Amino Acids (1999) 17: 175–183

Amino
Acids

Synthesis and enzymology of modified *N*-benzyloxycarbonyl-L-cysteinylglycyl-3,3-dimethylaminopropylamide disulphides as alternative substrates for trypanothione reductase from *Trypanosoma cruzi*: Part 3

C. T. Yuen[1], **J. Garforth**[1], **T. Besheya**[1], **R. Jaouhari**[1], **J. H. McKie**[1], **A. H. Fairlamb**[2], and **K. T. Douglas**[1]

[1] School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester, and [2] Department of Medical Parasitology, London School of Hygiene & Tropical Medicine, London, United Kingdom

Accepted June 15, 1998

Summary. Kinetic data for alternative substrates of recombinant trypanothione reductase from *Trypanosoma cruzi* were measured for a series of *N*-substituted-L-cysteinylglycyl-3-dimethylaminopropylamides, in which the cysteine N-substituent was either a variant of the benzyloxycarbonyl group or was L-phenylalanine or L-tryptophan. Replacing the benzylic ether oxygen atom by CH_2 or NH had relatively minor effects on k_{cat}, but raised the value of K_m 4.5- and 10-fold, respectively. Similarly, relative to the carbobenzoxy group, an *N*-L-phenylalanyl or *N*-L-tryptophanyl replacement on the cysteine hardly altered k_{cat}, but increased K_m values by 16.6 and 7.4 fold, respectively. These observations were consistent with the K_m values referring primarily to binding for this series of nonspecific substrates.

Keywords: Amino acids – Trypanothione – Glutathione – Benzyloxycarbonyl-reductase – Hydrocinnamoyl

Abbreviations: DCC, *N,N'*-dicyclohexylcarbodiimide; dmapa, dimethylaminopropylamine; DMF, dimethylformamide; GR, glutathione reductase; GSSG, glutathione disulphide; GSH, reduced glutathione; $T[S]_2$, trypanothione disulphide; Hbt, hydroxybenzotriazole; TFA, trifluoroacetic acid; TLC, thin layer chromatography; $T[SH]_2$, reduced trypanothione as dithiol; TR, trypanothione reductase; Z.cys.gly.dmapa, *N*-benzyloxycarbonyl-L-cysteinylglycyl-3-dimethylpropylamide

Introduction

The discovery of a crucial metabolic difference between man and the parasitic trypanosomes offered the possibility to design molecules which would com-

promise the parasite by inhibiting its cellular redox protection mechanism (Fairlamb et al., 1985). In contrast to their mammalian hosts, trypanosomes were found not to contain glutathione reductase (for glutathione (**1**)-based redox protection), but rather depended on trypanothione (**2**) (Fairlamb et al., 1985). Trypanothione reductase (TR), because of its important role in trypanosomes to reduce trypanothione disulphide ($T[S]_2$) to its cognate dithiol $T[SH]_2$, and its substrate specificity for $T[S]_2$, is an important target for drug chemotherapy against trypanosomiasis and leishmaniasis (Shames et al., 1986; Henderson et al., 1987). Consequently, efforts are required to define potential regions of the enzyme which can serve for rational inhibitor design.

The synthesis of trypanothione substrate analogues in which the spermidine moiety, as well as the glutamyl moieties, have been replaced by other functional groups has been reported (Henderson et al., 1987; El-Waer et al., 1991; Jaouhari et al., 1995a,b). TR binds these analogues and reduces them, although not as efficiently as its natural substrate, $T[S]_2$. Replacing the spermidine moiety of trypanothione by 3-dimethylaminopropylamine (dmapa), and the γ-glutamyl moieties by benzyloxycarbonyl groups (Z) (El-Waer et al., 1991), led to an alternative substrate for TR which was 18% as active as $T[S]_2$ itself based on k_{cat}/K_m values for *T. cruzi* TR (26% for TR from *Crithidia fasciculata*). The substrate Z.cys.gly.dmapa disulphide (**3**) was proposed as a cheaper, alternative assay substrate for studies of TR (El-Waer et al., 1991). The present investigation focuses on the benzyloxycarbonyl (Z) group and its role in the processing of Z-modified disulphides by TR. In particular, the role of the benzylic oxygen atom in this Z group is probed. To do so, we synthesised two additional novel substrates for TR, in which the oxygen atom in Z was substituted by NH(**4**) and CH_2 (**5**). Kinetic studies of these substrates with TR from *T. cruzi* are now reported, along with enzyme kinetic data for *N*-phenylalanyl (**9**) and *N*-tryptophanyl (**10**) derivatives of L-cysteinylglycyl-3-dimethylaminopropylamide disulphide.

