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DNA-Directed In Vitro Synthesis and Assembly of the Form II D-Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase from *Rhodopseudomonas sphaeroides*

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A biochemical analysis of the in vitro assembly of the form II ribulose-1,5-bisphosphate carboxylase/oxygenase from *Rhodopseudomonas sphaeroides* after transcription and translation from cloned DNA is presented. The predominant enzymatically active oligomeric forms of the in vitro-synthesized and -assembled ribulose-1,5-bisphosphate carboxylase are tetramers and hexamers. Assembly of the monomeric subunits to form active enzyme appears to be dependent on the presence of a minimum number of subunits in the cell extract. Assembly of ribulose-1,5-bisphosphate carboxylase also was observed when the protein-synthesizing extracts were prepared from cells which were partially derepressed for ribulose-1,5-bisphosphate carboxylase expression.

Rhodopseudomonas sphaeroides is a model organism for the study of anoxygenic photosynthesis. It is one of the most intensively studied and best understood photosynthetic systems with regard to the structural and functional events surrounding the entrapment of light energy and its conversion to chemical energy (3–5, 7, 10). In addition, photosynthetic enzyme activities, including the ribulose-1,5-bisphosphate (RuBP) carboxylase, have been physically and biochemically described in *Rhodopseudomonas sphaeroides* (9, 26; L. S. Sarles and F. R. Tabita, Fourth Int. Symp. Microbiol Growth on C_1 Compounds, Minneapolis, Minn., 1983), thus making this organism a system of choice for studies of the assembly of functional photosynthetic complexes as well as for studies of the expression of genetic determinants involved in photosynthesis.

In plants, algae, and most CO_2-fixing bacteria, the RuBP carboxylase is a heteromultimer of two nonidentical subunits, with a structure consisting of eight large subunits and eight small subunits (L_8S_8). However, two exceptions to this basic subunit structure exist in the purple nonsulfur photosynthetic bacteria: the homodimeric enzyme of *Rhodospirillum rubrum* (L_2) (25) and the homohexameric enzyme found in *Rhodopseudomonas sphaeroides* (L_6) (9). *Rhodopseudomonas sphaeroides* is unique in that it contains two distinct carboxylase activities, both the L_8S_8 enzyme (form I) and the L_6 enzyme (form II) (9).

Genes encoding the large and small subunits from plants and algae have been cloned (6, 8, 17, 22), and the large subunit gene from *Zea mays* has been expressed from a bacterial promoter (8), with formation of a 55,000-dalton polypeptide. However, the protein was not catalytically active. In comparison, the relatively simple quaternary structure of the *Rhodospirillum rubrum* and the *Rhodopseudomonas sphaeroides* form II enzymes makes them amenable to studies of expression and regulation from cloned DNA. Indeed, both the *Rhodospirillum rubrum* (23) and *Rhodopseudomonas sphaeroides* (18) cloned genes under the transcriptional control of an *Escherichia coli* promoter yielded active enzyme.

Rhodopseudomonas sphaeroides promoters are not expressed in *E. coli*, and this also appears to be true of other high-mole-percent guanine-plus-cytosine organisms, including *Streptomyces lividans* (21), *Myxobacteria* spp. (20), and *Caulobacter crescentus* (13). Therefore, we developed a cell-free DNA-directed protein-synthesizing system in *Rhodopseudomonas sphaeroides* to identify photosynthetic gene products from cloned genomic DNA (2). This system would also complement in vivo studies concerned with elucidating regulatory mechanisms of gene expression in *Rhodopseudomonas sphaeroides*.

As a step towards further understanding the factors involved in the assembly of functional photosynthetic complexes and oligomeric photosynthetic enzymes, we report here the physical and biochemical characterization of an enzymatically active form II RuBP carboxylase synthesized in vitro from cloned DNA. We have previously shown that a cloned 3-kilobase (kb) piece of *Rhodopseudomonas sphaeroides* DNA encodes the large subunit of the form II enzyme (18). We show here that no external factors need be added to the cell extracts for in vitro synthesis and assembly of this enzyme to occur.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *Rhodopseudomonas sphaeroides* wild-type 2.4.1 was used throughout this study. Cells were grown in Sistrom minimal medium A supplemented with 0.2% vitamin-free Casamino Acids (15). The cells (2.4 liters) for the preparation of S-30 fractions were grown photosynthetically with sparging (95% N_2–5% CO_2) or chemoheterotrophically (25% O_2) to a cell density of 0.8×10^9 to 1.0×10^9 cells per ml. Cell extracts from *Rhodopseudomonas sphaeroides* competent in in vitro protein synthesis were prepared as previously described (2) or anaerobically in a Freter anaerobic chamber (98% N_2–2% H_2).

