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Effect of Chloride and Glutamate Ions on In Vitro Protein Synthesis by the Moderate Halophile *Vibrio costicola*

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Vibrio costicola **grown in the presence of different NaCl concentrations contains cell-associated Na^+ and K^+ ions whose sum is equal to or greater than the external Na^+ concentration. In the presence of 0.5 M NaCl, virtually no in vitro protein is synthesized in extracts of cells grown in 1.0 M NaCl. However, we report here that active in vitro protein synthesis occurred in 0.6 M or higher concentrations of Na_2SO_4, sodium formate, sodium acetate, sodium aspartate, or sodium glutamate, whereas 0.6 M NaF, NaCl, or NaBr completely inhibited protein synthesis as measured by polyuridylic acid-directed incorporation of [^{14}C]phenylalanine. Sodium glutamate, sodium aspartate, and betaine (0.3 M) counteracted the inhibitory action of 0.6 M NaCl. The cell-associated Cl^- concentration was 0.22 mol/kg in cells grown in 1.0 M NaCl. Of this, the free intracellular Cl^- concentration was only 0.02 mol/kg. Cells contained 0.11 mol of glutamate per kg and small concentrations of other amino acids. All of the negative counterions for cell-associated Na^+ and K^+ have not yet been determined. In vitro protein synthesis by *Escherichia coli* was inhibited by sodium glutamate. Hybridization experiments with ribosomes and the soluble (S-100) fractions from extracts of *E. coli* and *V. costicola* showed that the glutamate-sensitive fraction was found in the soluble, not the ribosomal, part of the system. The phenylalanyl-tRNA synthetase of *V. costicola* was not inhibited by 0.5 M or higher concentrations of NaCl; it was slightly more sensitive to high concentrations of sodium glutamate. Therefore, this enzyme was not responsible for the salt response of the *V. costicola* in vitro protein-synthesizing system.**

The moderately halophilic bacterium *Vibrio costicola* contains substantial amounts of cell-associated Na^+ and K^+ ions when grown in media containing a wide range of NaCl concentrations; generally, the sum of these cell-associated ions is at least equal to the extracellular Na^+ concentration (17). However, when Wydro et al. (20) measured in vitro protein synthesis by extracts of this organism grown in the presence of 1.0 M NaCl, at different concentrations of NaCl, KCl, NH_4Cl, or mixtures of NaCl and KCl, they found that maximal activity occurs with about 0.2 M salts. However, with 0.5 M salts, virtually no in vitro protein synthesis takes place. Thus, it seemed to Wydro et al. that the salt concentrations present in the cell would prevent protein synthesis from taking place.

Wydro et al. (20) tacitly assumed that Na^+ and K^+ within *V. costicola* would be accompanied by Cl^- as the major counterion, which occurs in the extreme halophiles (4). This assumption, as should have been known at the time, was not justified.

In the present study, we determined the conditions under which high levels of protein synthesis could take place in extracts of *V. costicola*. These experiments gave us some clues to the internal environment in these bacteria. We made further measurements of cell-associated organic and inorganic anions that showed which anions were largely excluded, although not all that were present.

MATERIALS AND METHODS

Bacterial cultures. *V. costicola* NRC 37001 was grown with shaking at 27 to 28°C in a Fernbach flask containing 1/5 volume of PPT medium (1% Proteose Peptone no. 3 [Difco Laboratories], 1% Tryptone [Difco], 1 M NaCl [pH 7.0, without adjustment]). Cells at late exponential phase were harvested, washed with an isotonic washing buffer (1.2 M NaCl, 8 mM KCl, 0.41 mM $MgCl_2$, 0.05 M Tris [pH 7.5, adjusted with HCl]), and stored at −70°C.

Escherichia coli Q13 (Hfr *met tyr* RNase I^- $PNPase^-$) was grown in L broth (1% tryptone, 0.1% yeast extract, 0.5% NaCl) at 37°C until mid-exponential phase, harvested, washed with a buffer containing 60 mM NH_4Cl, 10 mM magnesium acetate, 6 mM 2-mercaptoethanol, and 10 mM Tris-hydrochloride (pH 7.8), and stored at −70°C.

Halobacterium halobium NRC 34003 was grown at 37°C in Sehgal and Gibbons complex medium (10) containing 25% (4.3 M) NaCl, harvested at late exponential phase, and subjected to analysis of Cl^- content as described below.

