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Direct trapping of formaldehyde formed via oxidative *N*-demethylation of *N*,*N*-dialkylarylamines by *Bacillus megaterium* using cysteamine derivatization

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Abstract

Oxidative *N*-demethylation was measured by incubation experiments using *Bacillus megaterium* isolated from topsoil as a biocatalyst for the *N*-demethylation of the *N*,*N*-dialkylarylamines *N*,*N*-dimethylaniline and *N*-ethyl-*N*-methylaniline. Formed formaldehyde, normally difficult to analyse in biological systems because of further metabolization, was successfully trapped and converted into thiazolidine by addition of cysteamine into the incubation media. Studies using *N*,*N*-di-(trideutero-methyl)-aniline and *N*-ethyl-*N*-(trideuteromethyl)-aniline as well as *N*,*N*-di-[methyl-^{13}C]-aniline and *N*-ethyl-*N*-[methyl-^{13}C]-aniline were performed to confirm that the *N*-demethylation proceeds via formaldehyde.

Keywords: *Bacillus megaterium*; Cysteamine; Formaldehyde; Microbial *N*-demethylation; Thiazolidine

1. Introduction

Microbial *N*-demethylation is known to be conducted by species of filamentous fungi and bacteria such as *Bacillus megaterium* (Cha et al., 2001; Taupp et al., 2005). Oxidative *N*-dealkylation is also a common process in the metabolization of xenobiotics and pharmaceuticals (Guengerich, 2001). Thus, biotransformation of *N*,*N*-dialkylarylamines has been investigated in in-vitro and in-vivo studies for a long time (Kiese and Renner, 1974; Gorrod et al., 1975a,b; 1979; Gorrod and Gooderham, 1981). Main metabolites were the corresponding *N*-oxides, *N*-dealkylated products and, using *N*,*N*-dimethylaniline (Ia) as substrate, formaldehyde (Willi and Bickel, 1973; Pandey et al., 1989). Formaldehyde, however, is difficult to analyze and quantify. A main problem is the known difficulty to extract this aldehyde with organic solvents from aqueous solutions usually occurring in biological processes. Another problem is the low boiling point (bp −19 °C) and the occurrence of formaldehyde as *para*-formaldehyde or even as a polymer molecule (Hayashi et al., 1985). In vivo, formaldehyde is further metabolized to formic acid and, ultimately, to carbon dioxide (Savenije-Chapel and Hordhoek, 1980).

Most commonly, formaldehyde is determined by colorimetry after derivatization to a coloured compound (Nash, 1953; Miksch et al., 1981; Georghiou et al., 1983); however, a major drawback is that many other compounds can interfere. Other methods involve

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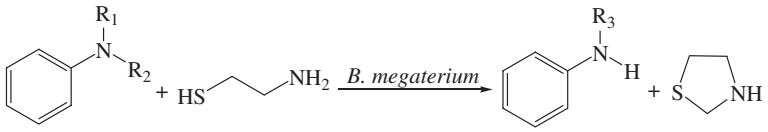

Fig. 1. *N*-Demethylation of *N*,*N*-dimethylaniline and *N*-ethyl-*N*-methylaniline by the isolated topsoil microorganism *Bacillus megaterium* (scheme). The formed formaldehyde is immediately trapped by addition of 2-aminoethanethiol (cysteamine) to form thiazolidine (III). $R_1=R_2=CH_3$: *N*,*N*-dimethylaniline (Ia); $R_3=CH_3$: *N*-methylaniline (Ib); $R_1=C_2H_5$, $R_2=CH_3$: *N*-ethyl-*N*-methylaniline (IIa); $R_3=C_2H_5$: *N*-ethylaniline (IIb).

derivatization with 2,4-dinitrophenylhydrazine (Van Hoof et al., 1985; Smith et al., 1989), *N*-benzylethanolamine (Kennedy and Hill, 1982) or *O*-(2,3,4,5,6-pentafluorobenzyl)oxyamine (Kobayashi et al., 1980; Le Lacheur et al., 1993). However, these methods require strong acidic and high-temperature conditions, which may cause undesirable reactions such as decomposition of carbohydrate, lipid and protein, and cannot be applied in biological systems (Yasuhara et al., 1998).

Unlike the other derivatization methods, formaldehyde reacts readily with cysteamine under mild conditions (room temperature and neutral pH) to form thiazolidine, which is much more stable and much less volatile (bp 164 °C) than formaldehyde, but is sufficiently volatile for gas chromatographic analysis (Hayashi et al., 1986). Analysis and quantification of aldehydes as thiazolidine derivatives have been performed previously in food such as coffee, overheated beef fat and in wine, brandy and sherry (Umano and Shibamoto, 1987; Lau et al., 1999). In addition, the technique has been used recently to measure atmospheric formaldehyde (Yu et al., 2005).

Measurement of formaldehyde as a product of oxidative *N*-demethylation, i.e., of *N*,*N*-dialkylarylamines containing at least one *N*-methyl group, using the mentioned cysteamine derivatization in a biological system, is not known to date. The procedure offers an easy to handle method under mild conditions trapping formaldehyde prior to further metabolization. A reaction scheme is outlined in Fig. 1.

2. Materials and methods

2.1. Chemicals

N,*N*-Dimethylaniline (Ia) was purchased from Merck (Darmstadt, Germany). *N*-Ethyl-*N*-methylaniline (IIa) and thiazolidine (III) as well as iodomethane-^{13}C and iodomethane-d_3 were from Aldrich (Taufkirchen, Germany). Cysteamine was obtained from Fluka (Deisenhofen, Germany). Organic solvents used for synthesis and extraction were distilled before use. Silica gel (Fluka, 60–100 mesh) was used for column chromatography. Thin-layer chromatography was performed on precoated silica gel $60F_{254}$ TLC plates from Merck (Darmstadt, Germany).

2.2. Microorganisms

B. megaterium was isolated from topsoil in a screening process and characterized by 16S rDNA and DNA/DNA hybridization against the *B. megaterium* type strain ATCC 14581 as recently described (Taupp et al., 2005). The isolated *B. megaterium* strain was deposited in the BCCM™/LMG, Bacteria Collection, Laboratorium voor Microbiologie, Universiteit Gent (RUG), B-9000 Gent, Belgium under the LMG number 23147.

2.3. Gaschromatography–mass spectrometry (GC-MS)

GC-MS analysis was performed using an Agilent 6890 gas chromatograph with split injector (1:20) coupled to an Agilent 5973 mass selective detector and an Agilent 7683 automatic liquid sampler. The system was equipped with the Chem Station software. A J&W DB Wax fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) was employed. The temperature program was 3 min isothermal at 50 °C and then increased with 4 °C/min to 220 °C using 1.0 ml/min helium constant flow. The MS operating values were as follows: ionization voltage 70 eV (electron impact ionization), ion source and interface temperatures 230 °C. Bacterial metabolites were identified by comparison with authentic reference compounds regarding retention times and mass spectra. Quantification was done using calibration curves of *N*-methylaniline, *N*-ethylaniline and thiazolidine.

2.4. Substrate acceptability tests

Liquid minimal media (75 ml) was prepared according to Dworkin and Foster (1958); 5 ml/l of a *Pseudomonas*-trace element solution were added and autoclaved at 121 °C for 16 min (Atlas, 1993). The culture was maintained under sterile conditions during