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MINI REVIEW

Glycoconjugate structures of parasitic protozoa

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Glycoconjugates are abundant and ubiquitous on the surface of many protozoan parasites. Their tremendous diversity has implicated their critical importance in the life cycle of these organisms. This review highlights our current knowledge of the major glycoconjugates, with particular emphasis on their structures, of representative protozoan parasites, including *Leishmania*, *Trypanosoma*, *Giardia*, *Plasmodia*, and others.

Key words: glycan/glycoconjugate/GPI anchors/parasite/pathogens

Introduction

Protozoan parasites have evolved unique lifestyles, often shuttling between their intermediate carriers and vertebrate hosts, encountering extremely harsh environments specifically designed to keep such microbial invaders at bay. Their survival strategies frequently involve the participation of glycoconjugates that form a protective barrier against hostile forces. In fact, a common feature of the parasite's cell surface architecture is the presence of an elaborate and often highly decorated glycocalyx that allows the parasite to interact with and respond to its external environment.

From the variant surface glycoprotein (VSG) of *Trypanosoma brucei* to the various phosphoglycans of *Leishmania*, these molecules are essential for parasite virulence. The diversity of the glycoconjugate structures and consequently the range of functions that have been ascribed to these molecules, from host cell invasion to the deception of the host's immune system, is simply astounding. Also interesting is the observation of parallels and similarities in structure that underscore evolutionary relationships between the different parasites. For example, an overwhelming number of these surface glycoconjugates are glycosylphosphatidyl inositol (GPI) anchored even though their functions are vastly different.

The purpose of this mini review is to highlight the known structures of the major glycoconjugates of representative protozoan parasites, with brief reference to their diverse roles in parasite virulence (Table I). We will not describe, however, the structures of GPI anchors in any great detail because several excellent reviews have been published on the subject (McConville and Ferguson, 1993; Ferguson, 1999) nor will we review the biosynthetic pathways.

African trypanosomes

The African trypanosomes of the species *brucei* are the etiologic agents of nagana disease in cattle and sleeping sickness in humans. The parasites are transmitted by blood-sucking tsetse flies and live extracellularly in the blood, lymph, and interstitial fluids of the mammalian host. Within the mammalian host, the parasites are polymorphic and are described as slender, intermediate, or stumpy trypomastigotes. In the insect the parasites migrate to the salivary glands, where they differentiate into epimastigotes and finally the metacyclic trypomastigote, which is infective. When the tsetse fly feeds, the parasites are inoculated into the host bloodstream, where they are exposed to the host immune system. Evasion of the host immune response relies on "antigenic variation" (Borst *et al.*, 1996; Rudenko *et al.*, 1998), a highly evolved strategy of survival that depends heavily on surface glycoproteins.

VSG

The cell surface of the metacyclic *T. brucei* trypomastigote is covered with a dense glycocalyx composed of approximately 10 million molecules of VSG. The parasite genome harbors about 1000 VSG genes that are expressed sequentially, allowing the parasite to evade recognition by the humoral immune system. Each VSG coat is encoded by a single VSG gene. As the parasites multiply in the host bloodstream and tissue spaces, the host immune system mounts an immune response that is effective against only a certain population of parasites, those expressing the antigenic VSG on their coat. Those that have "switched" to an alternative VSG coat escape the immune system. The glycocalyx also provides a diffusion barrier, thus preventing complement-mediated lysis.

The VSGs (Figure 1) are dimeric proteins, consisting of two 55-kDa monomers; each monomer carrying at least one occupied *N*-linked glycosylation site (Zamze *et al.*, 1991; Mehlert *et al.*, 1998; Ferguson, 1997, 1999). The amino-terminal domain represents roughly 75% of the mature protein and displays the most sequence diversity, while the carboxy-terminal domains are more similar. The diversity of the amino-termini gives the VSGs their unique immunological properties. VSGs are classified as class I, II, III, or IV based on peptide homology of the C-terminal domains (Carrington *et al.*, 1991). Most VSG variants belong to class I or II. Type I VSGs carry one conserved *N*-glycosylation

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Table I. Parasitic glycoconjugates

Parasite	Glycoconjugate	Representative references
Trypanosoma brucei	Variant surface glycoprotein (VSG)	Ferguson, 1997, 1999; Borst *et al.*, 1996
	Procyclic acidic repetitive protein (PARP)	Mehlert *et al.*, 1998; Ferguson *et al.*, 1993; Schenkman *et al.*, 1994
Trypanosoma cruzi	Mucins	Ferguson *et al.*, 1993; Previato *et al.*, 1994, 1995; Schenkmann *et al.*, 1993; Serrano *et al.*, 1993
	Glycosylinositolphospholipids (GIPLs)	Previato *et al.*, 1990; Carriera *et al.*, 1996
	Lipopeptidophosphoglycan (LPPG)	Previato *et al.*, 1990; Lederkremer *et al.*, 1991
Leishmania	Lipophosphoglycan (LPG)	Turco and Descoteaux, 1992; Descoteaux and Turco, 1999
	GIPLs	Turco, 1994; McConville *et al.*, 1994; McConville and Ferguson, 1993
	GP63	Funk *et al.*, 1997; Olafson *et al.*, 1990; Schlagenhauf *et al.*, 1998
	Secreted acid phosphatase (sAP)	Ilg *et al.*, 1999a, b; 1994a, b
	Proteophosphoglycan (PPG)	Ilg *et al.*, 1995, 1996, 1998, 1999a, b
	Phosphoglycan	Turco and Descoteaux, 1992; Descoteaux and Turco, 1999
Entamoeba	Gal-GalNAc lectin	Petri, 1996
	LPG/LPPG	Moody *et al.*, 1997; Moody-Haupt *et al.*, 2000
	Sialylated glycoconjugates	Stanley *et al.*, 1995; Chayen *et al.*, 1988; Arroyo-Begovich *et al.*, 1980, 1982
Crithidia	*D*-mannan	Previato *et al.*, 1979; Gorin *et al.*, 1979; Gottlieb *et al.*, 1978
	Lipoarabinogalactan	Schneider *et al.*, 1996; Previato *et al.*, 1979; Gorin *et al.*, 1979; Gottlieb *et al.*, 1978
Giardia	Cyst wall—GalNAc & GalN-protein complexes	Jarroll *et al.*, 1989; Manning *et al.*, 1992; Bulik *et al.*, 2000
	Variant surface protein (VSP)	Gillin *et al.*, 1990; Nash, 1992; Gillin and Reiner, 1996
	O-glycosylation	Papanastasiou *et al.*, 1997
Plasmodia	*N*-glycosylation	Murphy *et al.*, 1990; Kimura *et al.*, 1996; Burghaus *et al.*, 1999; Yang *et al.*, 1999
	O-glycosylation	Nasir-ud-Din *et al.*, 1992; Dieckmann-Schuppert *et al.*, 1993; Khan *et al.*, 1997
Trichomonads	LPG	Singh, 1993, 1994; Singh *et al.*, 1994; Singh *et al.*, 2000
	Adhesins	Corbeil *et al.*, 1989; Shaia *et al.*, 1998; Singh *et al.*, 1999
	Chitin	Kneipp *et al.*, 1998

site, 50 residues from the C-terminus, that is occupied by high mannose structures ($Man_{5–9}GlcNAc_2$, predominantly $Man_7GlcNAc_2$). The class II glycosylation sites are five to six residues in length and located 170 residues upstream of the C-terminus (Mehlert *et al.*, 1998). The C-terminal site is usually occupied by high mannose structures or by polylactosamine rich structures, and the inner site is modified by smaller oligosaccharides such as $Man_{3–4}GlcNAc_2$ or $GlcNAcMan_3GlcNAc_2$. One characterized class III VSG contains three putative *N*-glycosylation sites modified with either high mannose or biantennary structures. Both the complex and polylactosamine glycan chains may be terminated with an α1,3-linked Gal. Each VSG monomer is GPI anchored to the plasma membrane (Ferguson *et al.*, 1988). The GPI anchor is preassembled as a precursor structure that is then transferred to the mature C-terminal amino acid (aspartate for class I, serine for class II, asparagine for class III) with the concomitant loss of the C-terminal GPI anchor signal sequence (Englund, 1993). The mature anchor consists of the classical GPI core ethanolamine-HPO_4-6Man(α1,2)Man(α1,6)Man(α1,4)GlcN(α1,6)-inositoldimyristoylglycerol; however, in class I and II, the anchor is uniquely modified by Gal side chains (Holder, 1985; Ferguson *et al.*, 1988; Redman *et al.*, 1994). The class I VSG GPIs contain an average of three Gal residues, and class II VSGs contain up to six Gal residues. The class II VSGs usually contain an additional α-Gal attached to the 2-position of the nonreducing α-Man residue and one-third of the structures contain an additional β-Gal attached to the 3-position of the middle Man.