DNA template. Plasmids pLI4 and pLI10 which contain a 3-kb *Bam*HI insert (in opposite orientations) of *Rhodopseudomonas sphaeroides* DNA in vector pAS621 (14) were used to program the *Rhodopseudomonas sphaeroides* cell-

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free system. Two DNA templates, pRR116 and pRR2119, which contain the cloned *Rhodospirillum rubrum* large-subunit RuBP carboxylase gene also were used to prime the *Rhodopseudomonas sphaeroides* cell-free system (23). The construction, physical map, and characterization of the *Rhodopseudomonas sphaeroides* templates are described in an accompanying paper (18). Plasmid DNA was prepared by a Triton X-100 lysis procedure (15).

Conditions for in vitro protein synthesis by extracts of *Rhodopseudomonas sphaeroides*. The *Rhodopseudomonas sphaeroides* cell-free system that we have previously described for phage and heterologous plasmid templates was used (2). The volume of the reactions used here was 100 μl unless otherwise indicated. Synthesis was carried out in glass tubes (10 by 75 mm) or in 1-ml serum-stoppered vials previously brought to anaerobiosis. The counting efficiency of the L-[^{35}S]methionine incorporated into protein and precipitated onto the filter disks was determined to be 60%. The specific activity of the L-[^{35}S]methionine incorporated into protein on the filter disks, corrected for counting efficiency, was calculated to be 10^6 cpm/pmol. The results are expressed as picomoles of L-methionine incorporated per milligram of S-30 protein after subtraction of control blank values. The *Rhodopseudomonas sphaeroides* S-30 extracts had an average protein concentration of 16 mg/ml. RuBP carboxylase activity was measured immediately after termination of the in vitro synthesis reactions.

Enzyme assay. RuBP carboxylase activity was determined at 30°C as RuBP-dependent $^{14}CO_2$ fixation, and the assay conditions used here were as described previously (18) except that the assay was allowed to proceed for 5 min.

Gel electrophoresis. One-dimensional sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was used to identify the in vitro-synthesized protein products (12). Gel dimensions and running conditions either were as described by Cohen and Kaplan (5) or were 240 by 190 by 0.5 mm. Labeled proteins were visualized by fluorography with preflashed Kodak X-Omat AR-5 film (1). Molecular weight marker proteins used were: bovine serum albumin, 68,000; ovalbumin, 43,000; α-chymotrypsinogen, 25,700; β-lactoglobulin, 18,400; lysozyme, 14,300; cytochrome *c*, 12,300; and bovine trypsin inhibitor, 6,200.

Identification of various oligomeric forms of the RuBP carboxylase synthesized in vitro. To size the newly formed form II RuBP carboxylase, 1 ml of DNase-treated L-[^{35}S]methionine-labeled, as well as unlabeled extracts each were separately loaded onto a Bio-Gel A1.5 m gel filtration column (1.5 by 62 cm) equilibrated with 0.025 M Tris-hydrochloride buffer containing 1 mM EDTA, 5 mM 2-mercaptoethanol, and 0.1 M NaCl (pH 7.5), and the column was developed against gravity. Flow rates were maintained at 5 ml/h, and 1.5-ml fractions were collected. Molecular weight markers used to size the column eluate and which yielded a straight-line relationship between molecular weight and fraction volume were: thyroglobulin, 670,000; ferritin, 440,000; catalase, 247,500; bovine serum albumin, 68,000; ovalbumin, 44,000; and myoglobin, 16,800.

Materials. RuBP was the generous gift of W. L. Ogren, University of Illinois, Urbana, or was from Sigma Chemical Co., St. Louis, Mo. Acrylamide was obtained from Eastman Kodak Co., Rochester, N.Y., and was purified on a Bio-Rad AG501-X8(D) column (Bio-Rad Laboratories, Richmond, Calif.). Bio-Gel A1.5 m (200 to 400 mesh) was from Bio-Rad Laboratories. Translation-grade L-[^{35}S]methionine (1,445 Ci/mmol) was obtained from Amersham Corp., Arlington Heights, Ill. NaH[^{14}C]O_3 (53 mCi/mmol) and En^{3}Hance were from New England Nuclear Corp., Boston, Mass. Materials for cell-free protein synthesis were purchased from Calbiochem-Behring, La Jolla, Calif. All other chemicals were of reagent grade.

