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Life under Extreme Conditions - A Physico-Chemical VIEW

Remarkably, most of our biosphere on Earth is in the realm of environmental extremes, including the hydrothermal vents in the deep sea and the cold and high hydrostatic pressure habitats in the ocean depths. The ocean covers more than 70% of our planet's surface, and the average pressure encountered is 400 bar. Psychrophilic-piezophilic (cold- and pressure-adapted) species are even found on the deepest ocean floor (at 11,000 m) and in deep sea sediments, called the deep biosphere, where the pressure reaches 1100 bar or more [1, 2]. Recent drilling explorations indicate that marine and continental subsurface environments are habitable to depths greater than 5 km, and that this vast habitable environment may host a significant fraction of the total microbial biomass on Earth. Recent discussion of the temperature-pressure limits of life has been controversial. The upper temperature limit for life is currently standing at 122 °C. Claims that certain bacterial strains are viable up to 10 kbar and more at room temperature were first met with skepticism, but are now avidly supported. This leads us to the fundamental question of how the organisms that survive under such harsh conditions manage to do so. Although the history of pressure studies on biomolecular systems began 100 years ago when Nobel Prize laureate Piercy Bridgman reported the coagulation of egg white under high hydrostatic pressure at room temperature, a molecular understanding of the physical and chemical properties as well as elementary reactions of biomolecular systems under high hydrostatic pressure (HHP) conditions is still largely unknown, thus calling for detailed biophysical studies of biomolecular systems under such extreme conditions [2, 3]. In the search for habitable environments elsewhere in the cosmos, such as on Mars or on other planets and their moons in our solar system, one criterion is to look for liquid water, since non-water-based life has not yet been spotted [4]. A scientific question of great interest at present is the habitability of the Martian subsurface. However, the mere presence of liquid water does not make an environment habitable. How life responds to external physical and chemical factors, such as temperature, pressure, pH and salinity, also defines the limits of habitability. Below, we present some examples of physicochemical studies that address the effects of such extreme environmental conditions on the structure, dynamics and function of biomolecular systems, and also discuss possible mechanisms that could help mitigate the deleterious effects of such harsh conditions.

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For canonical double-stranded DNA structures (B-DNA), even kbar-pressures have been shown to have either a small stabilizing, destabilizing, or no effect on the structure of the DNA, depending on the temperature and salt concentration [5]. Recently it was found that non-canonical nucleic acid structure, such as DNA hairpins and G-quadruplexes, are much more pressure-sensitive. Single-molecule Förster resonance energy transfer (smFRET) measurements were used to directly measure their population distribution at HHP conditions. The pressure sensitivity of such structures is due to a conformational transition from a closed state to an open state, which is accompanied by a volume decrease in the order of -10 to -30 $cm^3\,mol^{-1}$ [6].

Biological membranes are among the most pressure-sensitive biomolecular systems. Their basic structural element consists of a phospholipid bilayer matrix, self-assembled by the hydrophobic effect. Membrane integrity and functionality is crucial for the cell, e.g., for energy generation, transport and signal transduction. The physicochemical properties of the membrane (e.g., lipid packing, fluidity, lateral organization, bending modulus), which modulate lipid-protein interactions and membrane protein function, are markedly affected by HHP. Phospholipids often exhibit lamellar phase transitions, such as a gel-to-liquid-crystalline (fluid) chain-melting transition. In the fluid-like phase, which is required for optimal physiological function, the lipids' acyl-chains are conformationally disordered ("melted"), whereas in the gel phase, the chains are ordered and densely packed (Figure 1). HHP leads to lipid chain ordering and eventually transition to a densely packed gel phase, compromising membrane protein function. A common slope of about 20 °C/kbar was observed for the gel/fluid phase boundary of saturated phospholipids (e.g., DMPC) using fluorescence, NMR and FTIR spectroscopic techniques which report on the membrane fluidity and the lipids' conformational state [3]. Conversely, phospholipids with *cis*-double bonds (e.g., DOPC) lead to very low chain-melting temperatures and smaller $T(p)$-phase transition slopes. They impose kinks in the linear conformations of the lipid acyl-chains, creating significant free volume fluctuations in the bilayer so that the ordering effect of HHP is reduced (Figure 1). Therefore, to maintain the physiologically relevant fluid-like state at high pressures, more of such cis-unsaturated lipids are incorporated into cellular membranes of deep sea organisms, which must survive at ~3 °C and pressures up to 1 kbar. Upregulation of the amount of unsaturatied lipids represents one type of homeoviscous adaptation. Similar adaptation mechanisms have also been observed for archaeal membranes through changes in their specific lipid chain structure. But nature is also able to control the fluidity of its membranes in other ways, such as by the incorporation of sterols and polyisoprenoids [1, 3].

