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EXPERIMENTAL ARTICLES

Relationships between the Osmoadaptation Strategy, Amino Acid Composition of Bulk Protein, and Properties of Certain Enzymes of Haloalkaliphilic Bacteria

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Abstract—Haloalkaliphilic microorganisms isolated from soda lakes were compared in terms of the amino acid composition of bulk protein and the reaction of a number of key enzymes to salts and pH of the medium. In the extremely haloalkaliphilic bacterium *Natroniella acetigena* (salt-in osmoadaptation strategy), acidic amino acids (glutamic and aspartic) made up 30.91 mol % of the total of bulk protein amino acids. In the moderate haloalkaliphiles *Tindallia magadiensis*, *Halomonas campisalis*, and *Halomonas* sp. AIR-2 (compatible-solutes osmoadaptation strategy), the proportion of acidic amino acids (24.36, 23.15, and 23.58 mol %, respectively) was lower than in *N. acetigena* but higher than in the freshwater *Acetobacterium paludosum* (20.77 mol %). The excess of acidic amino acids over basic amino acids (lysine and arginine) increased with the degree of halophily. The enzymes of haloalkaliphiles proved to be tolerant to salts and high pH values, although the degree of tolerance varied. The activity of *N. acetigena* CO dehydrogenase was maximum in the presence of 0.7 M NaCl, but it was virtually independent of the $NaHCO_3$ concentration. The hydrogenase and CO dehydrogenase of *T. magadiensis* exhibited maximum activity in the absence of NaCl; the CO dehydrogenase was most active at 0.25 M $NaHCO_3$, and hydrogenase activity was only weakly dependent on $NaHCO_3$ in the concentration range of 0–1.2 M. The nitrate reductases of *H. campisalis* and *Halomonas* sp. AIR-2 were active in broad ranges of NaCl and KCl concentrations; the activity maxima were recorded at moderate concentrations of these salts. The pH optima of most of the studied enzymes of haloalkaliphiles were in the alkaline zone. Thus, it was shown that the amino acid composition of bulk protein is determined by the osmoadaptation strategy employed by the bacterium. A correlation was found between the salt tolerance of enzymes and the proportion of acidic amino acids in the bulk protein. The ability of enzymes to function at high pH values is one of the mechanisms of adaptation of microorganisms to high pH values.

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Key words: soda lakes, haloalkaliphiles, osmoadaptation, amino acid composition of bulk protein, enzyme activity, salt-tolerance.

As distinct from saline lakes, soda lakes impose on microorganisms a double stress, caused by high salt concentrations and high pH values. In an environment with a high pH value and high Na^+ ion concentration, haloalkaliphiles, in addition to the necessity of osmoadaptation, face the problem of maintaining pH homeostasis in the cells [1]. Soda lakes are inhabited by microbial communities adapted to such conditions. Many microorganisms isolated from soda lakes have a pH optimum at pH 8–10 and require the presence of a considerable amount of salts in the medium; i.e., they are haloalkaliphiles. A natural question to ask is what mechanisms provide for the microorganism adaptation to the extreme conditions in these ecosystems.

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Microorganisms employ two fundamentally different strategies of adaptation to the osmotic stress imposed by high salt concentrations [2]:

(1) Cells may maintain a high intracellular concentration of salts, osmotically equivalent to the extracellular salt concentration (salt-in strategy); in this case, all intracellular systems are adapted to high salt concentrations.

(2) Cells may actively extrude salts, thus maintaining their low concentration in the cytoplasm. The osmotic pressure exerted by the medium is balanced by small organic molecules (compatible solutes strategy), which are either synthesized by the cells or taken up from the milieu. In this case, intracellular systems do not need to be specially adapted.