Materials and methods

Synthesis

For coupling reactions, anhydrous reactants and dry solvents were used. Aluminium oxide (active neutral, Brockmann Grade 1) was purchased from BDH Chemicals Ltd. Tlc was carried out on alumina and silica plates. Spots were made visible by UV and light or exposure to iodine vapor. Melting points were taken on a Gallenkamp apparatus with digital thermometer and are uncorrected. Z.cys.gly.dmapa disulphide **3** was prepared as described (El-Waer et al., 1991). 3-Phenylpropionyl chloride was obtained as a colourless oil by thionyl chloride treatment of 3-phenylpropionic acid (reflux, 80° for 2 h); IR (neat), 1,790 cm^{-1} ($\nu_{c=o}$); ^1H nmr ($CDCl_3$) δ_H: 7–7.4 (5H, m, Ar), 2.85–3.3 (4H, m, $C\underline{H}_2$-$C\underline{H}_2$). Derivatives **9** and **10** were from a previous study (Jaouhari et al., 1995a). NMR spectra were recorded either on Bruker WP803Y spectrometer at 80.1MHz for protons or a Jeol JNM Ex 270 spectrometer operating at 270MHz for ^1H and 68MHz for ^{13}C. Chemical shifts (δ) were reported in parts per million (ppm) relative to TMS, or the 7.25 ppm residual chloroform peak for ^1H nmr spectra. Splitting patterns are abbreviated as: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; m, unresolved multiplet. Fast atom

bombardment mass spectra (FAB-MS) were taken by means of a Kratos-Concept Instrument operating in the FAB mode (Xe-beam bombardment). Elemental analyses were performed on an EA 1108-Elemental Analyser (Carlo Erba Instruments) in the Department of Chemistry of the University of Manchester.

bis-(N-benzylaminocarbonyl)-L-cystine, **7**

To a solution of L-cystine (0.5 g, 2.08 mmol) in aqueous NaOH (3 ml), cooled to 0°C in an ice-bath, was added benzyl isocyanate (0.56 g, 4.21 mmol). The reaction mixture was stirred for 2.5 h at room temperature, and extracted with diethyl ether (3 × 50 ml). The aqueous layer was acidified cautiously to pH1 (Congo Red) with 2N HCl, the solution becoming cloudy. After 48 hours at room temperature, the precipitated white solid was filtered off, washed with ether and dried to give **7** (300 mg, 30%) R_f, 0.45 ($CHCl_3/CH_3OH$, 60/40, v/v). m.p. 245–248°C. ^1H nmr (CD_3OD) δ_H: 7–7.15 (10H, m, Ar), 4.45–4.55 (2H, m, 2xC<u>H</u>-CO), 4.15 (4H, s, 2xC<u>H</u>$_2$-NH), 2.93–3.1 (4H, m, 2xC<u>H</u>$_2$-S) ^{13}C nmr (CD_3OD) δ_c: 166.0 (COOH), 150.5 (<u>C</u>ONH), 131.0, 120.5, 118.5, 118.0 (C-Aryl), 44.0 (<u>C</u>H$_2$-C$_{arom.}$), 35.0 (<u>C</u>H-COOH), 32.4 (<u>C</u>H$_2$-S).

