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Purification and characterization of methionine γ-lyase from *Trichomonas vaginalis*

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Methionine γ-lyase (EC 4.4.1.11) was purified to homogeneity from the anaerobic protozoan parasite *Trichomonas vaginalis* by a series of f.p.l.c. procedures. The enzyme catalyses αγ- and αβ-elimination reactions of a number of derivatives of methionine and cysteine. It also catalyses γ-replacement reactions of the thiomethyl group of methionine, homocysteine and ethionine to yield the corresponding *S*-substituted homocysteine derivative. The enzyme is pyridoxal 5′-phosphate-dependent, has a native molecular mass of approx. 160 kDa and consists of four apparently identical subunits of molecular mass 43–45 kDa. The absorption spectrum of the enzyme is typical of those obtained for other pyridoxal 5′-phosphate-dependent enzymes, and the holoenzyme can be resolved to the apoenzyme by incubation with hydroxylamine and reconstituted by addition of the cofactor. The enzyme activity is significantly affected by carbonyl and thiol reagents, is competitively inhibited by a number of substrate analogues and is completely inactivated by the suicide inhibitor DL-propargylglycine. The *T. vaginalis* enzyme is similar, in terms of activity and properties, to the enzymes found in a number of species of bacteria that metabolize methionine under anaerobic conditions. It is suggested that methionine catabolism may be of particular importance to the survival of *T. vaginalis* under microaerophilic conditions in its host.

INTRODUCTION

Previous studies have shown that *Trichomonas vaginalis* rapidly catabolizes L-methionine to volatile thiols, including methanethiol (Thong *et al.*, 1987*a*,*b*). In mammals, methanethiol is a by-product of polyamine biosynthesis (Tabor & Tabor, 1984) or is produced directly from methionine by transamination to 2-oxo-4-methylthiobutyrate, followed by dethiomethylation (Steele & Benevenga, 1979). A similar mechanism has been proposed for aerobic bacteria and fungi (Segal & Starkey, 1969; Ruiz-Herrera & Starkey, 1969*a*). In contrast, methionine is deaminated and dethiomethylated simultaneously in some anaerobic and facultative bacteria capable of metabolizing methionine under anaerobic conditions (Tanaka *et al.*, 1977). The enzyme that catalyses this reaction was first detected in *Escherichia coli* and originally named methionase (Ohigashi *et al.*, 1951), but is now known as L-methionine γ-lyase or L-methionine methanethiol-lyase (deaminating) (EC 4.4.1.11) (Ito *et al.*, 1975). L-Methionine γ-lyase was subsequently demonstrated in extracts of a soil bacterium (Mitsuhashi & Matsuo, 1950), *Clostridium* sp. (Wiesendanger & Nisman, 1953), *Pseudomonas* sp. (Miwatani *et al.*, 1954; Kallio & Larson, 1955; Ito *et al.*, 1975; Tanaka *et al.*, 1977), some rumen bacteria (Merricks & Salsbury, 1974) and *Aeromonas* sp. (Nakayama *et al.*, 1984). The enzyme has since been purified from *Clostridium sporogenes* (Kreis & Hession, 1973), *Pseudomonas putida* (= *ovalis*) (Tanaka *et al.*, 1976, 1977; Esaki & Soda, 1987) and *Aeromonas* sp. (Nakayama *et al.*, 1984) and the physical and chemical properties have been studied in some detail. All of the bacterial enzymes are pyridoxal 5′-phosphate-dependent and catalyse αγ- and αβ-elimination in addition to γ- and β-exchange reactions.

The L-methionine-catabolizing activity previously demonstrated in cell-free extracts of *T. vaginalis* (Thong *et al.*, 1987*b*; Lockwood & Coombs, 1989) was shown to have many of the characteristics of the bacterial methionine γ-lyases, although the products of the reactions catalysed by the trichomonad enzyme were not all identified. It has been suggested that the methionine γ-lyase of *T. vaginalis* is also responsible for the parasite's homocysteine desulphurase activity, which results in the breakdown of homocysteine to 2-oxobutyrate, NH_3 and H_2S (Thong & Coombs, 1985, 1987; Lockwood & Coombs, 1989).

The presence of these two activities in *T. vaginalis* is particularly interesting, as neither apparently occurs in other species of trichomonads or in a range of other protozoan parasites (Thong *et al.*, 1987*b*; Thong & Coombs, 1987). The only other reported occurrence of protozoa which dethiomethylate methionine is for rumen species (Merricks & Salsbury, 1975). Methionine γ-lyase is absent from mammalian cells and, although homocysteine desulphurase activity has been detected, it was shown to be due to γ-cystathionase and thought to be of little or no significance in mammals (Cooper, 1983). Thus it appears that methionine γ-lyase of *T. vaginalis* performs a function peculiar to this parasite, and clearly the presence of the enzyme offers opportunities for chemotherapeutic attack, for example by prodrugs that will be activated by the parasite's enzyme. This paper describes the purification and characterization of methionine γ-lyase from *T. vaginalis* and compares the physicochemical properties with those of the bacterial enzymes.

