RESEARCH PROGRAM

AUSTRIA'S CHEMIE LINZ BEGINS BIOTECH R&D

LINZ, Austria—Chemie Linz AG, a large, state-owned chemical plant here, is initiating two biotechnology projects: transferring genes for herbicide and pesticide resistance into crop plants, and using biological catalysts to produce plant-protection components and pharmaceuticals.

Protests and public outrage recently forced Chemie Linz to abandon production of the herbicide trichlorophenoxyacetic acid (2,4,5-T). The compound is not degraded after field use, so it can contaminate the environment. Biodegradable plant-protection substances do exist, but they come with another serious disadvantage: they tend to kill many of the plants they are designed to protect. Genetic engineers at Chemie Linz hope to introduce genes for tolerance to these substances into sensitive crop plants.

Two recent research developments could help. The host range of the Ti plasmid from *Agrobacterium tumafaciens* has been extended to include some monocot species. Perhaps in the near future it will be adapted to transfer genes to agriculturally valuable monocots. Also, a locus-specific gene transfer is now possible with this system, albeit with low efficiencies.

Chemie Linz moves into biotech: The 100-liter fermentor in the background is used to grow microorganisms for biological synthesis of organic materials.

Chemie Linz's second project involves developing biological catalysts that can be used to produce various organic substances. Often they enable a cheap and efficient separation of optical isomers—not possible with classical procedures.

The company plans to invest at least 50 million schillings (about $2.3 million) in biotechnological research over the next five years but does not expect to profit from this research for at least 10 years. Chemie Linz is one of the 500 largest industrial corporations outside of the U.S., having 7600 employees and last reported annual sales of $760 million. It manufactures nitrogenous fertilizers, cattle feed components (urea), plant-protection substances, technical nitrogen chemicals, plastics and plasticizers, adhesives, fibers for technical fabrics, ropes and thread, and pharmaceutical products. Hans Kroath, a molecular biologist who previously did RNA-processing studies at the Roche Institute, manages the company's biotechnology project. His team consists of three additional scientists and five technicians.

—Stephen Sokoloff

SEPARATIONS TECHNOLOGY

CHROMATOGRAPHY: FROM HERE TO AFFINITY

ANAHEIM, Calif.—A new technology, fast affinity chromatography, could offer a faster, more efficient route to isolating and purifying proteins, receptors, immunoglobins, and other biological molecules of interest to the biotechnology community.

In affinity chromatography, a ligand attached to a column binds specifically to the molecule to be purified. The most popular support for ligand immobilization has been cyanogen bromide-activated, large-diameter agarose beads. These present problems, however: flow rates through the soft gels are limited, so separations require long time periods during which biological activity can be degraded. The ligands also leach from the support.

Perhaps most important, the soft gels change size under changing conditions of pH and ionic strength, trapping the macromolecule in the gel matrix; this precludes the extreme changes in solvent conditions necessary to break strong ligand-macromolecule interactions. The result is that the product is often obtained in a very dilute solution that must be greatly concentrated before purification can be attempted.

Fast affinity chromatography is based on a new support—developed by Donald Hollis of the Altex Scientific division of Beckman Instruments (Berkeley, CA) and Robert Shorr of SmithKline-Beckman Corp. (Philadelphia, PA)—composed of small-diameter, wide-pore silica particles coated with a hydrophilic layer that has epoxy functional groups scattered throughout. The epoxides easily form stable, covalently bonded linkages to ligands containing primary and secondary amines, hydroxyl groups, and sulfhydryl groups.

Because of the small size of the spherical silica particles, the mass transfer process—absorption and desorption of the macromolecule by the column—is much faster than with agarose beads. The physical strength of the silica permits the use of faster flow rates to take advantage of the improved mass transfer. Finally, the inertness of the support enables sharp changes in solvent composition for elution of the desired product. Most samples can be eluted in less than two column volumes. "In some cases," says Derek Southern of Beckman, "the proteins can become so concentrated that they tend to precipitate out of solution."

Shorr developed the new supports while attempting to isolate catecholamine receptors from brain cells. He found that conventional supports did not work well, especially because of variability in immobilization of the ligands. The new supports, however, have produced "spectacular results," he says. When he used a modified alprenolol as a ligand, Shorr says, he obtained the receptors in a pure form in one pass through the column—an 80,000-fold purification.

Enzymes can also be immobilized on the column. In one experiment, for example, Shorr immobilized the hydrolytic enzyme pronase and observed an 800-fold increase in enzyme activity.

Scott Ralston of the Salk Institute (La Jolla, CA) has used the support to isolate acetylcholinesterase, using procainamide as a ligand. He says it took him two hours to obtain about one milligram of the enzyme in 80 percent purity. He calls the supports a "tremendous asset" to his lab.

Beckman is marketing the supports—which are compatible with existing equipment—as Ultraffinity-EP.

—Thomas H. Maugh II