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On the occurrence of thioredoxin in *Trypanosoma cruzi*☆

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Abstract

The full coding sequence for thioredoxin from *Trypanosoma cruzi* (TcTRX) strain Tulahuen O has been cloned into the pRSETA vector. The protein was expressed in *Escherichia coli* with an N-terminal extension of six histidine residues for purification through metal ion chromatography. The biological activity of recombinant TcTRX was proved utilizing the insulin reduction assay. Amino acid sequence alignment indicates a high identity of TcTRX with thioredoxins from different sources. Immunocytochemistry assays showed that TcTRX is present in epimastigote forms of *T. cruzi*, thus, indicating that the gene is expressed in vivo, rather than being a pseudogene. The in vivo occurrence of TcTRX points out the necessity of considering this protein as a molecular component of the redox metabolism in trypanosomatids.

Keywords: *Trypanosoma cruzi*; Trypanosomatids; Redox metabolism; Thioredoxin

1. Introduction

Trypanosoma cruzi is the causative agent of Chagas' disease. As aerobic parasites, all trypanosomatids, are inevitably exposed to several reactive oxygen species (ROS), namely superoxide anions, hydrogen peroxide, and myeloperoxidase products. These chemical species are generated during the host defense reaction and also as byproducts of the aerobic metabolism. The ability of trypanosomatids to cope with such oxidative stress conditions appears oddly weak. Trypanosomatids possess an iron-containing superoxide dismutase (Le Trant et al., 1983) to scavenge phagocyte-derived superoxide anions, whereas they lack catalase and their glutathione peroxidase-like system exhibits low efficiency (Hillebrand et al., 2003; Schlecker et al., 2005). Catalase- and selenocysteine-containing glutathione peroxidases are the major hydroperoxide metabolizing enzymes in host organisms (Chance et al., 1979; Mezzetti et al., 1990).

In members of the family Trypanosomatidae, peroxide metabolism mainly involves a glutathionyl derivative of spermidine, trypanothione [N^1,N^8-bis (glutathionyl)-

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spermidine, $T(SH)_2$]. A system involving three distinctive oxidoreductases is able to catalyze the $T(SH)_2$-dependent hydroperoxide removal. These enzymes are trypanothione reductase (TR), homologous to glutathione reductase (Fairlamb and Cerami, 1992; Krauth-Siegel et al., 1987); a thioredoxin-related protein called tryparedoxin (TXN); tryparedoxin peroxidases (TXNPx), peroxiredoxin-type, or glutathione peroxidase-type proteins (Hillebrand et al., 2003; Nogoceke et al., 1997; Reckenfelderbaumer et al., 2000; Wilkinson et al., 2000a,b). It has been proposed that in trypanosomatids these redox components operate instead of the specific thioredoxin (TRX)-thioredoxin reductase (TRXR) system, found in different organisms (Fairlamb and Cerami, 1992). In fact, TRXR seems to be absent in these microorganisms (Hirt et al., 2002; Muller et al., 2003).

Classical TRXs have been characterized in several parasites: *Plasmodium falciparum* (Kanzok et al., 2002); *Fasciola hepatica* (Salazar-Calderon et al., 2001); *Schistosoma mansoni* (Alger et al., 2002). Also, TRX was identified and fully characterized in *Trypanosoma brucei brucei* (Reckenfelderbaumer et al., 2000; Schmidt et al., 2002; Schmidt and Krauth-Siegel, 2003). The presence of a gene encoding a putative TRX has been revealed by the genome sequencing project of *Leishmania major* (Myler et al., 1999). No evidence for the occurrence of TRX in *T. cruzi* is available, an issue being of relevance to fully understand the redox metabolism of this parasite. The *T. cruzi* CL Brener database (http://tcruzidb.org, http://www.genedb.org) show a putative *trx* gene. Here, we present results that add value to this database, reporting the cloning of a full gene (*trx*) coding for TRX from *T. cruzi* (Tulahuen O), its expression in *Escherichia coli*, and the chromatographic purification of the recombinant protein. Furthermore, the presence of TRX in epimastigote forms is evidenced immunologically.

2. Materials and methods

2.1. Parasites

Epimastigote forms of *T. cruzi* Tulahuen O strain were grown in CIEN medium (Barrios et al., 1980) at 28 °C. Collected parasites were washed three times with phosphate buffered saline (PBS), pH 7.4. Total RNA of *T. cruzi* Tulahuen O epimastigotes was isolated by using a commercial kit (SV total RNA isolation system, from Promega), whereas genomic DNA was prepared by standard methods (Maniatis et al., 1982).

2.2. Bacteria and plasmids

E. coli Top 10 F′ cells (Invitrogen) were used for cloning. The strain *E. coli* BL21 (DE3) was utilized in routine plasmid construction and expression experiments. The vector pGEM-T Easy (Promega) was selected for cloning and sequencing purposes. The expression vector was pRSET A (Invitrogen). DNA manipulations, *E. coli* cultures and transformations were performed according to standard protocols (Maniatis et al., 1982).

2.3. Amplification of the TcTRX gene

Specific oligonucleotide primers were designed from the *T. cruzi* CL Brener database (http://tcruzidb.org, http://www.genedb.org). The forward primer contained a *Bam*HI site and overlapped the 5′-end of the coding sequence (5′-GGATCCATGCCAGTGGTGGATGTGTACAGC-3′), and the reverse primer overlapped the 3′-end of the coding sequence and contained a *Hind*III site (5′-AAGCTTTTAACTATTGCTAATGATTTCCC-3′). A gene putatively encoding TRX in *T. cruzi* (*tctrx*) was amplified by PCR using genomic DNA of *T. cruzi* Tulahuen O (94 °C, 10 min; 94 °C, 1 min; 65 °C, 1 min; 72 °C, 2 min; 35 cycles; 72 °C, 10 min). The amplified DNA was cloned into the pGEM-T Easy vector and used to transform competent *E. coli* TOP 10 F′, as well as for sequencing. Total RNA (1.5 μg) from *T. cruzi* was reverse transcribed using a SuperScript™ II reverse transcriptase (Invitrogen) and amplified using a Taq DNA polymerase (Invitrogen). The reverse gene-specific primer was used for first strand cDNA synthesis and the forward gene-specific primer for the following amplification. The PCR products were electrophoretically separated on 2% agarose gel.

2.4. Southern blot

Genomic DNA from *T. cruzi* (10 μg) was digested with each of the following restriction enzymes (Promega): *Bam*HI, *Hind*III or *Eco*RI. The resulting restriction fragments were separated by 2% submerged agarose gel electrophoresis (SAGE) for 80 min at 90 V in standard TBE buffer, and subsequently blotted onto Hybond™-N+ membrane (Amersham Pharmacia Biotech) by capillary transfer. The DNA was cross-linked to the membrane by UV irradiation. Southern hybridization was performed according to "The DIG System User's Guide for Filter Hybridization" (Boehringer Mannhein). DIG-labeled *tctrx* gene was used as probe for hybridization assay.