Preparation of S-30 extracts. The frozen cell pellet (3 to 5 g) of *V. costicola* was thawed on a prechilled mortar, ground with twice its weight of alumina for 15 min, and extracted with 1 volume of an extraction buffer (120 mM NH_4Cl, 20 mM $MgCl_2$, 6 mM 2-mercaptoethanol, 3 mM spermidine, 10 mM Tris-hydrochloride [pH 7.5]) per g of pellet. RNase-free DNase I (Worthington Diagnostics) (final concentration, 3 μg/ml) was added to the ground material, which was centrifuged at 8,000 × g_{max} for 10 min; the supernatant was then centrifuged at 30,000 × g_{max} for 30 min at 4°C. The supernatant was dialyzed against 2 liters of extraction buffer for 2 h at 4°C. After dialysis, the cell extract was centrifuged at 30,000 × g_{max} for 30 min at 4°C to remove any insoluble material and stored in 0.5-ml aliquots at −70°C. The S-30 fraction from *E. coli* Q13 was extracted and dialyzed with a buffer containing 60 mM NH_4Cl, 10 mM magnesium acetate, 6 mM 2-mercaptoethanol, 3 mM spermidine, and 10 mM Tris-hydrochloride (pH 7.8).

Protein content determination. Samples were treated with cold acetone-methanol (5:2) (2), and their protein contents were determined by the procedure of Lowry et al. (12), using bovine serum albumin as a standard. The concentration of protein in S-30 preparations from *V. costicola* was 31 to 33 mg/ml. Absorbance at 260 nm was 300 to 350. The concentration of protein in *E. coli* S-30 was 63 mg/ml.

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Preparation of ribosome and S-100 fractions. Ribosomes were separated by centrifuging the S-30 fraction at 105,000 × g_{max} for 3 h. The upper two-thirds of the supernatant was aspirated carefully and dialyzed at 4°C against the extraction buffer. After dialysis, insoluble materials were removed by centrifugation to obtain a clear S-100 fraction, which was stored at −70°C in 0.5-ml aliquots. The crude ribosomal pellet was suspended gently in the same buffer and centrifuged again at 105,000 × g_{max}. The washed ribosomes were resuspended at a concentration of 83 mg/ml of the buffer and kept at −70°C.

Assay of in vitro protein synthesis activity. The reaction mixture (0.4 ml) contained 15 mM phosphoenolpyruvate, 2 mM ATP, 1 mM GTP, 124 mM NH_4Cl, 18 mM Mg^{2+}, 7.5 mM reduced glutathione, 82 mM Tris-hydrochloride (pH 7.6), 1.2 mg of polyuridylic acid [poly(U)] per ml, 1.96 μM [^{14}C]phenylalanine, various concentrations of salts as indicated below, and 0.2 volume (usually 80 μl) of S-30. In some experiments, 70 μl of S-100 and 10 μl of ribosome preparation were substituted for 80 μl of S-30. Incubation was at 30°C, and at 0, 1, 3, 5, 7, and 9 min, a 50-μl aliquot was spotted onto Whatman 3MM filter paper (12.5-cm diameter, divided into 2.2-cm squares) placed above a hot plate to accelerate evaporation of water. After drying, the filter paper was put into 10% trichloroacetic acid (TCA), heated at 90 to 95°C for 15 min, and washed with 5% TCA on a Büchner funnel. The paper was cut into squares and dried at 105°C for 1 h, and radioactivity was counted in 5 ml of ScintiVerse 1 (Fisher Scientific Co.) (7). Usually, incorporation of [^{14}C]phenylalanine into hot TCA-insoluble material reached a plateau after 7 min of incubation. Counts per minute after 9 min of incubation were used for calculation of amounts of [^{14}C]phenylalanine incorporated. Control experiments showed no difference between radioactivity trapped as TCA-insoluble material by this technique and that by the conventional test tube method (termination of reaction by the addition of TCA, heating, and filtration through a Millipore membrane filter).

Pyruvate kinase from rabbit muscle, used in previous studies (20), is activated by 0.1 M KCl or NH_4Cl (9). We found that in vitro protein synthesis in the absence of added muscle pyruvate kinase was about 80 to 90% of that in its presence. The enzyme was omitted in the experiments reported here.

