As a preliminary to the understanding of the function of the highly conserved *Escherichia coli* heat shock protein HtpG, the protein was purified and partially characterized. The *htpG* gene was subcloned into the inducible expression vector, pT7-6. Upon induction, the HtpG protein accumulated to approximately 30% of the total protein in the cell. A purification scheme was devised which involved column chromatography on DEAE-cellulose, hydroxylapatite, and Sephacryl S-200. The amino acid composition of the purified protein corresponded closely with the predicted amino acid composition derived from the DNA sequence, and the sequence of the 8 amino-terminal residues matched the predicted sequence exactly. The molecular weight of the desalted protein is 65,500, and the native molecular weight is 144,620, as calculated by using both the Stokes radius and the sedimentation coefficient. As the molecular weight predicted from the DNA sequence is 71,429, this indicates the HtpG protein is a dimer. The HtpG protein was found to be a phosphoprotein. Thus, HtpG is structurally similar to its eukaryotic homologue, hsp83, which is also a phosphoprotein and a dimer.

Relatively little is known about the *Escherichia coli* heat shock protein HtpG, originally named C62.5 for its coordinates on two-dimensional gels (Neidhardt et al., 1983; Phillips et al., 1987). It is highly conserved throughout evolution; 41% of its residues are identical with those of *Drosophila* hsp83, and 42% are identical with the human hsp83 (Bardwell and Craig, 1987). Some regions of the hsp83/HtpG proteins are greater than 90% conserved in all organisms examined (Bardwell and Craig, 1987). HtpG is encoded by the *htpG* gene which lies between *dnaZ* and *adh* at 11.1 min on the *E. coli* chromosome (Bardwell and Craig, 1987). The *htpG* gene can be detected from *E. coli* with little effect on viability; *htpG* deletion mutants are viable up to 1°C below the maximum temperature for viability of wild type cells (Bardwell and Craig, 1988). In contrast, yeast has two copies of the gene for hsp84, one of which is essential for growth at all temperatures (Finkestein and Farrelly, 1984). The *htpG* deletion mutant has a growth disadvantage in mixed cultures with wild type cells (Bardwell and Craig, 1988). Beginning with equal populations of *htpG* deletion mutants and wild type cells, the wild type cells outnumber the *htpG* deletion mutant cells 3 to 1 after 400 generations at 37°C (Bardwell and Craig, 1988).

The growth advantage of the wild type cells increases dramatically with an increase in temperature, suggesting that, although not essential for growth at these temperatures, the *htpG* gene product has a function in growth at higher temperatures (Bardwell and Craig, 1988). The *htpG* gene is induced by heat shock, treatment with ethanol, naldixic acid, or cadmium chloride, and during recovery from puromycin treatment (Van Bogelen et al., 1987). As a preliminary to the understanding of the function of this protein, we have overproduced the HtpG protein, devised a purification protocol, and partially characterized the purified protein.

**MATERIALS AND METHODS**

**Bacterial Strains, Phage, and Plasmids—**B178, a galE*, sup*, prototrophic *E. coli* W3110 strain is from our strain collection. MG1655, another *E. coli* K-12 prototrophic strain, and the plasmid, pMT1, were gifts from Drs. J. Bardwell and E. A. Craig (University of Wisconsin, Madison, WI). Bacterial strain K38 carrying the pGP1-1 plasmid was obtained from S. Tabor (Harvard Medical School) as was the pT7-8 cloning vector. The *E. coli* genomic library was constructed by Dr. S. Carinhour (University of Utah) using the λ cloning vector, L47.1, and approximately 10 kilobases of Sau3A-digested *E. coli* DNA fragments.

**Media—**L-broth contained 16 g of tryptone, 5 g of sodium chloride, and 5 g of yeast extract per liter. The pH was adjusted to 7.4 with NaOH. The low sulfur minimal medium contained 5.1 mM sodium citrate, 38 mM Na$_2$HPO$_4$, 22 mM KH$_2$PO$_4$, 9.2 mM NaCl, 18.7 mM NH$_4$Cl, 0.4 μM FeCl$_3$, 3 mM MgCl$_2$, 0.1 mM CaCl$_2$, 0.2% (w/v) glucose, 2 μg of vitamin B1/ml, 0.03 mM MgSO$_4$, and all L-amino acids except cysteine and methionine. Low phosphate medium contained 1.32 mM KH$_2$PO$_4$, 9.52 mM NH$_4$Cl, 0.276 mM K$_2$SO$_4$, 10 μM FeCl$_3$, 0.5 μM CaCl$_2$, 50 mM NaCl, 40 mM MOPS, and 4 mM Tricine.