Procyclic acidic repetitive proteins (PARPs) or procyclins

The surface coat of the procyclic form of *T. brucei* (the form found in the tsetse flies) is less densely packed and composed largely of acidic glycoproteins called the procyclins, also called the PARPs or procyclic acidic repetitive proteins (Treumann *et al.*, 1997; Mehlert *et al.*, 1998 and references therein) (Figure 1). Each cell expresses approximately 5 million copies of procyclins. The PARPs form a dense glycocalyx of GPI anchors with the polyanionic polypeptide repeat domains projecting above the membrane. There are two families of PARPs; the EP-PARPs bear glutamate-proline repeats and the GPEET-PARPs bear glycine-proline-glutamate-glutamate-threonine repeat sequences (Roditi *et al.*, 1987; Mowatt and Clayton, 1988). Some members of the EP-PARP family are *N*-glycosylated adjacent to the repeat domain. An unusual feature of this glycosylation site is that only a single type of *N*-glycan is found, a $Man_5GlcNAc_2$ oligosaccharide. The GPI

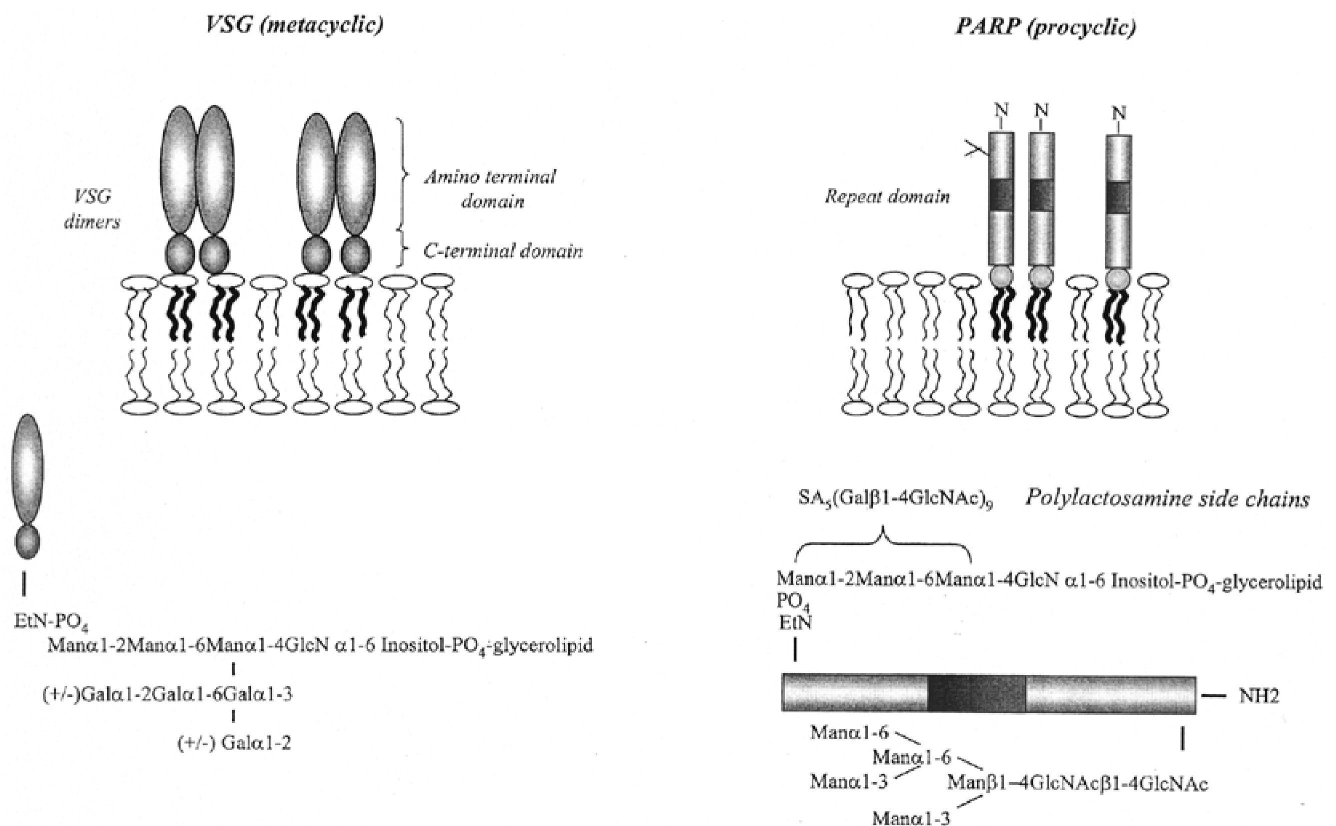


Fig. 1. A schematic representation of the major surface glycoconjugates of procyclic and metacyclic *T. brucei*. VSG (variant surface glycoprotein) is the major component of the metacyclic form, each molecule consisting of two GPI-anchored *N*-glycosylated monomers. The shaded ovals represent the protein component. The surface of the procyclic form is densely covered with PARPs, or procyclic acidic repetitive proteins. These are GPI-anchored polypeptides with polyanionic repeat domains (shaded in schematic). The anchor structures are detailed below the schematic.

anchors are modified with unusual branched polylactosamine [Gal(β1,4)GlcNAc] glycans (Ferguson *et al.*, 1993; Treumann *et al.*, 1997). Interestingly, when the parasites are grown in culture, the β-Gal termini of these side chains are substituted with sialic acid by the action of a trans-sialidase enzyme (Engstler *et al.*, 1993; Schenkman *et al.*, 1994). Recent work has shown that GPEET-PARP is phosphorylated (Medina-Acosta *et al.*, 1989; Butikofer *et al.*, 1999). The various PARPs appear to be expressed in a cell cycle–specific manner, and it has been hypothesized that the oligosaccharide chains may serve as lectin binding ligands within the tsetse fly midgut.

Trypanosoma cruzi

T. cruzi is the etiologic agent of Chagas' disease or South American trypanosomiasis and is transmitted by Reduviid bugs. The complex life cycle involves an intracellular dividing amastigote stage in mammalian host tissues and an extracellular nondividing bloodstream form, called the trypomastigote, which propagates the infection. The ingestion of the bloodstream parasite by the insect vector results in differentiation into epimastigotes that colonize the insect midgut. Migration to the hindgut promotes development into metacyclic trypomastigotes that are adapted for transmission via fecal contamination of fresh wounds or mucus membranes.

Mucins

T. cruzi has a dense and continuous coat composed of a layer of type I glycosylinositolphospholipids, or GIPLs (Previato *et al.*, 1990; Lederkremer *et al.*, 1991; Carriera *et al.*, 1996), and a family of small mucins (Schenkman *et al.*, 1993; Previato *et al.*, 1995; Serrano *et al.*, 1995) that project above the GIPL layer (Figure 2). The GIPLs have the same anchor structure as the *T. brucei* PARPs but are heavily substituted with Gal, GlcNAc, and sialic acid (Ferguson *et al.*, 1993). The mucins are rich in threonine, serine, and proline residues that are heavily glycosylated with *O*-linked GlcNAc as the internal residue and further modified with one to five galactosyl residues. The structures of the *O*-linked oligosaccharides are conserved between the epimastigotes and the metacyclic trypomastigotes. There are certain polymorphisms among the different strains (for example, the presence of galactofuranose [Gal_f] rather than galactopyranose [Gal_p]). Two groups of mucin-like molecules have been identified. Glycoproteins in the first group are 35–50 kDa and are expressed on the parasitic forms found in the insect (epimastigote and metacyclic trypomastigote). The second group consists of 80–200 kDa glycoproteins that are expressed in the cell culture–derived trypomastigotes (Almeida *et al.*, 1994).

