The Primitive Protozoon Trichomonas vaginalis Contains Two Methionine \(\gamma\)-Lyase Genes That Encode Members of the \(\gamma\)-Family of Pyridoxal 5’-Phosphate-dependent Enzymes*

(Received for publication, September 8, 1997, and in revised form, December 16, 1997)

Amanda E. McKie‡, Thomas Edlind§¶, John Walker‡**, Jeremy C. Mottram***, and Graham H. Coombs‡‡

From the ‡Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, the §§Welcome Unit of Molecular Parasitology, University of Glasgow, The Anderson College, Glasgow G11 6NU, Scotland, United Kingdom, and the ¶¶Department of Microbiology and Immunology, Allegheny University of the Health Sciences, Philadelphia, Pennsylvania 19129

Methionine \(\gamma\)-lyase, the enzyme that catalyzes the breakdown of methionine by an \(\alpha\),\(\gamma\)-elimination reaction and is a member of the \(\gamma\)-family of pyridoxal 5’-phosphate-dependent enzymes, is present in high activity in the primitive protozoon parasite Trichomonas vaginalis but is absent from mammals. Two genes, mgl1 and mgl2, encoding methionine \(\gamma\)-lyase, have now been isolated from T. vaginalis. They are both single copy, encode predicted proteins (MGL1 and MGL2) of 43 kDa, have 69% sequence identity with each other, and show a high degree of sequence identity to methionine \(\gamma\)-lyase from Pseudomonas putida (44%) and other related pyridoxal \(\gamma\)-lyase (30%), mgf1 and mgf2 have been expressed in E. coli as a fusion with a six-histidine tag and the recombiant proteins (rMGL1 and rMGL2) purified by metal-chelate affinity chromatography, rMGL1 and rMGL2 were found to have high activity toward methionine (10.4 and 0.67 \(\mu\)mol/min/mg of protein, respectively), homocysteine (370 and 128 \(\mu\)mol/min/mg of protein), cysteine (6.92 and 1.06 \(\mu\)mol/min/mg of protein), and O-acetylserine (3.74 and 1.51 \(\mu\)mol/min/mg of protein), but to be inactive toward cystathionine. Site-directed mutagenesis of an active site cysteine (C113G for MGL1 and C116G for MGL2) reduced the activity of the recombinant enzymes toward both methionine and homocysteine by approximately 80% (rMGL1) and 90% (rMGL2). In contrast, the activity of mutated rMGL2 toward cysteine and O-acetylserine was increased (to 214 and 142%, respectively), whereas that of mutated rMGL1 was reduced to 39 and 49%, respectively. These findings demonstrate the importance of this cysteine residue in the \(\alpha\),\(\beta\)-elimination and \(\alpha\),\(\gamma\)-elimination reactions catalyzed by trichomonad methionine \(\gamma\)-lyase.

\* This work was supported in part by the Wellcome Trust. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AJ000486 and AJ000487 for mgl1 and mgl2, respectively.

‡ Supported by a travel grant from the Burroughs Wellcome Fund.

*** Medical Research Council (United Kingdom) Senior Fellow.

** To whom correspondence should be addressed: Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow, Joseph Black Bldg., Glasgow G12 8QO, Scotland, U. K. Tel.: 44-141-330-4777; Fax: 44-141-330-3516; E-mail: g.coombs@bio.gla.ac.uk.

1 T. D. Edlind, J. Walker, G. H. Coombs, and J. C. Mottram, unpublished data.
information available for members of the γ-family has been limited until recently such that their evolutionary relationships were unclear. The sequences of several genes are now available which has allowed a phylogenetic analysis, but only one crystal structure (cystathionine β-lyase from Escherichia coli) has been solved (21) and the reaction mechanism elucidated (22).

The methionine γ-lyases of Pseudomonas putida and T. vaginalis have been characterized in some detail at the biochemical level (13, 14, 23–25) and, in the case of P. putida, the molecular level (26, 27). They share some biochemical properties, including subunit composition and the variety of substrates that can be catabolized. The methionine γ-lyase of T. vaginalis has been purified and shown to be a tetramer that has activity not only toward methionine but also related compounds including homocysteine, cysteine, and O-acetylseryne (13). The functional significance of methionine γ-lyase activity to the parasite is uncertain, but it has been proposed that the conversion of α-ketobutyrate to propionate with the concomitant production of ATP could make an important contribution to energy metabolism and also that the methanethiol may play a role in enabling the parasite to avoid damage from the immune response of the host (28). Whether trichomonads contain other enzymes of the γ-family of pyridoxal 5’-phosphate-dependent enzymes and are able to interconvert cysteine and homocysteine via the trans-sulfuration pathway are unknown. Mammals apparently lack methionine γ-lyase (17), and there is evidence that this difference between the parasite and host can be exploited by pro-drugs that are activated by the enzyme (29). In addition, inhibitors are likely to be detrimental to the survival of the parasite in its host and so have potential as chemotherapeutic agents against pathogens that have methionine γ-lyase activity.

