The hydrophobic mannoside Manα1-6Manα1-S-$(CH_2)_7$-CH_3 acts as an acceptor for the UDP-Gal:glycosylphosphatidylinositol anchor α1,3-galactosyltransferase of *Trypanosoma brucei*

Sabine PINGEL,*§ Robert A. FIELD,†‡|| Maria Lucia S. GÜTHER,† Michael DUSZENKO* and Michael A. J. FERGUSON†¶

†Department of Biochemistry and ‡Department of Chemistry, University of Dundee, Dundee DD1 4HN, Scotland, U.K.
and *Physiologisch-chemisches Institut der Universität Tübingen, Hoppe-Seyler-Str. 4, 72076 Tübingen, Federal Republic of Germany

The variant surface glycoproteins (VSGs) of *Trypanosoma brucei* are attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) membrane anchor. This anchor contains the core sequence ethanolamine-PO_4-6Manα1-2Manα1-6Manα1-4GlcNα1-6*myo*-inositol, which is conserved in all GPI anchors, and a unique αGal side chain attached to the 3-position of the αMan residue adjacent to the αGlcN residue. Here we report that trypanosome membranes can catalyse the transfer of Gal from UDP-Gal to the hydrophobic thioglycoside Manα1-6Manα1-S-$(CH_2)_7$-CH_3. Characterization of the galactosylated products by electrospray mass spectrometry, exoglycosidase digestion and periodate-oxidation studies revealed that the major product was Manα1-6(Galα1-3)Manα1-S-$(CH_2)_7$-CH_3. The similarity of this product to part of the mature VSG GPI anchor suggests that the thioglycoside is able to act as an acceptor for the trypanosome-specific UDP-Gal–GPI anchor α1,3-galactosyltransferase.

INTRODUCTION

The causative agent of African sleeping sickness and livestock diseases, the protozoan parasite *Trypanosoma brucei*, evades the host immune response by means of antigenic variation [1]. The variant antigens [variant surface glycoproteins (VSGs)] are tightly packed on the plasma membrane of each trypanosome providing a protective barrier for the parasite. In common with a wide variety of eukaryotic cell-surface glycoproteins, VSG molecules are anchored to the cell surface by a glycosylphosphatidylinositol (GPI) anchor [2]. All GPI anchors appear to have a common core structure of ethanolamine-PO_4-6Manα1-2Manα1-6Manα1-4GlcNα1-6*myo*-inositol, where the ethanolamine is in amide linkage to the C-terminus of the mature protein [3,4]. This conserved core structure may be variously substituted in a cell- and species-specific manner with extra ethanolamine phosphate and carbohydrate moieties. A unique feature of the VSG anchor is the αGal side chain which has the structure Galα1-2Galα1-6(Galα1-2)Galα1- [2]. The side chain is linked to the conserved core structure by a Galα1-3Man linkage to the αMan residue adjacent to the αGlcN residue. The presence of this unique side chain suggests the existence of trypanosome-specific α-galactosyltransferases [5]. Recently, a cell-free assay for GPI α-galactosylation has been reported using UDP-[^{14}C]Gal as the donor and endogenous VSG as the acceptor [6].

In this paper, we describe the galactosylation of the simple hydrophobic thioglycoside Manα1-6Manα1-S-$(CH_2)_7$-CH_3 by trypanosome membranes and UDP-Gal and the characterization of the major product of this reaction. The structure of this product strongly suggests that the thioglycoside can be used as a simple acceptor for the assay of the trypanosome-specific UDP-Gal:GPI anchor α1,3-galactosyltransferase.

MATERIALS AND METHODS

Materials

UDP-[4,5-^{3}H]Gal (41.7 Ci/mmol) and En^{3}Hance spray were from New England Nuclear. Coffee-bean α-galactosidase, bovine testes β-galactosidase and *Canavalia ensiformis* (jack bean) α-mannosidase were purchased from Boehringer (Mannheim, Germany). Aluminium-backed silica-gel 60 high-performance TLC (HPTLC) plates (Art. 5547) were obtained from Merck (Darmstadt, Germany). All other reagents were of analytical grade, and solvents were HPLC grade.

