Enzymes from extremophiles

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The industrial application of enzymes that can withstand harsh conditions has greatly increased over the past decade. This is mainly a result of the discovery of novel enzymes from extremophilic microorganisms. Recent advances in the study of extremozymes point to the acceleration of this trend. In particular, enzymes from thermophilic organisms have found the most practical commercial use to date because of their overall inherent stability. This has also led to a greater understanding of stability factors involved in adaptation of these enzymes to their unusual environments.

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Introduction: extremophiles and industry

The synthesis of polymer intermediates, pharmaceuticals, specialty chemicals and agrochemicals is often hampered by expensive processes that suffer from low selectivity and undesired byproducts. Mesophilic enzymes are often not well suited for the harsh reaction conditions required in industrial processes because of the lack of enzyme stability. For this reason, the use of biocatalysts in organic reactions represented only a small fraction of the potential industrial market in the past.

The discovery of new extremophilic microorganisms and their enzymes has had a great impact on the field of biocatalysis. Extremophiles are organisms that have evolved to exist in a variety of extreme environments and fall into a number of different classes that include thermophiles, acidophiles, alkalophiles, psychrophiles, and barophiles (piezophiles) and others (see Table 1). They have adapted to thrive in ecological niches such as deep-sea hydrothermal vents, hot springs, and sulfataric fields [1,2••]. As a result, these microorganisms produce unique biocatalysts that function under conditions in which their mesophilic counterparts could not survive, permitting the development of additional industrial processes.

Ten years ago, extremophiles were exotic organisms, explored by only a few research groups throughout the world. Now, although they still retain some of their eccentric status, they are often routinely used as the sources of novel enzymes at enzyme-discovery companies. The capabilities of extremophilic microorganisms have been the subject of many recent reviews [1,2••,3–6], articles, and a new journal (*Extremophiles*; Springer–Verlag) is entirely devoted to the topic. Table 1 lists extremophiles by habitat and some applications of their enzymes. Most of the work has been devoted to thermophiles and hyperthermophiles, but other groups have received more attention recently because of their biotechnological potential.

Psychrophilic enzymes produced by cold-adapted microorganisms display a high catalytic efficiency that offers considerable potential to the biotechnology industry, for example, in the detergent and food industries, and for the production of fine chemicals [7••]. The industrial potential for halophilic enzymes is increasing as the approaches to study the genetic processes of halophiles and our understanding of

Table 1

Industrial applications of enzymes isolated from extremophiles.

Extremophile	Habitat	Enzymes	Representative applications
Thermophile	High temperature Moderate thermophiles (45–65°C) Thermophiles (65–85°C) Hyperthermophiles (<85°C)	Amylases Xylanases Proteases DNA polymerases	Glucose, fructose for sweeteners Paper bleaching Baking, brewing, detergents Genetic engineering
Psychrophile	Low temperature	Proteases Dehydrogenases Amylases	Cheese maturation, dairy production Biosensors Polymer degradation in detergents
Acidophile	Low pH	Sulfur oxidation Chalcopyrite concentrate	Desulfurization of coal Valuable metals recovery
Alkalophile Halophile	High pH High salt concentration	Cellulases	Polymer degradation in detergents Ion exchange resin regenerant disposal, producing poly(γ-glutamic acid) (PGA) and poly(β-hydroxy butyric acid) (PHB)
Piezophile	High pressure	Whole microorganism	Formation of gels and starch granules
Metalophile	High metal concentration	Whole microorganism	Ore-bioleaching, bioremediation, biomineralization
Radiophile	High radiation levels	Whole microorganism	Bioremediation of radionuclide contaminated sites
Microaerophile	Growth in <21% O_2		