(N-benzylaminocarbonyl)-L-cysteinylglycyl-3,3-dimethylaminopropylamide disulphide, **4**

To a stirred solution of 7 (0.28 g, 0.55 mmol) and H-Gly.dmapa (0.19 g, 1.2 mmol, El-Waer et al., 1991) in anhydrous DMF (3 ml) was added N-hydroxybenzotriazole (0.15 g, 1.2 mmol), followed by N,N′-dicyclohexylcarbodiimide (0.23 g, 1.2 mmol). The reaction mixture was stirred for 1 hour at 0°C, and then for 24 hours at room temperature, after which the precipitate formed was filtered off. The solvent was removed from the filtrate under high vacuum for several hours at 30–40°C. The oily residue from this was dissolved in the minimum of dichloromethane and purified by neutral alumina chromatography using a mixture of CH_2Cl_2/CH_3OH (80/20, v/v) as eluent. Evaporation of the appropriate fractions (as detected by tlc) gave a sticky solid, which was triturated with hot ethyl acetate and on cooling yielded a white solid, which was filtered off, kept for 1 hour under high vacuum and identified as **4**, (100 mg, 23% yield). R_f, 0.62 ($CHCl_3$/CH_3OH, 80/20, v/v). m.p. 157–160°C. Calc. for $C_{36}H_{56}N_{10}O_6S_2$(788), C, 54.8; H, 7.10; N, 17.8. Found: C, 54.2, H. 6.9; N, 17.1. ^1H nmr (DMSO-d_6) δ_H: 8.36 (2H, 2xN<u>H</u>-CH$_2$, Gly), 7.8 (2H, t, J = 2.47, Hz 2N<u>H</u>-CH$_2$, dmapa), 7.05–7.35 (10H, m, Ar), 6.7 (2H, t, J = 1.35 Hz, 2xN<u>H</u>-CH$_2$, Z_{NH}), 6.42 (2H, d, J = 1.66 Hz, 2xN<u>H</u>-CH), 4.4 (2H, m, 2xC<u>H</u>-CO), 4.2 (4H, d, J = 1.8 Hz, 2xC<u>H</u>$_2$-CO), 3.62 (4H, d, 2xC<u>H</u>$_2$-Ar), 2.9–3.1 (8H, m, 2x C<u>H</u>$_2$-S plus 2x C<u>H</u>$_2$-NH(dmapa)), 2.15 (4H. t, J = 6.52 Hz, 2C<u>H</u>$_2$-N), 2.05 (12H, s, 2xN(C<u>H</u>$_3$)$_2$), 1.45 (4H, p, J = 5.6 Hz, 2xC<u>H</u>$_2$-CH$_2$-N). MS(FAB-Xe beam): 789(M + 1)$^+$/(100%), 395((M/2) + 1) (60%).

bis-(N-3-phenylpropionyl)-L-cysteine disulphide, **8**

To a solution of L-cystine (4.2 g, 17.4 mmol) in aqueous NaOH (30 ml) at 0°C in an ice-bath was added dropwise a toluene (30 ml) solution of 3-phenylpropionyl chloride (5.86 g, 34.8 mmol) and the reaction mixture stirred overnight at room temperature. Extraction with ether (2 × 25 ml) was carried out, and the aqueous layer acidified cautiously to pH1 with 2N HCl, which caused the solution to go cloudy. After 24 hours at room temperature, a solid precipitated, was filtered off, washed with ether and dried to give **8** (3.32 g, 36% yield). R_f, 0.26 ($CHCl_3/CH_3OH$, 60/40, v/v). m.p. 114–116°C (125–126°C: Lustig et al., 1974). ^1H nmr δ_H (CD_3OD): 6.9–7.1 (10H, m, 2Ar), 4.5–4.6 (2H, m, 2xC<u>H</u>-CO), 3.0–3.2 (4H, m, 2xC<u>H</u>$_2$-S), 2.62–2.8 (4H, m, 2xC<u>H</u>$_2$-CO), 2.18 (4H, t, J = 8.5 Hz, 2xC<u>H</u>$_2$-Ar). ^{13}C nmr (CD_3OD) δ_c: 177.0 (COOH), 173.5 (CONH), 141.0, 130.0, 129.4, (C-Aryl), 53.1 (<u>C</u>H.COOH), 41.0 (<u>C</u>H-C$_{arom}$), 38.0 (<u>C</u>H$_2$-COOH), 32.4 (<u>C</u>H$_2$-S).

bis-(N-3-Phenylpropionyl)-L-cysteinylglycyl-3,3-dimethylaminopropylamide disulphide, **5**