Synthesis of thio-octyl glycosides

Manα1-S-$(CH_2)_7$-CH_3, Manβ1-S-$(CH_2)_7$-CH_3 and Manα1-6-Manα1-S-$(CH_2)_7$-CH_3 were prepared from per-O-acetylated Man or per-O-acetylated Manα1-6Man and thio-octanol using the coupling procedure described by Ferrier and Furneaux [7]. Per-O-acetylated Manβ1-S-$(CH_2)_7$-CH_3 glycoside was obtained as a minor by-product in the preparation of the corresponding α-glycoside. All three per-O-acetylated glycosides gave ^{13}C NMR data consistent with their proposed structures; anomeric con-

Abbreviations used: GPI, glycosylphosphatidylinositol; HPTLC, high-performance thin-layer chromatography; VSG, variant surface glycoprotein; CID, collision-induced dissociation.

§ Present address: Department of Medicine and Microbiology/Immunology, University of California, San Francisco, CA 94143-0654, U.S.A.
|| Present address: Department of Chemistry, St Andrews University, Scotland, U.K.
¶ To whom correspondence should be addressed.

figurations were confirmed on the basis of one-bond C_1-H coupling constants:

Manα1-S-$(CH_2)_7$-CH_3 glycoside per-O-acetate:

δ_C (p.p.m.) (C^2HCl_3) 14.2, 21.0, 22.8, 28.9, 29.2, 29.3 (2), 31.4, 31.9, 62.6, 66.5, 69.1, 69.6, 71.3, 82.7 (C-1; $J_{C\text{-}H}$ 167.9 Hz), 169.8–170.5.

Manβ1-S-$(CH_2)_7$-CH_3 glycoside per-O-acetate:

δ_C (p.p.m.) (C^2HCl_3) 14.5, 21.1, 23.0, 29.2, 29.6 (2), 30.2, 32.2 (2), 63.3, 66.4, 71.0, 72.4, 76.9, 83.3 (C-1; $J_{C\text{-}H}$ 152.2 Hz), 169.9–170.5.

Manα1-6-Manα1-S-$(CH_2)_7$-CH_3 glycoside per-O-acetate:

δ_C (p.p.m.) (C^2HCl_3) 14.1, 21.0, 22.6, 28.6, 29.1 (3), 31.0, 31.8, 62.4, 66.0, 66.6, 66.7, 68.6, 69.0, 69.4, 69.6 (2), 71.1, 81.9 (C-1; $J_{C\text{-}H}$ 169.5 Hz), 97.5 (C-1′; $J_{C\text{-}H}$ 165.5 Hz), 169.4–170.5.

Per-O-acetylated thioglycosides were deacetylated (sodium methoxide/methanol) and desalted [Dowex AG-50 (X8) eluted with methanol] to give the corresponding thioglycosides.

Preparation of trypanosome cell-free system

Bloodstream forms of *T. brucei* strain 427 variant MITat 1.4 were isolated from infected rats [8]. Lysates of trypanosomes were prepared and stored as described previously [9], except that the incubation with tunicamycin before the hypotonic lysis was omitted. Membranes for galactosyltransferase assays were prepared by thawing an aliquot of lysate (1 ml containing 5×10^8 cell equivalents) and washing as described [9]. Membranes were finally resuspended in 250 μl of 100 mM sodium Hepes, pH 7.4, containing 50 mM KCl, 10 mM $MgCl_2$, 10 mM $MnCl_2$, 2 mM dithiothreitol, 2 mM ATP, 2.5 μg/ml tunicamycin, 0.05% Triton X-100, 0.2 mM *N*-tosyl-L-lysine chloromethyl ketone and 2 mg/ml leupeptin (2 × incubation buffer).

Galactosyltransferase assay

First, 50 μl of UDP-[4,5-^{3}H]Gal (5 μCi) was dried in an Eppendorf tube and redissolved in 25 μl of 2 mM acceptor substrate [Manα1-S-$(CH_2)_7$-CH_3, Manβ1-S-$(CH_2)_7$-CH_3 or Manα1-6Manα1-S-$(CH_2)_7$-CH_3], followed by the addition of 25 μl of trypanosome membranes (equivalent to 5×10^7 cells). After incubation for 60 min at 35 °C, the reaction was stopped by the addition of 333 μl of chloroform/methanol (1:1, v/v) and lipid was extracted for 16 h at 4 °C. After centrifugation, the lipid extract was dried under a stream of N_2, dissolved in 100 μl of water-saturated butan-1-ol and partitioned with 100 μl of water saturated with butan-1-ol. After vortexing and centrifugation, the [^{3}H]galactosylated acceptors were recovered in the upper butanol phase. The water phase was re-extracted three times with butan-1-ol. The combined butanol phases were back-extracted twice with 100 μl of water, dried in a Speed-Vac evaporator and analysed by HPTLC.

