Biotechnology Advances xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Some like it hot, some like it cold: Temperature dependent biotechnological applications and improvements in extremophilic enzymes

Khawar Sohail Siddiqui

Life Sciences Department, King Fahd University of Petroleum and Minerals (KFUPM), Dhahran, Saudi Arabia

ARTICLE INFO

Article history:
Received 15 July 2015
Received in revised form 12 November 2015
Accepted 12 November 2015
Available online xxxx

Keywords:
Psychrophilic
Cold-adapted
Thermophilic
Protein structure, function, stability
Computational, genetic and chemical modification
Magnetic nanoparticles

ABSTRACT

The full biotechnological exploitation of enzymes is still hampered by their low activity, low stability and high cost. Temperature-dependent catalytic properties of enzymes are a key to efficient and cost-effective translation to commercial applications. Organisms adapted to temperature extremes are a rich source of enzymes with broad ranging thermal properties which, if isolated, characterized and their structure–function–stability relationship elucidated, could underpin a variety of technologies. Enzymes from thermally-adapted organisms such as psychrophiles (low-temperature) and thermophiles (high-temperature) are a vast natural resource that is already under scrutiny for their biotechnological potential. However, psychrophilic and thermophilic enzymes show an activity–stability trade-off that necessitates the use of various genetic and chemical modifications to further improve their properties to suit various industrial applications. This review describes in detail the properties and biotechnological applications of both cold-adapted and thermophilic enzymes. Furthermore, the review critically examines ways to improve their value for biotechnology, concluding by proposing an integrated approach involving thermally-adapted, genetically and magnetically modified enzymes to make biocatalysis more efficient and cost-effective.

Contents

Abbreviations: DE, directed evolution; SDM, site-directed mutagenesis; ISM, iterative site-saturation mutagenesis; CM, chemical modification; GM, genetic modification/genetically modified; MNP, magnetic nanoparticles.

E-mail addresses: ksiddiqui@kfupm.edu.sa, sohailsiddiqui1995@yahoo.com.

http://dx.doi.org/10.1016/j.biotechadv.2015.11.001
0734-9750/

1. Introduction

Temperature is one of the most critical parameters that shape as well as constrain life on Earth and possibly beyond. Our biosphere is characterized by extremes of temperatures that can range from 400 °C (hydrothermal vents on the deep seafloor, Jupp and Schultz, 2000) to −89 °C (Antarctica, Siddiqui et al., 2013). Metabolically active organisms have been found living at 120 °C (hyperthermophiles) in geysers, hot springs, boiling mud-pots and hydrothermal vents (Takai et al., 2008; Dalmaso et al., 2015). The extreme thermostability of some cellular components suggests that the upper limit for survival may approach close to 250 °C (White, 1984; Fushida et al., 2014). For example, rubredoxin from *Pyrococcus furiosus* is the most thermostable protein found to date with an extrapolated melting temperature (T_m) of 200 °C (Hiller et al., 1997). On the other hand, at the lower temperature extreme, psychrophilic organisms have been found living between −15 to −25 °C in brine films (Bakermans et al., 2011; Mykytczuk et al., 2013). The majority of the biosphere is permanently cold, which includes deep-sea (covers 75% of the Earth), alpine and polar regions (Cavicchioli et al., 2002, 2011; Siddiqui and Cavicchioli, 2006; Siddiqui et al., 2013) and cold-appliances (Ertan et al., 2015). The thermally-adapted extremophilic organisms have been found to belong to all three domains of life (Bacteria, Archaea and Eukarya) and including both plants and animals (Margesin et al., 2007; Cavicchioli, 2006; Dalmaso et al., 2015).

In 2013, the global market for enzymes was approximately US$ 4.8 billion and is projected to achieve the US$ 7 billion target by 2018 (BCC Research, 2014). Their ability to withstand extremes of temperatures, gives enzymes from thermally-adapted organisms huge potential for applications in various biotechnological sectors such as industrial, medical, food/feed, environmental and agricultural (Damhus et al., 2013). Presently, the widespread industrial applications of enzymes are disadvantaged by their low activity and stability with concomitant high enzyme cost.

There are excellent reviews on the screening and structure–function–stability relationships in psychrophilic (Feller and Gerday, 2003; Siddiqui and Cavicchioli, 2006; Piette et al., 2011; De Santi et al., 2012; Feller, 2013; De Maayer et al., 2014; Fields et al., 2015; Berg et al., 2015; Cowan et al., 2015) as well as thermophilic (Vieille and Zeikus, 2001; Daniel et al., 2008; Feller, 2010, Yu et al., 2014; Cowan et al., 2015) enzymes. The current review specifically focuses on the properties and selected biotechnological applications of enzymes isolated from organisms that span a broad thermal range from below freezing point to beyond boiling point of water. This review critically examines how to further improve the properties (activity and stability) of thermally-adapted enzymes by genetic and chemical modification methods. The review is concluded by proposing a unified approach for the cost-effective biotechnological applications of thermally-adapted enzymes.