To a stirred solution of **8** (0.28 g, 0.55 mmol) and H-Gly.dmapa (0.19 g, 1.2 mmol, El-Waer et al., 1991) in anhydrous DMF (3 ml) was added N-hydroxybenzotriazole (0.15 g, 1.2 mmol), followed by N,N′-dicyclohexylcarbodiimide (0.23 g, 1.2 mmol). Work-up of the reaction mixture, as described for **4**, gave **5** (150 mg, 34%) R_f, 0.73 ($CHCl_3/CH_3OH$, 80/20, v/v). m.p. 160–163°C. Cacl. for $C_{38}H_{58}N_8O_6S_2$ (786): C, 58.0; H, 7.4; N, 14.3. Found: C, 57.8, H. 7.2; N, 14.0 ^{1}H nmr (DMSO-d_6) δ_H: 8.36 (2H, 2xN<u>H</u>-CH_2, Gly), 7.8 (2H, t, J = 2.75 Hz, 2N<u>H</u>-CH_2, DMAPA), 7.05–7.35 (10H, m, Ar), 6.7 (2H, t, 2xN<u>H</u>-CH_2, Z_{NH}), 6.42 (2H, d, J = 2.35 Hz, 2xNH-CH), 4.4 (2H, m, 2xC<u>H</u>-CO), 3.62 (4H, d, J = 6.8 Hz, 2xC<u>H_2</u>-Ar), 2.9–3.1 (8H, m, 2x C<u>H_2</u>-S plus 2x CH_2-NH(dmapa)), 2.65–2.80 (4H, m, 2xC<u>H_2</u>CO), 2.15 (4H. t, J = 6.56 Hz, 2x2CH_2-H), 2.05 (12H, s, 2xN(C<u>H_3</u>)$_2$), 1.45 (4H, p, J = 7.56 Hz, 2xC<u>H_2</u>-C<u>H_2</u>-N). MS(FAB-Xe beam): 787(M + 1)$^+$ (100%), 394((M/2) + 1) (36%).

Enzyme isolation and assay

Trypanothione reductase from *T. cruzi* was isolated by means of overexpression of the gene in *E. coli* JM109 cells bearing the expression vection PBSTNAV (Meinnel et al., 1988) as previously described (Benson et al., 1992). The enzyme was homogenous by the criterion of SDS PAGE and had a specific activity identical to wild-type TR (Krauth-Siegel et al., 1987). Enzyme activity was assayed at 25°C in 0.02 M HEPES buffer, pH 7.25, containing 0.15 M KCl, 1 mM EDTA, 0.12 mM TSST and 0.1 mM NADPH (Krauth-Siegel et al., 1987) at an enzyme concentration of approximately 0.3 $\mu g \cdot ml^{-1}$. Values of V_{max} and K_m were obtained by least squares non-linear regression analysis using the Enzfitter programme (distributed by Elsevier Biosoft).