This study of trichomonad methionine γ-lyase was undertaken with two main aims: to analyze the similarity of γ-family pyridoxal 5’-phosphate-dependent enzymes of this primitive euukaryote with their bacterial homologs; and to obtain data that will, in the longer term, enable the design of drugs that exploit the presence of methionine γ-lyase in microbial pathogens. To this end we have analyzed the structure and organization of genes that encode methionine γ-lyase in T. vaginalis, expressed the genes in E. coli, examined the biochemical properties of the recombinant enzymes, and analyzed the functional importance of an active site cysteine residue.

MATERIALS AND METHODS

Growth of Parasites and Preparation of DNA, RNA, and First Strand cDNA—A clonal cell line (G3) of T. vaginalis was grown axenically in modified Diamond’s medium as described previously (13). Cells were harvested in late log phase of growth (1–2 × 106/ml) and washed twice in 0.25 M sucrose. DNA was isolated using a Nucleon II kit (Scotlab, Coatbridge, Scotland). Total RNA was isolated using Trizol (Life Technologies, Paisley, Scotland), and poly(A) RNA was isolated using poly(A)+ mRNA columns (Stratagene). First strand cDNA for PCR was generated in a 50-μl reaction using 1 μg of poly(A)+ RNA, 100 ng of oligo(dt) primer, and 10 units of Moloney murine leukemia virus reverse transcriptase (Promega). After synthesis the reaction was diluted to 500 μl with 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA and heated to 65 °C for 15 min.

cDNA Cloning of mgl1 and mgl2.—The degenerate oligonucleotide primers used for the PCR of T. vaginalis methionine γ-lyase genes were based on consensus sequences from cystathionine γ-lyase from human (30), rat (31), and yeast (32). The sequence of the 5′- and 3′-oligonucleotides (using IUB codes) were: 5′-CGACAGTTTCGTTGGAGACGCCCAACNA-3′ and 5′-GCCTCGGCGCTTATRYTTGNTGC-3′. Inosine (I) residues were introduced at some positions of 4-fold degeneracy, and HindIII and XhoI restriction sites (underlined) were added to the 5′-ends of the oligonucleotides to facilitate cloning. The degenerate primers were used in PCRs with T. vaginalis first strand cDNA as the template. The 50-μl reaction mixture contained 5 ng of T. vaginalis cDNA, 5 ng of each of the primers, nucleotides at a final concentration of 2 mM, and 5 units of Taq DNA polymerase (Promega). The following amplification protocol was employed. An initial denaturation step of 4 min at 95 °C was followed by 30 cycles at 94 °C for 1 min, 42 °C for 1 min, and 72 °C for 1 min. A final step of 5 min at 72 °C was used to complete the extension. The amplified product was cloned into HindIII/XhoI-digested pBluescript (SK–) (Stratagene) and sequenced using Sequenase 2.0 (Amersham). Sequence data were analyzed using the Wisconsin GCG sequence software. Two distinct groups of PCR products were found to have homology to yeast cystathionine γ-lyase. These were used to screen a [lamda]ZapII T. vaginalis cDNA library as described previously (33). Positive plaques were isolated, and the inserts were rescued with R408 helper phage into pBluescript. Two positive clones, pMGL106 and pMGL5100, each of which hybridized to one of the 200-bp PCR products, were selected for further analysis. These cDNA clones were sequenced on both strands as described above.

5′-RACE and Inverse PCR—The 5′-ends of the mRNA transcribed from mgl1 and mgl2 were determined using a 5′-RACE System Kit (Life Technologies, Inc.). For RACE-PCR, total cellular RNA from T. vaginalis (1 μg) was transcribed into single-stranded cDNA using oligo(dt) as primer. cDNAs dNTPs and primer were removed, and a homopolymeric tail of dCs was added to the 3′-end. 5 μl of tagged cDNA was used for the PCRs containing the components of the 5′-RACE System Kit and a gene-specific primer.

The following gene-specific primers were used: mgl1, 5′-ATCGGAGATTAACTGATCTCCGCGCC-3′; and mgl2, 5′-AACACCGTGGGGAGGTGTCACCAGAGGC-3′. 30 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, and then a final step of 10 min at 72 °C were applied. Products from the first round of amplification were then used as template for PCRs with a nested primer: mgl1, 5′-GCAATGGCCACCATCGGAGAGATG-3′; and mgl2, 5′-GGACGAAATAGCACCATGCCAGAAG-3′. The amplification protocol was the same as outlined above except that 35 cycles were carried out. The 5′-RACE products were cloned directly into pTag vector (Ingenus) and sequenced.

