NEW METHODS FOR LARGE SCALE ISOLATION OF PROTEINS

Laboratory experiments with genetically engineered microorganisms begin on the scale of milliliters. In order for the products to be marketed, the scale of operation may have to increase to 10^5 liters or more. Expertise in fermentation scale-up, which first evolved for the production of penicillin during World War II, is currently supporting a major industry producing pharmaceuticals, food additives, and specialty chemicals. The same technology is now being expanded and applied to a whole new generation of biotechnology-based products.

Charles L. Cooney of MIT and Thomas Murphy of Abbott Laboratories reviewed the problems associated with "Large scale recovery of proteins from complex mixtures." These include the rate and stability of biocatalysis, mass and heat transfers, conversion yields, cost (especially of raw materials), and recovery. While recent advances in genetic engineering have facilitated the synthesis of many high value biomedical products, *e.g.* interferon and insulin, they have also generated new recovery problems for the biotechnologist.

Gary J. Calton of Purification Engineering, Inc., described his system for large scale isolation of proteins from complex mixtures. He emphasized the importance of rapid isolation for many biotechnology products. The three major problems in recovering these proteins are the labile nature of the proteins themselves; the action of proteases in the mixture, which may attack them; and the effects of insufficient cooling, which may lead to denaturation.

Calton reported a single-step purification procedure for the recovery of urokinase, a protein used for treating stroke patients, from urine and from fermentation broth. A monoclonal antibody to urokinase was immobilized to provide an affinity chromatography reagent. One million liters of urine were concentrated 50-fold, added to 10 grams of monoclonal antibody, and passed over a five-liter immunosorbent column. One recovery cycle requires 8.3 days, and 20 of these cycles yield 200 grams of pure urokinase. This amount of the enzyme has a market value of $18 million and is sufficient to treat more than 6,000 stroke patients. The conventional recovery procedure for urokinase from urine consists of 15 steps and yields a product that is only 50 percent pure.

Calton stressed the advantages of immunosorbent chromatography for rapid isolation of a single protein from a complex mixture of similar material and inhibitors. The high specificity of monoclonal antibodies facilitates recovery of proteins present in concentrations as low as 0.001 percent in fermentation broths. In addition, the procedure is easily scaled to industrial size. Calton pointed out the recovery costs can be reduced by using the immunosorbent column for additional recovery cycles. Columns can be used for 100 cycles or more without affecting product quality. The technique has been shown to efficiently recover a desired protein from a dilute solution sufficiently rapidly to minimize degradation of its pharmacological activity and provide an excellent tool for the manufacture of a relatively low cost drug for the treatment of stroke patients. Calton concluded, "The use of monoclonal antibodies is only limited by your imagination."

Paul M. Cino, Ph.D., is a senior research investigator at E. R. Squibb & Sons, New Brunswick, NJ.

RESEARCH PAPER ANALYSES

MINI-Ti: A TRUNCATED PLASMID SIMPLIFIES GENE TRANSFER

One of the most rapidly progressing areas of biotechnology is plant molecular biology. Several recent reports in BIO/TECHNOLOGY have focused on the manipulation of the Ti plasmid, a large plasmid found in *Agrobacteria*. Laboratories in the United States and Europe have announced successful exploitation of Ti plasmids to express bacterial genes in plant cells at the 15th Miami Winter Symposium[1]. Schell[2] recently reviewed the use of Ti plasmids as experimental gene vectors in plants in this journal.

When *Agrobacteria* infect a plant, they induce the formation of crown gall tumors. The tumor cells contain a segment of bacterial DNA called T-DNA. T-DNA, which is part of the Ti plasmid, "jumps" from the Ti plasmid and inserts itself in the plant chromosomal DNA, directing the tumor cell to synthesize various derivatives of arginine. Another region of the Ti plasmid, VIR, consists of the genes coding for transfer of T-DNA from the bacteria to the plant.

Most commonly used plasmids range between 3 and 15 kb; pBR322, for example, is 4.4 kb. Ti plasmids are commonly 200 kb, which makes them difficult to manipulate experimentally. Efforts are underway to reduce the size of the Ti plasmid in order to facilitate its use as a gene vector in plants. In this issue of BIO/TECHNOLOGY Mary-Dell Chilton's group reports success in constructing a truncated Ti plasmid, called a MINI-Ti, that can function in plants (see p. 262).

The original Ti plasmid was pTi-T37, which has a 23 kb T-DNA and codes for the arginine derivative nopaline. Chilton and coworkers separated the T-DNA and VIR functions, then constructed a MINI-Ti that contains only T-DNA genes. This MINI-Ti was then cloned into the wide host range vector pRK290. Although the MINI-Ti replicated in *Escherichia coli*, it was non-virulent, *i.e.* it did not induce nopaline synthesis, in plant cells. However, when MINI-Ti was transferred to cells in the presence of a helper plasmid containing VIR-DNA but no T-DNA, virulence was expressed.

Chilton and coworkers point out that while the MINI-Ti/pRK replicon they have constructed is still too large for easy manipulation, smaller derivatives of this plasmid should not be difficult to construct. They propose to delete all T-DNA functions, except those necessary for gene transfer, and to replace them with foreign genes or genetic markers. They are well on their way to developing a simple, multipurpose vector for genetic engineering in dicotyledonous plants.

References

1. Chilton, Mary-Dell. 1983. Crown Gall Gene as a Vector for Plants. BIO/TECHNOLOGY **1:** 163.
2. Schell, J. and Van Montagu, M. 1983. The Ti Plasmids as Natural and as Practical Gene Vectors for Plants. BIO/TECHNOLOGY **1:** 175–180.

NEW VECTORS FOR GENE CLONING IN BACTERIA & FUNGI

Most gene cloning vectors have been developed for the enteric bacteria, specifically *Escherichia coli*, and many of them cannot be maintained in other species. The perennial favorite pBR322, for instance, cannot be maintained in *Pseudomonas* species. Many important industrial fermentations utilize bacteria from genera other than *Enterobacteriacae* or fungi, and there is a great deal of commercial