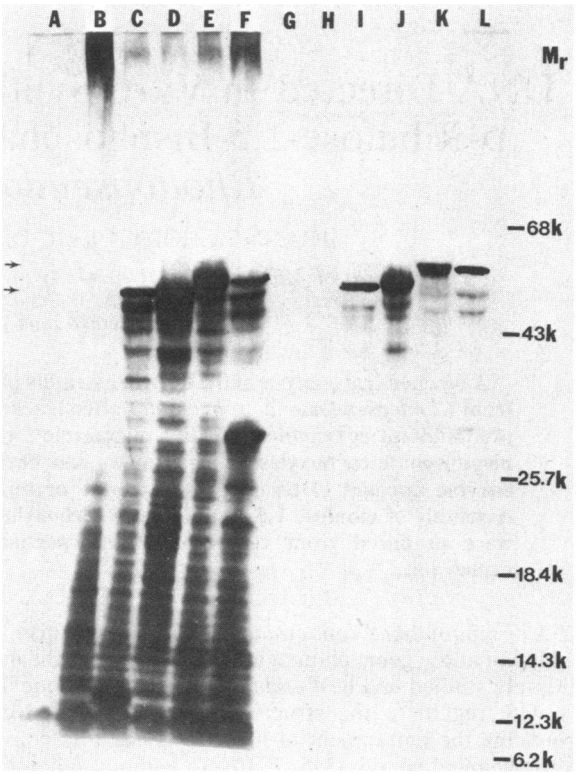


FIG. 1. Fluorograph of a 10 to 14% linear acrylamide gradient SDS gel of cell-free protein-synthesizing extracts of *Rhodopseudomonas sphaeroides* programmed with the various RuBP carboxylase DNA templates (lanes A to F) and immunoprecipitated with antibody prepared against the purified *Rhodospirillum rubrum* RuBP carboxylase (lanes G to L). The large subunits at ca. 52 and 55 kilodaltons are indicated by the arrows. The samples are (per lane) no DNA control (A and G), pAS621 (B and H), pLI4 (C and I), pLI10 (D and J), pRR2119 (E and K), and pRR116 (F and L).

RESULTS

Synthesis of the large subunit of the form II RuBP carboxylase in vitro. The gene for the RuBP carboxylase from *Rhodopseudomonas sphaeroides* has been previously cloned and identified as the form II gene (18). To test for the presence of the transcriptional regulatory sequences in the clones of the RuBP carboxylase gene and to further characterize the gene products of the cloned DNA in a homologous system, we utilized a *Rhodopseudomonas sphaeroides* in vitro-coupled transcription-translation system (2).

The *Rhodopseudomonas sphaeroides* cell-free system was programmed with plasmids pAS621, pLI4, pLI10, pRR2119, and pRR116, and the protein products were immunoprecipitated with antibody prepared against the *Rhodospirillum rubrum* RuBP carboxylase and served as positive controls for the antibody reaction. Figure 1 shows the total and precipitated in vitro-synthesized proteins electrophoresed on a SDS-polyacrylamide gel. No proteins were precipitated from cell extracts programmed with either endogenous message or plasmid pAS621 (Fig. 1, lanes G and H). However both pLI10 and pLI4 directed the synthesis of a 52,000-molecular-weight protein that cross-reacted with antibody to

Rhodospirillum rubrum RuBP carboxylase (Fig. 1, lanes I and J). The size of this product agrees precisely with the subunit molecular weight of 52,000, previously determined for the form II RuBP carboxylase (9). The smaller polypeptides immunoprecipitated from the cell extracts are most likely abbreviated forms of the large subunit caused by either premature termination of transcription or translation. Since expression of the RuBP carboxylase gene in vitro in an homologous cell-free system was independent of gene orientation, we conclude that the promoter region for the RuBP carboxylase gene is intact and present on the subcloned 3.0-kb fragment.