Trypanosomes are unable to synthesize sialic acid *de novo* and have the unusual ability to acquire it from host glyco-

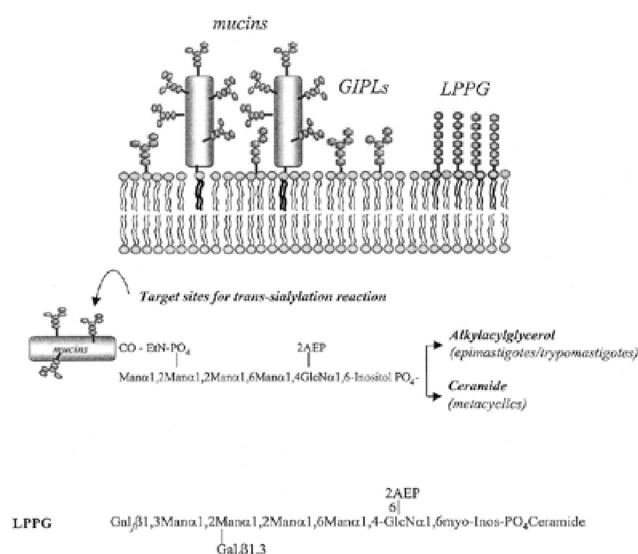


Fig. 2. Schematic representation of the surface coat of *Trypanosoma cruzi*. The cell surface of *T. cruzi* is covered with a dense layer of GIPLs (glycosylinositolphospholipids), mucins, and LPPG (lipopeptidophosphoglycan). The structures of the mucin anchors and the predominant LPPG species are outlined. Aminoethylphosphonate is abbreviated AEP.

conjugates. The galactose-rich mucin is the acceptor for the trans-sialylation reaction in which α2,3-linked sialic acid residues are transferred from host glycoconjugates by a trans-sialidase enzyme to the terminal β-Gal residues of the mucin. *Endotrypanum*, a trypanosomatid parasite of sloths, also has the ability to incorporate host-derived sialic acid into molecules on its own surface. The surface coat seems to have a primarily protective function and the sialylation of the mucins provides the parasite with the ability to survive in different environments. Sialylation is proposed to reduce the susceptibility of the parasite to anti-α-Gal antibodies present in the mammalian bloodstream (Pereira-Chioccola *et al.*, 2000). The heavily sialylated coat may also provide a structural barrier to other lytic agents encountered by the parasite as well as promote adherence to the macrophage (de Diego *et al.*, 1997) and modulate the production of NO and cytokines (de Diego *et al.*, 1997; Camargo *et al.*, 1997).

The structures of the GPI anchors of the mucins undergo modifications with parasite differentiation. The epimastigote mucin GPIs contain *sn*-1-alkyl-2-acylglycerol, and over 70% of the metacyclic mucins contain ceramide lipids (Serrano *et al.*, 1995; reviewed by Ferguson, 1997). Interestingly, the ceramide lipid species is found on lipopeptidophosphoglycan (LPPG), which is expressed in large amounts on the epimastigote surface. Recent work by Almeida *et al.* (2000) have established the role of GPI anchors from the trypomastigote stage of *T. cruzi* in the induction of NO, IL-12, and TNF-α. The trypomastigote GPIs were found to contain unsaturated fatty acids in the *sn-2* position of the glycolipid component.

LPPG

LPPG is the major cell surface glycan of the *T. cruzi* epimastigote with approximately 1.5×10^7 copies per cell. The expression of LPPG appears to be developmentally regulated as it is present in very low levels in the stages that infect the mammalian host (Golgher *et al.*, 1993). LPPG (Previato *et al.*, 1990; Lederkremer *et al.*, 1991) consists of a glycan linked to an inositol-phosphoceramide via a nonacetylated glucosamine (Figure 2). The glycan structure contains Man, nonreducing Gal_f and 2-amino-ethylphosphonate (2-AEP). The LPPG fraction extracted from *T. cruzi* epimastigotes contains three species that have slight variations in the glycan structure, with respect to the position of the nonreducing Gal_f. The major species (65%) bears two terminal Gal_f residues linked β1,3 to Man(α1,2)Man. The lipid component is an inositol phosphoceramide containing mainly palmitoylsphinganine, palmitoylsphingosine, and lignoceroyl-sphinganine. LPPG can be considered a member of the GPI family based on the presence of the Man(α1,4)GlcN(α1,6)*myo*-inositol-1-PO_4-lipid motif, the hallmark of all GPI anchors. It differs from the *Leishmania* GIPLs in the presence of the phosphoceramide moiety, a feature that has been found in the LPG of *Acanthamoeba castellanii* (Dearborn *et al.*, 1976) and in a lipid anchor found in *Dictyostelium discoideum* (Stadler *et al.*, 1989; Haynes *et al.*, 1993). Moreover, *T. cruzi* LPPG bears 2-AEP, as does the *A. castellanii* phosphoglycan.

Leishmania

Leishmania exist as flagellated, extracellular promastigotes in the midgut of their sand fly vector and as intracellular, aflagellar amastigotes in the phagolysosomes of the mammalian macrophage. The parasites are transmitted when a sand fly takes a blood meal from an infected individual. Within the sand fly midgut the promastigotes develop from the avirulent procyclic form to the virulent metacyclic form in a process called metacyclogenesis. Throughout their life cycle, *Leishmania* survive and proliferate in highly hostile environments and have evolved special mechanisms that enable them to endure these adverse conditions, including a dense cell surface glycocalyx composed of lipophosphoglycan (LPG), glycosylinositolphospholipids, or GIPLs (Orlandi and Turco, 1987; McConville and Blackwell, 1991) and secreted glycoconjugates, proteophosphoglycan (PPG) (Ilg *et al.*, 1994a,b, 1996), and secreted acid phosphatase (sAP) (Lovelace and Gottlieb, 1986).

LPG

LPG is the predominant cell surface glycoconjugate of *Leishmania* promastigotes. It is localized over the entire parasite surface, including the flagellum. Found in all species of *Leishmania* that infect humans, it is composed of four domains, (1) a 1-*O*-alkyl-2-*lyso*-phosphatidyl(*myo*)inositol lipid anchor, (2) a glycan core, (3) Gal(β1,4)Man(α1)-PO_4 backbone repeat units, and (4) an oligosaccharide cap structure (Turco and Descoteaux, 1992; Descoteaux and Turco, 1999 and references therein) (Figure 3A). Structural analysis of LPG from different species has revealed complete conservation of the lipid anchor, the glycan core, and the Gal(β1,4)Man(α1)-PO_4 backbone of repeat units. The distinguishing features of LPG are in the variations in the carbohydrate chains that branch off the main backbone and in the cap structures (McConville *et al.*, 1995). The C3 hydroxyl of the repeat unit Gal is the site of most side chain modifications. The LPG of *L. donovani* from Sudan does not possess any side chains, whereas the *L. donovani* LPG

from India possesses one to two β-Glc every four to five repeat units (Bray and Turco, 1999). The *L. major* backbone is galactosylated with one to four residues of β-Gal and often terminated with Ara. In *L. mexicana*, many of the repeat units are modified with β-Glc, whereas *L. tropica* has the most complex side chain modifications with over 19 different types of glycans. The most common *L. donovani* cap is the branched trisaccharide Gal(β1,4)[Man(α1,2)] Man(α1). *L. major* has the simplest cap structure, Man(α1,2)Man(α1).

LPG serves as the ligand for binding to lectins in the sand fly midgut, and thus structural variations correlate with infectivity and transmission by various sand fly species. Structural modifications are observed as the parasite progresses through various life stages. *L. donovani* attaches to its natural sand fly vector's midgut via the LPG cap structure, which terminates in a β-linked Gal and α-linked Man. Although both are required for binding, there is no information on the putative receptor or lectin. As the procyclic promastigotes undergo metacyclogenesis, the number of repeat units doubles from approximately 15 to 30 (Sacks *et al.*, 1995). This is believed to result in a conformational change that masks the terminal cap sequence and thus allows the parasite to detach from the midgut and migrate anteriorly. In *L. major* the doubling of the repeat units is accompanied by an alteration in the side chains that express fewer Gal residues and terminate with Ara (Sacks *et al.*, 1990). These structural changes are believed to obscure epitopes that promote adherence to the midgut and allow passage of the parasites to the mouth parts of the sand fly.

The number of LPG molecules expressed by the intracellular amastigotes is substantially down-regulated. Promastigotes express about 1–5 × 10^6 copies per cell and amastigotes express about 100 or 1000 copies per cell. The amastigote LPG from *L. major* is biochemically distinct from promastigote LPG (Glaser *et al.*, 1991; Moody , 1993). The glycan core and PI anchor structures are conserved among promastigotes and amastigotes with slight variations at the level of glucosylation. The amastigote LPG consists of an average of 36 repeat units that differ considerably from promastigote repeat units. Seventy percent of the repeat units are unsubstituted, and the rest contain Glc_p and Gal_p but no Ara. Also, side chains of up to 11 Gal residues have been found in contrast to the promastigote, where there are only 1–3 side chain Gal residues. The cap structure is predominantly Gal(β1,4)Man(α1-), whereas the major promastigote cap is Man(α1,2)Man(α1-).

LPG has been implicated in a number of functions within the mammalian host. In the blood stream, LPG prevents complement-mediated lysis by preventing insertion of the C5b-9 membrane attack complex into the promastigote membrane. It serves as a ligand for receptor-mediated endocytosis by the macrophage via complement receptors as well as the mannose receptor. Inside the macrophage LPG inhibits protein kinase C and the microbicidal oxidative burst as well as phagosome-endosome fusion (Descoteaux and Turco, 1999).