Poly(U) (sedimentation coefficient [s_{20}], 8.2) was a product of Miles Laboratories. L-[^{14}C]phenylalanine (uniformly labeled, 510 mCi/mmol) and L-[^{14}C]leucine (uniformly labeled, 333 mCi/mmol) were obtained from New England Nuclear Corp.

Assay of phenylalanyl-tRNA synthetase activity. Whatman no. 3 filter paper (12.5-cm diameter with 2.2-cm squares) was soaked in 10% TCA solution and dried overnight at room temperature. The enzyme reaction was performed in a mixture of the same composition as that for protein synthesis except that 1.2 mg of tRNA (type XXI [Sigma Chemical Co.], from *E. coli*) per ml was substituted for poly(U), and 0.2 volume of S-100 was used as the enzyme source. Prereaction mixture (320 μl) without S-100 was incubated at 30°C for 5 min, and a 40-μl aliquot was spotted on the center of a square of the presoaked filter paper. After the spot had dried for 1 to 2 h at room temperature, 10 μl of S-100 was added to it (zero time). To the residual 280 μl of prereaction mixture was added 70 μl of S-100. The mixture was incubated at 30°C, and at 0.5, 1, 1.5, 2, 2.5, and 3 min, a 50-μl aliquot was spotted on TCA-soaked filter paper. Radioactivity of TCA-insoluble phenylalanyl-tRNA was measured as described above for in vitro protein synthesis except that soaking in TCA was done at room temperature. Heating at 95°C for 15 min in TCA caused complete hydrolysis of phenylalanyl-tRNA.

Measurement of cell-associated Cl^- concentration. For determination of Cl^- content, cells were harvested at exponential phase at cell densities of 2.7 to 3.5 g (wet weight)/liter, washed twice with the washing buffer described above, and suspended to a concentration of about 0.3 g (wet weight)/ml of washing buffer.

Intercellular space (ICS), D (grams [dry weight] per gram [wet weight] of pellet), and water content of the ICS (W, grams per milliliter) were determined as described previously (17). The cell water content was calculated by the following formula: cell water = $1 - D - W \times$ ICS.

The Cl^- content of the pellet (A, moles of Cl^- per gram [wet pellet]) was determined as follows (6). Each sample was pelleted, and the supernatant was discarded. The inside wall of the tube was rinsed with distilled water (taking care not to touch the pellet) and wiped dry with tissue paper, and the wet weight was measured. The cell pellet was suspended in about 20 ml of distilled water, and to this suspension was added concentrated NaOH solution to a final concentration of about 0.3 N. The viscous suspension was heated at 90°C for 30 to 60 min to obtain a clear solution and diluted to 100 ml. A 20-ml portion was transferred to a porcelain crucible, ashed on a hot plate, and then ashed at 550 to 600°C. The white ash was dissolved in distilled water, and Cl^- content was estimated by the Mohr method (3). The Cl^- concentration of washing buffer (B, moles of Cl^- per milliliter) was also measured by the same method. Finally, the cell-associated Cl^- concentration (C, moles of Cl^- per kilogram of cell water) was calculated by the following formula: $C = (A - B \times \text{ICS})/(1 - D - W \times \text{ICS})$.

Measurement of intracellular amino acid concentration. Cells grown in 1 M NaCl-PPT medium were pelleted by centrifugation, and the inside wall of the tube was wiped dry with tissue paper. Intracellular free amino acids were extracted from this pellet (2 to 3 g [wet weight]) with cold 10% TCA three times for 2 h each time. Amino acid contents of combined cell extracts were analyzed by an automatic amino acid analyzer, and the amino acid content in the ICS of the pellet was subtracted from that in the cell extract. Finally, intracellular amino acid concentration was calculated by using the formula given above. All experiments were repeated at least twice, with similar results. Usually, representative values are shown.

TABLE 1. Effect of anions on in vitro protein synthesis by S-30 of *V. costicola*

Salt added (0.6 M)	Maximal incorporation (%)
None[a]	100[b]
NaF	0
NaCl	0
NaBr	0
Na_2SO_4	27
Sodium formate	52
Sodium acetate	40
Sodium lactate	24
Sodium aspartate	79
Sodium glutamate	121

[a] Reaction mixture with no added salt contained 0.124 M NH_4Cl, which supports the highest activity as shown previously (20).

