Purification and Characterization of the Chaperonin 10 and Chaperonin 60 Proteins from *Rhodobacter sphaeroides*†

Katherine C. Terlesky and F. Robert Tabita*

Department of Microbiology and The Biotechnology Center, The Ohio State University, 484 West 12th Avenue, Columbus, Ohio 43210

Received April 12, 1991; Revised Manuscript Received June 13, 1991

ABSTRACT: Two heat-shock proteins that show high identity with the *Escherichia coli* chaperonin 60 (groEL) and chaperonin 10 (groES) chaperonin proteins were purified and characterized from photolithoautotrophically grown *Rhodobacter sphaeroides*. The proteins were purified by using sucrose density gradient centrifugation and Mono-Q anion-exchange chromatography. In the presence of 1 mM ATP, the chaperonin 10 and chaperonin 60 proteins bound to each other and comigrated as a large complex during sucrose density gradient centrifugation. The native molecular weights of each protein as determined by gel filtration chromatography were 889 200 for chaperonin 60 and 60 000 for chaperonin 10. Chaperonin 60 is comprised of monomers with a molecular weight of 61 000 and chaperonin 10 is comprised of monomers with a molecular weight of 12 700 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Chaperonin 60 was 9.3% of the total soluble cell protein during photolithoautotrophic growth which increased to 28.5% following heat-shock treatment. When cells were grown photoheterotrophically or chemoheterotrophically, chaperonin 60 was reduced to 6.7% and 3.5%, respectively, of the total soluble protein. The N-terminal amino acid sequence of each protein was determined; chaperonin 60 of *R. sphaeroides* showed 72% identity to *E. coli* chaperonin 60 protein, and *R. sphaeroides* chaperonin 10 showed 45% identity with *E. coli* chaperonin 10. *R. sphaeroides* chaperonin 60 catalyzed ATP hydrolysis with a specific activity of 134 nmol min^{-1} mg^{-1} (k_{cat} = 0.13 s^{-1}) and was inhibited by *R. sphaeroides* chaperonin 10, but not *E. coli* chaperonin 10. The *E. coli* chaperonin 60 ATPase activity was inhibited by chaperonin 10 from both *R. sphaeroides* and *E. coli*.

The groEL and groES proteins are oligomeric proteins of the heat-shock regulon of *Escherichia coli* and facilitate the correct posttranslational assembly of oligomeric proteins. The groEL protein is a tetradecameric protein consisting of two stacked rings with seven subunits (M_r ~60 000) in each ring. Related proteins in prokaryotes and eukaryotes with high N-terminal amino acid identity are termed chaperonin 60 proteins (cpn60).[1] The native groES protein (M_r ~80 000) is a single ring of seven subunits (M_r ~10 000) and binds to cpn60 in the presence of Mg/ATP. The related group of proteins found in prokaryotes and mitochondria are designated cpn10 proteins.

Rhodobacter sphaeroides is a purple non-sulfur photosynthetic bacterium that can respond to the availability of macronutrients and energy sources in its environment using a diversity of complex metabolic processes. The photosynthetic pigments and proteins are regulated in response to light and the levels of oxygen; the organism can also repress the synthesis of proteins required for photosynthesis and grow chemoheterotrophically under aerobic conditions. *R. sphaeroides* is also capable of nitrogenase synthesis under conditions where the availability of fixed nitrogen is reduced, and carbon may be obtained from organic sources or fixed from CO_2 via the Calvin reductive pentose phosphate cycle (Tabita, 1988). Both processes require the synthesis of new proteins, many of which are complex oligomeric enzymes.

The first and key enzyme of the Calvin cycle is ribulose 1,5-bisphosphate(RuBP) carboxylase/oxygenase (RubisCO), which catalyzes the incorporation of CO_2 into the ene-diol of RuBP to yield two molecules of 3-phosphoglyceric acid; alternatively, the ene-diol may be attacked by O_2 to yield one molecule of 3-phosphoglyceric acid and one molecule of phosphoglycolic acid. *R. sphaeroides* is one of only a few photosynthetic bacteria that contain two forms of RubisCO (Gibson & Tabita, 1977). It contains the L_8S_8 (form I) oligomeric form composed of eight large subunits and eight small subunits, and a simpler L_x form (form II) composed of only large subunits that differ significantly in primary structure to form I large subunits (Gibson & Tabita, 1985). The genes encoding both forms of RubisCO have been isolated, expressed in *E. coli* (Gibson & Tabita, 1986; Muller et al., 1985; Quivey & Tabita, 1984), and sequenced (Wagner et al., 1988; Gibson et al., 1991). Recently, it was demonstrated that *E. coli* cpn60 and cpn10 promote in vivo assembly and/or folding of recombinant *Rhodospirillum rubrum* L_2 RubisCO and *Anacystis nidulans* L_8S_8 RubisCO (Goloubinoff et al., 1989a), and are required for reassembly of denatured *Rhodospirillum rubrum* L_2 RubisCO into active enzyme in vitro (Goloubinoff et al., 1989b).

