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Assimilatory Sulfur Metabolism in Marine Microorganisms: Sulfur Metabolism, Protein Synthesis, and Growth of *Alteromonas luteo-violaceus* and *Pseudomonas halodurans* During Perturbed Batch Growth†

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The antibiotic protein synthesis inhibitor chloramphenicol specifically blocked the incorporation of [^{35}S]sulfate into the residue protein of two marine bacteria, *Pseudomonas halodurans* and *Alteromonas luteo-violaceus*. Simultaneous inhibition of total protein synthesis occurred, but incorporation of ^{35}S into low-molecular-weight organic compounds continued. *A. luteo-violaceus* rapidly autolyzed, with similar reduction in cell counts, total culture protein and cellular sulfur, whereas *P. halodurans* remained viable. Treatment with chloramphenicol, growth during nitrogen and carbon limitation, and the carbon and energy sources used for growth did not alter the sulfur content of *P. halodurans* protein. The mean value (1.09%, by weight), representing a wide variety of environmentally relevant growth conditions, was in agreement with model protein composition. The variability of cellular composition of *P. halodurans* and *A. luteo-violaceus* is discussed with respect to the measurement of bacterial growth in natural environments. Total carbon and nitrogen per cell varied greatly (coefficient of variation, ca. 100%) depending on growth conditions. Variation in total sulfur and protein per cell was much less (coefficient of variation, <50%), but the least variation was found for sulfate incorporation into residue protein (coefficient of variation, ca. 15%). Thus, sulfate incorporation into residue protein can be used as an accurate measurement of de novo protein synthesis in these bacteria.

The complex and undefined nature of dissolved organic compounds in aquatic ecosystems and the extreme breadth of metabolic capabilities of the bacteria found in any water sample have made quantitative measurement of bacterial growth difficult. It is generally understood that the measurement of mineralization of individual organic carbon compounds cannot accurately represent the cumulative metabolism of diverse assemblages of microorganisms. For this reason, some ecologists have turned to the measurement of sulfate uptake by natural plankton communities in freshwater ecosystems (14, 16, 21). It was believed that sulfate uptake by whole cells might provide a measure of total bacterial heterotrophy if (i) sulfate is the only sulfur source and (ii) a predictable relationship exists between sulfate uptake and carbon metabolism. The first assumption is, in principle, correct, but there is no a priori reason why carbon and sulfur metabolism should be closely related. The wide distribution of carbon in biological molecules and its importance in storage products often formed in quantity during unbalanced growth argue against an invariant relationship between carbon and sulfur metabolism.

Sulfur is an essential element in protein. The sulfur-containing amino acid cysteine is largely responsible for the tertiary structure and stability of proteins through involvement as disulfide linkages. Cysteine residues at or near the active site of many enzymes are also important in catalysis (1, 15, 18, 24). The other sulfur-containing amino acid, methionine, is important in hydrophobic interactions with other amino acids and membranes. Of the major elements of biomass in bacteria (i.e., C, H, O, N, P, and S), sulfur has the most restricted distribution in the biochemical sense. Except for traces of the sulfur-containing RNA base 4-thiouracil (5, 20), organic sulfur is found only in low-molecular-weight (LMW) soluble materials (amino acids, vitamins, and coenzymes) and protein (9, 25). Sulfur metabolism and protein synthesis must therefore be closely related.

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Unperturbed batch growth experiments with *Pseudomonas halodurans* and *Alteromonas luteo-violaceus* demonstrated that the incorporation of sulfate into residue protein accurately represented de novo protein synthesis (7a) in spite of substantial variations in total protein per cell during the exponential phase and the onset of the stationary phase. The potential of using this method as an assay for natural marine bacterial protein synthesis will be greater if nutritional and environmental perturbations do not significantly affect the calculation of protein synthesis rates from sulfate incorporation into protein. Therefore, the relationship between sulfur assimilation and protein synthesis was investigated for *P. halodurans* during environmentally relevant nutritional stress. The influence of a bacterial protein synthesis inhibitor, chloramphenicol (CAP), was monitored for both *P. halodurans* and *A. luteo-violaceus* to provide further evidence for the specificity of sulfur metabolism for protein synthesis.

