FEBS Letters xxx (2013) xxx–xxx

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Commercial proteases: Present and future

Qing Li, Li Yi, Peter Marek, Brent L. Iverson *

Departments of Chemistry and Biochemistry, University of Texas, Austin, TX 78712, United States

ARTICLE INFO

Article history:
Received 15 November 2012
Revised 19 December 2012
Accepted 20 December 2012
Available online xxxx

Edited by Wilhelm Just

Keywords:
Commercial protease
Alkaline protease
Therapeutic protease
Protease engineering

ABSTRACT

This review presents a brief overview of the general categories of commercially used proteases, and critically surveys the successful strategies currently being used to improve the properties of proteases for various commercial purposes. We describe the broad application of proteases in laundry detergents, food processing, and the leather industry. The review also introduces the expanding development of proteases as a class of therapeutic agents, as well as highlighting recent progress in the field of protease engineering. The potential commercial applications of proteases are rapidly growing as recent technological advances are producing proteases with novel properties and substrate specificities.

1. Introduction

It might come as a surprise to some that proteases, enzymes that cleave other proteins or even themselves in catalytic fashion, make up the largest single family of enzymes, constituting an estimated 2% of the human genome [1,2]. Proteases are classified into six groups: aspartate, cysteine, glutamate, metallo, serine, and threonine [3] based on characteristic mechanistic features consistent within each member of a group. Through structural and functional diversity, proteases carry out a vast array of critical functions ranging from intracellular protein recycling to nutrient digestion to immune system cascade amplification. The diversification of the biological roles of proteases stem from the evolution of countless structural scaffolds to converge with similar active site geometries with varied substrate recognition motifs. Despite their many different forms and functions, the underlying theme of peptide bond scission by all proteases is the same: polarization of the scissile amide C=O bond as well as activation of a nucleophilic group to attack the carbonyl carbon leading to hydrolysis.

There is already a large array of commercially used proteases ranging from detergent additives to effective therapeutics. The therapeutic proteases have recently been nicely reviewed [4]. The present review expands coverage to include the large variety of other commercial protease classes. In general, current protease products rely on naturally evolved cleavage specificities, although other properties such as solubility and overall stability have been effectively engineered. Nevertheless, there is an incredible landscape of potential uses for engineered next generation proteases once the power and specificity of their individual hydrolysis reactions can be tailored for specific needs (Fig. 1). Thus, we will also focus on recent advances in the engineering of new specificities into existing proteases. The key idea here is that the apparent plasticity of protease active sites has enabled them to evolve with varying degrees of substrate specificity and selectivity, thus making proteases a promising framework with which to engineer unique and useful new activities.

Most naturally occurring proteases are initially produced as inactive precursors called zymogens. Some zymogens are activated by changes in the environment to induce a conformational change or the binding of a small molecule/peptide to produce an active conformation. A common strategy for zymogen activation involves expression of the protease fused to an activation segment, ranging in size from 2 to 100 residues, which prevents proteolytic activity until it is cleaved. The most common activation segments are N-terminal sequences that sterically block the active site. Beyond the role of sterically hindering the active site, activation segments are often important for the folding, stability, and sorting of the precursor protease [5]. Some interesting recent work has focused on small molecule activators of several zymogens, such as procaspases and zymogens of the fibrinolytic and coagulation systems [6,7]. Zymogen pro-domains have also been engineered onto enzymes via alternate frame folding or circular permutation, such as the conversion of the cytotoxic ribonuclease barnase into an artificial zymogen activated by HIV-1 protease [8,9].

* Corresponding author. Address: Department of Chemistry and Biochemistry, The University of Texas at Austin, 1 University Station, Austin, TX 78712, United States.

E-mail address: biverson@austin.utexas.edu (B.L. Iverson).

0014-5793/$36.00 © 2013 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
http://dx.doi.org/10.1016/j.febslet.2012.12.019

