POLYADENYLATED MESSENGER RNAs CODE FOR PHOTO REACTION CENTER AND LIGHT-HARVESTING ANTENNA POLYPEPTIDES OF *RHODOSPIRILLUM RUBRUM*

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1. Introduction

A reaction center complex that can be effectively solubilized from the chromatophores of purple bacteria by the detergent LDAO [1], contains three polypeptide subunits of 28, 24 and 21 kdalton with a stoichiometry of 1:1:1 [2–6]. Amino acid analysis indicates that the two smaller subunits are hydrophobic in composition [7]. Another 14 kdalton polypeptide which constitutes ~50% of the total chromatophore protein has been purified by extraction with organic solvent followed by column chromatography [8,9]. This polypeptide corresponds to the light-harvesting antenna [9]. Photobiological characteristics of the RC and LH are well established [6,9]. However, data concerning biogenesis and mode of assembly of these polypeptides in the membrane are nonexistent [10]. In the course of studying the sequences of 5 S and a novel 3 S ribosomal RNA of *Rhs rubrum* [11], the occurrence of polyadenylated mRNA was detected and characterized [12]. In view of this result, a pertinent question is: Are these polyadenylated mRNAs capable of coding for any of the polypeptides or RC and LH? Answers to the question are reported here.

2. Materials and methods

Rhodospirillum rubrum was grown anaerobically in the medium of [12] at 28°C in 500 ml Roux bottles in light. Cells were subcultured 4 times before the final culture and allowed to grow for 24 h. Total nucleic acid was extracted from 1 g cells by the procedure in [12]. Briefly:

1. The cells were washed in sterile, bidistilled water and disrupted in 15 ml buffer containing 10 mM Tris–HCl (pH 7.5), 3 mM $MgCl_2$ and 0.3 ml diethyl pyrocarbonate to inhibit ribonuclease activity, by 5 cycles of freezing in liquid nitrogen and thawing in water at 4°C.
2. Cellular RNA was precipitated by adjusting the supernatant to pH 5.2 by acetic acid. The precipitate was removed by centrifugation.
3. The pelleted RNA was then dissolved in 10 ml of 0.1 M Tris–HCl (pH 9), 0.12 M NaCl, 2 mM EDTA, 0.8% SDS, 0.1 ml diethyl pyrocarbonate and extracted 5 times with an equal volume of phenol–chloroform–iso amyl alcohol (50:49:1, v/v/v).

Phenol was washed out with anhydrous ether; the final aqueous phase was adjusted to 0.2 M sodium acetate and precipitated with 2.5 vol. 95% ethanol.

Total RNA (8 mg) thus obtained was washed with 90% ethanol, dried under nitrogen and dissolved in buffer containing 10 mM Tris–HCl (pH 7.5), 400 mM KCl and 1 mM EDTA and fractionated on an oligo-(dT)-cellulose column [12] under constant monitoring of A_{260} at 4°C. Flowthrough fraction was recycled thrice through the column. The material bound to the resin was eluted with sterile, bidistilled water and precipitated with 95% ethanol.

To verify that the oligo (dT)-cellulose column bound material indeed was polyadenylated RNA, aliquots of RNA as given in fig.2 were allowed to hybridize with ^{3}H-labelled poly (U) in a buffer which

Abbreviations: RC; reaction center; LHP; light-harvesting antenna polypeptide; LDAO; lauryl dimethylamine *N*-oxide; SDS–PAGE; sodium dodecyl sulfate-polyacrylamide gel; oligo (dT)-cellulose; oligo deoxythymidylic acid-cellulose; poly (U); poly(uridylic acid); poly (A); poly (adenylic acid); Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DDT, dithiothreitol; TCA, trichloroacetic acid

contained both pancreatic A and T_1 RNases as in [12–13]. After 45 min at 35°C, the reaction was terminated by cold TCA and material precipitated was trapped on Whatman GF/C filter disc, dried and counted.

