MEETING REPORT

FIRST CONFERENCE ON ADVANCES IN PURIFICATION OF RECOMBINANT PROTEINS

RAPID advances in molecular biology and genetic engineering techniques have led to a whole new industry that is concerned with industrial-scale production of biologically active substances. In contrast to chemical synthesis, where controlled reactions are carried out *in vitro* with defined reagents and catalysts, biotechnological synthesis takes place *in vivo* within living organisms—bacteria and yeast—or mammalian cells. The process makes use of recombinant DNA techniques. While these are becoming versatile, purification or 'downstream processing' of the recombinant products—proteins or glycoproteins—continues to throw up problems. The holding of an international meeting to discuss advances in purification of recombinant proteins highlights the importance of downstream processing methodology to the biotechnology industry. The meeting, held in Interlaken, Switzerland, from 14 to 17 March 1989, was organized by the Swiss Coordination Committee for Biotechnology (SCCB) and the Genie et Recherche sur les Biotechnologies des Proteines (GRBP), France, to focus on procedural developments in purification of recombinant proteins, and on the regulatory aspects of protein products derived from biotechnology. The subjects were covered in oral presentations, posters and round-table discussions, the summary of which is presented here.

A wide range of proteins and peptides, mainly those of therapeutic use, are being produced by novel biotechnological processes such as recombinant DNA methods and large-scale cell culture. Some of these products, such as interferons, human insulin, human growth hormone, a monoclonal antibody, hepatitis B vaccine and a tissue-plasminogen activator, have already been approved by the US Food and Drug Administration (FDA) for marketing in the United States, and several others are undergoing clinical trials (K. C. Zoon, USA).

Purification strategies are no longer solely dependent on inherent properties of the proteins, but are also influenced by upstream factors such as the recombinant DNA techniques used to express a protein in a given system. In the case of recombinant proteins synthesized in bacteria, generally, downstream processing seems to be much simpler for proteins that are secreted into the periplasm or the extracellular medium than for those that are retained in the cytoplasm (M. Scawen, UK). Under conditions of high expression the cytoplasmic proteins appear as insoluble 'inclusion bodies', which need to be subjected to mechanical disruption, solubilization and renaturation before purification—a process difficult on a large scale. Human γ-interferon, expressed in the yeast *Saccharomyces cerevisiae* as inclusion bodies, was solubilized with guanidine hydrochloride, and refolded simply by gradient dialysis or by a slow dilution with a buffer without denaturing agent (M. Casagli, Italy). In both cases a yield of 50–70% was obtained, with a ten-fold increase in specific activity.

After the bioreactor, centrifugation continues to be an important primary separation segment of downstream processing for the removal of cells, clarification of cell lysates, and isolation of inclusion bodies. However, it is highly energy-intensive on the large scale, and alternative technologies are being worked out. Most of the recent developments have been in the area of membrane filter separations. Examples include broader, new applications of cross-flow filtrations, membrane affinity filtrations, and new membrane materials such as ceramics, porous glass and other polymers (W. C. McGregor, USA). In these cases management of the fouling of the porous media is important.

The negative charges on cell surfaces, nucleic acids and endotoxins have been exploited for clarification of cell lysates using positively charged submicron polymer particles, called Biocryl® bioprocessing aids (BPAs), developed by Rohm and Haas Co., USA (K. Fletcher, USA). The energy and power required for centrifugation for the removal of cell debris treated with BPAs were reported to be reduced to 5 and 10% respectively of those required for centrifugation without BPAs.

