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The Photosystem I-like P840-reaction center of Green S-bacteria is a homodimer

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An operon encoding the P840 reaction center of *Chlorobium limicola* f.sp.*thiosulfatophilum* has been cloned and sequenced. It contains two structural genes coding for proteins of 730 and 232 amino acids. The first protein resembles the large subunits of the Photosystem I (PS I) reaction center. Putative binding elements for the primary donor, P840 in *Chlorobium* and P700 in PS I and for the acceptors A_0, A_1 and FeS-center X are conserved. The second protein is related to the PS I subunit carrying the FeS-centers A and B. Since all our efforts to find a gene for a second, large subunit failed, the P840 reaction center probably is homodimeric.

Introduction

Oxigenic photosynthesis involves two photosynthetic reaction centers which originate from two different, procaryotic ancestors: The reaction center of Photosystem II (PS II) is related to the one of purple bacteria [1,2], and the reaction center of Photosystem I (PS I) is related to the one of Green S-bacteria [2,3]. The latter is convincingly demonstrated by the characterization of a transcription unit in *Chlorobium limicola* f.sp. *thiosulfatophilum* [4]. This contains two structural genes coding for proteins of 730 and 232 amino acids. The first gene resembles the genes *psa*A and *psa*B which code for the large subunits of the PS I reaction center [3], the second resembles *psa*C, which encodes the PS I subunit carrying the FeS-centers A and B [3]. The crystal structure of the reaction center of purple bacteria revealed a heterodimeric architecture, the subunits L and M holding the pigments for the primary donor and the acceptors in pseudosymmetric array [1,5]. A similar heterodimeric structure is assumed for PS II [1,2], and is also favored for PS I [2,3]. It may not apply to the P840 reaction center of *Chlorobium*, however.

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Methods

Chlorobium limicola f.sp. *thiosulfatophilum* (Deutsche Sammlung von Mikroorganismen, 3400 Göttingen, Germany) was grown, and its P840 reaction center was prepared as described before [6]. From the major subunit at 65 kDa seven peptide sequences were obtained after proteolysis. This, and the methods for cloning and sequencing the P840 transcription unit are described in detail in Ref. 4.

Results and Discussion

A folding model of the P840 protein, which is encoded by the larger gene of the transcription unit in *Chlorobium* [4], is shown in Fig. 1. This model is in accordance with a fit of the P840 sequence into an alignment of the P700 proteins of PS I, the products of the genes *psa*A and -B [7]. It comprises 11 transmembrane helices, in accordance with the model for the P700 proteins [7]. Amino acids conserved in the aligment of the P840 protein and the two P700 proteins [4] are given in single letter code in Fig. 1. The identity is less than 15% each, but is high and particularly significant in the region binding FeS-center X [3]. The model in Fig.1 follows the 'positive inside rule' [8], with a surplus of positive charges on the cytoplasmic surface,

