The thioredoxin reductase system of mycoplasmas

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Representative species of the Mollicutes possess a thioredoxin reductase system (NTS) composed of a low-molecular-mass thioredoxin (TRX) and NADPH-binding thioredoxin reductase (NTR). The TRXs of *Mycoplasma pneumoniae* and *M. capricolum* have molecular masses of 11.2 and 12 kDa, respectively, and are stable at 90 °C for 10 min. Both TRXs reacted with monospecific polyclonal antibodies generated against the *Bacillus subtilis* TRX, but not with anti-*Escherichia coli* TRX antisera. The *M. capricolum* and *M. pneumoniae* NTRs were partially purified and were found to be active with the homologous TRX, but not with the TRX of *B. subtilis* or *E. coli*. The NTS activity had an optimal pH of 6.5–7.5 and was dependent on NADPH as an electron donor, a requirement which could not be fulfilled by NADH. The genes encoding the TRX and NTR (*trxA* and *trxB*) of *M. pneumoniae* were cloned and sequenced. The comparative analysis of the predicted amino acid sequence of *trxA* showed that the 11.2 kDa protein (102 aa) shared 26–68% sequence similarity with products of other known *trxA* genes and contained the conserved active site Cys-Gly-Pro-Cys. The predicted amino acid sequence of *trxB* contained 315 residues with a conserved NADPH binding domain and FAD binding domains I and II. The cysteine dithiol redox active region had isoleucine rather than threonine at the active site, as compared with other NTRs. The high activity of the NTS in mycoplasmas suggests that mycoplasmas may have evolved the NTS to protect themselves from the consequences of their self-generated oxidative challenge.

Keywords: thioredoxin, NADP-thioredoxin reductase, *Mycoplasma capricolum*, *Mycoplasma pneumoniae*, Mollicutes

INTRODUCTION

Thioredoxins (TRXs) are widely distributed in eukaryotic cells and are also found in prokaryotes (Gleason & Holmgren, 1988; Buchanan *et al.*, 1994). In the NADP⁺/thioredoxin system (NTS), the low-molecular-mass TRX proteins undergo reversible reduction which is linked to NADPH via a flavin enzyme, NADPH-thioredoxin reductase (NTR; Gleason & Holmgren, 1988). TRX and NTR catalyse the transfer of electrons from NADPH to disulfides in proteins and low-molecular-

mass compounds. TRX contains a pair of vicinal redox-active cysteine thiols (-CGPC-) that undergo reversible oxidation to form a disulfide bridge. Oxidized TRX is reduced by NTR which possesses a similar dithiol redox active site. In *Escherichia coli*, NTR is a dimeric protein with two identical subunits (Luthman & Holmgren, 1982); the flavin moiety (FAD) mediates transfer of electrons from NADPH to the redox-active site. Evidence from a previous study suggests that the NADP-thioredoxin system occupies a central position in controlling the division and proliferation of prokaryotes (Gleason & Holmgren, 1988). Principal sites of TRX action in eukaryotes lie at the levels of transcription (Matthews *et al.*, 1992; Schenk *et al.*, 1994), DNA replication (Buchanan *et al.*, 1994) and hormone action (Holmgren, 1979a). In addition to its regulatory activity, current evidence suggests that the NTS plays a role in

Abbreviations: DTNB, 5,5'-dithio-bis(1-nitrobenzoic acid); mBBBr, monobromobimane; NTR, NADPH-thioredoxin reductase; NTS, NADP⁺-thioredoxin reductase system; TRX, thioredoxin.

The GenBank accession numbers for the nucleotide sequences reported in this paper are U51987 (*trxA*) and U51988 (*trxB*).

correcting the effects of oxidative stress on proteins, maintaining them in their active state (Fernando *et al.*, 1992; Derman *et al.*, 1993) and may be involved in protein folding through the proper organization of disulfide bonds during the folding process (Pigiet & Schuster, 1986). While there is a wealth of information on the NTS in eukaryotes, much less is known about prokaryotes, where most studies have been performed with *E. coli* (Gleason & Holmgren, 1988) and with *Streptomyces clavuligerus* (Cohen *et al.*, 1993, 1994; Aharonowitz *et al.*, 1993).