Table 1. Subjects of investigation and their properties

Microorganism	Order Family	pH range/optimum	Reaction to Na^+	Na^+ range/optimum (M)	Reference
Acetobacterium paludosum	*Clostridiales* *Eubacreriaceae*	5.0–8.0/7.0 neutrophile	freshwater organism	–	[14]
Halomonas campisalis	*Oceanospirillales* *Halomonadaceae*	7.5–10.4/8.8–9.5 alkaliphile	moderate halophile	0.16–3.1/1.0	[15]
Halomonas sp. AIR-2	*Oceanospirillales* *Halomonadaceae*	7.5–10.6/9.5 alkaliphile	facultative moderate halophile	0.04–2.2/0.5–1.2	[16]
Tindallia magadiensis	*Clostridiales* *Clostridiaceae*	7.5–10.5/8.5 alkaliphile	moderate halophile	0.17–1.7/0.51–1.0	[10]
Halanaerobium saccharolyticum	*Halanaerobiales* *Halanaerobiaceae*	6.0–8.0/7.5 neutrophile	moderate halophile	0.5–5.1/1.7	[13]
Natroniella acetigena	*Halanaerobiales* *Halobacteroidaceae*	8.1–10.7/9.7–10.0 alkaliphile	extreme halophile	1.7–4.4/2.0–2.56	[9]

The salt-in strategy is primarily employed by two groups of organisms: the aerobic extremely halophilic archaea of the order *Halobacteriales* and the anaerobic halophilic bacteria of the order *Halanaerobiales.* Most other halophilic and halotolerant microorganisms employ the compatible solutes strategy. However, some halophiles that accumulate organic osmoregulators can simultaneously exhibit moderately high intracellular concentrations of Na^+, K^+, and Cl^- ions, thus combining both adaptation strategies [3].

A moderate or high concentration of KCl and NaCl in the cytoplasm necessitates the adaptation of all the intracellular enzyme systems [2, 4–6]. To achieve salt tolerance, proteins of halophiles must undergo evolutionary modifications in their amino acid composition, which make them "halophilic" due to the acquisition of additional residues of acidic amino acids [3, 5, 7]; most of these residues occur on the protein surface. Acidic amino acid residues are more strongly hydrated than other amino acid residues; therefore, an increase in their number decreases the hydrophobicity of the protein and helps to prevent the collapse of the protein structure or protein conglomeration. Thus, the amino acid composition of bulk protein plays an important role in haloadaptation.

Currently, data are available on the amino acid composition of the bulk protein of neutrophilic halophiles [3, 5, 8]. However, no analogous studies have been conducted with haloalkaliphilic microorganisms. The aim of the present work was to perform a comparative investigation of the amino acid composition of bulk protein of haloalkaliphiles and neutrophilic halophiles, as well to study the effect of salts and pH on the activity of certain metabolic enzymes of haloalkaliphiles.