RubisCO's from higher plants which are similar to the form I L_8S_8 are not active or assembled when cloned into *E. coli* (Gatenby & Ellis, 1990). This could indicate a requirement for a plant RubisCO "assembling protein"; indeed, a RubisCO "binding protein" was described in pea that binds to newly

† This work was supported by a grant from the National Institutes of Health (GM-24497) to F.R.T. and by a postdoctoral fellowship to K.C.T. from the Graduate School of The Ohio State University and the National Science Foundation Plant Biology Program (DMB-900837).

* Correspondence should be addressed to this author at the Department of Microbiology, The Ohio State University.

[1] Abbreviations: cpn60, chaperonin 60 (previously designated groEL); cpn10, chaperonin 10 (previously designated groES); RuBP, ribulose 1,5-bisphosphate; RubisCO, RuBP carboxylase/oxygenase; LB, Luria broth; IPTG, isopropyl β-D-thiogalactopyranoside; TES, *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonate; FPLC, fast protein liquid chromatography; SDS, sodium dodecyl sulfate; SDS-PAGE, SDS-polyacrylamide gel electrophoresis.

0006-2960/91/0430-8181$02.50/0 © 1991 American Chemical Society

synthesized large subunits (Barraclough & Ellis, 1980; Roy et al., 1982). This protein is an evolutionary homologue to the cpn60 of *E. coli* and has been shown to possess significant homology to other chaperonins from various prokaryotes and eukaryotes. A groEL-type protein (cpn60) that has 15 of 19 similar N-terminal amino acids to *E. coli* cpn60 was recently identified in *R. sphaeroides* though no physiological or catalytic properties of the protein were described (Watson et al., 1990).

R. sphaeroides, with its two forms of RubisCO, is an ideal organism in which to study RubisCO assembly. Moreover, RubisCO synthesis is highly regulated (Tabita, 1988), so the factors that promote its synthesis may also regulate the levels of chaperonin proteins required for the assembly of the high intracellular levels of RubisCO which may be produced. This organism also provides a system in which chaperonin synthesis may be regulated by factors other than heat and stress. This investigation describes the characterization of the cpn10 and cpn60 proteins from *R. sphaeroides* and compares the activity and properties of the chaperonin-associated ATPase activity with similar proteins from *E. coli*.[2]

Experimental Procedures

Bacterial Strains and Plasmids. *R. sphaeroides* strain HR is a streptomycin-resistant derivative of strain ATCC 17023, capable of growth at 42 °C (Weaver & Tabita, 1983). *E. coli* strain MC 1061 was the host strain harboring the pGroESL plasmid which was a gift from Drs. A. Gatenby and G. Lorimer.

Medium and Growth Conditions. For photolithoautotrophic growth, *R. sphaeroides* strain HR was grown at 30 °C in mineral salts medium containing no added carbon source. The medium was supplemented with 10 μM $NiCl_2$ and bubbled with 1.5% CO_2 in H_2 as described previously (Jouanneau & Tabita, 1986). For heat-shocked cultures, the culture flasks were maintained at 40 °C for at least 12 h prior to being harvested. For photoheterotrophic growth, the mineral medium was supplemented with 0.2% malate and 0.1% $NaHCO_3$, and cultures were grown in screw-capped tubes as described previously (Gibson & Tabita, 1977). *R. sphaeroides* was also cultured chemoheterotrophically under aerobic conditions in mineral medium containing 0.2% malate. All cultures were harvested in mid-exponential phase. *E. coli* strain MC 1061 (pGroESL) was cultured in Luria broth (LB) (Maniatis et al., 1982) containing chloramphenicol (20 μg/mL) and 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) at 37 °C for non-heat-shocked cultures, and at 42 °C for heat-shocked cultures.

Preparation of Cell Extracts. Cultures were harvested at 6000*g* and resuspended with breakage buffer to 5% of the original culture volume. Breakage buffer consisted of 50 mM *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonate buffer (TES) (pH 7.0) containing 10 mM $MgCl_2$ and 0.02 mg of DNase I (Sigma Chemical Co., St. Louis, MO) per milliliter. Cells were lysed by passage through a French pressure cell twice at 20000 lb/in.^{2}. Whole cells and cell debris were removed by centrifugation at 6000*g* for 10 min.

Purification of Chaperonins. Cpn60 was initially identified by immunoblotting of *R. sphaeroides* extracts probed with antibodies to purified cpn60 from *E. coli*. The M_r 61 000 protein that reacted with the antibody was detected throughout the purification steps by SDS-polyacrylamide gel electrophoresis and immunoblotting. The cpn10 protein was identified as an approximate M_r 10 000 protein that had altered migration on sucrose gradients in the presence of 5 mM ATP. Positive identification of each protein was confirmed by N-terminal amino acid analysis.

