

CYTOCHROME P-450 OF *STREPTOMYCES GRISEUS* AND XENOBIOTIC METABOLISM

F. Sima Sariaslani*, M. K. Trower; M. Ramachandra; S. E. Buchholz;
Ralph Stahl and C. A. Omer
Central Research & Development, E. I. duPont de Nemours &
Co., Wilmington, DE 19880-0228, USA
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INTRODUCTION

Streptomyces are aerobic prokaryotic microorganisms that are present in terrestrial and aquatic environments.¹ These organisms have long been exploited as producers of antibiotics and enzymes. In addition, because of their remarkable versatility and metabolic capability *Streptomyces* are instrumental in the breakdown of organic matter in the environment and therefore in recycling carbon in nature.¹

Streptomyces griseus

We have been studying the enzymatic system employed by *Streptomyces griseus* for oxidation of a diverse array of xenobiotics. We have shown that growth in a medium enriched with soybean flour induces a multicomponent cytochrome P-450 (P-450_{soy}) enzyme system in *S. griseus*.^{2,3} P-450_{soy} resembles its mammalian counterparts in its broad substrate specificity. The reactions performed by P-450_{soy} include, but are not limited to, aromatic and alicyclic hydroxylations (biphenyl, phenol, benzene, chlorobenzene, Benzo(a)pyrene, cyclohexane), O-dealkylation (7-ethoxycoumarin), epoxidation (precocene II) and N-acetylation (aniline), and N-oxidation (pyridine).⁴

S. griseus cells enriched in P-450_{soy} exhibit lack of stereo- and regioselectivity during camphor oxidation.^{5,6} This is in contrast to camphor oxidation by P-450_{cam} of *Pseudomonas putida* which shows a high degree of regio- and stereospecificity resulting in the production of 5-*exo*-hydroxycamphor as the sole reaction product.^{5,7} The strict specificity of P-450_{cam} has been attributed to the presence

of tyrosine-96 in the structure of this enzyme.^{8,9} The major product formed during oxidation of camphor by *S. griseus* cells containing P-450_{soy} is 6-*endo*-hydroxycamphor together with 5-*endo*-, 3-*endo*- and 5-*exo*-hydroxy derivatives as minor products.⁶ Multiple hydroxycamphor product formation is also observed in reconstituted assays containing homogeneous preparations of P-450_{soy}.¹⁰ Alignment of the amino acid sequence of P-450_{soy} with the *P. putida* enzyme indicates that tyrosine-96 is not conserved in P-450_{soy}. We have proposed that lack of this residue in P-450_{soy} contributes to its lack of specificity during camphor oxidation.¹⁰

In search for the other ancillary proteins of P-450_{soy} system we have identified a soybean flour-inducible, 14,000 molecular weight 7Fe ferredoxin (*S. griseus* 7Fe ferredoxin) in crude extracts of *S. griseus*.¹¹ This ferredoxin couples electron flow between spinach ferredoxin reductase and cytochrome P-450_{soy} for NADPH-dependent substrate oxidation. We have determined the primary structure of this ferredoxin and have shown that it contains a [3Fe-4S] and a [4Fe-4S] cluster.¹² The amino acid sequence of this protein, which consists of 105 amino acids with a calculated molecular weight of 12,291, shows high homology to the other reported 7Fe ferredoxins.¹²

We have recently isolated an FAD-containing and highly unstable soybean flour-inducible ferredoxin reductase (*S. griseus* ferredoxin reductase) from *S. griseus* crude extracts.¹³ This 60,000 molecular weight protein requires the presence of 20% glycerol and 5mM dithiothreitol for stabilization. *S. griseus* ferredoxin reductase is an NADH-dependent flavoprotein which requires Mg²⁺ for *in vitro* activity. A consensus FAD binding sequence, which possesses a high degree of homology to the other FAD-containing ferredoxin reductases, starts at residue 7 of the N-terminus of this flavoprotein. In reconstituted assays, the *S. griseus* ferredoxin reductase can use a variety of ferredoxins such as *Clostridium pasteurianum* ferredoxin, spinach ferredoxin and adrenodoxin for coupling electron transfer from NADH to cytochrome P-450_{soy}. We have observed catalytic activities of 20-40 nmol product for med/min/mg P-450_{soy} when the *S. griseus* 7Fe ferredoxin and the ferredoxin reductase were used in reconstituted assays.

P-450_{soy} is encoded by *soyC* (CYP105D) gene. This gene encodes a 413 amino acid protein with a molecular weight of 45,400.¹⁰ Five base pairs downstream of the stop codon of *soyC* is a translationally coupled open reading frame for a ferredoxin-like

protein of 6,600 molecular weight. The amino acid sequence of this ferredoxin-like protein is dissimilar to the soybean flour-inducible *S. griseus* 7Fe ferredoxin. Due to its proximity to the *soyC* gene we think that this open reading frame probably encodes the *in vivo* ancillary ferredoxin for P-450_{soy} and have therefore named this gene *soyB*.

To date we have shown that a recombinant *S. lividans* strain which contains the *soyC,B* gene oxidizes 7-ethoxycoumarin and precocene II. We are currently investigating metabolism of a wide array of xenobiotics by this recombinant strain. In addition, we are involved in over-expression of *soyC,B* genes which would allow isolation and characterization of the *soyB* gene product.

We have exploited the broad substrate specificity of P-450_{soy} and its resemblance to its mammalian counterparts to activate promutagenic chemicals.¹⁴ In a modified version of the *Salmonella*/gene mutation (Ames) assay, *Salmonella typhimurium* strains TA98 and TA1538 were reverted by mutagenic metabolites that were produced by *S. griseus* cells enriched in cytochrome P-450_{soy}. Promutagens that were activated included a variety of aromatic amines (benzidine; 2,4-diaminotoluene; 4-chloro-2-nitroaniline), polycyclic aromatics (benzo(a)pyrene), and small aliphatics (chloropicrin).

In another test system we have used two genetically engineered strains of *S. griseus* which can activate promutagenic chemicals and detect the presence of their mutagenic metabolites.¹⁵ One recombinant strain detects point mutations, while the other strain is sensitive to frame shift mutations. To our knowledge this is the first reported single-organism test system in which the enzymatic machinery for activation of promutagens has been linked to a detection system within the same organism.

We have been studying the feasibility of using artificial electron donors for transfer of reducing power from NADH to the P-450_{soy} component. We have shown that, in reconstituted assays, redox mediators such as phenazine methosulfate (PMS) and phenazine ethosulfate (PES) effectively replace the ferredoxin reductase and the ferredoxin and affect the NADH-dependent substrate oxidation by P-450_{soy}.¹⁶ We have shown reduction of P-450_{soy}, O-dealkylation of 7-ethoxycoumarin and epoxidation of precocene II by the NADH/PMS/ or NADH/PMS/P-450_{soy} system.¹⁶

As summarized above, P-450_{soy} of *Streptomyces griseus* mimics its mammalian counterparts in its broad substrate specificity

and the ability to activate promutagenic chemicals. Biochemical and molecular studies are currently underway to unravel the mechanism of the action of P-450_{soy} and the nature of the active site of this interesting enzyme.

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