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ISOLATION AND AMINO-TERMINAL SEQUENCES OF SUBUNITS FROM THE PHOTOSYNTHETIC REACTION CENTER OF *RHODOPSEUDOMONAS CAPSULATA*

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The amino-terminal sequences have been determined by Edman degradation for the reaction center polypeptides from a carotenoidless mutant of *Rhodopseudomonas capsulata*. Individual polypeptides were isolated by preparative electrophoresis and electroelution. By comparison with the sequences deduced from the DNA (Youvan, D.C., Alberti, M., Begush, H., Bylina, E.J. and Hearst, J.E. (1984) Proc. Natl. Acad. Sci. USA 81, 189–192) we conclude that the M and L subunits are processed so as to remove the amino-terminal methionine, whereas the H subunit is not processed at the amino-terminus after translation. None of the subunits is synthesized with a significant amino-terminal extension peptide.

Photosynthetic reaction centers catalyze the conversion of electromagnetic radiation into chemical potential. This conversion is accomplished by a series of light-driven electron-transfer reactions. The primary oxidants and reductants are protein-bound cofactors, including (bacterio)chlorophylls, (bacterio)pheophytins and quinones [2]. The protein matrix serves to maintain the cofactors at the appropriate distances and in the proper orientations to permit photochemistry to occur. The proteins in bacterial reaction centers and Photosystem II of higher plants probably have the function of stabilizing the semiquinone anion form of the secondary quinone acceptor (for a review, see Ref. 3). A fuller understanding of the role of the reaction-center protein requires knowledge of its structure as well as knowledge of any interactions between protein and cofactors. A prerequisite to this detailed understanding is a determination of the amino-acid sequence of the reaction center proteins. Sequence data obtained from isolated proteins complement the more extensive data available from DNA sequences. We have determined the amino-terminal sequences (7–16 amino acids) of the three reaction-center polypeptides (referred to as H, M, and L [2]) from a carotenoidless mutant of *Rhodopseudomonas capsulata*, a purple photosynthetic bacterium. Polypeptides, electroeluted from preparative SDS polyacrylamide gels, were subjected to automated Edman degradation in a gas-phase sequenator. The reaction center genes in *Rps. capsulata* have recently been sequenced by Youvan et al. [1,4]. Exact correspondence is seen between the DNA sequences and the protein sequences reported here. (A preliminary account of this work was presented at the Cold Spring Harbor meeting on Molecular Biology of the Photosynthetic Apparatus, 1984.)

The source of reaction centers for this study was *Rps. capsulata*, strain KZR8A1. This strain, constructed by transposon mutagenesis of wild-type strain SB1003 by Zsebo and Hearst [5], contains a transposon insert in the carotenoid E gene. KZR8A1 lacks colored carotenoids and fails to synthesize the 14 kDa protein typically found in

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Abbreviation: BChl, bacteriochlorophyll.

the B800–850 antenna complex of *Rps. capsulata* [6]. The near-infrared spectrum of KZR8A1 is characteristic of mutants containing only reaction center and B870 complexes [7].

Chromatophore membranes were depleted of most non-BChl proteins by resuspending to an absorbance of 10 at 870 nm, adding SDS to 0.05% final concentration, and centrifuging at 250 000 × *g* (60Ti rotor, Beckman) for 1 h. The resulting pellet was resuspended in a minimum volume of 10 mM Tris-HCl (pH 8.0) and applied to the preparative gel after solubilization (see below). Alternatively, the resuspended pellet was treated with an equal volume of glycerol and stored at −20°C. No effect of storage was seen over six months.

Preparative electrophoresis and electroelution were performed as in Ref. 8, using the buffer system of Laemmli [9]. Chromatophores containing 250 nmol BChl, as estimated at 375 nm [10], were solubilized in 75 mM SDS/65 mM dithiothreitol and loaded without heating onto a slab gel which was 3 mm thick and 14 cm wide. After stacking, proteins were separated in 12.2% acrylamide at 25 W constant power, using 4 l anode buffer as a thermal reservoir. Electrophoresis was continued for 20 min after the bromophenol blue tracking dye exited the gel, for a total time in the separating gel of approx. 200 min. After staining for 3 min and destaining for 5–7 min, the three reaction-center bands were cut out by hand using a sharp razor blade. Each gel slice retained was approx. 3 mm wide. Slices of the gel (4–5 mm) between H and M and between M and L were discarded. A detailed description of the gel-separation system has been provided elsewhere [11].

