**EUCLAS**

**Week 8: Biocatalysts**

**Answers to questions due 11/8/06**

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**Bugs as catalysts**

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Industry is having a hard time finding new catalysts to supercharge many of today’s complex reactions. So researchers are scouring the world for bacteria and fungi that can do the job biologically.

IN MANY biological and industrial processes, reactions must proceed rapidly or they are useless. That is why catalysts are so important. These agents hasten reactions, without themselves being consumed by them. This neat trick also makes them very cost-effective. Modern industrial catalysis began in Germany in 1909, when Fritz Haber managed to synthesise ammonia from nitrogen and hydrogen, using iron as a catalyst. Today’s chemical and oil industries depend mightily on a wide variety of synthetic catalysts to make fuels, clothing, consumer products and, perhaps most familiar of all, to clean up the noxious fumes from car exhausts.

The problem with conventional catalysts is that, although they work well much of the time, the process of getting from compound A to compound B is becoming increasingly complex and hard to do using standard chemistry. Chemical synthesis is a lengthy process with many steps. Those steps require nasty chemicals and solvents, generate a lot of waste, consume energy and chip away at the product.

Catalysis can also be based on enzymes—the proteins that accelerate all of life’s reactions, from digesting food to making DNA. In the 1850s, Louis Pasteur demonstrated that making alcohol from sugar is promoted by entities he called “ferments”, later named enzymes after the Greek for “in yeast”. Used since antiquity in brewing beer and making cheese, enzymes boast impressive advantages: they operate at room temperature, they prefer water to some fancy solvent and they generate few bothersome by-products. Used individually or as part of whole cells, these “bio” catalysts often far exceed the efficiency of synthetic catalysts, owing to their ability to distinguish subtleties of shape and location.

Global sales of enzymes for use in detergents, animal feed and textiles amounted to $1.8 billion in 2001, according to Sandra Erb of Technology Catalysts International (TCI) in Falls Church, Virginia. By comparison, sales in the “other market” for biocatalysts, which includes pharmaceuticals, industrial chemicals and speciality products such as flavours, were a modest $130m. Now, regulatory pressures, and global initiatives towards sustainable development, may give this other market a boost.

**Smart enzymes**

Biocatalysis has already replaced chemistry in several high-volume applications. For example, high-fructose corn syrup (the natural sweetener in soft drinks) is produced on a multi-tonne scale, using two enzymes to convert maize-starch to glucose and then to fructose. No conventional alternative to the process exists. Similarly, DSM, a Dutch chemical firm, and Tosoh in Japan have developed a biocatalytic route for producing aspartame, a high-intensity sweetener. An enzyme correctly selects the desired structure from among four possible variants.

The ability of enzymes to discriminate to that extent makes them extremely attractive to the pharmaceutical industry. Unlike the enzymes used in laundry detergents, which are designed to interact with generic soil—eg, blood and egg—making medicines requires very choosy catalysts.
Laboratory reactions often produce compounds in a mix of mirror-image forms that react differently in the body. Enzymes do a superb job of telling the forms apart, which is important since one is commonly inactive or, worse, may even be harmful.

Search for fresh sources

Although several thousand enzymes are known, the actual number that industry has to work with is not that large. Historically, enzymes have suffered from being in limited supply, which has constrained the transformations that can be performed. Sometimes, the enzymes themselves have been either too selective or not selective enough; or they have proved unstable outside a narrow range of conditions. Poor stability leads to decreased efficiency, which results in longer processing times, more replacement of catalysts, reduced yields and higher costs.

Happily, most of microbial life is virgin territory. Diversa of San Diego, California, and a handful of other companies have made a business out of combing the world to find novel genomes as sources for biocatalysts—harvesting robust samples near deep-sea thermal vents, or in caustic-soda lakes in Kenya. Because 99% of microbes cannot be grown outside their own niche, the bugs’ DNA is extracted and put into more lab-friendly organisms to look for desired activity. This approach is beginning to bear fruit. Diversa’s Mark Burk reckons that the firm has discovered roughly 200 nitrolases (enzymes that act as specialised clipping shears) from samples collected around the world, yielding several biocatalysts that halve the number of steps needed to make a building-block of Lipitor, a cholesterol-lowering drug.

