Ribosomes exist in large excess over the apparent demand for protein synthesis during carbon starvation in marine Vibrio sp. strain CCUG 15956.

Klas Flär dh
Paul S. Cohen
University of Rhode Island, pscohen@uri.edu
Staffan Kjelleberg

Follow this and additional works at: https://digitalcommons.uri.edu/cels_past_depts_facpubs
Terms of Use
All rights reserved under copyright.

Citation/Publisher Attribution
Available at: http://dx.doi.org/10.1128/jb.174.21.6780-6788.1992

This Article is brought to you for free and open access by the College of the Environment and Life Sciences at DigitalCommons@URI. It has been accepted for inclusion in Past Departments Faculty Publications (CELS) by an authorized administrator of DigitalCommons@URI. For more information, please contact digitalcommons-group@uri.edu.
Ribosomes exist in large excess over the apparent demand for protein synthesis during carbon starvation in marine Vibrio sp. strain CCUG 15956.

Terms of Use
All rights reserved under copyright.

This article is available at DigitalCommons@URI: https://digitalcommons.uri.edu/cels_past_depts_facpubs/54
Ribosomes Exist in Large Excess over the Apparent Demand for Protein Synthesis during Carbon Starvation in Marine Vibrio sp. Strain CCUG 15956

KLAS FLÄRDH,1 PAUL S. COHEN,2 AND STAFFAN KELLEBERG1*

Department of General and Marine Microbiology, University of Göteborg, Carl Skottsbergs gata 22, S-413 19 Göteborg, Sweden,1 and Department of Microbiology, University of Rhode Island, Kingston, Rhode Island 028812

Received 29 April 1992/Accepted 18 August 1992

Carbon starvation induces the development of a starvation- and stress-resistant cell state in marine Vibrio sp. strain S14 (CCUG 15956). The starved cells remain highly responsive to nutrients during prolonged starvation and exhibit instantaneous severalfold increases in the rates of protein synthesis and RNA synthesis when substrate is added. In order to elucidate the physiological basis for the survival of cells that are starved for a long time, as well as the capacity of these cells for rapid and efficient recovery, we analyzed the ribosome content of carbon-starved Vibrio sp. strain S14 cells. By using direct chemical measurements of the amounts of ribosomal particles in carbon-starved cultures, we demonstrated that ribosomes were lost relatively slowly (half life, 79 h) and that they existed in large excess over the apparent demand for protein synthesis. After 24 h of starvation the total rate of protein synthesis was 2.3% of the rate during growth, and after 3 days this rate was 0.75% of the rate during growth; the relative amounts of ribosomal particles at these times were 81 and 52%, respectively. The ribosome population consisted of 90% 70S monoribosomes, and no polyribosomes were detected in the starved cells. The 70S monoribosomes were responsible for the bulk of the protein synthesis during carbon starvation; some activity was also detected in the polyribosome size region on sucrose density gradients. We suggest that nongrowing carbon-starved Vibrio sp. strain S14 cells possess an excess protein synthesis capacity, which may be essential for their ability to immediately initiate an upshift program when substrate is added.

It is well established that bacterial growth in natural environments is restricted by energy and/or nutrient shortages (40, 57), and there is a growing interest in the adaptations and responses of bacteria to nutrient limitation (see references 15 and 25). In particular, many marine waters are oligotrophic and are characterized by extremely low concentrations of bioavailable organic carbon (39, 40). Growth of the heterotrophic bacterial populations that inhabit these waters is frequently limited because of energy and nutrient depletion (21, 22, 60), and the life cycles of the bacteria may include intermittent periods of growth and starvation-induced non-growth, as well as unbalanced growth (7, 23). In order to better understand the autecology of marine bacteria and to evaluate the participation of these organisms in nutrient cycling in marine environments, an improved knowledge of the physiological state induced by energy and nutrient starvation is needed. Bacteria such as Vibrio species, which are commonly isolated from a variety of marine ecosystems (5, 8), should have evolved adequate responses to nutrient limitation and are suitable model organisms for such studies.