Gel slices were washed and proteins electroeluted at 4°C in ammonium bicarbonate as in [8]. After elution each sample was lyophilized, resuspended in a minimum volume of water, and treated with 9 volumes of absolute ethanol at −20°C. After 48 h at −20°C the precipitated protein was recovered by centrifugation. At this point the proteins would not dissolve in water. H and M were redissolved in 0.1% SDS. L was redissolved in 0.1% SDS/50 mM dithiothreitol. The requirement for dithiothreitol to resuspend L probably arises from the five cysteine residues in this subunit [4]. The resuspended proteins were frozen until applied to the sequenator.

Amino-terminal sequences were determined on a gas-phase sequenator (Applied Biosystems, Foster City, CA). Approx. 10 μg of protein was applied per run. Phenylthiohydantoins were resolved by HPLC [12] on a Cyano column (IBM Instruments) and assigned without prior knowledge of the DNA sequence.

Fig. 1A shows an electrophoretogram of the chromatophores obtained after extraction with 0.05% SDS. The three reaction-center subunits are the most prominent bands. Proteins of the light-harvesting complexes are visible below the M_r = 14 000 standard. Acrylamide gradient gels resolve at least three light-harvesting proteins in this region [13]. The presence of material responsible for the faint bands visible near the reaction-center subunits did not prevent sequencing of the material prepared electrophoretically.

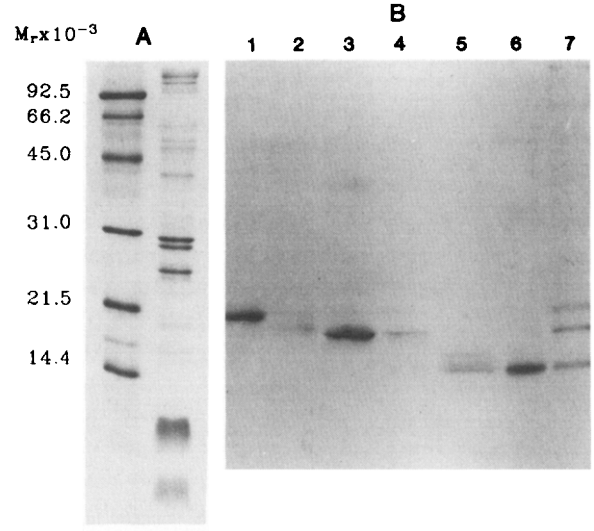

Fig. 1. SDS-polyacrylamide gel electrophoresis of chromatophore proteins and isolated reaction-center polypeptides. (A) (16% acrylamide) left, molecular-weight markers; right, proteins remaining in chromatophores depleted of most non-BChl binding proteins by centrifugation in the presence of 0.05% SDS. (B) (12% acrylamide) reaction-center subunits isolated by preparative electrophoresis. Lane 1, H subunit; lane 2, discarded material migrating between the H and M subunits; lane 3, M subunit; lane 4, discarded material migrating directly below the M subunit; lane 5, discarded material migrating directly above the L subunit; lane 6, L subunit; lane 7, sample loaded onto preparative gel (depleted chromatophores). The amount of material loaded into the 'discarded' lanes corresponds to 4-times as much preparative gel as was loaded into the lanes containing the retained subunits.

An electrophoretogram of the isolated subunits is shown in Fig. 1B. No cross-contamination between the three subunits is visible. Heavily loaded gels reveal another protein in the M fraction, which nearly comigrates with M on the preparative gel. The discarded regions of the gel are also shown. The material loaded into the 'discarded' lanes corresponds to 4-times as much of the preparative gel as was loaded into the lanes containing retained subunits. It is clear that the amount of material lost due to this precautionary step is small. Separation equivalent to that shown in Fig. 1 was obtained with loads up to 1 mg reaction center. This corresponds to approx. 350 μg of each subunit per 3 mm gel.

The amino-terminal sequences determined on the subunits after recovery from the gel are shown in Scheme I. Residual Coomassie blue remaining from the staining procedure prevented an accurate assignment of the amino acid released during the first cycle of the Edman degradation. The protein sequences deduced from the DNA sequences [1] for each subunit are shown for comparison. Exact agreement is seen between the residues assigned by Edman degradation and those deduced from the DNA sequences.

