Phase I and phase II enzymes produced by *Cunninghamella elegans* for the metabolism of xenobiotics

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Abstract

The filamentous fungus *Cunninghamella elegans* has the ability to metabolize xenobiotics, including polycyclic aromatic hydrocarbons and pharmaceutical drugs, by both phase I and II biotransformations. Cytosolic and microsomal fractions were assayed for activities of cytochrome P450 monoxygenase, aryl sulfotransferase, glutathione S-transferase, UDP-glucuronosyltransferase, UDP-glucosyltransferase, and N-acetyltransferase. The cytosolic preparations contained activities of an aryl sulfotransferase (15.0 nmol min\(^{-1}\) mg\(^{-1}\)), UDP-glucosyltransferase (0.27 nmol min\(^{-1}\) mg\(^{-1}\)) and glutathione S-transferase (20.8 nmol min\(^{-1}\) mg\(^{-1}\)). In contrast, the microsomal preparations contained cytochrome P450 monoxygenase activities for aromatic hydroxylation (0.15 nmol min\(^{-1}\) mg\(^{-1}\)) and N-demethylation (0.17 nmol min\(^{-1}\) mg\(^{-1}\)) of cyclobenzaprine. UDP-glucuronosyltransferase activity was detected in both the cytosol (0.09 nmol min\(^{-1}\) mg\(^{-1}\)) and the microsomes (0.13 nmol min\(^{-1}\) mg\(^{-1}\)). N-Acetyltransferase was not detected. The results from these experiments provide enzymatic mechanism data to support earlier studies and further indicate that *C. elegans* has a broad physiological versatility in the metabolism of xenobiotics.

Keywords: Metabolism; Enzyme; PAPS sulfotransferase; Cytochrome P450; *Cunninghamella elegans*

1. Introduction

Microbial systems have been used as models for mammalian metabolism since many metabolites formed from xenobiotics are similar to those formed in mammals [1,2]. The zygomycete fungi of the genus *Cunninghamella* have the ability to metabolize xenobiotics including polycyclic aromatic hydrocarbons and pharmaceutical drugs [3–8]. The metabolites produced are derived from phase I oxidation reactions such as aromatic and aliphatic hydroxylation, heterocarot oxidation, and N- or O-demethylation, which are also known in mammals [3,6,8]. The isolation of the sulfate, glucoside, glucuronide and acetylated metabolites from the fungal metabolism [4,9–13] indicated that *C. elegans* also has the potential for phase II biotransformations.

Little is known about the enzymes involved in the biotransformation of xenobiotics by *C. elegans*. A few published data are available on the enzymatic mechanisms for fungal xenobiotic metabolism
Since *Cunninghamella* spp. have been proposed as a model microbial system to mimic mammalian metabolism of xenobiotics [1,2], we wanted to provide enzymatic data for the formation of the observed metabolites and to provide enzymatic mechanisms for support this zygomycete to be used as a model for mammalian metabolism. In this study, we detected both phase I and phase II enzymes in *C. elegans.*

2. Materials and methods

2.1. Chemicals and methods

NADPH, *p*-toluenesulfonyl fluoride, dithiothreitol, 1-naphthyl β-D-glucuronide, 1-naphthyl β-D-glucoside, 1-naphthylsulfate, [1-14C]1-naphthol (7 μCi/μmol), 1-chloro-2,4-dinitrobenzene, glutathione (reduced form), acetyl coenzyme A, procainamide, *p*-aminobenzoic acid, PAPS, UDPGA, UDPG, and cyclobenzaprine were purchased from Sigma Chemical Co. (St. Louis, MO). 2-Hydroxycyclobenzaprine and N-desmethylcyclobenzaprine were prepared as described previously [20]. Protein concentrations were determined with a Coomassie brilliant blue protein assay reagent kit (Pierce, Rockford, IL) using bovine serum albumin as the standard. The reduced carbon monoxide difference spectrum was determined as described by Omura and Sato [21]. UV spectra were determined on a Shimadzu UV-VIS scanning spectrophotometer (UV-2101PC). Radioactivity was measured on a Packard Tri-carb 2000CA liquid scintillation analyzer (Packard Instrument Company, Meriden, CA).

2.2. Fungi and preparation of cell-free extracts

Cultures of *C. elegans* ATCC 36112 were grown in 30 ml of Sabouraud dextrose broth (Difeo Laboratories, Detroit, MI) in 125 ml Erlenmeyer flasks at 27°C and 150 rpm. Mycelia from 50 h old cultures (after depletion of glucose) were harvested by filtration and washed with deionized H2O.

Cell-free extracts of *C. elegans* was prepared as described by Cerniglia and Gibson [15]. 15 g of blended mycelia was disrupted in 25 ml buffer (50 mM phosphate, pH 7.4, 1 mM dithiothreitol, 20% glycerol) with 2 mM of *p*-toluen sulfonyl fluoride. The crude homogenate was centrifuged at 12000 × *g* for 15 min and then ultracentrifuged at 120000 × *g* for 90 min. The microsomal pellet was resuspended in the buffer and used immediately. The supernatant can be stored at −70°C for up to 2 months without loss of enzyme activities.

2.3. Enzyme assays

Aryl sulfotransferase activity was assayed using [1-14C]1-naphthol by the method of Leakey et al. [22] with the following modifications. The 500 μl incubation mixture contained 1 mM [1-14C]1-naphthol (0.1 μCi/μmol), 50 or 100 μM PAPS and cell extract (100–500 μg of protein), and was incubated at 37°C for 15 min. The aqueous layer was washed twice with 2 ml chloroform. Control incubations contained either boiled cell extract or no PAPS. Portions of the washed aqueous phase either were counted by liquid scintillation counting or were further analyzed by TLC using silica-gel plates developed with isopropanol:chloroform:28% ammonia (70/30/15).

