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Recombinant Protein Expression at the Zurich University of Applied Sciences Winterthur

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Abstract: In recent years recombinant enzymes have found widespread application in industrial uses ranging from organic synthesis to food industry. Recombinant proteins, including some enzymes, are nowadays used in the clinic to treat a broad spectrum of diseases. The elucidation of the human genome and current efforts in functional genomics and proteomics will greatly increase the number of therapeutic proteins. This article presents the R&D activities in the biochemistry lab at the Zurich University of Applied Sciences Winterthur, which recently has initiated a focus on recombinant protein expression.

Keywords: Downstream processing · Genomics · Protein analytics · Proteomics · Recombinant protein expression

The author studied biology at the Georg-August-University of Göttingen and completed her PhD in the group of Prof. Dr. H.G. Schlegel on the purification and characterization of enzymes at the institute of microbiology. She is co-author of the textbook 'Allgemeine Mikrobiologie'. She then joined the Institut für Chemo- and Biosensorik, Münster, Germany, where she realized R&D projects with partners from industry and academic institutions. In fall 2000 she was appointed lecturer for biochemistry at the Zurich University of Applied Sciences Winterthur.

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Industrial Uses of Recombinant Proteins

The protease trypsin, purified from bovine pancreas, was the first purified protein available commercially for use as a detergent in 1914. It proved to be so powerful compared to traditional washing powders that the original small package size made housewives suspicious so that the product had to be reformulated and sold in larger packages.

Since then, purified proteins, and especially enzymes, have conquered a tremendous range of applications in industry. The estimated value of the world enzyme market is presently about US $1.3 billion. Technical enzymes often replace chemical processes and thus incur environmental benefits. Detergents are by far the largest application for technical enzymes today, but enzymes are also used in many other fields, including textiles, starch, fuels, leather, personal care, and pulp and paper.

In addition, the food industry applies enzymes in baking and dairy products and for the production of beer, wine, beverage alcohol, fruit juice, oil and fats. Enzymes are also included in feed additives [1].

The introduction of genetic engineering allowed a further spread of enzyme applications. This is nicely illustrated by xylanase from the fungus *Trichoderma*, an enzyme that degrades the complex polysaccharide xylan present in plant cell walls. Xylanase is used in the pulp industry – at an alkaline pH – to liberate the wooden lignin parts from the pulp. It is also included in feed additives to increase the metabolizable energy from plant biomass. During the feed manufacturing process the xylanase has to withstand high temperature. Xylanase mutants with 2000-fold increased thermal stability at 70 °C and the pH-optimum shifted towards alkaline by one pH-unit were specifically designed and usable in both applications [2].

Enyzme-catalyzed chemical transformations are now widely recognized as practical and economic alternatives to traditional (non-biological) organic synthesis. Especially the unparalleled chiral and regional selectivity of enzymes is

used in chemical synthesis with remarkable rate acceleration. Examples are enantiopure alcohols and the sweetener aspartame which are produced with the use of enzymes in thousands of tons yearly (reviewed in [3]).

Proteins as Drugs and Drug Targets in the Post-genome Era

In Switzerland 48 recombinant proteins produced by genetic engineering are approved for medical use by Swissmedic, the Schweizer Heilmittelinstitut (formerly called the Interkantonale Kontrollstelle für Heilmittel IKS). They include hormones such as insulin, growth hormone and erythropoetin, cytokines such as interferon-α, monoclonal antibodies [4], therapeutic enzymes such as tissue plasminogen activator and others [5].

The completion of the sequence of the human genome has set the stage for a dramatic change in how modern medicines are developed. New technologies enable the use of systematic screening and selection of drug targets, making the drug discovery process significantly better and faster. The ultimate hope is to use these large-scale techniques to discover correlations of genes and gene products with disease, thus identifying the potential drug targets for a given disease and then model designer drugs for each one of these.

