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Short communication

Trypanosoma rangeli expresses a gene of the group II *trans*-sialidase superfamily☆

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Trypanosoma cruzi, the etiological agent of Chagas disease, and *Trypanosoma rangeli*, a nonpathogenic protozoa for mammals, present surface glycoproteins of the *trans*-sialidase superfamily (TSASF). According to sequence identity, molecular weight, and function [1–3], members of TSASF are classified into four groups. The first group includes *T. cruzi trans*-sialidase (TcTS) and *T. rangeli* sialidase (TrSial). TrSial expressed in *T. rangeli* epimastigotes forms is a strict hydrolytic enzyme that releases sialic acid residues from the host cell surface glycoconjugates [4–6]. In contrast, *T. cruzi trans*-sialidase transfers sialic residues from the host surface onto mucin molecules on the parasite's surface [3]. Although TrSial has been well characterized [4–9], its biological role remains unknown.

Members of group II *TSASF*, collectively known as gp85 (or *gp85*/*trans*-sialidase), are expressed in *T. cruzi* infective trypomastigotes forms, and intracellular amastigotes stages [1–3]. This group, which has only been described in *T. cruzi*, includes a set of heterogeneous GPI-anchored surface glycoproteins with similar molecular masses but different electrical charges. *gp85*/*trans*-sialidase proteins have been implicated in adhesion and/or internalization of the parasite to host cells [10,11], but none of its members have sialidase or *trans*-sialidase activity.

In a previous work, we cloned telomeric sequences from a *T. rangeli* [12]. One of the recombinants obtained, namely TrTel 4 (3376 bp), had an ORF with high percent identity with all members of the gp85/*trans*-sialidase family at 1 kb from the telomeric end, and with the transcription sense oriented from the centromere towards the telomere.

The putative 1953-bp long gene (*TrGP*) (Fig. 1A) encoded for 651 aminoacids (aa) putative protein with estimated mass of 71 kDa. At the nucleotide level, *TrGP* sequence displayed 62–67% identity (83–96% in some blocks) with *T. cruzi gp85*/*trans*-sialidase genes. In addition, the translated sequence of *TrGP* exhibited 45–50% identity (reaching 60% considering conservative amino acid substitutions) with proteins encoded by group II *TSASF* genes [10,13–17], and to a lesser degree (25–30% identity) with group I members of this superfamily, including *T. rangeli* sialidases (GenBank U83180, L14943). Blocks of sequence identity between TrGP and gp85 proteins are shown in Fig. 1B.

TrGP shares with all members of the TSASF [3] the following features:

(i) Two conserved copies of the bacterial neuraminidase motif SxDxGxTW (Asp Box) (Fig. 1A and B).
(ii) A partially complete copy of the subterminal element VTVxNVfLYNR (Fig. 1A and B). This motif, known

Abbreviations: aa, aminoacids; bp, base pair; CHEF, clamped homogeneous gel electrophoresis; gp85, surface glycoprotein of 85 kDa; GPI, glycosylphosphatidylinositol; kb, kilobase; kDa, kilodaltons; TBS, Tris-buffered saline; TcTS, active *trans*-sialidase of *T. cruzi*; TrSial, sialidase of *T. rangeli*; TSA, *trans*-sialidase

☆ *Note:* Nucleotide sequence data reported in this paper have been submitted to the GenBank™ data base with accession number AF426022.

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Fig. 1. (A) Schematic representation of *T. rangeli* Trtel 4 clone. Blocks are to indicate major sequence features. Arrows indicate the sense of the coding sequence. Diagonal hatched bar represents TrGP-Nterm probe used in hybridization experiments. SubTR represents subterminal conserved region characteristics of *T. rangeli* telomere [12]. Relative positions of Asp-boxes and VTVxNVfLYNR motifs are shown (thick vertical lines). (B) Clustal W multiple alignment of deduced amino acid sequences from TrGP and four *T. cruzi* surface proteins of group II of *TSA* gene superfamily. Sequences are as follows: TrGP (AF426022), TcASP-2* (U77951), Tcsp-2* (AY186573), TcTSA-E2 (U02613) and Tc85KD (M64836). Conserved residues are in black (100% conservation), dark gray (75% conservation), and light gray (50% conservation). Overlining indicates the following motif of *gp85*/*trans*-sialidase in the TrGP: a predicted N-terminal signal peptide, two Asp boxes, VTVxNVfLYNR motif (⋆), and the partially conserved fRiP sialidase motif (✱). Sequence enclosed in pointed line is $TrGP^{Nterm}$ peptide. In order to improve the alignment, we did not consider a section of 38 amino acids 5′ the N-terminal signal peptide in TcASP-2 and Tcsp-2 sequences.

as peptide J, has been found in gp85 from *T. cruzi* bloodstream trypomastigotes, and it has been implicated in the binding to the mammalian host laminin [10].

(iii) At its N-terminus, it has a signal peptide to direct the protein to the endoplasmatic reticulum that shared a higher percent of identity with *T. cruzi* gp85/*trans*-sialidase members (77%) than with *T. rangeli* sialidase (45%) (Fig. 1B).

However, TrGP is devoid of a recognition site for the GPI anchor, a characteristic of many members of TSASF (Fig. 1B). Many residues regarded as important for TrSial