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The photosystem II reaction center protein D1'in *Synechocystis* 6803

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Introduction

The highly conserved photosystem II (PSII) reaction center protein D1 is encoded by the *psbA* gene. In the cyanobacterium *Synechocystis* 6803 (*S*. 6803), *psbA* exists as three copies, *psbA1*, *psbA2* and *psbA3* (Jansson et al., 1998). Transcription of *psbA2* and *psbA3* is positively stimulated by light, whereas *psbA1* is silent. The transcription start sites for *psbA2* and *psbA3* have previously been mapped by primer extension analysis. Sequence analysis of the *psbA2* and *psbA3* genes shows that they have –35 and –10 elements characteristic of bacterial sigma70 (σ^{70}) promoters. For *psbA1*, two putative promoters have been proposed (Osiewacz and McIntosch, 1987).

The presence of the *psbA1* gene in *S***.** 6803 is enigmatic. No conditions have been established under which the gene is transcribed. However, the promoter of the *psbA1* gene has been mapped by heterologous expression in *E. coli*, and two possible mechanisms for *psbA1* gene silencing in *S*. 6803, the presence of negative *cis*-regulatory elements, and attenuation, have been discussed (Eriksson and Jansson, unpublished results).

Interestingly, when the *psbA1* gene in was forced to expression in *S***.** 6803 by promoter engineering, it was shown to encode a novel but fully functional D1 protein (Salih and Jansson, 1997). The new D1 form, D1', differs from D1 in 54 out of 360 amino acids (Table 1). Two of the most notable amino acid substitutions in D1' are the replacements of F186 by a leucine and P162 by a serine. By construction and analyses of mutants in *S*. 6803, we aim at elucidating the importance, and possible function, of F186 and P162 in the D1 protein and the significance of the F186L and P162S substitutions in D1'.

Material and methods

S. 6803-G cells were cultured at a photon flux density of ~30 μE m^{-2} s^{-1} as described (Jansson et al., 1998).

Oxygen evolution assays were according to Tyystjärvi et al. (1994) using 0.5 mM Phenyl-p-benzoquinone (PPBQ) or 0.25 mM 2,6-dichloro-p-benzoquinone (DCBQ) as electron acceptors and 1 mM ferricyanide.

Whole cell chlorophyll a determination was done according to Funk et al. (1998), and in isolated thylakoid membranes according to the method of Arnon (1949).

Thylakoid preparation was performed mainly as described (Salih et al., 1996)

SDS-PAGE were performed as described (Salih et al., 1996). Immunodetection of the D1 protein was performed with the ECL chemiluminiscense kit from Amersham Pharmacia Biotech (RPN.21089), using a polyclonal antiserum against amino acids 297-313 (produced by custom antibody production by Research Genetics Inc., Huntsville, Alabama, USA). It was used at a 60,000 x times dilution. The secondary antibody was used at a 4000 x dilution.

Pulse-chase experiments were carried out as described (Salih et al., 1996).

Fluorescence decay measurements were performed according to Funk et al. (1998).

The pattern of oxygen yield per flash induced by a series of short (10μs) saturating flashes from a Xe lamp was monitored at 25 °C with a Joliot-type electrode (Joliot, 1972).

Results and Discussion

The F186 and P162 positions are located in the vicinity of the reaction center chlorophyll dimer P680 and the redox-active Tyr161 (TyrZ), and are, with the exception of D1', conserved in all (>100) database entries for D1 amino acid sequences (available through BLASTP search retrievals at NCBI, http://www.ncbi.nlm.nih.gov/ (Table 1). From the strategic position of the "conserved" F186, in close proximity to Tyr161 and P680, it is tempting to suggest that this aromatic amino acid plays an important role in the electron transfer on the donor side of PSII. This notion has been corroborated by the total loss of PSII activity in the F186Y mutant of *S.* 6803 (Mäenpääet al., 1995; Wiklund et al., 1995). However, activation of the *S.* 6803 *psbA1* gene led to the discovery of the D1' protein, containing L186, and hence to the realization that F186 is not fully conserved.

Whether or not it is fruitful to ask if F186 directly participates in electron transfer between Tyr161 and P680 is not yet obvious. A substantial amount of data has produced powerful theoretical formalisms for intraprotein electron transfer (Beratan et al., 1992; Moser et al., 1992; Gray and Winkler, 1996; Heifets et al., 1998; Page et al., 1999; Winkler et al., 1999). Although the overall packing properties of the intervening protein matrix will effect the electronic coupling of an electron transfer acceptor-donor pair, the influence of individual amino acid residues on the electron transfer rate is likely to be minor (Moser et al., 1992; Gray and Winkler, 1996). In fact, an extensive survey of electron-transfer proteins by Dutton and coworkers (Page et al., 1999), suggest that the major requirement for efficient intraprotein electron transfer is that the redox centers are located within distance of 14 Å, and that the properties of the individual amino acids connecting the redox centers is of less importance. The distance between Tyr161 and P680 in D1 is 10-13 Å (Svensson et al., 1996; Zouni et al., 2001) and thus fulfills the proximity criterion postulated by Page et al. (1999). The importance of electron tunneling for Tyr161 oxidation also gets support from experimental data (Tommos and Babcock, 2000; Westphal et al., 2000).