MATERIALS AND METHODS

Microorganisms and cultivation conditions. Pure cultures of the anaerobic bacteria *Natroniella acetigena* Z-7937^T (= DSM 9952) and *Halanaerobium saccharolyticum* Z-7787^T (= DSM 6645) and of the facultatively anaerobic bacterium *Halomonas campisalis* Z-7398-2 were obtained from T.N. Zhilina. The facultative anaerobe *Halomonas* sp. AIR-2 (=DSM 17331) was obtained from D.Yu. Sorokin. Pure cultures of the anaerobic bacteria *Tindallia magadiensis* Z-7934^T (= DSM 10318) and *Acetobacterium paludosum* Z-4092^T (= DSM 8237) were obtained from V.V. Kevbrin and O.R. Kotsyurbenko, respectively (Table 1). *N. acetigena* was grown on a medium with lactate at pH 9.7 [9]. *T. magadiensis* was cultured on a medium with pyruvate at pH 8.5 [10]. The composition of the medium used for the cultivation of *H. saccharolyticum* was as follows (g/l): NaCl, 150.0; NH_4Cl, 0.33; KH_2PO_4, 0.33; $MgCl_2 \cdot 6H_2O$, 0.33; $CaCl_2 \cdot 6H_2O$, 0.33; KCl, 0.33; $MgSO_4 \cdot 7H_2O$, 4.0; $NaHCO_3$, 2.0; $Na_2S \cdot 9H_2O$, 0.5; yeast extract, 0.05; Lippert trace element solution [11], 1 ml; Wolin vitamin solution [12], 10 ml; 0.04% resazurin solution, 2 ml; glucose, 5.0; pH 7.0 [13]. *A. paludosum* was grown on a medium of the following composition (g/l): NH_4Cl, 0.33; KH_2PO_4, 0.33; $MgCl_2 \cdot 6H_2O$, 0.33; $CaCl_2 \cdot 6H_2O$, 0.33; KCl, 0.33; $NaHCO_3$, 1.5; $Na_2S \cdot 9H_2O$, 0.5; yeast extract, 0.02; Lippert trace element solution, 1 ml; Wolin vitamin solution, 10 ml; 0.04% resazurin solution, 2 ml; sodium lactate, 5.0; pH 7.0 [14]. The compositions of the media used for cultivation of *Halomonas campisalis* Z-7398-2 and *Halomonas* sp. AIR-2 were described in [15, 16]. *H. saccharolyticum* and *A. paludosum* were grown in an atmosphere of $N_2 + CO_2$ (80 : 20); the other cultures were grown in an N_2 atmosphere. All cultivation media were prepared using the anaerobic technique. For inoculation, mid-log-phase cultures were used in a dose of 1–3 vol %. *A. paludosum* was grown at 20°C; the other cultures were grown at 36°C.

Obtaining of cell-free extracts. Cell-free extracts were obtained after disruption of cells with lysozyme (5 mg/ml) or ultrasound (UZDN-1, 0.4 mA) or in an X-press. *N. acetigena* and *T. magadiensis* cells were disrupted under strictly anaerobic conditions in buffer

solutions that were prepared using oxygen-free water and stored under nitrogen. Exponential-phase cells were sedimented by centrifugation at 5000 *g*; resuspended in 5 ml of 0.05 M Tris–HCl buffer containing 10 mM sodium formate, 25 mM sodium thioglycollate, 25 mg of lysozyme, and 5 μg of DNase; and incubated for 12 h at 37°C. Cells of *H. campisalis* and *Halomonas* sp. AIR-2 were disrupted aerobically in 0.1 M phosphate buffer, pH 7.0. Disintegrated cells were centrifuged at 5000 *g* for 40 min. Supernatants were used for the determination of enzyme activity.

Determination of the activity of enzymes. The activities of hydrogenase and CO dehydrogenase were determined spectrophotometrically from the rate of benzyl viologen reduction by hydrogen or CO, respectively (thermostated anaerobic cuvettes, 37°C, 600 nm, Specord UV-VIS recording spectrophotometer (Germany)) [17, 18]. Activity of the enzymes was expressed in μmoles of benzyl viologen reduced in 1 min. The molar extinction coefficient of benzyl viologen was taken to be 8.65 mM^{-1} cm^{-1}.

For determination of nitrate reductase activity, methyl viologen reduced by dithionite was used as the electron donor [19]. The reaction mixture (1.0 ml) contained (mM) potassium phosphate buffer (pH 7.0), 100; KNO_3, 10; methyl viologen, 0.8; sodium dithionite, 1.2; nitrate reductase preparation, 10–100 μl. The reaction was initiated by the introduction of dithionite. The mixture was incubated at 70°C for 15 min; then, the reaction was terminated, and 500 μl of a 0.6% solution of sulfanilic acid in 20% HCl and 500 μl of 2 mM *N*-(1-naphthyl)ethylenediamine were added to determine the amount of nitrite produced. The extinction at 548 nm was measured after 15 min—a period needed for the development of coloration. The nitrate reductase activity was expressed in nmoles of nitrite produced in 1 min of the fermentative reaction.

