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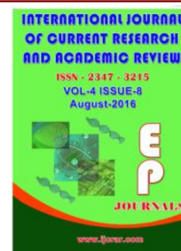

Thriving at High Temperatures: Molecular Adaptations in Hyperthermophic Microorganisms

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A B S T R A C T

The Hyperthermophilic microorgnisms thrive at high or very high temperature ranges, were first described in 1981 with the optimal temperatures higher than 80^{0}C. They are now in focus due to their unique thermo-stable enzymatic systems. The survival of these microbes at these high temperature ranges (80^{0}C or above) is highly correlated with the thermo-adaption molecular mechanisms, which ultimately help to combat with thermal inactivity of the cell components. The review focuses on a brief understanding about molecular part of the thermal stability of hyperthermophilic microbes, by concentrating on the genome and proteome levels. The mechanisms are very helpful in various biotechnological and fermentation processes as these processes most of the times carried out in high temperatures, thus requires thermostable enzyme systems.

Introduction

The growth of hyperthermophilic microorganisms is now very well understood that it established at temperatures well above 100°C, the boiling point of the water. The *Pyrolobus fumarii* is the described microorganism with the highest recorded growth temperature of 113.5°C, is the chemolithoautotrophic archaeon (Blochl *et al.*, 1997). These microorganisms have now been isolated from all types of natural and manmade marine and terrestrial hot environments,

which were actually discovered in 1967 by Thomas D. Brock from the hot spring of yellow stone national park (Vieille and Zeikus, 2001). They are widely distributed in hydrothermal environments in terrestrial as well marine and abyssal vent systems. The group includes 21 archaeal genera, and two bacterial genera with diverse growth physiology including both heterotrophs and chemoautotrophs (Stetter, 1996).

They grow optimally at temperatures between 80 and 110^{0}C, thus the enzymes from these unusual microorganisms usually withstand with temperatures that would rapidly denature most of the mesophilic or even some thermophilic (optimum growth temperature=<80^{0}C) proteins and enzymes (Pandey *et al*., 2016). Therefore the thermozymes having considerable biotechnological interest over mesophilic or psychrophilic enzymes due to their thermostability which is being associated with resistance to chemical denaturants, extraordinary performance and stability at high temperatures, easy purification by heat treatments after expression, often higher reaction rates due to decrease in viscosity at high temperature and even no risks of microbial contamination during product formation process (Danson *et al*., 1996; Adams and Kelly, 1998; Pandey *et al*., 2016). Following are the various molecular adaptation mechanisms that have been studied about stability in microbial cells of such harsh and very high temperature environments.

Adaptations in Hyperthermophiles at Proteome Level

Most of the proteins, produced by thermophilic and hyperthermophilic microorganisms are generally highly resistant to thermal and chemical denaturation. Although 20 natural amino acids of the mesophilic proteins have been found as building blocks of thermophilic and hyperthermophilic proteins, but the post synthetic modifications. *e.g.*, methylation of lysine, particularly in many proteins of *Sulfolobus* sp. as well as some other mechanisms observed in a number of thermophilic proteins (Robb and Clark, 1999). The thermostability in hyperthermophiles in the extreme thermal environments is the cumulative effect of packing efficiency (mainly through Van der Wall's interactions), networks of ion pairs and/ or hydrogen bonding (including α helix stabilization), the reduction of conformational strain (loop stabilization), and resistance to chemical modifications (Jaenick and Bohm, 1998; Robb and Clark, 1999). The more criteria for the stability enhancement appears to have multiple components such as optimized core packing, increased burial of hydrophobic surface area, more favorable helix-dipole interactions, and improvement in secondary structure propensity (Robb and Maeder, 1998). These mechanisms of adaptation in hyperthermophiles (including thermophiles) are described in the following sections.

Composition of Amino Acids

The composition of amino acids or the exchange of the amino acids in the hyperthermophilic proteins is the significant mechanism for the stabilization of the protein or polypeptide structures. As earlier reported that hyper stable proteins are composed of the same 20 amino acid set found in mesophiles but these amino acids undergo accelerated covalent modification at high temperature, pressures & extremes of pH that may thermophiles and hyperthermophiles can withstand (Hensel *et al*., 1992). Robb and Clark, 1999; were reported that the stability of the some amino acids, at the temperature beyond of

mesophiles and common thermophiles, declines in the following order (Val, Leu)> Ile > Tyr > Lys> His > Met > Thr > Ser > Trp> (Asp, Glu, Arg, Cys). Furthermore the sequence alignment of the thermophilic and mesophilic proteins revealed many substitutions in the amino acids as Lys to Arg, Ser to Ala, Gly to Ala, Ser to Thr, and Val to Ilu. Although the above substitutions in the amino acids are not giving any reliable idea according to the stability order of the amino acids, but it has also been reported that substitution in amino acids is responsible for increase in internal hydrophobicity and decrease in external hydrophobicity. Moreover, these substituents are reported as helix stabilizing residues and also decrease flexibility in the α-helical region (Jaenicke and Bohm, 1998). α-helics then become more rigid and are tightly packed by means of increased hydrophobic contacts. Thus to achieve thermal stability, increased hydrophobicity and decreased flexibility are the important factors. The Arg residue also increases the polar surface area of protein structure, thus provides the thermo-stability to hyperthermophilic proteins. Compatible solutes, some of which have recently been found to provide generic thermo-protection to proteins, may be acting by incrementally showing amino acid modification reactions at high temperatures (Hensel *et al*., 1992).