[b] A value of 100% represents 31.9 pmol of [^{14}C]phenylalanine (32,500 cpm) incorporated into hot TCA-insoluble material.

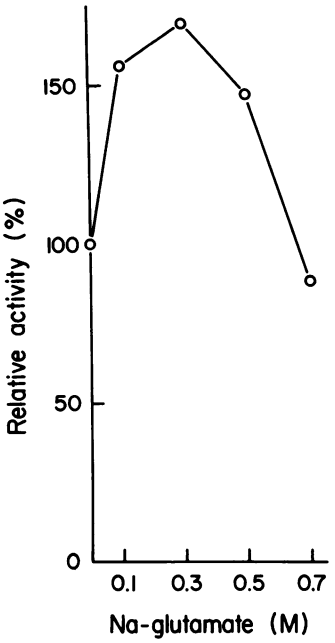

FIG. 1. Effect of sodium glutamate concentration on in vitro protein synthesis by S-30 of *V. costicola*; 100% activity represents incorporation of 38.7 pmol of [^{14}C]phenylalanine (39,500 cpm) into hot TCA-insoluble material per 10 μl of S-30 or 3 U of absorbance at 260 nm.

RESULTS

Effect of various anions on in vitro protein synthesis. As found previously (20), we also observed that when in vitro protein synthesis was measured in the presence of different concentrations of NaCl, KCl, and NH_4Cl, maximal phenylalanine incorporation occurred in 0.1 to 0.2 M salts, whereas no activity was observed in 0.6 M salts (data not shown). However, changing the anion could have a profound effect on protein synthesis in higher salt concentrations. No protein synthesis occurred in 0.6 M NaCl, NaF, or NaBr, but organic anions supported high levels of activity in the presence of 0.6 M Na^+ ions (Table 1). Significant activity was observed in 0.6 M Na_2SO_4, which is 1.2 M in Na^+ ions. The highest activity was found with 0.6 M sodium glutamate.

In vitro protein synthesis was also measured as the incorporation of [^{14}C]leucine in the presence of 19 other amino acids without added artificial messenger, i.e., using endogenous RNA as a messenger (14). A significant incorporation [3.3 pmol compared with 38.6 pmol of [^{14}C]phenylalanine in poly(U)-directed protein synthesis] was obtained in the presence of 0.6 M sodium glutamate, whereas no activity could be seen in the presence of 0.6 M NaCl.

In the presence of 0.124 M NH_4Cl, maximal activity was obtained with 0.3 M sodium glutamate, and activity was still high with 0.7 M sodium glutamate (Fig. 1). In contrast, in vitro protein synthesis by *E. coli* Q13 extract was inhibited by the addition of sodium glutamate and was completely inhibited by 0.5 M NaCl (Fig. 2). Potassium glutamate and ammonium glutamate had effects similar to those of sodium glutamate on *V. costicola* extracts (data not shown).

Compensation for inhibitory effects of Cl^- by organic anions. Our results thus far showed that Cl^- ions inhibit protein synthesis but that glutamate ions support this synthesis, even in the presence of high Na^+ ions. We also found (Fig. 3) that glutamate ions could, to some extent, counteract the inhibitory effects of 0.6 M Cl^- ions. The greatest compensatory effects of glutamate occurred in 0.3 to 0.5 M (total salt concentration, about 0.6 to 1.1 M), whereas higher sodium glutamate concentrations caused inhibition.

A number of other anions and the dipolar ion betaine can counteract the inhibitory effects of 0.6 M NaCl (Table 2). Glutamate was the most active, with potassium and ammonium glutamates being more so than sodium glutamate. Sodium aspartate and betaine were also effective; sodium acetate and Na_2SO_4 were less so, and glycerol, sodium lactate, and sodium formate were not at all.

Internal anions of *V. costicola*. Since protein synthesis took place in the presence of high concentrations of Na^+, K^+, or NH_4^+ ions and organic anions, especially glutamate, but not in such high concentrations of chlorides, and since glutamate and other organic anions could partly reverse the inhibitory effects of Cl^- ions, it seemed likely that low concentrations of Cl^- were maintained in the cytoplasm; or, if higher concentrations were present, they were accompanied by organic anions that could counteract their toxic effect. Measurement of cell-associated Cl^- and amino acids were carried out to investigate these possibilities.

