Biotechnology Advances 27 (2009) 297–306

Contents lists available at ScienceDirect

Biotechnology Advances

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

Research review paper

Production of recombinant proteins by microbes and higher organisms

Arnold L. Demain [a,*], Preeti Vaishnav [b]

[a] Research Institute for Scientists Emeriti (R.I.S.E.), Drew University, Madison, NJ 07940, USA
[b] 206 Akshardeep Apts., Near New Jain Temple, GIDC, Ankleshwar 393002, Gujarat, India

ARTICLE INFO

Article history:
Received 26 September 2008
Received in revised form 14 January 2009
Accepted 21 January 2009
Available online 31 January 2009

Keywords:
recombinant proteins
enzymes
bacteria
yeasts
filamentous fungi
insect cells
mammalian cells
transgenic animals
transgenic plants

ABSTRACT

Large proteins are usually expressed in a eukaryotic system while smaller ones are expressed in prokaryotic systems. For proteins that require glycosylation, mammalian cells, fungi or the baculovirus system is chosen. The least expensive, easiest and quickest expression of proteins can be carried out in *Escherichia coli*. However, this bacterium cannot express very large proteins. Also, for S–S rich proteins, and proteins that require post-translational modifications, *E. coli* is not the system of choice. The two most utilized yeasts are *Saccharomyces cerevisiae* and *Pichia pastoris*. Yeasts can produce high yields of proteins at low cost, proteins larger than 50 kD can be produced, signal sequences can be removed, and glycosylation can be carried out. The baculoviral system can carry out more complex post-translational modifications of proteins. The most popular system for producing recombinant mammalian glycosylated proteins is that of mammalian cells. Genetically modified animals secrete recombinant proteins in their milk, blood or urine. Similarly, transgenic plants such as *Arabidopsis thaliana* and others can generate many recombinant proteins.

Contents

1. Introduction

Proteins, the building blocks of life, are synthesized by all living forms as part of their natural metabolism. Some proteins, such as enzymes, serve as biocatalysts and increase the rate of metabolic reactions, while others form the cytoskeleton. Proteins play a significant role in cell signaling, immune responses, cell adhesion,

* Corresponding author. Drew University, R.I.S.E., HS-330, Madison, NJ 007940, USA. Tel.: +1 973 408 3937; fax: +1 973 408 3504.
E-mail address: ademain@drew.edu (A.L. Demain).

0734-9750/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.biotechadv.2009.01.008

and the cell cycle. They are commercially produced in industries with the aid of genetic engineering and protein engineering. Native and recombinant proteins benefit major sectors of the biopharmaceutical industry, the enzyme industry, and the agricultural industry. Products of these industries in turn augment the fields of medicine, diagnostics, food, nutrition, detergents, textiles, leather, paper, pulp, polymers and plastics. The first protein vaccine produced was the cow-pox vaccine by Jenner in 1796. The microbial fermentation industry was born in the early 1900s when the first large-scale anaerobic fermentations to manufacture chemicals such as acetone and butanol began, followed by the aerobic production of citric acid. Penicillin was discovered in 1927 but its development did not occur until the start of the 1940s, prior to the time that streptomycin was discovered. The first protein pharmaceutical produced was insulin by Banting and Best in 1922. The modern biotechnology era began in 1971 with the establishment of the Cetus Corporation in California about 1–2 years before the discovery of recombinant DNA by Berg, Cohen and Boyer in California. This was followed 5 years later by the start of Genentech, and then by other corporations such as Amgen and Biogen, etc.

By 2002, over 155 approved pharmaceuticals and vaccines had been developed by biopharmaceutical companies. Today, more than 200 approved peptide and protein pharmaceuticals are on the FDA list. Some of the recombinant protein pharmaceuticals produced are human insulin, albumin, human growth hormone (HGH), Factor VIII, and many more. Biopharmaceuticals have been instrumental in radically improving human health (Swartz, 1996): (i) diabetics no longer have to fear producing antibodies to animal insulin; (ii) children deficient in growth hormone no longer have to suffer from dwarfism or fear the risk of contracting Kreutzfeld–Jacob syndrome; (iii) children who have chronic granulomatous disease can lead a normal life by taking gamma interferon therapy; and (iv) patients undergoing cancer chemotherapy or radiation therapy can recover more quickly with fewer infections when they use granulocyte colony-stimulating factor (G-CSF). Many other examples of the conquest of disease could be mentioned.

