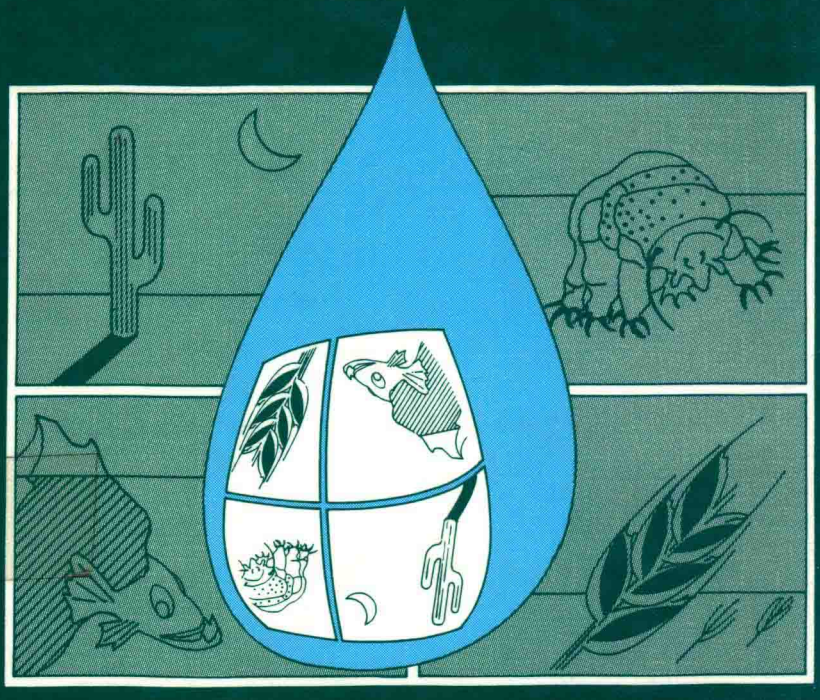
G. N. Somero C. B. Osmond
C. L. Bolis (Eds.)

Water and Life



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Water and Life

Comparative Analysis of Water Relationships at the Organismic, Cellular, and Molecular Levels

With 108 Figures

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Preface

Ich kenne die Weise, ich kenne den Text,
Ich kenn' auch die Herren Verfasser;
Ich weiß, sie tranken heimlich Wein
Und predigten öffentlich Wasser.
Heinrich Heine

The Tenth International Conference on Comparative Physiology was held on September 15–17, 1990 in Crans-sur-Sierre, Switzerland. Participants in this conference were charged with summarizing the achievements and the current state of the art for a wide variety of research areas in which water stress figures prominently. The objective of the conference organizers was to assemble in one place a group of scientists who share in common an interest in the water relationships of organisms, but whose research programs focus on widely diverse organisms – bacteria, fungi, plants, and animals – and levels of biological organization – anatomy, behavior, physiology, biophysics, biochemistry, and molecular biology. Through the formal presentations and the ensuing discussions, it was hoped that a great deal of creative cross-pollination could result. The chapters in this volume suggest that these objectives were well realized. The analyses of water relationships indeed include most of the major classes of organisms, and include analyses that range from behavioral escape from water stress to the fine-scale features of water-solute-protein interactions. A major theme of the conference was to show that common features of water structure and solute effects on water and macromolecular structure underlie many, perhaps most, of the water relationships of organisms. To the extent that this important general principle has been emphasized and further elucidated, the conference organizers will view their efforts as worthwhile.

This conference was made possible by very generous contributions from the Swiss Academy of Sciences, Swiss National Science Foundation, Swiss Physiology Society, International Commission for Comparative Physiology (IUPS, IUPAB, and IUBS), Fidia Research Laboratories (Italy), and Acqua (Italy). The editors, indeed, all participants, acknowledge too the hospitality and support shown by the Community of Chermignon (Valais). The editors also thank Dr. Jürgen Schmitt for drawing to our attention the above poem by Heine, which he – and the editors – believe summarizes especially well the ambience of this most enjoyable conference.

Summer 1991 N. SOMERO, C.B. OSMOND, C.L. BOLIS

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List of Contributors

Authors marked with an asterisk did not attend the symposium.
You will find the addresses at the beginning of the respective contributions.

