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Review

Preparative use of isolated CYP102 monooxygenases—A critical appraisal☆

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Abstract

Isolated P450 monooxygenases have for long been neglected catalysts in enzyme technology. This is surprising as they display a remarkable substrate specificity catalyzing reactions, which represent a challenge for classic organic chemistry. On the other hand, many P450 monooxygenases are membrane bound, depend on rather complicated electron transfer systems and require expensive cofactors such as NAD(P)H. Their activities are low, and stability leaves much to be desired. The use of bacterial P450 monooxygenases from CYP102 family allows overcoming some of these handicaps. They are soluble and their turnovers are high, presumably because their N-terminal heme monooxygenase and their C-terminal diflavin reductase domain are covalently linked. In recent years, protein engineering approaches have been successfully used to turn CYP102 monooxgenases into powerful biocatalysts.

Keywords: P450 monooxygenase; Enzyme technology; Enzyme stability; Co-factor regeneration

Contents

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1. Introduction

Heme-containing P450 monooxygenases constitute a major enzyme family (E.C.1.14...) comprising more than 4500 members from all kingdoms of life (http://drnelson.utmem.edu/CytochromeP450.html). Among their various functions, the oxidation of non-activated C–H bonds, often in a regio- and stereoselective manner, has attracted the interest of chemists.

In spite of this great potential, there are only few examples of P450-based selective microbial oxidations used in industrial processes, such as the 11β-hydroxylation of deoxycortisol to cortisol (Petzoldt et al., 1982), the terminal oxidation of fatty acids to dicarboxylic acids by a *Candida tropicalis* (Picataggio et al., 1992), the *p*-hydroxylation of 2-phenoxypropionic acid, an herbicide precursor, by *Beauveria bassiana* (Ladner et al., 1995), the subterminal hydroxylation of compactin to pravastatin by *Streptomyces carbophilus* (Serizawa and Matsuoka, 1991) and the regioselective hydroxylation of (*S*)-nicotine to 6-hydroxy-*S*-nicotine (Schmid et al., 2001). All these reactions are carried out using whole cell systems. Nevertheless, there are several drawbacks in using *in vivo* processes, such as poor substrate uptake, product degradation, product toxicity as well as product purification from the fermentation broth. On the other hand, attempts to use isolated P450 monooxygenases in biotransformation reactions are scarce and pose a considerable challenge.

P450 monooxygenases are intrinsically slow enzymes and exhibit rather poor stability. They require in general rather complex electron transfer systems such as ferredoxin and ferredoxin reductase or FMN/FAD containing reductases. Eukaryotic P450s though displaying interesting substrate spectra are mainly membrane associated. Additionally, as all catalyzed reactions depend on the supply of an equimolar amount of NAD(P)H, technical processes require a cofactor regeneration system or artificial electron donor to be economically advantageous. Some of the above problems can be alleviated by using microbial P450s: they are quite often soluble enzymes, relatively stable and may show high enzymatic activity.

In this brief review, we mainly focus on two promising bacterial P450 monooxygenases: CYP102A1 (P450 BM-3) from *Bacillus megaterium* (Narhi and Fulco, 1986) and CYP102A3 from *Bacillus subtilis* (Gutierrez et al., 2000; Lentz et al., 2004; Gustafsson et al., 2004). These proteins are natural fusion enzymes composed of an N-terminal heme monooxygenase linked to the C-terminal diflavin reductase domain. This protein organization may be responsible for the quite high activities of CYP102 monooxygenases compared to other known P450s. Moreover, they do not require additional electron transfer proteins.

The high sequence similarity to microsomal P450s and the unusual architecture of their reductase domain make CYP102A1 an interesting object to study reaction mechanism and electron transfer in eukaryotic monooxygenases.

CYP102A1 and CYP102A3 hydroxylate and epoxidize middle to long chain saturated, unsaturated and branched fatty acids at subterminal positions (Li et al., 1991; Gustafsson et al., 2004). The sequence identity between CYP102A1 and CYP102A3 is about 65% at protein level. Using improved expression protocols, recombinant CYP102 enzymes can be expressed in *Escherischia coli* to levels of up to $1000\,mg\,l^{-1}$ in fed-batch fermentation. Several purification protocols have been published, based on ion exchange chromatography, hydrophobic interaction chromatography or metal affinity chromatography, resulting in high levels of purity depending on the further application of the enzymes (Boddupalli et al., 1990; Li et al., 1991; Maurer et al., 2003).

The X-ray structure of the monooxygenase domain of CYP102A1 was first solved in 1993 (Ravichandran et al., 1993). Since then, several structures of CYP102A1 mutants with and without bound substrates (Li and Poulos, 1997; Haines et al., 2001) and one including the FMN binding domain (Sevrioukova et al., 1999) have been solved. Very recently, a fragment containing the FAD binding site could be crystallized and its structure was resolved by X-ray analysis (Warman et al., 2005). Detailed data are, however, still unpublished. These crystal structures provide the opportunity for rational protein design as well as for building homology models of the related enzymes CYP102A2 and CYP102A3. Based on this information, mutants of CYP102A1 and CYP102A3 have been engineered to meet the following needs: new substrate specificities, higher thermal stability and altered co-factor specificity.