EXPERIMENTAL

Parasites

Trichomonas vaginalis clone G3 was grown axenically in modified Diamond's medium as described previously (Coombs, 1976). Cells at late exponential phase of growth [$(1.5–2.0) \times 10^6$ cells/ml] were harvested by centrifugation at 2300 *g* for 10 min at 4 °C and washed twice with 0.25 M-sucrose. The washed cells were resuspended in ice-cold 0.25 M-sucrose to a density of approx. 10^8 cells/ml and disrupted by Potter homogenization as described previously (Lockwood *et al.*, 1988).

Subcellular fractionation

Four subcellular fractions (nuclear, large particle, small particle and non-sedimentable) were obtained by differential centrifugation by the method used by Steinbuchel & Muller (1986). Since the enzyme was found exclusively in the non-sedimentable

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Table 1. Subcellular localization of methionine γ-lyase in *T. vaginalis*

The results are from one experiment that is representative of at least three individual experiments to determine the distribution of each of the enzymes. The total activity is that recovered in all fractions as a percentage of that in the homogenate used as the starting material. Recovery in the fractions is given as a percentage of the total recovered in all four fractions.

	Recovery (%)				
	Nuclear	Large particle	Small particle	Non-sedimentable	Total
Proteinase	9.5	33.3	49.3	7.9	81.0
Acid phosphatase	9.7	22.5	56.4	11.3	108.8
Malate dehydrogenase	8.5	71.0	17.3	3.2	71.1
Lactate dehydrogenase	7.0	4.7	2.1	86.2	95.5
Methionine γ-lyase	4.7	2.2	1.9	91.1	126.8
Protein	36.5	12.2	9.5	41.7	94.7

fraction, the homogenate was centrifuged at 100000 *g* for 1 h at 4° C to obtain a non-sedimentable fraction which was used as the starting material for the enzyme purification. Pyridoxal 5′-phosphate was added to this fraction to give a final concentration of 20 μM, before storage at −70 °C.

Enzyme purification

The non-sedimentable fraction was clarified by filtration through a 0.22 μm-pore GS membrane (Millipore). Samples (1 ml) were injected on to a Mono Q HR 5/5 (Pharmacia) anion-exchange column, pre-equilibrated with 20 mM-potassium phosphate buffer, pH 7.5, containing 15 μM-2-mercaptoethanol, 1 mM-EDTA and 20 μM-pyridoxal 5′-phosphate. Protein was eluted with a 0–350 mM-NaCl gradient in the same buffer. Fractions containing the major peak of methionine γ-lyase activity were pooled and concentrated to approx. 1 ml by ultrafiltration through an XM 50 membrane filter (Amicon).

Solid $(NH_4)_2SO_4$ was added to the concentrated fractions (0.317 g/ml) to give a final concentration of 2.4 M, and the sample was filtered as above to remove any precipitated proteins before injection on to an Alkyl Superose HR 5/5 (Pharmacia) hydrophobic-interaction-chromatography column, equilibrated with 2.4 M-$(NH_4)_2SO_4$ dissolved in the same buffer used for the previous step. Bound protein was eluted by linearly decreasing the $(NH_4)_2SO_4$ concentration.

Fractions containing the methionine γ-lyase activity were pooled and injected on to a Superose 12 HR 10/30 column (Pharmacia) equilibrated with 0.2 M-potassium phosphate, pH 7.5, with the same additons used as in the buffers in the preceding steps. Protein was eluted with the same buffer, and the fractions containing the enzyme activity were pooled and concentrated as described above. The same column was calibrated with standards of known molecular mass (Blue Dextran, 2000000 Da; apoferritin, 443000 Da; β-amylase, 200000 Da; alcohol dehydrogenase, 150000 Da; BSA, 66000 Da; carbonic anhydrase, 29000 Da; cytochrome *c*, 12400 Da) and used for the estimation of the native molecular mass of the purified enzyme.

Resolution and reconstitution of the enzyme

Pyridoxal 5′-phosphate was removed from the holoenzyme by incubation with 1 mM-hydroxylamine in 10 mM-potassium phosphate buffer, pH 7.2, for 15 min at room temperature, followed by dialysis against three changes of the same buffer for 3 h at 4 °C. The absorption spectrum was recorded on a Philips PU8720 UV/Vis scanning spectrophotometer. The enzyme was reconstituted by the addition of various concentrations (0.01–1.0 mM) of pyridoxal 5′-phosphate.

Enzyme assays

The dethiomethylation of homocysteine (homocysteine desulphurase activity) was measured colorimetrically. The reaction mixture contained a final concentration of 2 mM-DL-homocysteine, 1 mM-lead acetate, 0.1 M-potassium phosphate buffer, pH 7.5, and 50 μl of fraction in a final volume of 150 μl. Incubation was at 37 °C, and the A_{340} after 10 min was measured with a Titertek Multistage MCC/340 e.l.i.s.a. plate reader.