Mycoplasmas (class Mollicutes) are the smallest and simplest self-replicating prokaryotes. They are widely distributed in nature as parasites and pathogens of man and other animals, insects and plants. The fact that mycoplasmas have the smallest genome of any living organism, are devoid of a rigid cell wall and are bound by a single membrane has made them attractive and useful tools for investigating basic problems in biology. The present study was prompted by the finding that the activity of a key 55 kDa phosphoprotein kinase present in a variety of mycoplasmas is very sensitive to the redox state of its cysteine residues (Z. Borovsky & S. Rottem, unpublished data) and activity can be detected only in the presence of excess thiol reagents. While looking for an enzyme system that would replace the need for thiol reagents, an NTS was detected and is thus implicated in keeping cysteines of mycoplasmal proteins in the reduced state. The aim of this study was to describe and characterize the NTS in two representative mycoplasmas, *M. capricolum*, a fast growing mycoplasma widely used in physiological studies and *M. pneumoniae*, a well-established human pathogen.

METHODS

Growth conditions and the isolation of cell fractions. *M. pneumoniae* (M129), *M. fermentans* and *M. penetrans* were grown in a modified Channock medium (Razin & Rottem, 1976) supplemented with 20% (v/v) inactivated horse serum (56 °C for 30 min). *M. capricolum*, *M. gallisepticum* (A5969) and *Acholeplasma laidlawii* were grown in a modified Edward medium (Razin & Rottem, 1976) supplemented with 4% (v/v) inactivated horse serum. *Spiroplasma melliferum* and *Sp. floricola* were grown in a modified Saglio medium (Shirazi *et al.*, 1995). The cultures were inoculated with a frozen inoculum at a level of 1–5% and then grown without aeration for 24–48 h. The *Mycoplasma* and *Acholeplasma* species were grown at 37 °C whereas the *Spiroplasma* species were grown at 32 °C. Growth was monitored by measuring the OD₆₄₀ of the culture and by recording pH changes in the growth medium. Cells were harvested by centrifugation at 12000 g for 15 min. The cells were washed twice in 0.25 M NaCl containing 10 mM Tris/HCl (pH 7.5) and incubated in the NaCl/Tris solution containing 15 mg digitonin ml⁻¹ for 15 min at 37 °C. The cells were then disrupted by ultrasonic treatment for 2–5 min in a W-350 Heat System Sonifier operated at 50% duty cycles at 160 W. Membranes were separated from the soluble fraction by centrifugation at 37000 g for 30 min. The supernatant was stored at -70 °C until used.

Purification of NTR and TRX. NTR and TRX were first fractionated by ammonium sulfate precipitation. Protein

fractions sedimented at 50–80% ammonium sulfate saturation (1 ml containing 5 mg protein ml⁻¹) were further purified by affinity chromatography on a blue Sepharose (Sigma) column (5 × 20 mm) prewashed with 10 ml 10 mM sodium phosphate buffer (pH 7.5). The column was eluted by a stepwise gradient of 0–1 M NaCl in sodium phosphate buffer and excess salts were removed by dialysis (2 h at 4 °C against sodium phosphate buffer).

Enriched TRX preparations of *M. capricolum* and *M. pneumoniae* were obtained by heating the 50–80% ammonium sulfate fraction to 80 °C for 15 min. TRX-enriched preparations of *Bacillus subtilis* or *E. coli* were obtained by heating the total soluble fraction to 80 °C for 15 min. The heated preparations were centrifuged at 37000 g for 10 min, and the TRX-enriched supernatant was separated from the sediment containing denatured proteins.

NTS activity. NTS activity was assayed using 5,5'-dithio-bis(1-nitrobenzoic acid) (DTNB; Holmgren, 1977). The reaction mixtures, in wells of an ELISA microplate, contained (in a total volume of 200 µl) 50 mM Tris/HCl (pH 8.0), 1 mM EDTA, 0.02 mM DTNB (5 µl of an ethanolic solution of 4 mg DTNB ml⁻¹), 10 µl of a TRX-enriched preparation (10–20 µg) and 10 µl of the partially purified NTR (5–50 µg). The reaction was started by the addition of 0.2 mM NADPH and activity was determined at 37 °C by measuring the increase in A₄₀₅ (ΔA₄₀₅) during the initial reaction period (2–5 min) using an ELISA plate reader and expressed as ΔA₄₀₅ min⁻¹ (units) or as ΔA₄₀₅ min⁻¹ (mg protein)⁻¹ (sp. act. units).

