Betaine Is the Main Compatible Solute of Halophilic Eubacteria

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A number of moderately halophilic bacteria of diverse taxonomic groups have been studied to determine the intracellular concentrations of organic compounds at various salt concentrations. Betaine was accumulated in all of these organisms in proportion to the salinity of the medium, suggesting that this compound plays a major role in osmoregulation.

The ability to adapt, within varying limits, to changes in the osmotic strength of the environment is a property inherent to most living cells. Adaptation to high solute concentrations is generally accompanied by the intracellular accumulation of solutes, which are different from the major solutes outside of the cells and which have to be compatible with the metabolic processes of cells. These solutes have been termed, therefore, “compatible solutes” (1). A variety of solutes is known to be involved in the osmotic adaptation of various microbes. In the extremely halophilic Halobacteriaceae archaeabacteria, KCl is the major intracellular solute, reaching an intracellular concentration of 5 eq/liter (2). The halophilic alga Dunaliella salina and some yeasts accumulate polyols (1,4). Some halotolerant and moderately halophilic euabacteria can adapt to wide ranges of salt concentrations (12), but the identity of their compatible solutes is unknown. It has been shown that the concentrations of some amino acids (most commonly glutamate and proline) increase in nonhalophilic and halotolerant euabacteria under osmotic stress (6,10), whereas freshwater and marine cyanobacteria accumulate sugars and sugar derivatives (9).

The presence of betaine in the halophilic phototrophic bacteria Ectothiorhodospira halochloris (3) and Synechococcus sp. strain DUN 52 (7) has been demonstrated recently as was the accumulation of this compound by nonhalophilic members of the family Enterobacteriaceae under solute stress (5). Since this compound has a high solubility and seems to have little effect on the activity of some enzymes (J. Imhoff, unpublished data), it could be an excellent compatible solute. In the present work, we studied the intracellular betaine concentration of a number of representative strains from a large collection of moderately halophilic euabacteria that have been characterized by numerical taxonomy (8,11). Some nonhalophilic euabacteria and extremely halophilic archaebacteria have also been included in our survey.

The moderately halophilic bacteria were grown in MH medium containing 3, 10, or 20% total salts as previously described (8). The 20% medium contained the following: NaCl, 2.8 M; MgCl₂, 0.15 M; MgSO₄, 0.16 M; CaCl₂, 6.5 mM; KCl, 53 mM; NaHCO₃, 1.4 mM; and NaBr, 0.5 mM. The nonhalophiles were grown in the same medium containing 0 or 3% salts, and the halobacteria were grown in a medium containing 0.5% (wt/vol) yeast extract (Difco Laboratories) and 25% salts. All were grown in 2-liter jackets aerated by a flow of humidified air through a glass sparger and incubated at 30°C; the halobacteria were incubated at 38°C. The cells were harvested at the end of the exponential phase, and the pellets were extracted with 10% perchloric acid. The extracts were neutralized with KOH, the precipitated potassium perchlorate was removed by centrifugation, and the organic solutes were determined in the supernatants. Glutamic acid was determined enzymatically (Boehringer Mannheim Biochemicals). Betaine was identified by thin-layer chromatography and quantitated by pyrolysis-gas chromatography (3). Cytoplasmic volumes were determined by the differential penetration of [³H]-water, and a nonpenetrating sugar, [¹⁴C]-ribose, sorbitol, and sucrose were used each time. A thick cell suspension was centrifuged through a layer of silicone oil of appropriate density, which was adjusted for each organism and salt concentrations. For this purpose, two silicone oils of different densities were used: DC550 and DC556 (Dow Corning Corp.). Brombencene was used to increase density. Usually, DC550 was used for the 3% grown cells, 5% brombencene in DC550 was used for the 5% cells, and 15 to 20% brombencene in DC550 was used for the 20% cells.

The intracellular concentrations of betaine and glutamic acid of cells grown at various salt concentrations are shown in Table 1. Changes in the cellular concentrations of glutamic acid did occur but did not correlate, or did so poorly, with the salt content of the medium. Among the cells of some strains, the concentrations of glutamate, which is the major amino acid in many, if not most, bacteria, approached or slightly exceeded those of betaine after growth in 3% salts. However, the concentrations of betaine increased consistently with the salinity of the media in all strains, and thus betaine became the major organic solute at the higher salt concentrations. In general, the intracellular concentrations of betaine were ca. 200 mM at 3% salts, ca. 650 to 850 mM at 10% salts, and ca. or greater than 1,000 mM at 20% salts. The increase of the betaine concentration correlated well with the increase of the salinity between 3 and 10% but did not do so in most of the strains between 10 and 20% salts. Since the optimal growth of the moderately halophilic strains investigated occurs at ca. 10% salts, this lack of correlation could point toward a limitation of growth due to the limited ability to synthesize betaine at these high salt concentrations. None of the moderately halophilic strains investigated showed substantial increases in the cellular pools of sugars or polyols with the increase of salt concentrations (data not shown), and the contribution of these compounds to the total intracellular solute was insignificant. ¹³C-nuclear magnetic resonance measurements with extracts of Vibrio costicola supported the chemical analyses showing betaine as the major organic solute and also substantial amounts of glutamic acid (data not shown).
In the three nonhalophilic bacteria, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus epidermidis*, the betaine content was small but significant, even in the cells grown in the absence of added salts. At 3% salts, the concentration of betaine rose to ca. 100 200 mM, which is in a range comparable to that of the moderately halophilic bacteria grown at this salinity. In contrast, we did not find significant concentrations of betaine in cells of *Halobacterium halobium* CCM 2090, *Halobacterium volcanii* DS-2, *Halobacterium saccharovorum* ATCC 29252, and *Halobacterium mediterranei* ATCC 33500, all of which are extremely halophilic archaebacteria.

We conclude, therefore, that betaine is of general importance for osmotic adaptation of most or all eubacteria. Other components could be involved, since counter ions for glutamic acid, particularly potassium ions, are accumulated to a certain extent (12). In addition, a significant contribution could be made by the additive effect of a number of metabolites, each present at a low concentration. However, betaine seems to play the major role (Table 1). It seems now that the three main branches of the microbial phylogenetic tree (13) have found three different kinds of compatible solutes: KCl in archaebacteria, polyols in eucaryotes, and betaine in eubacteria.

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**LITERATURE CITED**