Short communication

Characterization of microsomal glutathione S-transferases MGST1, MGST2, and MGST3 in cynomolgus macaque

Yasuhiro Uno, Norie Murayama, Mutsuki Kunori, and Hiroshi Yamazaki
Pharmacokinetics and Bioanalysis Center, Shin Nippon Biomedical Laboratories, Ltd., Kainan, Japan (Y.U.); Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University (N.M., M.K., H.Y.).
Running Title Page

Running title: characterization of cynomolgus MGSTs

Corresponding Author: Yasuhiro Uno, D.V.M., Ph.D.
Shin Nippon Biomedical Laboratories, Ltd., Pharmacokinetics and Bioanalysis Center, 16-1 Minami Akasaka, Kainan 642-0017, Japan
Phone: +81-73-483-8881. Fax: +81-73-483-7377. E-mail address: uno-yasuhiro@snbl.co.jp

Number of Text Pages: 17
Number of Tables: 2
Number of Figures: 2
Number of References: 13
Number of Words in Abstract: 248
Number of Words in Introduction: 408
Number of Words in Results and Discussion: 1010

Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; EPNPP, 1,2-epoxy-3-(p-nitrophenoxy)-propane; GST, glutathione S-transferase; MGST, microsomal GST; PCR, polymerase chain reaction; RT, reverse transcription.
Abstract

The glutathione S-transferase (GST) family is comprised of cytosolic, mitochondrial, and microsomal GSTs, essential drug-metabolizing enzymes metabolizing a wide range of endogenous and exogenous substrates. Among the microsomal GSTs in human, MGST1, MGST2, and MGST3 are involved in detoxification; however, MGSTs have not been fully investigated in cynomolgus macaque, an important primate species widely used in drug metabolism and toxicity studies. In the present study, cynomolgus MGST2 and MGST3 cDNAs were isolated from liver, and characterized along with previously isolated cynomolgus MGST1. For comparison with the cynomolgus cDNAs, MGST2 and MGST3 cDNAs were also isolated from rhesus macaque liver (closely related species to cynomolgus macaque). Cynomolgus MGST2 and MGST3, respectively, were highly identical (99% and 98%) to human MGST2 and MGST3 and identical to the amino acid sequences of the rhesus orthologs, and were closely clustered with human MGST2 and MGST3 by phylogenetic analysis. The analysis of genome data indicated that MGST1, MGST2, and MGST3, respectively, had similar gene structures and genomic organization in macaque and human. Therefore, cynomolgus MGSTs have molecular similarities to the corresponding human MGSTs. Cynomolgus MGST2 and MGST3 were expressed in liver, jejunum, and kidney, but at lower levels than MGST1. GST activities were measured with 1-chloro-2,4-dinitrobenzene and 1,2-epoxy-3-(p-nitrophenoxy)propane as substrates, using proteins heterologously expressed in Escherichia coli. Cynomolgus MGST1, MGST2, and MGST3 conjugated 1-chloro-2,4-dinitrobenzene and 1,2-epoxy-3-(p-nitrophenoxy)propane, indicating that cynomolgus MGST1, MGST2, and MGST3 are functional enzymes. These results suggest that these functional cynomolgus MGST enzymes and the corresponding human MGSTs are molecularly similar.
Introduction

Glutathione S-transferase (GST) catalyzes the conjugation of reduced glutathione to electrophilic substrates (e.g. halogenonitrobenzenes, arene oxides, and quinones), and some GST enzymes also exhibit peroxidase and isomerase activities (Hayes et al., 2005). The GST superfamily contains at least 16 members in human, comprised of cytosolic, mitochondrial, and microsomal GSTs (Hayes et al., 2005). The cytosolic and mitochondrial GSTs primarily metabolize foreign chemicals, such as drugs and environmental pollutants, and also play roles in the detoxification of reactive compounds (Hayes and Pulford, 1995).

