The trans-Sialidase from Trypanosoma cruzi a Putative Target for Trypanocidal Agents

Lucia Mendonça-Previato*, Adriane Regina Todeschini, Leonardo Freire de Lima and Jose Osvaldo Previato

Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde – Bloco G, Universidade Federal do Rio de Janeiro, 21944-970, Rio de Janeiro-RJ, Brasil

Abstract: Trypanosomatid protozoa are parasites of considerable medical and economical importance because they are the causative agents of chronic human and livestock diseases endemic in developing countries. Trypanosoma cruzi is the causative agent of Chagas’ disease, present in most of Latin America. The biology of this parasite presents some unusual features, one of which is the mechanism employed for the addition of sialic acid units to its own glycoproteins, the mucin-like molecules, or to exogenous glycoconjugates. This is mediated by a transglycosylase for sialic acid known as trans-sialidase and located on the external surface of the parasite, rather than by an intracellular CMP-sialic acid-dependent sialyltransferase. The Trypanosoma cruzi trans-sialidase is thought to play an important role in the pathogenesis of Chagas’ disease, and it represents a potential therapeutic target.

Keywords: Trypanosoma cruzi, Chagas’ disease, trans-sialidase, Mechanism of catalysis.

INTRODUCTION

Although sialic acid-containing glycoproteins have been described in T. cruzi, this parasite is unable to synthesize this monosaccharide from precursors such as acetate or N-acetylmannosamine [1]. The first evidence for the presence of sialic acid in T. cruzi was provided by Pereira et al. [2], who demonstrated that epimastigotes forms of parasite can be agglutinated by wheat germ agglutinin (WGA), a lectin that recognizes terminal sialic acids. Subsequent studies described the presence of sialic acid in tripomastigote forms of T. cruzi [3]. The question of how T. cruzi acquired a sialic acid-containing surface phenotype led our laboratory to search for an alternative pathway of sialic acid incorporation in this parasite. Previato et al. [4] proposed a novel pathway for the incorporation of sialic acid into T. cruzi glycoproteins, involving a transglycosylase for sialic acid, instead of a CMP-sialic acid-dependent sialyltransferase. In that work our group found that epimastigotes grown in the presence of fetal calf serum (FCS) displayed sialoglycoproteins, while those grown in the absence of FCS had only asialoglycoproteins. Treatment of epimastigotes grown in FCS with Clostridium perfringens neuraminidase abolishes WGA binding and instead enhances the binding of PNA, a lectin that recognizes terminal residues of β-galactose [2]. If the sialidase-treated parasites were incubated with sialyllactose or fetuin, but not with free sialic acid, they regained the ability to bind WGA, with concomitant reduction of PNA binding [4]. Then characterization of the sialic acid isolated from trypomastigotes retrieved from mouse infection showed that this was N-glycocolyneuraminic acid [5], the type of sialic acid found in mouse serum glycoproteins. The presence of trans-sialidase activity in trypomastigotes was later proven by Schenkman et al. [6] from the classical biochemical point of view. These and other findings [7, 8] led to the current model, in which T. cruzi has an obligate adaptation to obtain sialic acid from its hosts, fulfilling this need through trans-sialidase-mediated transfers from sources in its vicinity; this remains today one of the most interesting aspects of T. cruzi glycobiology. The present review deals with the biological aspects of the T. cruzi trans-sialidase relevant to this enzyme as a target, and with the current status of the efforts towards the rational design of drugs that inhibit the T. cruzi trans-sialidase (TcTS).

THE ROLES OF TcTS

trans-Sialidase is not a single protein. Rather, TS is a collective name that describes the products of a few hundred different genes with considerable sequence divergence among them. These genes encode both for molecules with trans-sialidase enzymatic activity and for proteins with functions unrelated to the transfer of sialic acid, such as the members of the 85 kDa glycoprotein family (Tc-85) [9] which is the subject of a recent review [10].