The maximum activity of each enzyme was taken as 100%. The protein content of the samples, determined by the Lowry method, was 2–5 mg/ml.

Analysis of the amino acid composition of proteins. Late-exponential-phase cells were sedimented by centrifugation at 5000 *g* for 40 min and subjected to acid hydrolysis in 6 N HCl at 110°C for 24 h. Hydrolysate was evaporated in a rotor evaporator; then the dry residue was washed with distilled water to remove hydrochloric acid and once more evaporated. Amino acids were analyzed in the form of N-trifluoroacetyl-*O*-isopropyl esters on a model 3700 chromatograph (Russia) equipped with a flame ionization detector and a capillary quartz column (25 m × 0.20 mm) containing the SE-30 phase. The temperature of the analysis was kept at 110°C for 5 min and then raised to 190°C at a rate of 5°C/min. Helium at a pressure of 0.9 atm in the column was used as the carrier gas. This procedure did not allow tryptophan and cysteine to be determined; neither could it determine methionine and histidine, decomposed during acid hydrolysis. Glutamic and aspartic acids are eluted as peaks common with glutamine and asparagine, respectively, and cannot be determined separately from them.

RESULTS

Amino Acid Composition of Bulk Protein of Haloalkaliphilic, Neutrophilic Halophilic, and Nonhalophilic Bacteria

The amino acid composition of bulk protein of *N. acetigena*, *T. magadiensis*, *H. campisalis*, *Halomonas* sp. AIR-2, *H. saccharolyticum*, and *A. paludosum* is presented in Table 2. It is evident from this table that the proportion of acidic amino acids (glutamic and aspartic) in the total amount of amino acids of cell protein and their excess over basic amino acids (lysine and arginine) increased with the degree of halophily of the microorganism. In the freshwater *A. paludosum*, the total of Glx + Asx comprised 20.77 mol %, the difference between the contents of acidic and basic amino acids, (Glx + Asx) – (Lys + Arg), equaling 12.41 mol %. These values were the lowest among the microorganisms that we investigated. In the moderately haloalkaliphilic *T. magadiensis, H. campisalis,* and *Halomonas* sp. AIR-2, and in the neutrophilic moderate halophile *H. saccharolyticum*, the proportions of acidic amino acids were higher than in the freshwater *A. paludosum*, amounting to 24.36, 23.15, 23.58, and 25.69 mol %, respectively. The difference between the contents of acidic and basic amino acids made up 15.00, 15.67, 15.62, and 18.97 mol %. The proportion of acidic amino acid was the greatest in *N. acetigena*: 30.91 mol %, the excess of acidic amino acids over basic ones equaling 23.99 mol % in this organism.

Effect of Salts on the Activity of Enzymes of Haloalkaliphilic Bacteria

We investigated the effect of salts on the activity of certain key enzymes of the extremely haloalkaliphilic bacterium *N. acetigena* and of the moderately haloalkaliphilic bacteria *T. magadiensis, H. campisalis*, and *Halomonas* sp. AIR-2.

The CO dehydrogenase of *N. acetigena* was active in a NaCl concentration range of 0–4.1 M, with an optimum at 0.7 M NaCl. In the presence of 0.7 M NaCl, a 20% stimulation of the CO dehydrogenase activity was observed. At NaCl concentrations higher than 3.5 M, the activity of the CO dehydrogenase decreased (Fig. 1). In the presence of 0–0.7 M $NaHCO_3$, the activity of the enzyme was constant; at higher concentrations of sodium bicarbonate, its activity decreased slightly (Fig. 2).