Furthermore, the observation that the antibody to the *Rhodospirillum rubrum* enzyme cross-reacted with the *Rhodopseudomonas sphaeroides* enzyme demonstrates that these two proteins share antigenic determinants. This cross-reaction is notable because antibodies to the *Rhodospirillum rubrum* do not cross-react with the RuBP carboxylase from spinach, chlorella, cyanobacteria, chemosynthetic bacteria, or purple sulfur bacteria which contain the form I type RuBP carboxylase (17). Since *Rhodospirillum rubrum* possesses the prototypical form II enzyme, these results are in agreement with the previous conclusion (18), that we have cloned the form II gene.

Synthesis of active enzyme in vitro from DNA templates from *Rhodopseudomonas sphaeroides* and *Rhodospirillum rubrum*. Table 1 shows the RuBP-dependent carboxylase activities of enzyme synthesized in vitro in the *Rhodopseudomonas sphaeroides* cell-free system when the form II *Rhodopseudomonas sphaeroides* carboxylase gene (pLI4, pLI10) or the *Rhodospirillum rubrum* gene (pRR116, pRR2119) was used to program the protein-synthesizing reactions. Although the *Rhodospirillum rubrum*-primed system gave lower activities than the *Rhodopseudomonas sphaeroides* DNA-primed system, in all cases the activity was significantly above background, and the results presented here are representative of similar results obtained from different S-30 preparations.

To further characterize the oligomeric form(s) of the in vitro-synthesized RuBP carboxylase, plasmid pLI4 was used as a source of template DNA for all further experiments because the RuBP carboxylase gene is transcribed from a *Rhodopseudomonas sphaeroides* promoter as defined with λ DNA templates in the S-30 extracts (18; J. Chory, E. D. Muller, and S. Kaplan, unpublished data). There are no known vector promoters available to promote transcription of the RuBP carboxylase gene in pLI4 (14).

Kinetics of active RuBP carboxylase cell-free synthesis. Figure 2 shows the kinetics of synthesis of new RuBP carboxylase subunits as well as the kinetics of active enzyme formation in cell extracts prepared from chemoheterotrophically grown *Rhodopseudomonas sphaeroides*. The results are presented as picomoles of [^{35}S]methionine incorporated into the 52,000-dalton large-subunit polypeptide and were determined from densitometric tracings of a fluorograh of an SDS-polyacrylamide gel of the total in vitro-synthesized products. The large subunit typically corresponded to 12 to 25% of the total newly synthesized polypeptide bands (data not shown). Although we can detect new subunits by 10 min into the assay and the synthesis is linear for 50 min, we cannot detect active enzyme until at least 40 min into the assay. When chloramphenicol was added at various times after the initiation of synthesis in vitro, it was observed that at least 35 min of uninterrupted synthesis was required before active enzyme was observed. Presumably, this was because the concentration of newly formed subunits was not sufficient to promote the formation of active enzyme. Since the inferred amino acid sequence for the *Rhodospirillum rubrum* large subunit has nine methionine residues, we calculated that 1.8×10^{11} subunits in a 100-μl reaction were minimally required for assembly to occur in vitro.

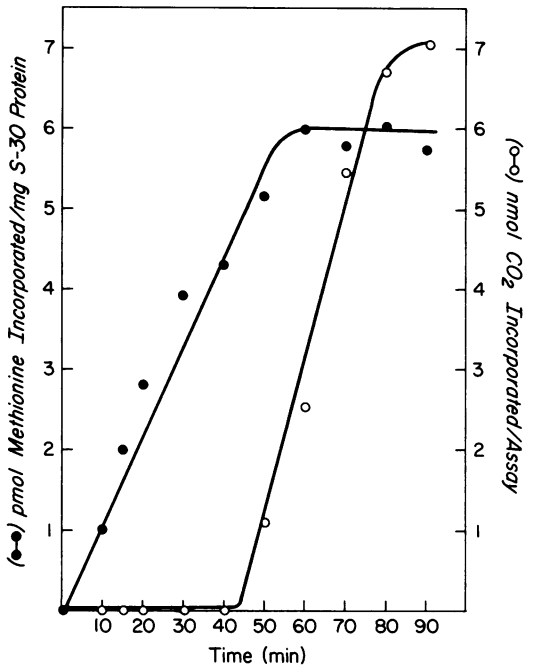


FIG. 2. Kinetics of L-[^{35}S]methionine incorporated into the 52,000-dalton monomeric subunits of the RuBP carboxylase. The values were calculated after the tracing of a densitometric scan of the total in vitro-synthesized products in a given 100-μl protein-synthesizing reaction and determined as a percentage (12 to 25%) of the total ^{35}S counts per minute incorporated into protein. Also plotted are the kinetics of detectable enzyme activity. The RuBP carboxylase was assayed for 5 min after the addition of RuBP. The specific activity of the $^{14}CO_2$ incorporated, corrected for counting efficiency, was 792 cpm/nmol of $NaHCO_3$.