The genome-wide sequencing of the human genome has revealed the players that make up a living organism. Every cell has in principle the same or very similar genetic repertoire, and what differentiates a diseased cell from a healthy one is which player is on the field, meaning which gene is expressed and translated into protein at which time and in which amount. *Functional genomics* approaches this difference by examining the differential expression of genes (mRNAs) using gene microarrays or PCR screening technologies. However, in general gene expression does not correlate too well with actual protein concentration as the stability of mRNA, RNA splicing, control of translation influence the production of proteins. In addition posttranslational modifications such as phosphorylations can be crucial in determining the activity of the protein. Here, *proteomics* comes into play, which describes the sum of the expressed proteins in a cell (proteome). Two-dimensional gel electrophoresis, HPLC separation and isotope-coded affinity tagging are the currently used methods to separate complex protein mixtures and allow the subsequent identification of the candidate protein by mass spectrometry.

It has been predicted that the combination of functional genomics and proteomics followed by rational drug design could potentially expand the drug discovery process to 10000 drugs as opposed to a meager 500 only a decade ago. It is estimated that among the 50000 genes identified by the human genome project are between 10000 and 15000 genes encoding different proteins involved in diseases. A huge requirement for recombinantly expressed and purified proteins either as therapeutically active drugs given directly to patients or recombinant proteins for use in high throughput screening for inhibitors or activators can thus be foreseen.

Recombinant Protein Production at the Zurich University of Applied Sciences Winterthur (ZHW)

With my appointment as lecturer for biochemistry at the ZHW in fall 2000, I have initiated a focus of the biochemistry lab on research and development of recombinant protein production and protein analytics.

The decision whether to use microorganisms or eukaryotic cells for protein production has to be made on the nature of the protein of interest. While prokaryotic expression systems normally allow for very high yield, they are not suited for proteins which carry posttranslational modifications such as glycosylations. In addition proper disulfide bond formation is often not found after cytosolic expression in microorganisms and may require refolding of the protein. As an alternative the protein can be expressed in the periplasm of bacteria or by secretion from eukaryotic cells, where helper proteins called chaperones assist proper folding and formation of disulfide bonds. Thus, prokaryotic and eukaryotic expression systems are being established in the lab. The integration of the biochemistry lab in the Section of Chemistry and Biological Chemistry enables a close collaboration with the bioengineering lab headed by B. Sonnleitner and the cell culture and tissue engineering lab headed by U. Graf (Fig. 1). The bioengineering lab

