

## CLASSICS

## There was more to osmolyte selection than just osmotic balance



Jason Treberg discusses the impact of Paul Yancey et al.'s classic paper 'Living with water stress: evolution of osmolyte systems', published in *Science* in 1982.

Occasionally a hypothesis comes along that is so broadly applicable to biological systems that it borders on being universal. Even less frequent is when the hypothesis involves linking knowledge from almost a century before with recent experimental observations on physiological and biochemical responses to environmental challenges. However, the seminal paper by Paul Yancey and colleagues (1982) was one such feat. The paper, entitled '*Living with water stress: evolution of osmolyte systems*', synthesized ideas into the now well-accepted notion that the small solutes that are accumulated within cells for osmotic balance were likely selected because they do not disrupt macromolecular structure or function.

The paper is well suited to those who are unfamiliar with osmoregulatory biology and physicochemical interactions because it summarizes the literature on the intracellular solutes common across taxa while also proposing a profoundly wide-reaching hypothesis linking the convergence of intracellular osmoregulatory strategies across diverse organisms.

The authors noted how, out of all the possible low molecular weight molecules 'available' for intracellular accumulation, only a small number have been selected by most taxa. Moreover, most of the selected solutes were small organic compounds. Perhaps more remarkable was the narrow range of chemical classes

that the accumulated 'organic osmolytes' fell into: they were virtually all polyhydric alcohols (polyols such as glycerol or mannitol), free amino acids and their derivatives or methylamines [a group of molecules with one or more methyl moiety attached to an amine group, such as trimethylamine-*N*-oxide (TMAO) and glycine-betaine]. In addition, urea – a solute also found across diverse animal taxa – was viewed as an unusual evolutionary option, because at concentrations of hundreds of millimoles per litre, which can be found *in vivo*, it is known to denature proteins and disturb function.

Most of the intracellular organic osmolytes found across organisms could be further described as 'compatible osmolytes' [reviewed in Yancey et al. (1982) but also see Brown (1976)], which are solutes that can accumulate in the cell without significantly disturbing macromolecular function and cellular processes. Yancey and co-workers then went on to demonstrate the concept of osmolyte compatibility using examples from barley, algae, a decapod crab and a teleost fish, where increasing amounts of compatible solutes do not alter enzyme kinetics or substrate binding, whereas increasing ion concentrations (NaCl or KCl) markedly decreased either enzyme activity or substrate affinity (see Yancey et al., 1982).

Having considered the role of compatible osmolytes in protein stabilisation, Yancey and his co-authors went on to consider how these organic compounds may counteract the effects of other potentially damaging osmolytes. Previously, Yancey and his mentor George Somero had recognized that some vertebrates accumulate high levels of urea in the order of several hundred millimoles per litre, which are high enough to destabilize many proteins. Yet, despite the apparent evolutionary pressure to evolve proteins that could resist the detrimental effects of urea, urea-accumulating species such as the chondrichthyans (elasmobranchs and Holocephali) have not, in general, evolved proteins that are urea resistant. So what was stabilizing the protein structure?

Previously, Yancey and Somero had also noted that species that have high levels of urea also tend to accumulate high levels of methylamines. Knowing that mixing stabilizing methylamines with the destabilizing osmolyte urea could counteract the detrimental effects of destabilising solutes (Yancey and Somero, 1980), Yancey and colleagues proposed the 'counteracting solute' hypothesis, where the opposing effects of different solutes could lead to no net impact on macromolecular function (Yancey et al., 1982).

Subsequently, the paper inspired a substantial body of research expanding on the protein–solute compatibility hypothesis, which continues to this day (Canchi and García, 2013); and some of the 1982 discussion, questioning whether the solutes directly interacted with proteins or mediated their effects via solvents, now, to me, seem ahead of their time. The team also noted the structural similarity between many of the stabilizing and destabilizing solutes that they had found within organisms and the ions in the Hofmeister series – where Hofmeister's 'salting out' ions and the 'salting in' ions, some of which could potentially stabilize protein structures, could also be mixed to counteract each other's influence (Hofmeister, 1888; Yancey et al., 1982; Clark and Zounes, 1977). The ideas laid out in Yancey et al. have more than stood the test of time, yielding >2000 citations in most scientific database searches and registering a Hirsch-index (h-index) value of well over 100. An even more compelling measure of the impact of this paper is the diverse nature of the journals citing the work, ranging from plant biology, physiology, medicine, evolution and physicochemical publications.

The authors also refer to the hypothesis as a case for 'genetic simplicity' in evolutionary adaptation, which – in my opinion – seems to have been overlooked, despite Yancey et al.'s compelling arguments. Solute influences are general to most proteins, where stabilizing solutes favour a more compact/stable protein structure regardless of the genetic

Classics is an occasional column, featuring historic publications from the literature. These articles, written by modern experts in the field, discuss each classic paper's impact on the field of biology and their own work.

background. So, over the course of evolution, instead of modifying each individual protein to adapt to and tolerate high-salinity stress, most organisms have evolved a few genetic modifications to facilitate the synthesis and regulation of a small number of metabolites to avoid perturbing protein structure and function, providing a strategy for tolerance to osmotic stress that does not require the creation of multiple isoforms for each protein, each of which is tailored to an individual osmotic situation.

Indeed, the overall concept of protein stabilization by cellular solutes has also been extended to include protection against salt stress, dehydration, temperature extremes, freezing and hydrostatic pressure (Yancey, 2005), demonstrating the wide utility of the core concept: that solutes selected for accumulation in cells may have functional importance far beyond simply acting as osmolytes.

Personally, this paper (Yancey et al., 1982) had a major influence on my scientific career. Having entered scientific research to ‘work on sharks’, I had something of an epiphany when I realized that the counteracting solute hypothesis was clearly demonstrated by the bizarre osmolyte selection found within

chondrichthyans. It illustrated to me how little I had, until then, appreciated that an understanding of comparative biochemistry can provide an explanation for biological mechanisms and their evolution. This led to me working on organic osmolytes for some time, including a period where I focused on my personal favourites, the chondrichthyans. And I now use Yancey et al.’s classic 1982 paper to demonstrate the ‘unity in diversity’ concept when teaching undergraduates about comparative approaches in biology.

The paper also used an integrative approach across all Domains of life and included data from many of the currently recognized kingdoms within the Eukarya. And yet, despite the diverse examples, the general hypothesis was supported. Even the ‘exceptions’, like the halophilic Archaea, which bucked the ‘genetic simplicity’ trend by accumulating ions rather than compatible solutes, ostensibly fit the model. This is because, with their proteins adapted to high intracellular ion strength, these biological outliers are also obligatorily restricted to high-salt environments, whereas some glycerol-accumulating algae can readily acclimate to high external ion concentrations but are by no means restricted to these extreme environments (Yancey et al., 1982).

Ultimately, it was the synthesis and hypotheses put forward by Yancey and colleagues in their 1982 Science paper that helped provide an important bridge between studies using solutes to probe protein stability/structure/function relationships at a protein chemistry level and natural selection’s ‘discovery’ of many of the same molecules for intracellular osmoregulation.

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