haloadaptation becomes more sophisticated [8]. Acidophiles typically share other extremophilic habitat properties such as thermophilicity, halophilicity or heavy-metal resistance, and they have found a niche in the bioprocessing of minerals [9••]. Alkaliphiles thrive in alkaline environments and have made a great impact in industrial applications, especially alkaline proteases and cellulases in biological detergents [10]. A novel subgroup of alkaliphiles that has been the focus of much attention recently is the anaerobic alkalithermophiles, whose most interesting feature noted so far is their short doubling time [11]. Deep-sea organisms are the source of piezophiles (barophiles), and research has focused mostly on the identification of pressure-regulated operons showing the relationship between pressure and microbial growth [12]. Only recently has attention been shifted to the potential biotechnological applications of piezophiles compared with those of other extremophiles [13]. Adaptation to high concentrations of heavy metals (otherwise essential as trace elements) is just beginning to be understood on the basis of homeostasis for metal ions [14••]. Obvious applications in the field of bioremediation and biomining are now being complemented by the biosynthesis of novel composites in the field of materials science [15]. Radiophiles are a class of extremophiles typically ignored but receiving a lot of attention recently, because of their ability to survive under conditions of starvation, oxidative stress, and high amounts of DNA damage [16••]. *Deinococcus radiodurans* is the most radiation-resistant organism known and is currently being engineered for remediation of radioactive waste [17]. Finally, aerobic life requires the presence of antioxidant enzymes, such as superoxide dismutase, catalase, and peroxidase, to eliminate deleterious oxygen derivatives. Microaerophilic organisms lack all of the abovementioned enzymes, but a new class of antioxidant systems is present to allow growth under low oxygen concentrations [18]. The existence of this alternate antioxidation system has been proven to confer aerotolerance to this class or extremophiles [19].

Biocatalysis with extremophilic enzymes

Much of the interest in extremophiles stems from their surprising properties. There has been extensive research on the structural proteins and key metabolic enzymes that are responsible for the organisms' unusual properties. Recent research has focused on the identification of extremozymes relevant for industrial biocatalysis. Table 2 shows many of the most interesting enzyme classes for use in industrial biotransformations that are active or stable under extreme conditions. These include esterases/lipases, glycosidases, aldolases, nitrilases/amidases, phosphatases and racemases. In general, it has been found that psychrophilic enzymes can help to enhance yields of heat-sensitive products, halophilic enzymes that are stable in high salt concentrations serve as models for biocatalysis in low-water media and thermophilic enzymes are highly resistant to proteases, detergents and chaotropic agents, which may also afford resistance to the effects of organic solvents [20••]. Although the number of extremophile enzymes that have commercial use is small, we have drawn on some recent work published in this field for enzymes that show industrial potential.

Esterases and lipases

Esterases and lipases are the most widely used biocatalysts in fine chemical applications, largely because of the advantages of these catalysts for the production of optically pure compounds. Large libraries of thermophilic esterases and lipases have been developed by a number of screening and enzyme-discovery methods [21•]. The industrial use of esterases and lipases is relatively mature compared with that of other enzyme classes, and rapid characterization tools are available for enzyme discovery and activity fingerprinting [22,23].

A lipase from *Bacillus thermocatenulatus* was expressed in *Escherichia coli* under the direction of a *Bacillus* promoter upstream of the lipase gene. The enzyme was stable up to pH 11 and maximum activity was found at pH 8.0–9.0 and at 60–70°C. This lipase also exhibited high stability in various detergents and organic solvents [24]. An esterase from *Bacillus licheniformis* was expressed from an *E. coli* recombinant strain [25]. The enzyme was stable at pH 7–8.5 with optimum activity at pH 8–8.5. The optimum temperature for activity was 45°C and the half-life was 1 hr at 64°C. A new esterase from the thermoacidophile *Bacillus acidocaldarius* was expressed in *E. coli* and its properties for organic synthesis were evaluated by measuring product enantioselectivities for various substrates [26]. Substantial enantioselectivity was observed only in the resolution of (+/–)-3-bromo-5-(hydroxymethyl)-δ2-isoxazoline, for which the (*R*)-product was obtained with an 84% enantiomeric excess at 36% conversion. The enzyme was also able to synthesize acetyl esters when tested in vinyl acetate and toluene.