Chaperonin proteins from *E. coli* and *R. sphaeroides* were purified in an identical manner with the exception that the *E. coli* proteins were not subjected to green A-agarose chromatography. The first step in the purification was sucrose density gradient centrifugation in the presence of 5 mM ATP. Stepwise sucrose gradients were prepared in 40-mL polyallomer centrifuge tubes. All sucrose solutions were prepared in breakage buffer containing 5 mM ATP. The gradient contained the following sucrose layers: 9.0 mL of 0.2 M sucrose, 8.5 mL of 0.4 M sucrose, 8.5 mL of 0.6 M sucrose, and 9.0 mL of 0.8 M sucrose. ATP was added to cell extracts to a final concentration of 5 mM and incubated for 30–60 min at 4 °C. The extract was layered over the gradients and centrifuged in a Beckman SW28 swinging-bucket rotor (20 h; 25 000 rpm; 4 °C). Fractions were collected, and the distribution of proteins in each fraction was examined by SDS-polyacrylamide gel electrophoresis. Fractions containing the high molecular weight cpn60–cpn10 complex were pooled. To remove any contaminating RubisCO enzyme, green A-agarose chromatography was performed. The column (10-mL bed volume) was equilibrated with buffer A [buffer A consisted of 50 mM TES (pH 7.0), 10 mM $MgCl_2$, and 10% ethylene glycol], and the sucrose gradient cpn60–cpn10 pool was loaded onto the column. The column was washed with 80 mL of buffer A and the effluent collected. Any RubisCO present in the sucrose gradient fractions remained bound to the green A matrix (Jouanneau & Tabita, 1987). The proteins in the effluent were then fractionated by fast protein liquid chromatography (FPLC) using a Model GP-250 gradient programmer (Pharmacia LKB Biotechnology Inc.) and a Mono-Q HR 10/10 anion-exchange column. The column was developed with a linear gradient of 0.0–1.0 M KCl in buffer A. Fractions were collected and analyzed for protein content by SDS-polyacrylamide gel electrophoresis. Pure cpn10 and cpn60 proteins were usually obtained at this point. However, in some preparations, a Superose-6 gel filtration column was used as a final purification step.

Molecular Weight Determination. The native molecular weight of the *R. sphaeroides* cpn60 was determined by Superose-6 gel filtration chromatography, and the molecular weight of cpn10 was determined by Superose-12 chromatography. Molecular weight standards (Bio-Rad Laboratories, Richmond, CA) included bovine thyroglobulin (670000), bovine γ-globulin (158000), chicken ovalbumin (44000), horse myoglobin (17000), and cyanocobalamin (1350).

The subunit molecular weight of each protein was determined by SDS-polyacrylamide gel electrophoresis. Phosphorylase *b* (97400), bovine serum albumin (66200), ovalbumin (42699), carbonic anhydrase (31000), soybean trypsin inhibitor (21500), and lysozyme (14400) were used as the molecular weight standards (Bio-Rad). In addition, carbonic anhydrase (29000), trypsin inhibitor (20400), lysozyme (14400), aprotinin (6100), and insulin chain B (3500) were used for the molecular weight determination of the cpn10 protein (Integrated Separation Systems, Hyde Park, MA).

N-Terminal Amino Acid Analysis. All samples used in the N-terminal amino acid analysis were electrophoresed in an SDS-polyacrylamide gel and electroblotted onto an Immobilon P membrane (Millipore Corp., Bedford, MA). The N-terminal sequence of each chaperonin protein was determined at the Ohio State University Biochemistry Instrument Center using an Applied Biosystems (Foster City, CA) 470A protein se-

[2] A preliminary report of this work has appeared (Terlesky, 1990).

quencer with an on-line 120A PTH analyzer and 900A data analysis module.

ATPase Assays. The ATPase activity of the cpn60 protein was determined by measuring the amount of radioactive inorganic phosphate released from [γ-^{32}P]ATP as modified from previous reports (Lill et al., 1989; Viitanen et al., 1990). The basic reaction mixture contained 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM KCl, and 0.5 μM cpn60 protomer. Reactions were initiated by the addition of [γ-^{32}P]ATP (7000–9000 mCi/nmol) to a final concentration of 1.0 mM. Reaction vials were placed in a 37 °C water bath. At the indicated time intervals, samples (5 μL) were removed to a microcentrifuge tube containing 175 μL of 1 M perchloric acid and 1 mM sodium phosphate which stopped the reaction. Next, 0.4 mL of 20 mM ammonium molybdate and 0.4 mL of isopropyl acetate were added. After mixing vigorously, the phases were allowed to separate, and 100 μL of the top organic phase was removed to a scintillation vial. The amount of ^{32}P was determined by liquid scintillation counting in a Beckman LS 5000 TD liquid scintillation counter. Control reactions in which no protein was added were routinely performed to correct for the small amount of acid hydrolysis of ATP that occurred during the duration of the experiment.

Immunological Techniques. Antibodies were produced to the native cpn60 of *R. sphaeroides* in a New Zealand White rabbit at Cocalico Biologicals, Inc. (Reamstown, PA). A preimmune sample was taken prior to any injections. Rocket immunoelectrophoresis (Jouanneau & Tabita, 1986) was used for quantification of cpn60 protein levels.