Methionine γ-lyase activity was assayed either by measuring the production of volatile thiols by the method described by Thong *et al.* (1987*a*,*b*) or by measuring 2-oxo acid production, as described by Esaki & Soda (1987). Buffers used to investigate the pH-sensitivity were 0.1 M-sodium acetate/acetic acid (pH 4.0–6.0), 0.1 M-potassium phosphate (pH 7.0–8.0) and 0.1 M-glycine/NaOH (pH 9.0).

K_m values were determined by Lineweaver–Burk plots. The range of substrate concentrations used were 0.1–5.0 mM for homocysteine and 0.5–20 mM for methionine.

Oxo acids were identified by h.p.l.c., using a Gilson system. After incubation of the enzyme with the appropriate substrate at 37 °C for 30 min, protein was precipitated with an equal volume of ice-cold ethanol and removed by centrifugation (10000 *g* for 5 min), and 20 μl of the supernatant was injected on to a Polypore H column (Brownlee). Oxo acids were eluted isocratically with 0.01 M-H_2SO_4 at a flow rate of 1.0 ml/min with an operating pressure of 2.1 MPa (300 lb/in^2) and a column temperature of 70 °C. Detection was at 210 nm.

Ammonia production was measured by the method of Horn & Squire (1967), with NH_4Cl as standard.

Methods used for the detection of subcellular marker enzymes were: proteinase with azocasein as substrate, Lockwood *et al.* (1984); malate dehydrogenase (decarboxylating) (EC 1.1.1.40), Steinbuchel & Muller (1986); lactate dehydrogenase (EC 1.1.1.27), Lockwood *et al.* (1988); and acid phosphatase (EC 3.1.3.2), Bergmeyer *et al.* (1974).

Protein was measured by the method of Sedmak & Grossberg (1977).

PAGE

Discontinuous dissociating SDS/PAGE was carried out exactly as described by Hames (1981). Native gels were identical with the denaturing gels, except that the SDS was omitted from the gel and buffers. Molecular masses were determined in the absence of denaturing agents by using Ferguson plots as described by Hedrick & Smith (1968). Native gels were stained for homocysteine desulphurase activity by immersion in a reaction