I Osmotic Solutes: Evolution, Function, and Regulation

Chapter 1

Adapting to Water Stress: Convergence on Common Solutions

G.N. SOMERO

Introduction

Because all cells, at least those that are metabolically active, are approximately 85–95% water, it is a truism to state that any environmental factor that affects the activity, structure, or physical state of water poses a threat to life. The primary focus of this symposium is on the ways in which diverse organisms – archaebacteria, eubacteria, fungi, plants, and animals – cope with water stress that may arise from a wide variety of environmental phenomena, including the salinity of the medium bathing aquatic organisms, desiccation in terrestrial habitats due to elevated temperatures or low humidity, and the threat – or reality – of freezing due to extremely low air or water temperatures. Despite the diversity of organisms considered, and the varied and complex environmental stresses that affect water relationships, it will be seen that a small number of fundamental adaptive strategies are followed in virtually all cases. Thus, an important conclusion from the investigations reported in this volume is that the notion of "unity in diversity" that is a hallmark of the discipline of comparative biochemistry (Baldwin 1970) applies strikingly well to the adaptations used by diverse organisms to cope with water stress. The title of this opening chapter is, then, an attempt to emphasize that, in the preservation of a physiologically appropriate intracellular solution for metabolic activities and macromolecular structure, there appear to be but a few acceptable alternatives in terms of osmotic solute (osmolyte) composition. Convergent evolution in different groups of organism has consistently "discovered" the types of adaptations that are needed to ensure that the aqueous portion of the cell is a "fit" environment for life – a concept developed in 1913 by L.J. Henderson in his classic work, *The Fitness of the Environment.*

One goal of this symposium is to develop a detailed understanding of the basis for evolutionary selection of a limited suite of low molecular weight solutes for use as osmotic agents in the intracellular fluid. The selective basis for using only a few classes of osmolytes will be seen to involve some of the most fundamental properties of biological systems, properties that were established at the dawn of biological evolution when the development of metabolic systems based on aqueous solutions

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of macromolecules commenced. To understand the selective basis for osmolyte evolution is to understand some of the most fundamental properties governing the thermodynamic relationships of aqueous phase macromolecular systems. We consider these fundamental properties after reviewing briefly the classes of osmolytes that have been selected in diverse organisms, and noting their effects – or noneffects – on cellular macromolecules.

Organic Osmolytes: The Commonest Solutions to Osmotic Stress

Table 1 lists the commonly occurring organic osmolytes found in different procaryotic and eucaryotic cells (for detailed treatment of these distribution patterns, see Yancey et al. 1982; Borowitzka 1985). These families of organic solutes are the dominant contributors to the intracellular osmolyte pool in most osmotically concentrated cells. Inorganic ions, e.g., K^+, tend not to be the major contributors to

Table 1. Distributions of organic osmolytes

Type of organism	Major Osmolytes
	Sugars and polyhydric alcohols
Cyanobacteria	Trehalose, sucrose, glycerol, mannitol, glucosylglycerol
Fungi	Glycerol, mannitol, arabitol
Algae	Glycerol, mannitol, glucose, sorbitol, sucrose
Vascular plants	Sorbitol, glucose, sucrose, mannitol
Animals	Trehalose and glycerol (dormant forms) sorbitol (mammalian kidney)
	Amino acids and amino acid derivatives
Eubacteria	Glutamate, proline, GABA
Vascular plants	Proline
Marine invertebrates	Various amino acids
Cyclostome fishes	Various amino acids
	Methylamines
Halophilic eubacteria	Glycine betaine
Vascular plants	Glycine betaine
Marine invertebrates	Glycine betaine, TMAO
Marine cartilaginous fishes	Glycine betaine, TMAO, sarcosine
Coelacanth	Glycine betaine, TMAO
Mammals (kidney)	Glycine betaine, glycerophosphorylcholine
	Urea
Marine cartilaginous fishes, coelacanth, crab-eating frogs, mammalian kidney, estivating amphibians, lungfishes and snails	
	β-Dimethylsulfoniopropionate
Unicellular marine algae and marine macroalgae	

intracellular osmolarity in most water-stressed cells, the halophilic archaebacteria being a noted exception. When the concentration of K^+ is built up in response to osmotic stress, as in certain eubacteria (see Csonka 1989), this response may be only a transient adaptation that is superseded by the accumulation of organic solutes like proline or glycine betaine (N′N′N′-trimethylglycine; Fig. 1).