GIPLs

The GIPLs are a major family of low molecular weight glycolipids synthesized by *Leishmania* parasites, which are not attached to either proteins or polysaccharides (McConville and Ferguson, 1993; McConville *et al.*, 1994; Turco, 1996; Ferguson, 1999 and references therein). These are expressed in very high copy numbers, approximately 10^7 copies per cell on both promastigote and amastigote surfaces. There are three major lineages of GIPLs that are expressed to different levels in different species or developmental stages. Based on the pattern of their glycan headgroups they are classified as type I (analogous to protein GPI anchors and based on the structure Manα1,6Manα1,4GlcNα1,6-PI), type II (analogous to LPG anchors and based on the structure Manα1,3Manα1,4GlcNα1,6-PI), or hybrid (contain features of both and based on the Manα1,6(Manα1,3)Manα1,4GlcNα1,6-PI motif) (Figure 3). The lipid components of the hybrid and type I GIPLs are rich in alkyl-acyl-PI with shorter (C18:0) alkyl chains. The type II GIPLs are more heterogeneous and contain longer alkyl chains (C24:0 or C26:0).

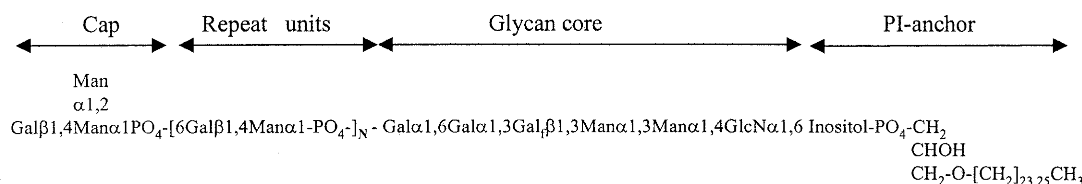
Not much is known about the functions of the GIPLs. The use of the mannose receptor in parasite attachment to the macrophage suggests that the mannose-rich GIPLs may play a role in macrophage invasion. Because the levels of LPG and the major promastigote surface protease, GP63, are dramatically down-regulated, the GIPLs are the major constituents of the amastigote surface and are presumably involved in protecting the parasite from environmental hazards as well as playing some role in parasite–host interactions, especially in the mammalian stage. In fact there is evidence that GIPLs are involved in modulating signaling events in the macrophage such as NO synthesis and the oxidative burst (McNeely *et al.*, 1989; Proudfoot *et al.*, 1995; Tachado *et al.*, 1997, 1999). There is also recent data to show that enzymes involved in GPI biosynthesis are essential for parasite virulence (Ilgoutz *et al.*, 1999), thus emphasizing the importance of protein-free GPI glycolipids in parasite viability.

GIPLs that are structurally related to the *Leishmania* type II and/or hybrid GIPLs and the *T. cruzi* LPPG have been identified in *Leptomonas samueli*, *Endotrypanum schaudinni*, *Herpetomonas samuelpessoai*, and *Crithidia fasciculata* (Previato *et al.*, 1992; Routier *et al.*, 1995; Schneider *et al.*, 1996). The *Leptomonas* glycophosphosphingolipid resembles *Leishmania* in the α-linked Man in the GPI core and resembles *T. cruzi* in the presence of aminoethylphosphonate and ceramide lipids. The *Endotrypanum* GIPLs are similar to *Leishmania* type II and hybrid GIPLs but are sphingolipids rather than glycerol lipids. Several *Endotrypanum* GIPLs contain D-Ara, and the terminal saccharide chains resemble the phosphoglycan side chains of *L. major* LPG. The *Leishmania* GIPLs contain predominantly α-linked Gal, whereas *Endotrypanum* contains β-linked Gal. Overall structural similarities are consistent with the close evolutionary relationship between *Leishmania*, trypanosomes, *Leptomonas*, and *Endotrypanum*.

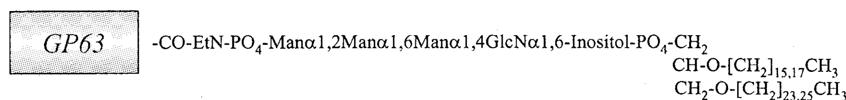
GP63

GP63 (Figure 3B) is the major cell surface glycoprotein of *Leishmania* promastigotes with 500,000 copies per cell and accounting for 1% of all cellular proteins. In amastigotes GP63 is expressed to a lower level, and the bulk of it is found in the flagellar pocket as opposed to covering the entire surface, as in promastigotes (Medina-Acosta *et al.*, 1989). It is a 63-kDa zinc metalloprotease and is anchored to the cell surface via a myristic acid containing GPI anchor. The amastigote GP63 subpopulation found in the flagellar pocket lacks a membrane anchor. Recently the crystal structure was solved and found to contain an active site structural motif found in other zinc proteases that may aid design of specific inhibitors (Schlagenhauf *et al.*,

A. ***LPG***



B. ***Protein anchors***



C. ***Hybrid GIPLs***

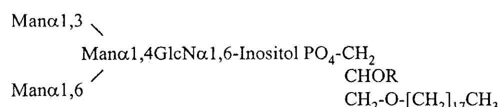


Fig. 3. Structures of *Leishmania* surface glycoconjugates. (A) LPG (lipophosphoglycan), the predominant glycoconjugate of promastigotes, consists of a Gal-Man-PO_4 repeat unit backbone attached to a lipid anchor via a glycan core. The structure of *L. donovani* promastigote LPG is shown above. Type II GIPLs share the LPG anchor structure. (B) Structure of GP63/glycoprotein anchors, also shared by type I GIPLs. (C) Structure of hybrid GIPLs.

1998). Primary sequence analysis has shown that GP63 contains three potential glycosylation sites (Button and McMaster, 1988). The *N*-linked glycans have been characterized in *L. mexicana* and *L. major* (Olafson *et al.*, 1990; Funk *et al.*, 1997). The glycans are biantennary high mannose-type, and some bear a terminal Glc in α1,3 linkage. The two major structures found in all promastigote species examined are $Man_6GlcNAc_2$ and $GlcMan_6GlcNAc_2$. In amastigotes the structures are more variable, and in *L. donovani* there appear to be no *N*-linked glycans. The presence of terminal Glc in the GP63 glycan is highly unusual with respect to oligomannose structures found in glycoproteins. Whether the stage-specific changes in glycan structure affect parasite infectivity and development is unknown.

The importance of GP63 in parasite life cycle is not well defined. GP63 has been shown to be proteolytically active against a number of substrates and thus may be involved in degradation of host macromolecules. It may also serve as a ligand for the macrophage receptor via complement components and protect the parasite against complement mediated lysis (Alexander and Russell, 1992). Attempts to obtain mutants that are defective in GP63 by targeted gene deletion is made difficult by the fact that it is encoded by a multigene family. Targeted deletion of six out of seven GP63 genes did not affect growth of the parasite *in vitro* or prevent formation of disease in mice (Joshi *et al.*, 1998). In a recent study, Hilley *et al.* (2000) generated a knockout of GPI8, the GPI:protein transaminidase that eliminated the expression of GP63 along with other GPI-anchored proteins. The knockout grew normally in culture, and its ability to infect macrophages *in vitro* was unaffected. More importantly the ΔGPI8 mutant was able to establish infection in mice, suggesting that GP63 is not essential for growth or infectivity in mammals.

Secreted glycoconjugates

In addition to cell surface LPG and GIPLs, *Leishmania* secrete a family of heavily glycosylated proteins and proteoglycans that are important for parasite virulence. Most of these express glycans that are similar in structure to those found on LPG, notably the Gal-Man-PO_4 repeat unit motif. The structural features of secreted acid phosphatase, phosphoglycan and proteophosphoglycan are briefly outlined below (Ilg *et al.*, 1999a).

sAP

With the exception of *L. major*, all *Leishmania* promastigotes secrete sAP (Figure 4A) from the flagellar pocket, their chief secretory organelle (Lovelace and Gottlieb, 1986; Bates *et al.*, 1990; Stierhof *et al.*, 1994). The secreted glycoproteins and proteoglycans tend to form distinct macromolecular complexes found both in the flagellar pocket as well as the culture media. Old World species, such as *L. donovani*, *L. tropica*, and *L. aethiopica* secrete mono- or oligomeric sAPs, whereas the South American species, such as *L. mexicana*, *L. braziliensis*, and *L. amazonensis* secrete sAPs that aggregate into large pearl-like filamentous polymers (Ilg *et al.*, 1994a,b, 1999a). The sAPs are encoded by multiple genes that have very high levels of sequence identity, even within different species. The *L. donovani* sAP peptides are heavily glycosylated

