining part. The A-B loop was suggested to bind the Mn-cluster from distance estimations based on magnetic interaction criteria [10]. Thus, one might hypothesize that the Mn-ions in the A-B loop region constitute the part of the Mn-cluster that dominates the magnetic interactions with the tyrosine radicals while the Mn site close to Tyr_Z is part of the electron transfer pathway from the water oxidizing site to Tyr_Z. A model where one Mn-ion functions as a "gateway" Mn has recently been discussed [89].

The putative metal-binding site (Fig. 2A) is situated approximately 7 Å from the center of Tyr_Z. The distance to D1-His 190, which has been suggested to form a hydrogenbond to Tyr_Z [10], is also approximately 7 Å. In this respect it is interesting that illumination of PS II, with a reversibly inhibited Mn-cluster, might result in oxidation of a side-chain that is thought to be a histidine residue [79]. Recently, Baumgarten *et al.* [89] calculated the distance between the Mn-cluster and the oxidizable side-chain to 7 Å. It is tempting to speculate that this reflects the 7 Å distance between a Mn-atom in the putative metal-site described here and D1-His 190. When no electrons from the Mn-cluster are available to reduce Tyr_Z^+, the highly oxidizing tyrosine radical instead would oxidize the nearby situated D1-His 190.

An alternative hypothesis is that the site close to Tyr_Z, composed of D1-Asp 170, D1-Glu 189 and D1-Gln 165, constitutes a Ca^{2+} site. Ca^{2+} is a necessary cofactor in the electron transfer reactions at the donor side of PS II and several binding sites might exist for Ca^{2+} [90, 91]. Also in this case an elimination of the site by site-directed mutagenesis is likely to inhibit both the Mn binding and the oxygen evolution since Ca^{2+} is a necessary cofactor both in the photoactivation process that lead to Mn binding [92] and in the oxygen evolving reactions [90].

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[1] J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, Nature **318,** 618–624 (1985).
[2] J. P. Allen, G. Feher, T. O. Yeates, H. Komiya, and D. C. Rees, Proc. Natl. Acad. Sci. U.S.A. **84,** 6162–6166 (1987).
[3] O. Nanba and K. Satoh, Proc. Natl. Acad. Sci. U.S.A. **84,** 109–112 (1987).
[4] J. C. Williams, L. A. Steiner, R. C. Ogden, M. I. Simon, and G. Feher, Proc. Natl. Acad. Sci. U.S.A. **80,** 6505–6509 (1983).
[5] J. C. Williams, L. A. Steiner, G. Feher, and M. I. Simon, Proc. Natl. Acad. Sci. U.S.A. **81,** 7303–7307 (1984).
[6] D. C. Youvan, E. J. Bylina, M. Alberti, H. Begusch, and J. E. Hearst, Cell **37,** 949–957 (1984).
[7] H. Michel, K. A. Weyer, H. Gruenberg, I. Dunger, D. Oesterhelt, and F. Lottspeich, EMBO J. **5,** 1149–1158 (1986).
[8] R. T. Sayre, B. Andersson, and L. Bogorad, Cell **47,** 601–608 (1986).
[9] A. Trebst, Z. Naturforsch. **41 c,** 240–245 (1986).
[10] B. Svensson, I. Vass, E. Cedergren, and S. Styring, EMBO J. **9,** 2051–2059 (1990).
[11] H. Michel and J. Deisenhofer, Biochemistry **27,** 1–7 (1988).
[12] B. Andersson and S. Styring, in: Current Topics in Bioenergetics (C. P. Lee, ed.), **Vol. 16,** pp. 1–81, Academic Press Inc., Orlando, Florida 1991.
[13] A. W. Rutherford, in: Progress in Photosynthesis Research (J. Biggins, ed.), **Vol. I,** pp. 277–283, Martinus Nijhoff Publishers, Dordrecht, The Netherlands 1987.
[14] R. J. Debus, B. A. Barry, G. T. Babcock, and L. McIntosh, Proc. Natl. Acad. Sci. U.S.A. **85,** 427–430 (1988).