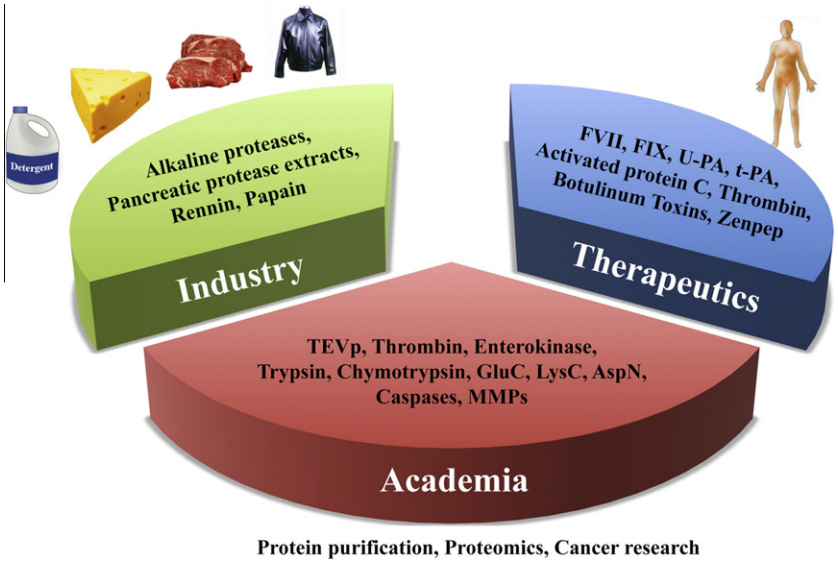

Fig. 1. An overview of protease applications.

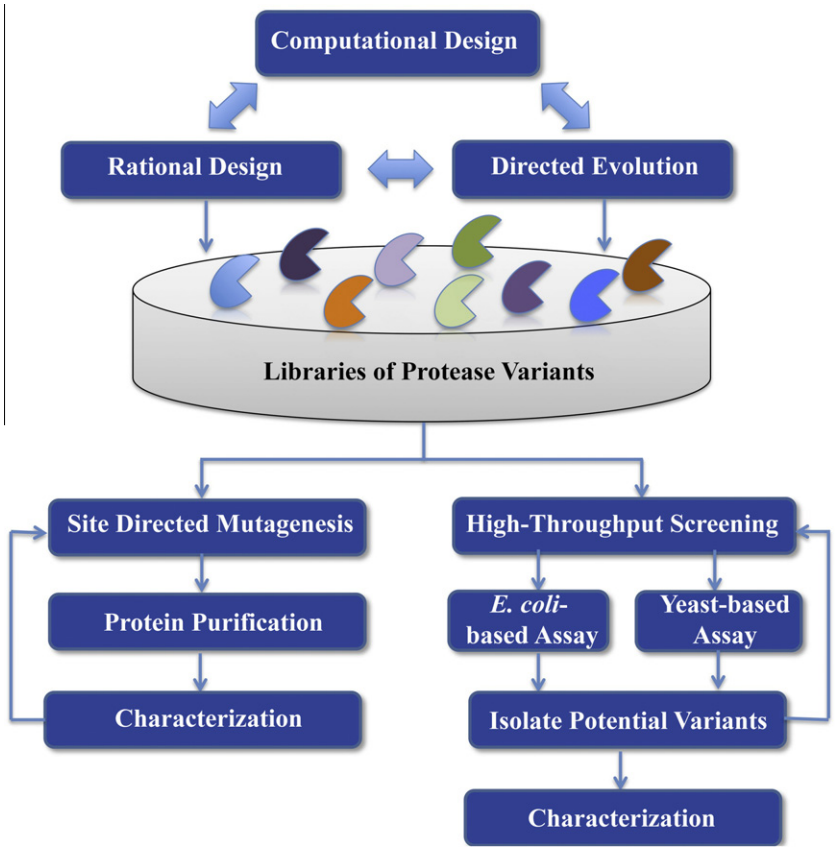

Fig. 2. Schematic diagram outlining the general approaches of protease engineering.

Proteases act within a context of complex networks comprised of small molecule activators and inhibitors, peptides, receptors, substrates, and binding domains, which also influence the spatial and temporal localization of their activity. The human genome contains over 550 presumed protease genes, the most abundant of which are metallo, serine and cysteine proteases represented by 191, 178 and 161 genes, respectively [10]. Threonine and aspartic acid proteases are of a relatively low abundance with only 27 and 21 genes, respectively [10]. Recent advances in proteomics have allowed researchers to gain a more comprehensive understanding of protease expression, regulation and activity that is now referred to as the degradome [11].

The goal of this review is to discuss the general categories of commercial proteases then survey the successful strategies (Fig. 2) now being used to improve protease properties, including the engineering of entirely new substrate selectivities, for industrial and therapeutic applications. The market for engineered proteases is already large, but with the advent of a more widespread ability to tailor stability, specificity and selectivity, the commercial future of proteases appears very bright indeed.

Please cite this article in press as: Li, Q., et al. Commercial proteases: Present and future. FEBS Lett. (2013), http://dx.doi.org/10.1016/j.febslet.2012.12.019