For inverse PCR, 1 μg of T. vaginalis genomic DNA was cleaved to completion with ClaI in a volume of 10 μl. After heat inactivation of the ClaI (68 °C for 30 min), the DNA was circularized in a 50 μl of T4 DNA ligase reaction and then purified (Wizard DNA Clean-Up, Promega) with elution in 40 μl of water. PCR was performed on 4-μl aliquots with Expand polymerase (Boehringer Mannheim) according to the manufacturer’s instructions; 33 cycles were performed at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, and then a final step of 10 min at 72 °C. TA-cloned products sizes agree with the ClaI sizes for the Southern hybridizations. The degenerate oligonucleotides were used to screen a [lamda]ZapII T. vaginalis genomic library (21) and the reaction mechanism elucidated. The following primers were used to screen the library: mgl1, 5′-CATGGATCCGTATGCTTGGTC-3′ with 5′-TGATGAGATCCGGCGCAATCTC-3′; and 5′-GGAAATGTGTCCTGTATGTTGTG-3′ with 5′-GCGTTGAGAACCTGATT-3′. Gel electrophoresis of the inverse PCR products revealed bands of approximately 2.4 and 1.6 kb (data not shown). With the addition of approximately 1.1 kb corresponding to the distance between each of the primer pairs, these products size agree with the ClaI fragment sizes for mgl1 and mgl2 (3.5 and 2.6 kb, respectively) observed by Southern blotting (see Fig. 2). The inverse PCR products were cloned and sequenced on both strands by primer walking.

Southern and Northern Blot Analyses—T. vaginalis genomic DNA (2 μg) was restricted, size fractionated on a 0.7% agarose gel, and blotted to Hybond N membrane as described (34). A 1.3-kb KpnI/SpeI fragment from PMGL1400, which contained the open reading frame for mgl1, and a 1.3-kb KpnI/SpeI fragment from PMGL5100, which was one nucleotide short at the 5′-end of being full-length for mgl2, were labeled by random priming (Stratagene). Hybridizations to T. vaginalis genomic DNA and subsequent washing steps were performed as described above for the library screen. T. vaginalis total (10 μg) and poly (A)+ (1 μg) RNA were denatured and run on a 1.5% agarose/formaldehyde gel as described previously (34). RNA was transferred to Hybond N membrane. A 1-kb EcoRI/XhoI fragment from PMGL1400 and an 800-bp EcoRI fragment from PMGL5100 were labeled by random priming. Hybridizations and washing steps were carried out under high stringency as for the Southern analyses.

Production of Recombinant MGL1 and MGL2—rMGL1 and rMGL2 were produced in E. coli using the pQE vectors (Qiagen). The T. vaginalis coding region was amplified by PCR from the cDNA clone pMGL1400 using the oligonucleotides 5′-GCAGATCTTTAAAAAGGCGGGCTTTAAGGC-3′ and 5′-GCAGATCTTTAAAAAGGCGGGCTTTAAGGC-3′. The amplified PCR product was cloned into NovéBide II pQE60 to give plasmid clone
The cultures were then induced with 2 mM and 0.2 mM isopropyl-
~proteinase inhibitors (100% v/v glycerol, 40 mM phosphate buffer, pH 6.5) and stored at 20 °C. After sample application, the column was washed with 30 ml of sonication buffer and with 30 ml of wash buffer (50 mM sodium phosphate buffer, pH 6.5, 300 mM NaCl, 20% v/v glycerol, 20 μM pyridoxal 5'-phosphate, 30 μM dithiothreitol, 100 mM sodium phosphate buffer, pH 6.5) and stored at 0 °C. Soluble fraction was loaded on to a 4-ml N7-NTA resin column (Qiagen) that had been pre-equilibrated with 20% v/v glycerol and by SDS-PAGE. Peak fractions were pooled and dialyzed against 100 mM imidazole buffer, pH 6.5. The effect of propargylglycine (Sigma) on homocysteine desulfurase detection mixture. Native PAGE using 12% gels was the same as for SDS-PAGE except that SDS was omitted from the gel and buffers, reducing agent was omitted from the sample buffer, and the sample was not boiled.