[15] R. J. Debus, B. A. Barry, I. Sithole, G. T. Babcock, and L. McIntosh, Biochemistry **27,** 9071–9074 (1988).
[16] W. F. J. Vermaas, A. W. Rutherford, and Ö. Hansson, Proc. Natl. Acad. Sci. U.S.A. **85,** 8477–8481 (1988).
[17] J. G. Metz, P. J. Nixon, M. Rögner, G. W. Brudvig, and B. A. Diner, Biochemistry **28,** 6960–6969 (1989).
[18] I. Virgin, S. Styring, and B. Andersson, FEBS Lett. **233,** 408–412 (1988).
[19] J. M. Erickson, J.-D. Rochaix, and P. Delepelaire, in: Molecular Biology of the Photosynthetic Apparatus (K. E. Steinback, S. Bonitz, C. J. Arntzen, and L. Bogorad, eds.), pp. 53–65, Cold Spring Harbour Laboratory, New York 1985.
[20] J. Devereux, P. Haeberti, and O. Smithies, Nucl. Acids Res. **12,** 387–395 (1984).
[21] G. Zurawski, H. J. Bohnert, P. R. Whitfeld, and W. Bottomley, Proc. Natl. Acad. Sci. U.S.A. **79,** 7699–7703 (1982).
[22] M. Sugita and M. Sugiura, Mol. Gen. Genet. **195,** 308–313 (1984).
[23] A. Pay, M. A. Smith, F. Nagy, and L. Marton, Nucl. Acids Res. **16,** 8176 (1988).
[24] T. N. Ulmasov, M. K. Gulov, K. A. Aliev, V. M. Andrianov, and E. S. Piruzian, Nucl. Acids Res. **18,** 186 (1990).
[25] P. Goloubinoff, M. Edelman, and R. B. Hallick, Nucl. Acids Res. **12,** 9489–9496 (1984).
[26] P. Bettini, S. McNally, M. Sevignac, H. Darmency, J. Gasquez, and M. Dron, Plant Physiol. **84,** 1442–1446 (1987).
[27] A. Spielmann and E. Stutz, Nucl. Acids Res. **11,** 7157–7167 (1983).
[28] G. Link and U. Langridge, Nucl. Acids Res. **12,** 945–958 (1984).
[29] J. Aldrich, B. Cherney, and E. Merlin, Nucl. Acids Res. **14,** 9537 (1986).
[30] M. Reith and N. A. Straus, Theor. Appl. Genet. **73,** 357–363 (1987).
[31] N.-H. Wu, J.-C. Cote, and R. Wu, Dev. Genet. **8,** 339–350 (1987).
[32] K. K. Oishi, D. R. Shapiro, and K. K. Tewari, Mol. Cell. Biol. **4,** 2556–2563 (1984).
[33] K. Ko, Nucl. Acids Res. **18,** 375 (1990).
[34] J. Aldrich, B. Cherney, E. Merlin, L. A. Christopherson, and C. Williams, Nucl. Acids Res. **14,** 9536 (1986).
[35] J. Hirschberg and L. McIntosh, Science **222,** 1346–1349 (1983).
[36] V. A. Efimov, A. V. Andreeva, S. V. Reverdatto, R. Jung, and O. G. Chakhmakhcheva, Nucl. Acids Res. **16,** 5685 (1988).
[37] L. Hanley-Bowdoin and N.-H. Chua, Plant Mol. Biol. **10,** 303–310 (1988).
[38] V. L. Kolosov, A. A. Bukharov, and A. S. Zolotarev, Nucl. Acids Res. **17,** 1759 (1989).
[39] J. Liedholm, S. Jansson, and P. Gustafsson, in: Kempe Symp. Report **8,** Swedish Univ. Agric. Sciences, pp. 115–126 (1988).
[40] K. Ohyama, H. Fukuzawa, T. Kohchi, H. Shirai, T. Sano, S. Sano, K. Umesono, Y. Shiki, M. Takeuchi, Z. Chang, S. Aota, H. Inokuchi, and H. Ozeki, Nature **322,** 572–574 (1986).
[41] J. M. Erickson, M. Rahire, and J.-D. Rochaix, EMBO J. **3,** 2753–2762 (1984).
