Serrano, L., & Fersht, A. R. (1989) *Nature 342*, 296-299.
Shoemaker, K., Kim, P. S., York, E. J., Stewart, J. M., & Baldwin, R. L. (1987) *Nature 326*, 563-567.
Smeaton, J. R., & Elliott, W. H. (1967) *Biochim. Biophys. Acta 145*, 547-560.
Studier, F. W., & Moffatt, B. A. (1986) *J. Mol. Biol. 189*, 113-130.
Tanford, C. (1969) *Adv. Protein Chem. 24*, 1-95.
Thomas, D. G., Russell, A. J., & Fersht, A. R. (1985) *Nature 318*, 375-376.
Warshel, A., & Russel, S. T. (1984) *Q. Rev. Biophys. 17*, 283-422.

Purification of Recombinant Ribulose-1,5-bisphosphate Carboxylase/Oxygenase Large Subunits Suitable for Reconstitution and Assembly of Active L_8S_8 Enzyme†

Bonggeun Lee and F. Robert Tabita*
Department of Microbiology and The Biotechnology Center, The Ohio State University, 484 West 12th Avenue, Columbus, Ohio 43210
Received April 18, 1990; Revised Manuscript Received July 9, 1990

ABSTRACT: Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) from *Anacystis nidulans* was reconstituted in vitro from extracts of *Escherichia coli* strains that separately express large and small subunits. This reconstitution system was shown to be useful for monitoring the appearance of dissociated or fractionated subunit preparations. Recombinant large subunits were purified to a state of homogeneity and retained reconstitution capacity in the presence of added small subunits. The purified large subunits appeared to be in the form of an octamer, probably an L_8 structure, and showed 0.15% of the carboxylase activity of the purified L_8S_8 enzyme. Purified large subunit octamers are disrupted by nondenaturing PAGE; however, the octamer is stable to electrophoresis in the presence of exogenous protein.

Ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (RubisCO)[1] is an important enzyme which catalyzes the initial reaction of the two competing metabolic pathways of photosynthetic CO_2 fixation and photorespiratory carbon oxidation (Tabita, 1988). This bifunctional enzyme catalyzes both the carboxylation and the oxygenation of RuBP. Considerable research on this enzyme has given productive information about its catalysis and structure (Andrews & Lorimer, 1987). In plants, algae, and most bacteria, RubisCO is a hexadecameric protein (L_8S_8) which is composed of eight large subunits of about 55 kDa and eight small subunits of about 13 kDa. While the large subunit bears the sites for activation and catalysis, the role of the small subunit is not fully understood despite its requirement for full enzyme activity (Andrews & Ballment, 1983). In the case of the L_8S_8 enzyme, the small subunits are attached to the top and the bottom of the large subunit octamer (Andersson et al., 1989; Chapman et al., 1988). In plants and green algae, the large and small subunits are encoded by the chloroplast and nuclear genomes, respectively. Unlike the situation in most eucaryotic organisms, the large and small subunit genes of RubisCO from the cyanobacterium *Anacystis nidulans*, which performs an oxygenic photosynthesis, are linked together and cotranscribed (Shinozaki & Sugiura, 1985). Because of the procaryotic nature of the *Anacystis* RubisCO genes, active and correctly assembled recombinant hexadecameric enzyme may be synthesized in *Escherichia coli* (Christeller et al., 1985; Gatenby et al., 1985; Tabita & Small, 1985). When the large subunit gene was separately expressed in *E. coli*, large subunits were synthesized, presumably in the form of L_8 octamers (Andrews, 1988). This crude L_8 RubisCO showed weak but measurable RubisCO activity; however, the protein was labile and resisted further attempts at purification.

In this investigation, we have shown that recombinant large and small subunits from separately expressed *rbcL* and *rbcS* genes of *A. nidulans* were active in reconstitution of RubisCO activity in vitro. Furthermore, the large subunits were purified to homogeneity in a stable L_8 form.[2]

Experimental Procedures

Bacterial Strains and Plasmids. *E. coli* strain MV1190 [Δ(*lac-pro* AB), *thi*, *sup* E, Δ(*srl-recA*)306::Tn10(tet^r)[F′*tra* D36, *pro* AB, *lac*I^{q}ZΔM15])] was used as the host strain for plasmids employed in this study. Plasmids constructed for this study were derived from plasmid pCS75 (Tabita & Small, 1985). *E. coli* MV1190 was purchased from Bio-Rad, Richmond, CA. Plasmids pTZ18R (Mead et al., 1986), pUC9 (Vieira & Messing, 1982), and pUC18 (Yanisch-Perron et al., 1985) were used as vectors for cloning.

Cell Culture and Preparation of Crude Extracts. *E. coli* plasmid-containing strains were grown for 8-9 h in Luria-Bertani medium (Maniatis et al., 1982) containing 100 μg/mL ampicillin; 5 mL of this culture was used to inoculate 1 L of Luria-Bertani medium containing 100 μg/mL ampicillin. This

† This work was supported by a grant from the National Institutes of Health (GM-24497).
* Correspondence should be addressed to this author at the Department of Microbiology, The Ohio State University.

[1] Abbreviations: RubisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; IPTG, isopropyl β-D-thiogalactopyranoside; TEM, 25 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 5 mM β-mercaptoethanol; TEMMB, 25 mM Tris-HCl, pH 8.0, 1 mM EDTA, 5 mM β-mercaptoethanol, 10 mM $MgCl_2$, and 50 mM $NaHCO_3$; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; DPM, disintegrations per minute; L, large subunit(s); S, small subunit(s).