Electrophoresis and Protein Determination. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed by the method of Laemmli (1970). Protein was determined with a protein dye reagent (Bio-Rad Laboratories, Richmond, CA) using bovine serum albumin as the standard.

Chemicals. The following were of reagent grade and obtained from Sigma Chemical Co. (St. Louis, MO): ethylene glycol, DNase I, TES, ATP, and IPTG. Propyl acetate was obtained from Aldrich Chemical Co. (Milwaukee, WI). Ammonium molybdate was obtained from Merck & Co. (Rahway, NJ). Green A–agarose was obtained from Amicon Division, W.R. Grace & Co. (Danvers, MA).

RESULTS

Chaperonin Purification. The cpn10 and cpn60 proteins were purified to homogeneity from *E. coli* and *R. sphaeroides* by using sucrose density gradient centrifugation and FPLC anion-exchange chromatography. Since the *E. coli* cpn60 and cpn10 chaperonins bind to each other in the presence of ATP, this was utilized to facilitate the identification and localization of the *R. sphaeroides* chaperonin proteins. Figure 1 is a SDS–polyacrylamide gel (11%) of each fraction from the sucrose gradient in the presence or absence of ATP. The cpn60 protein was located near the bottom of the gradient (fractions 1–8). Large subunit RubisCO oligomers also migrated to this location, however, the form I large subunit (M_r = 53 681) and form II large subunit (M_r = 58 721) are partly masked by the overabundant cpn60. The small subunit of RubisCO (M_r = 13 000) can be seen in fractions 4–10. The cpn10 protein, which is smaller than the small subunit of RubisCO, can be seen distributed throughout the gradient. However, in the presence of ATP, it is clear that a larger portion of this protein remained bound to the cpn60 and hence migrated near the bottom of the gradient (fractions 4–8). In the absence of ATP, most of the cpn10 protein migrated as its native M_r 60 000 form and was distributed in fractions 11–16. Following green A–agarose chromatography to remove contaminating Rubis-

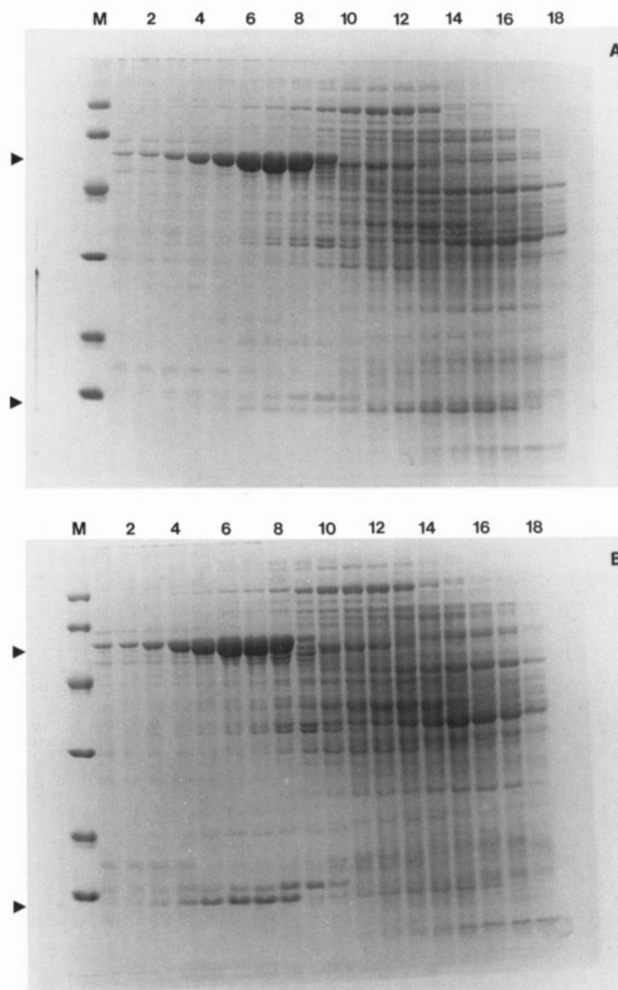


FIGURE 1: Sucrose density gradient centrifugation of cell extracts from heat-shocked (40 °C) photolithoautotrophically grown *R. sphaeroides* HR in the presence and absence of 5 mM ATP. (A) SDS–PAGE, using 11.5% acrylamide gels of samples taken from fractions after sucrose density gradient centrifugation of cell extract (12.3 mg of protein) in the absence of ATP. (B) SDS–PAGE of samples of fractions isolated following sucrose density gradient centrifugation of cell extracts in the presence of 5 mM ATP. Fraction 1 is from the bottom of the gradient. Arrows indicate the location of cpn60 and cpn10. M denotes the molecular weight standards (see text).

