Cytochrome P450 Proteins and Potential Utilization in Biodegradation

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The cytochrome P450 enzymes are major catalysts involved in the oxidations of xenobiotic chemicals in microorganisms as well as higher animals and plants. Because of their functional roles, they offer potential in biodegradation technology. A number of microbial P450s have already been characterized and offer advantages in terms of their high catalytic rates and facile expression in microorganisms. One approach to extending the catalytic selectivity to more compounds in the environment is rational design. In three cases, the three-dimensional structures of bacterial cytochrome P450 enzymes are available and can be further understood through studies with molecular dynamics. Many mammalian cytochrome P450 enzymes have been studied extensively and have potential for biodegradation because of their broad catalytic selectivities (e.g., P4502E1). Several advances have been made in the heterologous expression of these proteins in microorganisms. Improvements under development include electron transfer from flavodoxin and the use of cytochrome P450-NADPH-cytochrome P450 reductase fusion proteins. Random mutagenesis offers the potential of improving the catalytic activities of some of these proteins. Future challenges include the use of cytochrome P450 expression vectors in microorganisms capable of thriving in the environment; recent success in expression of vectors in *Salmonella* genotoxicity tester strains may be encouraging in this regard. — Environ Health Perspect 103(Suppl 5):25–28 (1999)

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Introduction

The cytochrome P450 enzymes are spread through the phylogenetic spectrum and, collectively, are major catalysts involved in the aerobic oxidation of almost all classes of organic molecules (for a recent review, see the entire January 1992 issue of FASEB J). The list of monooxygenation reactions that are catalyzed includes aliphatic hydroxylation, desaturation, heteroatom oxidation and dealkylation, epoxidation, oxidative group migration, and various modes of mechanism-based inactivation (1). Most of these different modes of oxidation may be understood in terms of some basic and common chemistry involving the activation of O₂ to generate a reactive FeO₃⁺ entity, the abstraction of a hydrogen atom or nonbonding or π electron (by FeO₃⁺), and radical recombination (Figure 1) (2). A key to our understanding of catalytic action of the P450s is the view that the FeO₃⁺ moiety is an inherently strong oxidant and that a great variety of oxidation reactions are accessible to the P450s if the proteins position specific moieties of a compound close enough to this entity (1). As pointed out above, the P450s appear in most organisms, and within each mammalian organism there are more than 40 different P450 genes expressed (4). Collectively, several hundred P450s from various P450s have now been characterized at the level of their primary sequences (4) so there is a wide repertoire of potential catalysts available for biodegradation work.

Use of Natural P450s

Microbial systems are of greatest utility for biodegradation work. Some bacterial and fungal P450s have already been characterized and are known to degrade certain chemicals of interest. Foremost among these is P450 101 from *Pseudomonas putida*, which has been reviewed by Gunsalus et al. (5) and Peterson and Lu (6). It should be pointed out however that the list of characterized bacterial and fungal P450s is still relatively short (4) and may not be particularly useful as a source for future biodegradation work.

Considerable potential exists for the isolation of new bacterial P450s and other microbial P450s capable of oxidizing particular substrates. The approach can involve searches for growth with a particular chemical as a carbon source (6). This approach is probably most applicable with the Pseudomonads (5).

Rational Redesign of P450s

An alternate approach is that of redesigning a particular known P450 enzyme to alter its catalytic specificity by changing amino acids in the protein. This approach requires knowledge of a (static) threedimensional structure of a protein. Currently, only two such P450 structures are available, the bacterial P450 101 and 102. Also of use is dynamic modeling in which computer programs are used to predict the motion of each atom in the protein (or at least in the active site) and project the spatial course of the substrate as a function of time (usually on the order of picoseconds).

Such an approach has been applied to P450 101 by Paulsen et al. (7). The approach was applied to the hydroxylation of norcamphor, and replacement of a Tyr residue with Phe led to decreased mobility of the substrate and an altered pattern of hydroxylation (7). This general approach will have the greatest potential in cases in which the mutants predicted to have altered properties can be produced and analyzed. Of course, appropriate vector systems will be required to introduce such P450s into field situations.
Heterologous Expression of P450 Proteins

Several microbial approaches to the expression of P450s have been employed. Bacterial P450s have been expressed in other bacteria at higher levels (6,8), and Candida tropicalis P450s have been expressed in Saccharomyces cerevisiae (9).

Mammalian P450s offer some advantages over the microbial P450s in terms of development for biodegradation work. The major advantages are that some of these enzymes have been characterized and more is already known about their catalytic specificities. Some are known to be rather nonselective in their substrate selectivity and can process compounds that are either small (e.g., P450 2E1) or large (e.g., P450 3A4) (10). The cDNAs are readily available since polymerase chain reaction and other technologies can be used to rapidly obtain the vectors from crude tissues. However, expression in microbial vectors is probably essential for the development of these proteins for biodegradation work.