2. Thermally-adapted enzymes and biotechnology

Thermally-adapted extremophilic enzymes have been adapted for use in a wide range of industries that span all six enzyme classes and are categorized either as low-temperature (LT), high- temperature (HT) or both LT/HT based on their current and/or potential biotechnological applications (Supplementary Table 1). This interest has been driven by increasing regulatory requirements and financial pressures to improve environmental sustainability and cost-effective productivity respectively. The overall target is to produce both high value, low volume (e.g. fine-chemical synthesis) and low value, high volume products (e.g. fuels) while minimising changes to existing industrial processes (Aehle, 2007; Cavicchioli et al., 2011; Margesin and Feller, 2010; Elleuche et al., 2014; Raddadi et al., 2015; Salle et al., 2015). Due to their flexible structures psychrophilic enzymes show high intrinsic activity and thermolability (Siddiqui and Cavicchioli, 2006; Piette et al., 2011; Feller, 2013; De Maayer et al., 2014; Fields et al., 2015; Berg et al., 2015; Supplementary Table 2) whereas thermophilic and hyperthermophilic enzymes, with their rigid tertiary structures, show little activity at low temperatures (Vieille and Zeikus, 2001; Feller, 2010; Daniel et al., 2008; Wells et al., 2014; Yu et al., 2014; Supplementary Table 3) in accordance with the activity–stability trade-off model (Siddiqui and Cavicchioli, 2006). The properties of mesophilic enzymes lie between those of psychrophilic and thermophilic homologues (Feller, 2010). Discussed below are some of the key biotechnological applications of psychrophilic and thermophilic enzymes.

2.1. Cold-adapted (psychrophilic) enzymes and their biotechnological applications

Cold-adapted enzymes work best around moderate temperatures and below (4–25 °C) where they bestow economic advantages due to energy savings (Cavicchioli et al., 2002, 2011). Reactions catalysed by cold-adapted enzymes can be efficiently accomplished by eliminating energy-requiring expensive heating steps, carrying out reactions during winter season and in permanently cold regions. Due to their intrinsic high activity (Supplementary Table 2), these enzymes provide high product yield and stereo-specificity at low temperatures. Importantly, low temperatures restrict unwanted chemical side-reactions that can occur at higher temperatures. This is especially critical in food processing, fine-chemical organic synthesis and pharmaceutical industries where alterations to heat-sensitive unstable substrates and product must be avoided. For example, low temperature enzyme reactions, typically used during food processing, retain volatile molecules and prevent modifications to flavouring and other compounds in order to avoid undesirable changes in taste and nutritional value. A further advantage of psychrophilic enzymes is their heat-lability (Supplementary Table 2) where there is a requirement for enzyme inactivation with a minimal rise in temperature in place of a harsh chemical step. For example, due to its high activity between 0 and 25 °C, cold-active collagenase has been shown to tenderize beef at low-temperature and is then inactivated at 40 °C as a result of rapid autolysis (Zhao et al., 2012). Food processing plants require frequent “cleaning-in-place” of equipment and fouled membranes where the use of thermolabile enzymes not only saves energy but also has the advantage of rapid inactivation at moderate temperature. This prevents the degradation of food that might occur due to residual enzyme activities (Lowry, 2010). Apart from food industry and fine-chemical synthesis, heat-inactivation is essential for sequential reactions in molecular biology in order to carry out subsequent steps (Cavicchioli et al., 2011).

In the chemical synthesis industry, hydrolytic enzymes need to work efficiently in pure organic or mixed aqueous-organic-solvents. For this purpose, enzymes in organic mixtures require a water hydration shell around their structure to support flexibility. Consequently, stripping the hydration shell by water-miscible organic solvents leads to inactivation of enzymes due to loss of flexibility, aggregation and/or denaturation (Cavicchioli et al., 2011). Cold-adapted enzymes show higher intrinsic flexibility due to the formation of numerous hydrogen bonds between the surface residues and solvent molecules (Siddiqui and Cavicchioli, 2006), which also offsets the effect of reduced viscosity at low temperatures (Siddiqui et al., 2004a). Additionally, higher surface hydrophilicity and hydrophobicity (Siddiqui and Cavicchioli, 2006) maintain a tight hydration shell even under low-water conditions thus promoting catalysis in organic solvents (Owusu Apenten, 1999 and Karan et al., 2012). Water molecules interact differently with polar and non-polar groups; polar groups promote direct contacts with water molecules whereas non-polar groups enhance interactions between water molecules. Polar, charged and non-polar groups on the protein surface form hydrogen bonds, electrostatic interactions and “hydrophobic-interactions” respectively with water (Raschke, 2006). Water molecules arrange themselves into ice-like cage structures

Please cite this article as: Siddiqui, K.S., Some like it hot, some like it cold: Temperature dependent biotechnological applications and improvements in extremophilic enzymes, Biotechnol Adv (2015), http://dx.doi.org/10.1016/j.biotechadv.2015.11.001