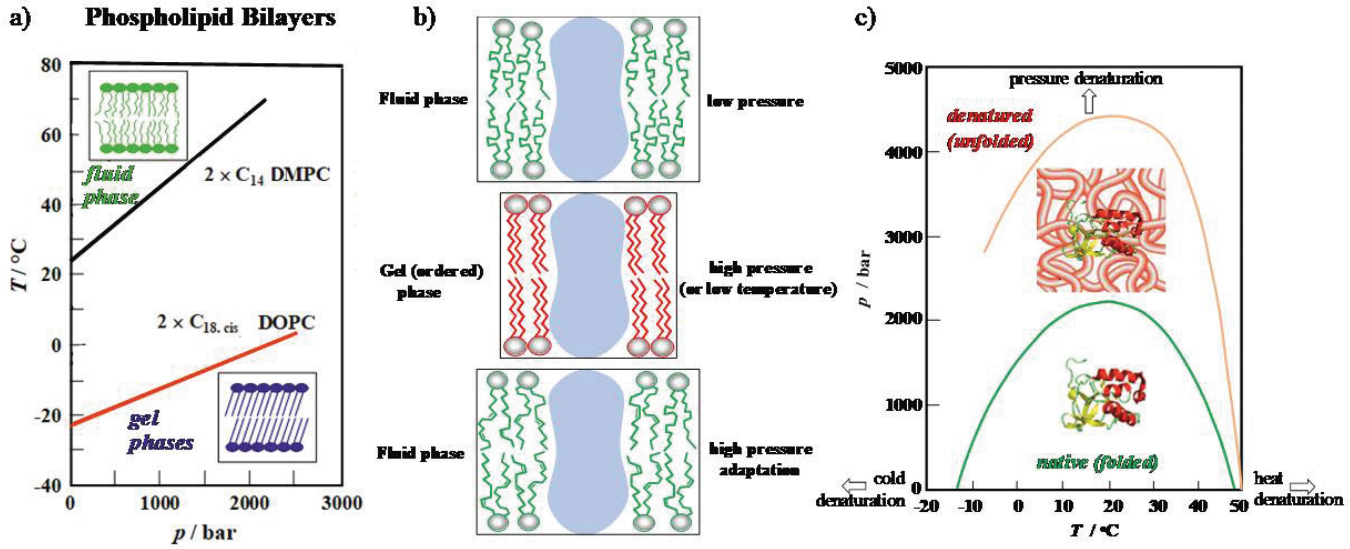

Fig. 1: a) (*T*, *p*)-phase diagram of a saturated (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, DMPC) and an unsaturated (1,2-dioleoyl-*sn*-glycero-3-phosphocholine, DOPC) phospholipid bilayer membrane. b) Schematics of the packing of a lipid bilayer with embedded membrane protein at ambient (low) pressure, at high pressure (or low temperature), and at high pressure in the presence of unsaturated lipids (adaptation). c) (*p*, *T*)-stability phase diagram of SNase (staphylococcal nuclease) in buffer solution (green line) and the effect of 30 wt% of the macromolecular crowding agent Ficoll (a polysaccharide) mimicking-cell-like crowding conditions (orange line) on the stability diagram (modified from [3]).

While protein folding and unfolding has been studied primarily in terms of perturbations by temperature or chemical denaturants (such as urea), a complete thermodynamic description of the stability of proteins also includes characterization of the response of protein structure to pressure. The stability curve of proteins in the (*p*, *T*)-plane has an elliptic-like shape as shown in the example illustrated in Figure 1c, which has been obtained invoking conformation-sensitive techniques such as SAXS, FT-IR, NMR and fluorescence spectroscopies [3, 5]. At pressures of about 2-8 kbar, most monomeric proteins unfold reversibly. Oligomeric proteins and multiprotein assemblies (including polymeric actin and tubulin) often dissociate into individual subunits already at much lower pressures. Next to release of void volume which is filled by water upon unfolding, negative volume changes driving the unfolding reaction also result from the disruption of polar and ionic bonds and subsequent hydration (electrostrictive effect) [3, 5]. As indicated in Figure 1c, proteins may also unfold in the cold, which often occurs between −30 and 0 °C. Since the freezing point of protein solutions can be as low as −21 °C (or even lower in salt solutions) at elevated pressure, proteins are still able to function at sub-0 °C temperatures in HHP environments. Of note, the (*p*, *T*)-phase diagram of life resembles that of proteins. Both exhibit similar, curved (*p*, *T*)- phase diagrams, whereas lipids and nucleic acids generally exhibit linear (*p*, *T*)-phase diagrams.