The hydrogenase and CO dehydrogenase of *T. magadiensis* were most active in the absence of NaCl; with an increase in the NaCl concentration from 0 to 4.3 M, their activity decreased (Fig. 1). Despite the inhibitory effect of NaCl, the enzymes retained about 40% of their

Table 2. Amino acid composition of bulk protein in haloalkaliphilic, haloneutrophilic, and nonhalophilic bacteria

Amino acid	*Acetobacterium paludosum*	*Halomonas campisalis*	*Halomonas* sp. AIR-2	*Tindallia magadiensis*	*Halanaerobium saccharolyticum*	*Natroniella acetigena*
Ala	11.94	13.35	12.57	11.17	10.73	10.02
Gly	10.95	12.14	10.66	9.60	9.80	9.84
Thr	6.94	5.61	5.39	5.69	5.69	4.71
Ser	6.54	5.00	5.03	4.88	5.33	3.92
Val	9.56	8.71	9.11	9.54	9.36	9.39
Leu	7.70	8.82	9.12	8.16	8.66	8.35
Ile	7.73	4.95	5.83	7.37	8.47	7.33
Pro	3.89	5.15	4.71	4.06	3.66	3.88
Asx	**10.50**	**10.94**	**10.87**	**12.31**	**13.33**	**13.66**
Glx	**10.27**	**12.21**	**12.71**	**12.05**	**12.36**	**17.25**
Phe	3.21	3.37	3.88	3.64	3.54	3.01
Lys	**5.58**	**4.42**	**3.03**	**5.26**	**4.02**	**4.84**
Tyr	2.41	2.26	2.17	2.16	2.35	1.70
Arg	**2.78**	**3.06**	**4.93**	**4.10**	**2.70**	**2.08**
Glx + Asx	**20.77**	**23.15**	**23.58**	**24.36**	**25.69**	**30.91**
Lys + Arg	**8.36**	**7.48**	**7.96**	**9.36**	**6.72**	**6.92**
(Glx + Asx) – (Lys + Arg)	**12.41**	**15.67**	**15.62**	**15.00**	**18.97**	**23.99**

Note: The data are averaged over at least three experiments. Glx and Asx signify total contents of Glu + Gln and Asp + Asn, respectively.

activity at a NaCl concentration higher that 4.0 M. The activity of CO dehydrogenase was maximum at 0.25 M $NaHCO_3$ and decreased upon further increase in the bicarbonate concentration (Fig. 2). The activity of *T. magadiensis* hydrogenase was virtually constant in the $NaHCO_3$ concentration range of 0–1.2 M (Fig. 2).

Nitrate reductases of *H. campisalis* and *Halomonas* sp. AIR-2 were highly resistant to NaCl and KCl. The activity of the enzyme was retained over the entire concentration ranges examined (0–4.1 M NaCl and 0–3.2 M KCl) (Figs. 1, 3). The enzyme of *H. campisalis* was most active at 0.3–2.0 M NaCl and 0.2–2.1 M

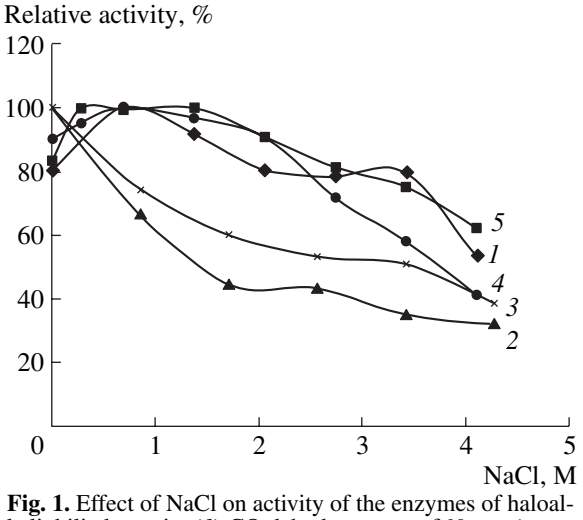

Fig. 1. Effect of NaCl on activity of the enzymes of haloalkaliphilic bacteria: (*1*) CO dehydrogenase of *N. acetigena*, (*2*) CO dehydrogenase of *T. magadiensis*, (*3*) hydrogenase of *T. magadiensis*, (*4*) nitrate reductase of *H. campisalis*, and (*5*) nitrate reductase of *Halomonas* sp. AIR-2.