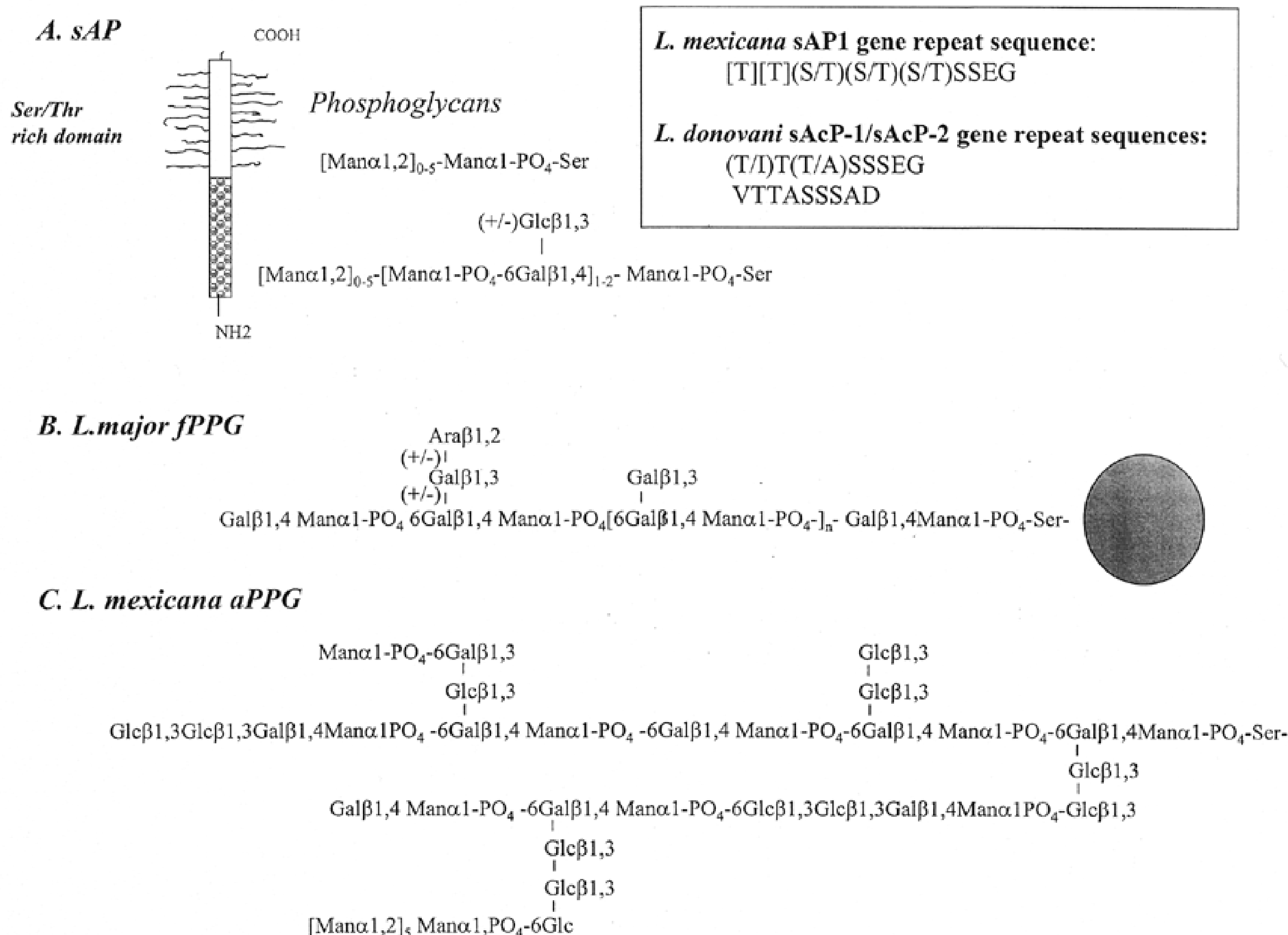


Fig. 4. Structures of secreted *Leishmania* glycoconjugates. (A) Acid phosphatase (sAP). The acid phosphatases are composed of Ser/Thr-rich peptides that are heavily glycosylated on serine with phosphoglycan chains, similar to those found on LPG. The consensus sequences of the repeat domains where phosphoglycosylation occurs are shown in the box. (B) *L major* fPPG or filamentous proteophosphoglycan is a mucin-like molecule with extensive phosphoglycosylation on serine residues. (C) *L. mexicana* aPPG or amastigote-specific PPG is distinct from fPPG and although it shares phosphoglycan structures with LPG and sAP, it is also modified with unique branched glycans.

on C-terminal serine/threonine-rich domains. The glycans are phosphodiester-linked to serine residues and commonly consist of the 6Gal(β1,4)Man(α1-)PO_4 repeat units found on LPG. The average number of repeat units is 32. The target sites of phosphoglycosylation are not random, rather, they are composed of repetitive motifs, with modifications on select serine residues. *L. mexicana* sAP is extensively modified with Man(α1-)PO_4 residues that may be further elaborated with α1,2-linked Man oligosaccharides or short Gal-Man-PO_4 repeats. Often the phosphoglycan chains are terminated with a diverse set of oligomannose cap structures.

PPG

Proteophosphoglycans identified to date in promastigotes include filamentous PPG or fPPG (Figure 4B) and a putative GPI-anchored cell-associated form or mPPG (Ilg *et al.*, 1994b, 1999b; Stierhof *et al.*, 1994). Amastigotes secrete their own nonfilamentous and stage-specific form termed aPPG (Ilg *et al.*, 1995). The filamentous form, fPPG, is secreted by promastigotes of all *Leishmania* species and forms a highly viscous mesh within which the parasites lie embedded. Compositionally fPPG consists of 95% phosphoglycans, with an abundance of serine, alanine, and proline in the peptide component. Over 80–90% of the serine residues are phosphoglycosylated with short Gal-Man-PO_4 repeats attached via phosphodiester bonds, which are terminated by small oligosaccharide cap structures (Ilg *et al.*, 1996). Although there is no direct evidence for the function of fPPG, it is believed that the gel-like matrix, formed by the interlocking filaments, traps the parasites in the sand fly anterior gut. Furthermore it has been hypothesized that the presence of the parasite plug deters the ingestion of a second bloodmeal, thereby encouraging the sand fly to probe several hosts and in the process improve the chances of transmission (Killick-Kendrick *et al.*, 1988).

L. mexicana amastigotes secrete copious amounts of aPPG (Figure 4C) into the phagolysosomal compartment. Structurally

it consists of a defined polypeptide backbone modified with phosphoglycans on serine residues (Ilg *et al.*, 1998). The glycans include previously identified structures common to LPG and sAP but also include a large number of novel highly branched structures, as opposed to fPPG. On average each molecule contains six mono- or multiphospho-oligosaccharide chains, capped by four neutral oligosaccharides. The glycans include neutral structures like $[Glc(\beta 1,3)]_{1-2}$ Gal(β1,4)Man, monophosphorylated glycans containing the conserved Gal-Man-PO_4 backbone but incorporating unusual stage specific modifications such as Gal(β1,3) or $[Glc(\beta 1,3)]_{1-2}$ Glcβ1, and monophosphorylated tri- and tetrasaccharides that are monophosphorylated on the terminal hexose, as well as tri- and tetraphosphorylated glycans. In parasite-containing vacuoles aPPG is found in mg/ml concentrations (Ilg *et al.*, 1995). Within the macrophage, aPPG is believed to contribute to the formation of the parasitophorous vacuole, thus participating in the maintenance of infection in the mammalian host (Peters *et al.*, 1997a,b). Amastigote PPG is believed to activate the complement system via the mannose-binding pathway by virtue of the large number of potential mannose-binding lectin binding sites. There is also evidence that PPG may contribute to the binding of *Leishmania* to host cells and may play a role in modulating the biology of the infected macrophage at the early stage of infection. Recently a membrane-associated form of PPG (mPPG) was identified in *L. major* promastigotes (Ilg *et al.*, 1999b). Analysis of the gene predicts a protein of 2300 amino acids with a large central repeat domain that is the target site for phosphoglycosylation and a C-terminal domain with a putative GPI anchor addition signal sequence.

Phosphoglycan

Culture supernatants of *Leishmania* promastigotes contain a hydrophilic phosphoglycan consisting of capped oligosaccharide repeat units identical to those found on LPG, but minus the GPI anchor and the glycan core (Turco and Descoteaux, 1992). The structure precludes the possibility of PI-PLC–mediated release from LPG, rather it is thought to have been released from the flagellar pocket via exocytosis.