The starvation-induced responses of marine Vibrio spp. have been extensively studied and described (for reviews, see references 22, 23, and 38). Vibrio spp. respond to energy and nutrient deprivation by a time-dependent series of adaptive physiological and molecular realignments (46). These responses are similar to those of other nondifferentiating bacteria, such as Escherichia coli and Salmonella typhimurium (35, 56). While the morphological mani-

* Corresponding author.
detectable rates (45, 49). The metabolically active ultramicro-cells are capable of rapidly recovering from starvation and respond instantaneously to the addition of nutrients by increasing the rates of RNA synthesis and protein synthesis severalfold (3). The upshift initiates a temporally ordered program of protein synthesis, including repression of starvation-induced (Sti) proteins (47), transient expression of some maturation specific (Mat) proteins, and induction of growth-related proteins. After a lag period or maturation phase, the length of which depends on starvation time, DNA synthesis and cell division resume, and culture growth resumes (3).

Unfortunately, detailed information about the physiologi- cal status of cells that are starved for a long time and how these cells are prepared for rapid recovery is lacking. How- ever, it may be that the nature and the functionality of the protein synthesis system in itself are of utmost importance for maintaining viability and substrate responsiveness. Albertson et al. (3) suggested that starved Vibrio sp. strain S14 possesses an excess of stable RNA and ribosomes that can be used in the rapid recovery process. It has also been proposed that extremely slowly growing E. coli cells contain extra RNA that is not used for protein synthesis, but can be put into action in response to increased nutrient supplies (24). Davis et al. (10) observed a correlation between loss of ribosomes and loss of viability during phosphate deprivation (39) and suggested that ribosome degradation is a major cause of cell death during starvation. Initially, it would be beneficial to starved cells to degrade ribosomes because such degra- dation would provide the cells with an endogenous source of the essential nutrients. However, if too much of the protein synthesis capacity was lost, the cells would have difficulty in recovering from starvation and would eventually die (10). Extensive and rapid degradation of ribosomes in E. coli has been observed repeatedly during stationary phase and during starvation for various nutrients (see references 10, 20, 30, 33, 34, 41, and 51). In addition, correlations among starvation survival, maximal growth rate, and functional characteristics of the ribosomes have been described for a series of natural isolates of E. coli (37).

In this paper we describe a study of the protein synthesis machinery during carbon starvation of Vibrio sp. strain S14. By directly measuring the amounts of ribosomal particles in carbon-deprived cells, we found that ribosomes were very slowly degraded and existed in large excess in relation to the actual rate of protein synthesis in the starved cells. Polyri- bosomes were not detected in the starved cells, and ribo- somes appeared to exist mainly as monomeric 70S particles. The role of ribosome stability in the ability of the organism to survive and to recover from starvation is discussed below.

MATERIALS AND METHODS

Bacterium, growth, and starvation conditions. Marine Vibrio sp. strain S14 (CCUG 15956) has been described previously (1, 31). On the basis of its 16S rRNA sequence, this strain resembles species that reside in the core of the genus Vibrio (11, 14). The cells were grown in batches of marine minimal medium (MMM) (52) at 26°C with rotary shaking. For each experiment, cultures were grown over- night in liquid MMM supplemented with glucose (1 g liter⁻¹) and were diluted 100-fold into fresh MMM supplemented with 0.15 g of glucose per liter. The cells grew exponentially (generation time, 75 min) in this medium until glucose deprivation caused cessation of growth. The resulting star- vation cultures were incubated for 9 days at 26°C with slow rotary shaking. Viability was assessed by plating serial dilutions in a nine-salt solution (artificial seawater) on VNNS agar (32).