NTS activity was also assayed by following the reduction of insulin disulfides in the presence of NADPH (Holmgren, 1979b). Reaction mixtures (in a total volume of 120 µl) contained 260 mM HEPES buffer (pH 7.6), 10 mM EDTA, 2 mM NADPH, 1 mM bovine insulin and preparations containing TRX and NTR. The reaction mixtures were incubated at 37 °C and stopped by the addition of 0.5 ml of a solution containing 2 mg DTNB and 6 M guanidine.HCl (pH 8.0). The reduction resulted in the precipitation of the β-chains and thus turbidity in the reaction mixture. Activity was determined spectrophotometrically.

Analytical methods. Protein in the cell fractions was determined by the method of Bradford (1976). Labelling cell proteins by monobromobimane (mBBBr) was performed by a modification of a procedure described by Wong *et al.* (1995). In brief, 20 µl 2.0 mM mBBBr in 100 mM Tris/HCl (pH 7.9) was added to 175 µl *M. capricolum* cell suspensions (containing 5 mg cell protein ml⁻¹) harvested at the various phases of growth and incubated at room temperature for 15 min. The cells were then solubilized by the addition of 200 µl 2% SDS in 10% glycerol containing 0.001% bromophenol blue and analysed by SDS-PAGE (Laemmli, 1970). The gels (16%, 1.5 mm thick) were developed for 16 h at a constant current of 9 mA. The gels were then placed in 12% (w/v) trichloroacetic acid and soaked for 4–6 h with one change of solution to fix the proteins; gels were then transferred to a solution of 40% methanol/10% acetic acid for 8–10 h to remove excess mBBBr and visualized under UV light (365 nm).

For immunoblotting, the SDS-PAGE gels were washed three times for 10 min in distilled water and proteins were transferred to nitrocellulose paper (Wong *et al.*, 1995). The blot was then blocked by soaking twice for 30 min in 200 ml of a buffer consisting of 20 mM Tris/HCl (pH 7.5) and 0.15 M NaCl (TBS buffer) containing 5% non-fat dried milk. The blocked blot was incubated for 4 h with a 1:500 dilution of rabbit antiserum generated against *E. coli* and *B. subtilis* TRX

in 5% non-fat dried milk in TBS buffer and then washed twice for 15 min with TBS buffer. Goat anti-rabbit IgG (alkaline phosphatase conjugate, Bio-Rad) was utilized to probe the blots. The alkaline phosphatase conjugate was diluted 1:3000 with 5% non-fat milk in TBS buffer and incubated with the blots for 1 h. The blots were then washed twice in TBS buffer and developed with the BCIP/NBT colour development solution (Bio-Rad).

Identification of genes and cloning. *M. pneumoniae* M129 was the source of genomic DNA used for amplification by PCR. Unless otherwise stated a cosmid library (Wenzel & Herrmann, 1989) comprising the complete genome of *M. pneumoniae* M129 was used for subcloning of genes and Southern blot analysis of *M. pneumoniae* DNA. The cosmids of the ordered *M. pneumoniae* gene library were individually digested with *Eco*RI. The fragments were separated by agarose gel electrophoresis and analysed using standard protocols for Southern blotting (Sambrook *et al.*, 1989). Sets of degenerate oligonucleotides derived from conserved regions were used as *trxA* and *trxB* gene-specific probes. Fragments which gave a positive signal under the most stringent hybridization conditions were subcloned from the corresponding cosmid in the plasmid vector pBC (Stratagene). As an alternative to subcloning from cosmid DNA, regions of interest were amplified by PCR from genomic *M. pneumoniae* DNA and then cloned either in the plasmid pBC or bacteriophage M13 DNA.

Oligonucleotides. The oligonucleotides were synthesized using phosphoramidite chemistry with a solid carrier on a model 364 DNA/RNA synthesizer from Applied Biosystems (Caruthers, 1982; Ferretti *et al.*, 1986). The oligonucleotides o.THR1 [5' GA(CT)TT(CT)TG(GA)GC(ACG)GA(GA)TG-(GA)TG], o.THR2 [5' GA(CT)TT(CT)TG(GA)GC(TA)GA-(GA)G(GA)TG], o.THRSE1 [5' TG(CT)GC(CG)AC(AGG-T)TG(CT)GA(CT)CG] and o.THRSE2 [5' TG(CT)GC(TA)-AC(AGCT)TG(CT)GA(CT)GG] are *trxA* and *trxB* gene-specific probes. o.3605 (5' GCAACAATGCGAACTCCT-GA) and o.4257 (5' GGGTATCCTGGTTTTCGGGTCAC) were used for amplification of the *trxB* gene.