MATERIALS AND METHODS

Organisms and culture conditions. *P. halodurans* was obtained from Galen E. Jones (University of New Hampshire, Durham). It is a halotolerant bacterium possessing a wide nutritional versatility. *A. luteo-violaceus* was isolated from Puerto Rico trench surface waters (6). It was identified on the basis of its characteristic violet pigment, limited nutritional versatility, extracellular protease production, and DNA base composition (12). The organisms were grown and maintained in RLC-water (6), an artificial seawater with relatively low contamination of sulfate. The complete medium used for routine culture maintenance and growth contained 10 mM sodium glutamate, 1 mM sodium sulfate, 500 μM ammonia, 40 μM potassium phosphate, and trace elements (11). Medium was usually inoculated with late-exponential- or early-stationary-phase cells at a final density of 1×10^4 to 5×10^4 cells ml^{-1}. Washed cell suspensions in RLC-water were used as inocula when necessary to avoid nutrient carry-over. The cultures were incubated at room temperature (20 to 23°C) on a Gyrotory shaker (New Brunswick Scientific Co., Edison, N.J.) at 250 rpm.

Sulfate incorporation during growth of *P. halodurans* on various carbon and energy sources was measured as follows: 1 liter of RLC-water containing inorganic nutrients (6) and 1 mM $Na_2{}^{35}SO_4$ (2 dpm $pmol^{-1}$) was inoculated with exponentially growing cells to a final density of about 3×10^4 cells ml^{-1}. Aliquots of 100 ml were aseptically transferred into sterile flasks and supplemented with sterile concentrated stock solutions to give a final concentration of 10 mM (acetate, citrate, fructose, glucose, lactate, pyruvate, and glutamate) or 207 mM (ethanol). Samples filtered at intervals for total ^{35}S were used to determine the growth rate. During exponential growth and again in the stationary phase, samples were collected for total protein, direct counts, and ^{35}S distribution (7a).

Fractionation of radioactively labeled cells. The procedure for separation of major biochemical fractions has been modified from the method of Roberts et al. (25) only in the use of glass fiber filters as an added pellet and site for the adsorption of precipitated macromolecules. In brief, it is a serial extraction procedure in which a filtered sample containing radioactively labeled cells is treated with cold 10% trichloroacetic acid (TCA), warm 80% ethanol and 80% ethanol–diethyl ether (1:1), and hot 10% TCA. The extracts from each step are centrifuged ($5,000 \times g$, 20 min), the residue is rinsed with fresh solvent and centrifuged, and like supernatants are combined. The respective extracts contain the following: LMW compounds; lipids plus alcohol-soluble protein; a complex mixture of hot acid-soluble materials containing RNA, DNA, and acid-labile polymers; and residue protein (25). Lipids and alcohol-soluble protein were separated by addition of water to effect phase separation, removal of the organic phase, and washing of the aqueous phase once with ether. The ethanol-ether solution was always separated into lipids and alcohol-soluble protein for ^{14}C-labeled samples and on occasion for ^{35}S-labeled samples, but lipid sulfur was always negligible (<2% of the total S). Inorganic sulfate was separated from LMW organic sulfur by precipitation with $BaCl_2$ in the acid extract.

Other methods. Protein was measured on the 10% TCA-insoluble material precipitated directly from the cell suspension and on the residue protein by the method of Bradford (3), using bovine serum albumin as a standard. Direct counts were made by epifluorescence microscopy according to Daley and Hobbie (8). Viability was determined by plating on complete medium containing 1.5% agar. All radioisotopic samples were filtered onto Whatman GF/F filters, using a punch funnel designed to reduce radioisotope adsorption blanks (6). Samples in Aquasol were counted in a Beckman LS-100C liquid scintillation counter, using the channels ratio method of quench correction.

RESULTS

Effect of CAP on sulfur incorporation and protein synthesis. The high percentage of the total cellular sulfur in protein (7a, 7b, 25) indicated that this was the major sink for reduced sulfur. Abrupt termination of protein synthesis through the action of an external agent (e.g., antibiotic protein synthesis inhibitors) can provide information on both the incorporation of sulfur into protein and the regulation of LMW organic sulfur pools. In addition, the use of an inoculum previously labeled with ^{35}S to equilibrium provided information on events during the lag phase of growth resulting from dilution of a stationary-phase culture into fresh medium. During the first 2 h of incubation, no change in viable or direct cell counts was observed with *P. halodurans*, but total protein increased, accompanied by sulfate incorporation into all fractions (Fig. 1). Since the LMW organic sulfur and the protein sulfur together constituted over 90% of the total sulfur, only these fractions will be shown in this and subsequent experiments. Total protein increased by 60% and total sulfur increased by 90% during the lag phase. Sulfur