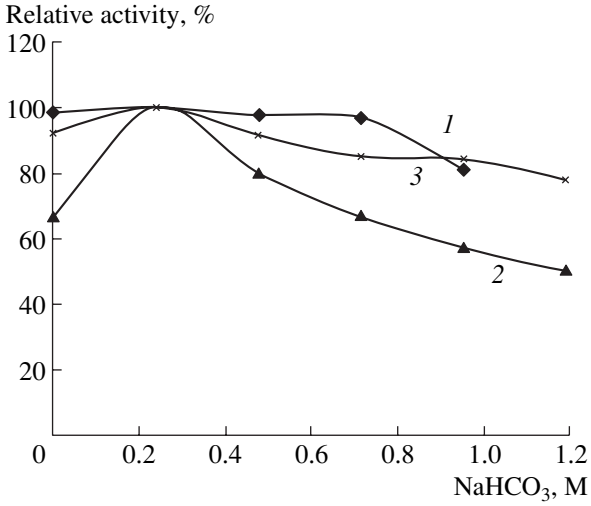

Fig. 2. Effect of $NaHCO_3$ on activity of the enzymes of haloalkaliphilic bacteria: (*1*) CO dehydrogenase of *N. acetigena,* (*2*) CO dehydrogenase of *T. magadiensis*, and (*3*) hydrogenase of *T. magadiensis.*

KCl. The nitrate reductase of *Halomonas* sp. AIR-2 showed maximum activity at 0.3–2.0 M NaCl and 0.2–1.6 M KCl. At 4.1 M NaCl, the nitrate reductases of *H. campisalis* and *Halomonas* sp. AIR-2 retained 40 and 60% of their activity, respectively; at 3.2 M KCl, these values were 70 and 60%.

Effect of pH on the Activity of Enzymes of Haloalkaliphilic Bacteria

The activity of the CO dehydrogenases of *N. acetigena* and *T. magadiensis*, the hydrogenase of *T. magadiensis*, and the nitrate reductase of *H. campisalis* were determined in buffers with various pH values (from 5.0 to 12.0). The CO dehydrogenase of *N. acetigena* was active in a pH range of 6.0–12.0, with an optimum at 9.0 (Fig. 4), a pH value somewhat lower than the pH optimal for the growth of this bacterium. The activity of this enzyme increased fourfold with an increase in pH from 7.0 to 9.0. The CO dehydrogenase and hydrogenase of *T. magadiensis* showed maximum activity at pH 10.0 (Fig. 4), a value that is higher than that optimal for the bacterium growth. The pH limits in the alkaline region were somewhat different for these enzymes—pH 11.5 and pH 12.0, respectively. The nitrate reductase of *H. campisalis* was active in the pH range of 5.0–11.0. Although the maximum activity was observed at pH 7, the enzyme retained a rather high activity under alkaline conditions (about 45% of the maximum value was retained at pH 9 and 20% was retained at pH 10).

DISCUSSION

Thus, we have shown that the amino acid composition of bulk protein is determined by the osmoadaptation strategy chosen by the organism. The proportion of acidic amino acids and their excess over basic amino acids increase with the degree of the microorganism halophily. The properties of the enzymes of haloalkaliphiles can to a considerable extent be predicted based on the amino acid composition of the bulk protein [3].