Measurement of the rates of total protein synthesis and RNA synthesis. The rate of total protein synthesis was determined as the rate of radioactive leucine incorporation into hot trichloroacetic acid (TCA)-insoluble material. Subvolumes of an experimental culture were given 1-[4,5-³H]leucine (Amersham) and unlabeled carrier leucine (1.2 µM; 98 Gbq mmol⁻¹). At appropriate intervals, 200 µl was removed and added to 2 ml of ice-cold 5% TCA, and the resulting preparations were heated at 90°C for 15 min and kept on ice for 1 h prior to collection of the precipitated material on polycarbonate membrane filters (pore size, 0.2 µm; Poretics Corp., Livermore, Calif.). The precipitates were washed three times with ice-cold 5% TCA and then transferred to scintillation vials. Aquassure (Dupont, NEN Research Prod- ucts, Boston, Mass.) was used as the scintillation cocktail, and the radioactivity was determined with a Beckman model 6000LL liquid scintillation counter. The rates of leucine incorporation were determined from the slopes of the linear incorporation graphs, as described by Nyström et al. (49), and were expressed as picomoles per minute per microgram of cellular protein. The rate of total RNA synthesis was determined by pulse-labelling with pre Label (0.13 mM [5,6-³H]uridine (1.4 TBq mmol⁻¹; Dupont, NEN Research Products). The rate of incorporation was determined by measuring the amount of radioactivity that was precipi- tated in ice-cold 5% TCA, as described above for leucine incorporation, except that the samples were not heat treated.

Chemical analyses of RNA and protein concentrations. RNA concentrations were determined by using the orcinol reaction, as described by Cooper (9), and yeast RNA as the standard. Samples were placed into equal volumes of 10% TCA on ice, and precipitates were collected by centrifuga- tion prior to analysis. Protein concentrations were deter- mined by using the Peterson modification of the method of Lowry et al. (53) and a protein assay kit (procedure no. P5656; Sigma Diagnostics, St. Louis, Mo.), including the optional TCA-deoxycholate precipitation. Bovine serum albumin was used as the standard.

Preparation and quantitation of ribosomal particles. In order to determine the ribosome content in Vibrio sp. strain S14 during long-term carbon starvation, growing and starved cells were harvested, lysed, and fractionated by differential centrifugations. Subvolumes (250 ml) of the experimental cultures were withdrawn at the onset of starvation and after different times of carbon starvation. The cells were har- vested by centrifugation at 10,000 × g max and 4°C for 10 min and were stored frozen at −70°C. The pellets were thawed, resuspended in 1 ml of buffer A (10 mM Tris-HCl [pH 7.5], 1 mM EDTA, 6 mM 2-mercaptoethanol, 1.25 mM dithiothre- itol), frozen, and thawed again, and 1 ml of buffer B (10 mM Tris-HCl [pH 7.5], 21 mM magnesium acetate, 60 mM KCl, 6 mM 2-mercaptoethanol, 1.25 mM dithiothreitol) was added together with lysozyme (300 µg ml⁻¹). After 15 min of incubation, 80 µl of 10% Triton X-100 was added. The lysates were kept on ice for another 15 min, and then 6 ml of buffer C (10 mM Tris-HCl [pH 7.5], 10.5 mM magnesium acetate, 0.5 mM EDTA, 30 mM KCl, 6 mM 2-mercaptoeth- anol, 1.25 mM dithiothreitol) was added together with DNase I (5 µg ml⁻¹). The lysates were cleared by pelleting unlysed cells by centrifugation at 7,000 × g max and 4°C for 10 min. The supernatants were centrifuged twice at 30,000 × g max and 4°C for 30 min, and the resulting extracts were designated the S-30 extracts. The ribosomal particles in the S-30 extracts were pelleted by centrifuging the extracts at
150,000 × gmax and 4°C for 60 min in a Beckman type 80Ti rotor. The crude ribosome pellets (designated P-150) were resuspended in buffer C and, along with the supernatants (designated S-150) and subsamples of the lysates, cleared lysates, and S-30 extracts, were stored frozen at −70°C until the protein and RNA concentrations were determined chemically. The terms particulate RNA and soluble RNA are used below for the pelleted RNA (P-150) and the RNA that stayed in the supernatant (S-150), respectively. The amounts of ribosomal particles after the different lengths of starvation were expressed as the amounts of particulate RNA per volume of cell suspension. The efficiency of the lysis procedure was determined for each sample as the percentage of RNA that was retained in the cleared lysates when unlysed cells had been removed. The chemically determined RNA and protein concentrations were corrected for the efficiency of lysis in each individual sample. This efficiency varied from 90 to 100% at the onset of starvation to about 40% for cells that were starved for a long time.