**RESULTS**

**Isolation and Characterization of *T. vaginalis* mgl1 and mgl2**—In the absence of data on the protein sequence of the trichomonad methionine γ-lyase or the gene sequence of methionine γ-lyases from other organisms, the approach we adopted to clone the trichomonad methionine γ-lyase gene was to use PCR with degenerate oligonucleotide primers based on regions of homology identified in the related enzyme cystathionine γ-lyase. This is another member of the γ-family of pyridoxal 5'-phosphate-dependent enzymes, one for which sequence information was available from yeast, rat, and human (30–32). The peptide sequences chosen were VVWIPTTNP-1 (3'-primer) and ATKY(M/I)NG (3'- primer). The 3'-primer corresponds to the pyridoxal 5'-phosphate binding domain, and the 5'-primer corresponds to a region highly conserved in cystathionine γ-lyase molecules and differing from other known pyridoxal 5'-phosphate-dependent enzymes of the α- and β-elimination families (20). PCR using *T. vaginalis* first strand cDNA resulted in the amplification of 200-bp DNA fragments that were of the expected size. Cloning and sequencing of these revealed that they belonged to two independent groups, both of which had predicted peptide similarity to yeast cystathionine γ-lyase but only about 50% identity with each other. The 200-bp insert of a representative clone from each group was used to screen a *T. vaginalis* cDNA library. Of the clones isolated, pMLG4100 contained a 1.3-kb insert with the complete open reading frame of the methionine γ-lyase 1 gene (*mgl1*), whereas pMLG5100 contained a truncated gene (*mgl2*) missing the 5'-end including the ATG start codon.

Genomic clones containing the *mgl1* or *mgl2* genes were isolated by inverse PCR. A 2.4-kb fragment containing part of the *mgl1* gene and flanking sequence was amplified from a 3.5-kb *ClaI* (see Fig. 2A, lane 1) fragment, while a 1.6-kb fragment containing part of the *mgl2* gene and flanking sequence was amplified from a 2.5-kb *ClaI* fragment (see Fig. 2B, lane 1). Sequence data for the PCR fragments were combined with the cDNA sequences determined from pMLG4100 and pMLG5100 to give the complete sequence of the *mgl1* and *mgl2* genes. 5'-RACE was used to map the putative site of initiation of transcription for *mgl1* and *mgl2*. *mgl1* and *mgl2* were found to have very short 5'- untranslated regions of 13 and 14 bp, respectively. The sequences from the 5'-termini to the A of the ATG start codon were ATTATTAGACAC (mgl1) and ACCTTTATATAAAAG (mgl2).

*mgl1* is predicted to encode a protein (MGL1) of 396 amino acids with a molecular mass of 42.9 kDa, and *mgl2* predicts a protein (MGL2) of 396 amino acids with a molecular mass of 43.1 kDa (Fig. 1). Amino acid sequence comparison of MGL1 and MGL2 revealed that the two trichomonad proteins had 69% identity. Data bank searches revealed the highest level of amino acid sequence identity for the two trichomonad proteins with the two cystathionine γ-lyases from *P. putida* (26, 27) (44–45%) and cystathionine γ-lyases from yeast (32) and human (30) (43–44%). Significant levels of sequence identity (30–38%) were also observed for MGL1 and MGL2 with a number of other sulfur amino acid-metabolizing enzymes, such as *O-acetylhomoserine sulfhydrylase* from yeast (38) and cystathionine γ-synthase from *E. coli* (39), which are also members of

**Enzyme Assays**—The activity of RML1 and RML2 toward a variety of substrates was measured by monitoring 2-ketoacid production as described (36) or the production of homocysteine (13). The breakdown of cysteine and O-acetylsyreine (both at 10 mM) by the two recombinant proteins was determined by assaying for the production of pyruvate as described (37). All assays were carried out at 37 °C using 0.1 mM homocysteine buffer, pH 6.5. Kinetic calculations were performed using the computer program Grafit (Erithacus Software). Homocysteine desulfurase activity in native polyacrylamide gels was detected by immersion at 37 °C in a reaction mixture containing 3.3 mM th-homocysteine, 0.33 mM l-cysteine, 10 mM 2-mercaptoethanol, and 100 mM imidazole buffer, pH 6.5. The effect of propargylglycine (Sigma) was determined by immersion of the gel in 10 mM propargylglycine in 0.1 M imidazole buffer, pH 6.5, for 5 min at 37 °C before immersion in the homocysteine desulfurase detection mixture. Native PAGE using 12% gels was the same as for SDS-PAGE except that SDS was omitted from the gel and buffers, reducing agent was omitted from the sample buffer, and the sample was not boiled.
the γ-family of pyridoxal 5′-phosphate-dependent enzymes (20). An alignment of methionine γ-lyases from *T. vaginalis* and *P. putida* and cystathionine γ-lyases from human (HsCCL (30)) and yeast (ScCYS3 (32)) is shown in Fig. 1. The two underlined sequences correspond to the areas of consensus to which the PCR primers were designed. 3/8 residues of the 5′-consensus site in MGL1 and MGL2 differed from those of yeast cystathionine γ-lyase, whereas all 8 residues were the same in the 3′-consensus site which corresponds to the pyridoxal 5′-phosphate binding region and contains the essential lysine residue. 35 residues that
are conserved in the four methionine γ-lyase sequences but absent from the cystathionine γ-lyases are indicated. These residues may play a role in defining the substrate specificity of the enzymes. One of these residues, cysteine 113 (numbering for MGL1, starred in Fig. 1), is of particular interest because it has been shown to be important for methionine γ-lyase activity in P. putida (25, 40). In addition, the MGL sequences have a 7-amino acid insertion relative to the cystathionine γ-lyase toward the NH2 terminus (residues 49–55 in MGL1).