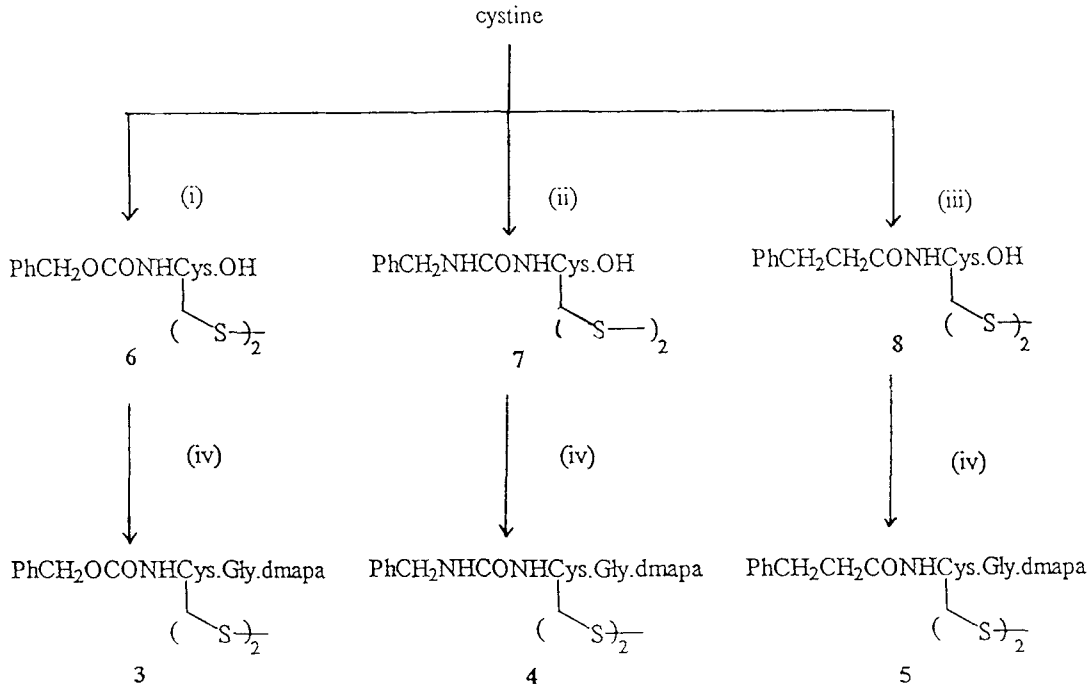
Results and discussion

The synthesis of substrate analogues **4** and **5** (Scheme 1) was based on coupling an amino-protected L-cystine and H-Gly.dmapa (El-Waer et al., 1991). Substrate **2** had already been synthesised (El-Waer et al., 1991) and substrates **7** and **8** were approached synthetically by acylation of the free amino groups in alkaline L-cystine. The protected-amino L-cystines were stable, white solids. Coupling **7** and **8** with H-Gly.dmapa was carried out using standard conditions (N-hydroxybenzotriazole/DCC) to give **4** and **5** (El-Waer et al., 1991).

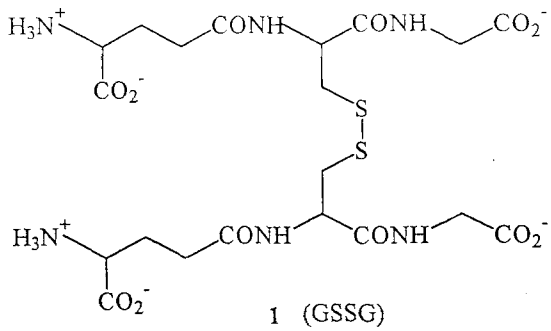
Table 1 collects kinetic parameters for **3–5** as substrates for recombinant TR from *T. cruzi*, measured at 25°C in pH 7.25 0.02 M HEPES buffer containing 0.15 M KCl, 1 mM EDTA and 0.1 mM NADPH. These compounds were not substrates of yeast glutathione reductase.

Comparison of Z.cys.gly.dmapa disulphide (**3**) with $T[S]_2$, the natural substrate, shows that the change in substrate structure led to a small change in k_{cat}/K_m, arising almost exclusively from the k_{cat} term (for TR from both *T. cruzi* and *C. fasciculata*) (El-Waer et al., 1991). This is in contrast to the effects of variation of structure of the series of R groups on the cysteinyl amino site for the series of compounds in Table 1. These compounds (**3–5**, **9**, **10**) all incorporate a major hydrophobic moiety.

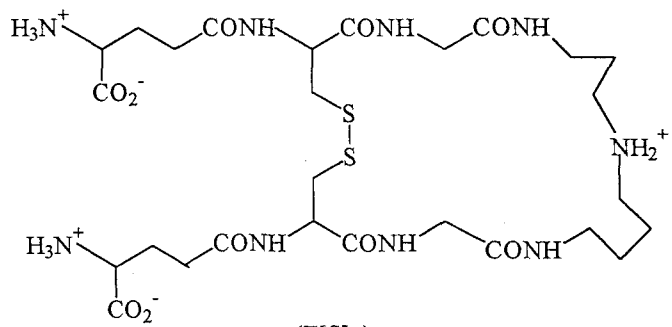
An *N*-benzyloxycarbonyl binding site has been found previously for another enzyme whose substrate involves the glutathione framework, glyoxalase I, although for that study the *N*-benzyloxycarbonyl site was located on the



Reagents and conditions: (i) $PhCH_2OCOCl$,aq. NaOH ; (ii) $PhCH_2NCO$, aq.NaOH ;
(iii) $PhCH_2CH_2COCl$, aq.NaOH (iv) H.Gly.dmapa, Hbt, DCC, DMF.