Analysis of ribosomal particles by density gradient centrifugation. Resuspended ribosomal pellets (P-150) were dialyzed against buffer D (same as buffer C, except that the magnesium acetate concentration was 0.105 mM and the EDTA concentration was 0.005 mM) to dissociate the ribosomes into 30S and 50S subunits. Samples were loaded onto 17-ml sucrose density gradients (5 to 20% sucrose in 10 mM Tris-HCl [pH 7.5]–0.10 mM magnesium acetate–30 mM KCl) and spun at 26,000 rpm in a Beckman type SW28.1 rotor for 8 h at 4°C. Prior to centrifugation, a small portion of radioactively labeled reference ribosomes was added to each sample to indicate the positions of intact 30S and 50S subunits. Reference ribosomes were obtained by labeling exponentially growing cells in MEM for two generations with [5,6-3H]uridine (0.3 μM; 1.4 TBq·mmol⁻¹; Dupont, NEN Research Products) and preparing an S-30 extract in buffer D as described above. The bottoms of the gradient tubes were punctured, and the contents of the tubes were pumped through the flow cuvette of a Beckman model DU-65 spectrophotometer and into a fraction collector, which divided each gradient into 34 fractions. The absorbance profiles were read at 254 nm. The positions of the reference ribosomal subunits were located by determining the radioactivity in the fractions.

Extraction and analysis of polyribosomes. The method which we used for polysome extraction was adapted from the freeze-thaw-lysozyme lysis procedure described by Ron et al. (54). Chloramphenicol (final concentration, 200 μg ml⁻¹) was added to 50 ml of log-phase cells that were grown to an optical density at 610 nm (OD610) of 0.2 in MEM supplemented with 1 g of glucose per liter, to 50 ml of cells that had been starved for 24 h, or to 75 ml of cells that had been starved for 144 h. After 30 s, the cells were poured over crushed ice and centrifuged at 10,000 × gmax and 2°C for 10 min. The resulting pellets were resuspended in 0.5 ml of a solution containing 10 mM Tris-HCl [pH 7.5], 30 mM KCl, 5.25 mM magnesium acetate, 0.25 mM EDTA, 6 mM 2-mercaptoethanol, 100 μg of chloramphenicol per ml, and 1.0 mg of lysozyme per ml and were twice frozen in liquid nitrogen and thawed in a 4°C water bath. Deoxycholate (final concentration, 0.3%) was added to the suspensions, as were DNase I (final concentration, 5 μg ml⁻¹) and dithiothreitol (final concentration, 1.25 mM), before the preparations were incubated on ice for 10 min. The extracts were cleared by centrifugation for 12 min in a Beckman type TL-A100.2 rotor at 30,000 × gmax and then were loaded onto 17-ml 10 to 30% sucrose density gradients (10 mM Tris-HCl [pH 7.5], 30 mM KCl, 5.0 mM magnesium acetate). The gradients were centrifuged in a Beckman type SW 28.1 rotor at 26,000 rpm and 4°C for 3 h. The gradients were analyzed as described above.

In order to compare the protein synthesis activities on the different classes of mono- and polyribosomes after different starvation times, the cells were pulsed with 1-[4,5-3H]leucine (final concentration, 70 mM; Amersham) for 10 s before quenching with chloramphenicol, harvesting of the cells, and polysome extraction. The specific activities were 0.16 TBq mmol⁻¹ for log-phase cells and cells that were starved for 24 h and 1.6 TBq mmol⁻¹ for cells that were starved for 144 h. The levels of hot TCA-insoluble radioactivity in the fractions were determined as described above after 0.1 mg of bovine serum albumin was added to each fraction.

We tested the possibility that starved cells contained RNases that degraded polysomes during the extraction procedure. Radioactive log-phase polyribosomes were used as probes for RNase activity in starved cells. Such polysomes were obtained by labeling exponentially growing cells for two generations with [2-3H]uridine (final concentration, 5 μM; 0.13 GBq·mol⁻¹; Amersham). The labeled cells were extracted separately or after they were mixed with equal amounts of unlabeled cells that had been starved for 24 or 144 h, and the polysomes were analyzed as described above.

