Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental Parasitology 111 (2005) 198–206
Experimental Parasitology
www.elsevier.com/locate/yexpr

Research brief

Trypanosoma cruzi: Sequence polymorphism of the gene encoding the Tc52 immunoregulatory-released factor in relation to the phylogenetic diversity of the species ☆

Bruno Oury [b,*], Frédérique Tarrieu [b], Adriano Monte-Alegre [a], Ali Ouaissi [a]

[a] *Institut de Recherche pour le Développement (IRD), Unité de Recherche no 8 "Pathogénie des Trypanosomatidae," Montpellier, France*
[b] *Institut de Recherche pour le Développement, Unité de Recherche IRD no 165 "Génétique et Evolution des Maladies Infectieuses," Unité Mixte de Recherche CNRS (Centre National de la Recherche Scientifique)-IRD no 2724, Montpellier, France*

Received 6 August 2004; received in revised form 4 July 2005; accepted 15 July 2005

Abstract

We have previously identified a *Trypanosoma cruzi* gene encoding a protein named Tc52 sharing structural and functional properties with the thioredoxin and glutaredoxin family involved in thiol-disulfide redox reactions. Gene targeting strategy and immunological studies allowed showing that Tc52 is among *T. cruzi* virulence factors. Taking into account that *T. cruzi* has a genetic variability that might be important determinant that governs the different behaviour of *T. cruzi* clones in vitro and in vivo, we thought it was of interest to analyse the sequence polymorphism of Tc52 gene in several reference clones. The DNA sequences of 12 clones which represent the whole genetic diversity of *T. cruzi* allowed showing that 40 amino-acid positions over 400 analysed are targets for mutations. A number of residues corresponding to putative amino-acids playing a role in GSH binding and/or enzymatic function and others located nearby are subject to mutations. Although the immunological analysis showed that Tc52 is present in parasite extracts from different clones, it is possible that the amino-acid differences could affect the enzymatic and/or the immunomodulatory function of Tc52 variants and therefore the parasite phenotype.

Index Descriptors and Abbreviations: *Trypanosoma cruzi*; Tc52; Immunomodulatory factor; Sequence polymorphism; aa, amino-acid(s); Ala, alanine; Arg, arginine; bp, base pair(s); kbp, kilobase pair(s); cDNA, DNA complementary to RNA; dNTP, dinucleotide tri-phosphate; DTU, discrete typing unit; FCS, foetal calf serum; Gln, glutamine; Glu, glutamic acid; Gly, glycine; GSH, reduced tripeptide glutathione; GST, glutathione-*S*-transferase; Ile, isoleucine; kDa, kilodalton(s); *L. infantum*, *Leishmania infantum*; *L. lainsoni*, *Leishmania lainsoni*; *L. major*, *Leishmania major*; LIT, liver infusion tryptose; Lys, lysine; Met, methionine; PBS, phosphate buffer saline; PCR, polymerase chain reaction; *P. discolor*, *Phyllostomum discolor*; Pro, proline; RPMI 1640, culture medium no 1640 developed at Roswell Park Memorial Institute; SDS, sodium dodecyl sulphate; Ser, serine; *T. infestans*, *Triatoma infestans*; *T. brucei gambiense*, *Trypanosoma brucei gambiense*; *T. cruzi*, *Trypanosoma cruzi*; *T. marinkellei*, *Trypanosoma marinkellei*; *T. rangeli*, *Trypanosoma rangeli*

Trypanosoma cruzi, the etiological agent of Chagas'disease, is an obligate intracellular parasite causing chronic infections in humans and a large number of other mammalian species. This protozoan parasite is transmitted to man in the faeces of hematophagous bugs of the Reduviidae family. The complex lifecycle of *T. cruzi* includes different stages in the insect vector and the vertebrate host. There are two parasite stages in the vector: epimastigotes and metacyclic trypomastigotes, whereas the vertebrate stages are bloodstream trypomastigotes and intracellular amastigotes. These series of transitions involve major modifications in morphology,

☆ Accession numbers: AY195575; AY238345; AY238346; AY238347; AY238348; AY238349; L07510; AY238350; AY238351; AY238352; AY238353; AY238354; AY238355; AY238356; AY238357; AJ582069.

* Corresponding author. Fax: +33 4 67 41 61 47.
E-mail address: Bruno.Oury@mpl.ird.fr (B. Oury).

0014-4894/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2005.07.001

gene expression, and cell cycle (Bonaldo et al., 1988; Gonzales-Perdomo et al., 1988).

In previous studies, we reported the identification of a cDNA encoding a *T. cruzi* Tc52 protein sharing motifs similar to those found in glutathione-*S*-transferase (GSTs) sequences and a set of small proteins mostly induced by different stress conditions (Schöneck et al., 1994). The Tc52 protein was found by gene knockout approach to be among factors crucial for parasite survival and virulence (Allaoui et al., 1999).