Perhaps the most noteworthy feature of the osmolyte distribution data in Table 1 is the occurrence throughout the different kingdoms of only a few classes of organic osmolytes: sugars, polyhydric alcohols, amino acids and their derivatives (e.g., taurine and β-alanine), β-dimethylsulfoniopropionate (DMSP), methylamines [glycine betaine and trimethylamine-N-oxide (TMAO)], and urea. There is no phylogenetic barrier to the accumulation of most types of organic osmolytes. For example, methylamines contribute importantly to osmotic balance in eubacteria, plants, fishes, invertebrates, and the mammalian kidney. Polyol osmolytes likewise exhibit a wide distribution among diverse organisms. These striking examples of convergent evolution reflect the common and fundamental types of interactions that occur among low molecular solutes, macromolecules and large molecular assemblages like membranes, and cellular water in all organisms.

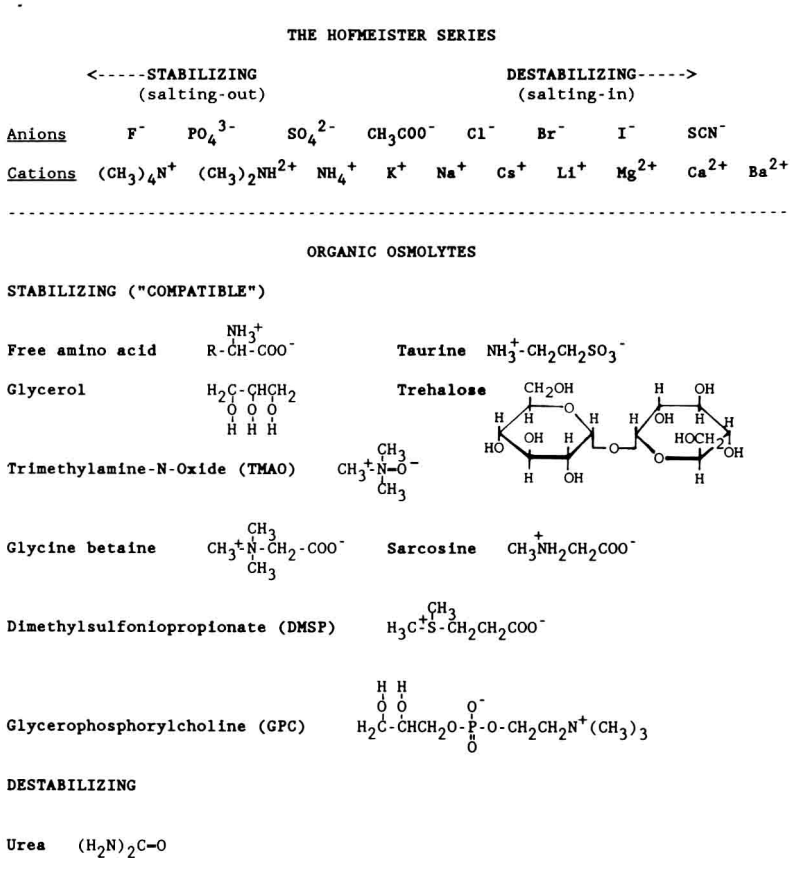

Fig. 1. The Hofmeister series and structures of commonly occurring organic osmolytes

Solute-Sensitive Processes: What Biochemical Properties Must Be Conserved in the Face of Water Stress?