CO (Jouanneau & Tabita, 1987), the chaperonin proteins were fractionated by Mono-Q HR 10/10 anion-exchange chromatography. The proteins were eluted from the Mono-Q column with a linear gradient of KCl from 0.0 to 1.0 M. Cpn10 eluted early in the gradient at approximately 0.10 M KCl. Cpn60 eluted from the column at approximately 0.25 M KCl. The proteins were judged to be homogeneous following this step (Figure 2).

N-Terminal Amino Acid Sequencing. To confirm the identity of the purified chaperonins, N-terminal amino acid sequences were determined by using purified preparations of cpn10 and cpn60. Only one N-terminal amino acid signal was detected for each purified chaperonin, further confirming the purity of the preparations. The N-terminal amino acid sequences for the *R. sphaeroides* cpn60 and cpn10 proteins are shown (Figure 3) and compared to known chaperonin proteins. The *R. sphaeroides* cpn60 N-terminal amino acid sequence showed 72% identity with *E. coli* cpn60, while the *R. sphaeroides* cpn10 exhibited 45% identity with *E. coli* cpn10 and 32% identity with the *Synechococcus* sp. strain PCC 7942 cpn10. The first five N-terminal amino acids were also determined for the purified *E. coli* chaperonins and were identical

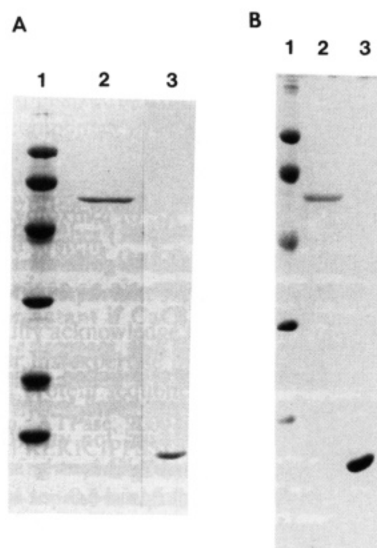
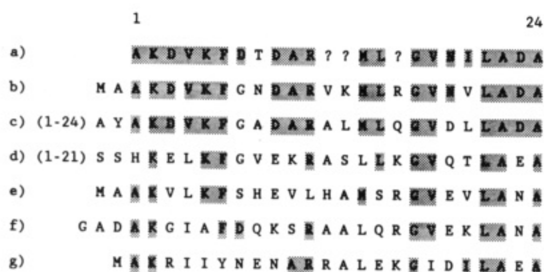


FIGURE 2: SDS-PAGE of purified cpn60 (lane 2) and cpn10 (lane 3) from *R. sphaeroides* (A) and *E. coli* (B). In lane 1 of both (A) and (B) are commercial molecular weight standards (see Experimental Procedures).



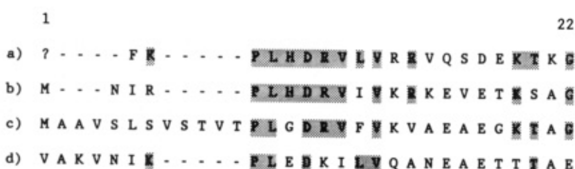


FIGURE 3: N-Terminal amino acid sequence comparison of cpn60 and cpn10 proteins from various prokaryotic and eukaryotic sources. (A) Sequence comparison of cpn60 chaperonins: (a) *R. sphaeroides* cpn60; (b) *E. coli* cpn60 (Hemmingsen et al., 1988); (c) yeast hsp60 (Reading, 1989); (d) 62-kDa antigen from *C. burnetii* (Vodkin & Williams, 1988); (e) human P1 protein (Jindal et al., 1989); (f) RubisCO binding protein from wheat chloroplasts (Hemmingsen et al., 1988); (g) P1 related protein from cyanobacteria (Cozens & Walker, 1987). (B) Sequence comparison of cpn10 chaperonins: (a) *R. sphaeroides* cpn10; (b) *E. coli* cpn10 (Hemmingsen et al., 1988); (c) *M. tuberculosis* cpn10 (Baird et al., 1989); (d) *Synechococcus* cpn10 (Webb et al., 1990). (–) indicates insertions in the sequence for maximum alignment. The shaded amino acids indicate residues identical with the *R. sphaeroides* chaperonin proteins. (?) indicates residues that were not identified with certainty. Numbers above the sequence refer to the amino acid residues that were resolved after N-terminal analysis. Numbers in parentheses refer to leader sequences prior to the cpn10 sequence in rows c and d of (A).

with the published sequence, confirming the identity of these proteins.

Molecular Weight Determination. The native molecular weight of *R. sphaeroides* cpn60, as determined by gel filtration chromatography, was 889 200. The retention volume of *R. sphaeroides* cpn60 on the gel filtration column was less than that for the *E. coli* cpn60 (M_r = 812 000). The native molecular weight of the cpn10 protein was 60 000. SDS-PAGE revealed that cpn60 had a subunit molecular weight of 61 000; cpn10 had a subunit molecular weight of 12 700.