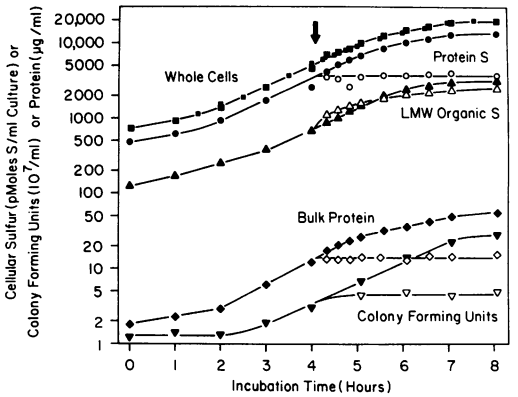

FIG. 1. Effects of CAP on growth, protein synthesis, and total uptake and distribution of sulfur in major biochemical fractions of *P. halodurans*. Cells were grown in complete medium containing 10 mM glutamate and 1 mM $Na_2{}^{35}SO_4{}^{2-}$ (final specific activity, 9 dpm pmol^{-1}) for 10 generations to obtain isotopic equilibrium. Shortly after the onset of the stationary phase, fresh medium of the same composition was inoculated to a final density of about 10^7 cells ml^{-1}. Samples were withdrawn for direct and viable cell counts, total (bulk) protein, and fractionation into major biochemical components. At 245 min (arrow), the culture was divided into two portions, one receiving CAP (25-μg ml^{-1} final concentration) and the other receiving an equal amount of distilled water as a placebo; samples were taken for the same parameters for an additional 8 h. Symbols: (open) CAP treated; (closed) control culture.

incorporation and bulk protein synthesis then proceeded with doubling times characteristic of growth in this medium, and cell division resumed shortly thereafter. Growth rates determined from increases in total protein (1.06 h^{-1}), total sulfur uptake (0.91 h^{-1}), and direct cell counts (0.91 h^{-1}) were similar.

The culture was split in the midexponential phase, and one portion was treated with CAP (25-μg ml^{-1} final concentration). An identical set of samples was taken from each flask at appropriate intervals. An effect was exerted specifically on total protein synthesis and incorporation of sulfate into protein (Fig. 1). Incorporation of sulfate into LMW organic sulfur compounds did not respond in the same fashion. In fact, the initial uptake of sulfate into this fraction was greater for CAP-treated cells than for the control culture. These effects were also manifest in the distribution of sulfur between the protein and LMW fractions. Table 1 shows that the proportion of the total S in the LMW organic sulfur fraction doubled as a result of CAP treatment.

It was not practical to take samples for direct counts and total protein at the exact time of CAP addition, but the onset of inhibition may be deduced from the intersection of the line describing the mean value of the CAP-treated total protein and residue protein-S, with the regression line delineating the same parameters in the control culture. The line drawn for the mean value of residue protein-S in the CAP-treated culture intercepted the regression line for the control culture at 2 min after CAP addition. The effects of CAP on total protein synthesis also took place 2 min after addition. In contrast, the colony-forming-unit average intercepted the control at 24 min, suggesting that cells near to division were competent to complete the process. That the colony-forming units remained constant verifies the reversible, bacteriostatic nature of this antibiotic.

The data for the LMW organic sulfur component shown in Fig. 1 and Table 1 demonstrated a rapid initial pooling in this fraction. A much clearer view of the phenomenon may be obtained by comparing the size of the LMW organic sulfur pool of control and CAP-treated cultures on a per-cell basis (Fig. 2). The LMW organic sulfur content of *P. halodurans* in the control culture remains relatively constant during the exponential growth phase, but addition of CAP results in a linear increase in pool size to a value over twice that of the control cells.

If only whole-cell uptake data had been taken, the action of CAP would have appeared to be

TABLE 1. Total uptake of $^{35}SO_4{}^{2-}$ and its distribution in biochemical fractions of *P. halodurans*: effects of CAP[a]

Expt	Incubation (min)	S pmol ml^{-1}	% of total radioactivity			
			LMW organic	Alcohol-ether soluble	Hot TCA soluble	Residue protein
Control	240	4,484	14.8	24.0	4.3	56.9
	305	9,673	14.8	14.1	2.5	68.6
	485	19,136	16.4	13.0	2.6	68.0
+CAP	305	6,363	23.8	17.2	3.6	55.4
	485	7,563	31.9	16.5	2.9	48.7

[a] Data from Fig. 1.

