A simple purification of procyclic acidic repetitive protein and demonstration of a sialylated glycosyl-phosphatidylinositol membrane anchor

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The procyclic acidic repetitive protein is the major cell-surface glycoprotein of the insect-dwelling procyclic forms of the *Trypanosoma brucei* species of African trypanosomes. The glycoprotein contains an acidic Glu-Pro repeat domain, a glycosyl-phosphatidylinositol membrane anchor and a putative asparagine glycosylation site. In this paper we describe a rapid purification scheme for this glycoprotein, using solvent extraction and hydrophobic interaction chromatography, and a partial

characterization of the glycosylphosphatidylinositol membrane anchor. The carbohydrate composition of the anchor is extremely unusual; it contains on average nine GlcNAc, nine Gal and five sialic acid residues. This is the first description of such a heavily substituted and negatively charged anchor. A comparison between the trypanosome procyclic surface and the *Leishmania* promastigote surface is also presented.

INTRODUCTION

The African trypanosomes *Trypanosoma brucei brucei* and *T. b. rhodesiense* are closely related species which differ in host range. Both undergo a complex life-cycle between a tsetse fly vector and a number of mammalian hosts, including many of the African wild game animals. *T. b. brucei* is generally non-infective to humans and causes the disease nagana in cattle, whereas *T. b. rhodesiense* is responsible for the most acute form of human African sleeping sickness. These parasites live extracellularly in the bloodstream, lymph and interstitial fluids of the mammalian host. They are protected against serum complement components by a dense surface coat of variant surface glycoprotein (VSG). The parasites evade the immune response of the host by antigenic variation, a process whereby a small number of parasites switch the expression of one VSG gene for another from a repertoire of several hundred VSG genes encoding immunologically distinct coat glycoproteins [reviewed by Cross (1990a)]. Upon ingestion by the tsetse fly vector in a bloodmeal the bloodstream form of trypanosomes rapidly differentiates into the morphologically and biochemically distinct procyclic forms which colonize the insect mid-gut. This differentiation process can be mimicked *in vitro* and has been shown to occur with the concomitant loss of the VSG coat and acquisition of a new cell-surface glycoprotein called procyclin or procyclic acidic repetitive protein (PARP) (Roditi et al., 1989; Ziegelbauer et al., 1990). Genes encoding PARP glycoproteins have been cloned and sequenced (Roditi et al., 1987; Mowatt and Clayton, 1987) and it has been shown that PARP genes are polymorphic and exist as a multigene family [reviewed by Roditi and Pearson (1990)]. The PARP glycoprotein has been purified from procyclic forms of *T. b. rhodesiense* by concanavalin A lectin affinity chromatography followed by gel-filtration chromatography (Richardson et al., 1988), and from procyclic forms of *T. b. brucei* by ion-exchange chromatography followed by concanavalin A lectin affinity chromatography (Clayton and Mowatt, 1989). The most striking feature of PARP glycoproteins is the presence of 22–29 consecutive Glu-Pro repeats, which are predicted to form an extended rod-like domain

(Roditi et al., 1989). To the N-terminal side of this repeat domain is a small protein domain of 35 amino acid residues, which ends with an N-glycosylation sequon and two Asp-Pro repeats immediately before the Glu-Pro repeat domain. The C-terminus of the Glu-Pro repeat domain terminates with a glycine residue, which is linked to a glycosyl-phosphatidylinositol (GPI) membrane anchor (Clayton and Mowatt, 1989). The lipid moiety of the GPI anchor was recently shown to be an *sn*-1-stearoyl-2-lysophosphatidylinositol which contains an additional fatty acid (palmitate) in hydroxy-ester linkage to the *myo*-inositol ring (Field et al., 1991b). The position of this palmitate residue has been localized to the 2- and/or 3-position of the inositol ring (Ferguson, 1992a). The biosynthesis of GPI precursors in *T. brucei* procyclic forms has also been studied (Field et al., 1991a,b; 1992). These studies suggest that the GPI precursor, which is transferred to PARP, has the structure ethanolamine-PO₄-6Man α 1-2Man α 1-6Man α 1-GlcN-PI (where the PI group is a palmitoylated *sn*-1-stearoyl-2-lysophosphatidylinositol, as described above). The structure, biosynthesis and functions of GPI anchors in general have been extensively reviewed (Ferguson and Williams, 1988; Low, 1989; Thomas et al., 1990; Cross, 1990b; Ferguson, 1991, 1992b).