Entamoeba

Entamoeba histolytica, the etiologic agent of amebic dysentery and hepatic abscess, has a life cycle that includes two stages, the disease-inducing amebic or trophozoite form and the infectious cyst stage. Infection is transmitted by the ingestion of the cyst via contaminated food or water. Within the intestine the cysts undergo excystation and develop into metacystic trophozoites that colonize the intestine by binding to colonic mucins and rapidly multiply. The trophozoites may multiply asymptomatically, causing mild intestinal symptoms, or, in more severe situations, invade the mucosal tissues resulting in ulceration and finally liver damage.

The Gal-GalNAc lectin

Binding to the colonic mucins is mediated by the well-characterized Gal-GalNAc lectin, a GPI-anchored protein (reviewed by Petri, 1996) with a critical role in parasite viability. The Gal-GalNAc lectin is a 260-kDa heterodimeric glycoprotein consisting of heavy (170 kDa) and light subunits (either 35 or 31 kDa) disulfide-bonded together. The 170-kDa subunit contains a carboxy terminal cytoplasmic domain adjacent to the cysteine-rich extracellular domain. The amino acid compositions of the two light subunits are similar with size differences attributed to carbohydrate and lipid modifications. Both contain conserved transmembrane domains and GPI anchors (McCoy *et al.*, 1993; Huston and Petri, 1998).

LPG and LPPG

Entamoeba trophozoites synthesize a cell surface lipoglycoconjugate, named lipophosphoglycan (LPG) based on its structural similarity with *Leishmania* LPG. The molecules were studied in various *Entamoeba* strains and found to consist of a lipid anchor and a phosphoglycan component that resembles the phosphoglycans of *Leishmania* LPG (Stanley *et al.*, 1992; Bhattacharya *et al.*, 1992; Moody *et al.*, 1997). A second species purported to have a protein component was named lipopeptidophosphoglycan or LPPG. The molecules are of importance as virulence factors because antibodies raised against them inhibited the ability of the parasites to kill target cells while vaccination retarded the development of liver abscess in animal models (Marinets *et al.*, 1997). Moreover, antigenically different molecules were found in different species and the polymorphisms appeared to be related to virulence (Moody *et al.*, 1997). Early studies provided no evidence of a protein component but showed the presence of a phosphoglycan composed of Gal and Glc attached to an undefined backbone. In a recent paper, Moody-Haupt *et al.* (2000) further characterize the surface antigens and show that the LPGs are actually two populations of GPI-anchored proteophosphoglycans. Like the *Leishmania* PPGs, these contain a highly acidic polypeptide component rich in aspartate, glutamate, and phosphoserine. The serine residues are extensively modified with chains of 2–23 Glc(β1,6)Gal disaccharide repeats that are presumably attached to the peptide backbone via a Gal-1-PO_4-serine linkage, that has previously not been reported (Figure 5). The PPG GPI anchors are unique in that they have a novel glycan backbone that contains the sequence Gal_1Man_2GlcN-(*myo*)inositol, the terminal α1,2 mannose residues of other protein anchors being replaced by the α-Gal residue. The anchor is also modified with 1–20 α-Gal residues. The trophozoite surface coat has an estimated 80 million copies and presumably plays an important role in modulating cell surface interactions, either by serving as ligands for host

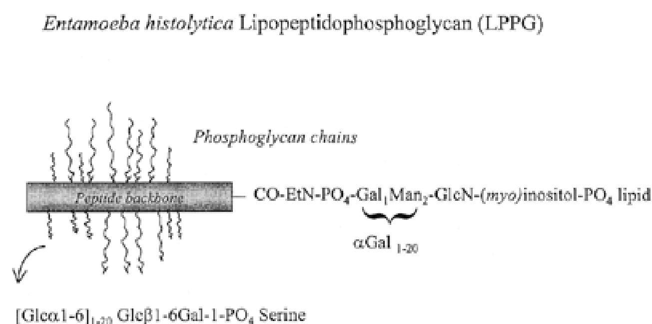


Fig. 5. Structure of *Entamoeba histolytica* lipopeptidophosphoglycan (LPPG).

receptors or by regulating the interactions of other cell surface molecules.

Sialylation- and encystation-specific glycoproteins

The *Entamoeba* cyst wall is mostly composed of chitin (Arroyo-Begovich and Carbez-Trejo, 1982), and there is evidence that the encystation process is accompanied by the appearance of sialylated glycoconjugates on the surface. Sialic acid is found on glycolipids in *E. invadens* trophozoites and as part of glycoproteins on cysts (Ribiero *et al.*, 1989; Avron *et al.*, 1986, 1987). In *E. histolytica* sialic acid has been reported on cyst specific glycoproteins (Chayen *et al.*, 1988; Stanley *et al.*, 1995). Thus the pattern of sialylation on the cell surface changes during encystation. Although numerous roles have been described for sialylation of mammalian glycoconjugates, a specific role cannot, as yet, be ascribed in the case of *Entamoeba*. It has been hypothesized, however, that the negative charge on the cyst surface afforded by the sialylated glycoconjugates may repel the cyst from the intestinal mucosa and thus promote expulsion from the host's intestine. Recently a novel encystation-specific glycoprotein called Jacob was identified as an abundant component of the *E. invadens* cyst wall (Frisardi *et al.*, 2000). A putative chitin-binding lectin, Jacob is a 45-kDa protein with a series of cysteine-rich domains and is bound by concanavalin A, wheat germ agglutinin, and ricin. It is believed to be involved in the formation of the cyst wall during encystation in conjunction with other encystation specific glycoproteins and the Gal/GalNAc lectin.

Crithidia

Crithidia are monogenetic members of the trypanosomatid family that colonize the digestive tract of flies. Two major types of glycans (Gottleib, 1979; Gorin *et al.*, 1979; Previato *et al.*, 1979) have been identified and characterized in *Crithidia*; a β1,2-linked D-mannan, which is a polymer of 50 or more Man residues, and a lipoarabinogalactan that consists of a lipid anchored β1,3 linked D-galactan modified with Ara residues (Figure 6). The lipid anchor is similar but not identical to the *Leishmania* LPG anchor and consists of a glucosaminylinositol phosphoceramide (Schneider *et al.*, 1996). The deduced structure of the arabinogalactan of *Crithidia* is $[Ara_p1\text{-}2]_x\text{-}[Gal\beta1,3]_n$-Gal(β1,3)Gal(α1,3)$Gal_f$(β1,3)Man(α1,3)Man(α1,4)GlcN(α1,6)-inositol phosphoceramide. The unusual D-Ara_p has been found in *E. schaudinni* (Xavier Da Silveira *et al.*, 1998) and the LPG of *Leishmania*. Earlier studies reported the presence of soluble arabinogalactan (Gorin *et al.*, 1979), however, purification procedures used at the time probably resulted in hydrolysis of the molecule at the Gal_f residue. Thus, the soluble glycan probably corresponds to the lipid-anchored lipoarabinogalactan reported by Schneider *et al.* (1996).

In a recent study, sialic acid–bearing glycoconjugates have been identified on the surface of *Crithidia* (Valle Matta *et al.*, 1996), although the structures of these molecules are unknown. Interestingly, the presence of sialoglycoconjugates in *Crithidia* growing in sialic acid–free chemically defined media suggests that the residues are synthesized *de novo* and not transferred from existing glycoconjugates by a trans-sialidase, as observed in trypanosomes.

Giardia

Protozoan parasites of the genus *Giardia,* the etiologic agents of diarrhea illness worldwide, represent one of the most ancient extant eukaryotes. The flagellated, binucleate, and actively dividing trophozoite colonizes the small intestine of a variety of mammalian hosts. In the intestine some trophozoites are induced to encyst, and the mature cysts are passed in excrement. The highly infective cysts live freely outside their host until they are introduced to another host, most commonly via contaminated drinking water.

Cyst

During cyst formation trophozoites become encased in a thick cyst wall, which helps the parasite survive the extremes of pH, temperature, and osmolarity between microenvironments. The cyst wall is distinguishable by an outer wall composed largely of a filamentous-type network. Once believed to be composed of chitin (Ward *et al.*, 1985), the cyst wall filaments of *G. lamblia* (Jarroll *et al.*, 1989; Bulik *et al.*, 2000) and *G. muris* (Manning *et al.*, 1992) are actually composed of GalNAc and galactosamine-protein complexes with GalNAc being the major sugar. However, the exact chemical structure is currently unknown.