The cloning and overexpression of another thermophilic esterase from *Archaeoglobus fulgidus* has been reported [27]. Among the *p*-nitrophenyl esters tested, the best substrate was *p*-nitrophenyl hexanoate with maximum activity observed at 70°C and pH 7.1. A lipase from *Bacillus stearothermophilus* was expressed in *E. coli* under IPTG induction [28]. Its optimum temperature for the hydrolysis of olive oil was 68°C and it was stable up to 55°C for 30 minutes and its thermostability increased by about 8–10°C in the presence of calcium ions.

Hyperthermophilic enzymes may have potential in specific applications that require high temperature or other special conditions. An esterase from *Pyrococcus furiosus* has been recombinantly expressed in *E. coli*, displaying optimal activity at 100°C [29]. The psychrophillic strain *Moxarella* TA144 is the source of three recombinant cold-adapted lipases that show high activity towards butyrate rather than longer-chain esters (especially in one of the lipases) [30]. A lipase from *Pseudomonas P38* with psychrophilic properties has been used in the synthesis of the flavor compound butyl caprilate in n-heptane (75% yield) [31]. Another psychrophilic lipase from *Pseudomonas B11-1* was recombinantly expressed and

Table 2

Biocatalytically relevant extremozymes.

Enzyme	Organism	Host/induction	Stability/activity	Reference
Hyperthermophilic esterase	*Pyrococcus furiosus*	*E. coli*/heterologous (own promoter)	T opt = 100°C $t_{1/2}$ = 50 min at 126°C	[29]
Thermophilic esterase	*Bacillus lincheniformis*	*E. coli*	T opt = 45°C $t_{1/2}$= 1 hr at 64°C	[25]
Thermophilic esterase	*Bacillus acidocaldarius*	*E. coli*	Active at 70°C	[26]
Thermophilic esterase	*Archaeoglobus fulgidus*	*E. coli*	Active at 70°C	[26]
Thermophilic lipase	*Bacillus Stearothermophilus*	*E. coli*/IPTG	T opt = 68°C stable 30 min at 55°C	[28]
Thermophilic lipase	*Bacillus thermocatenulatus*	*E. coli* DH5α/pUC18	T opt = 60–70°C	[24]
Psychrophilic lipase	*Moraxella* TA144	*E. coli*/pULG	T opt = 35°C*, 45°C† stable only as whole cell	[30]
Psychrophilic lipase	*Pseudomonas* sp. B11-1	*E. coli*/pUC118, IPTG	Topt = 45°C, activated by MeOH, EtOH, DMSO, DMF	[32]
Hyperthermophilic pullulanase	*Thermococcus aggregans*	*E. coli*	T opt = 95°C $t_{1/2}$= 2.5 h at 100°C	[37•]
Thermophilic pullulanase	*Bacillus acidopullulyticus*	*Bacillus acidopullulyticus*	55% active after 30 min at 60°C/pH 5.5	[35]
Thermophilic and acidophilic α-amylase	*Alicyclobacillus acidocaldarius*	*E. coli*	Optimum activity at 75°C and pH = 3	[62]
Halophilic β-galactosidase	*Haloferax alicantei*	*Haloferax alicantei*	Active only at 4 M NaCl	[38]
Halophilic class I fructose aldolase	*Haloarcula vallismortis*	*Haloarcula vallismortis*	Optimal activity at 2.5 M KCl	[44]
Hyperthermophilic fructose aldolase (Type II)	*Thermus aquaticus*	*Thermus aquaticus* YT-1	Active and stable at 90°C for >2 hr	[42]
Hyperthermophilic Fructose aldolase (Type I)	*Staphylococcus aureus*	*Staphylococcus aureus*	Stable at 97°C for 1.6 hr T opt = 37°C	[43]
Thermophilic 2-keto-3-deoxygluconate aldolase	*Sulfolobus solfataricus* nalidixic acid	*E. coli* JM109/pREC7	$t_{1/2}$ = 2.5 hr at 100°C	[45••]
Psychrophilic protease	*Bacillus TA39*	*Bacillus TA39*	Low temperature optimum	[48]
Halophilic protease	*Halobacterium halobium*	*Halobacterium halobium*	Max activity at 4 M NaCl	[49]
Thermophilic nitrile hydratase-amidase (whole cell)	*Bacillus* spp.	*Bacillus* spp.	Optimal growth at 65°C	[46•]
Thermophilic nitrile hydratase	*Bacillus pallidus*	*Bacillus pallidus*	Thermostable up to 55°C	[47•]
Hyperthermophilic alcohol dehydrogenase	*Pyrococcus furiosus*	*Pyrococcus furiosus*	$t_{1/2}$ = 160 hr at 85°C; 7 hr at 95°C	[50•]
Barophilic glutamate dehydrogenase	*Pyrococcus furiosus*		36 times more stable at 105° and 750 atm	[51]
Psychrophilic phosphatase	*Shewanella* sp.	*E. coli*	Low temperature optimum	[52]
Psychrophilic alanine racemase	*Bacillus psychrosaccharolyticus*	*E. coli*/pYOK3	Low temperature optimum (0°C)	[53••]