To understand the fundamental bases for the selection of these particular organic osmolytes, it is helpful to review some of the properties of cells that are highly sensitive to solute, e.g., salt, perturbation, yet which must be conserved if the cell is to remain metabolically active. Among the key properties of cells that are strongly disrupted by elevated salt concentrations are rates of enzymatic catalysis, binding of ligands by enzymes, protein subunit assembly, protein compartmentation, protein solubility, interactions between phospholipid bilayers and peripheral membrane proteins, and membrane fluidity and phase separation. Each of these key properties of macromolecular systems may be disrupted when intracellular salt concentrations reach high levels, e.g., concentrations greater than a few tenths molar (Yancey et al. 1982). A primary accomplishment of adaptation to water stress, then, is the avoidance of these multiple types of salt perturbation of critical biochemical systems.

A second major aspect of desiccation stress, one which will not be considered further in this chapter, but which will be the focus of the analyses of Drs. Crowe and Hand, is the accumulation of a water substitute in cells that undergo extremes of desiccation, e.g., cysts of the brine shrimp *Artemia*, which are almost fully desiccated. In these cysts, the integrity of proteins and membranes depends on the accumulation of trehalose (Fig. 1) which can provide a "water-like" physical environment which allows macromolecules and membranes to retain their native structures even in the near-absence of water. Thus, adaptation to desiccating conditions involves the selective accumulations of solutes that either allow the metabolic apparatus to function well in a concentrated intracellular milieu, or enable this apparatus to retain its native structure under conditions where most of the water is withdrawn from the cells, and the organism assumes a state of quiescence ("anhydrobiosis").

Preserving Metabolic Function in a Concentrated Intracellular Milieu: the Halophilic Archaebacterial Solution

There appear to be only two basic types of adaptations that allow metabolic activity and macromolecular structure to be retained under conditions of elevated intracellular osmolarity. The less common of these two strategies of adaptation is manifested only in the halophilic archaebacteria (Lanyi 1974). In these salt-tolerant and salt-requiring cells, the intracellular concentration of inorganic ions (chiefly K^+ and Na^+) has been estimated to reach approximately 7 molal. At these salt concentrations the proteins of all other types of organisms would be grossly altered in structure, function, and solubility, and normal cellular structure and metabolic activity would be impossible. What adaptations allow the halophilic bacteria to thrive under – yet only under – these enormously high intracellular concentrations of inorganic ions?

Studies of a variety of different proteins from halophilic archaebacteria have revealed that these proteins typically require high salt concentrations for their normal structure and function. Figure 2 illustrates the extreme salt tolerance of malate dehydrogenase (MDH) from *Halobacterium halobium*, as contrasted with

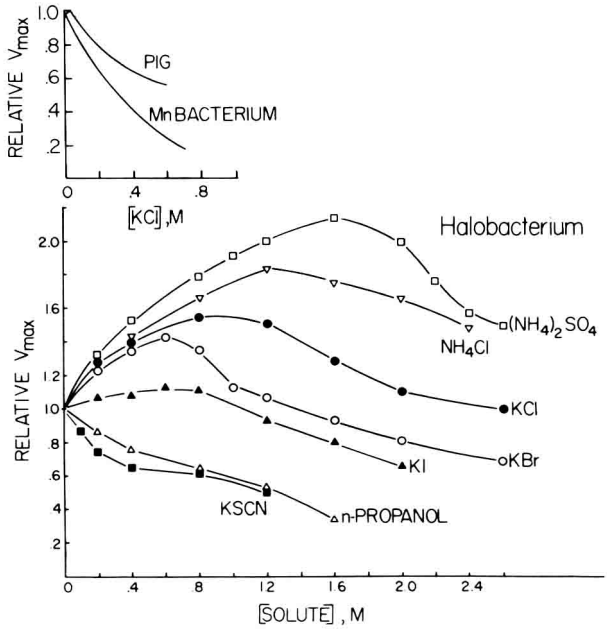

Fig. 2. The effects of different salts and n-propanol on the maximal velocity of the malate dehydrogenase (MDH) reaction of the halophilic archaebacterium *Halobacterium halobium*, and KCl effects on the activity of pig heart MDH (unpublished data of L.J. Borowitzka, S.L. French, and G.N. Somero)

the effects of KCl on MDHs of the pig and a marine manganese oxidizing eubacterium. The MDH of the halophilic bacterium not only tolerates vastly higher salt concentrations than the MDHs of the other two species, but it also exhibits peak activity at salt concentrations above 1 M in the case of certain salts, e.g., KCl, NH_4Cl, and $(NH_4)SO_4$. The halophile's MDH is just attaining peak function when the MDHs of the nonhalophiles are losing their activities.