TABLE 1. RuBP carboxylase activity after in vitro synthesis in cell extracts from *Rhodopseudomonas sphaeroides*

DNA template	CO_2 (nmol) incorporated per Assay[a]
None	0.5
pAS612	0.5
pLI4	5.77
pLI10	8.82
pRR2119	2.01
pRR116	1.44

[a] The conditions for cell-free synthesis and enzyme assay are described in the text. The specific activity of the $^{14}CO_2$ incorporated, corrected for counting efficiency, was 792 cpm/nmol of $NaHCO_3$.

Analysis of oligomeric forms of RuBP carboxylase synthesized in vitro. We showed that RuBP carboxylase activity in vitro is due to de novo-synthesized enzyme subunits. Since the pLI4 DNA template gave rise to active enzyme in vitro, this implied that at least a fraction of the subunits must be in the dimeric form (9). To further characterize the structural form of the in vitro-synthesized RuBP carboxylase, both L-[^{35}S]methionine-labeled as well as unlabeled synthesis products were chromatographed separately over a calibrated

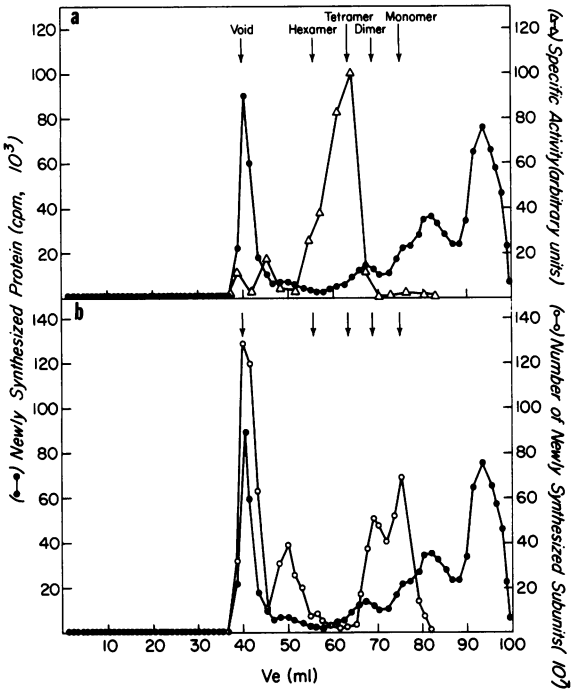
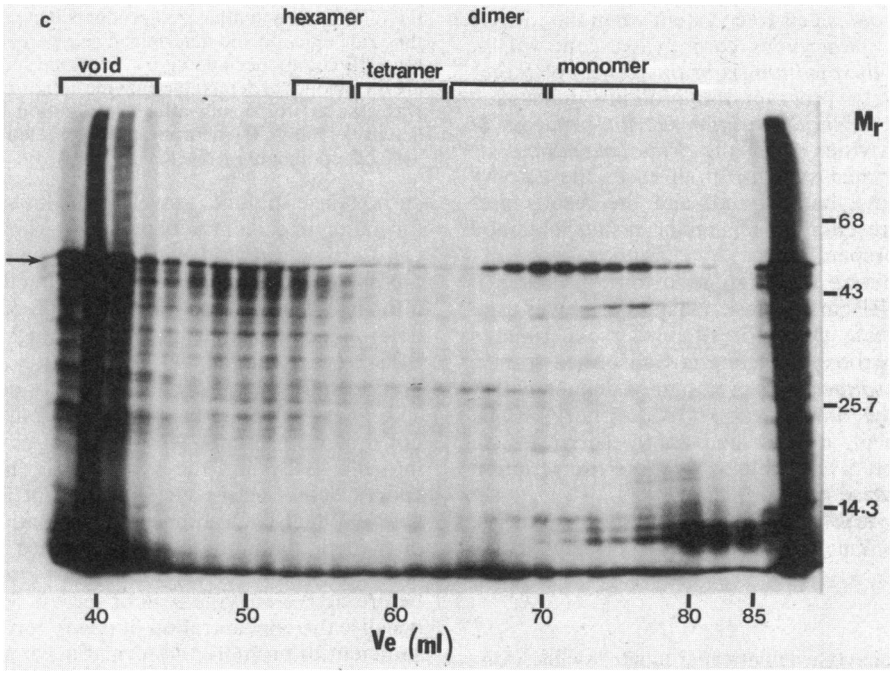