RESULTS

Carbon starvation conditions, survival, and endogenous metabolism. Since carbon starvation is the determinant for the development of starvation and stress resistance (50), a defined glucose starvation condition was chosen for these experiments. The way in which starvation was imposed on the cells, as well as the minimal medium (MMM), has not been used in previous studies of Vibrio sp. strain S14, and therefore, the carbon starvation response under these conditions was investigated. The Vibrio sp. strain S14 cultures that were grown in MMM supplemented with 0.15 g of glucose per liter stopped growing at an OD610 of 0.2. The growth arrest, which defined the onset of starvation, occurred immediately, and no deceleration phase could be distinguished (Fig. 1). No biomass increase was observed after the onset of starvation. Concomitantly, we observed abrupt decreases in the rates of total RNA synthesis and total protein synthesis (Fig. 1). As has been described previously for multiple-nutrient-starved Vibrio sp. strain S14 (47), the cells entered the second phase of starvation after 30 min, as demonstrated by the relative increases in the rates of macromolecular syntheses at that time. After 1.5 h of carbon deprivation, we observed a slow decrease in the total rate of protein synthesis that was continuous throughout the experimental period (Fig. 1). After 2 days the rate of leucine incorporation was less than 1% of the rate during growth (Table 1).

As Fig. 2A shows, the optical density decreased gradually during the starvation time which we studied. The level of viability was 100% for at least 5 days and thereafter decreased slowly (Fig. 2A). The time of onset and the rate of loss of viability varied slightly between the individual experiments, but the decrease in viability did not exceed 1 log after 9 days of starvation.

The decrease in the total cellular protein content was greatest during the first days, but the rate of decrease decelerated markedly after 4 days of starvation (Fig. 2B). The rate of degradation of total RNA, as determined by the orcinol method, was very low during the first 24 h and increased to about 1% h⁻¹ after 48 h of carbon starvation.
VOL. 174, 1992

RIBOSOMES IN A CARBON-STARVED MARINE Vibrio SP. 6783

FIG. 1. Effects of glucose deprivation on biomass (○) and the total rates of protein synthesis (■) and RNA synthesis (□). Cells were grown exponentially in minimal medium containing 0.15 g of glucose per liter until the carbon source was consumed and growth stopped. Biomass was determined by measuring OD 600. The rates of protein synthesis and RNA synthesis were determined as the rates of incorporation of [3H]leucine and [3H]uridine, respectively, and were compared with the rates prior to growth arrest, which were assigned values of 1.0. Representative data from one of two replicate experiments are shown.

Amount of ribosomes in carbon-deprived cells. We found that the loss of ribosomes during carbon starvation was a relatively slow process. The ribosome contents of the starving cell suspensions decreased during the first 3 days, with an apparent half-life of 79 h (Fig. 3). Subsequently, the rate of ribosome loss accelerated slightly (half-life, 40 to 50 h). The numbers of ribosomes per cell could be estimated by using the amounts of particulate RNA (Fig. 3), the molecular weight of the RNA in one E. coli ribosome (42), and the viability data (Fig. 2A). According to such calculations, the cells contained 20,000 to 35,000 ribosomes at the onset, 16,000 ribosomes after 24 h of starvation, and 8,000 ribosomes after 4 days of starvation.

The crude ribosome pellets used for these analyses contained only particulate RNA and insignificant amounts of potentially interfering substances, such as tRNA, polysaccharides, and DNA fragments. Thus, we expected that the orcinol method would give accurate measurements of the amounts of ribosomal particles. The validity of the data obtained from the chemical analysis of sedimentable RNA was further confirmed by the results of a density gradient centrifugation analysis of the P-150 preparations from cells that had been starved for various times. The amounts of particulate RNA that were loaded onto identical gradients were 109 μg for log-phase cells and cells that had been starved for 24, 48, and 72 h and 54.5 μg for cells that had been starved for 96 h. The absorbance profiles of a typical set of gradients are shown in Fig. 4. We detected no significant UV-absorbing material except the 30S and 50S particles. Integration of the 30S and 50S peaks demonstrated that the ratios of 30S particles to 50S particles ranged from 1:2.0 to 1:2.2 for all of the samples and that the sum of the areas in each gradient was directly proportional to the amount of RNA loaded (data not shown). Also evident from this analysis was the similarity in the shapes of the subunit peaks for all of the starvation times, which, in addition to the absence of particles other than 30S and 50S particles, indicated that abnormal ribosomal particles were not present.
in detectable amounts during the process of degradation (Fig. 4). In addition, the ratio of soluble RNA to particulate RNA (Table 2) indicated that only a minor fraction of the RNA from degraded ribosomal particles could have been present as soluble RNA. Throughout the starvation period, more than 70% of the total RNA was particulate, and there was only a minor, temporal increase in the amount of soluble RNA with greater starvation times (Table 2).