In human, microsomal GSTs constitute a superfamily called MAPEG (membrane-associated proteins in eicosanoid and glutathione metabolism). Of the six MAPEG members, 5-lipoxygenase activating protein, leukotriene C4 synthase, and microsomal prostaglandin E2 synthase 1 are largely involved in the production of leukotriene C4 and prostaglandin E2, which are essential for fever, pain, and inflammation, and do not contribute to drug metabolism or detoxification (Hayes et al., 2005). In contrast, MGST1, MGST2, and MGST3 play roles in detoxification of foreign compounds, and MGST2 and MGST3 are also capable of synthesizing leukotriene C4 (Hayes et al., 2005). Of the three human GSTs, MGST1, MGST2, and MGST3, MGST1 is most abundant in liver, and metabolizes hydrophobic substrates, such as phospholipid hydroperoxides and halogenated hydrocarbons (Morgenstern et al., 2011).

Cynomolgus macaque (Macaca fascicularis) is an important primate species in drug metabolism studies. A number of cytochromes P450 (P450s) have been identified and characterized in cynomolgus macaque, leading to the notion that the molecular properties of P450 enzymes in cynomolgus macaque and human are generally similar (Uno et al., 2011). The exception is CYP2C76, which is not orthologous to any human P450 and is partly responsible for species differences in drug metabolism between cynomolgus macaque and human (Uno et al., 2010). Therefore, characterization of drug-metabolizing enzymes corresponding to those essential for drug metabolism in human helps to better understand the similarities and differences of drug metabolism.
between model animals and humans. MGST1 cDNA has been identified in cynomolgus macaque, and the gene was shown to be expressed in liver, kidney, jejunum, and adrenal gland (Uno et al., 2009); however, the enzymatic properties of MGST1 have not been investigated.

In this study, cDNAs encoding MGST2 and MGST3 were isolated using total RNA extracted from cynomolgus macaque liver. These MGSTs, and previously identified MGST1, were characterized by sequence and phylogenetic analyses, quantitative polymerase chain reaction (qPCR) analysis for tissue expression patterns, and enzymatic analysis using proteins heterologously expressed in Escherichia coli. (E. coli).

Materials and Methods

Materials

1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-epoxy-3-\((p\)-nitrophenoxy)propane (EPNPP) were purchased from Wako Pure Chemicals (Osaka, Japan). Oligonucleotides were synthesized by Greiner (Tokyo, Japan), and TaqMan probes were synthesized by Biosearch Technology Japan (Tokyo, Japan). Anti-histidine tag antibody and horseradish peroxidase-conjugated goat anti-rabbit IgG were purchased from BioDynamics Lab. (Tokyo, Japan) and BD Gentest (Woburn, MA), respectively. All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) or Wako Pure Chemicals, unless otherwise specified.

Tissues and preparation of RNA and microsomes

Tissue samples, including brain, lung, heart, liver, kidney, adrenal gland, jejunum, testis, ovary, and uterus, were collected from six cynomolgus macaques (three males and three females from Indochina, 4-5 years of age, 3-5 kg), from which total RNA was extracted, as previously described (Uno et al., 2006). A pool of total RNA samples was used for qPCR analysis of each tissue type. Total RNA extracted from liver samples of six cynomolgus macaques (three males and three females...
from Cambodia, 4-5 years of age, 3-5 kg) and three rhesus macaques (three males from China, 7 years of age, weighing 3-5 kg) were used to isolate MGST cDNAs. This study was reviewed and approved by the Institutional Animal Care and Use Committee of Shin Nippon Biomedical Laboratories, Ltd.

**Molecular cloning**

Reverse transcription (RT)-PCR was performed as previously described (Uno et al., 2006) using total RNA extracted from cynomolgus and rhesus macaque livers. Briefly, RT was performed in a reaction containing 1 μg of total RNA, oligo (dT), and SuperScript III RT reverse transcriptase (Invitrogen, Tokyo, Japan) at 50°C for 1 h. The synthesized cDNAs were subsequently used as the templates for PCR reactions, which were performed using KOD-Plus-Neo DNA polymerase (Toyobo, Osaka, Japan) with a thermal cycler (Applied Biosystems, Tokyo, Japan) according to the manufacturer’s protocols. Thermal cycler conditions included an initial denaturation at 94°C for 2 min and 35 cycles at 98°C for 10 s, 65°C for 30 s, and 68°C for 30 s, followed by a final extension at 68°C for 5 min. The primers used were 5′-CGTTCTACAAATAGTTCCGTGAGAAAGATG-3′ and 5′-TTAGAATTGCCGCCTCAGTTTCTTG-3′ for MGST2; 5′-GCAAGATGGCTGTCCTCTAAGG-3′ and 5′-CTAAAATTCTTTATGGCAGCATTTGGA-3′ for MGST3. The amplified PCR products were cloned into pCR4 vectors using Zero Blunt TOPO PCR Cloning Kit for Sequencing (Invitrogen) according to the manufacturer’s instructions. The inserts were sequenced using ABI PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) with an ABI PRISM 3730 DNA Analyzer (Applied Biosystems).