[2] A preliminary report of this work has appeared (Tabita, 1989).

0006-2960/90/0429-9352$02.50/0

culture was grown at 37 °C with vigorous shaking, treated with 0.5 mM isopropyl β-D-thiogalactopyranoside (IPTG) at an A_{600} of about 0.4 to induce the *lac* promoter, and allowed to grow for 12–13 h prior to harvesting. Harvested cells were washed in TEM buffer (25 mM Tris-HCl, pH 8.0, 1 mM EDTA, and 5 mM β-mercaptoethanol), resuspended in TEM buffer containing 1 mM phenylmethanesulfonyl fluoride at 1 g (wet weight) of cells/mL, and then disrupted by sonicating 7 times for 20 s at 20-s intervals on ice. The extracts were centrifuged at 12000*g* for 10 min to remove cell debris and ultracentrifuged at 100000*g* for 1 h to yield a crude soluble extract.

Small Subunit Preparation for Reconstitution. The crude extract of *E. coli* MV1190(pBGL535) was precipitated by ammonium sulfate between 25% and 45% saturation. The resulting precipitate was resuspended in TEM buffer and dialyzed against this buffer overnight. This preparation was used for reconstitution studies.

Purification of Large Subunits of RubisCO. Using antibodies to the recombinant holoenzyme and rocket immunoelectrophoresis (Jonanneau & Tabita, 1986), it was determined that the large subunits represented about 6% of the total soluble protein of induced *E. coli* MV1190(pBGL520). This crude extract was loaded onto a green A–agarose column (Jouanneau & Tabita, 1986) equilibrated with TEMMB buffer (25 mM Tris-HCl, pH 8.0, 1 mM EDTA, 5 mM β-mercaptoethanol, 10 mM $MgCl_2$, and 50 mM $NaHCO_3$). A gradient of 0–1 M NaCl in TEMMB buffer was used to elute the bound large subunits. Fractions containing large subunits were detected by carboxylase assay after adding small subunits from *E. coli* MV1190(pBGL535) to reconstitute the activity. Those fractions capable of reconstitution were pooled and precipitated by adding ammonium sulfate to 70% saturation. The precipitate was resuspended and dialyzed against TEM buffer. The dialyzed material was then loaded onto a 0.2–0.8 M sucrose step gradient (Tabita & Small, 1985); fractions of 1 mL were collected, and portions were assayed for carboxylase activity after adding recombinant small subunits. Fractions from the sucrose gradient that were active in the reconstitution assay were subsequently loaded onto a Pharmacia (Piscataway, NJ) Mono Q column equilibrated with 20 mM Tris-HCl, pH 8.0. A gradient of 0–1 M NaCl in 20 mM Tris-HCl (pH 8.0) was passed through the column, and fractions were collected with a Pharmacia FPLC apparatus. In every purification step, the purity of the large subunit preparation was monitored by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) in 0.75-mm 14% polyacrylamide slab gels (Laemmli, 1970). If necessary, one more step of Mono Q column chromatography was performed to obtain a homogeneous preparation. Finally, the purified fractions were desalted by using an Amicon concentrator (Danvers, MA) and stored in aliquots at −70 °C after addition of glycerol to 20% (v/v).

RubisCO Assay. The RuBP-dependent incorporation of $^{14}CO_2$ into acid-stable 3-phosphoglyceric acid was employed according to previously reported procedures (Whitman & Tabita, 1976). Protein concentration was determined by the Lowry method (Lowry et al., 1951). Specific activity was expressed as nanomoles of CO_2 fixed per minute per milligram of protein in crude extracts or nanomoles of CO_2 fixed per minute per milligram of purified holoenzyme, or purified large subunit. When recombinant large or small subunit preparations were added in the reconstitution assay, the mixture was incubated at 30 °C for 5 min prior to activating the enzyme.

Results

Plasmid Constructs. The subclones were constructed from plasmid pCS75 (Tabita & Small, 1985) which had previously been shown to contain the *rbcL* and *rbcS* genes behind the *lac* promoter (Figure 1). Plasmid pCS75 was subsequently found to contain an extra 0.24 kilobase pair (kbp) *Pst*I fragment. The 2.2 kbp fragment, which codes for the large and the small subunit of RubisCO of *A. nidulans*, was liberated from pCS75 by digestion with *Pst*I and subsequently cloned into pTZ18R. The 1.5 kbp fragment containing the large subunit gene was liberated by partial digestion with *Hin*dIII and cloned into pUC18. The 0.7 kbp *Hin*dIII/*Pst*I fragment containing the small subunit gene was cloned into pUC9. In all plasmid constructs, neither the large nor the small subunit gene was found in the same reading frame as the β-galactosidase gene so that the expressed gene products are considered to be nonfusion wild-type proteins.

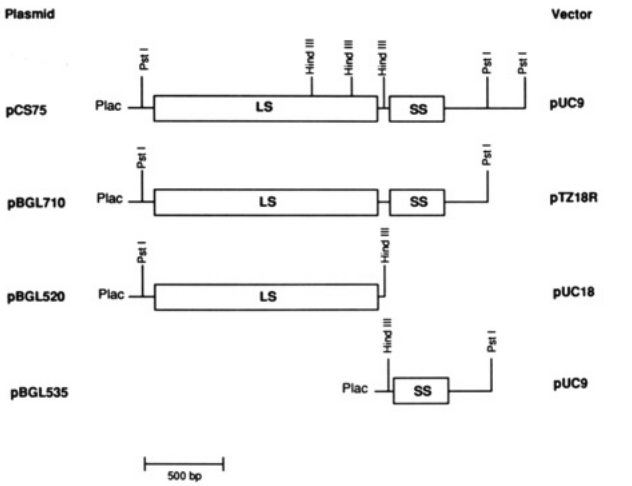

FIGURE 1: Plasmid constructions used in this study. All plasmids were derived from plasmid pCS75 (Tabita & Small, 1985).