Polyribosomes during carbon starvation. Polyribosomes were not detected in cells that were starved for either 24 or 144 h (Fig. 5B and C). The 70S monosomes accounted for 88% of the ribosome population in the starved cells, while 12% of the ribosomes were 30S or 50S subunits: 65% of the ribosomes in exponentially growing cells sedimented as polyribosomes, 23% sedimented as 70S monomers, and 12% dissociated into subunits (Fig. 5A).

The protein synthesis activities in the different classes of poly-, di-, and monosomes were analyzed by adding a short pulse of [³H]leucine prior to inhibition of peptide chain elongation with chloramphenicol and polyribosome extraction. The short [³H]leucine pulse revealed that protein synthesis in the cells that were starved for a long time took place mainly on 70S monosomes (Fig. 5B and C). Although no polyribosomes were detected by absorbance measurements, we observed a low rate of protein synthesis in the size region of the small polysomes in the density gradients (Fig. 5B and C). The specific leucine incorporation activity (i.e., amount of hot TCA-precipitable radioactivity per absorbance unit) of the 70S ribosomes in the cells that were starved for 24 h was about 200-fold lower than the activity in the growing cells. During growth, the specific activities of the poly- and monosome fractions were approximately equal (data not shown).

The absence of polyribosomes in the starved cells was not due to the presence of RNase activity in the cell extracts. This was demonstrated by the results of control experiments in which radioactively labeled polysomes from exponentially growing cells were used as probes for such enzyme activity. The polysome profiles of growing cells were identical, whether the labeled cells had been extracted and analyzed separately or after mixing with unlabeled cells that had been starved for 24 or 144 h (Fig. 6).

**DISCUSSION**

In this study we found that *Vibrio* sp. strain S14 cells that have been deprived of exogenous carbon for prolonged periods of time retain ribosomes in a large excess over the apparent demand for translation. The rates of total protein synthesis decreased immediately at the onset of starvation (Fig. 1) and after 48 h was less than 1% of the rate of synthesis during growth (Table 1). The content of ribosomal particles decreased slowly, and after 72 h of starvation 52% of the ribosomes were still intact (Fig. 3). This is a significantly lower degradation rate than that reported previously for *E. coli* strains, which lose ribosomes and rRNA relatively rapidly during stationary phase and starvation (see references 10, 20, 30, 33, 41, and 51). Nath and Koch (41) reported that rRNA is degraded at a rate of about 10% h⁻¹ for at least 6 h during glucose, nitrogen, or phosphorus

![Graph 3](image3.png)

**FIG. 3.** Stability of ribosomes during carbon starvation. The amount of ribosomal particles per unit volume of starved cell suspension was determined by measuring the amount of particulate RNA that sedimented at 150,000 × gₘₐₓ. Means and standard deviations from at least six determinations are shown.

![Graph 4](image4.png)

**FIG. 4.** Density gradient analysis of ribosomal subunits from cells that were carbon starved for 0 h (A), 24 h (B), 48 h (C), 72 h (D), and 96 h (E). Crude ribosomal particles were dialyzed against buffer D (0.1 mM MgCl₂) and were sedimented through 5 to 20% sucrose density gradients. Either 109 μg (A through D) or 54.5 μg (E) of particulate RNA was loaded onto each gradient. The A₂₆₀ profiles were determined by pumping the contents of each gradient through a spectrophotometer. Fractions were collected starting from the bottom of the tubes. A small amount of radioactively labeled ribosomes from growing cells was applied to each gradient; these labeled 30S and 50S subunits did not affect the A₂₆₀ profiles and were detected by liquid scintillation, and their positions in the gradients are indicated.
TABLE 2. Distribution of RNA and protein in soluble and particulate fractions at different times during carbon starvation