**Sequence analysis**

Sequence data were analyzed with DNASIS Pro (Hitachi Software, Tokyo, Japan), the Genetyx system (Software Development, Tokyo, Japan), and Sequencher (Gene Codes Corporation, Ann Arbor, MI). A phylogenetic tree was created by the neighbour-joining method. BLAST (National Center for Biotechnology Information) was used for the homology search, and BLAT (UCSC Genome
Bioinformatics) was used for the analysis of the human and macaque (rhesus) genome data. Amino acid sequences found in GenBank were used in this analysis, including human GSTA1 (NP_665683), MGST1 (NP_001247440), MGST2 (NP_002404), and MGST3 (NP_004519); cynomolgus MGST1 (ABO21637); rat MGST1 (NP_599176), MGST2 (NP_001099900), and MGST3 (NP_001178523); and mouse Mgst1 (NP_064330), Mgst2 (NP_778160), and Mgst3 (NP_079845). The amino acid sequence of rhesus MGST1 was deduced from a cDNA sequence (CO583554) highly identical to cynomolgus MGST1 cDNA. The amino acid sequences of cynomolgus and rhesus MGST2 and MGST3 were deduced from the cDNAs identified in this study. For analysis of gene structure and genomic organization, the cynomolgus MGST2 and MGST3 cDNAs identified in this study were used. The MGST cDNA sequences found in GenBank, were also used, including human MGST1 (NM_020300), MGST2 (NM_002413), and MGST3 (NM_004528); and cynomolgus MGST1 (EF104260).

**qPCR analysis**

Expression of cynomolgus MGST2 and MGST3 was quantified by real-time RT-PCR in brain, lung, heart, liver, kidney, adrenal gland, jejunum, testis, ovary, and uterus, as described previously, by using gene-specific primers and probes. Briefly, RT reactions were carried out as described above using random primers (Invitrogen), and one twenty-fifth of the reaction was used for the subsequent PCR. PCR amplification was conducted in a total volume of 20 μl using TaqMan Universal PCR Master Mix (Applied Biosystems) with the ABI PRISM 7500 sequence detection system (Applied Biosystems), according to the manufacturer’s protocols. The primers and the probe were used at a final concentration of 300 nM and 200 nM, respectively. The primers used were

5′-TGGAAAGGCAAGATTAAATAACAAAG-3′ and
5′-AGACAAGTAGCAAAAACTTGGTTGA-3′ for MGST2; 5′-CACCAGAACACGTTGGAAGT-3′ and 5′-TACGTTTGCTGGGTTCTCCT-3′ for MGST3. The probes used were

5′-FAM-TCGGGCCACAAACACTGTTGAGT-BHQ1-3′ for MGST2 and
5′-FAM-AGGTGTATCCACCCCCGGTATAGCTTCC-BHQ1-3′ for MGST3. To determine the
relative expression levels, the raw data were normalized to the 18S ribosomal RNA level. For each
tissue type, value is expressed as the average ± S.D. of three independent amplifications. *MGST1*
expression was also measured, as previously described (Uno et al., 2009), for comparison with
*MGST2* and *MGST3*.