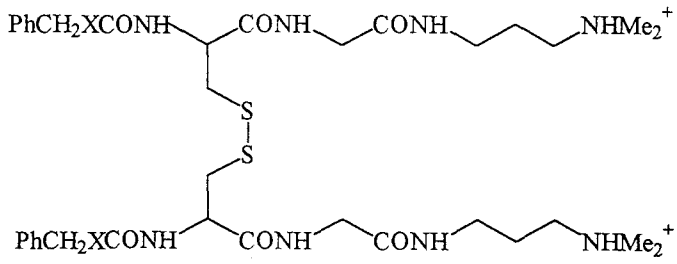


1 (GSSG)



2 (T[S]2)

Scheme 1



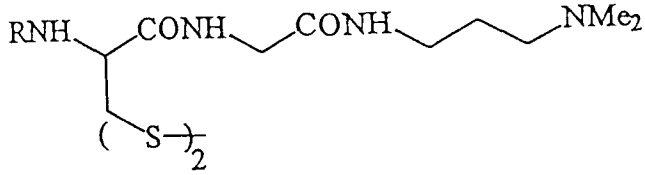
3 ; X = O

4 ; X = NH

5 ; X = CH_2

Scheme 1. Continued

Table 1. Kinetic parameters for trypanothione **2** and (*N*-substituted)-cysteinylglycyl-3,3-dimethylaminopropylamide disulphides (**3**, **4** and **5**) with recombinant *T. cruzi* trypanothione reductase at 25°C in pH 7.25 HEPES buffer (0.02 M) containing 0.15 mM KCl, 1 mM EDTA and 0.1 mM NADPH. Also included are the kinetic parameters for analogues **9** and **10** from a previous synthetic study (Jaouhari et al., 1995a)



Compound	R	k_{cat} (s^{-1})	K_m(μM)	$10^5 x k_{cat}/K_m$ ($M^{-1}s^{-1}$)
2	trypanothione[a]	214	55	39
3	$PhCH_2OCO$	4.94 ± 0.16	36.7 ± 2.8	1.35 ± 0.04
	lit.[a]	16.5	24	0.7
4	$PhCH_2NHCO$	10.5 ± 0.64	400 ± 56	0.26 ± 0.02
5	$PhCH_2CH_2CO$	3.43 ± 0.21	171 ± 30	0.20 ± 0.01
9	L-Phe	6.29 ± 0.34	610 ± 62	0.10 ± 0.01
10	L-Trp	2.35 ± 0.07	270 ± 17	0.09 ± 0.003

[a] data from El-Waer et al., 1991.

α-amino group of the glutathione (Douglas et al., 1982; Al-Timari and Douglas, 1986a). For mammalian glyoxalase I, the detailed effects of the *N*-benzyloxycarbonyl group ($PhCH_2XCO$; X = O) and its X = CH_2 and NH analogues provided evidence of coupling between binding interactions at this site and the region around the sulphur atom of the glutathione moiety i.e. in the catalytic site of the enzyme (Al-Timari and Douglas, 1986a). In yeast glyoxalase I, however, such linkage was not found (Douglas et al., 1982).

Effects on binding of *N*-acyl derivatives ($PhCH_2XCO$) of the glutathione substrate for glyoxalase II showed that for this enzyme there was little interaction between the enzyme and the *N*-acyl group (Al-Timari and Douglas, 1986b). In contrast, *N*-acylation of GSSG was found to affect both k_{cat} and K_m relative to GSSG for yeast glutathione reductase, with k_{cat} being decreased 72-fold: this was discussed in terms of possible distortion of the scissile disulphide bond of the substrate away from its optimal substrate location because of *N,N'*-disubstitution or to some non-productive binding (D'Silva and Douglas, 1983).

From Table 1, the replacement of the benzyloxycarbonyl oxygen (X=O) by NH or CH_2 has relatively minor effects on k_{cat}, but K_m is adversely affected by either change (raised 10-fold for **4** relative to **3**, and 4.5-fold for **5**). The net effect of these substitutions of X on k_{cat}/K_m are only 5–7 fold for **4** and **5**. Clearly, this region of the *Z*-group in **3** is not making crucial contacts with TR in terms of catalysis.

