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Chemistry and biotechnology: a productive union meets new challenges

Editorial overview

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Current Opinion in Biotechnology 2003, **14**:567–569

0958-1669/$ – see front matter

DOI 10.1016/j.copbio.2003.10.005

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Like a good marriage, the union of biotechnology and chemistry is fulfilling the needs of both parties and producing offspring that are new and unique in many aspects. Chemical biologists address long-standing problems in both chemistry and biology, from making new molecules to understanding the functions of complex networks inside living cells. Chemical biotechnologists are doing the same, with a view towards near- or long-term applications for the production of foods, pharmaceuticals, materials and consumer products or aimed at assisting researchers to discover more about nature. Their idiosyncratic blends of chemical and biological methods have strengthened and expanded the scope of both fields and opened whole new areas of research and technology.

In a single volume we cannot cover a field as vibrant and diverse as chemical biotechnology. We have therefore selected a few highlights that illustrate this ongoing, and fruitful, collaboration. Most of the reviews in this year's section address the chemist's fundamental interest in making molecules, using biological systems for synthesis in clever, new ways. The product molecules are extremely diverse and include secondary metabolites, polymers, and proteins that incorporate nonnatural amino acids and enable subsequent chemical modifications. The disparate biologically based approaches developed to make these molecules are adding important new capabilities in synthetic chemistry. In many cases these capabilities are not simply replacing an existing chemical method, but instead are leading to new classes of compounds unavailable by any other approach.

While making molecules has and will continue to offer lucrative and fulfilling employment for biologically oriented chemists ('biology in the service of chemistry'), chemical biotechnology also abounds with examples of chemistry in the service of biology. This section offers an excellent example of this, in which protein-based assays are used to probe cellular biochemical networks (reviewed by Michnick). The section also illustrates the fuzziness of the boundary between 'chemical' and 'pharmaceutical' biotechnology. Assays for protein–protein interactions in cells will clearly be important tools for drug discovery. Furthermore, many of the products of biotransformations — including secondary metabolites (reviewed by Mijts and Schmidt-Dannert) and some of the novel proteins incorporating nonnatural amino acids (as described in the review by Tirrell) — will be drugs themselves or will be used in other ways to treat disease and disabilities.

Making molecules: enzyme catalysis

Biocatalysis is one of the oldest branches of chemical biotechnology and continues to have significant industrial impact. Presenting at the BIO2003 conference in Washington DC, Rolf Bachmann (McKinsey and Company;

http://www.mckinsey.com/) explained that 5% of chemical sales depend on biotechnological procedures; this should increase to 10–20% by 2010. But, even maintaining the current level requires new biocatalytic processes and products that are competitive in an exceedingly competitive marketplace. Researchers from a variety of disciplines will need to cooperate to fulfill the demand for better products (e.g. with higher purity), in accordance with current economic, environmental and social needs. The pressure is on to perform, and chemical biotechnologists will have to meet this challenge.

Increasing biotechnology's contribution to industrial chemistry will mean discovering enzymes and new synthetic routes to fine and bulk chemicals. The biocatalytic carbon–carbon coupling and cleavage reactions reviewed by Breuer and Hauer have great potential for extending the spectrum of biocatalytic applications. Selective carbon–carbon coupling is central to synthetic organic chemistry, and these new biocatalytic systems (mainly employing aldolases and ketolases) are attracting considerable interest. Industrial bioconversions are still dominated by hydrolytic enzymes, but some lyases offer attractive alternatives, for example, to kinetic resolution processes, where product yields are often not higher than 50%. In addition to reviewing promising candidates for industrial application, Breuer and Hauer highlight recent findings regarding enzyme reaction mechanisms, ribozymes, catalytic antibodies and biomimetic catalysts.

Current industrial applications of enzyme biocatalysis are heavily dominated by enantioselective bioconversions for fine chemical production. But enzymes can serve in other synthetic roles, including the production, degradation and modification of polymers, as reviewed here by Gübitz and Cavaco Paulo. In addition to the well-established industrial treatment of cellulose fibres with cellulases, enzymes are useful for other applications in polymer chemistry. Robust and reliable enzymes will find applications in more environmentally friendly processes for bulk polymer production and for the regioselective functionalization of polymers. Such functionalized polymers promise improved materials properties and also open up new opportunities for medical applications. Enzymes that produce optically active polymers will provide materials for new separation technologies.

Enzymes that require expensive and labile cofactors for catalysis have a long history of scaring off potential industrial users. The perception that these enzymes are hard to use and even harder to establish in a viable biochemical process is still widespread, despite significant advances over the past decade. When cofactor-requiring enzymes are used, it is usually in the form of whole cell systems, despite the disadvantages of side-product formation and difficult product recovery. The fact that cofactor-requiring oxidoreductases and transferases offer such attractive biosynthetic opportunities — as they catalyze complex and difficult reactions — continues to drive creative research to solve 'the cofactor problem'. The proposed solutions, some of which have indeed been demonstrated on a commercial scale, include new bioreactor designs and a multitude of cofactor regeneration schemes, replacing the natural cofactor with a chemical alternative, and bioelectrocatalysis for redox enzymes. This section includes two reviews that address existing solutions as well as those much further out on the horizon. Zhao and van der Donk review activities aimed at the regeneration of ATP, sugar nucleotides and 3-phosphoadenosine-5′-phosphosulfate (PAPS), whereas Wong and Schwaneberg take a look at protein engineering opportunities in bioelectrocatalysis.