FIG. 3. Gel filtration of in vitro-synthesized and -assembled form II RuBP carboxylase. The cell extracts were treated with RNase-free DNase for 1 h to destroy the DNA template before loading the column. (a) Specific activity of the newly synthesized enzyme, plotted together with the total newly synthesized protein. The total activity was normalized to the amount of new ^{35}S-protein in the 52,000-dalton polypeptide band (c). (b) Plot of the number of newly synthesized subunits present in each oligomeric form of the RuBP carboxylase. These values also were calculated from the densitometric tracings from panel c and were determined as described in the legend to Fig. 1. Ve, Elution volume. The arrow in panel c designates the 52,000-dalton large subunit. The activity (nanomoles of CO_2 incorporated per assay) which was contained in each column fraction corresponding to each oligomeric form of the enzyme was: dodecamer, 21.00; hexamer, 12.02; tetramer, 22.65, dimer, 21.4; and monomer, <1.



Bio-Gel A1.5 m molecular sieve column and fractionated by size, and the different oligomeric forms of the enzyme were determined by the apparent molecular weight of the fractionated activity. Figure 3a shows the column profile of the newly synthesized protein as well as the specific activity profile of the assembled subunits. As a control, the endogenous incorporation (no added DNA) in cell extracts was also chromatographed, and the column profile consisted largely of one peak with an M_r of <10,000 (data not shown). The specific activity of the carboxylase is expressed in arbitrary units based on densitometer scans (Fig. 3c) of a radioautogram of an SDS-polyacrylamide gel of the newly synthesized labeled carboxylase subunits which have been fractionated on the same column. By using specific-activity measurements rather than total-activity measurements, we obtained more accurate estimates of which oligomeric form of the enzyme being fractionated is in fact the most active form of the enzyme made in vitro. The most active form of the enzyme in vitro chromatographed somewhere between the tetrameric form (M_r, 210,000) and the hexameric form (M_r, 350,000) of the enzyme. The dimeric form of the enzyme did not resolve well from the tetrameric form but also showed activity. There is a small peak of activity which comigrated with a dodecameric form (M_r, 650,000) of the enzyme. Although this is the region of the column which contained most of the total activity (56%), the specific activity of this

TABLE 2. In vitro assembly of RuBP carboxylase

Oligomeric form	% of total subunits	% of total activity per subunit (sp act)	% Total activity
Dodecamer	36	12	27
Hexamer	9	22	15
Tetramer	4	51	29
Dimer	21	15	27
Monomer	30	<1	<1

oligomeric form of the RuBP carboxylase is actually quite low (Table 2). The monomeric form of the enzyme has no activity, as has been previously shown for the purified form II RuBP carboxylase from *Rhodopseudomonas sphaeroides* (9). Figure 3b shows the number of subunits contained in each oligomeric form of the RuBP carboxylase. These data also were calculated from the densitometric scans of the various fractions from the column (Fig. 3c). The total enzyme activities from each fraction are indicated below the figure.

Physical characterization of in vitro conditions for the synthesis of RuBP carboxylase. Table 3 is a summary of the optimum conditions for the in vitro synthesis of the form II carboxylase with extracts of chemoheterotrophically grown *Rhodopseudomonas sphaeroides*. The optimal Mg^{2+} concentration for new carboxylase synthesis was 11 mM and corresponded closely to the value observed for the *Rhodopseudomonas sphaeroides* bacteriophage RS1 DNA template previously used to characterize the cell-free protein-synthesizing system from *Rhodopseudomonas sphaeroides* (2). The values observed here for the amount of DNA template and S-30 protein also correspond well with the previously reported values for RS1 in *Rhodopseudomonas sphaeroides* (2) and β-galactosidase synthesis in *E. coli* (24) and show that the system from chemoheterotrophically grown cells is comparable to the system described for *E. coli*.