The ability to trans-sialylate T. cruzi surface mucins [11, 12] and host glycoconjugates [5] determines that TS may function in modulating host-parasite interaction, both on the trypanomastigote surface or as a soluble mediator. Initial reports indicated that TcTS promoted host cell invasion [6], and precluded the action of lytic antibodies [13]. Alternatively, the enzyme was suggested to sialylate host cell glycomolecules, generating receptors used by the trypanosome for adherence to and penetration of target cells. Results with sialic acid-deficient mutants of Chinese Hamster Ovary cells support this hypothesis, as sialic acid-deficient cells are infected to a lesser degree than wild type cells [14, 15]. However, recent studies by Rubin-de-Celis et al. [16] suggest that

*Address correspondence to this author at the Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde – Bloco G, Universidade Federal do Rio de Janeiro – 21944 970, Cidade Universitária, Ilha do Fundão, Rio de Janeiro-RJ, Brasil. Tel: +552125026646; Fax: +552122808193; E-mail: luciamp@biof.ufrj.br
a major function of TcTS in host cell infection is to facilitate the escape from the parasitophorous vacuole into the cytosol; sialic acid may act as a barrier against parasite escape from the vacuole, as this escape was faster in sialic-acid-deficient cells.

The biological relevance of soluble TcTS could involve effects on the host immune system. Chenkova and Pereira [17] showed that in vivo injection of minute amounts of purified TcTS increases subsequent parasitemia and mortality in mice infected with T. cruzi. Exacerbation of virulence was abrogated by pre-incubation of the enzyme before administration with a monoclonal antibody that inhibits its enzymatic activity. These results have been verified more recently by Risso et al. [18]. However, the molecular mechanisms underlying parasite virulence associated with trans-sialidase activity have not been elucidated.

TcTS may have a role in immunomodulation of the infected host. Administration of the soluble enzyme to non-infected mice induces certain events that characterize the acute phase of Chagas’ disease, such as thrombocytopenia which is brought about by a reduction in the sialic acid content of platelets [19]; T and B cell activation [20, 21]; and a marked thymic involution [22]. With respect to T and B-cell activation, it should be mentioned that a non-specific, polyclonal, lymphocyte response constitutes one of the main factors hampering parasite control by the immune system during early infection [23].

Another intriguing and very well documented property of TcTS proteins is their capacity to induce host cell apoptosis [22, 24-26]. It has been shown that the pro-apoptotic effects of TS on T cells are directly associated with the resialylation of glycomolecules present in double negative thymocytes in the thymic cortex, and in spleen T cells [26]. However, a possibility that should not be ruled out is that thymocyte and T-cell apoptosis is brought about by the removal of sialic acid by the TcTS sialidase activity, together with the action of members of the galectin family. O and N-linked glycomolecules present in all leukocyte surfaces possess repetitive units of N-acetylglucosamine (GlcNAc) and β1,4 linked galactose (Gal). This disaccharide is a minimal ligand for galectin-family members [27]. Furthermore, the repetitive Galα1,4GlcNAc units, forming linear structures knowing as polylectosamine chains, can be ligands for the galectins [28], in particular for galectin-1, the pro-apoptotic effects of which are very well documented in a range of cell types [29-33]. The terminal Gal residue of such lactosamine chains can be modified by the action of sialyltransferases [34], resulting in terminally sialylated chains that are no longer galectin ligands. It is therefore possible to speculate that the removal of α2,3-linked sialic acid decorating terminal Gal residues in lactosamine chains by the sialidase activity in TcTS could generate a significant number of sites for galectin-1 binding, thus contributing to apoptosis.

Recently, our group demonstrated that T. cruzi uses its TS enzyme to resialylated the CD8+ T cell surface, dampening Ag-specific CD8+ T cell responses thus favouring its own persistence in the mammalian host [35].