Table I: Levels of *R. sphaeroides* Cpn60 under Different Conditions of Growth

growth condition[a]	levels of cpn60[b] (% soluble protein)
photolithioautotrophic	
30 °C	9.3 ± 2.0
40 °C	28.5 ± 0.5
photoheterotrophic	6.7 ± 0.7
chemoheterotrophic	3.5 ± 1.5

[a] Cells were grown as described under Experimental Procedures. [b] The amount of cpn60 expressed by these cells was determined immunologically as described under Experimental Procedures.

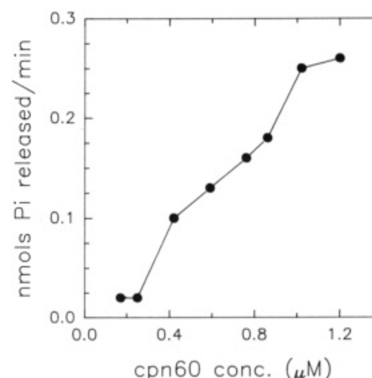


FIGURE 4: ATPase activity of *R. sphaeroides* cpn60. ATPase activity was assayed as described under Experimental Procedures. The reaction was terminated after 10 min by the addition of perchloric acid, and the amount of inorganic phosphate released per minute was determined.

Regulation of Expression of Cpn60. Antibodies were produced to the native cpn60 protein in order to study the expression of the protein under different growth conditions. Increasing the temperature of the culture from 30 to 40 °C resulted in a 3-fold increase in cpn60 protein levels in cell extracts (Table I). This effect was observed after 8 h at the elevated temperature. Furthermore, the carbon and energy source with which the organism was cultured had an effect on the expression of cpn60 (Table I). Growth under photolithoautotrophic conditions in a 1.5% CO_2/98.5% H_2 atmosphere yielded the highest levels of cpn60 protein expression; a lesser amount was observed under photoheterotrophic conditions. The levels of cpn60 were markedly reduced in cultures grown chemoheterotrophically in the dark.

ATPase Activity. R. sphaeroides cpn60 catalyzed the hydrolysis at ATP at 37 °C with a specific activity of 134 nmol min^{-1} (mg of protein)$^{-1}$ and a k_{cat} of 0.13 s^{-1}. The rate of hydrolysis increased with increasing concentrations of cpn60 up to 1.0 μM cpn60 protomer (Figure 4). The *E. coli* cpn60 preparation catalyzed ATP hydrolysis with a specific activity of 751 nmol min^{-1} mg^{-1} and a turnover number of 0.75 s^{-1} at 37 °C.

The ability of cpn10 to inhibit the ATPase activity of the cpn60 proteins was examined. *R. sphaeroides* cpn10 inhibited *R. sphaeroides* cpn60 ATPase activity; however, *E. coli* cpn10 did not inhibit the activity of *R. sphaeroides* cpn60 (Figure 5B). The converse experiment indicated that both cpn10 proteins inhibited the ATPase activity of *E. coli* cpn60 (Figure 5A). Each cpn60 was titrated with increasing amounts of cpn10, and the results clearly indicated that *R. sphaeroides*

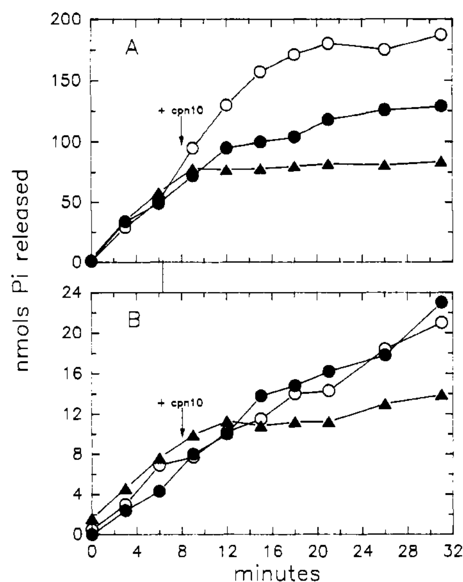


FIGURE 5: Inhibition of *R. sphaeroides* and *E. coli* cpn60 ATPase activity by cpn10 proteins. At the designated time intervals, samples (5 μL) were removed from the assay mix (250 μL) and assayed for inorganic phosphate. (A) Inhibition of *E. coli* cpn60 (0.5 nmol of protomer) by cpn10 (1.0 nmol of protomer) proteins: (○) ATPase activity in the absence of cpn10 proteins; (●) ATPase activity in the presence of *E. coli* cpn10, (▲) ATPase activity in the presence of *R. sphaeroides* cpn10. (B) Inhibition of *R. sphaeroides* cpn60 (0.5 nmol of protomer) by cpn10 (1.0 nmol of protomer) proteins: (○) ATPase activity in the absence of cpn10 proteins; (●) ATPase activity in the presence of *E. coli* cpn10; (▲) ATPase activity in the presence of *R. sphaeroides* cpn10.