Zhao and van der Donk describe a series of ingenious schemes for regenerating expensive substrates and cofactors. They advocate evaluating the various cofactor regeneration systems against a host of criteria, including the costs of the individual components and the total turnover number (i.e. how much product is made with a given amount of cofactor). When this is done, many of the new systems proposed for sugar nucleotide and ATP regeneration show considerable room for improvement. Although progress has been made for establishing regeneration systems using isolated enzymes (one or more different enzymes, as needed) in solution or immobilized on carriers, whole-cell methods (bacteria containing the assembled regeneration pathway) are in many cases more cost effective. Such coupled bacterial systems are in fact being used for moderate-scale regeneration of sugar nucleotides during the production of high-value, pharmaceutically important oligosaccharides. The authors point out that reducing current complex, multienzyme regeneration systems down to more simple schemes will be key to the development of viable standard procedures. Efficient and robust regeneration systems will facilitate cofactor-dependent enzymatic bioconversions as well as other applications such as cell-free protein synthesis, where inexpensive ATP is needed for cost-effective protein production.

Wong and Schwaneberg review protein engineering for bioelectrocatalysis and discuss opportunities to increase electron transport rates from electrode surfaces to redox proteins. Among the challenges they identify for establishing viable electrocatalytic systems, especially for biocatalysis, are finding systems for electrochemical regeneration of cofactors, improvement of enzyme stability by avoiding oxidative damage of the biocatalyst, obtaining a good distribution of the enzyme on an electrode, and understanding the dynamics and mechanisms of electron transfer in redox enzymes. Looking to past experience with directed enzyme evolution and rational protein design, where the improvement of technological features has been demonstrated for many enzymes, one can imagine that

protein engineering will be useful in solving some of the current problems of bioelectrocatalysis.

Making molecules: synthesis *in vivo*

An enormous range of molecules, both natural and synthetic, can be made using biocatalysts, either single enzymes or assembled in pathways. Natural products — mostly secondary metabolites — have been prime targets for biosynthesis, owing to their rich biological activities and the difficulty of synthesizing them chemically. In their review, Mijts and Schmidt-Dannert focus on recent efforts to engineer biosynthetic pathways to produce isoprenoids, polyketides and biopolymers in microbial hosts. Engineering aimed at controlling metabolic fluxes can dramatically increase production levels of given metabolites, obviously critical for any commercial application. Particularly exciting are the novel compounds — analogs of natural metabolites — that can be made with engineered pathways. By combining biosynthetic genes from different sources in new ways inside the engineered host, pathways to rare, and even previously unknown, metabolites have been constructed. This review also illustrates how bioinformatics, proteome analyses and this 'combinatorial' pathway engineering can work together for the production of new biopolymers.

The area reviewed by Tirrell takes biotransformation in a whole new direction: he describes recent efforts to engineer the cellular protein synthesis machinery to incorporate novel amino acids into proteins *in vitro* and *in vivo*. The development of aminoacyl-tRNA synthetases that will activate and charge novel amino acids, together with schemes for encoding the amino acids, have allowed researchers to build artificial or engineered proteins that include nonnatural amino acids in their sequence. Replacing all the leucine residues in a protein with a new amino acid (e.g. a heavily fluorinated analog) can change the overall physical behavior of a protein or a designed, protein-like macromolecule. Site-specific replacement of amino acids allows researchers to modulate the chemistry at specific locations, which is useful, for example, for subsequent specific chemical modification or for modulating active-site chemistry. The protein products of these engineered cells and *in vitro* pathways offer extraordinary new opportunities for chemists to intervene in cellular behaviors and to make protein-based materials with previously unimagined properties.

Probing protein networks

A particularly dynamic application of chemistry in biotechnology is in elucidating the structures and pathways of communication within biochemical networks in living cells. Many important, new analytical tools have been developed for probing cells at the systems level, and in his article Michnick reviews one such class of tools: protein fragment complementation assays (PCA) for identifying and studying the modulation of protein–protein interactions. The need for such tools in drug discovery is apparent: with them one can isolate the mode of action of a drug to a specific point in the network. Such pathway-based drug discovery will overcome problems associated with more traditional screening methods, allowing one to focus on selected targets and, of key importance, allowing off-pathway, non-specific or toxic effects of potential drugs to be assessed much earlier in the drug development process. Although the pharmaceutical applications are driving commercial development of such technologies, the potential impact of these tools in chemical biotechnology extends well beyond drug discovery and development. At a fundamental level, chemical biotechnology is aimed at developing strategies to define gene function at the level of entire genomes. Michnick describes how a PCA strategy can be used to infer gene function, addressing the problems that genomics has traditionally sought to answer, such as establishing common and unique traits to determine phylogenetic and evolutionary relationships among organisms. The same strategies can also be used to achieve a deeper appreciation of the biochemical organization of living cells at a systems level and to provide insight into the molecular schemes that all living things share and those that make individual cells and organisms unique.