In this paper we describe a simple and rapid purification of PARP and a partial characterization of its GPI-anchor glycan.

EXPERIMENTAL

Cells

T. b. rhodesiense procyclic cells (EATRO 2340) were a generous gift of Dr. Ian Maudlin, Tsetse Research Centre, University of Bristol, Bristol, U.K.

Purification of the PARP glycoprotein

A cell pellet of procyclic forms of *T. b. rhodesiense* (1×10^{10} cells) was extracted twice with 8 ml of chloroform/methanol/water (1:2:0.8, by vol.). The insoluble delipidated residue was extracted further by sonication twice with 5 ml of 9% (v/v) butan-1-ol in

Abbreviations used: VSG, variant surface glycoprotein; PARP, procyclic acidic repetitive protein; GPI, glycosyl-phosphatidylinositol; AHM, 2,5-anhydromannitol; LPG, lipophosphoglycan; PI, phosphatidylinositol.

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Table 1 Compositional analyses of PARP and the GPI-peptide

The theoretical amino-acid composition is taken from Clayton and Mowatt (1989). Abbreviations: unk, unknown; n.d., not determined; SA, sialic acid; EtN, ethanolamine.

Component	Theoretical	PARP	GPI-peptide
Asx	6.0	7.1	0.0
Glx	32.0	26.9	2.8
Ser	0.0	0.0	0.0
Gly	9.0	11.5	1.8
His	0.0	0.6	0.0
Arg	0.0	2.7	0.0
Thr	4.0	4.5	0.0
Ala	2.0	5.0	0.0
Pro	32.0	30.9	4.7
Tyr	0.0	1.2	0.0
Val	1.0	3.1	0.0
Met	0.0	0.0	0.0
Ile	0.0	2.0	0.0
Leu	1.0	3.2	0.0
Phe	0.0	1.3	0.0
Lys	6.0	9.6	0.0
EtN	1.0	1.0	1.1
Ins	1.0	1.0	1.0
Man	unk	5.3	1.9
Gal	unk	8.5	9.1
GlcNAc	unk	9.3	9.1
SA	unk	n.d.	4.9

water. The supernatants obtained after centrifugation (4000 *g* for 15 min) were pooled, concentrated by rotary evaporation, freeze-dried and redissolved in 5 ml of 0.1 M ammonium acetate/5% (v/v) propan-1-ol (buffer A). This fraction was applied to a column (1 cm × 10 cm) of octyl-Sepharose (Pharmacia), pre-equilibrated in buffer A, at 4 ml/h. The column was eluted with 20 ml of buffer A at a flow rate of 4 ml/h followed by a linear gradient to a final eluant composition of 60% (v/v) propan-1-ol in water over 100 ml at 12 ml/h. Fractions (1 ml) were collected and aliquots (2 µl) were spotted on to an aluminium-backed silica h.p.t.l.c. plate (Merck), which was stained with orcinol/sulphuric acid to detect the presence of carbohydrate. Carbohydrate material was detected in the column breakthrough and as a discrete peak eluting at 26% (v/v) propan-1-ol. Analysis of aliquots (50 µl) of the fractions across and adjacent to this carbohydrate peak for inositol, by g.c.-m.s., revealed a peak of *myo*-inositol coincident with the carbohydrate (total content 55 nmol of *myo*-inositol). These fractions were pooled, concentrated by rotary evaporation, and freeze-dried to remove ammonium acetate before further analysis. SDS/PAGE of this material and silver staining revealed a single diffuse band of approx. 35 kDa (results not shown). Amino acid analysis of this preparation confirmed its identity as PARP, see Table 1.

Purification of the PARP GPI-peptide

The octyl-Sepharose-purified PARP was digested extensively with the enzyme prolidase [50 units of enzyme in 40 mM Tris/HCl (pH 8.0)/25 mM MnCl₂/1 mM GSH] for 20 h at 37 °C. The digest was adjusted to 5% (v/v) with respect to propan-1-ol and rechromatographed on an octyl-Sepharose column as described above. A carbohydrate- and *myo*-inositol-containing peak was eluted at 27% (v/v) propan-1-ol, concentrated by rotary evaporation and freeze-dried to remove ammonium acetate.