2. Enzyme production

The enzyme industry flourished in the 1980s and 1990s when microbial enzymes came onto the scene. In the 1970s, most of the enzymes used were traditionally derived from plant and animal sources, which resulted in a low level of availability, high prices, and stunted growth of the enzyme industry. Microbial enzymes proved economically favorable since cultivation of microbes was much simpler and faster than that of plants and animals and the producing organisms could be easily manipulated genetically to produce desired qualities and quantities of enzymes. Some of the major industrial uses of enzymes in manufacturing include (1) *Escherichia coli* amidase to produce 6-aminopenicillanic acid (6-APA) at 40,000 tons/year; (2) *Streptomyces* xylose isomerase to isomerize D-glucose to D-fructose at 100,000 tons/year; and (3) *Pseudomonas chlorapis* nitrile hydratase to produce acrylamide from acrylonitrile at 30,000 tons/year (Jaeger et al., 2002). Amylases are produced at an annual rate of 95,000 tons per year. The total market for industrial enzymes reached $2 billion in 2000 and has risen to $2.5 billion today. The leading enzyme is protease which accounts for 57% of the market. Others include amylase, glucoamylase, xylose isomerase, lactase, lipase, cellulase, pullulanase and xylanase. The food and feed industries are the largest customers for industrial enzymes. Over half of the industrial enzymes are made by yeasts and molds, with bacteria producing about 30%. Animals provide 8% and plants 4%. Enzymes also play a key role in catalyzing reactions which lead to the microbial formation of antibiotics and other secondary metabolites.

Over the years, higher titers of enzymes were obtained using "brute force" mutagenesis and random screening of microorganisms. Recombinant DNA technology acted as a boon for the enzyme industry in the following ways (Falch, 1991): (i) plant and animal enzymes could be made by microbial fermentations, e.g., chymosin; (ii) enzymes from organisms difficult to grow or handle genetically were now produced by industrial organisms such as species of *Aspergillus* and *Trichoderma*, and *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica* and *Bacillus licheniformis* (e.g., thermophilic lipase was produced by *Aspergillus oryzae* and *Thermoanaerobacter* cyclodextrin glycosyl transferase by *Bacillus*); (iii) enzyme productivity was increased by the use of multiple gene copies, strong promoters and efficient signal sequences; (iv) production of a useful enzyme from a pathogenic or toxin-producing species could now be done in a safe host; and (v) protein engineering was employed to improve the stability, activity and/or specificity of an enzyme.

By the 1990s, many enzymes were produced by recombinant techniques. In 1993, over 50% of the industrial enzyme market was provided by recombinant processes (Hodgson, 1994); sales were $140 million (Stroh, 1994). Plant phytase, produced in recombinant *Aspergillus niger* was used as a feed for 50% of all pigs in Holland. A 1000-fold increase in phytase production was achieved in *A. niger* by the use of recombinant technology (Van Hartingsveldt et al., 1993). Industrial lipases were cloned in *Humicola* and industrially produced by *A. oryzae*. They are used for laundry cleaning, inter-esterification of lipids and esterification of glucosides, producing glycolipids which have applications as biodegradable non-ionic surfactants for detergents, skin care products, contact lenses and as food emulsifiers. Mammalian chymosin was cloned and produced by *A. niger* or *E. coli* and recombinant chymosin was approved in the USA; its price was half that of natural calf chymosin. Over 60% of the enzymes used in the detergent, food and starch processing industries were recombinant products as far back as the mid-1990s (Cowan, 1996).

Today, with the aid of recombinant DNA technology and protein engineering, enzymes can be tailor-made to suit the requirements of the users or of the process. It is no longer necessary to settle for an enzyme's natural properties. Enzymes of superior quality have been obtained by protein engineering, specifically by site-directed mutagenesis. Single changes in amino acid sequences yielded changes in pH optimum, thermostability, feedback inhibition, carbon source inhibition, substrate specificity, Vmax, K_m and K_i. A new and important method for improving enzymes was directed evolution (also known as applied molecular evolution or directed molecular evolution) (Kuchner and Arnold, 1997; Arnold, 1998; Johannes and Zhao, 2006). Unlike site directed mutagenesis, this method of pooling and recombining parts of similar genes from different species or strains yields remarkable improvements in enzymes in a very short amount of time. The procedure actually mimics nature in that mutation, selection and recombination are used to evolve highly adapted proteins, but it is much faster than nature. The technique can be used to improve protein pharmaceuticals, small molecule pharmaceuticals, gene therapy, DNA vaccines, recombinant protein vaccines, viral vaccines and to evolve viruses. Proteins from directed evolution work were already on the market in 2000 (Tobin et al., 2000).

Many enzymes are used as therapeutic agents to treat gastrointestinal and rheumatic diseases, thromboses, cystic fibrosis, metabolic disease and cancer. Sales of therapeutic enzymes were $2.3 billion in 1996 while in 1998 markets for therapeutic enzymes were as follows (Stroh, 1999): Pulmozyme (DNase) for cystic fibrosis, acute myocardial infarction and ischemic stroke, $350 million; Ceredase® and Cerezyme® (r-DNA version) for Gaucher's disease, $387 million. By 2007, the market for Cerezyme® reached $1.1 billion. The therapeutic market is in addition to the industrial enzyme market discussed above.

3. Systems for producing recombinant proteins

By means of genetic engineering, desired proteins are massively generated to meet the copious demands of industry. Hence, most