In vitro synthesis of RuBP carboxylase in S-30 extracts prepared from cells grown in different growth modes. *Rhodopseudomonas sphaeroides* is capable of growth in a wide variety of growth modes (11), and RuBP carboxylase is normally detected when the cells are growing anaerobically in either the photoautotrophic or the photoheterotrophic growth modes (25). The characterizations of the in vitro expression of the RuBP carboxylase gene that have been presented thus far have used extracts prepared from chemoheterotrophically grown cells on a succinate minimal medium, and the cell-free protein-synthesizing reactions have been performed in the presence of air. These are culture conditions for which there is normally little or no detectable carboxylase activity in vivo; therefore, presumably the gene for the carboxylase is not being expressed due to some unknown regulatory mechanism, or alternatively, the enzyme is in an inactive state. However, we have found assembly of active enzyme in these extracts. To gain further insight into the synthesis and assembly of form II carboxylase, we prepared extracts from *Rhodopseudomonas sphaeroides* grown in several different growth modes.

Table 4 summarizes the different conditions of growth, preparation of extracts, and cell-free synthesis of the carboxylase. For these studies, cells of *Rhodopseudomonas sphaeroides* were always grown on Sistrom succinate-containing minimal medium (15). In the absence of O_2, on succinate minimal medium, low levels of RuBP carboxylase activity can be detected in vivo (14 nmol/mg per min) (23; J. Chory and S. Kaplan, unpublished data). However, the enzyme activity is not fully derepressed, unless cells are grown either on butyric acid, which is obligatorily coupled to CO_2 fixation, or in the photoautotrophic mode on CO_2 and H_2 (23).

There is a small but significant stimulation of RuBP-dependent carboxylase activity when the pLI4 DNA was used to program the various types of *Rhodopseudomonas sphaeroides* S-30 extracts (Table 4). A complication of the analysis shown here is the fact that the photoheterotrophic extracts have substantial levels of endogenous RuBP carboxylase activity: 6.2 and 6.8 nmol of CO_2 incorporated per assay, respectively. Moreover, analysis of new enzyme activity from photoheterotrophically grown cells is complicated by the fact that at least a fraction of the endogenous RuBP carboxylase activity must be due to the form I enzyme (24). Finally, the chemoheterotrophically grown, aerobically prepared S-30 extracts were compared to chemoheterotrophically grown, but anaerobically induced, S-30 extracts (Table 4). The levels of enzyme activity were comparable to those obtained from chemoheterotrophic extracts. Since the endogenous enzyme activity in photoheterotrophically grown succinate cells complicated the in vitro analysis of carboxylase expression, we did not attempt to grow cells under culture conditions in which the activity of the RuBP carboxylases would be fully derepressed, i.e., cells grown photoheterotrophically on butyrate or photoautotrophically on CO_2 and H_2. The increased levels of endogenous activity

TABLE 3. Summary of physical characterization of in vitro-synthesized form II RuBP carboxylase

Characterization	Value[a]
Optimal concn of DNA template	10 μg/ml
Optimal amt of S-30	0.4 mg of protein
Optimal Mg^{2+} concn	11 mM
No. of molecules of monomer per molecule of DNA	13.0
Minimum no. of subunits required for assembly	1.8×10^{11}
Total no. of molecules of enzyme (hexamer) synthesized	8×10^{11}
Total no. of monomeric subunits synthesized	2.6×10^{12}

[a] These values were calculated for a 100-μl coupled synthesis reaction.

TABLE 4. In vitro synthesis of RuBP carboxylase in extracts prepared from cells grown in different growth modes

Growth of cells	Preparation of extract[a]	Synthesis conditions[a]	CO_2 (nmol) incorporated per Assay with:	
			No DNA	pLI4
Chemoheterotrophic (25% O_2)	Aerobic	Aerobic	0.5	5.77
Photoheterotrophic (5% CO_2–95% N_2, moderate light)	Aerobic	Aerobic	6.20	9.04
Photoheterotrophic (5% CO_2–95% N_2, moderate light)	Anaerobic	Anaerobic	6.80	10.22
Chemoheterotrophic (25% O_2) to anaerobic (5% CO_2–95% N_2)	Anaerobic	Anaerobic	0.5	4.42

[a] Aerobic, standard atmospheric pO_2; anaerobic, extracts were prepared and syntheses were performed in a Freter anaerobic chamber (98% N_2–2% H_2).

would tend to mask the amount of activity synthesized in vitro.

