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# Trans-sialidase and mucins of *Trypanosoma cruzi*: an important interplay for the parasite

M. Eugenia Giorgi, Rosa M. de Lederkremer *

*CIHIDECAR, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 1428 Buenos Aires, Argentina*

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ABSTRACT

A dense glycocalix covers the surface of *Trypanosoma cruzi*, the agent of Chagas disease. Sialic acid in the surface of the parasite plays an important role in the infectious process, however, *T. cruzi* is unable to synthesize sialic acid or the usual donor CMP-sialic acid. Instead, *T. cruzi* expresses a unique enzyme, the trans-sialidase (TcTS) involved in the transfer of sialic acid from host glycoconjugates to mucins of the parasite. The mucins are the major glycoproteins in the insect stage epimastigotes and in the infective trypomastigotes. Both, the mucins and the TcTS are anchored to the plasma membrane by a glycosylphosphatidylinositol anchor. Thus, TcTS may be shed into the bloodstream of the mammal host by the action of a parasite phosphatidylinositol-phospholipase C, affecting the immune system. The composition and structure of the sugars in the parasite mucins is characteristic of each differentiation stage, also, interstrain variations were described for epimastigote mucins. This review focus on the characteristics of the interplay between the trans-sialidase and the mucins of *T. cruzi* and summarizes the known carbohydrate structures of the mucins.



## 1. Trypanosoma cruzi

*Trypanosoma cruzi* is the etiologic agent of Chagas disease, the American trypanosomiasis which is transmitted to animals, including humans, by insect vectors of the family Reduviidae.[1,2] *T. cruzi* may be also transmitted from mother to fetus or by blood transfusions and organ transplants; therefore, with increasing northbound immigration of Latin Americans the disease is now of concern for the northern countries. Chagas disease presents variable clinical manifestations; usually, an initial acute phase sometimes asymptomatic is followed by a chronic condition that could lead to gastrointestinal and heart lesions.

*T. cruzi* differentiates during its life cycle as it travels from insects to humans, undergoing biochemical and morphological changes. Epimastigotes replicate in the midgut of a triatomine insect and in the intestine they transform into non-proliferative metacyclic trypomastigotes that pass from the hindgut to the feces. The infective trypomastigotes enter the vertebrate host through a skin wound or mucosal membranes, invade cells and differentiate into amastigotes which, after several cycles of binary division, differentiate back into trypomastigotes. These non-replicative forms are released into circulation upon host–cell rupture. Trypomastigotes may then infect other cells or be taken by the insect vector on blood sucking. The ingested trypomastigotes transform into epimastigotes, closing the cycle.

The biological, biochemical and genetic diversity of *T. cruzi* strains led to the recognition in 1999 of two major groups, I and II.[3] A sylvatic origin was recognized in strains grouped as *T. cruzi* I less infective than strains belonging to group II from the domestic cycle. Recently, by international consensus, strains were reclassified into six discrete typing units (DTU I-VI).[4]

## 2. *T. cruzi* surface glycoconjugates

A dense glycocalix covers the surface of the parasite and its composition is characteristic of each differentiation stage.[5] Most of the structural studies have been performed with the replicative, easily cultured, epimastigote forms. Free glycoinositolphospholipids (GIPLs) are the major components together with the mucins. These glycoproteins as other proteins of *T. cruzi* are anchored to the plasma membrane by a glycosylphosphatidylinositol (GPI).[6,7]

### 2.1. Trans-sialidase activity in *T. cruzi*

The interplay between the enzymatically active trans-sialidase (TcTS) and the mucins in the surface of *T. cruzi* trypomastigotes is crucial for pathogenesis.

For the acquisition of sialic acid, *T. cruzi* expresses at its surface a TcTS, capable of transferring sialic acid residues from host

* Corresponding author. Tel./fax: +54 11 4576 3352.
*E-mail address:* lederk@qo.fcen.uba.ar (R.M. de Lederkremer).