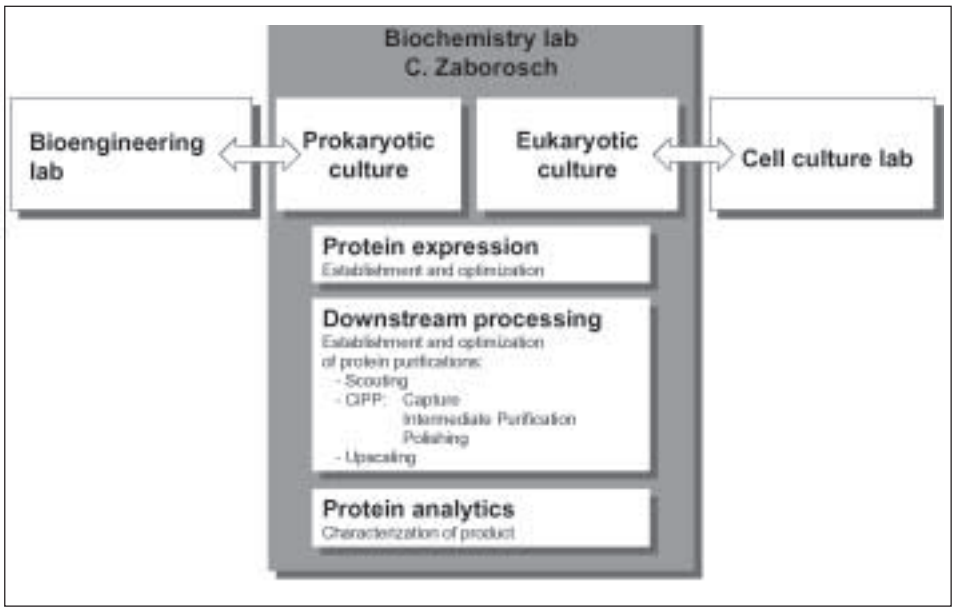

Fig. 1

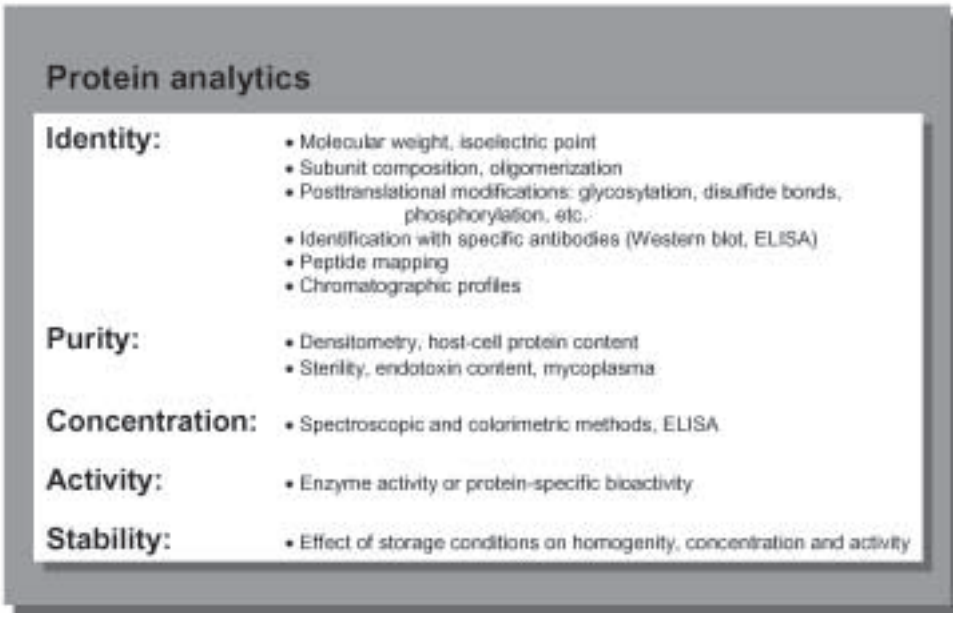

Fig. 2

is equipped with fermentors for large-scale cultivation of microorganisms and has well-recorded experience in process optimization. The same is true for the cultivation of eukaryotic cells in collaboration with U. Graf.

The next step after optimized protein expression is downstream processing which is performed on state-of-the-art instruments that also allow upscaling by the industrial partner. After purification the product analytics is performed by a variety of methods (Fig. 2).

A package from cultivation to protein purification and analytics of the final product can be offered to R&D partners and also allows teaching of students in modern biotechnology. Two projects on recombinant protein expression with industrial partners have been performed in the course of diploma work. In these projects expression levels of proteins (identity not disclosed) in *Escherichia coli* were optimized. Proteins were purified *via* an affinity tag on a metal chelate matrix and characterized.

Protein analytics was also realized within a CTI project of U. Graf on tissue engineering of cartilage tissue in which the biochemistry lab developed and validated the analytical methods to characterize the quality of the engineered cartilage constructs. In a further project together with the Section of Physics and Mathematics protein–protein interactions were investigated with an optical biosensor developed at the CSEM Neuchatel. The Departement of Technology, Computer Science and Natural Sciences with the Section of Chemistry and Biological Chemistry at the ZHW encompasses an unique possibility to combine the different expertises within the department. This enables a multidisciplinary approach to solve complex problems. Furthermore, we are engaged in the Swiss BioteCHnet, a partnership of the Swiss Universities of Applied Sciences in the field of biotechnology.

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[1] T. Godfrey, S. West, 'Industrial Enzymology', Macmillan Publishers Ltd, Houndmills, **1996**.
[2] O. Turunen, K. Etuaho, F. Fenel, J. Vehmaanpera, X. Wu, J. Rouvinen, M. Leisola, *J. Biotechnol.* **2001,** *88*, 37.
[3] A. Schmid, J.S. Dordick, B. Hauer, A. Kiener, W. Wubbolts, B. Witholt, *Nature* **2001**, *409*, 258.
[4] J.M. Reichert, *Nature Biotechnology* **2001**, *19*, 819.
[5] *www.swissmedic.ch*