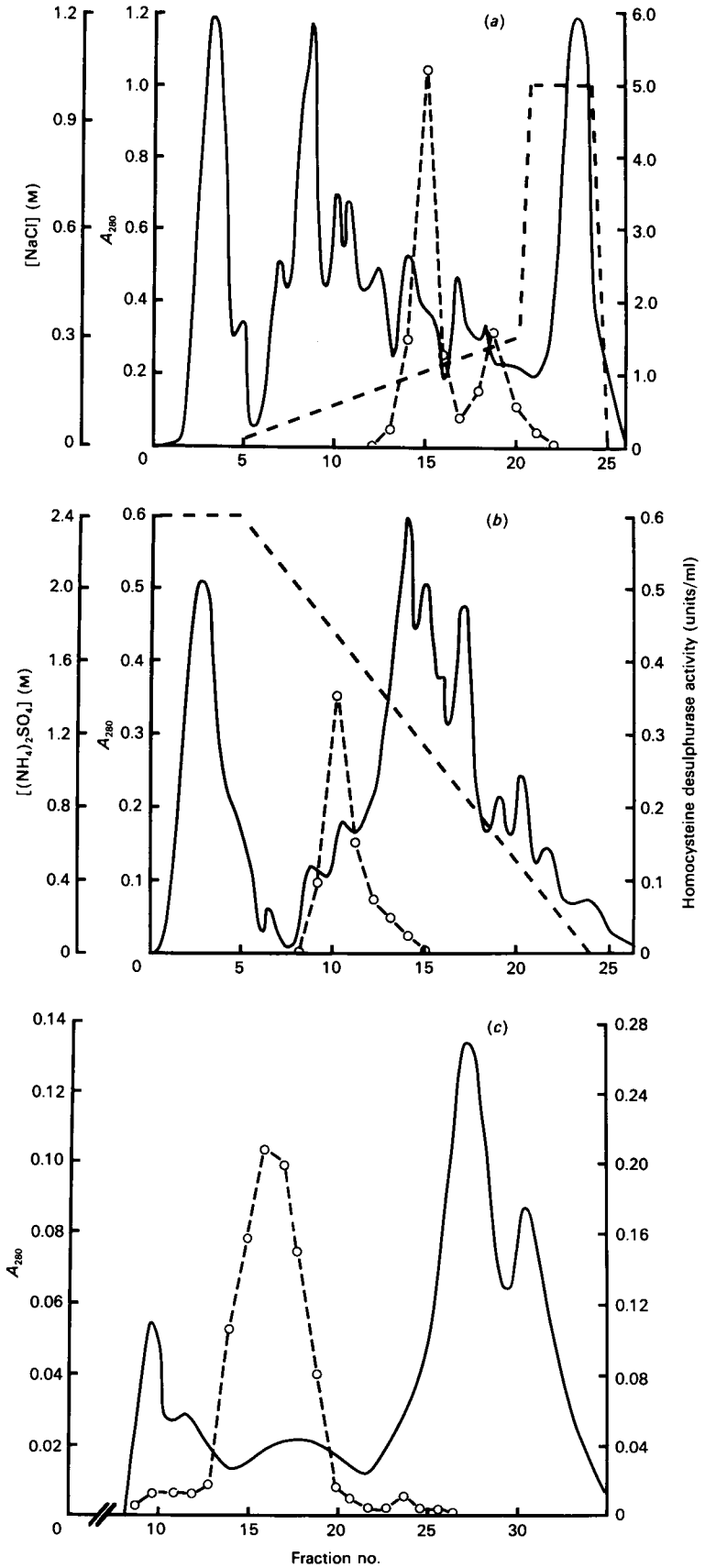

Fig. 1. Purification of methionine γ-lyase/homocysteine desulphurase from *T. vaginalis*

Methionine γ-lyase was purified by f.p.l.c. as described in the Experimental section. (*a*) Ion-exchange chromatography on a Mono Q column; (*b*) hydrophobic interaction chromatography on an Alkyl Superose column; (*c*) gel filtration on a Superose 12 column. Column fractions (1 ml) were assayed for protein (——) and homocysteine desulphurase activity (○): ----, salt gradients.

mixture containing 3.3 mM-DL-homocysteine, 0.33 mM-lead acetate, 28.4 mM-2-mercaptoethanol and 100 mM-Tris/HCl buffer, pH 7.5. Gels were stained for protein with Coomassie Blue or silver-stained by using Sigma Silver Staining Kit (AG 25).

Inhibitor studies

The carbonyl reagents L-cycloserine, hydroxylamine and DL-penicillamine and the suicide inhibitor DL-propargylglycine were made up as 10 mM stock solutions in buffer and added to the assay mixture to give a final concentation of 1 mM immediately before starting the reaction. The enzyme was preincubated with 15 μl of 10 mM-iodoacetamide, 0.1 mM-*p*-chloromercuribenzoic acid and 1 mM-5,5'-dithiobis-(2-nitrobenzoic acid), all dissolved in buffer, for 30 min at room temperature before assaying for activity.

Compounds tested as competitive inhibitors were added to the assay mixture, without substrate, at a final concentration of 25 mM and incubated at 37 °C for 10 min before addition of the substrate to start the reaction.

Materials

Columns for f.p.l.c. were obtained from Pharmacia, Milton Keynes, Bucks., U.K. Brownlee Polypore H column and Fisons h.p.l.c.-grade solvents were obtained from Anachem, Luton, Beds., U.K. All other reagents were obtained from either Sigma Chemical Co., Poole, Dorset, U.K., or Aldrich Chemical Co., Gillingham, Dorset, U.K.

RESULTS

Subcellular localization of methionine γ-lyase

Analysis of the four fractions obtained by differential centrifugation showed that over 90% of the methionine γ-lyase was contained within the non-sedimentable fraction (Table 1). Latency of the lysosomal marker enzymes and hydrogenosomal marker enzyme was investigated by assaying fractions in 250 mM-sucrose with and without detergent. Addition of 0.25% Triton X-100 gave an increase in activities of 4.9 ± 1.2-fold and 5.9 ± 2.1-fold ($n = 6$) for proteinase and acid phosphatase respectively, and of 2.4 ± 0.7-fold ($n = 3$) for malate dehydrogenase (decarboxylating), showing that most of these organelles were isolated intact. No increase in the methionine γ-lyase activity in the particulate fractions was obtained on addition of Triton X-100, suggesting that the enzyme is entirely cytosolic.

Purification of methionine γ-lyase

Methionine γ-lyase in the non-sedimentable fraction was found to be unstable, with 25% of the activity lost during a single freeze–thaw cycle. A variety of conditions were used in attempts to retain activity, including changes in ionic strength and pH of buffers, addition of reducing agents, thiol reagents, chelating agents and proteinase inhibitors. Most had no significant effect on the stability of the enzyme. It was found, however, that the addition together of pyridoxal 5'-phosphate (20 μM), 2-mercaptoethanol (15 μM) and the cysteine-proteinase inhibitor leupeptin (10 μg/ml) to the non-sedimentable fraction protected the enzyme activity so that there was no significant loss of activity during 1 week at −20 °C. These conditions were used routinely. The purified enzyme was relatively stable and retained approx. 90% of its activity when stored at 4 °C for 1 week in 0.2 M-potassium phosphate buffer, pH 7.5, with pyridoxal 5'-phosphate and 2-mercaptoethanol at the concentrations given above. Approx. 20% of the activity was lost on freezing and thawing the purified enzyme preparation.