G.c.-m.s. analysis

Analyses were performed with a Hewlett-Packard 5890-MSD system equipped with a bonded phase SE-54 column (30 m × 0.25 mm, SE-54 Econocap column, Alltech Associates). Analyses for *myo*-inositol and monosaccharides were as previously described (Schneider et al., 1990; Güther et al., 1992).

Amino acid analysis

Amino acid residues and ethanolamine were quantified following 6 M HCl hydrolysis (110 °C, 16 h) using a Waters Pico-Tag system, as described previously (Schneider et al., 1990).

Radiolabelling and isolation of the GPI glycan

The purified PARP GPI peptide (10 nmol) was subjected to nitrous acid deamination and NaB³H₄ reduction, to convert the GPI-glycan GlcN residue into [1-³H]2,5-anhydromannitol (AHM), using the conditions described in Güther et al. (1992). Following the paper-chromatography step the deaminated and reduced material recovered was subjected to aqueous HF dephosphorylation followed by re-N-acetylation, as described in Schneider et al. (1990), except that the products were desalted by passage through 1 ml of AG50-X12(H⁺) followed by three evaporations with 50 µl of toluene. The terminal sialic acid residues were removed by mild acid hydrolysis in 250 µl of 40 mM trifluoroacetic acid at 80 °C for 1 h. After drying, the sample was applied to Whatman 3MM paper and subjected to high-voltage electrophoresis to remove residual radiochemical contaminants, as described in Güther et al. (1992). The radiochemical purity of the neutral glycan preparation was checked by Dionex h.p.l.c.: a small aliquot of the preparation was hydrolysed (2 M trifluoroacetic acid, 100 °C, 4 h) and the products analysed by Dionex h.p.l.c. using a CarboPac PA-1 column eluted with 15 mM NaOH. All of the radioactivity was recovered in a single peak which co-chromatographed with an authentic AHM (Sigma) standard (results not shown). This result confirmed the presence of a non-N-acetylated glucosamine residue in the original PARP GPI anchor.

Bio-Gel P-4 chromatography and glycosidase digestions

Chromatography on Bio-Gel P-4 (–400 mesh) was performed at 55 °C using a column (1 m × 1.5 cm) eluted with water at 12 ml/h. Samples were co-injected with dextran oligomer internal standards as previously described (Schneider et al., 1990; Güther et al., 1992). Fractions (1 ml) were collected and aliquots (50 µl) were taken for liquid scintillation counting.

Digestion with *Bacteroides fragilis* endo-β-galactosidase (Boehringer) was performed in 40 µl of 50 mM sodium acetate, pH 5.8, containing 40 m-units of enzyme, for 16 h or 60 h at 37 °C. The reaction was terminated by heating (100 °C, 5 min) and the products desalted by passage through 0.2 ml of AG50-X12(H⁺) and two evaporations with 50 µl of toluene. Jack-bean β-hexosaminidase and Jack-bean β-galactosidase (Sigma) digestions were performed in 20 µl of 0.1 M citrate/phosphate buffer, pH 4.2, containing 80 m-units of enzyme and 10 mM D-mannonic acid γ-lactone, for 16 h at 37 °C. The reactions were terminated by heating (100 °C, 5 min) and the products desalted by passage through 0.2 ml of AG50-X12(H⁺) over 0.2 ml of AG3-X4(OH[–]) over 0.1 ml of QAE-Sephadex-A25. Jack-bean α-mannosidase (Boehringer) digestions were performed in 30 µl of 0.1 M sodium acetate, pH 5.0, containing 750 m-units of enzyme, for 16 h at 37 °C. Coffee-bean α-galactosidase (Oxford Glycosystems) digestions were performed in 20 µl of 0.1 M

sodium acetate, pH 6.0, containing 400 m-units of enzyme, for 16 h at 37 °C. The reactions were terminated by heating (100 °C, 5 min) and the products desalted by passage through 0.2 ml of AG50-X12(H⁺) and two evaporations with 50 µl of toluene.

RESULTS AND DISCUSSION

Purification of PARP

The purification protocol described in this paper is very similar to that used for the purification of lipophosphoglycan (LPG) from *Leishmania major* promastigotes (McConville et al., 1987, 1990b). It involves the delipidation of the cell pellet, using a single-phase chloroform/methanol/water solvent, followed by extraction of the insoluble residue with water saturated with butan-1-ol. Chromatography of this extract on an octyl-Sepharose column revealed a carbohydrate-containing peak eluting at 26% (v/v) propan-1-ol which coincided with a peak of *myo*-inositol. Amino acid analysis of this peak material showed that it contained a glycoprotein rich in Glu and Pro and with a similar composition to that predicted for PARP (Table 1). The presence of ethanolamine and *myo*-inositol, at about 1 residue per 30 Glu and Pro residues, was consistent with the previous identification of a GPI membrane anchor on PARP glycoprotein (Clayton and Mowatt, 1989).