The cyst wall proteins, CWP-1 and CWP–2, are 26 kDa and 39 kDa proteins, respectively, and combine to form a stable complex within the cyst wall of mature *G. lamblia* cysts, possibly through disulfide cross-linking. CWP-1 and CWP-2 each have one potential *N*-glycosylation site (Lujan *et al.*, 1997), although no oligosaccharide structures have been detected in these proteins. The presence of GalNAc and Gal as well as an abundance of serine/threonine residues (potential *O*-glycosylation sites) along with the enzymes GalN, GalNAc and GlcNAc transferases (Das and Gillin, 1996; Lujan *et al.*,

Crithidia fasciculata Lipoarabinogalactan (LAG)

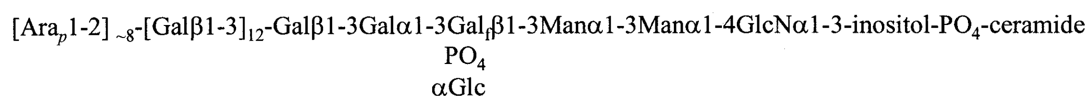


Fig. 6. Structure of lipoarabinogalactan (LAG) from *Crithidia fasciculata*.

1997) are suggestive of the presence *O*-glycosylation, however none has been reported in the cyst wall of *G. lamblia*.

Variant surface protein

The trophozoite surface, which has direct interaction with its host, is covered by a dense coat composed of one of a family of variant surface proteins (VSPs) (Gillin *et al.*, 1990; Nash, 1992; Gillin and Reiner, 1996). Although antigenic variation is generally very important in immune evasion, here VSPs are most likely involved in intestinal protease resistance. Much like VSGs of trypanosomes, *G. lamblia* VSPs undergo *in situ* type antigenic switching occurring about every 6–13 generations (Svard *et al.*, 1998). This enables the parasite to survive its mammalian host's intestinal milieu. VSPs vary greatly in size (50–250 kDa) and sequence but share common structural components. They have a highly conserved 26–27 amino acid carboxy terminal membrane-spanning segment followed by a 5 amino acid invariant region. They are rich in cysteine residues, most of which are repeats of CXXC, as well as a large quantity of serine and threonine residues (Gillin and Reiner, 1996; Svard *et al.*, 1998). Current thought is that the cysteine residues form tight intramolecular disulfide bonds and/or coordinate metal ions, which suggests a very compact structure such that the trophozoite is shielded from intestinal proteases. Random sequencing of about 9% of the *G. lamblia* genome identified 53–55 of the 205–236 putative protein genes as VSP genes (Gillin and Reiner, 1996; Svard *et al.*, 1998). The first VSP cloned, TSA-417, is a 66-kDa type 1 integral membrane protein that covers the entire surface of the trophozoite, including the characteristic adhesive disk and flagella (Gillin and Reiner, 1996). Of the 82 cysteine residues 52 belong to the CXXC consensus sequence. TSA-417 is shed from the trophozoite surface during encystation and subsequently packaged into lysosomal-type compartments. The disappearance of TSA-417 is concomitant with the appearance of another dominant surface VSP (VSP-1EX). This process of antigen switching appears to be transcriptionally regulated as evidenced by the simultaneous fall and rise of TSA-417 and VSP-1EX mRNAs, respectively (Svard *et al.*, 1998).

It was once believed that *G. lamblia* VSPs lacked carbohydrate modification, but recent work has verified that VSP4A1 is *O*-linked with short di- and/or trisaccharide chains with GlcNAc at the nonreducing end and Glc at the reducing end (Papanastasiou *et al.*, 1997). There are an estimated 5×10^6 copies of VSP4A1 per parasite with each molecule containing one occupied *O*-glycan, suggesting *O*-glycosylation represents a major carbohydrate modification of *G. lamblia* parasites. Currently, no *Giardia* VSPs have been identified with *N*-linked glycans (Nash *et al.*, 1983; Lujan *et al.*, 1995; Papanastasiou *et al.*, 1997). At least one protein (GP49) has been identified that carries a GPI anchor (Das *et al.*, 1991). Preliminary work suggests that *G. lamblia* is unable to synthesize its own fatty acids and must scavenge needed GPI anchor precursors from its host's intestinal contents (Subramanian *et al.*, 2000).

Plasmodia

Bloodborne parasites of the genus *Plasmodia* are the causative agents of malaria, the world's most medically important parasitic disease. Malaria kills over 1 million people annually, mostly young children (World Health Organization). Anophelene mosquitoes are the carriers of *Plasmodia* to humans and other mammals.

Plasmodia lead a very complicated life cycle, alternating between a sexual stage within the mosquito and an asexual stage within the mammalian tissues and blood. The life cycle of *P. falciparum* begins when a female *Anopheles* mosquito takes a blood meal. Sporozoites are injected into the mammalian host's blood stream where they selectively invade the host's liver cells. The parasites rapidly divide mitotically causing the hepatocytes to burst, spilling out a new infective merozoite form that attacks red blood cells. Within the red blood cell the merozoites transform into trophozoites and adhere to the vascular endothelium. Male and female gametes (or gametocytes) are produced within the red blood cells and passed back to the mosquito with another blood meal. Sexual reproduction occurs in the midgut of the mosquito with the union of the gametocytes. The newly formed ookinetes encyst within the gut lining of the mosquito and develop into infective sporozoites, completing the life cycle.

N- and O-glycosylation

The presence of glycosylated proteins in the asexual stages of *P. falciparum* has been demonstrated by various methods including metabolic labeling, lectin binding, and enzymatic methods. Some of the identified proteins include merozoite surface protein-1 (MSP-1), merozoite surface protein-2 (MSP-2), a heat shock–type protein (HSP-72), an erythrocyte binding antigen (EBA-175), merozoite rhoptry antigen, transferrin receptor, and a serine protease. However, the extent and nature of the modifications are highly controversial.

Merozoite surface proteins of *P. falciparum* play a critical role in the invasion of human erythrocytes by the malaria parasite. High mannose-type *N*-glycans have been discovered on the parasite protein MSP-1 when the protein is expressed in HeLa cells (Burghaus *et al.*, 1999) or the baculovirus system in insect cells (Murphy *et al.*, 1990). High mannose-type structures have also been detected in the early ring stage of *P. falciparum* (Kimura *et al.*, 1996) and merozoites (Yang *et al.*, 1999). Although others argue that the parasite bears "potential" *N*-glycosylation sites, the oligosaccharide structures are either not expressed or expressed at very low levels in native *P. falciparum* (Dieckmann-Schuppert *et al.*, 1992; Gowda *et al.*, 1997). For example, native EBA-175 is unglycosylated but contains five potential *N*-glycosylation sites. When EBA-175 is expressed in a baculovirus system, 20.6% of the protein is modified with the glycan Asn-$GlcNAc_2$-Man_3-Fuc, the principal saccharide species present in baculovirus. The glycosylated form neither lessened the binding of the protein to erythrocytes or affected immunogenicity arguing that *N*-glycosylation is not needed (Liang *et al.*, 2000). Given that *Plasmodia* proteins (MSP-1, MSP-2, and EBA-175) are believed to be unmodified *in vivo*, even though potential glycosylation sites may be present, expressing plasmodial proteins in mammalian cell lines warrants caution.

O-linked glycans have been detected in intraerythrocytic trophozoite and schizont stages (Dieckmann-Schuppert *et al.*, 1993; Nasir-ud-Din *et al.*, 1992). Support for *O*-glycosylation in *P. falciparum* was the direct presence of *O*-GlcNAc residues (Nasir-ud-Din *et al.*, 1992) as well as the expression of the enzyme *O*-GlcNAc transferase during the intraerythrocytic

stages (Dieckmann-Schuppert *et al.*, 1993). This is consistent with the finding of Joshi *et al.* (1998) that MSP-2 is substituted with *O*-glycans linked to the protein backbone via GlcNAc and GalNAc to serine/threonine residues. In contrast, one study demonstrated that *O*-linkages containing GlcNAc or GalNAc were not detected in any *P. falciparum* developmental stage (Gowda *et al.*, 1997), nor was GlcNAc transferase activity. A lack of detectable levels of either *N*- and *O*-linked glycans in the merozoite proteins MSP-1 and MSP-2 also has been reported (Berhe *et al.*, 2000). It has been proposed that GPI anchors are the major mechanism of protein glycosylation (Gowda *et al.*, 1997).

GPIs

Two *P. falciparum* GPIs have been reported: ethanolamine-phosphate-Manα(1,2)Man(α1,2)Man(α1,6)Man(α1,4)GlcN-phosphatidyl(*myo*)inositol-diacylglycerol and ethanolamine-phosphate-Man(α1,2)Man(α1,6)Man(α1,4)GlcN-phosphatidyl(*myo*)inositol- diacylglycerol, named Pfglα and Pfglβ, respectively (Gerold *et al.*, 1994; Tachado *et al.*, 1999). The GPI anchor is similar to most other protozoan GPI anchors described (McConville and Ferguson, 1993) except for a unique myristoyl moiety linked to the inositol ring (Gerold *et al.*, 1999). GPI anchors have been implicated in the activation of the host's macrophages leading to the production of inflammatory cytokines as well as cell adhesion molecules. According to Tachado *et al.* (1999), *P. falciparum* GPIs may mimic its host cell's own GPIs and activate GPI-associated signaling pathways, such as S*rc*-related protein tyrosine kinases. During invasion of erythrocytes, MSP-1 undergoes proteolytic processing and free glycolipids (possibly GPIs) are released, which have been implicated in the formation of a deep pit within the erythrocyte membrane whereby merozoites gain access to the cell (Gowda *et al.*, 1997).

