Enzyme-mediated dichloromethane toxicity and mutagenicity of bacterial and mammalian dichloromethane-active glutathione S-transferases

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Abstract The kinetic properties of bacterial and rat liver glutathione S-transferases (GST) active with dichloromethane (DCM) were compared. The theta class glutathione S-transferase (rGSTT1-1) from rat liver had an affinity for dihalomethanes lower by three orders of magnitude ($K_{\text{app}} > 50 \text{ mM}$) than the bacterial DCM dehalogenase/GST from Methylophilus sp. DM11. Unlike the bacterial DCM dehalogenase, the rat enzyme was unable to support growth of the dehalogenase minus Methylobacterium sp. DM4-2 cr mutant with DCM. Moreover, the presence of DCM inhibited growth with methanol of the DM4-2 cr transconjugant expressing the rat liver GSTT1-1. In Salmonella typhimurium TA1535, expression of rat and bacterial DCM-active GST from a plasmid in the presence of DCM yielded up to 5.3 times more reversions to histidine prototrophy in the transconjugant expressing the rat enzyme. Under the same conditions, however, GST-mediated conversion of DCM to formaldehyde was lower in cell-free extracts of the transconjugant expressing the rat GSTT1 than in the corresponding strain expressing the bacterial DCM dehalogenase. This provided new evidence that formaldehyde was not the main toxicant associated with GST-mediated DCM conversion, and indicated that an intermediate in the transformation of DCM by GST, presumably S-chloromethylglutathione, was responsible for the observed effects. The marked differences in substrate affinity of rat and bacterial DCM-active GST, as well as in the toxicity and genotoxicity associated with expression of these enzymes in bacteria, suggest that bacterial DCM dehalogenases/GST have evolved to minimise the toxic effects associated with glutathione-mediated catalysis of DCM conversion.

Key words Dichloromethane · Dichloromethane dehalogenase · Formaldehyde · Glutathione S-transferase · Bacterial genotoxicity

Introduction

Dichloromethane (DCM) is widely used as an industrial solvent (Howard 1990). DCM has been the subject of extensive toxicological studies because of its demonstrated tumorigenicity in mouse lung and liver (Green 1997), and of its suspected carcinogenicity in human erythrocytes, liver and kidney (Thier et al. 1998). Such tumours are believed to result from DCM conversion to formaldehyde by glutathione S-transferases (GST), via a short-lived glutathione conjugate, S-chloromethylglutathione (reviewed in Green 1997). The genotoxic potential of formaldehyde is well documented (O'Donovan and Mee 1993; Graves et al. 1996; Casanova et al. 1997). S-chloromethylglutathione, a known DNA-alkylating agent (Dechert 1995; Green 1997), is most probably also mutagenic (Graves et al. 1994, 1996).

Mammalian GST enzymes active with DCM (GSTT1-1) have been purified from rat (Meyer et al. 1991), mouse (Mainwaring et al. 1996a) and human (Schröder et al. 1996), and the corresponding genes were cloned and sequenced (Pemble et al. 1994; Mainwaring et al. 1996b; Schröder et al. 1996). Dihalomethane-specific GST, so-called dichloromethane dehalogenases, had been characterised previously in methylotrophic bacteria growing with DCM as the sole carbon source (reviewed in Leisinger et al. 1994). The gene encoding these enzymes was characterised in Methylobacterium sp. DM4 (La Roche and Leisinger 1990) and in Methylophilus sp. DM11 (Bader and Leisinger 1994). Bacterial dichloromethane dehalogenases/GST are thought to transform DCM by the same mechanism as the mammalian DCM-active GST enzymes (Blocki et al. 1994). Bacterial and mammalian DCM-active GST share ~25% sequence identity at the protein level, while the mammalian sequences are ~80% identical to one
another. DM4 and DM11 DCM dehalogenases are most closely related to each other in sequence databases (56% amino acid identity), suggesting that bacterial dihalomethane-specific GST diverged from a common precursor hundreds of millions of years ago already (Bader and Leisinger 1994). Even though DCM may have appeared in large quantities on earth only since industrial times, sufficient amounts may have been generated naturally to exert selective pressure for the evolution of DCM-specific GST. Production of DCM in volcanic eruptions has been postulated (Isidorov 1990), and other plausible mechanisms for the synthesis of DCM by natural processes have recently been reviewed (Urhanh and Ballschmitter 1998). Alternatively, DCM dehalogenases may have originally evolved to degrade other naturally occurring substrates structurally related to DCM, such as dibromomethane (Goodwin et al. 1997) or methyl chloride (Harper 1997).

There is an ongoing debate on the relevance of the mouse model for assessing DCM tumorigenicity in humans. Rats and hamsters, unlike mice, do not form liver tumours when exposed to DCM (Mainwaring et al. 1996b, 1998; Green 1997; Liteplo et al. 1998). DCM-active GST appeared to be predominantly located in the nucleus in the mouse but not in the rat (Mainwaring et al. 1996b, 1998), although recent data indicate that the cellular location of the GSTT1-1 enzyme may depend on the conditions that induce its expression (Sherratt et al. 1998). Bacteria, lacking a nuclear compartment would also be expected to be highly susceptible to DCM turnover by DCM-active GST enzymes. Hence, DCM exposure of Salmonella sp. Ames tester strains expressing rat liver GSTT1-1 as intracellular enzyme from plasmids had marked mutagenic effects (Thier et al. 1993; Oda et al. 1996).