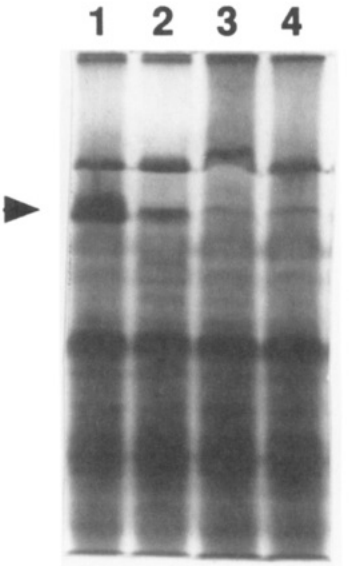

FIGURE 2: Nondenaturing polyacrylamide slab gel electrophoresis of *E. coli* crude extracts. Polyacrylamide slab gel electrophoresis was performed in 5% acylamide gels with about 200 μg of each crude extract using the Laemmli (1970) gel system in the absence of SDS. Crude extract was obtained from *E. coli* MV1190(pBGL710) (lane 1), *E. coli* MV1190(pBGL520) (lane 2), *E. coli* MV1190(pBGL535) (lane 3), and *E. coli* MV1190(pTZ18R) (lane 4). The arrow points to the position of the recombinant L_8S_8 or L_8 RubisCO.

Reconstitution of RubisCO Activity from E. coli Extracts. Crude extracts were prepared from plasmid containing *E. coli* strains, and nondenaturing PAGE was performed with each crude extract (Figure 2). The large subunits from crude extracts of *E. coli* MV1190(pBGL520) assembled to a position

Table I: Reconstitution of RubisCO Activity in Crude Extracts of *Escherichia coli*

crude extract source	nmol of CO_2 fixed min^{-1} (mg of protein)$^{-1}$
E. coli MV1190(pTZ18R)	0.0
E. coli MV1190(pBGL520)	0.8
E. coli MV1190(pBGL535)	0.0
E. coli MV1190(pBGL520) + *E. coli* MV1190(pBGL535)[a]	20.0
E. coli MV1190(pBGL710)	320.0

[a] Equal amounts of protein from each crude extract were mixed; no attempt was made to use saturating amounts of small subunits in this initial attempt at reconsitution.

Table II: Relative Activities of Recombinant RubisCO Preparations

enzyme source[a]	specific activity
purified L_8S_8	2470.0[b]
purified L_8 + crude S	310.0[c]
purified L_8	3.6[c]

[a] The L_8S_8 and L_8 *A. nidulans* RubisCO proteins were purified as described under Experimental Procedures. The small subunits were prepared as described under Experimental Procedures and were supplied at saturating concentrations. [b] Specific activity is expressed as nanomoles of CO_2 fixed per minute per milligram of enzyme. [c] Specific activity is expressed as nanomoles of CO_2 fixed per minute per milligram of L.

that approximated that of the holoenzyme in this gel system (Figure 2). Carboxylase activity was determined in each crude extract, and a trace amount of carboxylase activity was found in the *E. coli* MV1190(pBGL520) crude extract, but no activity was detected in *E. coli* MV1190(pBGL535) (Table I). When equal amounts of protein from *E. coli* MV1190(pBGL520) and *E. coli* MV1190(pBGL535) were mixed, considerable carboxylase activity was detected (Table I). Extracts from cells not containing small subunits did not stimulate activity. These results indicated that the active holoenzyme was reconstituted from the separately expressed recombinant large and small subunits. This reconstitution system was also employed to localize large or small subunits on sucrose gradients of crude extracts obtained from *E. coli* MV1190(pBGL520) or *E. coli* MV1190(pBGL535), respectively (Figure 3). Compared to the sucrose gradient profile of the L_8S_8 holoenzyme, large subunits were slightly displaced toward the top of the gradient, consistent with the expected position of octamers of large subunits (van der Vies et al., 1986). Small subunits were detected as a broad peak at the upper part of the sucrose gradient and were readily apparent in these fractions after SDS–PAGE.

Purification of Recombinant Large Subunits. Large subunits were purified from *E. coli* MV1190(pBGL520) crude extracts. Crude extracts were fractionated by green A–agarose column chromatography, sucrose density gradient fractionation, and FPLC column chromatography using a Mono-Q column (Figure 4). A single A_{280} peak from the FPLC column contained purified large subunits as shown by SDS–PAGE (Figure 5). However, on nondenaturing gels, the purified large subunits did not exhibit a distinct band like the one observed in *E. coli* MV1190(pBGL520) crude extracts, without the addition of either bovine serum albumin or *E. coli* crude extract (Figure 5). In the absence of exogenous protein, dissociation of the L_8 protein was observed from purified large subunit preparations, which appeared as a diffuse area at the lower part of a nondenaturing gel (Figure 5B, lane 10). It is not clear, at this time, what causes the apparent dissociation of the large subunits during electrophoresis in the absence of added protein.