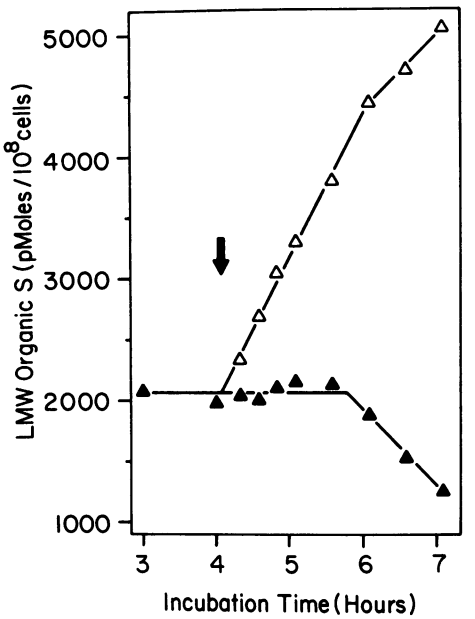

FIG. 2. Effects of CAP on the LMW organic sulfur pool in *P. halodurans*. Experimental details are given in the legend to Fig. 1. Cell density was taken from the regression line through the direct cell count data when no actual count was made. The arrow indicates the time of CAP addition. Symbols: (△) CAP treated; (▲) control culture.

rapid but incomplete; during the treatment period a net increase in total cellular sulfur of 68% occurred in CAP-treated cells. This represents only 79% inhibition relative to the control culture. CAP had no effect on the relationship between residue protein-S and total protein (Table 2), but the total sulfur per cell increased as a result of the inhibition of protein synthesis.

The response of *A. luteo-violaceus* to CAP was strikingly different from that of *P. halodurans* and suggested the presence of a powerful extracellular proteolytic activity associated with this microorganism. The addition of CAP to a midexponential-phase culture resulted not only in rapid blockage of protein synthesis, but also in cell autolysis and protein hydrolysis (Fig. 3). During the first 30 min of CAP treatment residue protein sulfur and total protein remained constant, whereas a rapid incorporation of sulfate into LMW organic compounds occurred, as observed for *P. halodurans*. By 60 min of CAP treatment, however, all components had begun to decline, and the viable counts had dropped to $<5 \times 10^5$ cells ml^{-1}. Autolysis was indicated by a decrease in direct cell counts and a visual increase in the viscosity of the culture medium. Autolysis could not explain the loss of total protein from the culture because the analysis of protein was carried out on TCA-precipitable material in the cell suspension rather than on filter-retainable material. The percentage of the total sulfur incorporated into protein remained high throughout CAP treatment because the loss of sulfur-containing compounds was uniform among the fractions. The sulfur distribution at 15 and 60 min of CAP treatment was identical, although the total sulfur was >30% less by 60 min.

Growth on various carbon and energy sources. In the natural environment, microorganisms are exposed to a large array of dissolved organic carbon compounds which may be used for growth and energy requirements. Therefore, the influence of the growth substrate on the sulfur content of protein was investigated. Eight substrates which supported growth of the organism were used, with growth rates ranging from 0.44 to 1.03 h^{-1}. The distribution of sulfur in the major biochemical fractions during growth on these compounds was identical within experimental error. There was no consistent influence of growth substrate on the residue protein-S–

TABLE 2. Cell number, protein, and sulfur relationships for *P. halodurans* during perturbed batch growth[a]

Expt	*n*	Per 10^8 cells		Protein-S (wt %) (protein-S/ total protein)
		Total protein (μg)	Total sulfur (ng)	
CAP				
Control	14	26.1 ± 9.6	325.9 ± 112.2	0.83 ± 0.08
CAP treated	9	31.2 ± 2.9	501.5 ± 39.4	0.81 ± 0.07
Carbon limited	10	18.6 ± 7.3	221.2 ± 69.2	0.89 ± 0.10
Multiple carbon sources				
Exponential phase	8	23.9 ± 7.8	291.0 ± 87.4	0.91 ± 0.05
Stationary phase	7	12.3 ± 4.3	157.7 ± 53.4	0.94 ± 0.06

[a] Experimental details are given in the text. Data are the mean ± 1 standard deviation for the indicated number of analyses.