**Construction of the λ: htpG Transducing Phage—**pMT1, a pBR322 derivative containing the *htpG* gene, was used to screen a λ library as described in Maniatis et al., 1982. Preparative amounts of bacteriophage λ were grown and purified as described in Maniatis et al., 1982. **Plasmid Constructions—**All reaction conditions and techniques for restriction enzyme digestions, ligations, electrophoresis of DNA, elution of DNA from agarose gels, and DNA transformations were as described by Maniatis et al., 1982, or as specified by the manufacturer.

**Overproduction of HtpG—**The procedure followed was that devised by Tabor and Richardson, 1985. Six liters of JS41, the *HtpG* overproducer cells (see "Results and Discussion") were grown in L-broth with 40 μg of each ampicillin and kanamycin/ml at 30°C to an *A$_{660}$* of 1.5 in an air shaker. The cells were shifted to 42°C in a shaking water bath for 30 min. Rifampicin was added to a final concentration of 100 μg/ml and the cells were then incubated at 37°C for an additional 2 h. The cells were harvested by centrifugation, and the cell pellet was stored at -70°C.

**Buffers—**Buffer K: 180 mM spermidine-C (Sigma), 50 mM DTT, 50 mM EDTA, and 0.9 M ammonium sulfate. Buffer A: 100 mM potassium phosphate (pH 7.0), 100 mM sodium sulfate, 5 mM β-

* The abbreviations used are: MOPS, 4-morpholinopropanesulfonic acid; SDS, sodium dodecyl sulfate; BSA, bovine serum albumin; DTT, dithiothreitol; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; HA, hydroxyapatite.

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mercaptopethanol, 5 mM EDTA, and 10% (v/v) glycerol. Buffer HA: 50 mM Tris (pH 7.5), 100 mM potassium chloride, 10 mM β-mercaptoethanol, and 10% (v/v) glycerol. Buffer D: 50 mM Tris (pH 8.0), 5 mM EDTA, 5 mM β-mercaptoethanol, 50 mM potassium chloride, 0.05% (v/v) Triton X-100, and 10% (v/v) glycerol. Buffer S: 50 mM Tris (pH 8.0), 0.2 M sodium chloride, 5 mM EDTA, 5 mM β-mercaptoethanol, 0.05% (v/v) Triton X-100, 10% (v/v) Ampholines (pH 5-7), 0.4% (v/v) Ampholines (pH 3.5-10), and 5% β-mercaptoethanol.

Protein Determination — The protein concentration was estimated by the Bio-Rad protein assay. A solution of known concentration of BSA was used as a standard.

Glycerol Gradient Sedimentation — Twenty µg each of the purified HtpG protein and the molecular weight markers (yeast alcohol dehydrogenase, BSA, ovalbumin, and lysozyme) were loaded onto a 5 to 20% linear glycerol gradient in 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 M EDTA, and 5 mM DTT. The gradient was centrifuged at 48,000 rpm in a Beckman SW 50.1 rotor for 24 h at 2 °C. Three drop fractions were collected from the bottom and analyzed on 12.5% SDS-polyacrylamide gels (Georgopoulos and Hohn, 1978).

Column Chromatography — Two mg each of apoferritin, the purified HtpG protein, catalase, yeast alcohol dehydrogenase, BSA, and ovalbumin (containing a Sephacyr S-column (1.8 cm x 40 cm) equilibrated with buffer S and eluted with the same buffer at a flow rate of 4 ml per h. One-mL fractions were collected. The void volume was determined by using blue dextran 2000, and the elution volume was determined by adding NaCl to the sample to a final concentration of 1 M and was located by measuring conductivity. Molecular weight markers for column chromatography and sedimentation analysis were obtained from Sigma.