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sialoglycoconjugates to parasite mucins (Fig. 1).[8–11] This process is fundamental for *T. cruzi* which is unable to synthesize CMP-sialic acid, the common nucleotide donor of sialic acid used by bacterial and eukaryotic sialyltransferases.[12] The transfer is specific and involves the sialic acid α-(2→3)-linked in the donor and the formation of the same linkage, with a terminal β-galactopyranosyl group, in the acceptor substrate (Fig. 2). TcTS also transfers, efficiently, α-(2→3)-linked *N*-glycolylneuraminic acid to terminal β-galactopyranosyl groups.[13,14]

The specific activity of the TcTS in epimastigotes is 17% of that found in trypomastigotes.[15] The enzyme is expressed in epimastigotes at the stationary phase and is structurally different from the trypomastigote enzyme.[16] On the other hand, the lower sialylation of the insect parasites is due to the low concentration of sialic acid donors in the gut lumen of the vector *Triatoma infestans*. A sialidase activity in the anterior midgut is responsible for the hydrolysis of sialyl residues in linkage α-(2→3)-β-Gal*p* and thus, they would not be available for the TcTS.[17] Recombinant TcTS was expressed in *Escherichia coli* and used for most in vitro studies.[18–20] The three dimensional structure of TcTS was determined by crystallographic studies on a fully active TcTS mutant and consists of two domains.[21–23] The N-terminal catalytic domain is connected through a long α helix to the C-terminal lectin-like domain which does not participate in the enzymatic reaction. The catalytic domain shares several features with the common sialidases, however, TcTS exhibits some distinct units that could be responsible for the different enzymatic activities. Two tyrosine residues (Tyr119 and Tyr248) together with Trp120, Val203 and Trp312 define a hydrophobic environment in the binding pocket favoring transglycosylation to hydrolysis.

Studies on crystals of TcTS in complex with different ligands, as the acceptor substrate lactose and the sialidase inhibitor 2,3-dehydro-3-deoxy-*N*-acetylneuraminic acid (DANA) showed that TcTS has two sites in the active center: the sialic acid-binding site and the galactose-binding site. The sialic acid site is present in all sialidases but in TcTS is somewhat different.

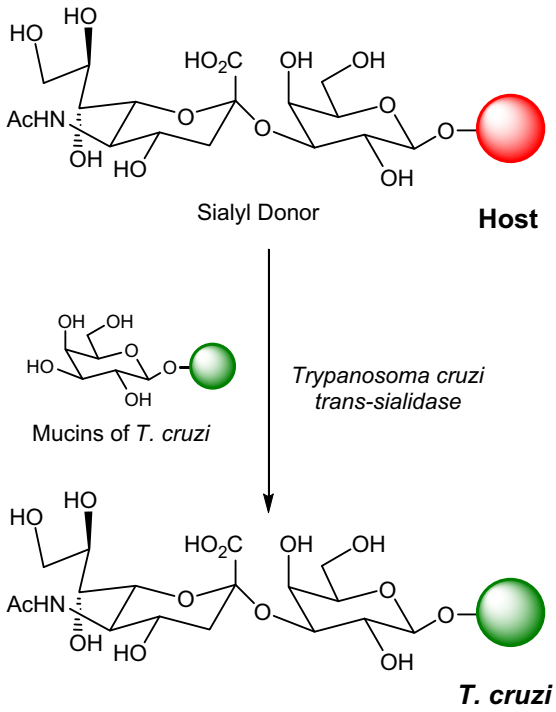


**Figure 2.** Transfer of sialic acid from a host glycoconjugate to β-galactopyranosyl units of the mucins. The transfer is specific to the 3-position of a terminal β-Gal*p* unit. oligosaccharide linked in a mammal glycoconjugate. oligosaccharide in a mucin of *T. cruzi*.