<table>
<thead>
<tr>
<th>Length of starvation (h)</th>
<th>Soluble RNA*</th>
<th>Particulate RNA*</th>
<th>Soluble protein*</th>
<th>Particulate protein*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (µg ml⁻¹)</td>
<td>% of total RNA</td>
<td>Concentration (µg ml⁻¹)</td>
<td>% of total RNA</td>
</tr>
<tr>
<td>0</td>
<td>1.2</td>
<td>8.1</td>
<td>14</td>
<td>92</td>
</tr>
<tr>
<td>24</td>
<td>2.4</td>
<td>16</td>
<td>13</td>
<td>84</td>
</tr>
<tr>
<td>48</td>
<td>2.6</td>
<td>21</td>
<td>9.9</td>
<td>79</td>
</tr>
<tr>
<td>72</td>
<td>2.2</td>
<td>23</td>
<td>7.5</td>
<td>77</td>
</tr>
<tr>
<td>96</td>
<td>1.7</td>
<td>27</td>
<td>4.6</td>
<td>73</td>
</tr>
<tr>
<td>144</td>
<td>1.0</td>
<td>26</td>
<td>2.8</td>
<td>74</td>
</tr>
<tr>
<td>216</td>
<td>0.3</td>
<td>22</td>
<td>1.0</td>
<td>78</td>
</tr>
</tbody>
</table>

* Soluble RNA or protein was the RNA or protein that was not sedimented by centrifugation at 150,000 x g_{max} (S-150).
* Particulate RNA or protein was the RNA or protein that was sedimented by centrifugation at 150,000 x g_{max} (P-150).
* Micrograms per milliliter of starved cell suspension.
* The total RNA was the sum of the soluble and particulate RNA fractions.
* The total protein was the sum of the soluble and particulate protein fractions.

starvation. Okamura et al. (51) reported that more than 90% of labeled sedimentable ribosomes were lost after 6 h of carbon deprivation. However, two marine Vibrio spp. were recently reported to retain 10 to 26% of their original 16S rRNA for 15 days in stationary phase or during multiple-nutrient starvation (25).

Davis and coworkers (10) observed a correlation between the loss of ribosomes and the relatively rapid decrease in viability displayed by E. coli cells that had been deprived of phosphate. When 20 to 50% of the cells were viable, one-half or more of the nucleic acid, including the majority of the ribosomes, had been lost. Similar results were reported to have been obtained for carbon and nitrogen starvation as well. It was proposed that ribosome degradation is a major cause of cell death during starvation (10). The slow and continuous loss of ribosomal particles in carbon-deprived Vibrio sp. strain S14 is not paralleled by a decrease in viability, at least not for the first 5 days of starvation (this study).

Abnormal ribosomal particles were not observed during starvation of Vibrio sp. strain S14; such particles have been reported to be formed during magnesium starvation (36) and inhibition of protein synthesis (27) in E. coli. Ribosome degradation in carbon-starved Vibrio sp. strain S14 seems to occur in accordance with the model presented by Kaplan and Apirion (20), who suggested that after the initial disintegration of the ribosome subunits, further degradation of the rRNA proceeds rapidly to nucleotides. Wada et al. (59) have reported that during the transition of E. coli to stationary phase, a decrease in the overall rate of protein synthesis is accompanied by the dimerization of 70S ribosomes into 100S particles. These authors proposed that the 100S particles may be a storage form of unoccupied ribosomes and that they constituted 40% of the total ribosomal particles during stationary phase (59). Such ribosome dimers were not detected in the ribosome population after Vibrio sp. strain S14 cells were deprived of carbon (Fig. 5).