Preparation of Antisera — Two female New Zealand white rabbits were injected with 1 mg of purified HtpG protein emulsified in Freund’s complete adjuvant. Four weeks later, the rabbits were injected with 500 µg of purified HtpG emulsified in Freund’s incomplete adjuvant. The rabbits were bled from the ear 1 week following the second injection and 1 week following booster injections.

Immunoprecipitation — For immunoprecipitation of [35S]methionine-labeled cells, B175 cells were grown in M9 medium with low sucrose (0.6 at 30 °C and then shifted to 42 °C for 10 min. The cells were then labeled for 5 min with 20 µCi of [35S]methionine (ICN). The cells were harvested by centrifugation for 2 min in an Eppendorf Microfuge. The cell pellet was resuspended in 50 µl of 50 mM Tris (pH 8.0), 3.5 M NaCl, 0.1 mM EDTA, and 5 mM DTT. The gradient was centrifuged at 48,000 rpm in a Beckman SW 50.1 rotor for 24 h at 2 °C. Three drop fractions were collected from the bottom and analyzed on 12.5% SDS-polyacrylamide gels (Georgopoulos and Hohn, 1978).

RESULTS AND DISCUSSION

Subcloning of the htpG Gene Into the High Expression Vector pTT76 — It was observed that overexpression of the HtpG protein was deleterious to the cell; most notably, E. coli strains that overexpress HtpG had an extremely delayed lag time upon resumption of growth after storage in the cold relative to the lag exhibited by wild type cells. Because of the possibility that overproduction of HtpG may lead to the selection of htpG mutations or extragenic suppressors, an htpG transducing phage was cloned from an E. coli genomic library by using an htpG-containing plasmid as a probe (a gift from Drs. J. Bardwell and E. Craig, University of Wisconsin). The htpG gene was subcloned from the transducing phage into the inducible pT7-6 high expression vector (Taber and Richardson, 1985). The background level of htpG transcription was greatly reduced by using the XmnI site located in the ~35 sequence of the second of two overlapping htpG promoters to obtain the DNA fragment for cloning (Cowling et al., 1985). The transducing phage was also digested with EcoRI which digests in the middle of the ade gene that is directly adjacent to the 3' end of the htpG gene (Bardwell and Craig, 1987). The XmnI to EcoRI fragment was purified by gel electrophoresis and subcloned into pT7-6 previously digested with EcoRI and Smal. By subcloning into this vector, the htpG gene was placed under the control of the powerful T7 promoter (Taber and Richardson, 1985). This plasmid was used to transform K38, which contains a λ lysogen, carrying the cI857 thermolabile repressor, and the plasmid, pGP1-1, which carries the gene for the T7 RNA polymerase under the control of the λ P8 promoter (Taber and Richardson, 1985). The resulting HtpG overproducing strain is referred to as JS41 in the text. The protocol devised by Taber and Richardson, 1985, was used for the induction of T7 RNA polymerase and subsequently htpG. After induction, the HtpG protein accumulated in these cells to 27% of the total protein in the cell as determined by scanning the gel shown in Fig. 1. The induced cells were harvested by centrifugation and stored as a cell paste at ~70 °C.

Purification of the HtpG Protein — The cells were lysed, and the cell extract was clarified as described in Shlomai and Kornberg, 1980. The frozen cell paste (11 g) was resuspended in 40 ml of 10% (w/v) sucrose in 50 mM Tris-HCl (pH 8.0) and allowed to thaw on ice. Five ml of buffer K with fresh lysozyme (2 mg/ml) was added. After 45 min on ice, the mixture was incubated at 37 °C for 4 min and then returned to ice for an additional 10 min. The cell extract was centrifuged at 12,000 rpm in a Beckman Type 35 rotor at 30,000 rpm for 30 min at 4 °C. All subsequent purification steps were performed at 4 °C. To remove excess DNA, the clarified cell extract (43 ml) was passed directly over a DEAE-52 cellulose column (36 x 2.5 cm) which was equilibrated with buffer A at a flow rate of 5 ml per 10 min. The void volume was monitored by A 280 nm, and the eluted protein fractions were 50 mM sodium pyrophosphate, and 0.1 mM phenylmethylsulfonyl fluoride. The cells were disrupted by three freeze-thaw cycles with liquid nitrogen. The cell lysate was clarified by a 15-min centrifugation in an Eppendorf Microfuge, and the supernatant fluid was processed as described for [35S]methionine-labeled cells.