Althouhg several works demonstrate that the biological effects induced by TcTS proteins are restricted to the active enzymes, other results showed that the enzymatically inactive TcTS [36] is a sialic acid-binding lectin that co-stimulates host T cells through leucosialin (CD43) engagement [37]. In addition, Dias et al. [38] observed that inactive TcTS is able to bind to sialyl residues present in endothelial cells, triggering NF-kappaB activation and subsequent expression of adhesion molecules, thus facilitating parasite entry into host cells.

MECHANISM OF CATALYSIS OF TcTS

TcTS is a member of the family number 33 of glycoside hydrolases (GH-33) that essentially includes the bacterial and eukaryotic exo-alpha sialidases (http://www.cazy.org) [39]. However, TS preferentially catalyzes the transfer of sialic acid residues from Siaα2-3Galβ1-x-containing donors to terminal β-galactopyranosyl (β-Galp) acceptors, attaching them in α2-3 linkage [40]. Terminal α-Gal, Galβ1-4(Fucα1-3)GlcNAc and Galβ1-3(Fucα1-4)GlcNAc are not acceptors [41]. Incorporation of one N-acetylneuraminic acid (Neu5Ac) residue onto an acceptor appears to hinder the entry of a second residue when two potential acceptor sites are present on the same oligosaccharide [12]. In the absence of a suitable carbohydrate acceptor, TS irreversibly transfers sialic acid to a water molecule, thus functioning as a sialidase similar to viral, mammalian and bacterial sialidases. Although the enzyme differs from those sialidases in acceptor specificity, it shares the same six-bladed β-propeller fold for the catalytic domain, and has the same key catalytic amino acids found in sialidases from bacteria [42-44], viruses [45-47], trypanosomes [48, 49], leech [50] and humans [51]. The crystal structure obtained a few years ago confirms that the molecular architecture of the TcTS active site preserves several conserved features of microbial sialidases: a) the arginine triad (Arg35, Arg245, Arg314) that interacts with the carboxylate group present in the sialic acid derivatives, and b) the Glu557 residue that stabilizes Arg35, a Tyr342, a Glu230 and an Asp290 at the reaction center, necessary for the catalytic activity [49]. All sialidases so far studied, as well as TcTS, catalyze sialoside hydrolysis with retention of configuration [52]. However, despite being glycosylases, these enzymes do not possess an aspartate or glutamate residue appropriately positioned to act as a nucleophile in a double-displacement mechanism [53], which suggests an alternative mechanism of hydrolysis. The evidence of production of traces of 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (Neu5Ac2en) after prolonged incubation of Ω(2-3)-sialyllactose [52], together with the TS crystal structure obtained by Buschiazzo et al. [49], pointed towards a transition-state involving oxocarbonium ion intermediate. Such an intermediate was previously proposed for sialoside hydrolysis by viral sialidases [46], which like bacterial sialidases are known to use an SN1 reaction in their mechanisms of catalysis [54].

Concurrent works based on deuterium and carbon isotope effects presented evidence that TS catalyzes sialoside hydrolysis through a pathway involving a sialyl-enzyme covalent intermediary [55]. Further, Watts et al. [56], using the α2,3-difuoro-N-acetylneuraminic acid to inactivate TcTS have shown by mass spectrometry that the enzyme becomes covalently modified on the active site Tyr residue by the 3-fluorosialyl moiety, consistent with the Tyr230 residue being