Cells grown in 1.0 M NaCl medium maintain a much lower concentration of Cl^- ions (0.22 mol/kg) than in the external medium (Table 3). Christian and Waltho (4) found earlier that *V. costicola* grown in 1 M NaCl medium to stationary phase contains 139 ± 25 mmol of Cl^- per kg.

It was not known what proportion of the Cl^- in these cells was associated with the cell-associated Na^+ and K^+ ions and what was associated with organic cations. It was thought that the latter Cl^- ions would be lost on ashing the cells, but would be retained if NaOH were added before ashing took place. This was shown to be true by using arginine hydrochloride and cysteine hydrochloride as model compounds. To test for the presence of such Cl^- ions we harvested *V. costicola* in the late exponential phase of growth and digested the washed cell pellets in 0.3 N NaOH before ashing them as described above. Other pellets were ashed directly without NaOH treatment. Similar experiments were done with pellets of *H. halobium*. Cells of *V. costicola* contained a substantial proportion of Cl^- ions that were lost on direct ashing (0.16 of 0.37 mmol/g of pellet). Total Cl^- repre-

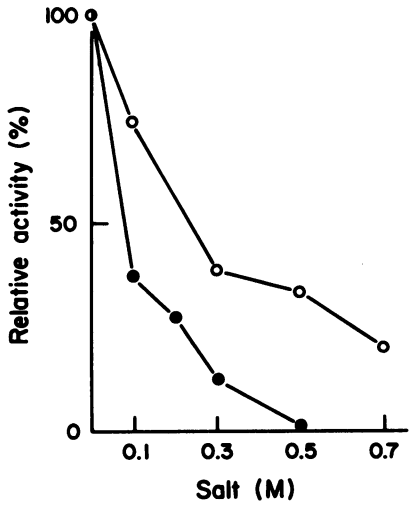

FIG. 2. Effect of sodium glutamate (○) and NaCl (●) concentrations on in vitro protein synthesis by S-30 of *E. coli* Q13; 100% activity represents incorporation of 90.5 pmol of [^{14}C]phenylalanine.

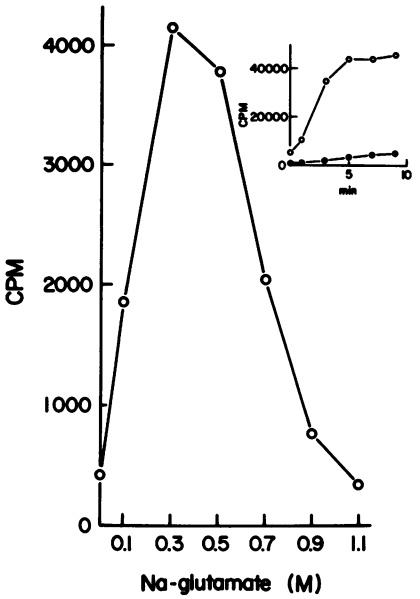

FIG. 3. Compensation of inhibitory effect of Cl^- with glutamate ion. The reaction mixture contained 0.6 M NaCl and various concentrations of sodium glutamate. Inserted figure shows a time course of [^{14}C]phenylalanine incorporation in reaction mixtures containing 0.6 M sodium glutamate (○) or 0.6 M NaCl plus 0.3 M sodium glutamate (●).

sented both intracellular and intercellular Cl^- ions. Subtraction of Cl^- in the ICS gave the very low value of 0.02 mol/kg as free intracellular Cl^- ions. In contrast, all of the Cl^- in *H. halobium* appeared to be associated with Na^+ or K^+ ions since no more was lost on direct ashing than on ashing after alkali treatment. Table 3 also shows intracellular concentrations of acidic and basic amino acids. These amino acids accounted for 62% of the total intracellular amino acids. A sum of free Cl^- (0.02 M), aspartate, and glutamate does not seem enough to act as counteranions of cell-associated Na^+ and K^+. Further investigation of ion balance in *V. costicola* is now in progress.

Site of action of NaCl and sodium glutamate. Our results showed that high sodium glutamate concentrations activated in vitro protein synthesis by *V. costicola* but inhibited that by *E. coli* (Fig. 1 and 2). To localize the salt-sensitive and salt-requiring components of these systems, we studied the effects of different sodium glutamate concentrations on in vitro protein synthesis by hybrid mixtures of the ribosomal and soluble (S-100) portions of extracts from these two bacteria.