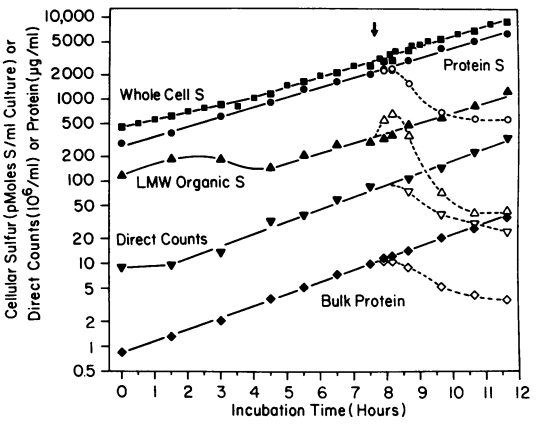

FIG. 3. Effects of CAP on growth, protein synthesis, and total uptake and distribution of sulfur in major biochemical fractions of *A. luteo-violaceus*. Cells were grown in complete medium containing 10 mM glutamate and 100 µM $Na_2{}^{35}SO_4{}^{2-}$ (final specific activity, 10 dpm $pmol^{-1}$) for 10 generations to obtain isotopic equilibrium. Shortly after the onset of the stationary phase, fresh medium of the same composition was inoculated to a final density of about 9×10^6 cells ml^{-1}. Samples were withdrawn for direct and viable cell counts, total (bulk) protein, and fractionation into major biochemical components. At 460 min (arrow), the culture was divided into two portions, one receiving CAP (25-µg ml^{-1} final concentration) and the other receiving an equal amount of distilled water as a placebo; samples were taken for the same parameters for an additional 8 h. Symbols: (open) CAP treated; (closed) control culture.

total protein relationship (Table 2) in either late-exponential-phase or stationary-phase cultures.

A surprising result of the growth of *P. halodurans* on various carbon and energy sources was that the final total protein content of the cultures, using non-nitrogen-containing compounds, was strikingly similar (23.1 ± 1.4 µg ml of culture^{-1}), as were the final values for total sulfur (296.6 ± 9.4 ng of S ml of culture^{-1}). Routine culture of *P. halodurans* was carried out in medium containing 500 µM ammonia and 10 mM sodium glutamate, with an effective nitrogen concentration of 10.5 mM and final protein concentrations reaching 80 µg ml^{-1}. With a protein-N content of 17% by weight (17), the final protein in the low-nitrogen cultures contained almost 60% of the added ammonia-N. Since nitrogen is an important component of nucleic acids and LMW compounds, it is likely that the higher-density sample of cultures grown with 500 µM available N had reached a nitrogen-limited stationary phase. A later experiment with glucose as the carbon and energy source verified that nitrogen limits the amount of protein production at 500 µM N, although the cell numbers attain nearly the normal stationary-phase density, leading to markedly decreased protein per cell (data not shown).

Influence of carbon and energy limitation. Carbon and energy sources are often thought to be major growth-limiting factors for bacteria in marine environments due to the naturally low concentrations of labile dissolved organic compounds in seawater. Figure 4 shows total, LMW, and protein-S as well as direct counts and total protein for three points in exponential growth and several hours of a stationary phase induced by carbon limitation. At 9.5 h, the total carbon metabolized was 4.70 mM (as carbon, determined from radioisotope metabolism), equal to 93.9% of the total available glutamate.

It was again observed that residue protein-sulfur and total protein synthesis declined in rate about 1 to 1.5 generations before the very abrupt cessation of cell division. Protein sulfur was the dominant sulfur-containing fraction, and the distribution of both carbon and sulfur in the major biochemical fractions was similar to that for normal batch growth (7b). The number of dividing pairs of cells observed during the direct

