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Novel biocatalysts: Recent developments

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Abstract

The limited number of suitably well characterized biocatalysts continues to limit progress in the application of biological routes in the synthesis of compounds for novel pharmaceuticals, materials, or performance chemicals. In this situation, the discovery of novel biocatalysts or novel functionalities or substrates on existing ones is an important task. This work describes a range of novel biocatalysts obtained recently through one of three techniques: environmental sampling or screening, protein engineering on existing enzymes, or extension of the catalytic profile of existing catalysts.
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1. Introduction and scope

Biocatalysts are important for the production of pharmaceuticals and fine chemicals because of their high enantio- and regio-selectivity and as an alternative to chemical catalysts in more environmentally friendly processes. However, the number of biocatalysts that are suitable for industrial uses is still very limited. Thus, the search for biocatalysts with higher enantioselectivity and novel activity is the subject of an increasing number of research papers.

This review describes examples for the three most common ways to identify novel activity: (i) screening of environmental samples, usually for an organism which can catabolize a certain compound, (ii) protein engineering of an existing biocatalyst, or (iii) testing an existing biocatalyst for novel functionality or an extended substrate specificity in comparison with the current range. A combination of techniques can be used in conjunction to create enzymes with the desired properties.

Screening of environmental samples has the most utility when the reaction in question could potentially be part of the metabolism of the organism in question. One example covered in this review is the hydroxyaminobenzene (HAB) mutase reaction where the reaction step is part of a catabolic pathway to degrade nitrobenzene, a xenotoxic compound to the organism. Additionally, environmental screening can be used in cases where no enzyme currently exists for the reaction because in contrast to protein engineering, no template enzyme is required to begin development. Further details on environmental screening can be found in reviews on the subject (such as Ogawa and Shimizu, 1999).

Testing an existing biocatalyst, such as the example of NADPH oxidase functionality found in an NADH oxidase, is the simplest technique to implement as it can be performed with biocatalysts already on hand. This strategy is most successful when the substrate being tested is similar to the natural substrate of the biocatalyst.

Finally, protein engineering, or improvement of an existing biocatalyst can be used to change substrate specificity or increase overall reactivity of an existing biocatalyst. The creation of a secondary amine oxidase from a primary amine oxidase is one such example covered in this review. Protein engineering is most suited to the case when a template

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enzyme with some measurable level of the activity in question exists and when a good method of identifying improved proteins (screening or selection) is in place (for practical advice on how to perform different mutagenesis techniques please refer to Braman, 2002). Many excellent reviews of protein engineering by recombination or directed evolution also exist such as (Powell et al., 2001; Reetz, 2004; Farinas et al., 2001; Lutz and Patrick, 2004).

This review focuses on a handful of very interesting biocatalysts with novel functionality which have been selected as examples for illustrative purposes. It is not meant to serve as an encyclopedic review of biocatalyst development. In addition, improvements in production processes of biocatalysts, such as protein expression or purification issues, as well as a discussion of detailed techniques of novel biocatalyst discovery are outside the scope of this review.

2. New catalysts through screening of enviromental samples

2.1. HAB mutase

HAB mutase catalyzes the rearrangement of hydroxyaminobenzene to 2-aminophenol (Fig. 1a), with specificity for production of the ortho-product in contrast with the typical Bamberger rearrangement, which gives primarily the para- or 4-substituted product. The most well-studied example is the HAB mutase from *Pseudomonas pseudoalcaligenes* JS45, which was identified by screening soil samples for organisms that could grow on nitrobenzene as a sole carbon source. The organism was shown to catabolize nitrobenzene reductively, rather than oxidatively; first transforming nitrobenzene into hydroxyaminobenzene and then to 2-aminophenol, which then undergoes ring fission to the semi-aldehyde (Nishino and Spain, 1993). Similar mutase activity on hydroxyamino-derivatized aromatics occurs in *Mycobacterium* sp in the rearrangement of 4-hydroxyl amino toluene to 6-amino-m-cresol (Spiess et al., 1998), in *Ralstonia eutropha* (Schenzle et al., 1999) and *Pseudomonas putida* on various compounds (Park et al., 1999), and in the metabolic pathway to catabolize 4-chloronitrobenzene in *Rhodosporidium* sp. (Corbett and Corbett, 1981). 3-nitrotoluene, 1-nitronaphthalene, and 2-nitrotoluene also serve as substrates for the *P. pseudoalcaligenes* enzyme, although to a lesser degree (Kadiyala et al., 2003).