Bonding Interactions

The different types of bonding interactions (Mainly electrostatic interactions and hydrogen bonds) are also playing the important role in thermostability of the hyperthermophoilic proteins. The increased number of the hydrogen bonds is responsible for increased hydrophobic internal packing. It is also reported that all the thermotolerant molecular structures not only having significant increased hydrogen bonding interactions but also increased number of ion pairs and salt bridges in protein structure (Karshikoff and Ladenstein, 2001). The basic and acidic side chains on the surface of proteins interact to form ionic networks that are able to hold domains and subunits together through electrostatic interactions. As the hydrogen bonding interactions increase the thermo-stability, the ionic networks help to prevent hydrolysis at high temperature, consequently wide spread ionic networks were observed in the proteins of the more extreme hyperthermophiles (Robb and Maeder, 1998; Robb and Clark, 1999; Mukaiyama and Takano, 2009). The ionic network of *Pyrococcus furiosus* (an extremophilic archaea) glutamate dehydrogenase (GDH) demonstrate extensive clustering of spatially alternating positive and negative charges, which have a component of hydrogen bonding (Robb and Clark, 1999). Moreover, the another report on the comparison of the structure of the glutamate dehydrogenase from *Pyrococcus furiosus* and its homolog form *Clostridium symbiosum* (a gram positive, obligate anaerobic bacteria) is revealed that the increase number of ion pairs and ion pair networks in the hyperthermophilic proteins revealed their thermostability in those extreme environments. Similar observations have also been reported in same proteins of *Thermotoga maritima* (a hyperthermophilic bacterium), *Sulfolobus solfataricus* (a hyperthermophilic archaeon), and *Aquifex pyrophilus* (a hyperthermophilic bacterium) (Xiao and Honig, 1999). Surprisingly the protein with the highest temperature stability on record is the *Pyrococcus furiosus* ferredoxin retains structure at temperatures up to 200^0C, features an ion-pair at the amino terminus (Robb and Clark, 1999). The salt bridges along with hydrogen bonds and ion networks have been proved to stabilize α- helical conformation of certain peptides. The salt bridges also appear to

stabilize the tertiary structure of certain proteins if the residues contributing to the bridge residue on the adjacent structural elements (Hennig *et al*., 1995).

Nature of Proteins

The loss of integrity of the protein molecules is dependent on the unfolding of the monomeric proteins into the denatured form at high growth temperatures. Many proteins of hyperthermophiles, that are monomeric in mesophiles, are found to form not only the oligomeric structures but also they bind to large substrate to form a complex. These types of cooperative associations allow the strong intramolecular association for protein stability (Robb and Maeder, 1998; Robb and Clark, 1999). The proteins, that form oligomers or bind to larger substrates then themselves, dissociation is thought to precede the irreversible unraveling of monomers. So the many proteins that are monomeric in mesophiles are found to form oligomers in extreme thermophiles or hyperthermophiles. The example is chorismate mutase from hyperthermophilic methanogenic archaea, *Methanococcus jannaschii*, appears to have developed a dimeric quarternary organization as an adaptation to stability and the compact beta-alpha TIM barrel of phosphoribosyl anthranilate isomerase, which is monomeric in enteric bacteria, and is dimeric in *Thermotoga maritima* (Hennig *et al*., 1997).

Absence of Loose Ends

In many hyperthermophilic proteins, the amino- and carboxy- termini are not free to skirmish and initiate loosing of polypeptide. In a specific case of phosphoribosyl anthranilate isomerase from *Thermotoga maritima*, not only the termini are buried in hydrophobic pockets (as described earlier), but a disordered loop found in the homologous protein form *E. coli* is replaced with an alpha-helix, thereby inhibiting the nucleation of melting (Hennig *et al*., 1997; Robb and Clark, 1999). Loose ends must be handled somewhat differently in multiple proteins from hyperthermophiles that have inteins. The example reported that the rib nucleotide reductase from *Pyrococcus furiosus* has been shown to have two inteins, and the mechanisms of intein excision have been shown to proceed at optimal growth temperatures, implying that there is an end-stabilizing mechanism for these protein splicing reactions.