To assess the importance of the F186 position in the D1 protein, and the F186L substitution in D1', engineered forms of the D1 proteins, where the aromaticity, hydrophobicity and physical size of the side chain were changed, have been constructed (Wiklund et al, 2001). F186 was replaced by leucine, serine, alanine, tyrosine or tryptophane. The F186L/P162S double substitution was constructed to mimic the composition in the D1' protein. Finally, the F186Y/P162S double substitution was made to probe for possible compensatory effects of the P162S replacement on the F186Y substitution.

The PSII competence in the different F186 mutants was assessed by steady-state and flash-induced oxygen evolution measurements, fluorescence analyses, western blot analyses and pulse-chase experiments. The data revealed that only the leucine replacement yielded a functional D1 protein. The results show that a leucine can efficiently, although not entirely, replace the phenylalanine at position 186 but they also clearly demonstrate that other substitutions severely affect the functional and/or structural integrity of the D1 protein. We conclude that the hydrophobicity and van der Waals volume are the most important features of the residue at position 186 (Wiklund et al, 2001). Exchanging P162 for a serine yielded no observable phenotype. This is in agreement with results by Kless and Vermaas (1996).

T286 in D1 is postulated to be hydrogen-bonded to an ester oxygen in the P680 dimer, and the T286A substitution in D1' could potentially reposition one of the chlorophyll ring planes in P680 relative the situation in D1. In addition to D1', position 286 is occupied by an alanine also in a *psbA* ORF from the marine cyanobacterium *Synechococcus* WH 7803 and in a deduced amino acid sequence from the *psbA2* gene in the thermophilic cyanobacterium *Synechococcus elongatus naegeli* (Table 1). We do not know whether any D1 protein is produced from these two *psbA* genes. *Synechococcus elongatus naegeli* contains two continuous *psbA* genes, *psbA1* and *psbA2*. It has been shown that the *psbA1* gene, encoding a D1 with a threonine in position 286, is active (Motoki et al., 1995).

It should be pointed out that D1' in *Synechocystis* 6803 is not related to D1:2 in *Synechococcus* 7942, which is transiently produced under stress conditions (Schaefer and Golden 1989; Campbell et al., 1996).The presence of the *psbA1* gene in *Synechocystis* 6803 is enigmatic (Salih and Jansson, 1997; Jansson et al., 1998), and whether or not the D1’ protein serves a function in the *Synechocystis* 6803 cells under specific environmental conditions is still an open question.

Table 1. **Divergent amino acids between D1 and D1' in *S.* 6803.**

D1	D1'	Comments	D1	D1'	Comments
L5	Q5		F186	L186	F186 is located in the vicinity of Y161 and P680; conserved in all D1 forms except D1'.
Q6	L6		I192	V192	
Q7	G7		S209	A209	
R8	L8		S212	A212	
E9	Q9		V224	I224	
S10	E10		Y235	Q235	
A11	Q11		S270	A270	
E15	S15		I281	V281	
Q16	R16		T286	A286	T286 is postulated to ligand P680; conserved in all D1 forms except D1' and D1 from *Synechococcus* WH 7803 and a hypothetical D1 from *Synechococcus elongatus naegeli*, where it also is A286.
Q19	C19		M288	L288	
V21	I21		G289	A289	
N25	S25		S291	C291	
I28	L28		T292	C292	
V30	I30		M293	F293	
T35	V35		S309	A309	
V67	I67		V313	P313	
S79	T79		I314	V314	
G80	A80		G315	S315	
A100	H100		L321	I321	
V115	I115		A336	V336	
M127	L127		E347	D347	
S152	A152		Q348	A348	
V157	T157	V157 is conserved in all D1 forms except D1' and D1 in *Acorus gramineus*, where it also is T157.	A349	Q349	
F158	L158	F158 is located in the neighborhood of Y161; conserved in all D1 forms except D1'.	P350	M350	
P162	S162	P162 is located in the vicinity of Y161 and P680; conserved in all D1 forms except D1'	T354	N354	
M172	L172		V358	I358	
I184	L184		N359	E359	

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