Biotechnology and the production of proteins

from A.J. MacLeod

THE idea of using recombinant DNA techniques to construct bacterial strains capable of synthesizing therapeutic proteins is currently being widely promoted. It is not, though, the only possibility for large scale production of proteins and the advantages and disadvantages of different methods need to be considered. Particularly for proteins intended for therapeutic use there are severe constraints on the precise nature and purity of the products.

Bacterial methods would seem to have the advantage of almost limitless production with recovery from a relatively simple mixture. However, apart from albumin, virtually all of the protein species in plasma are glycoproteins or lipoproteins. Modulation of the antigenicity or pharmacokinetics of the proteins is likely to be important *in vivo* and the ability of bacteria to reproduce these features faithfully is doubtful. This problem may be reduced by *in vitro* modification (Marshall & Humphreys, *J. appl. biochem.* **1**, 88; 1979). As bacteria degrade abnormal proteins efficiently (Goldberg & St John *Ann. Rev. Biochem.* **45**, 747; 1976) it is probable that partial digestion of the product will occur with effects on biological activity, even if antigenic loci remain intact.

Should synthesis of significant quantities of biologically active protein be achieved in a bacterial system, its recovery will still present major difficulties. In systems where secretion of human proteins, with or without a bacterial protein carrier, has been attempted, secretion has occurred into the periplasmic layer and the products have not successfully passed into the culture supernatant. (Villa-Komaroff *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 3727; 1978. Fraser & Bruce *Proc. natn. Acad. Sci. U.S.A.* **75**, 5936; 1978). Protoplasts have to be prepared to release the proteins but this has the disadvantage of releasing a mass of other bacterial products and debris into the crude preparation. If the cells have to be lysed to release accumulated protein this problem will be much more severe. Even if secretion into the culture supernatant can be achieved bacterial products and debris will still be present as contaminants.

Bacterial proteins are intensely antigenic in mammals and cell debris is pyrogenic. Even trace levels of bacterial contamination repeatedly administered over a long period, as in the control of haemophillia or in cancer therapy, may be dangerous. Products from bacterial cultures will have to approach absolute purity. However, as greater purity is sought with large-volume processes costs rise exponentially and consequently the processing cost may offset the initial production cost advantage of bacterial systems. A particular difficulty in this case, as opposed to the production of antibiotics or viral vaccines, is that some of the impurities will be very similar, chemically and physically, to the product.

Rather than using bacterial systems for human protein production it might be better to manipulate human cells so that they can be cultured more easily and be more productive. The plasminogen activator urokinase produced by normal human kidney cells *in vitro* is already available for clinical use (Lewis *Thrombos. Haemostas.* **42**; 895; 1979). Transformation of mammalian cells with genes from prokaryotes and eukaryotes has been demonstrated (Wigler *et al. Cell* **16**, 777; 1979). A more direct route to a satisfactory cell line might be to construct cell hybrids as has been done with leukocyte-lymphoblastoid hybridomas (Galfre *et al. Nature* **266**, 550; 1977). A step in this direction was the recent announcement by Widman *et al.* (*J. Cell. Phys.* **100**, 391; 1979) of the immortalization of a normal differentiated liver function in a hepatoma cell hybrid. The liver is the site of production of many of the plasma proteins. Systems capable of supporting very large-scale cultures of animal cells have been developed and are being introduced in association with established fermentation technology (*Eur. Soc. Anim. Cell Technol.* 3rd Meeting, *Develop. biol. Stand* **46**; 1980).

The advantages of human cell systems are that the products are likely to be correctly modified, they should be secreted into the medium, and antigenicity and pyrogenicity will be much reduced. Cell lines have the advantage that the cultures will not age as do normal cells and it may even be possible to construct lines that will grow and function in suspension. The disadvantages with cell lines include the incorporation of tumour cells that could release oncogenic material. However, methods of overcoming this problem have been suggested (Hillman *Advances Exp. Med. Bio.* **118**, 47; 1978).

The absolute homogeneity of the products from cloned systems may cause considerable complications in their use. Genetic polymorphism of plasma proteins resulting in multiple allelic forms is now well established with more than 20 variants of human albumin identified to date. Adminstration of such products over a prolonged period, if they do not match the recipients serum type, could induce the production of antibodies. Avoiding this could require production from several cell strains, each producing material of a different serum protein-type. The plasma proteins currently available minimize this complication because they are prepared from pooled donation and thus contain a variety of serum protein types. Even so the development of antibodies to factor VIII in haemophiliacs receiving therapy can occur (Shapiro *Clinics in Haematology* **8**, 207; 1979).

Human protein has already been in use for some 30 years from products of the industrial fractionation of plasma from routine blood donations. Improvements in process control, fluid handling and separation techniques applied to the established cold-ethanol precipitation process have facilitated the design of a continuous, sequential precipitation process that yields defined fractions from large volumes of plasma. The products currently available include the albuminoid stable plasma protein solution, various immunoglobulin preparations and coagulation factor concentrates (Watt & Dickson *Proc. Int. Workshop on Technology for Protein Separation and Improvement of Blood Plasma Fractionation, U.S.D.H.E.W.* Publ. No. (NIH) 78 — 1422, 245; 1978). These products are widely used but their quality is affected by damage to the proteins during processing and by contamination. The latter include both exogenous material such as hepatitis B virus and bacterial pyrogens, and redundant plasma proteins which may be biologically active, provoking, for instance, thrombogenic or hypotensive side-effects. These problems can largely be attributed to the inherent difficulty of recovering a substantial proportion of a particular component from a milieu as complex as plasma.

An attractive possibility might be to exploit the well established techniques of plasma fractionation along with developments in human cell culture. Currently, substantial quantities of human protein pastes are produced as by-products of plasma fractionation. These byproducts consist largely of α and β globulins, and low molecular weight proteins and have already been shown to contain many of the specific components required for serum-free culture of animal cells *in vitro* (MacLeod & Drummond *Develop. Biol. Stand.* **46**, 17; 1980) The preparation of a nutrient culture medium for human cells based on human plasma proteins would avoid exposure of the cells to heterologous proteins which could subsequently contaminate the product (Bonin *et al. J. Biol. Stand.* **1**, 187; 1973). Thus it is possible to envisage a situation where human plasma would be collected and processed to yield albumin and possibly some immunoglobulins. The remainder would be processed to support the synthesis of a wide range of products by human cells *in vitro*, the whole constituting an integrated unit based on technology of which much is already well developed. □

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0028-0836/80/200136-01$01.00