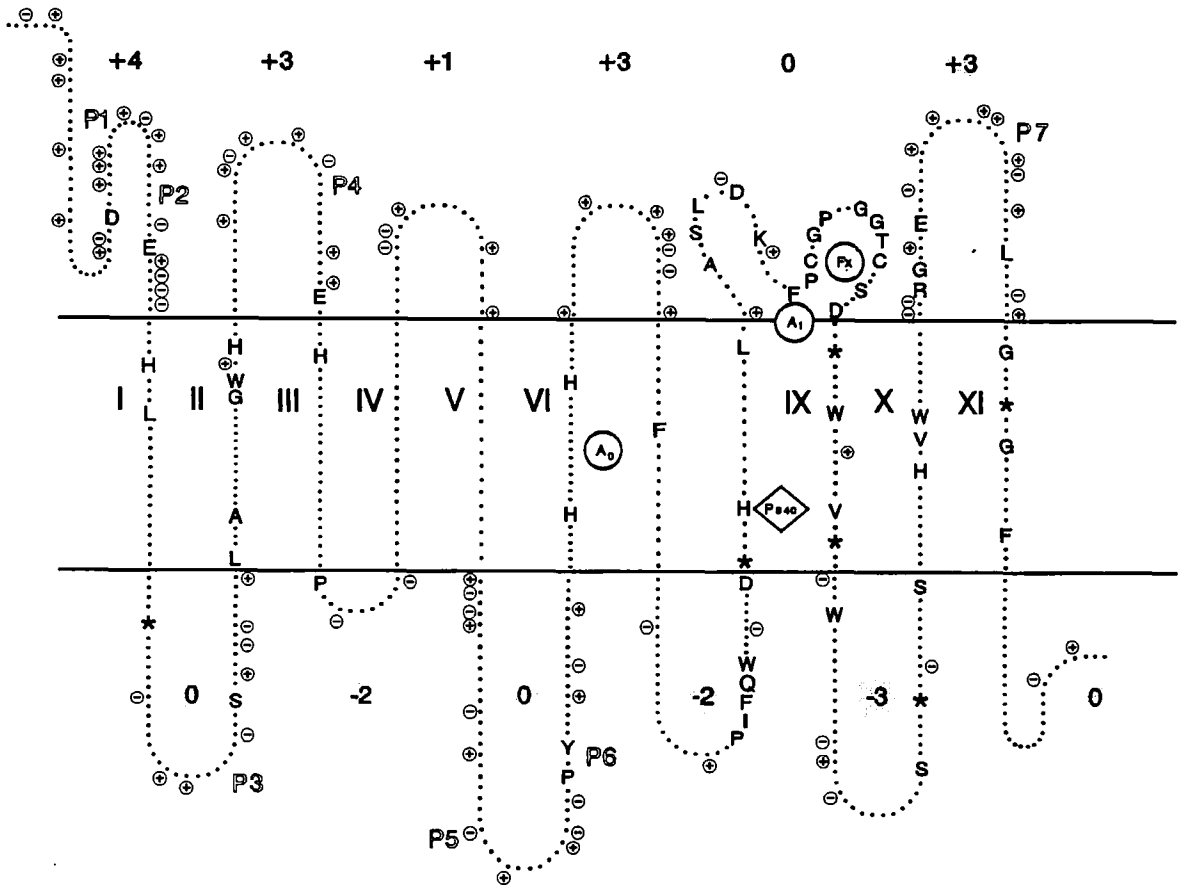

Fig. 1. Folding model of the P840-protein. The 11 putative transmembrane helices are indicated by roman numbers. Numbers on top and bottom give the net charge of the helix interconnecting loops and of the termini. Conserved amino acids in an alignment with the P700 proteins PS IA and PS IB [4] are given in single letter code. Asterisks represent conserved aromatic residues. P840, A_0, A_1 and F stand for the primary electron donor, the early electron acceptors and the FeS-center X, respectively. P1–P7 stand for the seven peptides obtained from the 65-kDa polypeptide. All are tryptic peptides, except P2, which resulted from V8-treatment. The amino-acid sequences in single letter code are FLFQR for P1 (position 49–53 in the protein), TRSTKWYQIFDTEKIDDEQV for P2 (position 56–75), VSLGIDTYSTK for P3 (position 122–132), FGDMVFSGTSAK for P4 (position 189–200), VSFNFVEQGGK for P5 (position 327–337), EFAEFPAYAILPR for P6 (position 344–356) and KFLN for P7 (position 654–657).

which is expected to be the one where FeS-center X is located. Fig.1 also indicates the locations of the primary electron donor P840, probably a 'special pair' of bacteriochlorophylls *a*, and of the early electron acceptors A_0, another bacteriochlorophyll, and A_1, probably a menaquinone, which is discussed at length elsewhere [4]. The peptide binds FeS-center X by 2 cysteines. Since a total of 4 cysteines are required, the P840 reaction center, like the P700 reaction center of PS I [3], has to be a dimer.

The transcription unit for the P840 reaction center contains only 1 gene for a large subunit in the P840 reaction center. For PS I reaction centers two genes are known, called *psa*A and *psa*B, usually organized in one transcription unit [3]. In *Chlorobium*, however, we found no signs for a second, related gene in Southern blots with 16 different restriction enzymes, even under very low stringency. Cross hybridization occurs between *psa*A and B of PS I and therefore is also expected for the two *Chlorobium* genes which would encode a heterodimer of the reaction center. A homodimeric reaction center in *Chlorobium* is also indicated by the finding of the nucleotide sequences for all seven peptides in the large gene of the transcription unit (P1–P7 in Fig.1). Since these peptides were obtained from the 65-kDa band of a photoactive P840 reaction center preparation [6], the presence of a second protein in this band is highly unlikely. The probability is only 1 in 128 for two very different proteins of equal access to proteinases. It would be higher for highly related proteins, but then we should have observed cross hybridization in Southern blots.

Purple bacteria, PS II [1,5], and probably also PS I [3] contain heterodimeric reaction centers of pseudo-C2 symmetry. The redox centers for charge separation are organized in two branches across the membrane, one of them being favored by symmetry breaking elements. The advantage of such an arrangement is a matter of

intense discussion. At low light intensity the quantum yield may be increased in an asymmetric system, which allows the two quinone acceptors to act in series, the one in the preferred branch being reduced first and staying permanently bound [5]. At high light intensity photodamage may be avoided, because a permanently bound quinone in the preferred branch can accept two electrons, before triplet states are built by back reactions, causing the formation of singlet oxygen. Perhaps triplet states were less damaging for a strictly anaerobic ancestor to *Chlorobium*, and it could live with parallel branches of electron transfer to mobile quinones. This might have been kept after an FeS-center X had been acquired. Actually, it is not known whether the quinone in *Chlorobium* reflecting A_1 is tightly bound as in PS I [3], and whether its only function is to reduce FeS-center X. It may be pertinent and in accordance with a homodimeric structure, that the leucine zipper motif in front of the FeS-X binding peptide, which has been implicated for heterodimer formation in PS I [9], is not found in the P840 protein [4].

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