Long range PCR. The improved PCR method for the amplification of DNA fragments up to 45 kbp was used (Cheng *et al.*, 1994). The reactions were performed with the Gene Amp XLPCR kit (Perkin-Elmer) according to the manufacturer's protocol. Genomic *M. pneumoniae* DNA used for amplification was purified as described previously (Wenzel & Herrmann, 1988). The PCR products were purified by agarose gel electrophoresis on 0.5–1% agarose gels.

Hybridization with oligonucleotides. Nylon filters were preincubated for at least 4 h at 37 °C in a mixture containing 5 × SSC (1 × SSC is 150 mM NaCl in 15 mM sodium citrate, pH 7.2), 10 × Denhardt's solution (1 × Denhardt's solution is 0.02% Ficoll, 0.02% polyvinylpyrrolidone and 0.02% bovine serum albumin), 0.05% SDS and 100 µg denatured herring sperm DNA ml⁻¹. For hybridization, the labelled preheated (65 °C for 5 min) probe was mixed with the same buffer as used for prehybridization and adjusted to 5 × 10⁵ c.p.m. ml⁻¹ (0.1–0.5 pmol). Filters were hybridized overnight at 37 °C. Depending on the length and G + C content of the oligonucleotide between 0 and 25% formamide was added to the hybridization solution to optimize the conditions. The melting temperatures for the oligonucleotides with the highest and lowest G + C content were estimated and these values used for calculation of the formamide concentration in the hybridization mixture (Wenzel & Herrmann, 1988). After hybridization, the filters were washed twice for 20 min with 5 × SSC at 37 °C and 0.05% SDS, dried and autoradiographed.

DNA sequencing and computer-assisted analysis. The sequence data in this study were generated by the enzymic dideoxy chain-termination method described by Sanger *et al.* (1977). The radioactive label was substituted by a fluorescent label and *Taq* polymerase was used in the reaction (Ansoorge *et al.*, 1987). Computer analyses were done with the program package HUSAR (Heidelberg Unix Sequence Analysis Resources) release 4.0 at the German Cancer Research Centre, Heidelberg, Germany. This package is based on the GCG program package version Unix-8.01 of the Genetics Computer Group, Wisconsin. Multiple alignment was performed with the program package LASERGENE (Dnastar). Percentage divergence was calculated by comparing sequence pairs in relation to the phylogeny reconstructed by the program MEGALIGN (Dnastar). The similarity index compares sequences directly, without accounting for phylogenetic relationships.

RESULTS AND DISCUSSION

NTS activity in Mollicutes

The NTS activity of nine mollicutes was tested by following the reduction of DTNB in the presence of NADPH. High activities (0.09–0.25 sp. act. units) were found in the *Mycoplasma* species *M. capricolum* (kid), *M. pneumoniae* (M129), *M. gallisepticum* (A5969), *M. fermentans* and *M. penetrans*, whereas the activities found in *A. laidlawii*, *Sp. melliferum* and *Sp. floricola* were low (0.008–0.035 sp. act. units). All subsequent experiments were performed with *M. capricolum* and *M. pneumoniae*. HPLC analysis of mBBR derivatives of low-molecular-mass thiols obtained from *M. capricolum* and *M. pneumoniae* extracts revealed that these organisms lack glutathione, but contain substantial levels of another unidentified thiol metabolite (R. C. Fahey & S. Rottem, unpublished data). Furthermore, these organisms lack glutathione reductase (Ben-Menachem *et al.*, 1994), suggesting that the thiol metabolites are maintained in their reduced form mainly through the NTS.

Fig. 1. The effect of culture age on NTS activity. □, OD₆₄₀; ●, specific activity.

Fig. 2. Changes in fluorescence intensities of *M. capricolum* proteins upon ageing. *M. capricolum* cells were harvested at various times and labelled by the SDS-PAGE/mBBR procedure (Wong *et al.*, 1995). Lanes: A, 18 h, OD₆₄₀ 0.25; B, 22 h, OD₆₄₀ 0.40; C, 26 h, OD₆₄₀ 0.42; D, 32 h, OD₆₄₀ 0.30. Arrows point to bands with decreased labelling intensities.