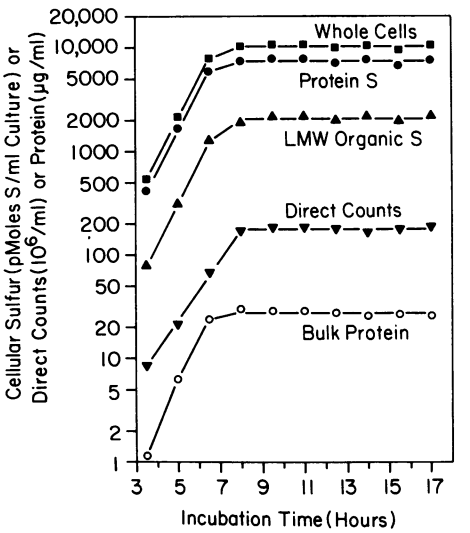

FIG. 4. Growth, protein synthesis, and total uptake and distribution of sulfur in major biochemical fractions of *P. halodurans* during a carbon-limited stationary phase. An overnight culture in complete medium containing 1 mM glutamate and 250 µM Na_2SO_4 was inoculated into 1,500 ml of fresh medium at a final density of about 2×10^6 cells ml^{-1}. After shaking for 10 min, the culture was aseptically divided into three portions: individual flasks received $Na_2{}^{35}SO_4{}^{2-}$ (final specific activity, 5 dpm $pmol^{-1}$), [*U*-^{14}C]glutamic acid (final specific activity, 285 dpm $nmol^{-1}$), or distilled water. Samples were withdrawn for direct counts, total (bulk) protein, and distribution of ^{14}C and ^{35}S.

counts declined continuously from 11% at 3.5 h of incubation to 0% at 9.5 h.

Total sulfur and total protein per cell (Table 2) were much lower than the values for normal batch growth, but remain quite stable during more than 7 h of the carbon-limited stationary phase. The weight percent sulfur was very similar to the values reported in the previous experiment and also showed no influence of carbon limitation (Table 2). The C/S (101.5 ± 76.7) and C/N (3.06 ± 0.20) ratios agreed with values from unperturbed batch growth (Cuhel et al., in press). The metabolism/incorporation ([incorporation + respiration] [incorporation]$^{-1}$ of [*U*-^{14}C]glutamic acid) ratio (2.39 ± 0.28) was 24% higher, possibly reflecting continued respiration to meet basal energy requirements at the expense of endogenous carbon compounds, but no effect of carbon limitation was noted on the distribution of ^{14}C during the stationary phase (data not shown).

A more complete tabulation of the data from these experiments has been published (R. L. Cuhel, Ph.D. thesis, Woods Hole Oceanographic Institution, Woods Hole, Mass., 1981).

DISCUSSION

Virtually all of the sulfur metabolism in *A. luteo-violaceus* and *P. halodurans* is concerned with the production and utilization of protein precursors. This fact is clearly demonstrated by the results of protein synthesis inhibition by CAP in *P. halodurans*, which produced an absolute cessation of both total protein synthesis and incorporation of sulfur into residue protein virtually instantaneously. The elimination of product formation results in a greater than twofold increase in the size of the LMW organic sulfur pool. The same pattern of events occurs in *A. luteo-violaceus* during the first few minutes of CAP treatment, before autolysis begins.

The results of the investigation of the flow of sulfur through major biochemical fractions relative to cell growth and protein synthesis strongly support the applicability of sulfur incorporation studies to marine bacteria in natural habitats. The only factor found to cause a significant alteration in the sulfur content of the total protein in *P. halodurans* or *A. luteo-violaceus* is variation in the external sulfate concentration (7a), but this variable is not encountered in seawater. Figure 5 shows the relationship between sulfate incorporation into residue protein and total (bulk) protein in *P. halodurans* for all experiments with sulfate concentrations of >500 μM. Although nitrogen-limited cultures have values above the regression line, the controls for the experiment also have unusually high residue protein/total protein ratios, reflecting a small degree of variability in the measurement. The

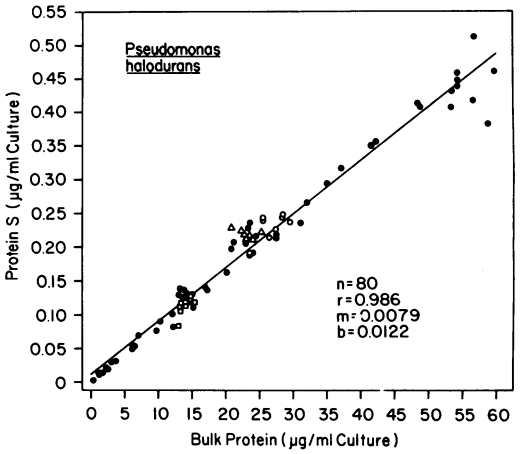

FIG. 5. Residue protein sulfur-total (bulk) protein relationship for *P. halodurans*. Data are shown for all cultures with sulfate concentrations of >500 μM. Symbols: (●) unperturbed batch growth and stationary phases; (△) nitrogen-limited cultures; (□) CAP-culture; (○) carbon-limited culture.

residue protein/total protein ratio does not describe the true sulfur content of protein because some protein and protein-S are solubilized by alcohol and hot TCA. This ratio is an operational one, i.e., the measurement which can be made with natural populations. The relationship between residue protein-S and total protein is similar for *A. luteo-violaceus* (Fig. 6), but problems encountered with protease activity in stationary phases prevented measurement of the ratio over as wide a set of conditions as those used with *P. halodurans*.