Genomic Organization and Expression of mgl1 and mgl2—To assess the copy number of mgl1 and mgl2 in T. vaginalis, genomic DNA was digested with restriction enzymes and analyzed by Southern blotting using the mgl1 and mgl2 cDNAs as probes (Fig. 2). For each of the five restriction enzymes used, a single major hybridizing band was detected with each of the mgl1 and mgl2 probes, which is consistent with the presence of single copy genes. For example, the 3.5-kb ClaI fragment detected by the mgl1 probe (Fig. 2A, lane 1), which was cloned by inverse PCR, revealed the presence of one open reading frame, and likewise one copy of mgl2 was found on the 2.6-kb ClaI fragment detected by the mgl2 probe (Fig. 2B, lane 1). At the high stringency conditions used for the Southern blot, the two genes did not cross-hybridize to a significant extent. Both mgl1 and mgl2 probes hybridized to a single 7.5-kb EcoRI band (Fig. 2, A and B, lane 2). This raises the possibility that the two genes are linked, but this has not been investigated further.

To examine the expression of mgl1 and mgl2, Northern blots were performed with total and poly(A)⁺ RNA. A single 1.3-kb transcript was detected with both the mgl1- and mgl2-specific probes (data not shown). The length of the mRNAs for the two genes corresponds well with the size of the cDNA clones isolated, the position determined for the addition of the poly[A] tails, and the sites mapped for transcription initiation.

Expression and Purification of Recombinant MGL1 and MGL2—The two T. vaginalis mgl1 genes were cloned into pQE vectors (Qiagen) for expression of recombinant enzyme with a six-histidine tag. The complete open reading frame of mgl1 was inserted by PCR into the pQE60 vector, which utilizes the normal ATG start codon of the gene and adds a six-histidine tag at the COOH terminus. The generation of an EcoRI band (Fig. 3, lanes 1 and 2) and likewise one copy of mgl2 were expressed in M15pREP4 vectors (Qiagen) for expression of recombinant enzyme with a six-histidine tag. The complete open reading frame of mgl1 was inserted by PCR into the pQE30 vector. This resulted in a fusion protein with 21 residues of vector-derived sequence, including a six-histidine tag at the COOH terminus.

MGL2 sequence. The pMGL5100 clone is not full-length, which is due in place of a serine at position 2 of the recombinant protein.

To examine the expression of mgl1 and mgl2, Northern blots were performed with total and poly(A)⁺ RNA. A single 1.3-kb transcript was detected with both the mgl1- and mgl2-specific probes (data not shown). The length of the mRNAs for the two genes corresponds well with the size of the cDNA clones isolated, the position determined for the addition of the poly[A] tails, and the sites mapped for transcription initiation.

Expression and Purification of Recombinant MGL1 and MGL2—The two T. vaginalis mgl1 genes were cloned into pQE vectors (Qiagen) for expression of recombinant enzyme with a six-histidine tag. The complete open reading frame of mgl1 was inserted by PCR into the pQE60 vector, which utilizes the normal ATG start codon of the gene and adds a six-histidine tag at the COOH terminus. The generation of an EcoRI band (Fig. 3, lanes 1 and 2) and likewise one copy of mgl2 were expressed in M15pREP4 E. coli and subsequently affinity purified by FPLC on Ni²⁺-NTA resin. About 10 mg of recombinant enzyme was purified from each 450 ml of culture. Denaturing SDS-PAGE analysis confirmed the predicted sizes of rMGL1 and rMGL2 (43.8 and 47.2 kDa) and showed a high degree of purity (Fig. 3A, lanes 1 and 2). The major protein detected by SDS-PAGE for each of the MGL1 and MGL2 preparations (Fig. 3B, lanes 1 and 2) was shown to be coincident with enzyme activity as detected by a native PAGE in situ assay for homocysteine desulfurase activity (Fig. 3B, lanes 3 and 4). Methionine γ-lyase purified from T. vaginalis has high homocysteine desulfurase activity (13). The in situ homocysteine desulfurase activity of the two recombinant
TABLE I
Comparison of enzymatic activities of mutated and wild type recombinant proteins