Throughout the purification procedures, the methionine γ-lyase and homocysteine desulphurase activities were co-purified. For convenience, enzyme elution was routinely monitored by the homocysteine desulphurase assay. Anion-exchange chromatography resulted in the separation of two peaks of homocysteine desulphurase/methionine γ-lyase activity (Fig. 1*a*). The major peak corresponded to about 90% of the total activity and was eluted at a lower salt concentration than the second peak. These peaks were shown to correspond to the major and minor bands of activity separated by native PAGE (Fig. 2*b*). Only the major peak of activity was purified further. Hydrophobic-interaction chromatography of the pooled and concentrated fractions containing the major peak of activity eluted from the Mono Q column resulted in the removal of most of the contaminating proteins (Fig. 1*b*) at the expense of a poor recovery of the enzyme activity. The remaining contaminating proteins were removed and the activity was desalted by gel filtration (Fig. 1*c*), which left the enzyme in a buffer in which it was stable. Table 2 gives the purification and recoveries obtained at each step. The amount of enzyme recovered in the soluble fraction was consistently higher than was apparently present in the homogenate. Addition of $(NH_4)_2SO_4$ to the enzyme before hydrophobic-interaction chromatography caused a decrease in the amount of activity detected, and hence the increase in recovery after the gel-filtration step was due to relief from this inhibition as a result of desalting of the enzyme. The values obtained probably represent an underestimation of the amount of enzyme recovered, owing to the loss of its activity during the purification. Approx. 0.05% of the original soluble protein remained after the purification procedure. The protein was judged to be homogeneous by SDS/PAGE (Fig. 2*a*) and no additional bands were detected by using silver staining.

Physical properties

The molecular mass of the native enzyme estimated by gel filtration and native PAGE is approx. 150–180 kDa and a subunit mass of 42–45 kDa as determined by SDS/PAGE (Fig. 2*a*).

Activity of the purified enzyme was optimal in the pH range 7.5–8.0 with both homocysteine and methionine as substrates and could not be detected below pH 6.5 or above pH 9.0. The apparent K_m values under the standard conditions of the enzyme assay were 0.5 ± 0.09 mM for homocysteine and 4.3 ± 1.1 mM for methionine.

The enzyme displayed properties typical of many pyridoxal 5'-phosphate-dependent enzymes. Dialysis against buffer not containing the cofactor resulted in the loss of 50% of the activity. No re-activation by pyridoxal 5'-phosphate occurred after loss of enzyme activity during storage or dialysis. Nor was activity restored by addition of dithiothreitol. The absorption spectra of the purified enzyme are shown in Fig. 3. After resolution of the enzyme with hydroxylamine to give the apoenzyme, no absorption peak at 420 nm was exhibited and no activity could be detected. The activity was restored by more than 90% by addition of 0.1 mM-pyridoxal 5'-phosphate. The activity is also completely inhibited by 1 mM-DL-propargylglycine, an irreversible inhibitor of many pyridoxal 5'-phosphate-dependent enzymes, under the same conditions.

Substrate specificity and reaction products

The purified enzyme catalysed the $\alpha\gamma$-elimination reaction with homocysteine, ethionine and methionine (Table 3) to give the appropriate thiol, 2-oxo acid and ammonia. By h.p.l.c., it was shown that 2-oxobutyrate was the sole 2-oxo acid produced from homocysteine, and pyruvate accounted for all the expected 2-oxo acid produced from the $\alpha\beta$-elimination of cysteine. Although ammonia production from methionine could not be