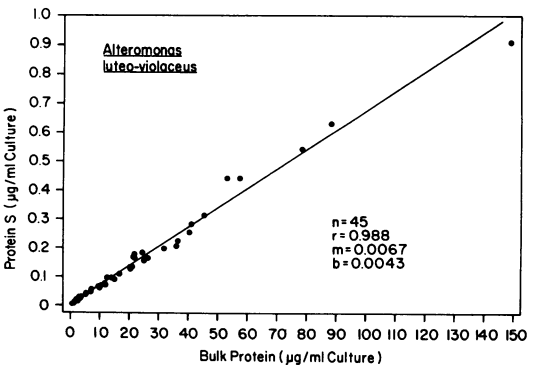

FIG. 6. Residue protein sulfur-total (bulk) protein relationship for *A. luteo-violaceus*. Data are shown for all cultures with sulfate concentrations of >100 μM. The correlation coefficient *r* is given for the linear regression of *n* points with calculated slope *m* and *y*-intercept *b*.

TABLE 3. Variability in cellular composition of *P. halodurans* and *A. luteo-violaceus*[a]

Parameter	*P. halodurans*				*A. luteo-violaceus*			
	n	Mean	CV (%)	Range	*n*	Mean	CV (%)	Range
μg 10^8 cells^{-1}								
Particulate organic carbon	28	18.8	84.7	3.6–62.4	21	19.3	104.7	3.4–71.5
Particulate nitrogen	28	5.4	104.1	0.9–23.2	21	4.9	91.4	1.1–15.4
Total protein	162	23.8	48.7	3.6–66.0	79	12.9	37.8	4.2–28.9
Total ^{14}C	23	31.5	51.3	12.3–63.7	23	8.1	29.1	3.6–14.3
Total ^{35}S	110	0.293	44.2	0.104–0.606	80	0.106	29.6	0.048–0.209
C/N (weight/weight)	28	3.8	19.9	2.7–5.1	21	3.8	21.0	2.8–6.0
C/S (weight/weight)	79	97	57.7	45–429	46	89	9.2	73–112
(g g of protein^{-1}) × 100								
Particulate organic carbon	24	96	91.1	22–466	20	83	47.6	46–226
Particulate nitrogen	26	37	178.5	6–324	20	23	59.8	13–75
Total ^{14}C	26	124	36.9	79–280	14	82	17.2	62–110
Total ^{35}S	95	1.20	16.2	0.78–1.73	59	0.94	25.4	0.53–1.65
TCA-insoluble ^{35}S	95	1.00	12.4	0.74–1.26	59	0.78	18.1	0.48–1.20
Protein ^{35}S[b]	80	0.86	14.3	0.64–1.13	45	0.72	15.9	0.57–1.09
Protein ^{14}C	18	58.0	15.8	49.2–79.9	14	46.4	7.1	40.6–53.5
Protein ^{35}S (residue protein)$^{-1}$[c]	60	1.07	15.6	0.80–1.45	10	0.92	15.9	0.75–1.20
Protein ^{14}C (residue protein)$^{-1}$[c]	15	61.2	22.5	48.9–88.3	6	68.6	3.8	65.6–72.1

[a] Combined data from all experiments. Particulate carbon and nitrogen data are from Perkin-Elmer CHN analyses. CV, Coefficient of variation.

[b] Data for cultures with sulfate concentrations of >500 μM (*P. halodurans*) or 100 μM (*A. luteo-violaceus*).

[c] Normalized to the amount of protein in the fractionation residue.

The work in this paper emphasizes the relative constancy of the residue protein-S/total protein ratio because of its potential application to the measurement of protein synthesis in natural marine bacterial populations. During the course of the study, other parameters indicative of nutritional status were measured as well to determine the variability of cellular composition as a function of environmental conditions. The concurrent measurement of cell counts, protein, ^{14}C and ^{35}S assimilation, and particulate organic carbon and nitrogen permits the calculation of a number of ratios relevant to interpretation of microbial growth studies.