| Substrate               | rMGL1 | MLGL1(C113G) | Mutant/wild type | μmol/min/mg protein | %   |
|-------------------------|-------|--------------|------------------|---------------------|-----|
| Homocysteine            | 370 ± 11 (8) | 34.5 ± 3.2 (14) | ND (8)          | 9.3                 |
| Methionine              | 10.4 ± 0.31 (4) | 0.79 ± 0.17 (8) | ND (8)         | 7.6                 |
| Cysteine                | 6.0 ± 0.39 (8) | 2.38 ± 0.35 (8) | ND (8)        | 39                  |
| O-Acetylserine          | 3.74 ± 0.10 (4) | 1.83 ± 0.12 (8) | ND (8)       | 49                  |
| Cystathionine           | ND (4) | ND (8)       |                  |                     |

| Substrate               | rMGL2 | MLGL2(C116G) | Mutant/wild type | μmol/min/mg protein | %   |
|-------------------------|-------|--------------|------------------|---------------------|-----|
| Homocysteine            | 128 ± 22 (14) | 27.5 ± 5.8 (19) | ND (8)          | 21                  |
| Methionine              | 0.67 ± 0.18 (11) | 0.15 ± 0.06 (14) | ND (8)       | 22                  |
| Cysteine                | 1.06 ± 0.42 (16) | 2.31 ± 0.71 (17) | ND (8)       | 217                 |
| O-Acetylserine          | 1.51 ± 0.49 (12) | 2.15 ± 0.17 (14) | ND (8)       | 142                 |
| Cystathionine           | ND (12) | ND (8)       |                  |                     |

TABLE II
Kinetic parameters of wild type and mutated rMGL1 and rMGL2 with respect to catabolism of homocysteine and cysteine

At least 10 different substrate concentrations were used, with at least three replicate assays.

| Substrate               | Homocysteine | Cysteine | K_m (μmol/min/mg protein) | V_max (μmol/min/mg protein) |
|-------------------------|--------------|----------|--------------------------|-----------------------------|
| rMGL1                   | 12.2         | 256      | 8.5                      | 14.9                        |
| rMGL1(C113G)            | 15.2         | 42       | 9.7                      | 4.6                         |
| rMGL2                   | 37.7         | 132      | 22.3                     | 2.4                         |
| rMGL2(C116G)            | 6.2          | 53       | 3.6                      | 4.8                         |

The activity of the mutated enzyme toward cysteine and O-acetylserine was increased to 217 and 142%, respectively. Neither of the mutated enzymes exhibited activity toward cystathionine.

Comparative kinetic analyses of the mutated and wild type recombinant enzymes with respect to the catabolism of homocysteine and cysteine were performed (Table II). The slightly higher K_m and markedly lower V_max values of rMGL1(C113G) compared with those of wild type rMGL1 suggest reduced substrate binding efficiency of this mutated enzyme. The chemically modified P. putida methionine γ-lyase also exhibited increased K_m values for a range of cysteine-analog substrates (25). In contrast, the apparent K_m of rMGL2(C116G) for cysteine was considerably lower than that of rMGL2, and this correlates with the enhanced activity (higher V_max) of the mutated enzyme toward this substrate. Curiously, the K_m of rMGL2(C116G) for homocysteine was also much reduced relative to that of the wild type enzyme, despite the significantly lower V_max of the mutated enzyme with this substrate.

DISCUSSION

A native methionine γ-lyase activity from T. vaginalis has been purified and characterized previously (13). The two genes mgl1 and mgl2 from T. vaginalis, as reported in this study, have methionine γ-lyase activity when expressed in E. coli. Recombinant MGL1 and MGL2 have substrate specificity profiles similar to that of the native enzyme, catalyzing the breakdown of methionine, homocysteine, cysteine, and O-acetylserine but being inactive toward cystathionine (Table I). mgl1 and mgl2 are both highly transcribed genes with similar sized mRNAs, and the protein products are both about 45 kDa (Fig. 3, C and D, lane 3), comparable with the native enzyme, which was estimated to have a molecular mass of 160 kDa composed of four apparently identical 43–45 kDa subunits (13). Because rMGL2 was estimated to be about 160 kDa, this suggests that the recombinant enzyme is also a tetramer composed of identical subunits. It is possible that the purified enzyme charac-
zymes is 69%, but they are significantly less similar (44%) to currently known how much each gene contributes toward the activity. Thus whereas it seems very likely that mgl1/mgl2 encode methionine γ-lyase activity in T. vaginalis, it is not currently known how much each gene contributes toward the total enzyme activity of the parasite.