Earlier, we showed that the moderate haloalkaliphiles *T. magadiensis*, *H. campisalis*, and *Halomonas* sp. AIR-2 employ the compatible-solutes osmoadaptation strategy ([16]; Kevbrin, unpublished data). These bacteria maintain moderate intracellular concentrations of Na^+, K^+, and Cl^- ions, which depend on the external concentration of NaCl. There are preliminary data showing that *T. magadiensis* cells contain glycine betaine. This compound is an osmoregulator widespread among halophiles [3]. *H. campisalis* cells contain ectoine as an osmolyte [16]. In these microorganisms, it is the organic osmolytes that are mainly responsible for the osmotic balance. The proteins of these microorganisms function in the presence of organic osmoregulators and do not need pronounced adaptation to salts. In most cases, the cytoplasmic enzymes exhibit maximum activity in the absence of salts. However,

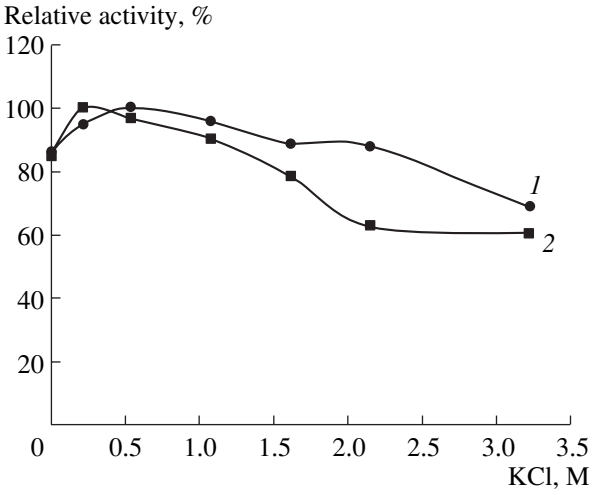

Fig. 3. Effect of KCl on activity of the enzymes of haloalkaliphilic bacteria: (*1*) nitrate reductase of *H. campisalis* and (*2*) nitrate reductase of *Halomonas* sp. AIR-2.

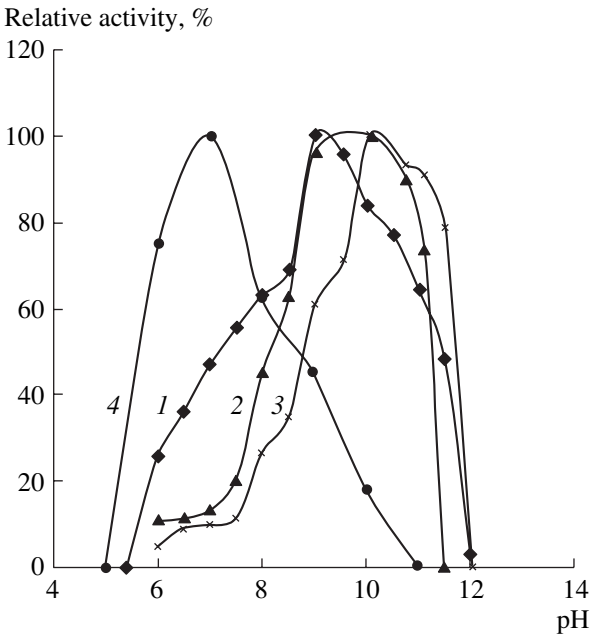

Fig. 4. Effect of pH on activity of the enzymes of haloalkaliphilic bacteria: (*1*) CO dehydrogenase of *N. acetigena*, (*2*) CO dehydrogenase of *T. magadiensis*, (*3*) hydrogenase of *T. magadiensis*, and (*4*) nitrate reductase of *H. campisalis.*

since the salt concentrations in cells of these microorganisms are moderately elevated, it can be anticipated that some of the enzymes of haloalkaliphiles, the membrane-bound ones first of all, may exhibit halophilic properties [3]. In our study, the halotolerance of enzymes correlated with the amino acid composition of cell proteins. In terms of the proportion of acidic amino acids in the bulk protein and their excess over basic amino acids, the haloalkaliphiles *T. magadiensis*,