Variability in cellular composition is summarized for both *P. halodurans* and *A. luteo-violaceus* in Table 3. These data combine the results presented in this paper with those for unperturbed batch growth and sulfur-limited growth (7a, 7b). Total ^{14}C data were only obtained during unperturbed batch growth experiments and delineate the relatively high degree of variability during normal growth. Particulate organic carbon and nitrogen, a measurement commonly made in phytoplankton studies, exhibited a 20-fold range of values in both organisms, with standard deviations nearly equal to or greater than the mean. The variation is predictable: most is a result of sulfur starvation and indicates storage of carbon reserves. It is well known that nitrogen limitation leads to the accumulation of carbon reserve polymers in bacteria (10, 13, 26) and algae (19). Starvation for sulfur results in even greater glycogen storage in bacteria (2). Accumulation of carbon and nitrogen is also observed for both *P. halodurans* and *A. luteo-violaceus* during the stationary phase if carbon and energy sources are abundant.

The protein content of *P. halodurans* fluctuates considerably, ranging over twofold during unperturbed exponential growth (7b). This characteristic is not shared by *A. luteo-violaceus*. The highly variable protein content of *P. halodurans* is emphasized by the >18-fold range of values, compared with <7-fold in *A. luteo-violaceus*. Nonetheless, the coefficient of variation is much less for total protein per cell than for either particulate carbon or nitrogen.

The variation in total cellular sulfur is very similar to that for total protein. The slightly lower coefficient of variation is a result of sulfur limitation experiments in which synthesis of sulfur-deficient protein occurred. Excluding these data, the agreement in the coefficients of variation for total protein and sulfur confirm the close relationship between protein synthesis and whole-cell sulfate uptake. However, there is still substantial variability due to fluctuating LMW pool size and divergent rates of cell division and protein synthesis. Therefore, whole-cell sulfate uptake is not a good measurement of growth in terms of cell numbers.

Whole-cell sulfate uptake has been used in freshwater ecosystems in the hope of finding a method for estimating bacterial carbon production for inclusion in carbon budgets (4, 14, 16, 21). However, laboratory studies demonstrated a 65-fold range in C/S uptake ratios (23), preventing unambiguous interpretation of carbon assimilation values derived from sulfate uptake studies (22). Indeed, the C/S ratio for *P. halodurans* (Table 3) spans the range of purportedly constant C/S ratios of 50:1 (14) to 500:1 (21) during exponential growth alone. A much smaller range is observed for *A. luteo-violaceus* because cell division and protein synthesis are more tightly coupled. The mean C/S ratios are similar and agree with the mean of 107 determined by Jordan and Peterson (16) for five freshwater bacteria grown in chemostats. It is clear that nonideal growth must be taken into account before applying a mean value derived from steady-state growth to a bacterial assemblage in a variable environment.

The physiology of bacteria growing in variable environments precludes the use of a simple relationship between whole-cell carbon and sulfur metabolism. If protein synthesis can be accepted as a useful index of bacterial growth, a more readily interpreted relationship becomes apparent (Table 3). A large array of nonprotein carbon and nitrogen compounds in bacteria is indicated by the great variability of these elements with respect to protein. The variability of total cellular sulfur per unit of protein is the smallest of the three major elements of biomass studies (^{14}C data are again for exponential growth only). All macromolecular sulfur parameters have a similar degree of variation when normalized to protein. The measurement of ^{35}S incorporation into cold 10% TCA-insoluble material therefore provides a simple and reliable method for quantitative protein synthesis measurement in pure cultures, with much greater sensitivity than chemical assays.

Finally, the data obtained in this study are in agreement with the model protein of Jukes et al. (17), which indicates a sulfur content of 1.1% in protein. The true weight percent S in protein, i.e., the residue protein-S/residue protein ratio, is 1.07 for *P. halodurans* and 0.92 for *A. luteo-violaceus*. Since the model protein composition is derived from numerous sources from all kingdoms, the agreement of the sulfur content of protein for the two marine bacteria examined strongly suggests that the relationship described above will be valid for natural assemblages of marine bacteria. This generalization was tested in a survey of the sulfur content of protein for a number of marine bacterial isolates and mixed populations representing diverse nutritional and taxonomic groups (7).

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