*Recombinant enzyme. †Wild-type enzyme.

its activity measured in the presence of organic solvents; the enzyme was activated by methanol, ethanol, dimethyl sulfoxide (DMSO) and *N*,*N*-dimethylformamide (DMF), whereas it was inactivated by acetonitrile [32].

Glycosidases and enzymes involved in sugar chemistry

The use of thermostable enzymes is well known in starch hydrolysis for glucose and fructose production [33] and advances are still being made in this area. An α-amylase-encoding gene from the extremely thermophilic archaea *Thermococcus hydrothermalis* was recently cloned and expressed in *E. coli* [34]. This recombinant α-amylase is optimally active at 75–85°C and at pH 5.0–5.5. In addition, a heat-stable pullulanase from *Bacillus acidopullulyticus* was characterized with respect to its stability against thermal and chemical denaturation and its reactivation after complete chemical unfolding [35]. The addition of sucrose, polyols, and Na_2SO_4 strongly stabilized the enzyme against thermal inactivation.

Glycosidases from hyperthermophiles are one of the most widely studied enzyme classes from these organisms [36].

A hyperthermostable glycosidase enzyme with pullulanase activity at 90°C from *Thermococcus aggregans* was cloned and expressed in *E. coli* [37•]. Unlike all other pullulan-hydrolyzing enzymes described to date, the enzyme is able to attack α-1,6- as well as α-1,4-glycosidic linkages in pullulan, affording a mixture of maltotriose, panose, maltose and glucose. The enzyme is also able to degrade starch, amylose and amylopectin, forming maltotriose and maltose as main products.

Halophilic glycosidases have also been recently identified. Purification of a halophilic β-galactosidase from *Haloferax alicantei* using ion-exchange chromatography was facilitated by addition of sorbitol as a stabilizer of enzyme activity in the absence of salt. This extremozyme cleaves several different β-galactoside substrates such as *o*-nitrophenyl-β-D-galactopyranoside, 5-bromo-4-chloro-3-indolyl-galactopyranoside (X-Gal), lactulose and β-D-fucosidase, but is not active towards lactose, nor does it show β-glucosidase, β-arabinosidase or β-xylosidase activity [38]. A gene encoding a β-galactosidase from the halotolerant psychrophile *Planococcus* sp. was cloned in an *E. coli* host [39]. The purified enzyme was most active at pH 6.5 at a temperature of 42°C in the absence of metals. Of special interest was the finding that the enzyme remained active at high salt concentrations, which makes it a possible reporter enzyme for halotolerant and halophilic organisms.