GSTs are ubiquitous housekeeping enzymes found in nearly all animals and some parasites. They belong to a super family of isoenzymes catalysing the conjugation of the reduced tripeptide glutathione (GSH) with a large variety of xenobiotics as well as endogenous substances like bilirubin, steroids, carcinogens, and some organic compounds. Some GSH-conjugates are known to be directly implicated in carcinogenesis but also in detoxification of those substances (Ketterer et al., 1988). Drug resistance mediated by GSTs has been reported in insects, some plants, and perhaps also in tumours and parasites (Hayes and Wolf, 1988).

Cytosolic GSTs are homo- or heterodimers composed of two subunits of 24–28 kDa, linked by non-covalent interactions. They are shared out among five classes, α, μ, π, θ, and σ (Landi, 2000). Identity between classes did not exceed 30% and is found mainly within their N-terminal region also named glutathione binding site (G-site). The non-specific hydrophobic C-terminal region (H-site) has a much more variability than the G-site.

Evolutionary relationships allowed drawing the hypothesis that an ancestor gene product used GSH as a cofactor to exert the reducer function, which could be its main role. The GSH binding does appear in the N-terminal region (G-region), whereas the individual function is related to the C-terminal hydrophobic region (H-region). From a progenitor protein all the others could have evolved by gene duplication and further divergence leading to the large diversity in function. Another common feature is that GSTs act as dimers, the only exception being the *T. cruzi* Tc52 protein, which seems to be ancestral. Indeed it is believed to be a result of gene duplication without further separation. The Tc52 protein is composed of two homologous domains comprising a GSH binding site (G-site) and a hydrophobic C-terminal region (H-site) (Ouaissi et al., 2002b). The molecule may act as a dimeric-like complex where the two "pseudo-subunits" areas are arranged in an antiparallel fashion separated by a strong β-turn motif (Ala225-Pro-Gly-Tyr228).

Biochemical studies have shown that the purified Tc52 protein could function in vitro as a thioltransferase (Moutiez et al., 1995). Finally, immune protection experiments in murine model suggest that Tc52 is among candidate molecules that may be used to design an optimal vaccine to control *T. cruzi* infection (Ouaissi et al., 2002a).

It is well known that *T. cruzi* isolates show high levels of genotypic diversity (Barnabé et al., 2000). Genetics studies have revealed that the population structure of *T. cruzi* is predominantly clonal and genetic exchanges could occur but were too infrequent to influence on the genetic structure and evolution of the species (Tibayrenc et al., 1986). Natural clones appear to be identical for a given set of genetic loci. Genetic diversity is caused by clonal divergence and rare genetic exchanges (Brisse et al., 1998; Gaunt et al., 2003; Machado and Ayala, 2001). Phylogenetic approaches have evidenced that the species is distributed into two main lineages which were referred to as discrete typing units (DTUs) I and II (Tibayrenc, 1998) or *T. cruzi* I and *T. cruzi* II (Momen, 1999). DTU II or *T. cruzi* II is subdivided into five discrete sub-lineages referred to as IIa–IIe (Barnabé et al., 2000).

Various biological parameters including growth kinetics, virulence, and tissue tropism in mice, in vitro drug sensitivity and vectorial transmissibility are highly linked to phylogenetic divergence between variants of the parasite (De Lana et al., 1998, 2000; Dvorak, 1984; Laurent et al., 1997; Pinto Da Silva et al., 1998; Revollo et al., 1998). Therefore, in the light of these observations, we thought it was of interest to examine the presence and the possible sequence variation of Tc52 gene in several reference variants of *T. cruzi*.

All parasite strains surveyed in this present work were cloned. *T. cruzi*, *Trypanosoma marinkellei*, and *Trypanosoma rangeli* epimastigotes were grown at high cell density at 27 °C in LIT medium supplemented with 10% heat-inactivated foetal calf serum (FCS) and 50 μg/ml gentamycin. *Trypanosoma brucei gambiense* procyclic forms were cultivated at 27 °C, in the Cunningham medium supplemented with 10% heat-inactivated FCS and 50 μg/ml gentamycin. *Leishmania infantum*, *Leishmania lainsoni*, and *Endotrypanum* clones were maintained at 26 °C in RPMI medium supplemented with 10% heat-inactivated FCS and 50 μg/ml gentamycin. When reaching the stationary phase, cultures were harvested by centrifugation at 750*g* for 10 min at 4 °C. Parasite pellets were then washed in phosphate buffer saline (PBS) by three successive centrifugations and then freezed at −80 °C until used.

To identify the Tc52 gene, we have used the polymerase chain reaction (PCR) to amplify a fragment encoding the domains of Tc52 protein containing the putative amino-acids (aa) involved in GSH binding (aa 20–418), followed by direct sequencing of individual PCR products. DNA was purified by phenol/chloroform extraction as described by Oury et al. (1997). Amplifications of the Tc52 gene were performed in a volume of 50 μl using the Tc52A sense primer (5′-ATGAAGGCTTTGAAA CTTTTTAAAG-3′) and the Tc52B antisense primer