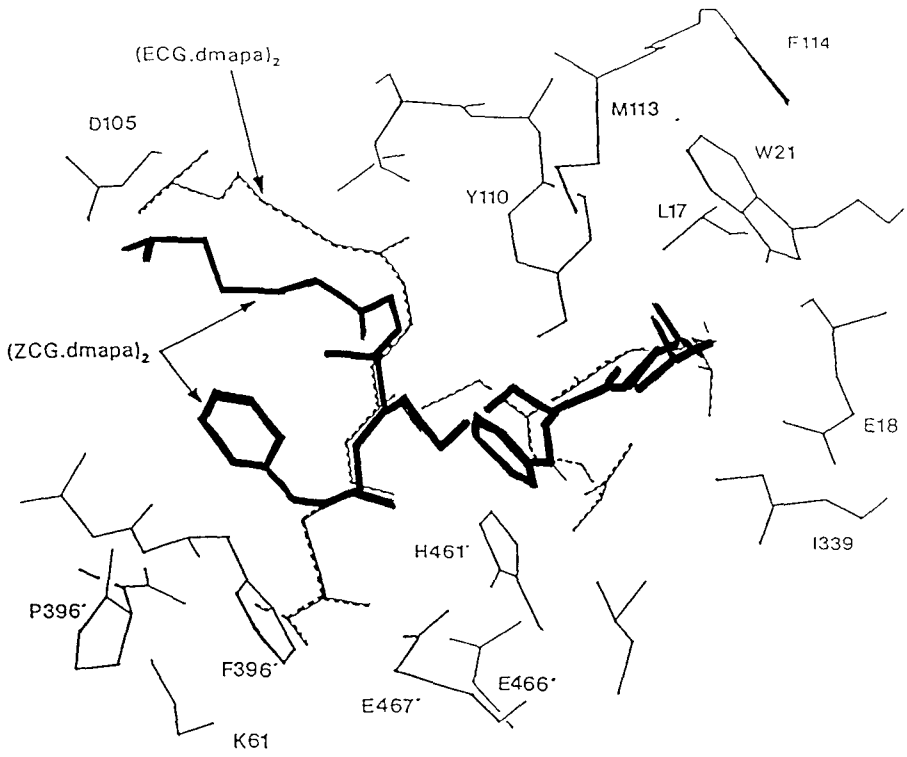


Fig. 1. The substrate-binding site of trypanothione reductase showing Z.cys.gly.dmapa disulphide [$(ZCG.dmapa)_2$], shown in bold, and L-γ-glutamyl-Lcysteinylglycyl dimethylaminopropylamide disulphide [*(ECG.dmapa)*$_2$], dotted, overlaid on one another in their proposed binding sites. The locations of the Z- and γ-glu groups are shown as orthogonal, the preferred regions for binding being separate for these two *N*-substituents. The *Z* group was predicted to bind in a hydrophobic pocket formed in part by proline-398′ and phenylalanine-396′, whereas the γ-glutamyl group in this model is in the region of glutamate-466′, glutamate-467′ and lysine-61

Also in Table 1 are the kinetic data for *T. cruzi* for symmetrical disulphides from a previous synthetic study (Jaouhari et al., 1995a). In these cases L-phenylalanine and L-tryptophan were used to replace the γ-L-Glu residues of γ-L-glu.cys.gly.dmapa disulphide. Relative to the *Z* group (**3**) these residues do not significantly alter k_{cat} but, as for **4** and **5**, the values of K_m are considerably higher. Overall the decrease in k_{cat}/K_m for **9** and **10** was only ~13-fold. As yet there is no experimental evidence that K_m refers to binding for TR substrates and so the effects of X-substitution on K_m are not easily interpretable. However, from the X-ray model of TR, the putative binding region for the benzyloxycarbonyl group is located some distance from the catalytic disulphide of TR. The region of the Z-site has been characterised using substituted phenothiazines (Chan et al., 1998). This Z-site is shown in Fig. 1 which also shows γ-glutamyl-L-cysteinylglycyl-dmapa disulphide, both superimposed into the active-site of TR indicating the potential binding site for the *N*-benzyloxycarbonyl group of Z.cys.gly.dmapa disulphide in the region of phenylalanine-396′. The simplest interpretation of the data for these alternative substrates is that K_m actually refers to binding, providing a family of structures that allows dissection and isolation of binding and catalysis for detailed kinetic studies.