The level of sequence identity between the trichomonad enzymes is 69%, but they are significantly less similar (44%) to the Pseudomonas enzymes. The trichomonad and bacterial methionine γ-lyases are more closely related phylogenetically to each other than to other members of the γ-family of pyridoxal 5′-phosphate-dependent enzymes (20), and they share several features. Both catalyze methionine and homocysteine; however, the Pseudomonas recombinant enzyme can also use cystathionine as a substrate (27) whereas the trichomonad enzyme cannot. Interestingly, it was reported that the native P. putida enzyme was not active toward cystathionine, whereas the Aeromonas enzyme had such activity (14). A low activity apparently of cystathionine γ-lyase was detected in crude lysates of T. vaginalis (41), but the enzyme responsible for this is unknown, and it could simply reflect a broad substrate specificity of another enzyme. The success of the strategy adopted for the cloning of the two trichomonad methionine γ-lyase genes and the finding that the use of the degenerate primers (based on conserved regions in cystathionine γ-lyases) did not lead to the amplification of fragments of other genes suggest that T. vaginalis does not contain cystathionine γ-lyase itself. There is little information on the presence, or otherwise, of other trans-sulfuration enzymes, and other members of the γ-family of pyridoxal 5′-phosphate-dependent enzymes, in trichomonads. They contain high levels of an activity designated serine sulfurylase (19) that catalyzes the conversion of cysteine to serine and hydrogen sulfide. This activity is thought to be mediated by cystathionine β-synthase, the first enzyme in the trans-sulfuration pathway, in many cells (15). Cystathionine β-synthase is a member of the β-family of pyridoxal 5′-phosphate-dependent enzymes (20). To date, however, cystathionine synthesis, and indeed trans-sulfuration reactions in general, have not been shown to occur in trichomonads. Thus the current data clearly suggest that sulfur amino acid metabolism of T. vaginalis differs considerably from that in mammals and that the mgl genes of T. vaginalis are more closely related to bacterial genes than any detected so far in eukaryotes.

Chemical modification studies on the P. putida methionine γ-lyase showed that a cysteine residue (Cys-116, P. putida numbering) was important, but not essential, for activity of the enzyme (25, 40). The equivalent cysteine residue is present in the two trichomonad methionine γ-lyases, but not in other γ-family pyridoxal 5′-phosphate-dependent enzymes (Fig. 1). For example, yeast cystathionine γ-lyase has a glycine in this position and indeed has no cysteine residues at all (32). This suggests that the cysteine residue at this position cannot be directly involved in the catalytic mechanism, although clearly it could be involved in mediating substrate binding and so determining specificity. Mutation of this cysteine residue to a glycine in rMGL1 reduced α,γ-elimination activity, for example the breakdown of homocysteine, by 90% and α, β-elimination activity, for example the breakdown of cysteine, by 61%, which correlates well with data obtained for P. putida (25). Mutation of this cysteine residue in rMGL2 to a glycine, however, although reducing α,γ-elimination activity by 80%, resulted in more than twice the α,β-elimination activity. These data confirm the importance of this cysteine residue in substrate specificity. They also show that despite the high similarity between the two trichomonad enzymes there clearly are differences in the active sites which have important implications for the catalytic mechanisms of α,β-elimination and α,γ-elimination reactions. We must await data on the three-dimensional structure of the active site of the two trichomonad enzymes for a thorough interpretation of these differences and the role of the cysteine residue. The recent success in solving the structure of the γ-family enzyme cystathionine β-lyase (21) should enable some useful modeling of the active site of trichomonad methionine γ-lyases. However, for a definitive study data on the crystal structure of the trichomonad protein are required. To this end, rMGL1 has recently been crystallized, and structural studies are under way.