Adaptation in Nucleic Acids in Hyperthermophiles:

Nucleic acids are other kinds of heat labile bio-molecules, highly susceptible for both denaturation and hydrolysis at high temperatures *i.e.*, there is a greater burden of both chemical degradation and duplex destabilization (Robb and Maeder, 1998; Robb and Clark, 1999). At these temperatures, not only the DNA double helix unwind, but it also induces various chemical modifications that are highly mutagenic, such as depurination or cytocine deamination. PCR, the base of the modern molecular biology, utilizes the denaturation step which consisting of heating the reaction to 94-98^{0}C for 20-30 seconds, causes DNA melting by disrupting the hydrogen bonds between the base pairs of two strands (en.wikipedia.org, 2015). Then how do these special types of microbes maintain the DNA double helical structure as such as other prokaryotic microbes at these high temperatures? Most of the archaea and some eubacteria of these unusual habitats can overcome the problem of DNA denaturation at up to 113^{0}C, by using certain characteristic features that might have enabled them to survive in these

environments (Trivedi *et al*., 2005). The concentration of various salts, partially the GC content, presence of polycationic polyamines, reverse gyrase, certain cationic proteins and modified nucleotides can play a major role in nucleic acid stability at high temperature.

CG Content

Stabilization of nucleic acid duplex structure may also be achieved by increasing the G–C ratio. But the cytidine and guanosine concentration (CG content) always is not a satisfactory mechanism for the nucleic acid protection (Forterr, 2001; Daniela and Cowan, 2000). DNA in hyperthermophiles is protected by all other mechanisms because many reports are showing that there is no correlation between the G+C content of the hyperthermophilic genome and their optimal growth temperature (Trevedi *et al*., 2005). Only the secondary structure of ribonucleic acids appears to be stabilized against thermo-degradation by an increased content of GC base pairs within the stem-loop areas and by post translational modifications (Stetter, 1996; Hickey, 2004).

Polyamines

The polyamines play a role in stabilization of DNA duplexes because they prefer to bind to the phosphate groups strongly in each strand of DNA duplex, and reduce the repulsion between the phosphate groups of DNA. Polyamines bind to the major groove of GC-rich duplexes and to the minor groove of AT-rich duplexes. These GC and AT rich regions allow not only in the stabilization of DNA, but also DNA–RNA hybrids, triple-helix DNA, as well as other double-helical secondary structures, such as stems and loops in rRNA, mRNA and tRNA (Hou *et al*., 2001). The concentration of the putrescine (H_2N-$(CH_2)_4$-NH_2), spermidine (H_2N-$(CH_2)_3$-NH-$(CH_2)_3$-NH_2), thermo-spermine (H_2N-$(CH_2)_3$-NH-$(CH_2)_3$-NH-$(CH_2)_4$-NH_2), and spermine (H_2N-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$-NH_2)were detected in various *Sulfolobus* strains (Daniel and Cowan, 2000).

Concentration of Ions and Salts

Ions thermally stabilize not only the secondary structure of nucleic acids but its primary structure as well. Metallic ions in the solution (mainly Na^+ ans Mg^{2+}) play a crucial role in thermostability and folding kinetics of nucleic acids (Grogan, 1998). The stability of DNA-DNA, RNA-DNA, and RNA-RNA duplexes decreased with decreasing the NaCl concentration, thus indicated linear dependency of their *Tm* values on the logarithm of the sodium ion concentration (Robb and Maeder, 1998; Robb and Clark, 1999). They also reported that monovalent cation (*e.g.* LiCl, NaCl, KCl, CsCl etc.) enhanced *Tm* of the duplex but divalent cations (such as $MgCl_2$, $CaCl_2$, $MnCl_2$, $BaCl_2$ etc.) stabilized the duplex more effectively. Another mechanism to stabilize nucleic acids is also to enhance the *Tm* of DNA and RNA by enhancing internal salt concentrations. By the mechanism *Tm* of DNA or RNA double helices increases with the ionic strength, due to the screening of the negative charges associated to the sugar-phosphate backbone. The molar concentrations of potassium di-inositol-1, 1-phosphate or tripotassium cyclic-2, 3-diphosphoglycerate present in most of the hyperthermophiles, suggested a possible mechanism for stabilization both of nucleic acids and protein conformation at high temperatures (Daniel and Cowan, 2000).

Reverse Gyrase

Statter (1999) reported that the thermal resistance to DNA double helix appears to

be improved in hyperthermophiles by the reverse gyrase, causes positive supercoiling for stabilization. *A. priori*, stabilization of DNA duplex and RNA hairpins can be partly achieved by increasing the GC content of the molecule, since GC base pairs (with three hydrogen bonds). However, this strategy has limitation, since a melting temperature (*Tm*) of 110^0C would require more than 95% GC for a linear DNA in physiological salt conditions.

Conclusion

The extraordinary ability of the hyperthermophilic microbes to tolerate or live in such harsh temperature conditions extremely depends upon the structural modifications on the biomolecules such as proteins and nucleic acids. Various molecular mechanisms of hyperthermophilic microorganisms described in this review offer a great solution for various industrial processes carried out at high temperatures. Thus the understanding of these molecular mechanisms together with biotechnological research should lead to the future availability of new hyperthermophilic enzymes to meet all types of industrial demands for a developed future.

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