APPLIED ENZYMOLOGY

NEW ENZYMES THE OLD-FASHIONED WAY: FIND THEM

KATATA, Japan—Traditional microbial screening programs continue to be important in the industrial development of novel enzymes as evidenced by presentations at the Taniguchi Foundation's Seventh International Symposium on the Life Sciences (Recent Advances in Applied Enzymology) held here in November. One highlight—a new fungal peroxidase—that might brighten the laboratories of biologists doing assays using hydrogen peroxide coupled reactions as detectors, was described by Teruo Amachi (Suntory Ltd., Osaka, Japan).

The ability of hydrogen peroxide oxidoreductases to couple hydrogen peroxide to a variety of substrates has been exploited in diagnostic and research laboratories in applications ranging from enzyme-linked immunosorbent assays (ELISAs), to nucleic acid hybridization, to tracing neuronal connections. These assays most frequently employ the peroxidase isolated from horseradish roots (HRP). But HRP's activity with some useful substrates is less than optimal, and most commercially available enzyme contains a number of isozymes.

A new fungal peroxidase isolated by researchers at Suntory, Ltd. (Osaka, Japan) is a potent catalyst of the chemiluminescent oxidation of luminol.

The Suntory scientists screened soil fungi and found a previously undescribed taxon of the Hyphomycetes (*Arthomyces ramosus*) that secreted large amounts of a very active peroxidase, which they have termed ARP. ARP's activity resides in a single-chain, heme-containing glycoprotein of 41 kD (as estimated from sedimentation equilibrium), with about five-percent carbohydrate content and one molecule of heme. According to Amachi, the researchers presently purify the enzyme from 2,000-liter fungal cultures, under conditions where the cells secrete 100 units/ml. Protein from the final gel-filtration is readily crystallized from a 60-percent saturated ammonium sulfate solution.

With the most common clinically used hydrogen donor substrate (4-aminoantipyrine), the V_{max} of ARP is three times that of the HRP isozyme mixture. And with the chemiluminescent substrate luminol, its V_{max} is 500 times greater. The Suntory group has used this differential to design glucose and cholesterol assays that are notably more sensitive than corresponding assays using HRP. As Amachi pointed out, it should be possible to develop enhanced-sensitivity ELISAs based on this "chemiluminescent potential." It will also be interesting to determine how the new enzyme compares to HRP in the oxidation of tetramethyl benzidine, the chromogen of choice in molecular biological applications.

—Harvey Bialy