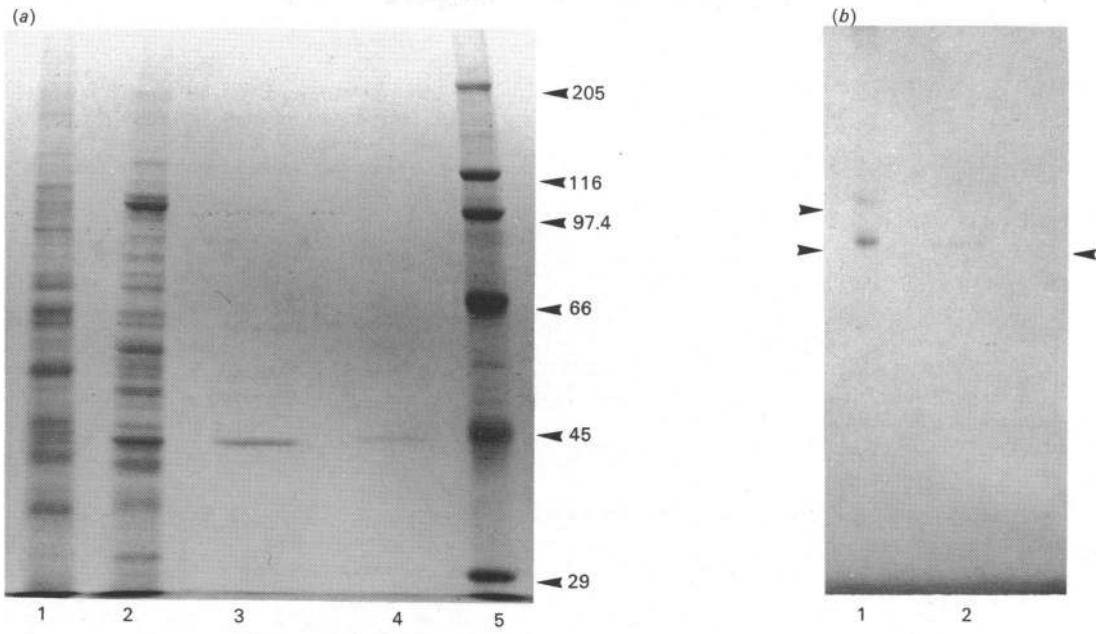

Fig. 2. PAGE analysis of different stages during the purification of methionine γ-lyase/homocysteine desulphurase

(*a*) Fractions containing enzyme activity were pooled and analysed by SDS/PAGE. The gel was stained with Coomassie Blue. Key to tracks: 1, soluble fraction (25 μg); 2, proteins eluted from Mono Q column (25 μg); 3, proteins eluted from Alkyl Superose column (2 μg); 4, proteins eluted from Superose 12 column (0.8 μg); 5, molecular-mass markers (kDa). (*b*) Native gel stained for homocysteine desulphurase activity (arrowed). Key to tracks: 1, soluble fraction (25 μg); 2, purified enzyme (0.5 μg).

Table 2. Purification of methionine γ-lyase from *T. vaginalis*

Activity was measured with homocysteine as the substrate. Specific activity is in μmol of PbS produced/min per mg of protein and total activity in μmol of PbS produced/min. The results are representative of more than 18 separate purifications. Methionine γ-lyase activity, measured as α-oxobutyrate produced from methionine, co-purified throughout the procedure.

	Volume (ml)	Total protein (mg)	Total activity	Specific activity	Purification (fold)	Recovery (%)
Homogenate	10	303	9.1	0.03	1.0	100
Soluble fraction	9	172	12.1	0.07	2.3	132
Mono Q eluate	27	3.7	4.9	1.31	43	54
Alkyl-Superose eluate	2	0.54	1.6	2.93	97	6.6
Superose 12 eluate	6	0.25	0.8	3.17	105	8.8

detected in cell-free lysates of *T. vaginalis* (limit of detection 10 nmol/min per mg of protein), the purified enzyme released ammonia in equimolar amounts to 2-oxo acid and thiols. The addition of 2-mercaptoethanol, however, caused a decrease in 2-oxobutyrate and ammonia production and an increase in thiols released by the enzyme. This suggests the occurrence of the exchange reactions between 2-mercaptoethanol and homocysteine and its *S*-substituted analogues (Table 3). The analogous reaction with cysteine and its *S*-substituted analogues also occurred (results not shown).

The D-enantiomers of methionine and cysteine are not substrates for the enzyme, nor are other L-amino acids, including norleucine, 2-aminobutyrate, norvaline, serine and cystathionine. *O*-Acetyl-L-serine is, however, a particularly good substrate for the enzyme (Table 4). Replacement of the sulphur atom of methionine and ethionine by selenium increased the activity towards these substrates; however, oxygen-substituted analogues (threonine and homoserine) were inert. The activity towards a number of *S*-substituted analogues was also determined and the results are shown in Table 4.

A range of cysteine analogues were also tested (Table 4). The disulphide L-cystine and the selenium analogue showed similar specificities to cysteine. Addition of a methyl group increased the activity 2-fold, but increasing the alkyl chain further (results not shown) did not affect the activity.

Inhibitors

The purified methionine γ-lyase activity was completely inhibited by incubation with the carbonyl reagents L-cycloserine, hydroxylamine and DL-penicillamine (all at 1 mM), which are known inhibitors of pyridoxal 5′-phosphate-dependent enzymes. It is also highly sensitive to thiol reagents and is inhibited by over

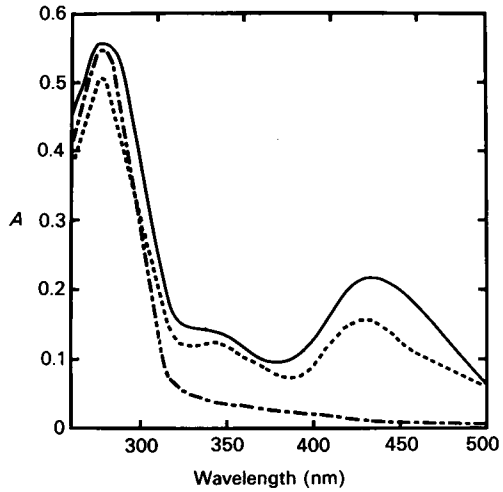

Fig. 3. Absorption spectra of L-methionine γ-lyase purified from *T. vaginalis*

——, Purified enzyme in 0.1 M-potassium phosphate buffer, pH 7.5; ----, purified enzyme dialysed against the same buffer; ---, purified enzyme after resolution to the apoenzyme with hydroxylamine.