Wheat germ S30 fractions were prepared and treated with Ca^{2+}-dependent micrococcal nuclease to reduce the background mRNA activity as in [14,15]. In vitro translation of poly(A)$^+$ mRNA was carried out at 26°C for 120 min in 50 μl reaction mixture containing: 20 μl S30 fraction, 22 mM Hepes-KOH buffer (pH 7.4), 2 mM DTT, 1 mM ATP, 75 μM GTP, 10 mM creatine phosphate, 45 μg/ml creatine phosphate kinase, 95 mM potassium acetate, 2.8 mM $MgCl_2$, 30 μM of each of the 19 unlabelled amino acids, 30 μCi [^{14}C]leucine (298 Ci/mmol) and 3 μg poly(A)$^+$ RNA. The optima for pH, potassium, magnesium and mRNA concentrations were determined in a series of pilot experiments. Analysis of the in vitro translation products was carried out by 5–12% SDS–PAGE using the discontinuous buffer system as in [16]. Gel was stained with Coomassie blue, destained, fixed in 2% acetic acid–25% 2-propanol, dried and photographed.

Authentic RC and LH polypeptides used as markers in the gel analysis, were derived from cells cultured in presence of [^{14}C]leucine. RC was purified by the method in [17]. The LH polypeptide was semi-purified from the reaction center depleted chromatophores as in [9].

All reagents and buffers whenever appropriate were recrystallized or autoclaved. Glass vessels were washed in 0.1 N NaOH and siliconized.

3. Results

Total nucleic acid extracted by the 3 step procedure from the phototrophically grown *Rhs. rubrum* has been separated into poly(A)$^+$ and poly(A)$^-$ fractions by oligo(dT)-cellulose chromatography (fig.1). From 10 measurements consistent yield of poly(A)$^+$ fraction was 4.8 ± 0.2% of 8 mg total nucleic acid fractionated. Preparation was essentially free of DNA contaminant since its presence could not be detected by digesting an aliquot in RNase free DNase I [12]. Analysis of 25 μg poly(A)$^+$ RNA on formamide–PAGE, results in >40 bands revealed by ethidium bromide stain (data not shown). Presence of polyadenylated RNA has also been confirmed by hybridization with ^{3}H-labelled poly(U), as depicted in fig.2.

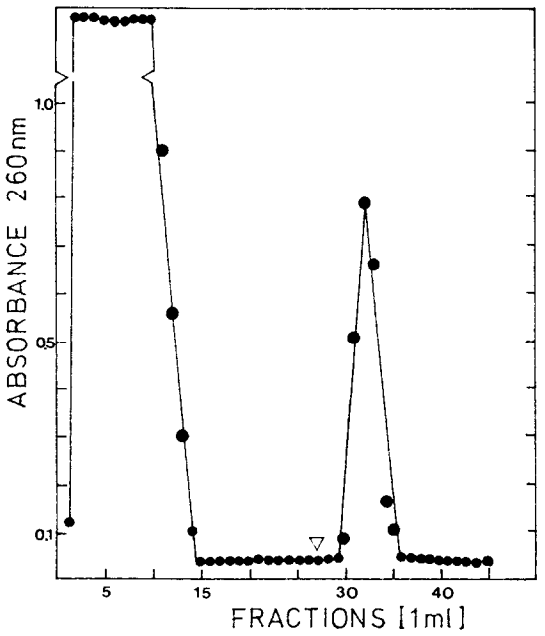

Fig.1. Oligo (dT)-cellulose column fractionation of total RNA purified from phototrophic cells of *Rhodospirillum rubrum*. About 8 mg total RNA was dissolved in buffer as in section 2 and applied in to the column at 4°C. Resin-bound poly(A)$^+$ RNA was eluted by water as indicated by the arrow