The responses of MDH of this halophilic archaebacterium to salts reflect several important characteristics of the enzyme itself, and of the general interactions between proteins, small solutes, and water structure. First, the requirement of enzymes of halophilic archaebacteria for high salt concentrations in order to attain maximal activity is a reflection of the unusual amino acid composition of these proteins (Lanyi 1974). The proteins of these bacteria are exceedingly enriched in acidic amino acid residues (glutamyl and aspartyl residues) and depleted in basic residues (arginyl and lysyl residues). Thus, these proteins bear a high net negative charge, which prevents the proteins from folding into the native, compact structure needed for activity, unless a cationic counterion like K^+ is present at adequate concentrations. The activation of halophilic enzymes by increasing salt concentration is partly a reflection of the overcoming of charge repulsion among the acidic residues by salt titration, with the concomitant folding of the protein into a native, functionally active form.

This type of salt activation is completed by relatively low, i.e., a few tenths M, salt concentrations, a reflection of specific and strong ion binding to the carboxylate groups on the protein.

The further stimulation of activity by higher salt concentration is thought to be a reflection of the salting-out of the weakly hydrophobic groups that characterize the proteins of these halophilic organisms. In addition to bearing a large excess of negatively charged amino acid residues, proteins of halophilic archaebacteria contain, relative to proteins from other organisms, small percentages of strongly hydrophobic residues and a large percentage of weakly hydrophobic residues. The adaptive significance of this difference in hydrophobicity may involve the need to retain a satisfactory degree of protein structural flexibility in the face of salt conditions that tend to strongly stabilize protein structure. The high intracellular levels of K^+, for instance, will act to salt-out hydrophobic groups, making the proteins very rigid and insoluble. By reducing the inherent hydrophobicity of the proteins, these salting-out effects of K^+ are reduced, and the proteins retain the flexibility needed, for example, to undergo modulator-induced changes in conformation. The solubility of the proteins is enhanced by their high levels of net charge.

The activation of the proteins by different salts reflects the salts' abilities to salt-out hydrophobic groups. Ammonium sulfate, the strongest salting-out salt used in the study shown in Fig. 2, has the highest activating effect, with maximal activation being found at approximately 1.6 M salt. The effects of the other inorganic salts reflect their ranking in the Hofmeister series, the empirical ranking of ions based on their abilities to solubilize ("salt-in") or precipitate ("salt-out") proteins (see von Hippel and Schleich 1969 for review). Salts that are salting-in of hydrophobic groups, e.g., KBr, KI and KSCN, prevent the enzyme from folding into its native conformation, and are generally inhibitory of enzymatic activity. Only at low concentrations of KI and KBr do the charge-neutralizing effects of K^+ noted at low salt concentrations overcome the denaturing effects of the salting-in anions, I^- and Br^-. For the strongly salting-in salt KSCN, the ability of the SCN^- ion to solubilize hydrophobic groups is so strong that even the titration of the carboxylate groups by K^+ is unable to lead to activation of the enzyme.

The success with which halophilic archaebacteria have been able to exploit environments of extreme salinity attests to the effectiveness of this strategy of osmotic adaptation, which entails wholesale modification of proteins to allow life under high intracellular salt concentrations. However, the evolutionary success of these extreme halophiles can be viewed as having occurred at a considerable cost: these organisms are absolutely dependent for their survival on a high salt environment, and they are unable to thrive – or to survive – in the face of widely fluctuating salinities. This latter ability is a hallmark of the wide variety of halotolerant species, bacteria, plants, and animals, that have taken a different evolutionary route in developing tolerance of concentrated media. Osmotic adaptation in these halotolerant, and frequently very euryhaline, species has not entailed wholesale modification of the proteins of the cells, as in the halophilic archaebacteria, but rather has involved the selective accumulation of low molecular weight organic molecules that can be accumulated to extremely high concentrations without perturbing proteins. These organic osmolytes have appropriately been termed "compatible" solutes (Brown and Simpson 1972).