Table 3. Rate of formation of the products of methionine γ-lyase purified from *T. vaginalis*

Activities are given as μmol of product/min per mg of protein, and are means ± S.D. from three independent determinations: ND, not detectable.

Substrate	Products		
	α-Oxo acid	Thiol	NH_3
Methionine	0.50 ± 0.08	0.36 ± 0.02	0.34 ± 0.10
Methionine + 10 mM-2-mercaptoethanol	0.21 ± 0.02	0.51 ± 0.11	0.08 ± 0.04
Homocysteine	4.1 ± 1.1	3.2 ± 0.4	2.6 ± 0.4
Homocysteine + 10 mM-2-mercaptoethanol	1.8 ± 0.6	4.0 ± 1.3	0.8 ± 0.5
Ethionine	0.70 ± 0.12	0.68 ± 0.13	0.91 ± 0.01
Ethionine + 10 mM-2-mercaptoethanol	0.18 ± 0.07	0.55 ± 0.06	ND

80% by 1 mM-iodoacetamide, 10 μM-*p*-chloromercuribenzoic acid and 0.1 mM-5,5′-dithiobis-(2-nitrobenzoic acid). These results confirm the previously published findings that were obtained with crude cell-free extracts (Thong *et al.*, 1987*b*).

A number of substrate analogues that were not hydrolysed by the enzyme were also tested for inhibition of 2-oxo acid production from methionine (Table 5). The D-enantiomers of methionine and cysteine and the L-forms of threonine and serine had no effect on activity. The L-amino acids homoserine, norleucine and 2-aminobutyrate did decrease the activity, but L-cystathionine gave very little inhibition. The most effective inhibitor was DL-homocysteic acid (Table 5).

Addition to the assay of methioninol, in which the carboxy group of methionine is replaced by an alcohol, resulted in approx. 50% inhibition. Removal of the amino group to give 4-methylthiobutan-1-ol decreased the inhibitory action only slightly, and shortening the carbon chain to 2-methylthioethanol had little additional effect. Methoxyethanol, which contains an oxygen atom in place of sulphur, was an effective inhibitor, but further decreases in the carbon chain or substitutions (e.g. methoxyacetic acid, methoxyethoxyethanol) completely abolished the inhibitory effect.

Table 4. Substrate specificity of methionine γ-lyase purified from *T. vaginalis*

Results are expressed as the activity relative to that towards methionine under the standard assay conditions. The values are means ± S.D. from at least three determinations using purified enzyme and confirm the results obtained with partially purified enzyme preparations.

Substrate	Relative activity
L-Methionine	100
L-Ethionine	144 ± 32
L-Methionine sulphone	296 ± 53
L-Methionine sulphoxide	86 ± 14
L-Methionine sulphoximine	115 ± 26
DL-Homocysteine	903 ± 114
L-Cysteine	99 ± 5
L-Cystine	108 ± 14
S-Methyl-L-cysteine	207 ± 43
O-Acetyl-L-serine	945 ± 56
DL-Selenomethionine	204 ± 16
DL-Selenoethionine	188 ± 61
DL-Selenocysteine	97 ± 15
S-Adenosylhomocysteine	38 ± 14
S-Adenosylmethionine	830 ± 126
DL-Homocysteic acid	14 ± 3
L-Djenkolic acid	174 ± 24

Table 5. Inhibitors of methionine γ-lyase purified from *T. vaginalis*

Results are given as the activity, measured as 2-oxo acid produced, in the presence of the test compound as a percentage of that in control samples and are means ± S.D. from at least three determinations using purified enzyme. Similar results were obtained with partially purified enzyme.

Compound	Activity remaining (%)
D-Methionine	96.3 ± 4.8
D-Cysteine	115.0 ± 16.3
L-Cystathionine	89.9 ± 11.1
DL-Homocysteic acid	31.1 ± 7.9
L-Homoserine	56.3 ± 8.9
L-Serine	89.0 ± 12.2
L-Threonine	97.5 ± 4.4
L-Norleucine	58.8 ± 9.7
L-Aminobutyrate	53.8 ± 8.6
Methioninol	48.5 ± 14.8
4-Methylthiobutan-1-ol	61.9 ± 5.3
2-Methylthioethanol	68.7 ± 7.8
Methoxyethanol	45.2 ± 1.9
Methoxyacetic acid	106.1 ± 21.9
3-Methylthiopropane-1,2-diol	100.6 ± 14.3
Methoxyethoxyethanol	99.5 ± 5.2

DISCUSSION

The results have shown that the homocysteine desulphurase and methionine-catabolizing activities previously described in *T. vaginalis* co-purify and are due to a single multifunctional enzyme. The enzyme catalyses the simultanous deamination and dethiomethylation of methionine and cysteine to give 2-oxobutyrate and pyruvate respectively. The only other products of the reaction that were detected were low-molecular-mass thiol compounds and ammonia; these were produced in equimolar amounts to the

2-oxo acids, as was predicted. Analysis by h.p.l.c. showed that the 2-oxo acid analogues of methionine (2-oxo-4-methiolbutyrate) or homocysteine (2-oxothiolpropionate) were not produced. These would be expected if the substrates were first deaminated as in the transamination pathway that occurs in mammalian tissues (Steele & Benevenga, 1979).