The yield of PARP from *T.b. rhodesiense* (55 nmol per 10¹⁰ procyclics) suggests a minimum copy number of about 3.5×10^6 PARP molecules/cell. This compares favourably with the estimate of 6×10^6 copies/cell for *T.b. brucei* PARP (Clayton and Mowatt, 1989) and suggests a good yield (> 60%), as compared with the yield of 30% for the procedure reported by Clayton and Mowatt (1989). The simple extraction and single-column purification described in this paper are extremely rapid, and

results in a salt- and detergent-free preparation of PARP which is ideal for structural analyses.

Carbohydrate analysis of PARP and the isolated GPI peptide

The PARP material was found to be rich in carbohydrate (Table 1), particularly Gal and GlcNAc. Since PARP is known to contain an Asn-Gly-Thr glycosylation sequon distal to the GPI anchor, at the N-terminal side of the Glu-Pro repeat domain, experiments were performed to distinguish which sugars were specifically associated with the GPI anchor. Previous studies with PARP purified by the conventional procedure (Clayton and Mowatt, 1989) have shown that the molecule is largely resistant to the actions of Pronase and *Staphylococcus aureus* V8 protease (M. A. J. Ferguson and C. E. Clayton, unpublished work). The octyl-Sepharose-purified PARP was digested extensively with the enzyme prolidase and rechromatographed on an octyl-Sepharose column. The GPI-peptide fragment eluted at 27% (v/v) propan-1-ol and this material was subjected to compositional analysis as described before (Table 1). This fraction contained *myo*-inositol, ethanolamine, glycine (the mature C-terminal amino acid residue) and a few residues of glutamine and proline, but none of the amino acid residues characteristic of the mature N-terminal domain, such as Lys and Thr. Most significantly, this GPI-peptide fraction did not contain any aspartic acid residues, which rules out the presence of the putative N-linked glycosylation site. Accordingly, the large amounts of Gal, GlcNAc and sialic acid must be associated with the GPI-anchor glycan. The prolidase-generated GPI-peptide fragment contains significantly less mannose than the intact glycoprotein; this supports the notion that the Asn-Gly-Thr sequon does contain an N-linked glycan, probably of the oligomannose type.

The presence of two mannose residues/mol of inositol in the GPI-peptide fragment is consistent with a conventional trimannosyl GPI-core glycan (Ferguson, 1991) where one of the three mannose residues is cryptic to the monosaccharide analysis due to the presence of the ethanolamine phosphate bridge (Ferguson et al., 1985, 1988). This is also consistent with the deduced structure of the GPI precursor isolated from *T. brucei* procyclic cells (Field et al., 1991b). The Gal, GlcNAc and sialic acid residues presumably form a complex side-chain on this core which is added after transfer of the GPI precursor to the protein. The presence of Gal and GlcNAc in approx. equimolar amounts suggested that this side-chain might be based on a polylactosamine type of structure.

Partial characterization of the GPI glycan

The GPI-peptide fraction was deaminated with nitrous acid and the released phosphatidylinositol (PI) moiety was recovered by butanol extraction for structural characterization (Ferguson, 1992a). The delipidated glycan was subsequently reduced with NaB³H₄ to yield a [1-³H]AHM-containing product. The presence of AHM, and the isolation of released PI, is indicative of a typical GlcN-PI glycosidic linkage common to all GPI membrane anchors (Ferguson, 1991, 1992b). After paper chromatography the radiolabelled glycan was dephosphorylated with cold 48% (v/v) aq. HF, re-N-acetylated, desalted and desialylated by mild acid hydrolysis to yield a neutral glycan fraction. This material was further purified from radiochemical contaminants by high-voltage paper electrophoresis and analysed by Bio-Gel P-4 chromatography (Figure 1a). The neutral glycan fraction eluted as a broad peak from around 15 or 16 glucose units up to and including the void volume of the column. The glycan fraction is clearly polydisperse, indicating a large degree of size heterogeneity. The compositional data in Table 1 suggest an average