**Heterologous expression of proteins in *Escherichia coli*.**

Expression plasmids were generated and cynomolgus MGST1, MGST2, and MGST3 proteins
were expressed in *E. coli*. To prepare expression plasmids, PCR was performed as described above
using each MGST cDNA as a template. To facilitate subcloning of the PCR products into vectors,
reverse primers containing the restriction enzyme site sequence for (XhoI) were used. The forward
primers used were; 5′-ATGGTTGACCTCACCCAGATAATGGA-3′ for MGST1,
5′-ATGGCCGGGAACACTCGATCCT-3′ for MGST2, and 5′-ATGTTCCAAGGCCGCAAGA-3′ for
MGST3. The reverse primers used were;
5′-CCGCTCGAGCTTTACAGGTACAATTTATTTTTCAGCAACCT-3′ for MGST1,
5′-CCGCTCGAGTTAGAATTGCCGCCTCAGTTTCTTG-3′ for MGST2, and
5′-CCGCTCGAGCTAAAATTCTTTAATGGCAGCATTTGGA-3′ for MGST3. PCR products were
digested using restriction enzymes (EcoRV and XhoI), and were subcloned into a pET30a vector
(Novagen, Madison, WI), which provides an N-terminus 6xHis-tag. Proteins were expressed in
*Escherichia coli* using the generated expression plasmids according to the manufacturer’s protocols.

Proteins were expressed in *Escherichia coli* using the expression plasmids according to the
manufacturer’s instructions. Briefly, starter Luria-Bertani medium/kanamycin (30 µg/mL) cultures
inoculated with single transformed colonies of *E. coli* strain BL21 were incubated, with continuous
shaking at 180 rpm, at 37°C for 12 h, and were diluted (1:100) into Terrific-Broth/kanamycin (30
µg/mL) medium. After incubation for 4 h, 0.4 mM isopropyl-D-thiogalactoside was added to the
cultures; and, incubation was continued at 25°C for 16 h. After harvesting the bacterial cells,
bacterial microsomal fractions were prepared by a series of fractionation and high-speed
centrifugation steps (Guengerich et al., 1996). Expressed MGST proteins were electrophoresed and
visualized on sodium dodecyl sulfate-polyacrylamide gels, and were quantified by immunoblotting using anti-histidine tag antibody.

**Enzyme assays**

The ability of cynomolgus MGST proteins to catalyze GST conjugation reactions were analyzed using typical GST substrates (CDNB and EPNPP) as described previously (Habig et al., 1974). Briefly, a typical reaction mixture (1.0 mL) contained bacterial microsomes expressing recombinant MGST protein, 5.0 mM GSH, and substrate (1.0 mM CDNB or 0.50 mM EPNPP) in 0.10 mM potassium phosphate buffer (pH 6.5). The product formation rates were recorded at 340 and 360 nm for CDNB and EPNPP at 25°C for 5 min in a spectrophotometer (Hitachi U-3000, Tokyo, Japan) and were calculated with the coefficients of 9.6 and 0.5 mM−1cm−1, respectively.

**Results and Discussion**

RT-PCR was used to successfully isolate MGST2 and MGST3 cDNAs from cynomolgus macaque liver, and for comparisons, rhesus macaque liver (a species closely related to cynomolgus macaque). Cynomolgus MGST2 and MGST3 cDNAs contained open reading frames of 147 and 152 amino acid residues, respectively, and shared high amino acid sequence identities (98-99%) with human MGST2 and MGST3, respectively, both of which were higher than with MGST1 (Table 1). Cynomolgus MGST2 and MGST3 amino acid sequences were identical to the corresponding rhesus orthologs. A phylogenetic analysis of human, cynomolgus, rhesus, rat, and mouse amino acid sequences indicated that cynomolgus MGST1, MGST2, and MGST3, together with the rhesus orthologs, were most closely clustered with the corresponding human orthologs, respectively (Fig. 1). Rat MGST1, MGST2, and MGST3, and the corresponding mouse orthologs were most closely clustered, respectively. The cDNA sequences identified in this study, including cynomolgus MGST2 (KC734513) and MGST3 (KC734515), and rhesus MGST2 (KC734514) and MGST3 (KC734516) were deposited into GenBank.
To determine exon-intron structures and genomic organization, the human and (rhesus) macaque genomes were analyzed using BLAT. The first exon (non-coding) of macaque MGST3 was predicted from the human MGST3 sequence because the identified cynomolgus/rhesus MGST cDNAs contained only the coding sequence. Among the macaque MGSTs analyzed, the largest was MGST2 (>37 kb), followed by MGST3 and MGST1, similar to human (Supplemental Fig. 1). Macaque MGST1, MGST2, and MGST3 contained 4, 5, and 6 exons, respectively, similar to human (Supplemental Fig. 1). Other than the first and last exons, whose sizes vary due to inclusion of non-coding sequences, the sizes of the MGST1, MGST2, and MGST3 exons were the same in macaque and human, ranging from 58 bp to 124 bp, except for exon 2 of MGST1 (158 bp in macaque and 148 bp in human). This discrepancy might be attributable to the use of a splice acceptor site (AG) in macaque MGST1, 10 bp upstream of that of human MGST1. The corresponding site in human MGST1 was AA, and thus would not readily serve as a splice acceptor site. Several human MGST1 transcript variants, due to alternative exon 1 sequences, were found in GenBank. Although such MGST1 transcript variants have not been identified in cynomolgus/rhesus macaque, sequences highly identical to the exon 1 sequences of those human MGST1 variants were found in the macaque genome. Therefore, the MGST1 transcripts with various exon 1 sequence sizes might also exist in macaque. Virtually all introns begin with the dinucleotide GU and end with AG, consistent with the consensus sequences for splice junctions in eukaryotic genes. Moreover, MGST1, MGST2, and MGST3 were located in corresponding regions of the macaque and human genomes in the same directions, respectively (Supplemental Fig. 2). These results suggest similarities between macaque and human MGST gene structure and genomic organization.