NTS activity and the redox state of proteins during growth

Fig. 1 shows the results of a representative experiment on the changes in NTS activity of *M. capricolum* cells during growth. Highest activity was found in cells harvested at the mid-exponential phase of growth, whereas at the stationary phase of growth, the activity was very low. Hence, mid-exponential phase cells were utilized throughout this study. Using the *in vivo* mBBR labelling technique, we obtained evidence for a change in the redox state of several *M. capricolum* cell proteins upon ageing. When intact cells, harvested at different phases of growth, were labelled with mBBR immediately after harvest the major change observed was a progressive decrease in mBBR binding to a 29 kDa and a 31 kDa protein upon ageing of the culture (Fig. 2). The relative amounts of the 29 kDa and 31 kDa proteins in stationary-phase cells and early-exponential-phase cells were apparently the same as suggested by the intensity of the Coomassie-blue-stained bands observed after SDS-PAGE analysis (data not shown). Therefore, it is suggested that the decrease in the redox state of these proteins upon ageing may be due to a decrease in the NTS activity (see Fig. 1).

The NTS was further analysed by following the reduction of the S-S bonds joining the α and β insulin chains (Holmgren, 1979b). The extent and kinetics of the insulin reduction in various cell preparations was similar to that obtained with the DTNB reduction assay

Fig. 3. The effect of divalent cations on *M. capricolum* NTS activity. ●, Mg²⁺; ◆, Ca²⁺; ▼, Zn²⁺; ▲, Fe²⁺; ■, Cu²⁺. All divalent cations were added as chlorides. The results presented are the means of three experiments done with different batches of cells.

(data not shown). The NTS activity was confined to the soluble fraction of the cells and was dependent on NADPH as an electron donor, a requirement which could not be fulfilled by NADH (data not shown). The optimal pH for activity was 6.5–7.5. Fig. 3 shows the effect of various cations on *M. capricolum* NTS activity. Whereas Ca²⁺ and Mg²⁺ had very little effect, Mn²⁺ (data not shown), Zn²⁺, Cu²⁺ and Fe²⁺ at concentrations of 1–4 mM inhibited the activity. NTS activity was markedly stimulated by high concentrations of monovalent cations, with highest activities observed at concentrations of Na⁺, K⁺ or NH₄⁺ greater than 150 mM (data not shown).

The partial purification of NTR and TRX

Partial purification of *M. capricolum* NTR was obtained by ammonium sulfate precipitation followed by affinity chromatography using a blue Sepharose column (Table 1). Very similar results were obtained with the *M. pneumoniae* NTR. The blue Sepharose step separated the TRX from the NTR. The TRX was exclusively eluted in the void volume whereas the NTR was eluted by 100 mM NaCl in 10 mM sodium phosphate buffer (pH 7.5). Therefore, the NTR-enriched blue Sepharose fractions did not show NTS activity unless an enriched TRX preparation, obtained by heating the 50–80% ammonium sulfate fraction to 80 °C for 15 min was added to the reaction mixture. The yield of activity at this stage was 80–85% and a purification of about 30-fold was obtained (Table 1). The partially purified *M. capricolum* and *M. pneumoniae* NTRs were active with

Table 1. Partial purification of the NTR of *M. capricolum* NTS

NTS activity was measured in reaction mixtures containing 5–20 µg protein of the various preparations.

Preparation	Protein (mg)	Total activity (units)	Yield (%)	Specific activity [units (mg protein) ⁻¹]	Purification (-fold)
Crude soluble fraction	32.40	7.09	100.0	0.22	0
50–80% Ammonium sulfate fraction	6.75	9.48	133.0	1.40	6.4
Blue Sepharose column*	1.00	6.00	84.5	6.00	27.4

* Activity was measured in the presence of an enriched *M. capricolum* TRX preparation (10 µg protein).

Fig. 4. Antigenic cross-reactivity of *M. capricolum* and *M. pneumoniae* TRX preparations with *E. coli* and *B. subtilis* TRX. Partially purified TRX preparations from *M. capricolum* (a) and *M. pneumoniae* (b) were separated on a 10–20% SDS-polyacrylamide gel, transferred to nitrocellulose and immunoblotted with either rabbit anti-*E. coli* TRX antibodies (1) or rabbit anti-*B. subtilis* TRX antibodies (2).

homologous TRX preparations, but almost no activity was obtained with the TRX of *E. coli* or *B. subtilis* (data not shown).