The use of enzymes for carrying out complex sugar synthesis is a topic well explored using mesophilic enzymes. Recently, thermophilic glycosidases have been employed in the synthesis of disaccharides such as β-D-fucopyranosyl-β-D-xylopyranosides as a mixture of 1→3 and 1→2 products, without formation of the 1→4 linkage [40•]. Dihydroxyacetone phosphate (DHAP)-dependent aldolases are also among the most potentially useful biocatalysts because they enable transformations without manipulating protecting groups [41]. A stable fructose aldolase (class II) has been isolated from *Thermus aquaticus* [42]. This thermostable enzyme remains stable after heating at 90°C for 2 hours but loses 80% of activity after 1 hr at 97°C. This compares with an early aldolase example (class I fructose 1,6-bisphosphate aldolase) that, although derived from a mesophile (*Staphylococcus aureus*), has a half-life of 1.6 hr at 97°C [43]. The activity of a class I fructose 1,6-bisphosphate aldolase from *Haloarcula vallismortis* was determined in the presence of various salts [44]. The aldolase displayed optimum activity in 2.5 M KCl and was partially active in NH_4Cl and LiCl. Polyvalent anions, such as arsenate, pyrophosphate and phosphate, caused further enhancement of enzyme activity when 1–10 mM was added to the standard KCl-containing medium. A new 2-keto-3-deoxygluconate aldolase (KDG-aldolase) from *Sulfolobus solfataricus* shows specificity towards non-phosphorylated substrates, yielding pyruvate and glyceraldehyde [45••]. This hyperstable enzyme has the ability to catalyse carbon–carbon-bond synthesis with non-phosphorylated metabolites, and its expression in *E. coli* gives a fully active enzyme.

Nitrile-degrading enzymes

Nitrile-degrading enzymes are of considerable importance in industrial biotransformations, with several examples of commercial implementation. A thermophilic *Bacillus* spp. capable of transforming aliphatic nitriles, cyclic nitriles and dinitriles was used as a free cell suspension and immobilized in alginate beads to study the utilization of acetonitrile and acrylonitrile in a buffered biotransformation medium [46•]. Interestingly, in the presence of urea or chloroacetone, amidase activity was inhibited and the amide intermediate accumulated, as demonstrated in continuous bioreactor experiments. Another thermophilic nitrile hydratase was identified in *Bacillus pallidus Dac521* [47•]. The enzyme hydrolysed a narrow range of aliphatic substrates and did not hydrolyse any of the cyclic, hydroxy-, di- or aromatic nitriles tested. The activity was irreversibly inhibited by benzonitrile.

Proteases and peptidases

Many extremophile proteases have been studied because of their utility in the laundry detergent industry. Of recent interest in this industry is the challenge of finding stable proteases that function in cold water. In addition, peptidyl synthesis studies with mesophilic enzymes have shown that low temperature favors high yields, because of reduced hydrolysis of the acyl-enzyme intermediate. In most other processes, reduced energy consumption due to low temperature operation will be a significant advantage. Psychrophilic subtilisin from the Antarctic *Bacillus* TA39 exhibits a low-temperature optimum as expected, and mutations aimed to increase rigidity produced an enzyme with thermostability similar to that of mesophilic subtilisin, with 2- and 20-times the activity of wild and mesophilic subtilisin respectively [48]. An extracellular protease from *Halobacterium halobium* (maximum activity at 4 M NaCl) was used in the synthesis of glycine-containing oligopeptides in yields up to 76%, taking advantage of increased stability in the presence of organic solvents [49]. Although combining high salt and organic solvent seems to enhance activity, the solubility of the salt limits the solvent concentration, which in turn can be overcome by enzyme immobilization.

