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Is FMO-protein related to PscA in the reaction center of green sulfur bacteria?

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Introduction

FMO-protein is a water-soluble protein found only in green sulfur bacteria. Each subunit (365/366 amino acids) contains 7 bacteriochlorophyll (BChl) *a* molecules wrapped in a string bag of protein consisting of 15 strands of β sheet, short lengths of α helix, and a few regions of irregular conformation. PscA is a membrane-bound protein (730/731 amino acids) with 11 transmembrane helices, 8 BChl *a* molecules and 2 chlorophyll *a* molecules esterified with Δ 2,6 phytandienol. When the FMO sequence is compared to those of PscA, PshA (RC protein of heliobacteria), CP43 and CP47 (core proteins of PS II), identity scores for the four proteins (570, 350, 195, and 140 respectively) indicate that FMO-protein is most closely related to PscA. In addition both FMO and PscA contain a signature sequence (L–HH) that contains two BChl *a* binding sites in FMO. We have mapped the FMO sequence onto the PscA sequence with PAM250 (modified for His binding-site identities) as a test of the hypothesis that FMO protein shares a common ancestor with PscA.

Methods

A consensus FMO-protein sequence was constructed from individual sequences of FMO-proteins from *Prosthecochloris (Pr.) aestuarii*, *Chlorobium (Cb.) limicola,* and *Cb. tepidum* (Daurat-Larroque et al. 1986, Hager-Braun et al. 1995, Dracheva et al. 1992). A consensus PscA sequence was constructed from individual sequences from *Cb. limicola* and *Cb. tepidum* (Büttner et al. 1992 revised by PEDANT 2001, DA Bryant unpublished data). The matrix PAM250 (Dayhoff et al. 1978) was modified by the introduction of the letter J to symbolize BChl *a*-binding His residues in FMO. The score for matching J-H pairs was set at +10, while the score for matching H-H pairs was left at +6. The gap initiation penalty was set at –10 and the gap extension penalty at –2. Alignments were generated by PAM250 using a Windows NT-based Pentium III-1GHz computer and Bioedit software (Hall 1999). In order for an alignment to be acceptable, it had to meet the following criteria:

1. The signature sequences, LVJJ in FMO and LXJJ in PscA, had to be aligned.
2. A third binding site, FJ–/YJG or SJX/NJI, also had to be aligned.
3. The identity score had to be at least 0.11.

Results

When the entire sequences of FMO and PscA were aligned, the result failed both criteria 2 and 3. The PscA sequence was then systematically shortened from the N-terminus until the alignments met all criteria. This first took place when the sequence was cut off between T170 and K200. The sequence was then trimmed from the C-terminus (A731) in order to eliminate large gaps and at the same time increase the local identity score at the C-terminus. This was achieved when the sequence was cut off between I670 and L680. The optimum overall alignment was taken to be G190-L680 (extended alignment, identities (excluding X/X) = 0.109) as shown in Figure 1. This alignment is characterized by a large (31 residue) gap between W225 and G257 and a third binding site at FJ–/YJG.

When the N-terminus was further shortened to A210, the large gap disappeared. The optimum overall sequence was taken to be A210-I670 (intermediate alignment, identities (excluding X/X) =0.112) as shown in Figure 1. Further shortening of the N-terminus to W220, F230, and N240 led to failing alignments. However when the N-terminus was cut at A250, an acceptable alignment appeared with the third binding site shifted from to FJ–/YJG to SJX/NJI. This alignment was further improved by cutting at W260, and the optimum overall sequence was taken to be W260-I670 (restricted alignment, identities (excluding X/X) = 0.110) as shown in Figure 1.

The robustness of the three optimum alignments was tested by trimming the individual sequences of PscA from *Cb. limicola* and *Cb. tepidum* appropriately and aligning them with the individual FMO sequences from *Pr. aestuarii, Cb. limicola,* and *Cb. tepidum.* The results are summarized in Table 1. Four of the six alignments based on the 190-680 sequences are similar to that for the consensus alignment, but only two of the six alignments based on the 210-670 sequences are similar to that for the consensus alignment. However, all six alignments based on the 250-670 sequences are similar to that of the consensus alignment.

Table 1. Alignments of FMO-proteins from *Pr. aestuarii, Cb. limicola* and *Cb. tepidum* with PscA segments from *Cb. limicola* and *Cb. tepidum.* The alignments of three residues at the third binding site are listed.