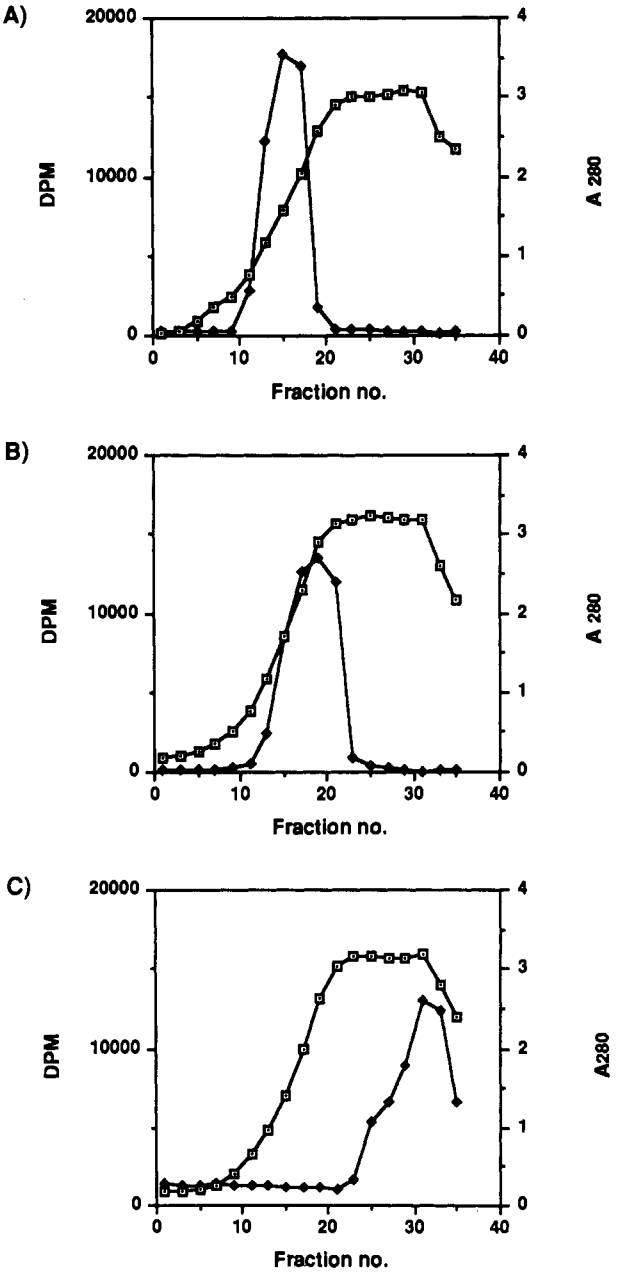

FIGURE 3: Localization of the large and the small subunits after sucrose gradient fractionation of *E. coli* crude extract containing recombinant large or small subunits. (A) 22.5 mg of *E. coli* MV1190(pBGL710) was loaded onto a 0.2–0.8 M sucrose step gradient. Fractions of 1 mL were collected, and samples were assayed for RubisCO activity. (B) 25 mg of *E. coli* MV1190(pBGL520) was loaded onto a sucrose gradient, and samples of each 1-mL fraction were assayed in the presence of 20 μL of *E. coli* MV1190(pBGL535) crude extract (43.0 mg/mL). (C) 25 mg of *E. coli* MV1190(pBGL535) crude extract was loaded onto a sucrose gradient, and samples of each 1-mL fraction were assayed for RubisCO activity in the presence of 20 μL of *E. coli* MV1190(pBGL520) crude extract (31.0 mg/mL). RubisCO activity was expressed as dpm of $^{14}CO_2$ fixed (♦); (⊡) absorbance at 280 nm.

When increasing amounts of crude small subunit preparations were added to purified large subunits, a saturation effect on RubisCO activity was noted (Figure 6). The L_8S_8 holoenzyme was purified from *E. coli* MV1190(pBGL710), by using the same procedure as that employed for large subunits, to a specific activity of 2470 nmol of CO_2 fixed min^{-1} (mg of enzyme)$^{-1}$ (Table II). By contrast, the specific activity of purified large subunits was only 3.6 nmol min^{-1} (mg of large subunit)$^{-1}$, but increased to 310 nmol min^{-1} (mg of large subunit)$^{-1}$ in the presence of saturating amounts of crude

