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Impact of high hydrostatic pressure on bacterial proteostasis

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ABSTRACT

High hydrostatic pressure (HHP) is an important factor that limits microbial growth in deep-sea ecosystems to specifically adapted piezophiles. Furthermore, HHP treatment is used as a novel food preservation technique because of its ability to inactivate pathogenic and spoilage bacteria while minimizing the loss of food quality. Disruption of protein homeostasis (*i.e.* proteostasis) as a result of HHP-induced conformational changes in ribosomes and proteins has been considered as one of the limiting factors for both microbial growth and survival under HHP conditions. This work therefore reviews the effects of sublethal (≤100 MPa) and lethal (>100 MPa) pressures on protein synthesis, structure, and functionality in bacteria. Furthermore, current understanding on the mechanisms adopted by piezophiles to maintain proteostasis in HHP environments and responses developed by atmospheric-adapted bacteria to protect or restore proteostasis after HHP exposure are discussed.

1. Introduction

Hydrostatic pressure is an important parameter in the biosphere. Deep-sea environments, incurring hydrostatic pressures up to 110 MPa, are populated by a high diversity of microorganisms, known as piezophiles, which often require these high pressures for optimal growth [1]. By contrast, growth of atmospherically adapted microorganisms, such as the mesophile *Escherichia coli*, progressively becomes compromised with increasing pressure and completely ceases around 50 MPa [2–3]. At exposure to pressures exceeding 100 MPa, microorganisms start suffering lethal injuries, a phenomenon currently exploited in modern food preservation. In fact, high hydrostatic pressure (HHP) processing is considered a promising non-thermal pasteurization approach that inactivates foodborne pathogens and spoilage microorganisms without compromising the nutritional and sensorial properties of the food [4]. Unfortunately, foodborne pathogens such as *E. coli* O157:H7 and *Listeria monocytogenes* can acquire extensive levels of HHP resistance, indicating that pressure adaptation is a readily evolvable trait [5–6].

The influence of HHP on biomolecules is essentially described by the thermodynamic principle of Le Châtelier and Braun, which states that a molecular system will counteract an increase of pressure by occupying a smaller volume [7–8]. In proteins, disruption of hydrophobic and electrostatic interactions, which play a major role in maintaining the quaternary and tertiary structure, are known as the main driving forces in the reduction of protein volume [8–9]. In general, pressures < 200 MPa result in dissociation of oligomeric proteins, while higher pressures cause unfolding of monomeric proteins because of water penetration in internal cavities, typically reaching irreversible unfolding at pressures > 400 MPa [9–10]. In contrast, double stranded structures of nucleic acids are stabilized under pressure because enhanced stacking of hydrophobic bases [11]. Pressure also induces better packing of the acyl chains within the phospholipid bilayer resulting in lateral shrinking and increased thickness, which causes a fluidity transition from liquid-crystalline to the gel phase [7,12].

At the integrated level of the growing and living cell, however, the manifold of HHP effects on biomolecules inevitably culminates into pleiotropic cellular defects and phenotypes, compromising DNA replication, transcription, translation, protein functionality, and membrane integrity. Since the cellular perception and impact of HHP are to an important extent driven by the effect of HHP on proteins, this review is focused on the effect of sublethal (≤100 MPa) and lethal (>100 MPa) pressures on the synthesis, structure, functionality, and management of proteins in both mesophilic and piezophilic bacteria.

2. Effect of sublethal HHP on bacterial growth

2.1. Cellular impact of sublethal HHP on proteostasis

Dissociation of protein complexes at pressures under 100 MPa may play a decisive role in growth inhibition of mesophilic bacteria, as many of these complexes are involved in essential cellular processes such as replication, transcription, and translation (Table 1). In this context, DNA replication has been shown to be one of the most pressure sensitive processes of macromolecule synthesis [2]. A detailed *in vivo* study on the effect of HHP on DNA, RNA, and protein synthesis was

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Table 1
Effect of sublethal (<100 MPa) and lethal (>100 MPa) HHP on bacterial proteostatis. Data were obtained in *E. coli* except where indicated.

Phenotype	Pressure (MPa)	Reference
Effect of sublethal HHP on bacterial growth (<100 MPa)		
Growth inhibition	50	[2–3]
Cell filamentation		
Depolymerization of FtsZ and inhibition of septum formation	50	[13]
Mrr-triggered DNA-damage response leading to SulA-mediated FtsZ inhibition	75–100	[35–36]
Inhibition of DNA replication	50–81	[2]
Inhibition of RNA synthesis	77–80	[2,14]
Alterations in the regulation of gene expression	30–70	[16–18]
Inhibition of protein translation	60–70	[2,19]
Alteration of transmembrane proteins		
Transient inhibition of proton transfer ATPase (*S. faecalis*)	50	[24]
Dimerization of ToxR (*V. cholerae*)	20–50	[27]
Effect of lethal HHP shock on bacterial survival (>100 MPa)		
Loss of membrane integrity and leakage of intracellular proteins	100–600	[89,100]
Irreversible ribosomal damage (*E. coli*, *L. monocytogenes*, *L. mesenteroides*)	100–500	[67–69]
Emergence of cytosolic aggregates (*E. coli*, *L. monocytogenes*, *L. mesenteroides*)	300–600	[68,74–75]
Inactivation of transmembrane proteins and disruption of pH homeostasis		
F0F1-ATPase (*L. plantarum, Lactococcus lactis*)	250	[25,65]
Arginine and glucose pH homeostasis transmembrane proteins	300	[66]
Disaggregation of pre-existing protein aggregates	200–400	[64,81]