Gel Electrophoresis — SDS-polyacrylamide (12.5% (w/v)) gels were employed according to the procedure of Laemmli (1970), and stained by Coomassie Blue R-250. Two-dimensional gels were used according to the procedure of O’Farrell (1975) as modified by Georgopoulos et al. (1982). The procedure involved isoelectric focusing in the first dimension (1.6% (v/v) Ampholines (pH 5 to 7), 0.4% (v/v) Ampholines (pH 5.5 to 10)) and 12.5% SDS-polyacrylamide gel electrophoresis in the second dimension. For autoradiography, the gels were dried and exposed to Kodak SB100 film (3S) or Kodak XAR-5 film (3P).
collected. The fractions were brought to 15% saturation by the addition of solid ammonium sulfate over 25 min. After an additional 15 min of slow stirring, the mixture was centrifuged for 30 min at 30,000 rpm in a Beckman fixed angle 35 rotor. The pellet was discarded, and the supernatant was brought to 60% saturation with the slow addition of solid ammonium sulfate. The solution was centrifuged as described above. The supernatant was then loaded onto a DEAE-52 cellulose column previously equilibrated with buffer D at a flow rate of 3 ml per h. Two-ml fractions were collected, and the HtpG protein was located by measurement of absorbance at 280 nm. The position of the HtpG protein was located by measurement of absorbance at 280 nm and by SDS-polyacrylamide gel electrophoresis of the fractions and was found to elute at approximately 100 mM NaCl (Fig. 1). Peak fractions were pooled and dialyzed for 4 h against 1 liter of HA buffer with one change. The dialyzed protein was diluted 1:2 with HA buffer and loaded onto a hydroxyapatite column (1.3 x 1.8 cm) previously equilibrated with buffer HA. The loaded column was washed with 200 ml of HA buffer, and then the protein was eluted with a 500-ml linear gradient of 0 to 400 mM K2HPO4/KH2PO4 (pH 7.5) in HA buffer at a flow rate of 30 ml per h. Two-ml fractions were collected, and the position of the HtpG protein was located by measurement at A280 nm and by analysis of fractions on SDS polyacrylamide gels. The elution curve for the HtpG protein showed a peak at 62 mM K2HPO4. The HtpG-enriched fractions were pooled, and the HtpG protein was precipitated by the slow addition of solid ammonium sulfate to 60% saturation as described previously. The pellet was resuspended in 3 ml of buffer S and dialyzed for 3 h against 1 liter of buffer S. The protein (58 mg) was applied to a Sephacryl S-200 column (1.8 x 180 cm) equilibrated with buffer S in three separate batches or preparations, so as not to overload the column. The column was eluted with buffer S at a flow rate of 4 ml per h, and 1-ml fractions were collected. The HtpG protein was located and precipitated with ammonium sulfate as described previously. The pellet was resuspended in buffer S and dialyzed against buffer S for 5 h. It was then stored in 50% (v/v) glycerol at -70 °C for further use. The yield of 24 mg of purified HtpG was approximately 8% of the total HtpG calculated to be in the initial cell extract. The purified protein was judged to be at least 95% pure as determined by a densitometry scan of a Coomassie Blue-stained gel with a Joyce-Loebl densitometer (Fig. 1). As an additional test of purity, the amino acid composition was determined and found to be very close to the amino acid composition predicted from the DNA sequence (Table I). The protein is particularly enriched for glutamic acid and aspartic acid, which is consistent with an acidic pi of 4.95 for the native protein (Neidhardt et al., 1983; Phillips et al., 1987). The amino-terminal amino acid sequence of the first 8 residues of the purified protein is exactly as predicted from the DNA sequence (Table I). The amino-terminal methionine was not removed.