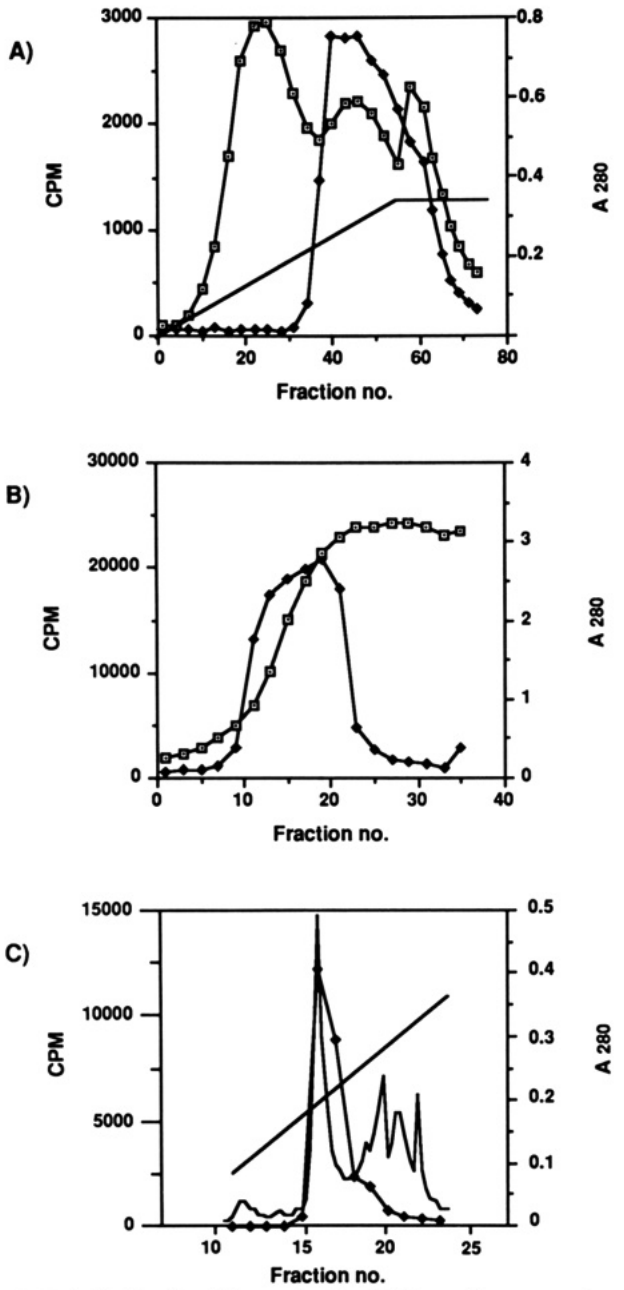

FIGURE 4: Purification of large subunits. (A) Green A–agarose column chromatography of *E. coli* MV1190(pBGL520) crude extract: crude extract from a 4-L culture of *E. coli* MV1190(pBGL520) was loaded onto a green A–agarose column. A 300-mL gradient of 0–1 M NaCl in TEMMB buffer was passed through the column. Fractions of 5.5 mL were collected, and portions of each fraction were assayed for RubisCO activity. (B) Sucrose gradient fractionation of green A fractions containing large subunits; samples of each 1-mL fraction were assayed for RubisCO activity. (C) FPLC Mono Q column chromatography of sucrose gradient fractions containing large subunits; samples of each 1-mL fraction were assayed for RubisCO activity. In all cases, assays were performed in the presence of 10 or 20 μL of *E. coli* MV1190(pBGL535) crude extract (37.3 mg/mL) for 10 min. RubisCO activity was expressed as cpm of $^{14}CO_2$ fixed (♦); (⊡) absorbance at 280 nm in (A) and (B); (—) absorbance at 280 nm in (C). The diagonal lines in (A) and (C) represent gradients from 0–1 and 0.25–0.58 M NaCl, respectively, used to elute large subunits.

recombinant small subunits and ranged from 200 to 400 nmol min^{-1} (mg of large subunit)$^{-1}$ with different preparations. Thus, the reconstituted RubisCO activity was not as great as that obtained with the native recombinant enzyme. Either the purified large subunits were not as functional as the large subunits of the octameric core of the L_8S_8 holoenzyme or the

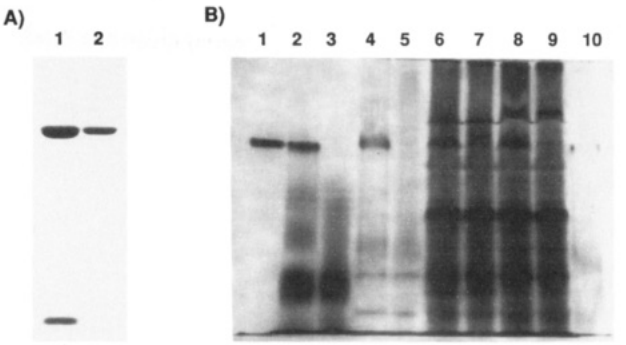

FIGURE 5: Gel electrophoresis of purified large subunits. (A) SDS–PAGE using 14% acrylamide gels of (lane 1) purified holoenzyme and (lane 2) purified large subunit. (B) PAGE, using 5% acrylamide gels, of purified large subunits in the absence of SDS: lane 1, purified holoenzyme; lane 2, 8.4 μg of purified large subunits and 200 μg of bovine serum albumin; lane 3, 200 μg of bovine serum albumin; lane 4, 8.4 μg of purified large subunits and 200 μg of boiled *E. coli* MV1190(pTZ18R) crude extract; lane 5, 200 μg of boiled *E. coli* MV1190(pTZ18R) crude extract; lane 6, 8.4 μg of purified large subunits and 200 μg of *E. coli* MV1190(pTZ18R) crude extract; lane 7, 200 μg of *E. coli* MV1190(pTZ18R) crude extract; lane 8, 8.4 μg of purified large subunits and 200 μg of *E. coli* MV1190-(pBGL535) crude extract; lane 9, 200 μg of *E. coli* MV1190-(pBGL535) crude extract; lane 10, 8.4 μg of purified large subunit.