SDS-PAGE analyses of partially purified TRX preparations on a 10–20% acrylamide gel in the presence of 0.1 M tricine revealed one major band with an apparent molecular mass of 12 kDa in *M. capricolum* and three bands with apparent molecular masses of 10, 11.5 and 14 kDa, respectively, in *M. pneumoniae*. N-terminal analysis of the *M. capricolum* 12 kDa band revealed an amino acid sequence of ADIHKIXSKEQFDKE, in complete agreement with the deduced amino acid sequence

of the *M. capricolum* *trxA* gene (Bork *et al.*, 1995). When the partially purified TRX preparations, separated by SDS-PAGE, were blotted onto nitrocellulose and probed with polyclonal antisera raised against *E. coli* and *B. subtilis* TRX, the antisera against *B. subtilis* reacted strongly with the 12 kDa protein in *M. capricolum* and with the 11.5 kDa protein in *M. pneumoniae*. No reaction was observed with antisera against *E. coli* TRX (Fig. 4).

Cloning and nucleotide sequence of the *trxA* gene

Southern blot analysis of the *EcoRI*-digested cosmid gene bank of *M. pneumoniae* with oligonucleotides o.THR1 and o.THR2 detected a 6.7 kbp *EcoRI* fragment. This fragment was present in the overlapping cosmids pcosMPK8 and pcosMPA65. After subcloning into the plasmid pBC the entire 6.7 kbp DNA fragment was sequenced.

The nucleotide sequence contained an ORF predicting a protein of 102 aa which shared significant homologies with TRXs from other species (Fig. 5). In particular, the characteristic cysteine dithiol redox-active region (-CGPC-) was conserved. The active segment in the *M. pneumoniae* TRX is located at positions 30–33. The calculated molecular mass for the translation product of the *trxA* gene was 11215 Da. This was in good agreement with the molecular mass of TRXs of other species and with the results obtained by immunoblot analysis (Fig. 4). The sequence analysis of the regions flanking the *trxA* gene did not detect an ORF encoding a *trxB* gene, indicating that, unlike in *Strep. clavuligerus* (Cohen *et al.*, 1993), the *trxB* and *trxA* genes are not organized in an operon. This resembled the organization of *E. coli* (Lim *et al.*, 1985), *Haemophilus influenzae* (Fleischmann *et al.*, 1995) and *M. genitalium* (Fraser *et al.*, 1995).

The multiple protein sequence alignment of *M. pneumoniae* TRX with some other known TRXs revealed similarity of 26–68% (Fig. 5). The highest score was obtained with *M. genitalium*. Despite the almost identical degree of similarity between *M. pneumoniae* TRX and the TRXs of *B. subtilis* and *E. coli* (34 and 31% respectively), antisera raised against *B. subtilis* TRX bound to *M. pneumoniae* TRX, whereas antisera raised

notion is supported by the corresponding sequence of the NTR of *Saccharomyces cerevisiae* where the threonine is replaced by valine (Fig. 6). The sequence variation in the dithiol redox-active region of *M. pneumoniae* may explain the failure of oligonucleotides o.THRSE1 and o.THRSE2 to bind as both were derived assuming the conserved amino acid sequence CATCDG.

The possible role of NTS in mycoplasmas

It has previously been shown that *M. pneumoniae*, as well as other pathogenic *Mycoplasma* species, produce reactive forms of oxygen such as hydrogen peroxide, superoxide radicals and hydroxy radicals (Lynch & Cole, 1980; Tryon & Baseman, 1992). It has been suggested that such reactive molecules cause injury to host cells by oxidizing membranes lipids as well as oxidizing host cell proteins, thus playing a major role in the pathogenesis of these organisms (Almagor *et al.*, 1984). The complete nucleotide sequence of *M. genitalium* and *M. pneumoniae* genomes (Fraser *et al.*, 1995; Himmelreich *et al.*, 1996) show that these mycoplasmas appear to lack catalase, peroxidase or oxygen dismutases known to function in the removal of the deleterious hydrogen peroxide or oxygen radicals. Nonetheless, these organisms seem to be resistant to the effect of oxidative molecules (Tryon & Baseman, 1992). It is likely that mycoplasmas have evolved other mechanisms to protect themselves from the consequences of their self-generated oxidative challenge. We suggest that the NTS described in the present study may function as a detoxifying system protecting mycoplasmas from reactive oxygen compounds. Such protection can be achieved either by direct catalysis of the reduction of protein disulfides and/or by maintaining intracellular pools of low molecular mass thiol metabolites in their reduced form.

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