Compatible Solutes: the Most Common Solution to Water Stress

Since the pioneering work by Brown and colleagues (e.g., Brown and Simpson 1972; Borowitzka and Brown 1974; Brown 1976), the widespread occurrence and physiological significance of compatible solutes has been documented in a wide array of osmotically concentrated organisms (Table 1). As illustrated in Fig. 3, solute compatibility with protein structure and function has been shown for polyhydric alcohols like glycerol, certain free amino acids and their derivatives (e.g., taurine), and methylamines like glycine betaine, osmolytes with widespread occurrence in the different kingdoms.

The effects (or noneffects) of compatible solutes on protein function are in contrast to the effects of even relatively benign inorganic salts like KCl and NaCl. Thus, for example, the activity of glucose-6-phosphate dehydrogenase of the halotolerant and euryhaline green alga *Dunaliella viridis* is strongly inhibited by KCl and NaCl, with virtually all activity lost by ~3 M combined salt concentration

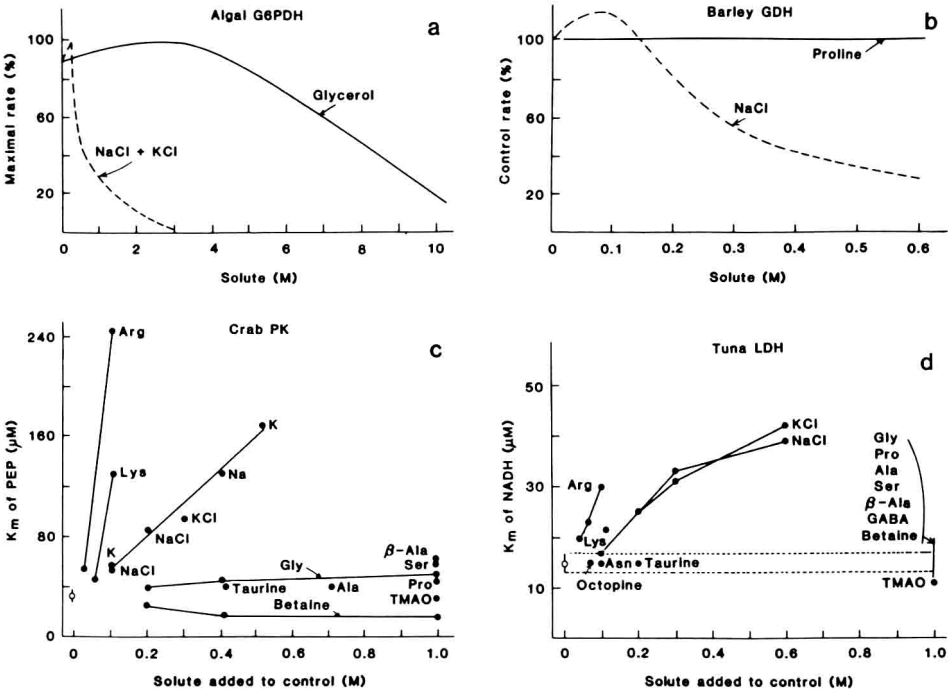

Fig. 3a-d. The effects of salts and organic osmolytes on enzymatic activities and Michaelis-Menten constants. **a** Salt and glycerol effects on the maximal velocity of the glucose-6-phosphate dehydrogenase reaction of the unicellular green alga *Dunaliella viridis* (Borowitzka and Brown 1974). **b** NaCl and proline effects on activity of glutamate dehydrogenase from barley (Stewart and Lee 1974). **c** Salt and organic solute effects on the K_m of phosphoenolpyruvate (PEP) for pyruvate kinase (PK) of the marine crab *Pachygrapsus crassipes* (Bowlus and Somero 1979). **d** Salt and organic solute effects on the K_m of NADH of muscle-type lactate dehydrogenase (LDH) from the bluefin tuna (Bowlus and Somero 1979). (Yancey et al. 1982)