HtpG Is a Phosphoprotein—Polyclonal antiserum to HtpG was prepared in New Zealand white rabbits and used to immunoprecipitate the HtpG protein. Fig. 2, A and B shows the results of immunoprecipitation of [32P]methionine-labeled cells. The heat shock protein GroEL was a contaminant with both preimmune and anti-HtpG antibodies. The nonspecific precipitation of GroEL could be caused by its identity as the common antigen in bacterial infections (Pardue, 1988), by its large decatameric structure or by its tendency to aggregate (Hendrix, 1979). The amount of the protein that migrated to the acidic side of GroEL during electrophoresis was variable, depending on the strain of bacteria. Although it migrated close to the position of NusA (Phillips et al., 1987), it is not

### Table I

**Table I**

| Amino acid | Chemical analysis | DNA sequence |
|------------|------------------|--------------|
| residues/molecule protein | | |
| Alanine | 50.2 | 47 |
| Arginine | 38.2 | 37 |
| Aspartic acid/asparagine | 66.1 | 70 |
| Cysteine | 0 | 0 |
| Glutamic acid/glutamine | 83.5 | 84 |
| Glycine | 32.7 | 30 |
| Histidine | 14.4 | 14 |
| Isoleucine | 34.7 | 31 |
| Leucine | 72.2 | 66 |
| Lysine | 49.8 | 46 |
| Methionine | 12.5 | 13 |
| Phenylyalanine | 26.2 | 26 |
| Proline | 15.7 | 16 |
| Serine | 37.4 | 44 |
| Threonine | 30.4 | 34 |
| Tyrosine | 17.8 | 18 |
| Valine | 37.8 | 38 |

**Amino-terminal sequence**

| Met-Lys-Glu-Arg-Gly | Met-Lys-Glu-Arg-Gly |
|---------------------|---------------------|
| Gln-Glu-Thr-Arg-Oly | Gln-Glu-Thr-Arg-Oly |
Purification and Properties of E. coli Heat Shock Protein, HtpG

FIG. 2. Immunoprecipitation and two-dimensional gel analysis of labeled cell extracts with anti-HtpG antibodies. The intact HtpG protein is marked with an arrow in B, D, and E. A, [35S]methionine-labeled B178 cells immunoprecipitated with preimmune rabbit antibodies; B, [35S]methionine labeled B178 cells immunoprecipitated with rabbit anti-HtpG antibodies; C, [32P]-labeled JS41 cells immunoprecipitated with preimmune rabbit antibodies; D, [32P]-labeled JS41 cells immunoprecipitated with rabbit anti-HtpG antibodies; E, [32P]-labeled MG1655 cells immunoprecipitated with rabbit anti-HtpG antibodies. For A and B, B178 cells were grown in minimal medium with low sulfur at 30 °C to A695 of 0.5 (Georgopoulos and Hohn, 1978). The cells were then shifted to 42 °C and labeled between 10 and 15 min after the shift with 20 μCi of [35S]methionine. The cells were harvested by centrifugation and then lysed by the procedure described by Chandrasekhar et al. (1986) except the cells were lysed by the addition of 62 mM fresh lysozyme. In C and D, JS41 cells were grown in low phosphate medium (Neidhardt et al., 1974), and then the expression of the HtpG protein was induced. At the time of induction, the cells were labeled with 100 μCi/ml [32P]. In E, B178 cells were grown in low phosphate (MOPS) medium (Neidhardt et al., 1974) at 30 °C to an A695 of 0.5, labeled for 1 h with 100 μCi/ml [32P], and then shifted to 42 °C for 15 min. Cells labeled with [32P] were resuspended in the buffer described by Corvera et al. (1986) to inhibit dephosphorylation. The cells were lysed by three freeze-thaw cycles, and the cell extract was clarified as described. Antigen-antibody complexes were recovered from [32P]-labeled and [35S]methionine-labeled cell lysates with formalin-fixed S. aureus cells (Cowan I strain) as previously described (Bardwell et al., 1986). The antigen-antibody complex was recovered from the S. aureus cells by resuspending the cells in the lysis buffer described for two-dimensional gels, heating to 56 °C for 5 min, and then centrifuging the cells for 3 min in an Eppendorf Microfuge. The supernatant fluid was loaded directly onto isoelectric focusing gels. Two dimensional gels were run according to the procedure of O'Farrell (1975), as modified by Georgopoulos et al. (1982). The arrows point to the position of the HtpG protein.

the NusA protein as it did not co-migrate with purified NusA, and HtpG was not immunoprecipitated with anti-NusA antibodies (data not shown). The purified HtpG protein was seen to focus in at least three spots suggesting that it had been modified. Both the GroEL and HtpG proteins exhibited a ladder of degradation products. A similar ladder was seen when purified HtpG was separated by electrophoresis on two-dimensional gels. Such a ladder of degradation products could indicate that these proteins undergo self-degradation as has been indicated for hsp70 (Mitchell et al., 1985), the eukaryotic homologue to the DnaK heat shock protein (Bardwell and Craig, 1984).