There is considerable precedent for the expression of mammalian P450s in yeast, beginning with the work of Sakaki et al. (11). The literature on expression of yeast has been reviewed recently (12). The systems have some advantages; however, Saccharomyces strains used to date are probably not suitable for transfer to the field. If satisfactory fungal vectors and strains could be developed, they could be considered.

Expression of enzymes in bacteria has many advantages. Bacterial expression of mammalian P450s was hampered by early problems with proteolysis of NADPH-P450 reductase (13) and problems in expression of native P450 sequences (14). However, with new vectors and slight modification of 5’ sequences, the high level expression of several bovine, rabbit, and human P450s has been achieved (Table 1). A typical Fe(3+)-CO versus Fe(2+) spectrum of human P450 3A4 produced in Escherichia coli is shown in Figure 2. The P450s most likely to be of use in biodegradation studies may be P450s 1A1, 1A2, 2E1, and 3A4 (Table 1); their catalytic specificities are known in some detail. P450 1A1 oxidizes polycyclic hydrocarbons, P450 1A2 oxidizes aromatic amines, P450 2E1 oxidizes small halocarbons and vinyl monomers, and P450 3A4 oxidizes a number of large molecules (10). A problem encountered in the bacterial expression of P450s is the lack of a P450-reductase activity, although with some P450s there is some reduction by a flavodoxin (MR Waterman and C Jenkins, personal com-

imunication). One approach to this problem would be the coexpression of NADPH-P450 reductase using a second plasmid; however, a more practical approach appears to be the construction of P450:NADPH-P450 reductase fusion proteins, for which there is precedent (16–18). Several of these have already been prepared (Table 1) although their functional activity in bacterial cells has not yet been characterized.

Improvement to P450s through Random Mutagenesis

The construction of P450:NADPH fusion proteins (Table 1), which should prove to be active within cells, enables a number of strategies to be considered for the improve-

Table 1. Mammalian P450s expressed in E. coli.*

| Human             | Others          |
|-------------------|-----------------|
| P450 1A1          | Rabbit P450 2B4 |
| P450 1A2          | Rabbit P450 2C3 |
| P450 2C10         | Rabbit P450 2E1 |
| P450 2E1          | Rat P450 7A     |
| P450 3A4          | Bovine P450 11A |
| P450 11A          | Bovine P450 17A |
| P450 17A          |                 |
| P450 1A1 NADPH-P450 reductase |         |
| P450 1A2 NADPH-P450 reductase |         |
| P450 3A4 NADPH-P450 reductase |         |

*Data from Barnes et al. (14), Gillam et al. (15), Richardson et al. (19), Wada et al. (20), Li and Chiang (21), Larson et al. (22), Shimada et al. (23), Sandhu et al. (24), and Fisher et al. (25).

Figure 2. Fe(3+)-CO vs Fe(2+) difference spectrum of E. coli membranes containing P450 3A4 expressed in the vector pC6W. The baseline is also shown from Gillam et al. (15).

Figure 1. Major oxidation reactions catalyzed by P450 enzymes. From Guengerich (3).
ment of the systems. As mentioned earlier, rational redesign can be done if three-dimensional structures can be obtained; however, even in the absence of such information, it is possible to consider improvement of catalytic activities through random mutagenesis approaches. This strategy is particularly attractive since many of the P450 substrates under consideration are toxic, and screening should not be difficult. A typical approach would involve replacing a large segment of the P450 construct with a portion in which mutations had been randomly introduced and then transfecting the resulting plasmids into E. coli. Colonies capable of growth on high levels of the substrate could be selected and the cDNA recovered for analysis of the sequence (26,27). The expressed protein could be purified and its catalytic activity analyzed in more detail. The same approach could be applied to other segments of the P450 to obtain a more active enzyme, which could then be used in a vector and organism more appropriate for use in the field.

**Propects and Problems**

The approaches outlined above are all technically possible today (e.g., Figure 2 and Table 1), and only the matters of catalysis within cells and improvement through random mutagenesis remain to be achieved. There is also hope that the general strategies may be enhanced with increasing knowledge about the characteristics of P450s found in microorganisms, plants, and insects where there has been considerable interest. The acquisition of more three-dimensional structures will also augment the work.

Some problems do need to be overcome. In particular, there will be a real need to move useful P450 constructs into vectors that can be used in the field. In the field, there are problems with low oxygen tension, limited nutrients, etc., that will need to be dealt with. Finally, it is unknown what regulatory problems will arise from the introduction of novel engineered microorganisms into the field.

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