These studies prompted us to investigate the toxicity and mutagenicity associated with DCM turnover by dichloromethane dehalogenase/GST during growth of methylotrophic bacteria with DCM as the sole carbon source. In this work, we compare the enzymatic properties of the bacterial DCM dehalogenase from Methylphilus sp. DM11 (Vuilleumier and Leisinger 1996) and of rat liver GSTT1-1 (Meyer et al. 1991), and the effects of the expression of these two enzymes in bacteria exposed to DCM.

Materials and methods

Reagents

Restriction and DNA-modifying enzymes were purchased from Fermentas (Maechler, Basel, Switzerland) if not noted otherwise. Oligonucleotides were purchased from Microsynth (Balgach, Switzerland). All other chemicals were analytical grade and were purchased from Fluka (Buchs, Switzerland) except where noted.

Bacterial strains

Escherichia coli XL1-Blue (Stratagene) was used for cloning, plasmid amplification, and expression of rat liver GSTT1-1, and E. coli BL21(DE3)pLysS for expression of bacterial DM11 dehalogenase from plasmid pME1919 (Vuilleumier and Leisinger 1996). Transconjugants of Methyllobacterium sp. DM4-2cr (La Roche and Leisinger 1990) were constructed by biparental mating using E. coli S17-1 (Simon et al. 1983) as the donor. Transconjugants of Salmonella typhimurium TA1535 (Maron and Ames 1983) were obtained by heat shock transformation with plasmids isolated from S. typhimurium LB5000 (Bullas and Ryu 1983) as the intermediate host. The identity of all new strains was verified by plating on selective media, polymerase chain reaction (PCR) amplification of the dcmA and rGSTT1 genes, and by measuring DCM dehalogenation in cell-free extracts as described below.

Molecular cloning

Standard recombinant DNA techniques were used (Ausubel et al. 1998). The broad host range plasmid pME1685 and the corresponding transconjugant strain Methyllobacterium sp. DM4-2cr(pME1685), designated here DM4-2cr(DM11), were described previously (Gisi et al. 1998). This transconjugant strain allowed the constitutive expression of the DCM dehalogenase dcmA gene of Methylphilus sp. DM11 in Methyllobacterium sp. DM4-2cr. Plasmid pME1690, a plasmid otherwise identical to pME1685 but carrying rGSTT1, the gene for the rat liver GSTT1-1 (Pemble and Taylor 1992), in place of the DM11 DCM dehalogenase, was constructed as follows. The HindIII site downstream of rGSTT1 in pKK233-2-GST5(+) (Thier et al. 1993) was removed and a BamH1 site inserted by digestion with HindIII, end-filling with Klenow fragment and subsequent insertion of a BamH1 linker. A 750 bp fragment carrying rGSTT1 was excised from the resulting plasmid by digestion with NcoI and mung bean nuclease (Boehringer Mannheim), followed by partial digestion with BamHI. The P2 promoter of the dcmA gene of Methyllobacterium sp. DM4 was PCR-amplified from plasmid pME1540 (Gisi et al. 1998) using the T3 universal primer ATTAACCTCTACTAAGG and primer CACGTATTCTCCTCATGCT. The resulting PCR product was treated with T4 DNA polymerase, cut with HindIII, and ligated together with the blunt-BamHI rGSTT1 fragment into the BamHI- and HindIII-restricted pBluescript-KSII(+) (Stratagene) derivative pME1673 previously used in the cloning of dcmA genes (Gisi et al. 1998), yielding plasmid pME1688. The fragment containing rGSTT1 obtained by sequential digestion of plasmid pME1688 with BspI1201, Klenow treatment and XbaI digestion was next ligated into the broad host range plasmid pJB3Km1 (Blatny et al. 1997) which had been sequentially cut with SpfI, treated with Klenow fragment and digested with XbaI, yielding plasmid pME1690. The transconjugant strain Methyllobacterium sp. DM4-2cr(pME1690) will hitherto be referred to as DM4-2cr(GSTT1) for simplicity.

Four derivatives of the expression vector pTrc99A (Pharmacia) were constructed, which featured either the DCM dehalogenase gene dcmA from Methylphilus sp. DM11 or rGSTT1 in both sense and antisense orientations behind the isopropyl-β-D-thiogalactoside (IPTG)-inducible Pev promoter. The dcmA gene from Methylphilus sp. DM11 was PCR-amplified with primers AGAGAAATTCCATGGGATCTAAGC and GACCATATTACGGTATCTTTCC containing NcoI and BamHI sites, respectively, and inserted as NcoI-BamHI fragment into pTrc99A, yielding plasmid pME1983. The latter encodes the S2G mutant of the DM11 DCM dehalogenase gene behind the Pev promoter of expression vector pTrc99A. The corresponding transconjugant strain was designated TA1535(DM11 +). Insertion of the NcoI-HindIII rGSTT1 fragment from pKK233-2-GST5(+) (Thier et al. 1993) yielding plasmid pME1895, and the corresponding strain TA1535(GSTT1 +). Control plasmids with dcmA and rGSTT1 genes in the antisense orientation behind the Pev promoter of vector pTrc99A were obtained by ligating the GST genes fragments from the (+) plasmids as NcoI-digested, T4 DNA polymerase-treated, and HindIII-digested fragments with DNA fragments from plasmids pME1983 and pME1895, which had been successively digested with PstI, treated with T4 DNA polymerase and cut with HindIII. The resulting control plasmids pME1984 and