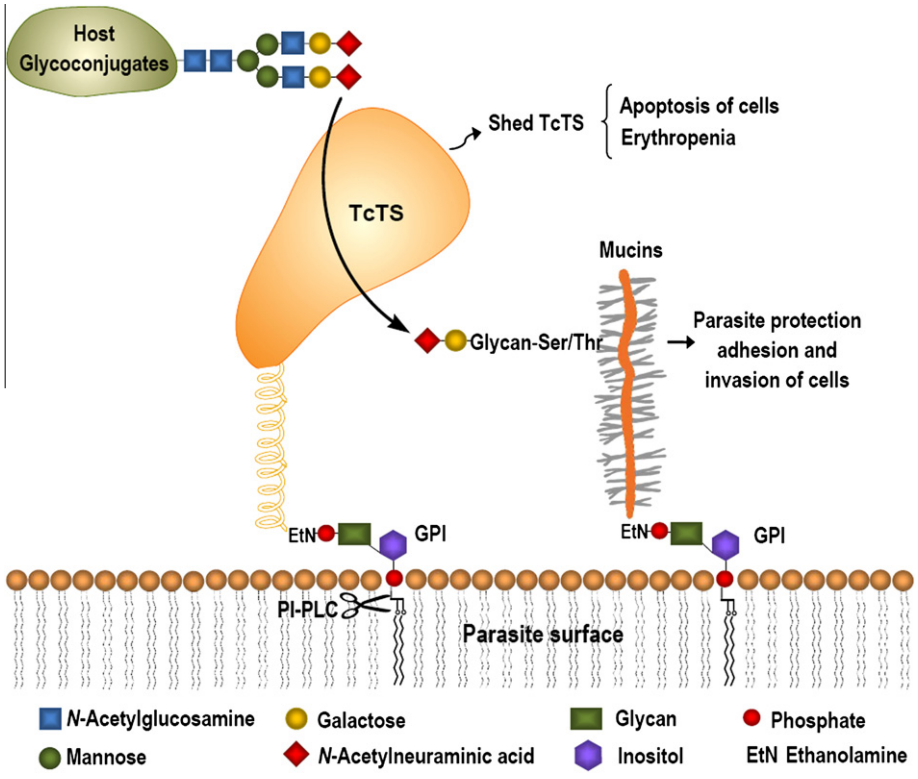


**Figure 1.** The glycosylphosphatidylinositol (GPI) anchored trans-sialidasa of *T. cruzi* (TcTS) transfers sialic acid from host–cell glycoconjugates to GPI-anchored mucins in the parasite surface. TcTS is cleaved by PI-PLC and shed to the medium. Some of the biological effects of TcTS in circulation are indicated.

Not all the members of the trans-sialidase family are enzymatically active. In vitro studies, using NMR spectroscopy, showed that recombinant inactive trans-sialidase still binds to terminal sialic acid and galactose residues, thus functioning as a lectin.[24] In fact there is a large superfamily of trans-sialidases in *T. cruzi* with a conserved peptide motif called FLY that binds to the surface of epithelial cells and increases parasite entry.[25] This sequence may contribute to tissue tropism with preference for the heart vasculature.[26] One of the members of the trans-sialidase family, ASP-1, is preferentially expressed in intracellular amastigote forms.[27] It was shown that ASP-1 does not bind to sialic acid and has not TS activity.

Since TcTS in trypomastigotes is anchored by a GPI[28,29] it may be shed by an endogenous PI-PLC into the bloodstream of the host, where it may produce several alterations in the immune system.[30–34]

#### 2.1.1. Role of parasite sialylation

The TcTS exerts a beneficial function for the parasite by acquisition of the sialic acid needed for infection.[8] In the epimastigote stage sialylation might be involved with the adhesion of the parasites to the epithelial cells in the rectal ampoule of the insect.[9] Important biological effects are mediated by the TcTS sialylation of trypomastigote mucins as protection against the alternative complement pathways as well as cell adhesion and invasion. TcTS is involved in the escape of the parasite from the parasitophorous vacuole.[35] Higher amounts of TcTS are expressed in the more pathogenic *T. cruzi* II strains.[36] Although lower parasitemias are obtained in mice infected with parasites of a *T. cruzi* I strain and lower amounts of sialic acid are found on their surface, I and II strains have the same potency for cell invasion in vitro.[37] The increased virulence related to higher sialylation would be due in fact to a modulation of the immune response of the host by interaction with siglecs which are sialic acid binding Ig-like lectins.[37,38] A method for the identification of the target immune cells in the surface was described.[39]

#### 2.1.2. Inhibitors of *T. cruzi* trans-sialidase activity

It is perceptible that TcTS, being a virulence factor in the mammal and/or insect stages of trypanosomes, specific for the parasite, with no equivalent in the human host, is an interesting target for drug design. Potential inhibitors for TcTS are usually classified depending on the active site region they target. In the last years several laboratories have been active in seeking inhibitors for TcTS, mainly directed to the sialic acid-binding site.[40–42] In particular, some natural products or synthetically modified natural products proved to be good inhibitors in vitro of the TcTS but the authors do not report studies on toxicity in animals.[43,44] Inhibitors of TcTS directed to the β-galactosyl acceptor site should be more specific, as other sialidases lack this interaction. Lactose derivatives effectively inhibited the transfer of sialic acid to *N*-acetyllactosamine, lactitol being the best.[20] The lactose analogs are only moderate inhibitors. However, they are non-toxic to animals. Fast clearance from blood was solved using lactitol releasing pellets which prevented apoptosis of spleen cells.[34] With the aim to improve the bioavailability lactose analogs have been conjugated with polyethylene glycol.[45]