Acknowledgements

We are grateful to the Wellcome Trust for support, to the Medical Research Council for a studentship (JG) and for financial support (RJ) from the UNDP/World Bank/WHO Special programme for Research and Training in Tropical Diseases.

References

Al-Timari A, Douglas KT (1986a) Inhibition of mammalian glyoxalase I (lactoylglutathione lyase) by *N*-acylated S-blocked glutathione derivatives as a probe for the role of the N-site of glutathione in glyoxalase I mechanism. Biochim Biophys Acta 870: 160–168

Al-Timari A, Douglas KT (1986b) Inhibition by glutathione derivatives of bovine liver glyoxalase II (hydroxyacylglutathione hydrolase) as a probe of the N- and S-sites for substrate binding. Biochim Biophys Acta 870: 219–225

Benson TM, McKie JH, Garforth J, Borges A, Fairlamb AH, Douglas KT (1992) Rationally designed selective inhibitors of trypanothione reductase. Biochem J 286: 9–11

Chan C, Yin H, Garforth J, McKie JH, Jaouhari R, Speers P, Douglas KT, Rock PJ, Yardley V, Croft SL, Fairlamb AH (1998) Phenothiazine inhibitors of trypanothione reductase as potential antitrypanosomal and antileishmanial drugs. J Med Chem 41: 148–156

D'Silva C, Douglas KT (1983) Photoaffinity labelling of yeast glutathione reductase by a bifunctional aryl azide derivative of oxidised glutathione. Photobiochem Photobiophys 5: 151–158

Douglas KT, Al-Timari A, D'Silva C, Gohel DI (1982) Role of the *N*-terminus of glutathione in the action of yeast glyoxalase I. Biochem J 207: 323–332

El-Waer AF, Douglas KT, Smith K, Fairlamb AH (1991) Synthesis of *N*-benzyloxycarbonyl-L-cysteinylglycine 3-dimethylaminopropylamide disulfide: a

cheap and convenient new assay for trypanothione reductase. Anal Biochem 198: 212–216

Fairlamb AH, Blackburn P, Ulrich P, Chait BT, Cerami A (1985) Trypanothione: a novel bis(glutathionyl)spermidine cofactor for glutathione reductase in trypanosomatids. Science 227: 1485–1487

Henderson GB, Fairlamb AH, Ulrich P, Cerami A (1987) Substrate specificity of the flavoproetin trypanothione disulfide reductase from *Crithidia fasciculata*. Biochemistry 26: 3023–3027

Jaouhari R, Besheya T, McKie JH, Douglas KT (1995a) Synthesis of symmetric disulphides as potential alternative substrates for trypanothione reductase and glutathione reductase: part 1. Amino Acids 7: 327–342

Jaouhari R, Besheya T, McKie JH, Douglas KT (1995b) Synthesis of asymmetric disulphides as potential alternative substrates for trypanothione reductase and glutathione reductase: part 2. Amino Acids 7: 343–351

Krauth-Siegel RL, Enders B, Henderson GB, Fairlamb AH, Schirmer RH (1987) Trypanothione reductase from *Trypanosoma cruzi* purification and characterization of the crystalline enzyme. Eur J Biochem 164: 123–128

Lustig N, Speigelstein-Klarfeld, Schneider E, Lichtenstein E (1974) Phenylacetyl and phenylpropionyl amino acids: their inhibitory effect on glutamine synthetase and their resistance to acylase. Israel J Chem 12: 757–763

Meinnel T, Mechulam Y, Fayat G (1988) Fast purification of a functional elongator tRNAmet expressed from a synthetic gene in vivo. Nucl Acids Res 16/16: 8095–8096

Shames SL, Fairlamb AH, Cerami A, Walsh CT (1986) Purification and characterization of trypanothione reductase from *Crithidia fasciculata*, a new member of the family of disulfide-containing flavoprotein reductases. Biochemistry 25: 3519–3526

Authors' address: Prof. K. T. Douglas, School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester M13 9PL, UK.

Received October 23, 1997