Polyribosomes were not detected by absorbance measurements of density gradients from carbon-starved cultures (Fig. 5). This finding is in accordance with observations of E. coli during short-term glucose starvation (12). Some active polysomes appeared to be present in the starved cells, however. Low protein synthesis activity was found in the polysome size region in the sucrose gradients (Fig. 5). The difference in the total rates of protein synthesis between growing and starved cells was reflected in the specific protein synthesis activity observed on the 70S ribosomes, which was approximately 200-fold lower after 24 h of starvation. This observation may be interpreted such that a small fraction (1/200) of the ribosomes were translating with the same rate as during growth, or that all ribosomes were elongating at a 200-fold-reduced rate. Naturally, an intermediate situation may exist. If nontranslating ribosomes are present, it is possible that they are bound to mRNA, and if they are, they may have paused in the process of initiation, elongation, or termination. The absence of polysomes in carbon-deprived cells indicates that either the availability of mRNA or the rate of translation initiation limits protein synthesis. The size of the mRNA pool during starvation is not known, but a starvation-induced increase in the mean mRNA stability and the presence of extremely long-lived starvation-specific messages have been reported previously for Vibrio sp. strain S14 (2). The initiation step or some step after initiation complex formation is believed to be the rate-limiting step in translation in exponentially growing cells (43). A similar situation may exist in carbon-starved Vibrio sp. strain S14, and a severe limitation at this step may explain the dearth of polyribosomes in such cells. By using a Vibrio sp. strain S14 cell-free protein-synthesizing system, the functional characteristics of the ribosomes in starved cells will be analyzed further in our laboratory.

The discussion about rate-limiting factors for protein synthesis in carbon-deprived cells is also relevant for understanding the recovery process. As mentioned above, multiple-nutrient-starved Vibrio sp. strain S14 cells respond instantaneously to nutrients by increasing the rate of protein synthesis. The length of the subsequent lag phase or maturation phase before DNA synthesis, cell division, and regrowth commence is dependent on the length of prestarvation (3). An ordered and sequential pattern of protein synthesis, as revealed by two-dimensional gel electrophoresis of pulse-labeled cells, was observed during the recovery process. This pattern included a set of maturation-specific (Mat) proteins (3). An understanding of the very fast initial response of the translation apparatus and how it is initiated may contribute to the elucidation of the sensing and signaling pathways involved in the induction of the Mat proteins.

The connection between the capacity for starvation survival and the characteristics of the translation machinery has recently been emphasized by Mikkola (37). Natural isolates of E. coli generally do not exhibit optimized ribosome performance characteristics as do commonly used laboratory strains and thus deviate from the optimization principle for rapid bacterial growth regarding ribosome efficiency and
mass investment in the translation apparatus (13, 29). It has been concluded that maximal bacterial growth rate and ribosome efficiency are not selected for in many natural bacterial populations. Natural isolates are also superior to laboratory strains in starvation survival, and a clear correlation has been demonstrated among slow loss of viability, slow growth, and slow ribosome phenotype. Interestingly, all of the natural isolates tested evolved the same characteristic as laboratory strains, including curtailed starvation resistance, after a few hundred generations in glucose-limited chemostats (37). As indicated by these data, the demands and selection pressures that operate on the translation apparatus may be very different in nongrowing starved cells compared with cells growing rapidly in laboratory media.

It has been suggested that the proportionality between bacterial growth rate and ribosome concentration can be exploited for autecological studies of the physiological status
of individual cells in environmental samples (see reference 42). The use of labeled probes against specific rRNA sequences and fluorescence microscopy should allow not only the detection of specific species or other taxa, but also estimates of growth rates in situ. Our data, as well as the data of Kramer and Singleton (26), have important implications for such analyses. Nongrowing carbon-starved bacterial cells contain significant amounts of ribosomal particles; in fact, they may harbor as many as 8,000 ribosomes per cell after 96 h of nongrowth (this study). This should be compared with the 6,700 ribosomes per cell in exponentially growing E. coli Br/ with a generation time of 100 min at 37°C (6). The natural E. coli isolates that had slow ribosomal performance characteristics and displayed a slow loss of viability during starvation could compensate for their lower ribosome efficiency by increasing ribosome concentrations during growth at low rates (37). Clearly, the relationships among physiological status, RNA and ribosome contents, ribosome stability, and starvation survival in natural bacterial isolates need further attention.

ACKNOWLEDGMENT

This study was supported by a grant from the Swedish Natural Science Research Council.

REFERENCES