Other extremozymes

Many other enzymes have been identified from extremophiles. Libraries of thermostable dehydrogenases have been identified that are useful in stereoselective transformation of ketones to alcohols [21•]. An NADP alcohol dehydrogenase from *P. furiosus* is reported to be thermostable under anaerobic conditions, but very labile in the presence of oxygen (half-life in air of about 1 hr at 23°C) [50•]. *P. furiosus* alcohol dehydrogenase utilizes a range of alcohols and aldehydes, including ethanol, 2-phenylethanol, tryptophol, 1,3-propanediol, acetaldehyde, phenylacetaldehyde, and methyl glyoxal. Kinetic analyses indicated a marked preference for catalyzing aldehyde reduction with NADPH as the electron donor. Two thermophilic glutamate dehydrogenases (native and recombinant) were found to be stablized by pressures up to 750 atm [51]. For both enzymes, the stabilizing

effect increased with temperature as well as pressure, reaching 36-fold for the recombinant version at 105°C and 750 atm, the largest pressure-induced thermostabilization of an enzyme reported to date. A cold-adapted phosphatase from *Shewanella* sp. was expressed in *E. coli*, exhibiting the same high catalytic activity at low temperature as the native phosphatase [52]. Alanine racemase from *Bacillus psychrosaccharolyticus* showed the highest catalytic activity at 0°C, while extremely labile over 35°C [53••].

Factors affecting extremozyme stability

There is no single factor that has been identified to cause stability of extremozymes to the harsh environments they must survive in, but the recent work on extremophilic enzymes, particularly thermophilic enzymes, helps to understand the types of general trends that factor into stability.

Extreme environmental conditions require optimized interactions within the protein, at the protein–solvent boundary, or with the influence of extrinsic factors such as metabolites, cofactors, and compatible solutes [1]. Factors that contribute to the remarkable stability of extremozymes include an increased number of ion pairs, reduction in the size of loops and in the number of cavities, reduced ratio of surface area to volume, changes in specific amino acid residues, increased hydrophobic interaction at subunit interfaces, changes in solvent-exposed surface areas, increase in the extent of secondary structure formation and truncated amino and carboxyl termini. Several of these potential stabilization mechanisms are highlighted below.

Changes in amino acid residues

Labile amino acids undergo accelerated covalent modification at extremes of temperature, pressure, and pH, thus facilitating protein denaturation. The overall protein stability may be increased upon internalization of the more labile amino acids in the hydrophobic core. The frequency of occurrence of labile amino acids such as cysteine, asparagine, and aspartic acid is significantly lowered in hyperstable proteins with respect to their mesophilic counterparts [54••]. It has also been shown that increments of stability can be achieved in proteins by introducing 'rigidifying' mutations. For example, Van den Burg [55] *et al.* were able to enhance the stability of a monomeric thermolysin-like protease by replacing glycine and alanine residues with alanine and proline, respectively, in flexible regions of secondary structure.

Ionic interactions

Crystal structures of extremophilic proteins indicate that ion pairs may play a prominent role in the stabilization of hyperthermophilic proteins in which the hydrophobic effect is minimal [56•]. The availability of crystal structures of homologous proteins from hyperthermophiles to mesophiles has facilitated structural comparisons, homology-based modeling studies, and site-directed mutagenesis [57]. Networks of ionic interactions that can act over a much longer range than hydrophobic interactions have been observed in proteins isolated from the more extreme hyperthermophiles [54••]. For example, Vetriani *et al.* [56•] completed homology-based modeling and direct structure comparison on hexameric glutamate dehydrogenases isolated from hyperthermophiles *P. furiosus* and *Thermococcus litoralis*. They postulated that the decreased thermostability of the *T. litoralis* enzyme was a result of the breakdown of a crucial intersubunit ion-pair network. By altering two residues in the less-stable enzyme, this interaction was restored and a significant elevation of thermostability was observed. Structural comparisons of β-glucosidases isolated from hyperthermophilic and mesophilic organisms also demonstrated that the hyperthermophilic protein has significantly more ion pairs on the protein surface, thus enhancing thermal stability [36].