Glucuronosyltransferase and glucosyltransferase were assayed by the same procedure as that for aryl sulfotransferase, except that PAPS was replaced by 1 mM UDPGA or 1 mM UDPG, respectively. Radioactivity in the aqueous layer was analyzed by silica-gel TLC developed with isopropanol:chloroform:28% ammonia:water (70/30/18/7) and isopropanol:chloroform:28% ammonia (70/50/15), respectively.

Glutathione S-transferase was assayed with 1-chloro-2,4-dinitrobenzene as described by Habig et al. [23]. The reaction mixture contained 1 mM 1-chloro-2,4-dinitrobenzene, 1 mM reduced glutathione and 40–400 μg of protein in 1 ml of a phosphate buffer (pH 6.5). The reaction was monitored by measuring absorption at 340 nm.

In *N*-acetyltransferase assays, the substrates were either 1 mM procainamide or *p*-aminobenzoic acid with 1 mM acetyl coenzyme A. After incubation of the substrates with the enzyme fractions (1 mg) at 37°C for 30 min, the decrease of the amount of primary amine was assayed colorimetrically with sodium nitrite and *N*-1-naphthylethylenediamine [22]. Rat liver cytosol fractions (100000 × *g*) were used as positive controls [22].
Cyclobenzaprine hydroxylase and N-demethylase were assayed as described previously [8]. Reaction mixture contained 1.5–3.0 mg of protein, 1 mM NADPH and 1 mM cyclobenzaprine in a final volume of 0.5 ml. After incubation at 37°C for 2 h, aliquots of the reaction mixtures were analyzed by HPLC.

3. Results and discussion

Table 1 shows the phase I and phase II enzyme activities in the cell extracts of *C. elegans*. These enzymatic reactions are shown in Fig. 1. The glutathione S-transferase and aryl PAPS sulfotransferase had the highest activities among all enzymes as-
Table I
Activities of phase I and phase II enzymes in C. elegans

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Sp. act. (nmol min⁻¹ mg⁻¹) *</th>
<th>Cytosol</th>
<th>Microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPS sulfotransferase</td>
<td>15.0 ± 1.4</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Glutathione S-transferase</td>
<td>20.8 ± 1.7</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>UDP-glucosyltransferase</td>
<td>0.27 ± 0.11</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>UDP-glucuronosyltransferase</td>
<td>0.09 ± 0.05</td>
<td>0.13 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>N-Acetyltransferase</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>Cyclobenzaprine 2-hydroxylase</td>
<td>nd</td>
<td>0.15 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Cyclobenzaprine N-demethylase</td>
<td>nd</td>
<td>0.17 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

* The assays were done in triplicate.

The aryl PAPS sulfotransferase was a cytosolic protein with an activity of 15.0 nmol min⁻¹ mg⁻¹ at 37°C. The TLC analysis showed that the radioactive product exclusively comigrated with authentic 1-naphthyl sulfate. 1-Chloro-2,4-dinitro benzene glutathione S-transferase activity in fungi has not been demonstrated before. The bacterial sulfotransferase known from Klebsiella K-36 and Eubacterium A-44 [24,25] catalyzes a different reaction which is a sulfate transfer from one phenolic sulfate to another phenolic compound. Detection of cytosolic and microsomal UDP-glucuronosyltransferase activities suggests that there may be multiple isoforms of this fungal enzyme. Alternatively, the cytosolic UDP-glucosyltransferase may also exhibit UDP-glucuronosyltransferase activity. The glutathione S-transferase activity we observed was about 50% higher than that reported by Wackett and Gibson [14].

The carbon monoxide difference spectrum of the microsomal protein (Fig. 2) showed a peak at 450 nm, but the major peak is at 420 nm, suggesting that significant degradation of constitutive cytochrome P450 may have occurred. The microsomal enzymes, but not the cytosolic protein, oxidized cyclobenzaprine to produce 2-hydroxycyclobenzaprine and N-desmethylcyclobenzaprine (Fig. 3). The specific activities for these oxidation reactions were 0.15 and 0.17 nmol min⁻¹ mg⁻¹, respectively (Table I). Together with several other cytochrome P450 monooxygenase activities detected in the microsomal fractions of Cunninghamella spp. [8,15–19,26], these results suggest that the zygomycetes exhibit a wide range of oxidative reactions similar to those in mammalian systems [2].

In a screening study, we found that among 20 fungal species tested, Cunninghamella elegans had the most efficient phase I metabolism. In addition, a 100-fold lower aryl PAPS sulfotransferase activity and a 20-fold lower UDP-glucuronosyltransferase activity were detected in the cytosolic preparations of the plant pathogen Fusarium oxysporum var.

![Fig. 2. The reduced CO difference spectrum of C. elegans microsomes.](https://academic.oup.com/femsle/article-abstract/138/2-3/221/541045)
pini, and the white-rot fungus Phanerochaete chrysosporium, respectively. Other enzyme activities were either much lower or not detected in the cell-free extracts (unpublished data). This report provides initial identifications of the enzymes in C. elegans for xenobiotic metabolism including hydroxylation, N-demethylation, sulfation, glucuronidation, glycosylation, and glutathione conjugation. These fungal enzymes showed comparable levels of activity with those found in rat liver for metabolism of xenobiotics [22]. Thus, data were provided to support the zygomycete as a model for mammalian xenobiotic metabolism at the enzyme level.

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