When *E. coli* or *V. costicola* ribosomes were combined with S-100 from *E. coli*, activity decreased with increasing sodium glutamate concentrations (although a slight activation in 0.1 M sodium glutamate was observed with a mixture of *V. costicola* ribosomes and *E. coli* S-100; Fig. 4). However, ribosomes from both bacteria functioned well in high sodium glutamate concentrations when combined with S-100 from *V. costicola*. These results suggest that tolerance to high sodium glutamate concentrations resides in the soluble, rather than the ribosomal, fractions of *V. costicola* cytoplasm.

One component of the S-100 fraction, the phenylalanyl-tRNA synthetase of *V. costicola*, was investigated as a possible site of action of salt on in vitro protein synthesis. We found, however, that the activity of this enzyme was only slightly inhibited by as much as 0.9 M NaCl and that its activity decreased with increasing concentrations of sodium glutamate (Fig. 5). Thus, this enzyme cannot account for the effects of NaCl and sodium glutamate on in vitro protein synthesis, although it would certainly function in high glutamate concentrations.

TABLE 2. Compensation for Cl^- inhibition with sodium salts of various anions and other solutes added to the reaction mixture containing 0.6 M NaCl

Salt (0.3 M)	Compensation effect (%)
NaF	0
NaCl	0
NaBr	0
Na_2SO_4	19
Sodium formate	0
Sodium acetate	17
Sodium lactate	0
Sodium aspartate	87
Sodium glutamate[a]	100
Potassium glutamate	138
Ammonium glutamate	125
Betaine	77
Glycerol	0

[a] Activity with sodium glutamate was set at 100% (4,150 cpm).

The demonstration by Hipkiss et al. (8) that pulse-labeled proteins of *V. costicola* are rapidly degraded in vivo suggests that the low rate of in vitro protein synthesis in higher NaCl or KCl concentrations might really be caused by a high rate of protein breakdown in these salts. However, proteins synthesized in vitro in the presence of 0.1 M NaCl and then exposed to 0.6 M NaCl (by adding solid NaCl) remained quite stable for at least 20 min. Thus, 0.6 M NaCl, and presumably other salts, act as true inhibitors of protein synthesis.

DISCUSSION

Halobacterium salinarium contains 1.3 M Na^+, 4.5 M K^+, and 3.6 M Cl^- (4). The in vitro amino acid-incorporating system of *Halobacterium cutirubrum*, now considered to be the same species as *H. salinarim* (H. Larsen, personal communication) requires 3.8 M KCl, 1.0 M NaCl, and 0.4 M NH_4Cl for maximal activity. KCl can be replaced to only a small extent by NaCl and not at all by NH_4Cl without a serious loss of activity (1).

In contrast to these extreme halophiles, which contain Cl^- as the major anion, the moderate halophile *V. costicola* excludes Cl^-, as shown earlier by Christian and Waltho (4)

TABLE 3. Internal Cl^- and charged amino acid concentrations of *V. costicola* grown in the presence of 1.0 M NaCl

Anion	Concn (mmol/kg) ± SE[a]	
Cl^-	220 ± 10	(n = 5)
Aspartate	2.0 ± 0.1	(n = 2)
Glutamate	114 ± 3	(n = 2)
Lysine	21 ± 2	(n = 2)
Histidine	0.6 ± 0.1	(n = 2)
Arginine	ND[b]	(n = 2)
Ornithine	39 ± 2	(n = 2)

[a] Mean values from n determinations. Cell water content was 0.477 ± 0.004 g/g (wet pellet) (n = 6). A 1-g wet pellet contained 235 mg of protein.
[b] ND, Not detected.

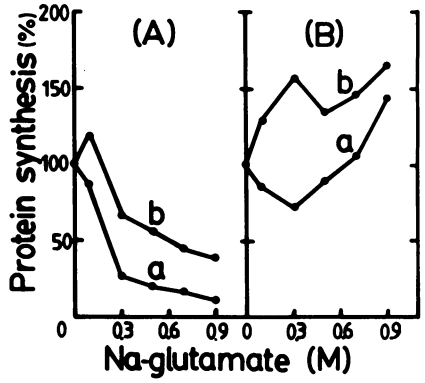

FIG. 4. Effect of sodium glutamate on poly(U)-directed in vitro protein synthesis by combination of ribosomes and S-100 from *V. costicola* or *E. coli*. Numbers in parentheses below are picomoles of [^{14}C]phenylalanine incorporated at 0 M sodium glutamate (100%). (A) Curve a, *E. coli* S-100 plus *E. coli* ribosomes (131.3); curve b, *E. coli* S-100 plus *V. costicola* ribosomes (56.8). (B) Curve a, *V. costicola* S-100 plus *E. coli* ribosomes (24.5); curve b, *V. costicola* S-100 plus *V. costicola* ribosomes (9.8).

for cells at stationary phase and confirmed by our present data for exponential-phase cells.