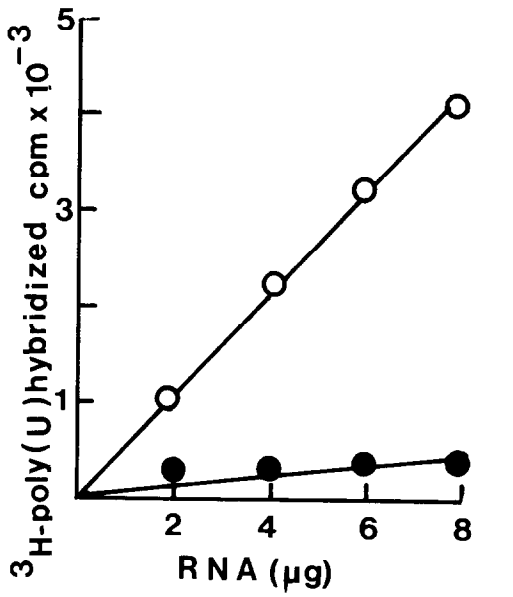

Fig.2. ^{3}H-labeled poly(U) hybridization with polyadenylated mRNA. Aliquot was hybridized with ^{3}H-labeled poly(U) with spec. act. 52.1 μCi/mol phosphate. Cold TCA-precipitable counts were measured on GF/C filters. Poly(A)$^+$ RNA (○); poly(A)$^-$ RNA (●).

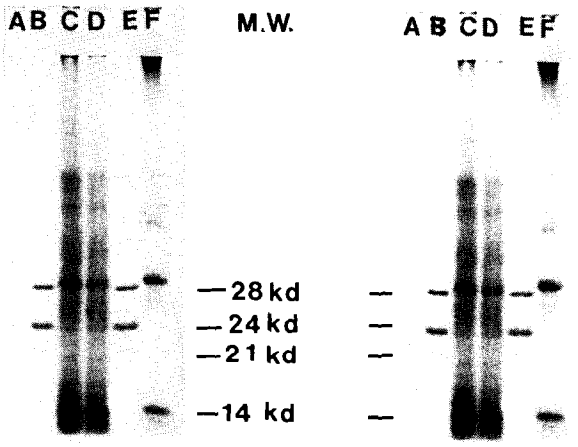

Fig.3. SDS–PAGE resolution of in vitro translation products. Lane A, background; lanes B,E, migrations of authentic RC polypeptides; lane F, the migration of authentic LH polypeptide of 14 kdalton. In lanes C,D 25 μl each of in vitro translation products were run after processing the samples as in [14,16].

In attempts to determine which polypeptide the total mRNA preferentially code for, the polyadenylated RNAs have been translated in a wheat germ cell-free extract. Analysis of the patterns of [^{14}C]leucine labelled polypeptides synthesized under the direction of poly(A)$^+$ RNA is shown in fig.3. Two prominent bands which co-migrate with authentic RC polypeptide markers are 28 and 24 kdalton although the 21 kdalton band in the product is barely visible. In addition, LH polypeptide is also formed in vitro and co-migrates well with the authentic LHP marker. The nature of the in vitro products and their electrophoretical mobilities clearly demonstrate for the first time that both RC and LH polypeptides are coded by polyadenylated messenger RNAs in *Rhs. rubrum*.

4. Discussion

Factors that control the biosynthesis and differentiation of the membrane system of photosynthetic bacteria have been critically reviewed [18]. It has also been suggested that protein complexes, among other components, are included into membrane by a multistep process. Our results demonstrate that biogenesis of RC and LH polypeptides starts at a very early growth phase in phototropic *Rhs. rubrum* with very little production of 65 kdalton ribulose biphosphate carboxylase. After induction of morphogenesis of photosynthetic apparatus the RC and LH polypeptides are synthesized first in *Rhs. capsulata* [19]. By comparison, in vitro translation assay has revealed that the insertion of LH chlorophyll *a/b* protein in higher plant thylakoid membrane occurs via a 29.5 kdalton precursor [20]. Identical co-migrations of the in vitro products and authentic markers in our results do not suggest the involvement of precursor as such. Nevertheless, based upon the information presented, further experiments could shape the debate into certitude.

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