[42] M. Turmel, J. Boulanger, and C. Lemieux, Nucl. Acids Res. **17,** 3875–3887 (1989).
[43] G. D. Karabin, M. Farley, and R. B. Hallick, Nucl. Acids Res. **12,** 5801–5812 (1984).
[44] M. Keller and E. Stutz, FEBS Lett. **175,** 173–177 (1984).
[45] I. Jansen, J. Jakowitsch, C. B. Michalowski, H. J. Bohnert, and W. Löffelhardt, Curr. Genet. **15,** 335–340 (1989).
[46] C. W. Morden and S. S. Golden, Nature **337,** 382–385 (1989).
[47] S. E. Curtis and R. Haselkorn, Plant Mol. Biol. **3,** 249–258 (1984).
[48] J. M. Vrba and S. E. Curtis, Plant Mol. Biol. **14,** 81–92 (1989).
[49] S. S. Golden, J. Brusslan, and R. Haselkorn, EMBO J. **5,** 2789–2798 (1986).
[50] B. Mulligan, N. Schultes, L. Chen, and L. Bogorad, Proc. Natl. Acad. Sci. U.S.A. **81,** 2693–2697 (1984).
[51] G. Ajlani, D. Kirilovsky, M. Picaud, and C. Astier, Plant Mol. Biol. **13,** 469–479 (1989).
[52] P. D. Ravnikar, R. Debus, J. Sevrinck, P. Saetaert, and L. McIntosh, Nucl. Acids Res. **17,** 3991 (1989).
[53] J. Metz, P. Nixon, and B. Diner, Nucl. Acids Res. **18,** 6715 (1990).

[54] H. D. Osiewacz and L. McIntosh, Nucl. Acids Res. **15,** 10585 (1987).
[55] K. Holschuh, W. Bottomley, and P. R. Whitfeld, Nucl. Acids Res. **12,** 8819–8834 (1984).
[56] J. Alt, J. Morris, P. Westhoff, and R. Herrmann, Curr. Genet. **8,** 597–606 (1984).
[57] K. Shinozaki, M. Ohme, M. Tanaka, T. Wakasugi, N. Hayashida, T. Matsubayashi, N. Zaita, J. Chunwongse, J. Obokata, K. Yamaguchi-Shinozaki, C. Ohto, K. Torazawa, B. Y. Meng, M. Sugita, H. Deno, T. Kamogashira, K. Yamada, J. Kusuda, F. Takaiwa, A. Kato, N. Tohdoh, H. Shimada, and M. Sugiura, EMBO J. **5,** 2043–2049 (1986).
[58] O. F. Rasmussen, G. Bookjans, B. M. Stummann, and K. W. Henningsen, Plant Mol. Biol. **3,** 191–199 (1984).
[59] J. Hiratsuka, H. Shimada, R. Whittier, T. Ishibashi, M. Sakamoto, M. Mori, C. Kondo, Y. Honji, C.-R. Sun, B.-Y. Meng, Y.-Q. Li, A. Kanno, Y. Nishizawa, A. Hirai, K. Shinozaki, and M. Sugiura, Mol. Gen. Genet. **217,** 185–194 (1989).
[60] V. A. Efimov, A. V. Andreeva, S. V. Reverdatto, and O. G. Chakhmakhcheva, Bioorg. Khim. **14,** 1573–1576 (1988).
[61] E. M. Neumann, Carlsberg Res. Commun. **53,** 259–275 (1988).
[62] A. A. Bukharov, V. L. Kolosov, O. N. Klezovich, and A. S. Zolotarev, Nucl. Acids Res. **17,** 798 (1989).
[63] C. J. Howe, R. F. Barker, C. M. Bowman, and T. A. Dyer, Curr. Genet. **13,** 343–349 (1988).
[64] J. M. Erickson, M. Rahire, P. Malnoe, J. Girard-Bascou, Y. Pierre, P. Bennoun, and J.-D. Rochaix, EMBO J. **5,** 1745–1754 (1986).
[65] S. S. Golden and G. W. Stearns, Gene **67,** 85–96 (1988).