The trichomonal enzyme closely resembles the methionine γ-lyase detected in and purified from a number of bacteria. The native molecular mass of the trichomonal enzyme is approx. 160 kDa, as compared with 150 kDa for the methionine γ-lyase purified from *Clostridium sporogenes* (Kreis & Hession, 1973) and 173 kDa for the enzyme from *Pseudomonas ovalis* (Tanaka *et al.*, 1977). Most of the bacterial enzymes consist of four identical subunits; the only exception is the *Clostridium* enzyme, which was reported to comprise two pairs of subunits of different molecular mass (Kreis & Hession, 1973). The single band detected under denaturing conditions with the trichomonal enzyme is indicative of there being four identical subunits in this case.

The trichomonal enzyme is also similar to the bacterial enzymes in being unstable with time and rapidly inactivated by freezing and thawing. All of the enzymes are optimally active around neutral or slightly alkaline pH. The trichomonal enzyme is also similar to the bacterial enzymes in that all of the purified enzymes are pyridoxal 5'-phosphate-dependent and possess properties typical of this group of enzymes. They all have characteristic absorption maxima around 420 nm. This suggests that the formyl group of the bound pyridoxal 5'-phosphate forms an azomethine linkage with an amino group of the protein, as in other pyridoxal 5'-phosphate enzymes studied so far (Martinez-Carrion, 1986). They are all inhibited by carbonyl or thiol reagents and DL-propargylglycine, a suicide inhibitor.

Bacterial methionine γ-lyase catalyse αγ- and αβ-elimination and γ- and β-replacement reactions. The purified trichomonad enzyme is similarly multifunctional. Only L-amino acids are active substrates; the D-enantiomers are not substrates, nor do they competitively inhibit the activity. This indicates that the L-configuration of the α-carbon is necessary for binding of the substrate to the active site. This is true of all other methionine γ-lyases except for an enzyme from *Aspergillus*, which was reported to also catalyse the γ-elimination of D-methionine (Ruiz-Herrera & Starkey, 1969*b*). Apart from amino acids with sulphur-containing substituents, only *O*-acetyl-L-serine was active. All other amino acids tested were inert, indicating the specificity of the enzyme for the C–S or C–O rather than C–C bonds of amino acids. Norleucine and L-2-aminobutyrate both inhibit the enzyme, however, and so may bind to the active site. Both α- and γ-elimination reactions are also observed in cystathionine-cleavage reactions, catalysed by cystathionase (EC 4.4.1.1) from rat liver (Matsuo & Greenberg, 1959). The purified methionine γ-lyase of *T. vaginalis* has no activity towards cystathionine, and so is clearly distinct from the mammalian enzyme.

Although the trichomonad enzyme is similar to all of the bacterial enzymes previously desctribed, it most closely resembles the *Clostridium* enzyme in specificity towards different substrates. In particular, both these enzymes have much higher activities towards homocysteine and cysteine relative to methionine than the *Pseudomonas* enzymes.

Methionine γ-lyase has been reported to occur only in anaerobic bacteria (*Clostridium* and *Aeromonas*), in species of aerobic bacteria that are capable of utilizing methionine under anaerobic conditions (*Pseudomonas*), and in anaerobic protozoa (*T. vaginalis* and rumen ciliates). Recently we have also detected the enzyme in *Entamoeba histolytica* and other rumen ciliates (B. C. Lockwood & G. H. Coombs, unpublished work). However, it is not found in other species of *Trichomonas* (Thong *et al.*, 1987*b*), *E. invadens* or another anaerobic protozoan, *Giardia lamblia* (B. C. Lockwood & G. H. Coombs, unpublished work). Thus there is not a strict correlation between the presence of the enzyme in protozoa and their anaerobic nature. The organisms concerned, however, are not considered to be closely related phylogenetically, and it is likely that the explanation for the occurrence of methionine γ-lyase in just some species will be clearer when its functional significance has been elucidated. There is some evidence that it may be involved in energy production in *T. vaginalis* (Lockwood & Coombs, 1989).

The presence of methionine γ-lyase in *T. vaginalis* provides a good target for chemotherapeutic attack. It is envisaged that the data presented here on substrate specificity and inhibitor binding are a step towards the design of compounds that will be specifically hydrolysed by this enzyme to give a product that is toxic to *T. vaginalis*. Such prodrugs should have a high chemotherapeutic index, as the enzyme is absent from the host, and so should have potential value against *T. vaginalis* and possibly some other protozoal and bacterial infections. To obtain such compounds is the long-term aim of this project.

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