Aqueous two-phase systems based on polymer/polymer or polymer/salt solutions can prove to be very useful during the initial phases of purification for the extraction of protein from cell debris, proteases, etc. (B. Mattiasson, Sweden). The extraction is mainly dependent on the surface properties of the protein and the phase composition, and is designed

in such a manner that the protein of interest partitions to the phase other than that in which the particulate matter and other contaminating proteins remain. Such systems require minimal inputs of time and energy, and are extremely easy to scale up. Tissue-plasminogen activator synthesized in yeast was isolated from 45 kg of wet cells (obtained from 1000 litres of fermentation broth) in a polyethylene glycol/phosphate two-phase system, with a recovery of 75–90% and purification factor of 1.2–1.3 (P. F. Fauquex, Switzerland). Aqueous two-phase partitioning was also employed for the isolation of an enzyme from a mammalian cell culture containing large amounts of bovine serum albumin (BSA). Most (98%) of the BSA partitioned to the salt-rich bottom phase. Recovery of the enzyme was improved from 50% to 80% after multiple extractions with fresh PEG phase (J.-P. Lenders, Switzerland).

Chromatographic procedures form a part of almost all purification schemes. There were several reports on purifications based on various combinations of hydrophobic interaction, ion-exchange, molecular sieve and other chromatographies. Examples include purification of recombinant stable toxin from *Escherichia coli* (R. Kaul, India) and that of recombinant AIDS virus protein gp 120 from a Chinese hamster ovary cell line (C. Scandella, USA). Several new fast flow resolution matrices have been developed. Pharmacia, Sweden, has added Superdex, a composite of cross-linked agarose and dextran, to its list of gel filtration matrices. The gel has a fractionation range of 3–70 kDa for peptides and proteins.

Although initially developed as analytical tools, both high-performance liquid chromatography (HPLC) and fast protein liquid chromatography (FPLC) are beginning to be turned into powerful production methods not only for small peptides but also for larger proteins. Even on a large scale (i.e. 10–100 g protein or more), purity levels of 99.99% or higher can be achieved. As a model study, purification of recombinant growth hormone and interferons was carried out using these methods (E. Rinderknecht, USA).

Purification of recombinant human superoxide dismutase was carried out in an automated three-step process on a Bio Pilot system, developed from the FPLC concept by Pharmacia, Sweden (N. T. Pettersson, Sweden). The whole process, comprising desalting on Sephadex G-25, ion exchange on Q Sepharose High Performance, and gel filtration on Superdex 75, was completed within 150 minutes, allowing up to 9 cycles per day, with a yield of 1 g of purified material per cycle.

Genetic engineering techniques allow the synthesis of fusion proteins, which are fusions of the protein of interest and another, readily purifiable, polypeptide. It is also possible to introduce specific cleavage sites into hybrid fusion proteins to allow subsequent separation of the two moieties. A synthetic IgG-binding peptide has been used as fusion partner for peptide growth factors like IGF-I (insulin-like growth factor I), IGF-II and brain-IGF. This enabled the use of IgG Sepharose as an affinity matrix for purification of the growth factors (H. Lundström, Sweden). A general purification method, based on selective interaction between a polyhistidine chain fused to the protein and a nickel–nitroloacetic acid absorbent, has been developed at Hoffmann–La Roche (E. Hochuli, Switzerland). A soluble fusion protein from *E. coli* containing four repeats of an immunodominant epitope of *Plasmodium falciparum* antigen Pf 155 has been purified to be tried as a vaccine against human malaria (Å. Danielsson, Sweden).

The quality and stability of recombinant-DNA derived proteins, produced by biotechnological processes, are strongly influenced by the processes themselves. Therefore validation of a biotechnological process designed for the production of a recombinant protein for therapeutic use is essential to ensure quality and safety of the final product (R. G. Werner, FRG). A series of documents, called 'Points to consider', has been drafted by FDA to discuss a number of issues that must be considered during the manufacture of biotechnology products.

For the validation of recovery and purification stages it is necessary to examine the yields and purification factors after essential process steps for removal of contaminating proteins, nucleic acids, endotoxins and potential viruses. Removal of contaminants that could be introduced during purification must also be considered. In addition the reproducibility of the quality of the formulated product must be determined by a number of protein analytical, immunological and biochemical test methods for identity, purity, safety and potency.

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