H. campisalis, and *Halomonas* sp. AIR-2 ranked above the freshwater *A. paludosum* but below the extremely haloalkaliphilic *N. acetigena* (Table 2). It is not surprising, therefore, that the enzymes of these haloalkaliphiles proved to be salt-tolerant, although the extent of their salt-tolerance varied considerably. Thus, the CO dehydrogenase and hydrogenase of *T. magadiensis* showed maximum activity in the absence of NaCl but were not inhibited by $NaHCO_3$. On the contrary, the nitrate reductases of *H. campisalis* and *Halomonas* sp. AIR-2 were most active in the presence of moderate concentrations of NaCl or KCl. It should be mentioned that not all of the nitrate reductases of denitrifying bacteria of the genus *Halomonas* exhibit such a high tolerance to salts. Thus, the nitrate reductase of the moderate halophile *Halomonas* (formerly, *Paracoccus*) *halodenitrificans* is active in a much narrower NaCl concentration range (0–1.7 M), and its maximum activity is observed in the absence of salt [20]. At the same time, the growth of this bacterium occurs at a NaCl concentration as high as 20% (3.4 M).

The extreme haloalkaliphile *N. acetigena*, which belongs to the order *Halanaerobiales*, does not synthesize osmoregulators but accumulates Na^+, K^+, and Cl^- to a very high concentration in the cytoplasm (which increases with the NaCl content in the medium) to counterbalance the osmotic pressure of the highly mineralized environment [16]. Therefore, its enzymes exhibit special features reflecting their adaptation to high salt concentrations. Indeed, the *N. acetigena* proteins contain a larger amount of acidic amino acids, and their excess over basic amino acids is close to that characteristic of halobacteria [5], which also employ the salt-in strategy. Not only do the enzymes of such microorganisms require salts for their maximum activity, but they may even be inactivated in the absence of salts. A typical example of a halophilic enzyme is the CO dehydrogenase of *N. acetigena*: it shows absolute dependence on NaCl and is highly resistant to it, analogously to the enzymes of other haloanaerobes [21]. Earlier, we showed that in the extremely halophilic eubacterium *Acetohalobium arabaticum*, belonging to the order *Halanaerobiales*, the activity of certain catabolic enzymes, including CO dehydrogenase, is stimulated by high concentrations of NaCl; the maximum activity of CO dehydrogenase was observed at 1 M NaCl [22].

The haloalkaliphile *N. acetigena* and the neutrophilic halophile *H. saccharolyticum* belong to the same order, *Halanaerobiales*, and employ the same salt-in osmoadaptation strategy. Therefore, it could be anticipated that these microorganisms have similar contents of acidic amino acids and similar contents of basic amino acids. Indeed, the proportions of basic amino acids in these haloanaerobes proved to be very close: 6.72 mol % in *H. saccharolyticum* and 6.92 mol % in *N. acetigena*. However, the proportion of acidic amino acid in the proteins of *N. acetigena* turned out to be considerably higher than that in the proteins of *H. saccharolyticum.* Possibly, such a large excess of acidic amino acids is necessary not only for osmoadaptation but also for the regulation of intracellular pH. Obligate alkaliphiles are known to maintain intracellular pH at values of about 8–9, which is at least 2 units lower than the pH of the environment [1].

Thus, we have demonstrated that the enzymes of the haloalkaliphiles *H. campisalis, N. acetigena*, and *T. magadiensis*, like the enzymes of other alkaliphilic microorganisms, are extremely tolerant to high pH values. Earlier, we showed that the hydrogenase of the haloalkaliphilic sulfate-reducing bacterium *Desulfonatronum thiodismutans* is active in the pH range of 7.5–11.5 with an optimum at pH 9.5 [23]. The hydrogenase of another haloalkaliphilic sulfate-reducer, *Desulfonatronovibrio hydrogenovorans*, has an activity optimum at pH 9.5 and retains 50% of the maximum activity level at pH 12 [24]. Thus, a conclusion can be made that one of the mechanisms of adaptation of microorganisms to highly alkaline environments consists in the ability of their enzymes to function at high pH values.

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