\[ \text{Gal} \text{N} \text{Ac} \text{2en} \]
the catalytic nucleophile of TS. On the basis of structural [57] and kinetic studies [58], it was proposed that the Asp$^{59}$ acts as a general acid/base catalyst in a double-displacement mechanism. In this mechanism, as the sialic acid unit approaches the enzyme, it displaces the Tyr$^{119}$ away from the binding site [49], and its carboxylate group interacts with the Arg triad, while its acetalid group interacts with Asp$^{66}$; this induces planarization of the sialic acid moiety around the oxygen ring, with C1, C2 and C3 assuming a $^3$H$_2$ conformation during the transition state. The C2 suffers nucleophilic attack by Tyr$^{342}$, assisted by Glu$^{330}$ acting as a general base, and a covalent linkage is formed. The covalent intermediate assumes a $^3$C$_3$ conformation. The aglycone leaves the catalytic cleft, thus making space for binding of the sialic acid acceptor. Transfer to the acceptor would then occur through attack of the C2 of the sialyl-enzyme intermediate by water or by the 3-OH group of a lactose moiety, which must be deprotonated by the residue acting as acid/base catalyst, Asp$^{58}$ [58]. This is a classical ping-pong mechanism similar to that of other glycosidases [53].

Nevertheless, this model would allow a water molecule to attack the sialosyl-enzyme intermediate before lactose reaches its binding site, resulting in simple hydrolysis rather than in efficient sugar transfer. A mechanism involving a ternary complex, with the lactose moiety located in the acceptor binding site before the transference of sialic acid, would support higher rates for the transference reaction, as the acceptor substrate would dislodge water molecules from the catalytic pocket. This mechanism would need additional conformational rearrangements in the TcTS, since the crystal structure of the enzyme shows a lactose binding site that is too narrow to accommodate the aglycone moiety of the donor substrate and the acceptor moiety simultaneously [49]. However, one cannot rule out the possibility that such rearrangements may take place in solution, as opposed to the crystallized TS. This hypothesis is given support by several experimental observations. The first are kinetic studies of both parasite-derived [41] and recombinant TS [59], which are consistent with a bisubstrate sequential mechanism. The second type of evidence arises from differences in optimal temperature between the trans-glycosylation and the hydrolysis reaction of TS, suggesting the presence of a binding site for the acceptor molecule [59]. The authors proposed that the stronger temperature dependence observed for the trans-glycosylation reaction as compared with the hydrolysis reaction may be explained by a requirement of close proximity between the acceptor and the donor bound to the catalytic site for the trans-glycosylation to take place. Thus, an increase in the reaction temperature might alter the enzyme conformation in a way that changes the distance between the binding sites, changing the relative rate between trans-glycosylation and hydrolysis reactions [59]. A third type of evidence comes from studies using NMR spectroscopy and an enzymatically inactive analog of TcTS (iTS) presenting a Tyr$^{342}$→Hys mutation. These studies demonstrated that iTS has the same specificity for potential sialic acid donors as active TcTS, i.e. it binds α2,3 sialic acid containing molecules [37], but it does not bind βGal-containing acceptors, unless a sialoside is also present. Unless a sialoside is also present, suggesting that iTS interacts with its ligands in a sequential ordered bi-ligand mechanism [60]. These results suggest that the correct binding of sialic acid donor to TS may trigger a conformational change in the enzyme that creates the conditions for the formation of a ternary complex. According to this hypothesis, acceptor binding to the enzyme would displace the water molecules from TcTS catalytic cleft before formation the sialyl-enzyme intermediate takes place, facilitating the selective transfer reaction [57]. Finally, surface plasmon resonance results show that lactose binds to an inactive mutant of TS (Asp$^{59}$→Asn) in the presence of α2,3-sialylactose [49]. This discussion provides that further structural data are needed to shed light into the reaction mechanism that underlies efficient sugar transfer activity rather than simply hydrolysis by TcTS. Given the importance of TS and sialidases as virulence factors in several infections [61], structural and mechanistic works must be relentless, and must take into account that mutations in key amino acids may produce important modifications in the TcTS mechanism catalysis.

Taking the above comments together, the inhibition of the catalytic activity of TcTS is a potential target for rational drug design against T. cruzi, especially in the interaction to and prevent of invasion of the parasite into mammalian host cells. The relevant aspects and recent results on chemical inhibitors of TcTS were recently published [62-64].

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