Bioprospecting in extreme environments can turn up novel micro-organisms for use as potential biocatalysts. But given that a pinch of typical soil contains around 100m microbes, enormous diversity can be found even in one’s own back garden, says Bob Holt of Avecia, a manufacturer of specialty chemicals based in Britain. Avecia has a collection of 6,000 organisms—ie, bacteria and fungi—and produces building-blocks for drugs in large quantities, using both biocatalytic methods and conventional chemistry.

Screening (ie, distinguishing useful catalysts from uninteresting ones, or the best of a good crop) is a critical part of discovery. According to Jean-Louis Reymond of the University of Bern, Switzerland, the challenge is to screen for the property you really want and not for something else. Many screens fail because they rely on highly reactive substances that respond equally well to strong and weak catalysts. Using such screens is akin to qualifying team members for an assault on Everest by getting them to step on a chair. Dr Reymond and his colleagues have developed chemical tests—including one patented jointly with Protéus in Nimes, France—for a variety of desirable properties that generate a colour-coded “fingerprint” of activity that is specific for each enzyme, and may prove useful in enzyme discovery.

Mimicking evolution

What nature cannot deliver, technology can help along. Genetic techniques developed in recent years make it possible to mimic in a laboratory the basic steps of Darwinian evolution—by tinkering with an enzyme’s genetic structure so as to optimise certain desired reactions. This “directed evolution” induces mutations in genes and selects for the best among them over several rounds, generating multitudes of new variants in a fraction of the time that nature would require.

An extension of directed evolution culls several different beneficial mutations and assembles them together in the same gene, with the aim of obtaining an additive or synergistic effect. The person who developed this “DNA shuffling” technology, Willem Stemmer of Maxygen in Redwood City, California, likens it to classical breeding of plants and animals, only for molecules, without having to go through the entire sexual cycle.
How much easier it would be if one could create ideal catalysts from scratch. Unfortunately, says Donald Hilvert, an organic chemist at the Swiss Federal Institute of Technology (ETH) in Zurich, enzymes are like car engines: easy to dismantle, but hard to build. Very little is known about how an enzyme's structure contributes to its function or how it interacts with other molecules. Dr Hilvert's research focuses on just such questions: how enzymes work, how to mimic them and how to adapt them for tasks that nature never thought of.

The patent situation for biocatalysis is a nightmare, notes Ms Erb. Many techniques for altering the genetic code of an enzyme are covered by patents, which means that a company that bioengineers the enzyme must be careful not to use patented techniques. The question of who owns the enzyme if a patented technique is used without permission has led to a number of companies licensing each other's proprietary technologies.

In the end, whether biocatalysis succeeds or not comes down to market economics. "Much is possible," says Bernard Witholt, a biotechnologist at ETH. "The technological platform is largely in place, and there is no shortage of research in the field." Chemists' traditional discomfort with biological processes—a mundane but surprisingly common bar—is giving way to an appreciation of the potential benefits of enzymes. As this grows, biocatalysis should become a more interesting option for industry. “What is needed,” says Dr Witholt, “is to identify where the mid-term market and technical possibilities lie.”

Questions.

#1. Most enzymes are catalysts, but are all catalysts enzymes? Name one catalyst that is not an enzyme.

#2. Name one advantage of enzyme catalysts.

#3. Say you find a new extremophile bacterium that you suspect makes a desired enzyme, but you can’t grow the organism in a lab because its requirements are too extreme. How can you study the enzyme?

#4. Enzymes, which are the products of genes, change over evolutionary time. How does “directed evolution” work to improve an enzyme’s usefulness in a very short period of time?