Glutamate dehydrogenase, which catalyzes the oxidative deamination of glutamate to 2-oxoglutarate with concomitant reduction of NADP, is ubiquitous in nature, and has been isolated in organisms ranging from psychrorophiles to hypertherophiles. The crystal structures of the multisubunit glutamate dehydrogenase isolated from two closely related hyperthermophiles *T. litoralis* (88°C) and *P. furiosus* (100°C) were compared. The less stable *T. litoralis* enzyme had a decreased number of ion-pair interactions, modified patterns of hydrogen bonding resulting from isosteric sequence changes, substitutions that decrease packing efficiency and substitutions that give rise to subtle shifts in main- and side-chain elements of the structure [58•]. Molecular mechanisms of stabilization may differ among related hyperthermophilic species. For example, ferredoxin isolated from the hyperthermophilic archaeon *P. furiosus* possesses unique properties in size and amino acid composition when compared with related ferredoxins from other hyperthermophilic archaea and bacteria [59].

Cavagnero *et al.* [60] have compared the temperature dependence of the unfolding kinetics of rubredoxins from the hyperthermophile *P. furiosus* and the mesophile *Clostridium pasteurianum* to probe kinetic stability factors. The results indicate that the more thermostable protein unfolds at a much slower rate, but exhibits increased sensitivity to pH variations. They concluded that ion pairs at key surface positions play a kinetic role in protein unfolding.

Cooperative association

In oligomeric proteins, enzyme denaturation under extreme conditions is generally initiated by subunit dissociation, followed by irreversible denaturation of the monomeric form. It has been found that the oligomer structure for some extremozymes can be more complex than their mesophilic protein counterparts that exist as monomers or dimers. For example, the iron hydrogenase isolated from the hyperthermophilic bacterium *Thermotoga maritime* exists as a homotetramer, whereas the corresponding dehydrogenases isolated from mesophilic organisms typically consist of either one or two subunits [61•]. In addition, the chorismate mutase from the hyperthermophile *Methanococcus jannaschii*

may have developed a dimeric quaternary organization as a stability adaptation (see [54••] and references therein). These oligomeric structures are often held together by extensive networks of acidic and basic sidechains.

Solvent-exposed surface area

Extremozymes are often characterized by unusual properties at the solvent-exposed surface area that lead to increased stability. Halophilic enzymes such as α-amylase isolated from *Alicyclobacillus acidocaldarius* consist of a highly negative surface charge that increases the solubility and renders them more flexible at high salt concentrations [62]. In addition, analysis of the crystal structure of an extremely acidophilic xylanase has shown numerous acidic residues located on the surface compared with on other xylanases of higher pH [63]. These highly charged surface areas are neutralized by tightly bound water molecules.

Catalytic mechanisms

Recent mechanistic studies support the general conclusion that catalytic mechanisms are shared between mesophile and extremophile counterparts. A recombinant β-glucosidase from the hyperthermophile *P. furiosus* and the mesophilie *Agrobacterium faecalis* [36] were compared. The enzymes were characterized by various methods, including substrate specificities, pH dependences, kinetic isotope effects, and linear free energy relationships. The enzymes were found to exhibit similar broad substrate specificities and nearly identical pH dependencies with several different substrates, as well as similar Bronsted plots, a high correlation coefficient for the linear free energy relationship, and similar inhibition constants with various inhibitor types. These results indicate that despite a large difference in temperature optima, the enzymes exhibit similar catalytic mechanisms. In addition, amide hydrogen exchange rates were measured on a millisecond time scale for rubredoxins from *P. furiosus* and the homologous *C. pasteurianum* protein [64]. The nearly identical exchange rates might explain the similarities of maximal catalytic rates at their physiological temperatures.

Conclusions

The recent advances in the discovery of stable enzymes from extremophiles have resulted in their increased use for applications such as organic synthesis and the production of specialty chemicals, pharmaceutical intermediates, and agrochemicals. The acceleration of enzyme discovery from this diverse class of organisms has helped facilitate the development of new industrial processes. Our understanding of the biochemical properties of these unique enzymes is finally starting to enable more creative applications. As extremophile enzyme discovery is coupled to enzyme modification by rational engineering or directed evolution, the development of economical bioprocesses will accelerate and be enabled on larger and larger scales.

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