## 3. Carbohydrate structures in mucins

The *T. cruzi* mucin gene repertoire is remarkable for its complexity and versatility. It was suggested that the variation of the mucin core polypeptide expressed in the mammal stages is one of the mechanisms by which *T. cruzi* may evade immune response.[46] The O-linked oligosaccharides in the mucins account for up to 60% of the molecular mass. Both, epimastigote and the infective trypomastigote forms have similar number of mucin molecules per surface area.[47] They play the crucial function of being acceptors of sialic acid needed by the parasite to build a negatively charged coat that protects trypomastigotes from killing by human anti-α-galactopyranosyl antibodies. In fact, the α-Gal units are a structural feature that distinguishes the carbohydrates of the trypomastigote stage.[48,49]

Variations in the carbohydrate structure of the mucins in different stages of the parasite have been observed. Also, inter-strain differences in the carbohydrates of mucins of the epimastigote stage have been described.[5] The structures of the O-linked oligosaccharides have been elucidated for the mucins of the epimastigote stages of the parasite. By comparing structural data on the mucins from different strains it followed that the O-linked oligosaccharides may be derived from two cores, β-Gal*p*-(1→4)-GlcNAc or β-Gal*f*-(1→4)-GlcNAc (Fig. 3). The cores are further branched with various units of Gal*f* and/or Gal*p*. The mechanism whereby the presence of Gal*f* correlates with the parasite inactivation was not fully elucidated.

A puzzling fact is that, depending on the strain, galactose may be found in the furanose configuration together with the more common galactopyranose. Galactofuranose was first described in mucins of the G strain, classified as *T. cruzi* I.[50,51] It was later found in other strains belonging to the same group[52,53] whereas in strain Y of group II galactose is only present in the pyranose form.[54] Also, only Gal*p* substituents are found in the mucins of the CL14 and CL Brener strains[55–57] previously included in group II and now reclassified as DTU VI.[4] It is interesting that the Tulahuen strain, now included in the new hybrid type VI expresses mucins with substitution of GlcNAc by either Gal*p* or Gal*f*.[58] The biosynthetic steps for the incorporation of Gal*f* in some mucins deserves further investigations since Gal*f* is an antigenic epitope,[59] which is not present in the mammalian host.

Although galactose is a constituent of all mucin oligosaccharides, the surface hexose transporter expressed by *T. cruzi* is unable to transport galactose.[60] This monosaccharide is not incorporated from the extracellular medium by the parasite which is able to transform the incorporated glucose, via the nucleotide, into the donor nucleotides for galactose. The UDP-Glc 4′-epimerase,[61] together with the UDP-Gal*p* mutase recently identified in *T. cruzi*,[62,63] provide the necessary Gal*p* and Gal*f* for the construction of the parasite glycoconjugates. The *T. cruzi* UDP-Glc 4′-epimerase is encoded by the *Tc GALE* gene. Null mutants could not be obtained, suggesting that the gene is essential. Under diminished supply of UDP-Gal*p*, incorporation of Gal*p* into the mucins was more affected than biosynthesis of the Gal*f* containing GIPLs, suggesting that they play an important role for parasite survival in culture.

Another remarkable feature of the O-linked chains is that they are linked to the protein by α-GlcNAc instead of the GalNAc common in vertebrate mucins. Indeed, GalNAc was not found in any of the *T. cruzi* glycoconjugates. The inability of the UDP-Glc 4′-epimerase of *T. cruzi* to convert UDP-GlcNAc into UDP-GalNAc, in contrast to the human epimerase, explains these findings.[61] It is also unusual for surface glycoproteins that, in some strains, a significant amount of non-substituted O-linked GlcNAc was found.[50,55] The enzyme that transfers the first GlcNAc from UDP-GlcNAc has been characterized,[64] but the glycosyltransferases which build the O-linked chains in the mucins were not yet studied.

Information on the fine structure of the carbohydrates in the mucins from mammal-derived stages (tGPI-mucins) is scarce. A complex mixture of oligosaccharides was obtained from mucins of cell derived trypomastigotes after reductive β-elimination.[48] The smallest structure was the trisaccharide alditol α-Gal*p*-(1→3)-β-Gal*p*-(1→4)-GlcNAc-ol. The larger structures are branched and arise from 4,6-disubstituted GlcNAc-ol with a variable number

