COMPATIBLE SOLUTES IN MICROORGANISMS THAT GROW AT HIGH TEMPERATURE

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Summary

The accumulation of organic solutes is a prerequisite for osmotic adjustment in the vast majority of halophilic microorganisms. Many thermophilic and hyperthermophilic microorganisms also live in saline environments and require mechanisms for osmotic adjustment. These organisms generally accumulate very unusual compatible solutes. Di-*myo*-inositol-phosphate, dimannosyl-di-*myo*-inositol-phosphate, diglycerol phosphate, mannosylglycerate, and manosylglyceramide have not been identified in bacteria or archaea that grow at lower temperatures. Some of these compatible solutes, namely mannosylglycerate, mannosylglyceramide, and diglycerol phosphate play an important role in osmotic adaptation of thermophilic and hyperthermophilic organisms. There is also the growing awareness that some of these compatible solutes may have a role in the protection of cell components against thermal denaturation. Di-*myo*-inositol-phosphate, for example, accumulates at temperatures above the optimum for growth and could have a role in thermoprotection. Mannosylglycerate and diglycerol-phosphate, in addition to

their role in osmotic adjustment, protect enzymes and proteins from thermal denaturation *in vitro* as well or better than other known compatible solutes. While the pathways leading to the synthesis of compatible solutes from thermophiles and hyperthermophiles are largely unknown, there has been progress in elucidating the biosynthesis of mannosylglycerate and di-*myo*-inositol-phosphate. Fundamental and applied interest in compatible solutes and osmotic adjustment in these organisms drives research, which will in the near future, allow us to understand better their role in osmotic protection and thermoprotection of some of the most fascinating organisms known on Earth.

1. Introduction

Marcus Porcius Cato ended many of his speeches in the Roman Senate with the now famous expression "*Censer Carthaginem esse delendam*" (I declare that Carthage must be destroyed) until war was ultimately declared on this city in 151 BC. The Third Punic War ended with the defeat and the complete destruction of Carthage a few years later. The Romans slaughtered its inhabitants, torched the city and, by order of the Senate, ploughed the ruins under and then sowed them with salt to symbolize the death of the city. Salt is frequently associated with death and infertility of soil or of water, the name Dead Sea being one example of the ancient idea that life is killed by large amounts of salt. Indeed, the Dead Sea is dead to the naked eye, but under the microscope the water is teeming with life that depends on large quantities of salt. This review does not deal with extremely halophilic microorganisms, which are adapted to life in water with high salt concentrations up to saturated brines. Rather, it deals "with a pinch of salt," but a pinch of salt in very hot water. The organisms that live in a little salt at very high temperatures also represent very special and enigmatic forms of life that deserve special attention. This review deals with the salt relations of thermophilic and hyperthermophilic organisms, the uniqueness and diversity of their osmolytes, and the effect of these osmolytes on cell components.

Thermophilic and hyperthermophilic organisms, like all organisms living in aqueous environments, are faced with alterations in the water activity, to which they must adjust, in order to grow. An increase in the concentration of low molecular mass solutes of an aqueous environment always results in a decrease in the water available to the microorganism. The decrease in the external water activity imposed by salts or sugars, for example, leads to a decrease in the cell volume and/or the turgor pressure ultimately affecting metabolic systems and macromolecules. To adjust to the higher solute concentrations of the environment, microorganisms must accumulate an intracellular solute to reestablish the cell turgor pressure and/or cell volume, and preserve enzyme activity at the same time. On the other hand, microorganisms must decrease the intracellular level of solutes to adapt to lower environmental solute concentrations imposed by dilution; this adjustment implies extrusion (or metabolic conversion) of the solute to decrease the turgor pressure and avoid lysis.

2. Strategies for Osmotic Adaptation in Microorganisms

Microorganisms have developed two main strategies for osmoadaptation that appear to be completely different from each other. One strategy for osmotic adaptation, which appears to be the rarer of the two, relies on the influx of ions from the environment to very high intracellular levels. However, the uptake of these ions is selective, since in contrast to the major environmental ions, namely Na^+ and Cl^-, the major intracellular cation is generally K^+. The extremely halophilic archaea of the family *Halobacteriaceae*, that includes the canonical extreme halophiles of well known genera such as *Halobacterium*, *Haloarcula*, *Natronobacterium*, and *Natronococcus*, and the lesser known halophilic and anaerobic bacteria of the order *Haloanaerobiales*, have developed this strategy for osmotic adjustment. These organisms have to compromise a very saline intracellular environment with extensive changes in the composition of proteins and other cell components. Most of the enzymes of these organisms have a negative charge due to a predominance of acidic amino acids and are strictly dependent on K^+ and/or Na^+ for activity. Although these organisms depend on a highly saline cytoplasm for osmotic adaptation, some species of *Natronococcus* and *Natronobacterium*, accumulate an organic anionic osmolyte identified as sulfotrehalose. This compatible solute helps to counterbalance the positive charge of K^+ and Na^+ and contributes to the osmotic balance of the cells.

The majority of microorganisms have not, however, undergone extensive genetic alterations as a prerequisite for adaptation to a saline environment. These organisms exclude NaCl and most small extracellular organic compounds from the cytoplasm through the accumulation of specific compatible solutes. Intracellular macromolecules have not undergone extensive modifications in these organisms and are, therefore, sensitive to high concentration of salts. This mechanism greatly reduces the necessity of extensive genetic modification providing a versatile means for rapid adaptation to osmotically changing environments. A large variety of microorganisms, ranging from bacteria to yeast, fungi, and algae, rely exclusively on compatible solutes for osmoadaptation indicating that this strategy is very successful. This idea is reinforced by the fact that some species, such as the microalgae of the genus *Dunaliella* that accumulate glycerol, represent some of the most halophilic organisms known. Moreover, yeast and fungi are unsurpassed in their ability to grow in environments with extremely high concentrations of sugars that, like saline conditions, require osmotic adjustment with exclusion of the extracellular solute.

Halotolerant and halophilic microorganisms generally prefer to uptake compatible solutes from the environment over *de novo* synthesis. For this reason, organisms grown in complex media often accumulate solutes such as trehalose and glycine betaine from the yeast extract in the medium. Presumably, the same preference for solute uptake occurs in nature where compatible solute scavenging is probably the obvious and most inexpensive source of osmolytes. Solutes can be supplied from the death of compatible solute producers or from their release due to decrease of environmental osmolarity. Many microorganisms are unable to metabolize their compatible solutes upon dilution of environmental salt (or sugar) and depend entirely on extrusion mechanisms to control the increase in the turgor pressure. The excreted compatible solutes can then become available to other organisms.

Many slightly and moderately halophilic archaea appear to possess a mixed type of osmoadaptation where K^+ accumulates to high levels along with an anionic organic compatible solute to counterbalance the positive charge.

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Biographical Sketches

Helena Santos is Associate Professor and Research Leader at the Instituto de Tecnologia Química e Biológica, the New University of Lisbon, Portugal. She received MSc and PhD degrees in Biophysics from the New University of Lisbon, and did postdoctoral training at the Chemistry/Biochemistry Departments of the University of Leicester, UK (1984). Her research interests involve the study of microbial physiology using *in vivo* and *in vitro* nuclear magnetic resonance as the main analytical tool. In the last six years, she became interested in the investigation of the biochemical strategies that allow some organisms to grow at temperatures near 100 ºC; her team characterized several new compatible solutes from hyperthermophiles, determined their stabilizing effects on enzymes, and elucidated biosynthetic pathways. She is a coauthor in over 120 papers in refereed international journals, and two European patents.

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