qPCR analysis was performed to measure expression of cynomolgus MGST2 and MGST3 in brain, lung, heart, liver, kidney, adrenal gland, jejunum, testis, ovary, and uterus. Cynomolgus MGST2 was expressed most abundantly in jejunum, followed by adrenal gland and kidney, while MGST3 was expressed most abundantly in heart, followed by jejunum and kidney (Fig. 2A). Similarly, in human, MGST2 is expressed in liver and small intestine (Jakobsson et al., 1996), and MGST3 is expressed in heart (Jakobsson et al., 1997). Cynomolgus MGST1 is expressed in liver,
jejenum, and kidney (Uno et al., 2009), similar to human MGST1 (Estonius et al., 1999). The results indicate similarities between cynomolgus macaque and human MGST tissue expression patterns.

Expression levels of MGSTs identified in this study were compared with the expression level of the previously identified cynomolgus MGST1. In liver, cynomolgus MGST1 was abundantly expressed, whereas the expression of MGST2 and MGST3 was negligible (Fig. 2B). Similarly, in jejunum and kidney, there was much higher expression of cynomolgus MGST1 than MGST2 or MGST3 (Fig. 2B). Therefore, among the cynomolgus MGSTs, cynomolgus MGST1 was expressed most abundantly in liver, jejunum, and kidney. In human, MGST1 is also abundantly expressed in liver (Morgenstern et al., 2011). In contrast, cynomolgus MGST3 was expressed most abundantly in brain and heart (Fig. 2B). Similarly, expression of human MGST3 was detected in these tissues, and in heart appears to be abundant (Jakobsson et al., 1997).

The ability of cynomolgus MGST1, MGST2, and MGST3 to catalyze conjugation reactions of typical GST substrates, CDNB and EPNPP, was analyzed using proteins heterologously expressed in E. coli. All cynomolgus MGSTs catalyzed the conjugation of CDNB and EPNPP (Table 2), indicating that all three MGSTs were functional enzymes in cynomolgus macaque. CDNB was metabolized most efficiently by MGST3, followed by MGST1 and MGST2, and EPNPP was most efficiently metabolized by MGST1, followed by MGST3 and MGST2 (Table 2). In human, MGST1, MGST2, and MGST3 also catalyze the conjugation of CDNB (Hayes et al., 2005), partly indicating similarities in cynomolgus macaque and human MGST1, MGST2, and MGST3 enzymatic properties, respectively. Although all three cynomolgus MGSTs are functional enzymes, abundant expression of MGST1 in liver, kidney, and jejunum, essential organs for drug metabolism and toxicity, suggests that MGST1 likely plays the major role in detoxification (among cynomolgus MGSTs), similar to human. In contrast, more ubiquitous expression of MGST2 and MGST3 might reflect their roles in synthesis of leukotriene C4.