The amino-terminal sequences of M, L, and H have been determined by Edman degradation in *Rps. sphaeroides* R-26 [14], using a large scale procedure for separation of the subunits. Likewise, the M and L subunits of *Rhodospirillum rubrum* G-9 have been isolated in large quantities and sequenced [15]. Sequence homologies among reaction center proteins from *Rps. capsulata, Rps. sphaeroides,* and *R. rubrum* have been presented previously [1,15]. The isolation procedure described here does not yield such large quantities as do the other procedures, but has the advantage of being applicable to any set of proteins which can be resolved by electrophoresis. The ability to separate H, M, and L, all of which migrate within a 1.5 cm section of the gel, demonstrates the very high resolution of the preparative gels. The optimal resolution of H, M, and L obtained by HPLC on a TSK SW2000 column (LKB) resulted in significant cross contamination (data not shown). Unlike Theiler et al. [15], we have found aqueous SDS to be an ideal agent for maintaining the hydrophobic reaction-center proteins in solution. We are currently developing fragmentation schemes for the reaction-center subunits dissolved in SDS.

The amino-terminal amino acid of each mature subunit can be identified by comparing the protein and DNA sequences (see Scheme I). For example, in the M subunit, the second residue in the protein

M Subunit

A)	NH_2X	Glu	Tyr	Gln	Asn	Phe	Phe	Asn	Gln	Val	Gln	Val	Ala	Gly	Ala	Pro	Glu	
B)	NH_2Met	Ala	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

L Subunit

A)	NH_2X	Leu	Leu	Ser	Phe	Glu	Arg	Lys	Tyr	Arg	Val
B)	NH_2Met	Ala	–	–	–	–	–	–	–	–	–

H Subunit

A)	NH_2X	Val	Gly	Val	Asn	Phe	Phe	Gly
B)	NH_2Met	–	–	–	–	–	–	–

Scheme I. Amino-terminal sequences for the reaction center subunits from *Rps. capsulata,* strain KZR8A1. (A) Sequences determined by automated Edman degradation on polypeptides eluted from preparative SDS-polyacrylamide gels. (B) Sequences deduced from the DNA (Ref. 1). Agreement between the two sequence determinations is indicated by a dash.

(the first assigned residue) is the third residue deduced from the DNA. Thus the first amino acid in the mature M subunit is alanine, and the amino-terminal methionine has been cleaved. Likewise, the methionine has been cleaved from the L subunit, leaving alanine as the first amino acid in the mature subunit. In the H subunit, the second residue in the protein is also the second residue deduced from the DNA sequence. Thus the amino-terminal methionine is retained in the H subunit. Prior to assembly, none of the subunits is processed at the amino-terminus so as to remove more than one amino acid.

The gene for the M subunit from *Rps. sphaeroides* has been sequenced by Williams et al. [16]. The amino-terminal sequence of the protein has been determined by Sutton et al. [14]. The situation with respect to processing is the same as in *Rps. capsulata.* The amino-terminal methionine is cleaved, leaving alanine as the first amino acid in the mature protein. The first amino acid in the mature L subunit from *Rps. sphaeroides* is alanine; the first residue in the mature H subunit is methionine [14]. Thus it appears that the processing of L and H is similar in *Rps. capsulata* and *Rps. sphaeroides,* but sequences for the L and H genes in *Rps. sphaeroides* are needed to confirm this.

The significance of the difference in processing for the M and L subunits versus the H subunit is not known. It is interesting to note other differences between these subunits. The genes for the M and L subunits are adjacent to each other in *Rps. capsulata* and follow directly the genes for the B870 antenna complex, while the gene for the H subunit is 20000 basepairs away [1]. The M and L subunits bind all of the known pigments and quinones, whereas the H subunit is not known to bind any cofactors [2]. Recently, Chory et al. [17] have demonstrated the presence, in cells completely devoid of intracytoplasmic membranes, of a protein thought to be the H subunit, based on apparent M_r and cross-reactivity to anti-reaction center antibodies. (The presence of M or L could not be absolutely excluded, because of their poor antigenicity.) These workers speculated on a role for the H subunit as a site of insertion of BChl or BChl-binding proteins into the chromatophore membrane. From the experiments concerning cofactor binding it is clear that the role of the H subunit is quite different from the role of the M and L subunits. The location of H on a different operon from that of M and L suggests a different history for H prior to insertion into the membrane.

It remains to be seen whether the different processing of the reaction center polypeptides is related to their different histories prior to assembly or to their different roles once inserted into the chromatophore membrane.

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