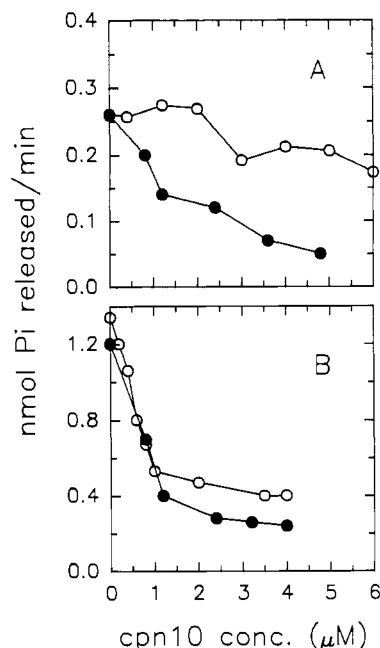


FIGURE 6: Titration of cpn60 ATPase activity with cpn10 proteins. The assay (25-μL volume) was terminated after 8 min by the addition of perchloric acid. The total amount of inorganic phosphate released per assay was then determined. (A) ATPase activity of *R. sphaeroides* cpn60 (1 μM) in the presence of increasing concentrations of *E. coli* cpn10 (○) or *R. sphaeroides* cpn10 (●). (B) ATPase activity of *E. coli* cpn60 (1 μM) in the presence of increasing concentrations of *E. coli* cpn10 (○) or *R. sphaeroides* cpn10 (●).

cpn60 ATPase activity was not inhibited by *E. coli* cpn10 at concentrations as high as 18 μM cpn10 protomer (Figure 6A). As little as 1.0 μM cpn10 from either source significantly inhibited *E. coli* cpn60 ATPase activity. In addition, there was no apparent binding of *E. coli* cpn10 to *R. sphaeroides* cpn60 upon sucrose density gradient centrifugation in the presence of 5 mM ATP (data not shown).

DISCUSSION

In this study, we have characterized two heat-shock proteins purified from photolithoautotrophically grown *R. sphaeroides*. The proteins were similar to the *E. coli* chaperonin proteins that facilitate correct posttranslational folding and/or assembly of recombinant *Anacystis nidulans* and *Rhodospirillum rubrum* RubisCO (Goloubinoff et al., 1989). Comparison of the N-termini of the *R. sphaeroides* cpn60 and cpn10 to the corresponding *E. coli* proteins revealed that the cpn60 proteins are more conserved than the cpn10 proteins. Several physical properties of *R. sphaeroides* cpn60, including native and subunit molecular weight and the N-terminal amino acid sequence, were similar to the protein isolated from *E. coli* (Hemmingsen et al., 1988). In comparison of the ATPase activities, the k_{cat} of *E. coli* cpn60 was 5.8-fold higher than the k_{cat} for *R. sphaeroides* cpn60, though the overall rates of ATP hydrolysis for both proteins were low compared to the activity of true ATPase enzymes. This low rate of ATP hydrolysis is due to the fact that the ATPase activity of cpn60 proteins is probably a secondary reaction to the proposed primary role in facilitating protein folding in vivo (Viitanen et al., 1990). The exact mechanism of protein folding is not known; however, it is hypothesized that a cpn60–cpn10 complex forms in the presence of ATP followed by binding of an unfolded oligomeric protein such as RubisCO. ATP hydrolysis is believed to occur simultaneous to release of the folded protein from the chaperonin complex. The two chaperonins would also separate at this point in the absence of ATP. Although the binding site of the unfolded oligomeric proteins to the chaperonin complex is not known, several recombinant bacterial proteins including β-lactamase and the small subunit of RubisCO have been shown to associate with the cpn60 of *E. coli* (Bochkareva et al., 1988; Landry & Bartlett, 1989). Recombinant RubisCO's from plant sources, however, are not assembled in *E. coli*, and this is believed to be due, at least in part, to incompatability between the host chaperonins and the foreign oligomeric protein. Proteins related to the cpn60 chaperonins have been isolated from plant chloroplasts (Hemmingsen et al., 1988), but no proteins related to cpn10 have thus far been described.

Comparison of the cpn10 proteins revealed less conservation among this family of proteins. This not only was seen at the level of the N-terminal amino acids in which the *R. sphaeroides* and *E. coli* proteins shared only 45% identity in the first 22 amino acids but also was reflected by the inhibition of cpn60 ATPase activity. Since *E. coli* cpn10 does not apparently bind to *R. sphaeroides* cpn60, it is likely that there is some specificity of the cpn10 protein for its homologous cpn60. In experiments examining the refolding of denatured *R. rubrum* RubisCO, the heterologous complexes of yeast cpn60–*E. coli* cpn10 and chloroplast cpn60 (RubisCO binding protein)–*E. coli* cpn10 were, respectively, only 10% and 25% as effective in facilitating RubisCO reconstitution compared to the homologus *E. coli* cpn60–cpn10 complex (Goloubinoff et al., 1989). Thus, the inability to assemble a recombinant protein may reflect a requirement for the homologous chaperonin proteins from the same source. Indeed, future studies of the assembly of the L_8S_8 (form I) and L_x (form II) RubisCO of *R. sphaeroides* may be greatly facilitated by using *R. sphaeroides* chaperonin proteins.