Figure 1 Bio-Gel P-4 chromatography of the desialated PARP GPI glycans

The radiolabelled desialated PARP-GPI-glycan fraction was analysed by Bio-Gel P-4 chromatography before (a) and after (b) exhaustive endo- β -galactosidase digestion. Radioactivity was detected by liquid scintillation counting of fraction aliquots. The numbers at the top of the figure are glucose units (Gu) and indicate the elution positions of co-injected glucose oligomer internal standards. V_0 indicates the column void volume.

formula of $\text{Gal}_6\text{GlcNAc}_6\text{Man}_3\text{AHM}_1$ for the desialylated glycans. These results suggest an average size of about 32 glucose units, estimating hexoses as 1 glucose unit each, *N*-acetyl hexosamines as 2.0 glucose units each (Yamashita et al., 1982) and AHM as 1.7 glucose units (Ferguson et al., 1988). Thus the Bio-Gel P-4 chromatogram suggests that the neutral glycan fraction could contain glycans ranging from about $\text{Gal}_4\text{GlcNAc}_4\text{Man}_3\text{AHM}_1$ to about $\text{Gal}_{14}\text{GlcNAc}_{14}\text{Man}_3\text{AHM}_1$.

The possible presence of *N*-acetylglucosamine ($\text{Gal}\beta 1-4\text{GlcNAc}$) repeats was investigated using the enzyme endo- β -galactosidase. The fractions containing radioactive glycans were pooled, dried, digested with endo- β -galactosidase for 16 h and rechromatographed on a Bio-Gel P-4 column. This resulted in a substantial shift in the glycan population towards smaller species (results not shown). The radioactive fractions were pooled again and re-digested with endo- β -galactosidase for 60 h. Analysis of this exhaustive digest by Bio-Gel P-4 chromatography (Figure 1b) revealed radioactive glycans ranging from the void volume to around 11 glucose units, and the formation of a discrete 9.2 glucose-unit peak. Further digestion of the products with endo- β -galactosidase caused only minimal additional digestion (results not shown). The results indicate that the neutral glycans contain at least some *N*-acetylglucosamine units, but that large regions of the glycans are inherently resistant to the enzyme. This could be due to the presence of some α -Gal side-chains and/or branched poly-*N*-acetylglucosamine units, both of which confer resistance to endo- β -galactosidase (Scudder et al., 1987). Both of these features have been reported recently as components of the complex N-linked oligosaccharides isolated from the type 2 VSGs of bloodstream-form *T.b. brucei* (Zamze et al., 1991). Resolution of these possibilities, and the location of the terminal sialic acid residues, must await more detailed structural analyses.

The 9.2 glucose-unit peak was isolated and could be digested to an oligosaccharide of 7.2 glucose units with jack-bean β -hexosaminidase, which could in turn be digested to 5.2 glucose units with jack-bean β -galactosidase. This 5.2 glucose-unit product was found to be resistant to digestion with jack-bean β -hexosaminidase, jack-bean α -mannosidase and coffee-bean α -galactosidase. These exoglycosidase-digestion results suggest that the 9.2 glucose-unit glycan contains the following non-reducing terminal sequence: β -D-GlcNAc-(β -D-Gal) $_2$ -X; where X is not α -Gal, β -Gal, β -GlcNAc or α -Man. The 5.2 glucose-unit glycan is presumably based on the conserved GPI-core structure, which after deamination, reduction and dephosphorylation yields a 4.2 glucose-unit glycan of $\text{Man}\alpha 1-2\text{Man}\alpha 1-6\text{Man}\alpha 1-4\text{AHM}$ (Ferguson et al., 1988; Schneider et al., 1990). This supposition would be consistent with the structure deduced for the GPI precursor molecule isolated from *T.b. brucei* procyclic cells (Field et al., 1991a). We can therefore tentatively conclude that 9.2 glucose-unit species, generated by endo- β -galactosidase digestion, has the structure: β -GlcNAc- β -Gal- β -Gal-X-[$\text{Man}\alpha 1-2\text{Man}\alpha 1-6\text{Man}\alpha 1-4\text{AHM}$], where the nature of the residue 'X' is currently unknown.