Segment	190-680	210-670	250-670
PscA *tepidum*			
FMO *aestuarii*	No match	FJ–/YJG	SJ–/NJI
FMO *tepidum*	FJ–/YJG	FJ–/YJG	SJ–/NJI
FMO *limicola*	FJ–/YJG	SJS/NJI	SJS/NJI
PscA *limicola*			
FMO *aestuarii*	FJ–/YJG	FJ–/YJG	SJS/NJI
FMO *tepidum*	FJ–/YJG	FJ–/YJG	SJ–/NJI
FMO *limicola*	SJS/NJI	SJS/NJI	SJS/NJI
PscA consensus			
FMO consensus	FJ–/YJG	SJX/NJI	SJX/NJI

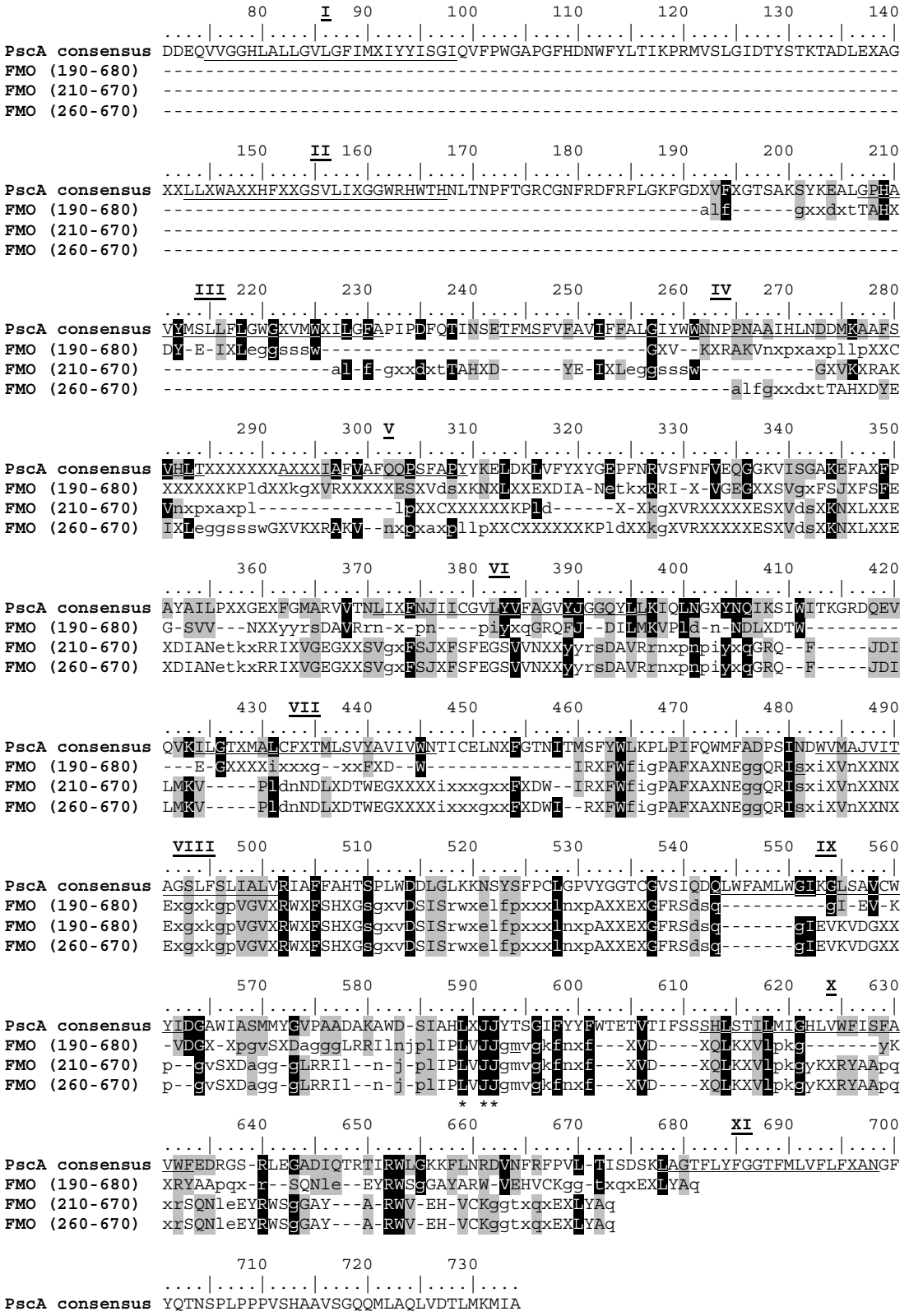

Fig. 1. Extended (190-680), intermediate (210-670) and restricted (260-670) maps of FMO-protein on PscA. Underlined segments in PscA denote α-helices labelled I-XI. Identities are shown in black, and similarities are shaded. Signature sequences are denoted by * **.

Discussion

We have mapped 3 of the 7 BChl *a*-binding sites of the FMO consensus sequence onto 3 different trimmed versions of the PscA consensus sequence. In the extended map of FMO on PscA the N-terminus and helices I and II of PscA have been cut off along with the C-terminal segment and most of helix XI. In the intermediate map loop 2 has also been deleted, and in the restricted map helix III and loop 3 are missing as well. This most robust map extends from the beginning of helix IV up to the beginning of helix XI. Although we cannot be certain which of the three maps most closely represents the relationship between FMO-protein and PscA, we believe that our results support the hypothesis that they share a common ancestor that was a reaction center protein like PscA.

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