REFERENCES

1. Gunderson, J., Hinkle, G., Leipe, D., Morrison, H. G., Stickel, S. K., Odelson, D. A., Breznak, J. A., Neoral, T. A., Muller, M., and Sugin, M. L. (1995) J. Bact. Microbiol. 42, 411–415
2. Bui, E. T. N., Bradley, P. J., and Johnson, P. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8651–8655
3. Hauler, T., Steier, Y., Blattner, J., and Clayton, C. F. (1997) Eur. J. Cell Biol. 73, 240–251
4. Coombs, G. H., and Muller, M. (1995) in Biochemistry and Molecular Biology of Parasites (Marr, J. J., and Muller, M., eds) pp. 407–417, Academic Press, London
5. Muller, M. (1998) in Evolutionary Relationships among Protozoa (Coombs, G. H., Nichkeman, K., Sleip, M. A., and Warren, A., eds) pp. 109–122, Chapman and Hall, Cambridge
6. Muller, M. (1993) J. Gen. Microbiol. 189, 2879–2889
7. Bui, E. T. N., and Johnson, P. J. (1998) Mol. Biochem. Parasitol. 76, 355–310
8. Hornier, D. S., Hirt, R. P., Clevinigton, S., Lloyd, D., and Embley, T. M. (1997) Proc. R. Soc. Lond. B Biol. Sci. 263, 1053–1059
9. Gernet, M., Philippe, H., and Le Guyader, H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14614–14618
10. Roger, A. J., Clark, C. G., and Doolittle, W. F. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14618–14622
11. Bradley, P. J., Lahbi, C. J., Plumper, E., and Johnson, P. J. (1997) EMBO J. 16, 4384–4393
12. Thong, K., Coombs, G. H., and Sanderson, B. E. (1987) Mol. Biochem. Parasitol. 23, 223–231
13. Lockwood, B. C., and Coombs, G. H. (1991) Biochem. J. 279, 675–682
14. Esaki, N., and Soda, K. (1997) Methods Enzymol. 143, 459–465
15. Cooper, A. J. L. (1983) Annu. Rev. Biochem. 52, 187–222
16. Giovanielli, J. (1987) Methods Enzymol. 143, 438–452
17. Griffith, O. W. (1987) Methods Enzymol. 143, 366–376
18. Soda, K. (1987) Methods Enzymol. 143, 452–459
19. Thong, K., and Coombs, G. H. (1987) Exp. Parasitol. 63, 143–151
20. Alexander, F. W., Sandenier, E., Mehta, P. K., and Christie, F. (1994) Eur. J. Biochem. 219, 953–960
21. Clausen, T., Huber, R., Laber, B., Pohlenz, H. D., and Messerschmidt, A. (1996) J. Mol. Biol. 262, 202–224
22. Clausen, T., Laber, B., and Messerschmidt, A. (1997) Biol. Chem. Hoppe-Seyer 378, 321–326
23. Tanaka, H., Esaki, N., Yamamoto, T., and Soda, K. (1976) FEBS Lett. 66, 307–311
24. Tanaka, H., Esaki, N., and Soda, K. (1977) Biochemistry 16, 100–106
25. Nakayama, T., Esaki, N., Tanaka, H., and Soda, K. (1988) Agric. Biol. Chem. 52, 177–183
26. Inoue, H., Inagaki, K., Sugimoto, M., Esaki, N., and Soda, K. (1985) J. Biochem. (Tokyo) 117, 1210–1210
27. Hori, H., Takabayashi, K., Orvis, L., Carson, D. A., and Nobori, T. (1996) Cancer Res. 56, 2116–2122
28. Lockwood, B. C., and Coombs, G. H. (1991) in Biochemical Protozoology (Coombs, G. H., and North, M. J., eds) pp. 113–122, Francis and Taylor Ltd, London
29. Coombs, G. H., and Mottram, J. C. (1997) Parasitology 114, 561–580
30. Lu, Y., O'Dowd, B. F., Orrego, H., and Israel, Y. (1992) Biochem. Biophys. Res. Commun. 189, 749–758
31. Erickson, P. F., Maxwell, I. H., Su, L.-J., Baumann, M., and Glode, L. M. (1990) *Biochem. J.* **269**, 335–340
32. Ono, B. I., Tanaka, K., Naito, K., Heike, C., Shinoda, S., Yamamoto, S., Ohmori, S., Oshima, T., and Toh-e, A. (1992) *J. Bacteriol.* **174**, 3339–2247
33. Mallinson, D. J., Lockwood, B. C., Coombs, G. H., and North, M. J. (1994) *J. Gen. Microbiol.* **140**, 2725–2735
34. Mettrem, J. C., Kinnaid, J., Shiels, B. R., Tait, A., and Barry, J. D. (1993) *J. Biol. Chem.* **268**, 21044–21051
35. Harlow, E., and Lane, D. (1988) *Antibodies: A Laboratory Manual*, pp. 53–138, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
36. Soda, K. (1968) *Anal. Biochem.* **25**, 228–235
37. Czok, R., and Lämprecht, W. (1974) in *Methods in Enzymatic Analysis* (Bergmeyer, H. U., ed) Vol. 3, pp. 1446–1448, Academic Press, New York
38. Kerjan, P., Cherest, H., and Surdin-Kerjan, Y. (1986) *Nucleic Acids Res.* **14**, 7861–7871
39. Duchange, N., Zakin, M. M., Ferrara, M., Saint-Girons, I., Park, I., Tran, S. V., Py, M. C., and Cohen, G. C. (1983) *J. Biol. Chem.* **258**, 14868–14871
40. Nakayama, T., Esaki, N., Tanaka, H., and Soda, K. (1988) *Biochemistry* **27**, 1587–1591
41. Thong, K., and Coombs, G. H. (1985) *IRCS Med. Sci.* **13**, 493–494