Immunoprecipitation of the [32P]-labeled cells demonstrated that HtpG is a phosphoprotein. Under these conditions, HtpG immunoprecipitated with another phosphoprotein of approximately 70 kDa (Fig. 2, D and E). This protein migrates very close to, but not coincident with, the σ32 protein (Phillips et al., 1987). The phosphorylated HtpG protein could easily be immunoprecipitated from the JS41 HtpG overproducer cells after induction (Fig. 2D). We were, however, unable to immunoprecipitate phosphorylated HtpG from B178 cells which carry a single copy of the htpG gene. Immunoprecipitation of another E. coli K-12 strain, MG1655, did result in a small amount of full size protein with an extensive ladder of degradation products, suggesting that the phosphorylated protein is very unstable (Fig. 2E). By superimposing the autoradiograph on the stained gel, it was determined that the phosphorylated HtpG is more acidic than the majority of the HtpG protein. It was estimated that the phosphorylated form of HtpG constitutes approximately 7% of the total htpG protein in JS41 cells by determining the percentage of [32P]methionine counts in the acidic form of the protein.

Native Molecular Weight Determination—The molecular weight of the denatured HtpG is 65,500 as determined on
Purification and Properties of E. coli Heat Shock Protein, HtpG

FIG. 3. Gel filtration of purified HtpG on a Sephacryl S-200 column. Two mg of purified HtpG was loaded onto a Sephacryl S-200 column (1.8 × 180 cm) with 2 mg each of the protein standards: horse spleen apoferritin (Stokes radius, 66 Å), bovine liver catalase (52 Å), yeast alcohol dehydrogenase (46 Å), bovine serum albumin (35 Å), and ovalbumin (27 Å) (Sigma). The column was run at a flow rate of 4 ml/h at 4 °C.

SDS polyacrylamide gels. This is slightly smaller than the molecular weight of 71,429 determined by nucleotide sequence and may be a function of the shape of the denatured protein or the acidic isoelectric point. In order to determine the native molecular weight, the purified HtpG protein was subjected to nondenaturing gel filtration and sedimentation analysis. The Stokes radius of the purified HtpG protein is 56.5 ± 0.5 Å (Fig. 3), as determined by the method of Siegel and Monty, 1966, with the same Sephacryl S-200 column that was used for protein purification (Fig. 3). This is comparable to the Stokes radius for a spherical protein of approximately 275,000.

In order to determine the sedimentation coefficient, purified HtpG was centrifuged through a 5-ml linear 20% (v/v) to 40% (v/v) glycerol gradient with various proteins as molecular weight markers. HtpG was found to have a sedimentation coefficient of 5.55 ± 0.05 s, which corresponds to the sedimentation coefficient of a spherical protein of 95,000 (Fig. 4). Because of the disparity of the molecular weights as determined by these methods, the native molecular weight was determined by the equation:

\[ M = R N 6 \pi \eta s/(1 - \nu p) \]

where \( R \) is the Stokes radius, \( N \) is Avogadro’s number, \( \eta \) is fluid viscosity, \( \nu \) is partial specific volume, \( s \) is the sedimentation coefficient, and \( \rho \) is the density of the solution (Siegel and Monty, 1966). An average partial specific volume of 0.72 cm³/g was used. With this equation, a native molecular weight of 144,620 was determined. As the molecular weight of the protein as determined by DNA sequence is 71,429, these results indicate that the native protein is most probably a dimer and that it is nonspherical.

These studies show that the HtpG protein is structurally similar to its eukaryotic homologue, hsp90, which is also a phosphoprotein (Welch et al., 1983) and a nonspherical dimer (Koyasu et al., 1986). The HtpG protein is smaller owing to the absence of two segments in the E. coli htpG gene that are present in the eukaryotic homologue (Bardwell and Craig, 1987). One segment begins at the position encoding hsp83 amino acid 220 and includes 50 hydrophilic amino acids (Bardwell and Craig, 1987). The other segment includes the carboxyl-terminal 35 amino acids (Bardwell and Craig, 1987).