[66] J. C. Gingrich, G. E. Gasparich, K. Sauer, and D. A. Bryant, Photosyn. Res. **24,** 137–150 (1990).
[67] J. G. K. Williams and D. A. Chisholm, in: Progress in Photosynthesis research (J. Biggins, ed.), **Vol. IV,** pp. 809–812, Martinus Nijhoff Publishers, Dordrecht, The Netherlands 1987.
[68] V. A. Efimov, A. V. Andreeva, S. V. Reverdatto, and O. G. Chakhmakhcheva, Nucl. Acids Res. **16,** 5686 (1988).
[69] R. M. Schwartz and M. O. Dayhoff, in: Atlas of Protein Sequence and Structure (M. O. Dayhoff, ed.), **Vol. 5,** pp. 101–110, National Biomedical Research Foundation, Washington D.C. 1979.
[70] M. Gribskov and R. R. Burgess, Nucl. Acids Res. **16,** 6745–6763 (1986).
[71] M. Degli Esposti, M. Crimi, and G. Venturoli, Eur. J. Biochem. **190,** 207–219 (1990).
[72] A. Mohamed and C. Jansson, Plant. Mol. Biol. **13,** 693–700 (1989).
[73] P. Moënne-Loccoz, B. Robert, and M. Lutz, Biochemistry **28,** 3641–3645 (1989).
[74] E. Nabedryk, S. Andrianambinitsoa, G. Berger, M. Leonhard, W. Mäntele, and J. Breton, Biochim. Biophys. Acta **1016,** 49–54 (1990).
[75] H. Michel, O. Epp, and J. Deisenhofer, EMBO J. **5,** 2445–2451 (1986).
[76] J. G. Metz, J. Wong, and N. I. Bishop, FEBS Lett. **114,** 61–66 (1980).
[77] B. A. Diner, D. F. Ries, B. N. Cohens, and J. G. Metz, J. Biol. Chem. **263,** 8972–8980 (1988).
[78] M. A. Taylor, J. C. L. Packer, and J. R. Bowyer, FEBS Lett. **237,** 229–233 (1988).
[79] A. Boussac, J.-L. Zimmermann, A. W. Rutherford, and J. Lavergne, Nature **347,** 303–306 (1990).
[80] G. C. Dismukes, Chemica Scr. **28A,** 99–104 (1988).
[81] M. Seibert, N. Tamura, and Y. Inoue, Biochim. Biophys. Acta **974,** 185–191 (1989).
[82] T. Ono and Y. Inoue, FEBS Lett. **278,** 183–186 (1991).
[83] J. Breton, D. L. Thibodeau, C. Bertomieu, W. Mäntele, A. Verméglio, and E. Nabedryk, FEBS Lett. **278,** 257–260 (1991).
[84] W. Vermaas, J. Charité, and G. Shen, Z. Naturforsch. **45c,** 359–365 (1990).
[85] I. Sinning, H. Michel, P. Mathis, and A. W. Rutherford, Biochemistry **28,** 5544–5553 (1989).
[86] I. Vass and S. Styring, Biochemistry **30,** 830–839 (1991).
[87] G. T. Babcock, B. A. Barry, R. J. Debus, C. W. Hoganson, M. Atamian, L. McIntosh, I. Sithole, and C. F. Yocum, Biochemistry **28,** 9557–9565 (1989).
[88] P. J. Nixon and B. A. Diner, in: Proceedings of the 12th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (P. C. Pedersen and B. Onard, eds.), pp. 1732–1734 (1990).
[89] M. Baumgarten, J. S. Philo, and G. C. Dismukes, Biochemistry **29,** 10814–10822 (1990).
[90] A. Boussac and A. W. Rutherford, Biochemistry **27,** 3476–3483 (1988).
[91] K. Kalosaka, W. F. Beck, G. Brudvig, and G. Cheniae, in: Current Research in Photosynthesis (M. Baltscheffsky, ed.), **Vol. I,** pp. 721–724, Kluwer Academic Publisher, Dordrecht, The Netherlands 1990.
[92] A.-F. Miller and G. W. Brudvig, Biochemistry **29,** 1385–1392 (1990).