Our results also show that *V. costicola* has good reasons to exclude Cl^- ions since high concentrations of these ions inhibit in vitro protein synthesis in these bacteria, as they do in other cells, including *Saccharomyces cerevisiae* (18), reticulocytes (16), HeLa cells, L cells, and wheat germ (19). Cl^- exclusion is common in plant and animal cells (15) and occurs in *E. coli* (15), although it is not known whether Cl^- ions inhibit protein synthesis in these bacteria.

Although Cl^- ions and, as shown here, Br^- and F^- ions, are toxic, other anions are not. High levels of in vitro protein synthesis can take place in 0.6 M or higher sodium glutamate, potassium glutamate, and in the sodium salts of other organic anions, as well as Na_2SO_4. Furthermore, a number of these compounds, including the dipolar ion betaine, which may play an important role in *V. costicola* and other salt-tolerant bacteria (11), can partially counteract the inhibitory effects of Cl^- ions.

What are the anions which balance the cell-associated Na^+ and K^+ in *V. costicola*? At present, we do not know. The sum of the anions measured, Cl^-, glutamate, and aspartate, is substantially below that of the cations. Furthermore, most of the Cl^- present seems associated with organic cations.

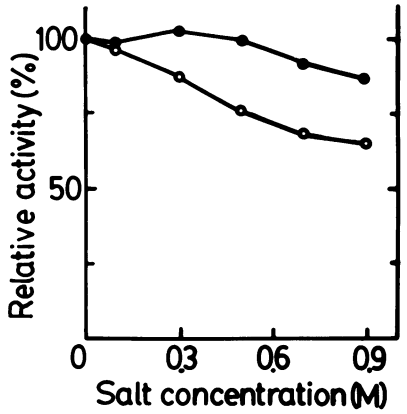

FIG. 5. Effect of sodium glutamate (○) and NaCl (●) concentrations on phenylalanyl-tRNA synthetase of S-100 of *V. costicola*; 100% relative activity represents 25.6 pmol of [^{14}C]phenylalanine.

Possibly, some of the cell-associated cations are neutralized by negative groups on proteins or other polyanions in the cell. Masui and Wada (13) found that isolated cell envelopes of the moderate halophile, *Pseudomonas halosaccharolytica* contain up to 4 M Na^+. Coleman (5) found that 80% of Na^+ and 96% of Cl^- was envelope bound in *Bacillus amyloliquefaciens*, but 95% of K^+ was found in the cytoplasm. Wydro (Ph.D. thesis, University of Ottawa, 1977) found that the ribosomal proteins of *V. costicola* are more acidic than those of *E. coli*, though less acidic than those of *H. cutirubrum*. Charges of some of the cell-associated cations in these bacteria might be balanced by anionic charges on cellular proteins. Concentrations of Na^+ and K^+ glutamate corresponding to those of the anions present would certainly permit protein synthesis, and it seems possible that organic anions might also partly counteract any toxic effects of Cl^- ions.

We do not know the site of action of the different salts tested. Hybridization experiments with ribosomal and soluble fractions from the cytoplasm of *V. costicola* and *E. coli* suggested that much of the characteristic response to high sodium glutamate concentrations is caused by the soluble, rather than the ribosomal, part of the system. Curiously, the ribosomes of *E. coli* could function in as high a sodium glutamate concentration as can those of *V. costicola*, provided that the soluble fraction from the latter bacteria is also present. We also showed that the phenylalanyl-tRNA synthetase of *V. costicola* can function very well in high NaCl concentrations, even better than in high sodium glutamate concentrations, so that this enzyme was not the site of Cl^- toxicity. Further experiments are now in progress to define the site of action of different salts on protein synthesis in *V. costicola*.

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