

The metabolic activation of 2-aminofluorene, 4-aminobiphenyl, and benzidine by cytochrome P-450-107S1 of *Pseudomonas aeruginosa*

Piyatilake Adris, Carlos Lopez-Estraño, King-Thom Chung *

Department of Biology, The University of Memphis, 3774 Walker Street, Memphis, TN 38152, United States

Received 23 February 2007; accepted 11 July 2007

Available online 1 August 2007

Abstract

Pseudomonas aeruginosa is an important opportunistic pathogen of the human urinary bladder. Similar to rat liver S9, the cell-free extract from *P. aeruginosa* caused significant increase of histidine reversion numbers with the *Salmonella typhimurium* tester strain TA98 in the Ames *Salmonella* mutagenicity assay in the presence of either 2-aminofluorene, 4-aminobiphenyl, or benzidine procarcinogens. The presence of cytochrome P-450 protein in the cell-free extract was demonstrated by the carbon monoxide difference spectrum. We employed gene knockout technology to inactivate one of the three known putative cytochrome P-450 genes of *P. aeruginosa*, namely CYP107S1, which we postulated to be the most likely to induce activation. The ampicillin resistant gene from PUC19 DNA confers carbenicillin resistance to *P. aeruginosa*. We inserted a synthetic ampicillin gene flanked by 40 base-pairs of the 5' and 3' untranslated region of the CYP gene by electroporating the synthetic gene into electrocompetent *P. aeruginosa* cells. CYP107S1 knockout strains were selected on 1000 µg/ml carbenicillin plates. A single cloned carbenicillin resistant colony was isolated and used to determine its mutagenic capacity using Ames *Salmonella* mutagenicity assay. The results showed that *Salmonella* TA98 tester strain returned the number of revertants to its baselines level indicating the lack of metabolic activation of procarcinogens in the *P. aeruginosa* CYP107S1 knockout cell-free extract. In addition, the characteristic cytochrome P-450 peak determined by the carbon monoxide difference spectrum was completely absent in the cell-free extract from this CYP107S1 knockout strain bacterium. Homologous recombination of the synthetic ampicillin gene on the CYP 107S1 P-450 locus was confirmed by PCR on purified genomic DNA extracted from the knockout bacterium. The metabolic activation of tested procarcinogens is, therefore, carried out by CYP107S1 in *P. aeruginosa*.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: *Pseudomonas aeruginosa*; Metabolic activation; Cytochrome P-450; Procarcinogens

1. Introduction

Of numerous xenobiotics, benzidine (BZ), and 4-aminobiphenyl (4-AB) (Fig. 1) are very important because of their inevitable potentials to reach the human body and cause toxicity despite all human efforts to curb and control their use. Both BZ and 4-AB are known to be carcinogenic to humans and a variety of mammalian species (IARC, 1972, 1982; DHHS, 1991; Choudhary, 1996). Benzidine is known to cause human bladder cancer (IARC, 1982; DHHS, 1991; Choudhary, 1996). Although the manufac-

ture of BZ for sale has been banned in the United States since the mid-1970s, there are about 90 different kinds of azo dyes based on BZ and BZ congeners still in use in the market (Castroni, 1997; Witt, 1980). Large amounts of these dyes have been released into the environment and eventually get into the biological food chain (Leisinger and Brunner, 1986). Through oral ingestion from contaminated water and other sources, these dyes can be reduced to their constituent aromatic amines such as BZ by intestinal microbiota (Chung et al., 1992). Environmental microorganisms can also generate BZ from these azo dyes by azo reduction (Chung and Stevens, 1993). Structurally, 4-AB is similar to BZ being its monodeaminated derivative (Fig. 1) and is also a carcinogen (IARC, 1972). It is known to

* Corresponding author. Tel.: +1 901 678 4458; fax: +1 901 678 4457.
E-mail address: kchung@memphis.edu (K.-T. Chung).

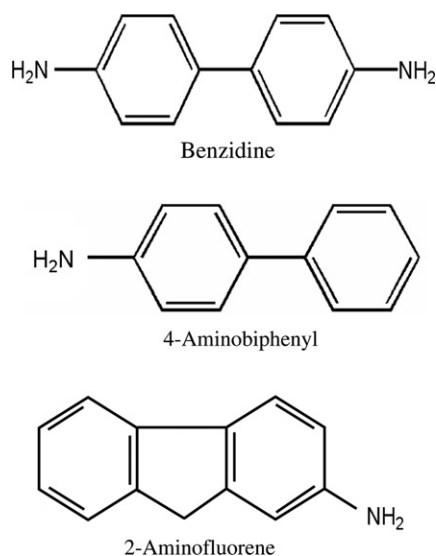


Fig. 1. The chemical structures of the procarcinogens used.