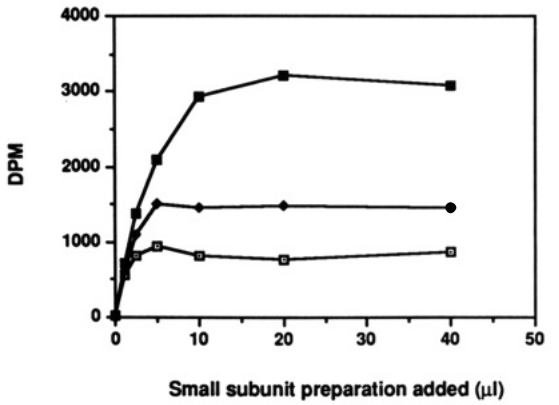

FIGURE 6: Carboxylase activity of purified large subunits in the presence of various amounts of small subunits. Carboxylase activity was measured after various amounts of *E. coli* MV1190(pBGL535) small subunit preparation (37.4 mg/mL) were added to a fixed amount of purified large subunits: (⊡) 1.7 μg of large subunits; (♦) 3.5 μg of large subunits; (■) 7 μg of large subunits; RubisCO activity was expressed in terms of the dpm of $^{14}CO_2$ fixed.

crude small subunit preparation introduced an additional complicating factor. Thus far, we have been unsuccessful in attempts to purify the recombinant small subunits (Lee and Tabita, unpublished results), and attempts to obtain purified small subunits from the *A. nidulans* L_8S_8 holoenzyme by the mild acid separation procedure (Andrews & Ballment, 1983) have not succeeded (Rai and Tabita, unpublished results). Yet this procedure was successful in our hands for separating small subunits from acid-treated RubisCO from *Agmenellum quadruplicatum* strain PR-6, an organism very similar to that previously employed for the in vitro isolation of small subunits (Andrews & Ballment, 1983). Despite the lower specific activity in the reconstitution assay, the purified large subunits obviously retained the capacity of reconstituting RubisCO activity by binding to small subunits. In the absence of small subunits, purified large subunits exhibited weak carboxylase activity, equivalent to about 0.15% of the activity of the L_8S_8 holoenzyme. The activity of the purified large subunits showed a linear dependence on the amount of protein and the reaction time (Figure 7). This activity was inhibited by the addition of the transition-state analogue 2-carboxyarabinitol 1,5-bis-

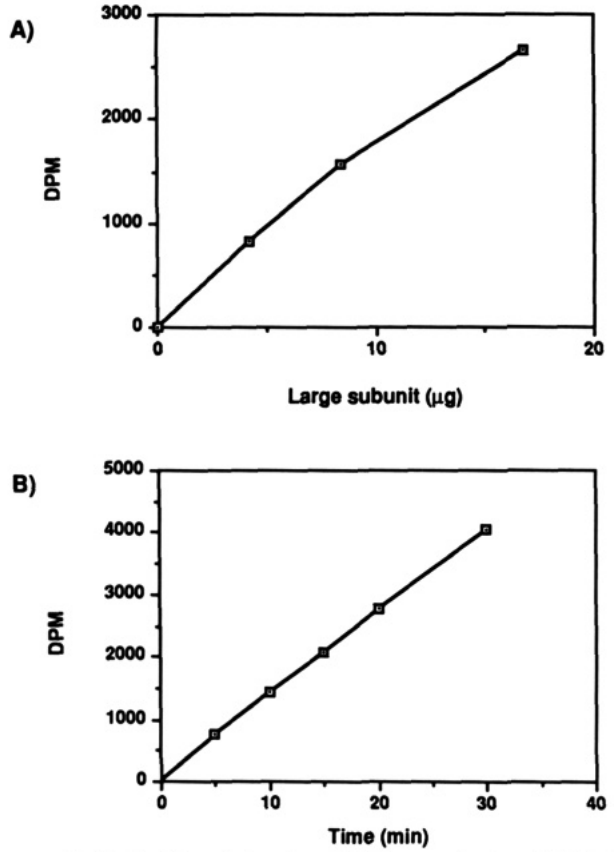

FIGURE 7: RubisCO activity of purified large subunits. (A) Carboxylase activity was measured for 10 min with increasing amounts of purified large subunit. (B) Carboxylase activity was measured with 8.4 μg of purified large subunits at various reaction times. All assays represent RuBP-dependent fixation into acid-stable product. The dpm of $^{14}CO_2$ fixed is plotted on the *y* axis.

phosphate. To measure this trace RubisCO activity, high specific radioactivity $NaH^{14}CO_3$ (152.5 Bq/nmol) was used, analogous to a recent report which reported that crude L_8 preparations contained 0.5% of the activity of the native L_8S_8 enzyme (Andrews, 1988).

When compared to purified L_8S_8 holoenzyme, the purified large subunit preparation migrated through a Superose-6 column at a position consistent with an L_8 structure (Figure 8). Unexpectedly, a second A_{280}-absorbing peak was noted in the elution profile of the purified large subunit preparation and to some extent in the elution profile of the recombinant L_8S_8 enzyme. However, this second peak was found not to contain protein, and spectrophotometric scanning of this material showed a maximum absorption at 256 nm, indicating that it may be some form of nucleotide. Only the first A_{280} peak of the large subunit preparation showed RubisCO activity in the presence of added small subunits, and only fractions from the first peak contained large subunit polypeptides, as noted by SDS–PAGE (Figure 8). When the purified holoenzyme and purified large subunit octamers were scanned in the ultraviolet, they showed a somewhat similar absorbance profile (Figure 9).