The GPI glycans isolated from PARP in this study represent the most complex structures described to date. Sialic acid has been shown to be a component of the GPI anchor of the scrapie prion protein (Stahl et al., 1992), but in this case it is present in only a small side-chain of sialic acid-Gal-GalNAc, linked to the conserved GPI core. In the case of the PARP GPI glycan, the sialic acid residues could be removed with *Clostridium perfringens* neuraminidase (results not shown), indicating that they are not O-acetylated forms. Due to the methanolysis conditions used for the monosaccharide analysis it was not possible to distinguish between *N*-acetyl and *N*-glycolyl forms of sialic acid. The presence of sialic acid in the PARP GPI anchor is quite surprising since

sialic acid is absent from all of the *T. brucei* VSG N-linked glycans and GPI-anchor glycans analysed to date (Ferguson et al., 1988; Zamze et al., 1990, 1991; Güther et al., 1992). It is generally thought that these organisms may be deficient in CMP-sialic acid synthesis and/or conventional sialyltransferase activities, although this has not been directly addressed. An alternative model for the sialylation of the PARP GPI anchor might involve a trans-sialidase on the cell surface of the procyclic forms of *T. brucei* which can utilize the sialoglycoproteins present in the fetal calf serum (used during cell culture) as sialic acid donor. This would be analogous to the situation in the trypomastigote form of the American trypanosome *Trypanosoma cruzi*, where such a trans-sialidase activity has been demonstrated (Prevato et al., 1985; Zingales et al., 1987; Schenkman et al., 1991). This possibility raises the question of whether the sialic acid content could be an artefact of the culture conditions, or whether similar sialylation of PARP could occur *in vivo* in the tsetse fly gut via trans-sialylation from ingested serum sialoglycoproteins.

Physico-chemical comparison between PARP and *Leishmania* LPG

The PARP purification procedure described here was originally applied to *T.b. rhodesiense* procyclics to search for the expression of an LPG homologue. *Leishmania* LPG molecules are made up of phosphorylated disaccharide repeat units, which can be substituted with mono- to tetra-saccharides according to the source of the LPG. These repeat domains are attached to a phosphoheptasaccharide core, which is linked to a lyso-alkylphosphatidylinositol (McConville et al., 1990b; Thomas et al., 1992; Ilg et al., 1992). They are related to GPI membrane anchors in that they share a common $\text{Man}\alpha 1-4\text{GlcNAc}\alpha 1-6\text{Ins}1$ -lipid motif. The observations described here suggest that the African trypanosomes do not express detectable quantities of LPG-like molecules. However, it is noteworthy that, like *Leishmania* LPG, the PARP molecule is a GPI-anchored polyanionic molecule with an extended conformation and similar charge density (one fixed negative charge per 0.5–1.0 nm of length) (Roditi et al., 1989; Ferguson et al., 1991; Homans et al., 1992). This general physico-chemical similarity between PARP and LPG could explain the ability to purify both molecules by the same extraction and chromatography protocol. The current study indicates a minimum copy number of 3.5×10^6 PARP molecules/procyclic trypanosome, which is similar to the estimates for LPG on *Leishmania* promastigotes (Orlandi and Turco, 1987; McConville and Bacic, 1989; Ilg et al., 1992). Taking all these similarities into account, it is conceivable that these two molecules perform similar protective and/or adhesive (Davies et al., 1990; Pimenta et al., 1992; McConville et al., 1992) roles for the insect mid-gut-dwelling forms of these two distantly related kinetoplastid parasites.

In addition to the polyanionic rod structure, PARP also contains a large glycan proximal to the plasma membrane as part of its GPI anchor. Given the high copy number of PARP per cell, this carbohydrate could form a glycocalyx covering a substantial proportion of the plasma membrane. This could have a generally protective function, shielding the membrane from the hydrolases of the insect gut. Preliminary studies (M. J. McConville and P. Murray, unpublished work) indicate a paucity of glycolipids in *T.b. rhodesiense* procyclics, whereas *Leishmania* promastigotes are rich in glycoinositol-phospholipids (McConville et al., 1990a; McConville and Blackwell, 1991). In this respect the large GPI glycan of the PARP molecule may take the place of these smaller cell-surface glycolipids. Thus it is possible that PARP has adopted a structure with which *T.b. rhodesiense* presents to tsetse fly gut

a surface not dissimilar to the combined effects of LPG and the glycoinositol phospholipids on the surface of *Leishmania* promastigotes.

Note added in proof (Received 17 December 1992)

Trans-sialidase activities on the surface of *T. brucei* procyclics have been found by the groups of Victor Nussenzweig (New York University) and Roland Schaur (Christian-Albrechts Universität, Kiel), (personal communications).

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