performed by Yayanos and Pollard [2] in *E. coli*, whose cell division is inhibited at 50 MPa accompanied by a characteristic filamentous growth phenotype. These authors showed that intracellular incorporation of radioactively ^{14}C-labelled thymine at pressures between 50 MPa and 81 MPa only occurred for an initial period and then ceased, suggesting that initiated DNA replication rounds were completed but initiation of new DNA replication rounds was compromised. Although it currently remains unclear how these pressures molecularly preclude the initiation of DNA replication, it was recently shown that destabilization of the clamp loading complex (by deleting the χ or HolC subunit) or disruption of the replication restart primosome (by deleting DnaT or PriA) inhibited growth of *E. coli* already at 30 MPa [3]. Aside from the replisome, divisome functionality has been shown to become compromised as well. In fact, Isshi et al. [13] demonstrated that the septum forming FtsZ polymers of *E. coli* undergo dissociation *in vitro* at 50 MPa, while *E. coli* cells displaying filamentous growth at 50 MPa lacked an FtsZ ring.

Exposure of mesophilic microorganisms to sublethal pressures also progressively impairs the process of protein production at both the transcriptional and translational level. According to the incorporation rate of ^{14}C-labelled uracyl in *E. coli* under pressure, Yayanos and Pollard [2] showed that RNA synthesis became impaired at 21 MPa and was completely inhibited at 77 MPa. In line with these findings, an *in vitro* study on *E. coli* RNA polymerase (RNAP) activity under pressure showed that transcriptional elongation was progressively delayed from 20 MPa upwards and halted at pressures exceeding 80 MPa, although the atmospheric rate of elongation could be completely recovered after decompression [14]. Interestingly, RNAP complexes actively involved in elongation were even able to resume transcription after exposure to 150 MPa for 30 min, whereas 80% of free RNAP complexes were irreversibly inactivated at such pressure. Aside from structural RNAP effects, however, HHP can also affect gene expression in a regulatory fashion due to its impact on the interaction between DNA and activator or repressor proteins [15]. As such, the activity of the *E. coli lac* operon promoter was shown to increase 78-fold at 30 MPa in the absence of the inducer isopropyl-beta-D-thiogalactopyranoside (IPTG) [16], presumably because of the dissociation of the tetrameric LacI repressor protein from the *lac* operator [17–18].

In turn, protein translation is completely inhibited in *E. coli* at pressures of 60–70 MPa, although it can be rapidly resumed after decompression [2,19]. This inhibition has been linked *in vitro* and *in vivo* to ribosome subunit disassembly upon exposure to sublethal pressures [19–20]. Under high pressure, the association-dissociation equilibrium of the voluminous multimeric 70S complex shifts to the corresponding 30S and 50S constituents [21]. Furthermore, an *in vitro* study has suggested that the post-translocational complex is the most HHP sensitive intermediate of the translation elongation cycle [20].

A number of membrane proteins have been found to suffer from <100 MPa exposure as well, although it remains unclear to which extent this can be attributed to HHP-mediated changes in lipid bilayer fluidity [22–23]. For instance, the proton translocating activity of the F0F1-ATPase of *Streptococcus faecalis* is arrested at 50 MPa [24], which may lead to ATP depletion and disruption of pH homeostasis [25–26]. Furthermore, the dissociation of the transmembrane dimer ToxR, which initiates the signalling cascade for transcription of *Vibrio cholerae* toxins, is induced at 20–50 MPa [27], likely because of conformational changes in the ToxR protein itself and independently of the lipid membrane state [23]. In this context, it is also noteworthy that the ToxR homologue of the deep-sea bacterium *Photobacterium profundum* is involved in the signalling of the pressure adaptation response [28].

In contrast to the previous effects that mainly stem from a compromised protein functionality, the activity and specificity of some enzymes can be directly modulated by pressures low enough to maintain their stability. Enzymatic activity under pressure can be favoured by a negative volume change associated with the chemical reaction and by partial substrate unfolding allowing a better enzymatic access [29]. For example, an *in vitro* assay demonstrated that the hydrolysis rate of β-lactoglobulin by the thermolysin protease produced by *Bacillus thermoproteolyticus* increased by 22-fold with pressure upshift from 0.1 MPa to 200 MPa, while it decreased at pressures above 300 MPa because of enzyme conformational changes [29–30]. Furthermore, thermolysin activity on non-specific protein substrates, such as alcohol dehydrogenase and haemoglobin, was accelerated by pressure increase up to 200 MPa, which was attributed not only to the pressure effect on the reaction equilibrium but also to partial substrate unfolding [29,31].

Enhanced enzymatic activity upon pressurization has also been reported *in vivo* in the case of the enigmatic endogenous Type IV restriction endonuclease Mrr of *E. coli* K-12. Mrr-mediated restriction of the host chromosome and concomitant induction of the DNA damage (SOS) response was originally observed upon the heterologous expression of certain foreign methyl transferases in *E. coli* K-12 [32−33]. Strangely, however, exposure of this strain to a HHP shock of approximately 100 MPa appears to trigger Mrr activity even in the absence of foreign methyl transferases, in turn generating double strand breaks in the host chromosome that result in a RecBCD-dependent activation

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