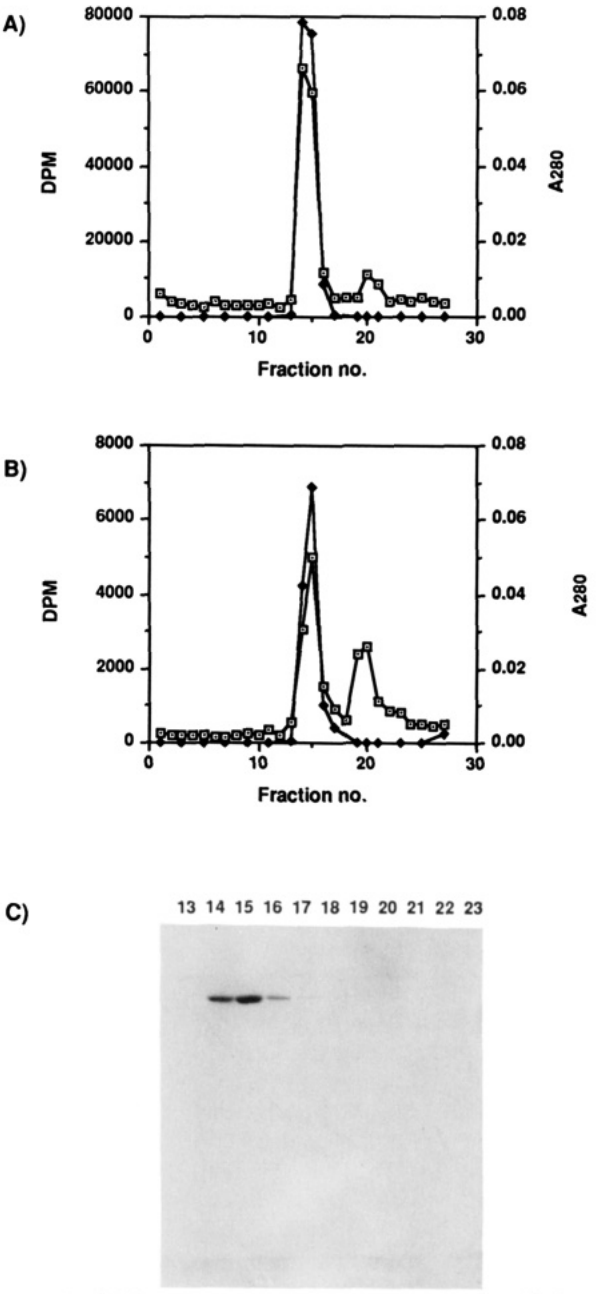

FIGURE 8: Gel filtration column chromatography of purified holoenzyme and purified large subunits. (A) 110 μg of purified holoenzyme was loaded onto a Pharmacia FPLC Superose-6 column and eluted with 25 mM Tris-HCl (pH 8.0) containing 0.1 M NaCl. Fractions of 1 mL were collected, and portions of each fraction were taken for carboxylase assay. (B) 84 μg of purified large subunits was chromatographed on the same column, and fractions of 1 mL were collected; portions of each fraction were taken for carboxylase assay in the presence of 20 μL of *E. coli* MV1190(pBGL 535) crude extract (43.0 mg/mL). RubisCO activity was expressed in terms of the dpm of $^{14}CO_2$ fixed (♦); (⊡) absorbance at 280 nm. (C) SDS–PAGE, using 14% acrylamide gels, was performed on fractions 13–23 of the column in (B).

Discussion

In this investigation, we have demonstrated that RubisCO activity may be reconstituted by using extracts prepared from *E. coli* strains that separately express large and small subunits. Using these parparations, it was convenient to localize large and small subunits on sucrose gradients or gel filtration columns, particularly in situations where the holoenzyme dissociates, as recently observed for various mutant forms of RubisCO (Lee and Tabita, unpublished results).

In crude extracts of *E. coli* MV1190(pBGL520), a distinct band was observed which migrated to a similar position as the L_8S_8 holoenzyme on nondenaturing gels. However, purified large subunits which eluted from gel filtration columns at a position consistent with an octameric structure (L_8) failed to exhibit a distinct band on nondenaturing gels and showed evidence of dissociation. The fact that the addition of exogenous bovine serum albumin or crude *E. coli* extract was capable of stabilizing the structure of L_8 octamers on

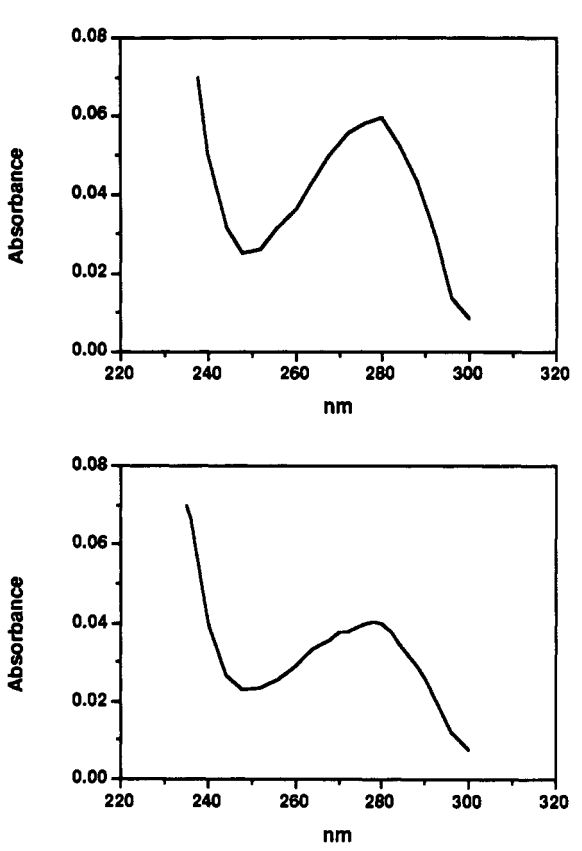

FIGURE 9: Ultraviolet absorption spectrum of purified holoenzyme and purified large subunit. (A) Holoenzyme from fraction 14 of Figure 8A. (B) Large subunit from fraction 15 of Figure 8B.