The HAB mutase from *P. pseudoalcaligenes* has been cloned, overexpressed in *E. coli*, and characterized. There are actually two isozymes, HAB mutase A and B, with 44% amino acid identity between them. The two enzymes are similar in size, but have different thermal and pH stability profiles and are transcribed divergently (Davis et al., 2000). Neither requires a cofactor. With one exception (Luckarift et al., 2005), the two appear to have the same broad substrate specificities.

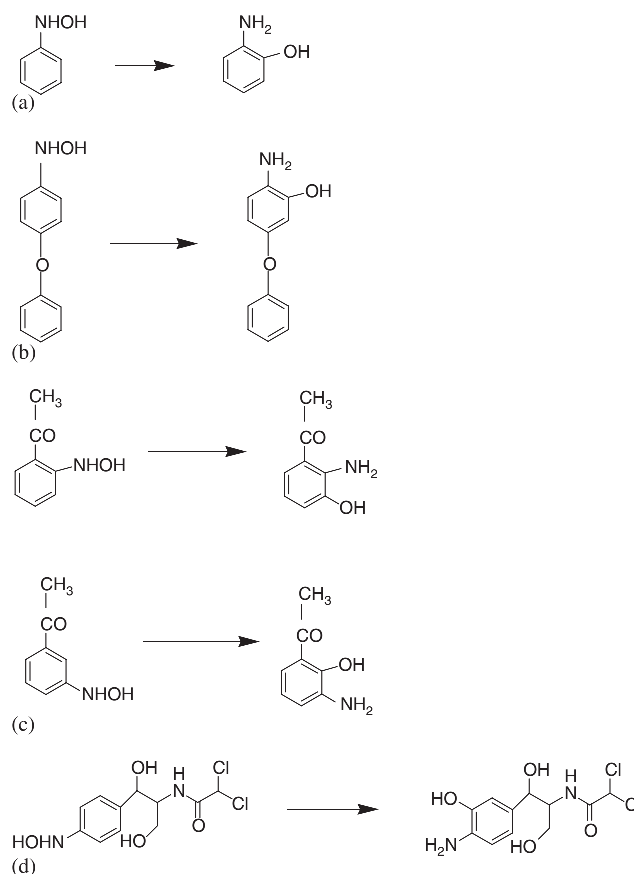

Fig. 1. The reactions catalyzed by HAB mutase from *Pseudomonas pseudoalcaligenes* JS45: (a) the native reaction, rearrangement of hydroxylaminobenzene to 2-aminophenol, (b) the rearrangement of 4-hydroxylaminobiphenyl ether to 2-amino-5-phenyoxyphenol, (c) the rearrangement of 3-hydroxylamino-acetophenone to 3-amino-2-hydroxy-acetophenone (top) and 2-hydroxylamin oacetophenone to 2-amino-3-hydroxy-acetophenone, (d) the rearrangement of the hydroxylamino derivative of chloramphenicol to the aminophenol analog.

Explorations of the substrate specificity of the *P. pseudoalcaligenes* HAB mutase have revealed some interesting and useful activities. Nadeau et al. investigated the production of 2-amino-5-phenoxyphenol, a fluorescent whitening agent, photosensitizer, and intermediate in the synthesis of phenoxybenzoxazole, from 4-nitrobiphenyl ether using the naturally occurring nitroreductase from *P. pseudoalcaligenes* and HAB mutase B, in crude lysate and in partially purified form (Fig. 1b). This represents an alternative to the chemical process involving the nitration of phenol and then reduction via a metal catalyst to the amine. Yields for mononitration product are extremely low and the chemical process requires extreme conditions. While no optimization of the process was undertaken, a single product, 2-amino-5-phenoxyphenol was produced stoichiometrically in an anaerobic process. One disadvantage of this biocatalytic route is the NADPH requirement of the nitroreductase enzyme, necessitating a cofactor regeneration system if partially purified enzymes are used (Nadeau et al., 2000).

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