In the GST superfamily, many cytosolic GSTs have evolved through recent gene duplications (Sherratt and Hayes, 2002), probably under selective pressure from oxidative stress, including the GSTA, GSTM, GSTO, and GSTT subfamily genes, which comprise highly identical paralogous genes.
in the subfamily. In contrast, the MAPEG family (including \textit{MGST1}, \textit{MGST2}, and \textit{MGST3}) does not contain paralogs in the subfamily (Fig. 1), possibly reflecting their essential physiological roles in synthesis of leukotriene C4 established early on in their evolution (Sherratt and Hayes, 2002).

In summary, we identified MGST2 and MGST3 cDNAs from cynomolgus macaque liver. The amino acid sequences of these cynomolgus MGSTs were highly identical to those of the human orthologs. The gene structures and genomic organization of \textit{MGST1}, \textit{MGST2}, and \textit{MGST3} of macaque and human are highly conserved. Moreover, tissue expression patterns of cynomolgus \textit{MGST1}, \textit{MGST2}, and \textit{MGST3} and the corresponding human orthologs were similar. Cynomolgus MGST1, MGST2, and MGST3 catalyzed conjugation of typical GST substrates, CDNB and EPNPP. These results suggest that cynomolgus MGST1, MGST2, and MGST3 generally have molecular and functional characteristics similar to human MGST1, MGST2, and MGST3, respectively.
Acknowledgments

We greatly appreciate Mr. Masahiro Utoh, Dr. Koichirio Fukuzaki, and Dr. Ryoichi Nagata for their support of this study, and Mr. Patrick Gray for reviewing the manuscript.
Authorship contributions

Participated in research design: Uno and Yamazaki.

Conducted experiments: Uno, Kunori, and Murayama.

Performed data analysis: Uno, Kunori, and Murayama.

Wrote or contributed to the writing of the manuscript: Uno and Yamazaki.
References


Legends for figures

Fig. 1. Phylogenetic tree of MGSTs. The phylogenetic tree was created by the neighbour-joining method with GST amino acid sequences from human (h), cynomolgus macaque (mf), rhesus macaque (mm), rat (r), and mouse (m). Human GSTA1 was used as the outgroup. The scale bar indicates 0.1 amino acid substitutions per site for distance measurement.

Fig. 2. Expression of cynomolgus MGSTs in tissues. qPCR analysis was conducted as described in Materials and Methods. The expression level of each gene was normalized to the 18S rRNA level, and the greatest expression value was adjusted to 1, to which all other expression levels were adjusted accordingly. (A) Expression levels of cynomolgus MGST2 and MGST3 were measured in brain, lung, heart, liver, kidney, adrenal gland, jejunum, testis, ovary, and uterus. Values are shown as the average ± S.D. of three independent amplifications. (B) Expression levels of cynomolgus MGST2 and MGST3 were compared with the expression level of the previously identified MGST1 in liver, jejunum, kidney, brain, and heart. Values are shown as the average of three independent amplifications.
### Table 1

Cynomolgus and human, MGST cDNA and amino acid sequence identity.

<table>
<thead>
<tr>
<th>Cynomolgus</th>
<th>Human</th>
<th>Sequence identity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Amino acids</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cDNA</td>
</tr>
<tr>
<td>MGST1</td>
<td>MGST1</td>
<td>93</td>
</tr>
<tr>
<td>MGST2</td>
<td>MGST2</td>
<td>99</td>
</tr>
<tr>
<td>MGST3</td>
<td>MGST3</td>
<td>98</td>
</tr>
</tbody>
</table>

The cDNA and amino acid sequences of cynomolgus and human MGSTs were compared using BLAST as described in *Materials and Methods.*
Table 2

Catalytic activities of cynomolgus MGST enzymes heterologously expressed in *E. coli*.

<table>
<thead>
<tr>
<th>MGST</th>
<th>CDNB</th>
<th>EPNPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/min/nmol MGST</td>
<td>μmol/min/nmol MGST</td>
</tr>
<tr>
<td>MGST1</td>
<td>0.36</td>
<td>7.25</td>
</tr>
<tr>
<td>MGST 2</td>
<td>0.30</td>
<td>4.03</td>
</tr>
<tr>
<td>MGST 3</td>
<td>1.59</td>
<td>5.80</td>
</tr>
</tbody>
</table>

CDNB and EPNPP were incubated at concentrations of 1.0 mM and 0.5 μM, respectively, with recombinant MGST protein, as described in *Materials and Methods*. Results are presented as means of duplicate determinations.