Our current knowledge of proteins from extremophiles is still rather limited. Certain types of amino acids appear to be preferred in protein sequences from specific environments [2]. Proteins from thermophilic organisms tend to have a higher proportion of hydrophobic residues or ion pairs. Halophilic organisms, which must maintain high levels of intracellular ions, have proteins whose sequences exhibit a preference for surface-exposed acidic residues. Proteins from psychrophilic organisms have evolved to maintain appropriate levels of dynamic motion even in the cold and tend to have fewer stabilizing interactions. Adaptation of proteins to HHP habitats is even less clear. However, not only mutations of the amino acid sequence of proteins, but also the composition of the cytoplasm of the extremophile has a significant impact on protein stability. The cellular milieu is fairly crowded and contains a rather complex aqueous solution with a variety of different co-solutes (organic osmolytes). Organisms living under extreme conditions are able to accumulate particular cosolvents in their cells, such as sorbitol or trimethylamine-*N*-oxide (TMAO) to protect their proteins from denaturation. Interestingly, TMAO, one of the most potent cosolvents found to date, was found to be upregulated in deep sea organisms up to high levels [7-9], and acts as a chemical chaperone to counteract the deteriorating effect of pressure not only on proteins but also on non-canonical DNA and RNA structures. Also macromolecular crowding can help stabilize biomolecular systems. As shown in Figure 1c, temperature- and pressure-induced unfolding of SNase is shifted significantly to higher temperatures and pressures in 30 wt% polysaccharide solution mimicking intracellular crowding conditions [3]. Such stabilizing effects is largely due to the excluded volume effect imposed by the crowder molecules, which favors the more compact folded protein state. Interestingly, both cosolvents and crowding have been shown to strongly affect not only the stability but also the conformational and internal dynamics of proteins and thus their response to HHP [2].

Although the question of whether there is life on other planets like Mars remains speculative, we can ask questions about how physical and chemical conditions expected might theoretically influence their habitability. We can then use terrestrial organisms and biomolecules to explore the boundary space of habitability for known life under such conditions. The main candidates for habitable environments on Mars are deep aqueous environments. Next to sulfate, these cold and high-pressure environments are thought to contain high concentrations of perchlorate salts, due to their very low eutectic temperatures. Perchlorate salts have been found to have deleterious effects on microbial

life and therefore must be considered as a factor that will affect the habitability of these environments. We used well characterized biomolecular systems, such as the enzyme α-chymotrypsin (α-CT), to investigate the combined effects of perchlorate ions and high pressures as a proxy for biomolecular stability, organization and reactivity under these extreme conditions [10, 11]. Figure 2 shows the effect of these Martian salts on the (*p*, *T*)-stability diagram well as on the activity of α-CT. Increasing the perchlorate concentration reduces the temperature and pressure stability of the protein, shifting the boundary to lower values of temperature and pressure. However, the protein is still rather pressure stable at low temperature and in the presence of perchlorate. HHP increases the enzymatic activity of α-CT, even in the presence of high concentrations of the chaotropic salt $Mg(ClO_4)_2$, which is due to the negative activation volume ($\Delta V^{\neq} < 0$) of the reaction. On the other hand, $MgSO_4$ increases both the stability and activity of α-CT. The difference in the effect of both salts is mainly due to the fact that they preferentially interact with the peptide backbone (perchlorate) or are excluded from the peptide surface (sulfate). This indicates a potential beneficial interplay of salt, pressure, and activity. While concentrated perchlorate brines reduce the habitability of an aqueous environment, high pressures and lower temperatures may counteract the deleterious perchlorate effect and thus increase habitability. Similar to the enzymes of extremely halophilic archaea, changes in amino acid composition under such high perchlorate concentrations could also contribute to enhanced performance of the enzyme. It will be interesting to see if sub-0 °C temperatures favor survival of bacteria and archaea in perchlorate brines.

We now know how and why extreme physical and chemical conditions alter the functionally important structure and dynamics of proteins and many other biomolecules. However, to better understand the processes associated with the physical limits of life, far-reaching high-pressure studies of biological systems at different levels of complexity (genome, proteome, lipidome, metabolome, ...) are still needed, which should also take into account the particular cellular milieu of the extremophiles. These results could not only lead to a deeper understanding of fundamental life phenomena, but also reveal previously unknown adaptation mechanisms. The diversity of extremophile organisms and the biomolecules they synthesize will provide a database that can also be used for biotechnological applications that require enzymes with specific properties to achieve optimal target activity [2].

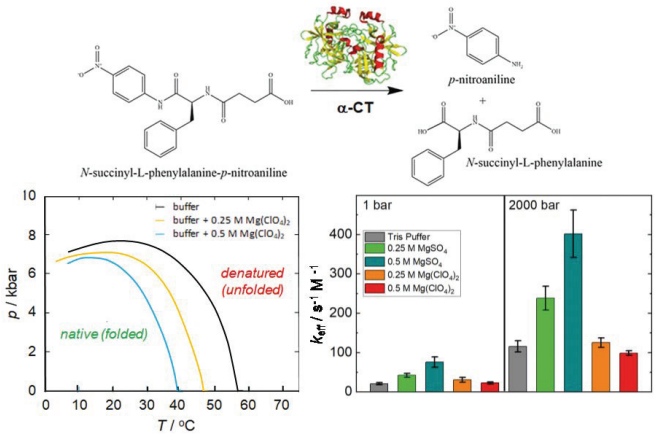

Fig. 2: Bottom, left: (*p*, *T*)-stability phase diagram of α-chymotrypsin. The lines indicate the transition from the native to the (partially) unfolded state. Bottom, right: Kinetic efficiency, k_{eff}, of the hydrolysis reaction shown above for different salt solutions obtained by Michaelis-Menten plots of the enzymatic activity of α-CT at T = 20 °C in different buffer solutions at two selected pressures, 1 bar and 2000 bar (adapted from [10]).

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