DISCUSSION

The results presented here show that an important photosynthetic enzyme, RuBP carboxylase/oxygenase, can be synthesized from cloned genomic DNA in a cell-free protein-synthesizing system developed from the photosynthetic procaryote, *Rhodopseudomonas sphaeroides*. This result, determined by the assay for RuBP-dependent CO_2 fixation activity, is significant because detection of RuBP carboxylase activity depends upon formation of active carboxylase monomers, oligomerization, and activation as well as coupled RNA and protein synthesis. To our knowledge, this is the first report of in vitro assembly and activation of an RuBP carboxylase.

We have characterized in some detail the biochemical events accompanying the in vitro synthesis reaction. From the inferred amino acid sequence of the *Rhodospirillum rubrum* large-subunit polypeptide derived from the DNA sequence, the activity of purified enzyme (23), and the number of input DNA molecules used to program synthesis, we have determined that there are roughly 13 subunits synthesized per molecule of DNA. Results from Zubay (26) and Pratt (19) have shown similar results with β-galactosidase and alkaline phosphatase, respectively.

One further calculation can be made from the data in Table 3, that it takes ca. 5 min to transcribe and translate one molecule of DNA to one subunit with an M_r of 52,000. It has been shown that transcription and translation of β-galactosidase in vitro in *E. coli* extracts proceed at about one-third the in vivo rate (26), and that value is approximately what we observe here.

The difference between the kinetic curves for active RuBP carboxylase enzyme and the synthesis of RuBP carboxylase monomers suggests that a threshold concentration of RuBP carboxylase subunits is required before formation of active enzyme can proceed. A similar phenomenon has been described in whole cells of *E. coli* for the synthesis of thiogalactoside transacetylase (16), and the same observation also was made in a cell-free coupled system in *E. coli* for the maturation of alkaline phosphatase dimers (19). The most obvious step that might be concentration limited is the formation of oligomers, and we conclude that there is a requirement for at least 1.8×10^{11} subunits in a 100-μl reaction before assembly into active oligomeric forms can be detected in the in vitro system.

The most active form of the enzyme in vitro was the tetrameric-hexameric form, with greater than 70% of the total specific activity corresponding to these forms. The purified native form II enzyme from *Rhodopseudomonas sphaeroides* is largely a hexamer with an M_r of 360,000 (8). We have observed assembly of the form II RuBP carboxylase into dimers, tetramers, hexamers, and dodecamers (Table 2). Gibson and Tabita have observed that dissociating the form II enzyme by electrophoresis at pH 9.5 results in the formation of various active oligomeric forms, including dimers, tetramers, and dodecamers, of the 52,000-dalton subunit (9).

We were able to detect enzyme activity (and, therefore, assembly) in vitro when extracts were prepared from cells grown chemoheterotrophically, photoheterotrophically, or from anaerobically induced cells. Therefore, all the necessary components for transcription, translation, and assembly of an active form II RuBP carboxylase are present in cells grown in a variety of conditions. Whether the in vitro systems will be useful for studies on the regulation of photosynthetic gene expression remains to be determined.

The *Rhodopseudomonas sphaeroides* extracts contain all the factors required for assembly of the enzyme derived from *Rhodospirillum rubrum* DNA. This result points to the general utility of the *Rhodopseudomonas sphaeroides* cell-free system to perform studies of DNA-directed assembly of photosynthetic gene products from other bacterial sources. In conclusion, the potentials for this system to identify other photosynthetically important gene products or for use in studies with heterologous DNA templates is now well established. The observation that the system, prepared from cells grown in a variety of different growth modes, works well enough to study the assembly of a functional photosynthetic enzyme now makes feasible studies on the DNA-directed assembly of functional photosynthetic complexes in membranes and synthetic phospholipid structures. In very recent studies from this laboratory (J. Hoger and S. Kaplan, unpublished data; P. Kiley and S. Kaplan, unpublished results), the in vitro synthesis of reaction center polypeptides and light-harvesting polypeptides, respectively, has been demonstrated.

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