Toxoplasma

Toxoplasma gondii is an Apicomplexan parasite with a complex life cycle that includes three distinct stages: sporozoites, which are the product of the sexual cycle within a cat, and the asexually dividing tachyzoites and bradyzoites within the parasite's mammalian host. The infectious sporozoite and the bradyzoite forms are encased within distinct cysts, which, unlike the trichomonads and *Entamoeba*, are not structurally composed of chitin or any other glycoconjugate. Tachyzoites multiply inside the host's tissue followed by rupture of the host cell and infection of a nearby cell. *Toxoplamsa* is very promiscuous, infecting many kinds of cells, including fibroblasts, epithelial and endothelial cells, macrophages, and cells of the central nervous system. Generally any nucleated cell may host *T. gondii*. Once the parasite establishes an infection, tachyzoites shift from rapid multiplication to the formation of tissue cysts filled with bradyzoites. Here, the parasite may live for long periods of time, up to many years. The infection is generally asymptomatic, however coinfection with HIV often leads to fatal encephalitis (Luft and Remington, 1992).

The first evidence for *N*-glycosylation in *Toxoplasma* was with the discovery of four *N*-linked structures on the tachyzoite glycoprotein gp23 (Odenthal-Schnittler *et al.*, 1993). These structures lack sialic acid or Fuc but possess GalNAc in terminal positions. At the same time, the production of the dolichol-linked precursors $Glc_3Man_9GlcNAc_2$ and $Man_9GlcNAc_2$ was discovered in *T. gondii* microsomes. At least one of the five major tachyzoite surface antigens, SAG1, is reported to have one potential *N*-glycosylation site (Odenthal-Schnittler *et al.*, 1993), however, the site remains unoccupied except when the protein is expressed in Chinese hamster ovary cells (Kim *et al.*, 1994). Recently *O*-glycans have been discovered in the tachyzoite dense granule antigen 2 (GRA2). This protein is modified with short mono- and disaccharides consisting of GalNAc and GalNAc-containing species (Zinecker *et al.*, 1998).

GPI anchors of *T. gondii* are essentially the same as those of African trypanosomes (McConville and Ferguson, 1993). The *T. gondii* GPI anchor is unique in that it carries a branching β-GalNAc on the core Man of the anchor (Tomavo *et al.*, 1992; McConville and Ferguson, 1993), which is required for immunogenicity (Striepen *et al.*, 1997).

Trichomonads

Trichomonas vaginalis and *Tritrichomonas foetus* are flagellated parasites that colonize the urogenital lining of humans and cattle, respectively. Although the exact mechanisms of infection are unknown, strong evidence exists for the involvement of surface carbohydrate-containing molecules in cytoadhesion and cell destruction (Arroyo *et al.*, 1992; Mirhaghani and Warton, 1998; Singh *et al.*, 1999; Kneipp *et al.*, 1999).

LPG-like molecules

Like *Leishmania,* the major surface glycoconjugate of trichomonad parasites is LPG, which plays a part in cytoadhesion to host vaginal epithelial cells (Singh, 1993). Approximately 2×10^6 and 3×10^6 copies of LPG-like molecules are expressed on the surface of *T. foetus* and *T. vaginalis*, respectively (Singh, 1994). The heterogeneous LPG of *T. vaginalis* is 30–78 kDa, while LPG from *T. foetus* appears homogeneous (58 kDa). The molecule consists of at least three conserved domains: an unusual ceramide-containing phosphatidylinositol anchor, a large glycan core, and oligosaccharide repeat units. LPG of *T. vaginalis* contains Rha, GalNAc, GlcNAc, Gal, Glc, and Xyl and repeating units of polylactosamine (Singh *et al.*, 2000). LPG of *T. foetus* also contains Rha, GalNAc, GlcNAc, Gal, Glc, and Xyl as well as GlcA and large amounts of Fuc and recently discovered rare internal deoxy hexose residues (Singh *et al.*, 2000). Mild acid hydrolysis of *T. foetus* LPG generated more than 14 components as analyzed by matrix-assisted laser desorption ionization–fourier transform ion-cyclotron resonance mass spectrometry. The released oligosaccharides indicated compositions, such as $HexNAc_2Hex_2deHex$, $HexNAc_4Hex_2deHex$, $HexNAc_4Hex_2HexAdeHex$, $HexNAc_3$-$HexNHex_2deHex$ and $HexNAc_3Hex_2deHex_2$, and so on (Singh *et al.*, 2000). Unlike other GPI-anchored molecules, LPGs of trichomonad parasites do not contain Man.

The phosphosphingolipid anchors of *T. foetus* and *T. vaginalis* have been partially characterized (Costello *et al.*, 1993). The inositol ring is 1-linked to the phosphosphingolipid, the phosphoethanolamine linked at the 3-postion, and a fucosyl residue at the 4-position. The *T. vaginalis* anchor does not contain the fucose linkage.

Adhesins

The surface of *T. foetus* is covered with glycoproteins called adhesins that aid the parasite in attachment and adhesion to host urogenital lining (Corbeil *et al.*, 1989; Singh *et al.*, 1999). The adhesin Tf190 is a dimer of 140 and 60 kDa subunits (Shaia *et al.*, 1998). Detection of the carbohydrate moieties on Tf190 by high-performance liquid chromatography confirmed the presence of Fuc, GalN, GlcN, Gal, Glu, and Man, which is similar to those of *T. foetus* LPG (Shaia *et al.*, 1998), however the exact structure or position of the carbohydrates remain unknown. Another adhesin TF1.17, a 50–70-kDa glycoprotein is so far uncharacterized, though the same monoclonal antibodies react strongly with LPG (Singh *et al.*, 1999) suggesting a close connection with each other.

Chitin and other surface glycoconjugates

T. vaginalis and *T. foetus* express β-linked GlcNAc polymers of chitin on their surface membrane (Kneipp *et al.*, 1999). Though chitin is known to add structural integrity as well as provide protection for short-lived periods of time outside the mammalian urogenital environment, the polymer may also be a part of a surface-exposed receptor that recognizes and binds to host cell lectins. There are an estimated 4.2×10^5 and 3.0×10^5 surface expressed lectin receptors on *T. vaginalis* and *T. foetus*, respectively (Kneipp *et al.*, 1999). Chitinolytic activity has been identified in *T. vaginalis* as well, although the role remains unknown (Sanon *et al.*, 1998). Other surface glycoconjugates with sialic acid (Benchimol *et al.*, 1981), Man (Mirhaghani and Warton, 1998; Crouch and Alderete, 1999), and GlcNAc-containing residues (Mirhaghani and Warton, 1998) have been implicated in cytoadhesion and cytopathogenicity (Gilbert *et al.*, 2000).

Concluding remarks

Protozoan parasites are among the earliest and most versatile members of the eukaryotic kingdom. As such, they have evolved over billions of years in concert with their hosts and adapted with ingenuity to circumvent the myriad threats posed by host defense mechanisms. The diversity of their lifestyles and habitat necessitates the application of highly individualized strategies which range from "antigenic variation" in *T. brucei* for evasion of the host complement system to the application of the Gal/GalNAc lectin in *Entamoeba* for adherence to the colonic mucins. As we continue to further our understanding of these parasites and their pathology, the important role of surface glycoconjugates emerges as a common theme. In the last decade or so it has become clear that cell surface molecules play a critical role in parasite infectivity. Although these surface glycoconjugates sometimes share structural features as well as biosynthetic strategies with host molecules, they are all unique in certain aspects. The hope for the future is that the comparison of host-pathogen molecules and better understanding of biosynthetic pathways will lead to the development of therapeutic strategies by exploiting the structural and biosynthetic differences between host and parasite.

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Abbreviations

2-AEP, 2-aminoethylphosphonate; EBA, erythrocyte binding antigen; Gal_f, galactofuranose; Gal_p, galactopyranose; GIPLs, glycosylinositolphospholipids; GPI, glycosylphosphatidyl inositol; HSP, heat shock protein; LPG, lipophosphoglycan; LPPG, lipopeptidophosphoglycan; MSP, merozoite surface protein; PARPs, procyclic acidic repetitive proteins; PPG, proteophosphoglycan; sAP, secreted acid phosphatase; VSG, variant surface glycoprotein; VSP, variant surface protein.

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