RESEARCH PAPER ANALYSIS

FIRST CRYSTALLIZATION OF CELLULASE REPORTED

In this issue of *Bio/Technology*, Jean-Paul Aubert and his colleagues at the Institute Pasteur (Paris) report a significant advance toward understanding how bacterial endoglucanases function in the degradation of cellulosic substrates.

The enzymes of *Clostridium thermocellum*, which can convert cellulose directly to ethanol, exist as a multiprotein extracellular aggregate. Determining the structure and functionally dissecting the components of this 6-million-Dalton complex is a major goal of researchers who hope to exploit these enzymes in biomass conversions. Aubert's group has approached the problem by screening a gene bank of *C. thermocellum* DNA for cellulolytic activities expressed in *Escherichia coli*.

After isolating 10 distinct recombinant plamids and identifing structural genes for three endoglucanases (*cel*A, B, and C), they met with a fortunate accident. In the course of subcloning a fourth enzyme, they found that *E. coli* transformed with a plasmid carrying a 3.5 kb Hind III insert overproduced an endoglucanase (EGD) to such an extent that crystallization of the protein was possible from granules that formed in the cells. X-ray analysis of these crystals has already begun.

Solving the structure of endoglucanase D should allow researchers to define the binding and catalytic domains of the enzyme, and to begin to understand how the aggregate—composed of about twenty polypeptide chains—is organized. Comparison of the active sites of EGD and lysozyme, the best characterized glycosidic enzyme, also promises to be instructive.

Exciting results can equally be expected from an analysis of the EGD-encoding clone at the nucleotide level. Possibly, the sequences responsible for the fortuitous overexpression of the gene can be identified, thus leading to the development of analogous systems from which other cellulases of the complex might be crystallized.

Another possibility is that sequence comparisons of the cloned genes will reveal basic functional motifs. One such motif could be represented by a reiterated sequence near the 3′ ends of *cel*A and B. These nucleotides encode a stretch of 23 amino acids that are repeated near the carboxyl-termini of endoglucanases A and B. Preliminary data indicate that the same repeat occurs in endoglucanase D.

—Harvey Bialy

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Crystals of endoglucanase D of the bacterium *Clostridium thermocellum*. The enzyme was produced and crystallized from an *Escherichia coli* expression system. In the long prismatic crystal (0.5 × 1.2 mm) the triagonal axis lies in the plane of the photograph. In the other crystal, it is nearly perpendicular to that plane.

GAMMA LINOLENIC ACID

U.K. USES FERMENTATION TO MAKE HEALTH FOOD

LONDON—Biotechnology has found a new and potentially highly profitable niche in the world of health food. Research by Colin Ratledge and his colleagues at the University of Hull has led to the development of a process, now being exploited by Sturge Biochemicals (Selby), for the production of gamma linolenic acid (GLA) by fermentation. Currently available as a constituent of Evening Primrose Oil, GLA is reported to be useful in alleviating pre-menstrual stress and skin disorders such as eczema. Research is now proceeding into its alleged benefits against multiple sclerosis, rheumatoid arthritis, and some forms of heart disease. Although the oil is expensive—and all of these health applications remain controversial—it is consumed in considerable quantities. In addition, GLA is incorporated into cosmetics, and it may have wider uses in the conventional food industry.

The process developed by Ratledge for Sturge Biochemicals yields oil containing 17–18 percent GLA; this contrasts with 7–8 percent GLA in oil made from the seeds of the Evening Primrose. The producer organism is undisclosed, but the technology is thought to be similar to that for citric acid manufacture by *Aspergillus niger*, in which the parent company, John and E. Sturge Ltd., has long experience. Many species among the Mucorales also synthesize significant quantities of GLA, so the workhorse is almost certainly a mold. The new bulk process is cheaper, yields oil more quickly than by seed-growing (just a few days instead of 18 months), and ensures high quality and constant composition. Toxicity tests carried out by the British Industrial Biological Research Association and the Huntingdon Research Centre have revealed no evidence of mycotoxins, mutagenicity, or adverse effects in rats up to dietary levels as high as 30,000 ppm. Nonetheless, Sturge is conscious that it may face difficulty in convincing organically inclined consumers that its biotechnology product is as "natural" as the Evening Primrose Oil on sale. **—Bernard Dixon**