nondenaturing gels suggests that interactions with heterologous proteins might be important to maintain the large subunits in the L_8 structure. Perhaps, small subunit binding sites on the large subunits, when exposed to solvent, contribute to the loose association of the large subunits under the conditions of electrophoresis. Under normal conditions, both subunit genes are cotranscribed in procaryotes, and the L_8S_8 enzyme is assembled immediately into a stable holoenzyme (Tabita, 1988). Thus, the effects we have observed here with the purified large subunits undoubtedly reflect the ability of small subunits to maintain the enzyme in its most stable conformation.

Various large subunit preparations, which retain the capacity to reconstitute with small subunits, have been obtained after stripping small subunits from large subunit octamers under rather gentle procedures (Andrews & Ballment, 1983; Asami et al., 1983; Incharoensakdi et al., 1985; Jordan & Chollet, 1985). However, all of these preparations appeared to retain residual amounts of small subunits. Andrews (1988) was able to show that recombinant large subunits prepared from extracts of *E. coli* catalyzed weak RubisCO activity, conclusively demonstrating that small subunits are not absolutely required for activity. In this earlier study, however, the large subunits were unstable, perhaps because of poor expression, and were not successfully purified. The procedures described in the current investigation, using *E. coli* cells that express over an order of magnitude more large subunits than obtained by Andrews (1988), result in stable and homogeneous preparations. This preparation will be employed in future investigations of the catalytic capacity of the L_8 core and should prove invaluable for investigations of the role of the *GroEL* and *GroES* chaperonin proteins (Goloubinoff et al., 1989a,b) in mediating assembly of the L_8 core. Recently, the structure of the L_8S_8 enzyme from plants has been described, and various residues on the large subunit which appear to be important for assembly with small subunits have been identified (Knight et al., 1989). The X-ray structure of L_8, in the complete absence of small subunits, as well as site-directed mutagenesis studies, should provide information relative to the conformation required for assembly and give insights to molecular changes that occur when small subunits interact with the L_8 core.

ACKNOWLEDGMENTS

We thank Dr. John Pierce for the gift of 2-carboxyarabinitol 1,5-bisphosphate.

Registry No. RubisCo, 9027-23-0.

REFERENCES

Andersson, I., Knight, S., Schneider, G., Lindqvist, Y., Lundqvist, T., Brändén, C. I., & Lorimer, G. H. (1989) *Nature 337*, 229–234.

Andrews, T. J. (1988) *J. Biol. Chem. 263*, 12213–12219.

Andrews, T. J., & Ballment, B. (1983) *J. Biol. Chem. 258*, 7514–7518.

Andrews, T. J., & Lorimer, G. H. (1987) *Biochem. Plants 10*, 131–218.

Asami, S., Takabe, T., Akazawa, T., & Codd, G. A. (1983) *Arch. Biochem. Biophys. 225*, 713–721.

Chapman, M. S., Suh, S. W., Carmi, P. M. G., Cascio, D., Smith, W. W., & Eisenberg, D. S. (1988) *Science 241*, 71–74.

Christeller, J. T., Terzaghi, B. E., Hill, D. F., & Laing, W. A. (1985) *Plant Mol. Biol. 5*, 257–263.

Gatenby, A. A., van der Vies, S. M., & Bradley, D. (1985) *Nature 314*, 617–620.

Goloubinoff, P., Gatenby, A. A., & Lorimer, G. H. (1989a) *Nature 337*, 44–47.

Goloubinoff, P., Christeller, J. T., Gatenby, A. A., & Lorimer, G. H. (1989b) *Nature 342*, 884–888.

Incharoensakdi, A., Takabe, T., & Akazawa, T. (1985) *Arch. Biochem. Biophys. 237*, 445–453.

Jordan, D. B., & Chollet, R. (1985) *Arch. Biochem. Biophys. 236*, 487–496.

Jouanneau, Y., & Tabita, F. R. (1986) *J. Bacteriol. 165*, 620–624.

Knight, S., Andersson, I., & Brändén, C. I. (1989) *Science 244*, 702–705.

Laemmli, U. K. (1970) *Nature 227*, 680–685.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, p 440, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Mead, D. A., Szczesna-Skorupa, E., & Kemper, B. (1986) *Protein Eng. 1*, 67–74.

Shinozaki, K., & Sugiura, M. (1985) *Mol. Gen. Genet. 200*, 27–32.

Tabita, F. R. (1988) *Microbiol. Rev. 52*, 155–189.

Tabita, F. R. (1989) *Microb. Growth C_1 Compd., Proc. Int. Symp., 6th*, V5–3.

Tabita, F. R., & Small, C. L. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 6100–6103.

van der Vies, S. M., Bradly, D., & Gatenby, A. A. (1986) *EMBO J. 5*, 2439–2444.

Vieira, J., & Messing, J. (1982) *Gene 19*, 259–268.

Whitman, W., & Tabita, F. R. (1976) *Biochem. Biophys. Res. Commun. 71*, 1034–1039.

Yanisch-Perron, C., Vieira, J., & Messing, J. (1985) *Gene 33*, 103–119.