Since the *E. coli* chaperonin proteins have been shown to be required for posttranslational assembly of recombinant RubisCO, the *R. sphaeroides* chaperonin proteins probably have a similar function. This is further supported by am-

plification of the protein levels during photolithoautotrophic growth, when the synthesis of Calvin cycle enzymes, particularly RubisCO, is markedly derepressed. In the unicellular alga *Chlamydomonas reinhardtii*, expression of the hsp-70 homologous heat-shock genes is regulated by both thermal stress and light (Gromoff et al., 1989). Thus, enhanced expression of *R. sphaeroides* cpn60 in response to autotrophic growth conditions may be an important mode of chaperonin regulation, and may facilitate the productive assembly of the large amounts of RubisCO that are required under these growth conditions (Jouanneau & Tabita, 1986).

Acknowledgments

We thank Drs. G. Lorimer and A. Gatenby for sending us strains and plasmids containing *E. coli groEL* and *groES* genes. We are also grateful to Jane Tolley at the Ohio State University Biochemical Instrumentation Center for the N-terminal analyses.

References

Baird, P. N., Hall, L. M. C., & Coates, A. R. M. (1989) *J. Gen. Microbiol. 135*, 931–939.

Barraclough, R., & Ellis, R. J. (1980) *Biochim. Biophys. Acta 608*, 19–31.

Bochkareva, E. S., Lissin, N. M., & Girshovich, A. S. (1988) *Nature 336*, 254–257.

Chandrasekhar, G. N., Tilly, K., Woolford, C., Hendrix, R., & Georgopoulos, G. (1986) *J. Biol. Chem. 261*, 12414–12419.

Cozens, A. L., & Walker, J. E. (1987) *J. Mol. Biol. 194*, 359–383.

Gatenby, A. A., & Ellis, R. J. (1990) *Annu. Rev. Cell Biol. 6*, 125–149.

Georgopoulos, C. P., Hendrix, R. W., & Ellis, R. J. (1988) *Nature 333*, 330–334.

Gibson, J. L., & Tabita, F. R. (1977) *J. Biol. Chem. 252*, 943–949.

Gibson, J. L., & Tabita, F. R. (1985) *J. Bacteriol. 164*, 1188–1193.

Gibson, J. L., & Tabita, F. R. (1986) *Gene 44*, 271–278.

Gibson, J. L., Falcone, D. L., & Tabita, F. R. (1991) *J. Biol. Chem. 266* (in press).

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*, 884–889.

Gromoff, E. D., Treier, U., & Beck, C. F. (1989) *Mol. Cell. Biol. 9*, 3911–3918.

Hemmingsen, S. M., Woolford, C., van der Vies, S. M., Tilly, K., & Dennis, D. T. (1988) *Nature 333*, 330–334.

Jindal, S., Dudani, A. K., Singh, B., Harley, C. B., & Gupta, R. S. (1989) *Mol. Cell. Biol. 9*, 2279–2283.

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

Jouanneau, Y., & Tabita, F. R. (1987) *Arch. Biochem. Biophys. 254*, 290–303.

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

Landry, S. J., & Bartlett, S. G. (1989) *J. Biol. Chem. 264*, 9090–9093.

Lill, R., Cunningham, K., Brundage, L. A., Ito, K., Oliver, D., & Wickner, W. (1989) *EMBO J. 8*, 961–966.

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

Muller, E. D., Chory, J., & Kaplan, S. (1985) *J. Bacteriol. 161*, 469–472.

Quivey, R. G., Jr., & Tabita, F. R. (1984) *Gene 31*, 91–101.

Reading, D. S., Hallberg, R. L., & Myers, A. M. (1989) *Nature 337*, 655–659.

Roy, H., Bloom, M., Milos, P., & Monroe, M. (1982) *J. Cell Biol. 94*, 20–27.

Tabita, F. R. (1988) *Microbiol. Rev. 55*, 155–158.

Terlesky, K. C. (1990) *Abstr. Annu. Meeting Am. Soc. Microbiol.*, K-96.

Viitanen, P. V., Ludden, T. M., Reed, J., Goloubinoff, P., O'Keefe, D. P., & Lorimer, G. H. (1990) *Biochemistry 29*, 5665–5671.

Vodkin, M. H., & Williams, J. C. (1988) *J. Bacteriol. 170*, 1227–1234.

Wagner, S. J., Stevens, S. E., Jr., Nixon, B. T., Lambert, D. H., Quivey, R. G., Jr., & Tabita, F. R. (1988) *FEMS Microbiol. Lett. 55*, 217–222.

Watson, G. M. F., Mann, N. H., MacDonald, G. A., & Dunbar, B. (1990) *FEMS Microbiol. Lett. 72*, 439–354.

Weaver, K. E., & Tabita, F. R. (1983) *J. Bacteriol. 156*, 507–515.

Webb, R., Reddy, K. J., & Sherman, L. A. (1990) *J. Bacteriol. 171*, 5079–5088.