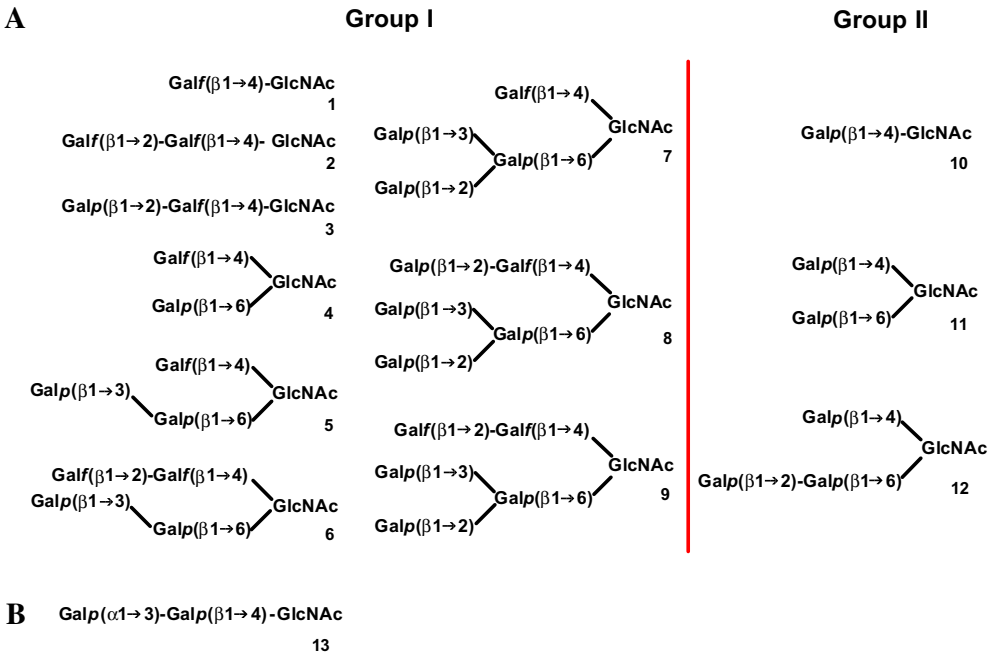


**Figure 3.** O-Linked oligosaccharides in the mucins of *T. cruzi*. (A) Structures determined in mucins of insect stages of strains belonging to group I or group II. (B) The smallest structure defined in mucins from cell derived trypomastigotes.

of Gal*p* residues, no Gal*f* was found. Unlike the trisaccharide, some of these structures were sialylated which indicates the presence of terminal β-Gal*p* units in some of the branches. The difference of the O-linked oligosaccharides in mucins of the insect and mammal stages is the presence of α-Gal*p*-(1→3)-β-Gal*p* epitopes in the latter.[48] These oligosaccharides are highly immunogenic and are the targets for lytic anti α-galactosyl antibodies found in sera from patients with chronic Chagas disease. Although mucin-type glycoproteins have been immunocharacterized in the intracellular amastigote stage[65] the structure of the carbohydrate component is not known.

The oligosaccharides in the mucins of the epimastigote stage have been chemically synthesized.[5,66] The major oligosaccharide in the mucins of *T. cruzi* G strain is pentasaccharide **7** (Fig. 3). From the two terminal β-D-Gal*p* units available for sialylation by TcTs, only the less hindered 1-3 linked Gal*p* is selectively sialylated.[67] By a comparative kinetic study it was shown that the presence of Gal*f* does not interfere with the acceptance of sialic acid by the Gal*p* in the oligosaccharides.[68] Thus, the diminished virulence of the strains belonging to group I is not caused by undersialylation. The antigenicity of Gal*f* could account for parasite inactivation by the mammalian immune system.[59]

In summary, two striking structural features characterize the mucins of the mammal and insect stages of *T. cruzi*: the presence of nonreducing terminal α-Gal*p* in trypomastigotes and of β-Gal*f* in strains of epimastigotes from sylvatic origin. The incorporation of antigenic α-Gal*p* or Gal*f* in mucins of the mammal and insect stages, respectively may be related to immunogenicity, but elucidation of the significance and timing of the process requires further studies. Since galactose in these configurations is not acceptor of sialic acid some of the O-linked chains in the mucins must have terminal β-Gal*p* in order to function as sialyl acceptors.

## 4. Perspective

The characterization of the glycosyltransferases that construct the oligosaccharide chains of the mucins may provide further tools to interfere with the biology of the parasite. The significance for the incorporation of the antigenic units β-Gal*f* or α-Gal*p*, in the *O*-chains of metacyclic or mammalian trypomastigotes, respectively, will give further insight into the infectious process.

On the other hand, a bi-substrate approach to target the donor and acceptor binding sites of TcTS may lead to the development of more efficient drugs for Chagas disease.

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