This is reflected in the lower sedimentation coefficient of 5.6 S rather than the 6.2 S for the murine homologue, hsp90 (Koyasu et al., 1986), and the smaller Stokes radius of 56 Å rather than the 65 Å for hsp90 (Koyasu et al., 1986). The homologous amino acid sequences and the similar structures suggest that HtpG and hsp93/hsp90 perform similar functions. However, the HtpG protein is not absolutely essential for growth in E. coli except at very high temperatures (Bardwell and Craig, 1988), whereas its eukaryotic homologue, hsp93, appears to be required at all temperatures (Finkelstein and Farrelly, 1984).

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REFERENCES

Bardwell, J. C., and Craig, E. A. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 848–852

FIG. 4. Glycerol gradient sedimentation. Twenty μg of purified HtpG was sedimented with protein molecular weight markers through a 5-ml linear 20% (v/v) to 40% (v/v) glycerol gradient (50 mM Tris (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 5 mM DTT) in a Beckman SW50.1 rotor for 24 h at 48,000 rpm at 2 °C. Three drop fractions were collected from the bottom and analyzed by SDS polyacrylamide gels. The molecular weight markers which were used are yeast alcohol dehydrogenase (sнес. 7.4), bovine serum albumin (sнес. 4.31), ovalbumin (sнес. 3.66), and lysozyme (sнес. 1.91) (Sigma).
Bardwell, J. C., and Craig, E. A. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 5177–5181
Bardwell, J. C., and Craig, E. A. (1988) J. Bacteriol. 170, 2977–2983
Bardwell, J. C. A., Tilly, K., Craig, E., King, J., Zylcz, M., and Georgopoulos, C. (1986) J. Biol. Chem. 261, 1782–1785
Chandrasekhar, G. N., Tilly, K., Woolford, C., Hendrix, R., and Georgopoulos, C. (1986) J. Biol. Chem. 261, 12414–12419
Corvera, S., Whitehead, R. E., Mottola, C., and Czech, M. P. (1986) J. Biol. Chem. 261, 7675–7679
Cowing, D. W., Bardwell, J. C. A., Craig, E. A., Woolford, C., Hendrix, R. W., and Gross, C. A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 2679–2683
Finkelstein, D. B., and Farrelly, F. W. (1984) Fed. Proc. 43, 1499
Georgopoulos, C. P., and Hohn, B. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 131–135
Georgopoulos, C., Tilly, K., Drahos, D., and Hendrix, R. (1982) J. Bacteriol. 149, 1175–1177
Hendrix, R. W. (1979) J. Mol. Biol. 129, 375–392
Koyasu, S., Nishida, E., Kadowaki, T., Matsuzaki, F., Iida, K., Harada, F., Kasuga, M., Sakai, H., and Yahara, I. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8054–8058
Laemmli, U. K. (1970) Nature 227, 680–685
Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Mitchell, H. K., Petersen, N. S., and Buzin, C. H. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4969–4973
Neidhardt, F. C., Bloch, P. L., and Smith, D. F. (1974) J. Bacteriol. 119, 736–747
Neidhardt, F. C., VanBogelen, R. A., and Lau, E. T. (1983) J. Bacteriol. 153, 597–603
O’Farrell, P. H. (1975) J. Biol. Chem. 250, 4007–4021
Pardue, M. L. (1988) Genes Dev. 2, 783–785
Phillips, T. A., Vaughn, V., Bloch, P. L., and Neidhardt, F. C. (1987) in Escherichia coli and Salmonella typhimurium (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) pp. 919–966, American Society for Microbiology, Washington, D. C.
Sblomai, J., and Kornberg, A. (1980) J. Biol. Chem. 255, 6788–6793
Siegel, L. M., and Monty, K. J. (1966) Biochim. Biophys. Acta 112, 346–362
Tabor, S., and Richardson, C. C. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 1074–1078
VanBogelen, R. A., Kelley, P. M., and Neidhardt, F. C. (1987) J. Bacteriol. 169, 26–32
Welch, W. J., Garrels, J. I., Thomas, G. P., Lin, J., and Feramisco, J. R. (1983) J. Biol. Chem. 258, 7102–7111