induce tumours in experimental animals (Clayson et al., 1967; Deichmann et al., 1965) and implicated in the causation of human bladder cancer (Gurein and Buchanan, 1988; Schulte et al., 1988; Vineis, 1994). This compound is released in cigarette smoke (Skipper and Tannenbaum, 1989; Palmiotto et al., 2001; Siegal, 1993), the burning of fossil fuels, in the chemical industry (Ambrosone et al., 1996; Melick et al., 1971), and in diesel exhaust fumes (Palmiotto et al., 2001). The inhalation route is important for entry of 4-AB into the human body (Skipper and Tannenbaum, 1989; Palmiotto et al., 2001; Siegal, 1993). It is known that cigarette smokers have an increased likelihood to develop lung cancer and cancer at other sites such as the breasts and bladder (Ambrosone et al., 1996). Hemoglobin and DNA-AB adducts have been detected in the blood of smokers (Melick et al., 1971; Bryant et al., 1988; Burger et al., 2001). These adducts are thought to be important in the pathogenesis of cancer. 4-Aminobiphenyl can also be metabolically generated from the deamination of BZ by the intestinal bacteria (Cerniglia et al., 1986; Chung, 2000).

2-Aminofluorene (2-AF) (Fig. 1) is a procarcinogen of experimental animals and causes bladder cancer in rats and rabbits (Irving, 1962; Uehleke, 1963). It is similar in structure to BZ and 4-AB and requires metabolic activation to become mutagenic (Irving, 1962; Uehleke, 1963).

It is generally recognized that the initial metabolic activation of aromatic amines is the conversion of these amines to *N*-hydroxylamines, which is reported to be accomplished by cytochrome P-450 isozymes. It has also been shown that the liver is the main site of hydroxylation (Irving, 1962; Kadlubar et al., 1977; Kato et al., 1983; Uehleke, 1963).

We have recently found that endogenous bacteria including the opportunistic pathogen *Pseudomonas aeruginosa* could metabolically activate the above-mentioned procarcinogens (Adris and Chung, 2006). The organism is known to occur in 24% of humans and is found mainly

in the gastrointestinal tract, skin surfaces, and mucous membranes of the oral cavity (Todar, 2005). *P. aeruginosa* is known to be an opportunistic pathogen of the human urinary bladder. NNIS (1999) in the United States reports that *P. aeruginosa* is responsible for 11% of urinary tract infections.

The genome of *P. aeruginosa* (PAO1) (Stover et al., 2000) has three cytochrome P-450 genes, namely CYP107S1, CYP168A1, and CYP169A1 (Nelson et al., 1996; Nelson, 2004). Little is known whether these genes are expressed and whether they can also carry out the oxidations. It is certain that at least one or more of these cytochrome proteins are expressed as we demonstrated the characteristic P-450 spectrum in the cell-free extract of *P. aeruginosa*. In order to examine whether cytochrome P-450 induces metabolic activation, we used gene knockout technology to inactivate one of the three known putative cytochrome P-450 genes of *P. aeruginosa*, namely CYP107S1. Based on sequence similarity this P-450 isoform conforms to the structure of closely related CYP families like CYP101, CYP108 (Nelson et al., 1996) or CYP111 (Ropp et al., 1993) that are known to hydroxylate aromatic xenobiotics. Our aim is to investigate whether the inactivation of the CYP107S1 gene would result in the abolition of metabolic activation of the procarcinogens by the absence of the gene product, the cytochrome P-450 protein, which we postulate to be the specific factor in the metabolic activation of the above mentioned procarcinogens in *P. aeruginosa*.

2. Material and methods

2.1. Chemicals

2-Aminofluorene (2-AF) (15-37-86), 4-aminobiphenyl (4-AB) (92-67-1), benzidine (BZ) (992-87-1), dimethyl sulfoxide (DMSO), nicotinamide adenine dinucleotide phosphate monosodium salt (NADP), D-glucose phosphate, [(Cholamidopropyl)dimethylammonio]-1-propane-sulfonate(CHAPS) and mutanolysin were obtained from Sigma Chemical Company (St. Louis, MO). 2-Aminofluorene, 4-AB, and BZ in DMSO were freshly prepared at proper concentrations.

Custom PCR primers were purchased from Invitrogen (Frederick, MD), PACYC Duet-1 vector from Novagen (Madison, WI), Taq polymerase, 1 kb DNA ladder, and dNTPs from New England Biolabs (Ipswich, MA). Carbenicillin disodium salt from Sigma-Aldrich (St. Louis, MO), *Eco*R1, PCR vector 2.1, PUC 19 DNA, bovine serum albumin, T4 ligase, calf intestinal phosphatase, Mg, and ampicillin were obtained from Invitrogen (Carlsbad, CA). QIAGEN DNA purification kits was also obtained (Valencia, CA).

2.2. Bacteria cultures

P. aeruginosa was provided by Dr. T.-Y. Wong, Department of Biology, Teaching Laboratory, The University of

Download English Version:

<https://daneshyari.com/en/article/2603726>

Download Persian Version:

<https://daneshyari.com/article/2603726>

[Daneshyari.com](https://daneshyari.com)