Exoglycosidase digestions

Galactosylated products (15000 c.p.m.) were dried and redissolved in 20 μl of 0.1 M sodium citrate/phosphate buffer, pH 6.0, containing 0.5 unit of coffee-bean α-galactosidase or in 20 μl of 0.1 M sodium citrate/phosphate buffer, pH 4.5, containing 9 munits of bovine testes β-galactosidase or in 20 μl of 0.1 M sodium acetate buffer, pH 5.0, containing 0.1% sodium taurodeoxycholate and 0.75 unit of jack-bean α-mannosidase. After incubation for 16 h at 37 °C the reactions were terminated by boiling for 5 min, and the samples partitioned between butan-1-ol and water for HPTLC analysis as described above.

HPTLC

Samples were applied in 4 μl of water-saturated butan-1-ol to aluminium-backed silica-gel 60 HPTLC plates (10 cm). The plates were developed in chloroform/methanol/1 M ammonium acetate/13 M NH_3/water (180:140:9:9:23, by vol.). For fluorography, HPTLC plates were sprayed with En^{3}Hance and exposed to Kodak X-Omat XAR-5 film at −70 °C, using an intensifying screen. Standard compounds Manα1-S-$(CH_2)_7$-CH_3 and Manα1-6Manα1-S-$(CH_2)_7$-CH_3 (5 μg each) were run alongside the samples. Lanes containing non-radioactive samples were cut out after development of the HPTLC, sprayed with orcinol reagent (20 mg/ml orcinol monohydrate in ethanol/conc. H_2SO_4/water, 75:10:5, by vol.) and heated for 5 min at 110 °C.

Generation of the Manα1-6(Gal-)Manα1-S-$(CH_2)_7$-CH_3 products

Membranes from 1.7×10^{10} trypanosomes were prepared by thawing and washing 34 ml of trypanosome lysate as described above. The membranes were resuspended in 8.5 ml of 2 × incubation buffer and a large-scale galactosyltransferase reaction was performed in a final volume of 16 ml containing 2 mM Manα1-6Manα1-S-$(CH_2)_7$-CH_3, 1 mM UDP-Gal and 8 ml of trypanosome membrane suspension (equivalent to 1.6×10^{10} cells). After incubation for 19 h at 35 °C, the reaction was stopped by the addition of 106.7 ml of chloroform/methanol (1:1, v/v) and extracted for 16 h at 4 °C. The lipid extract was dried in a rotary evaporator and partitioned between 30 ml of butan-1-ol and 30 ml of water, as described above. The combined butanol phases were back-extracted with water, dried in a rotary evaporator and resuspended in 380 μl of butan-1-ol. In parallel, to generate a radioactive tracer, 1.6 ml of UDP-[^{3}H]Gal (160 μCi) was dried and redissolved in 400 μl of 2 mM Manα1-6Manα1-S-$(CH_2)_7$-CH_3, followed by 400 μl of trypanosome membrane suspension. After incubation for 60 min at 35 °C, the reaction was stopped by the addition of 5.33 ml of chloroform/methanol (1:1, v/v) and extracted for 16 h at 4 °C. The lipid extract was dried under a stream of N_2 and partitioned between water-saturated butan-1-ol and water saturated with butan-1-ol (using 500 μl volumes), as described above. The combined butanol phases, containing typically 2×10^6 c.p.m., were dried under a stream of N_2 and resuspended in 40 μl of butan-1-ol. The products of the large-scale galactosyltransferase reaction were applied to three aluminium-backed silica-gel 60 HPTLC plates (20 cm × 20 cm; total lane lengths 50 cm), alongside 0.5 cm radioactive tracer lanes and the HPTLC plates were developed as described above. Immediately after the development the radioactive Manα1-6([^{3}H]Gal-)Manα1-S-$(CH_2)_7$-CH_3 tracer product was detected using a Raytest RITA TLC linear-analyser. The regions of the HPTLC plates corresponding to the major α-galactosylated product, Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3, were scraped off and extracted with 15 ml of methanol/pyridine/water (2:1:1, by vol.). The extract (containing the bulk product and 240000 c.p.m. of tracer) was dried and partitioned between 1.5 ml each of butan-1-ol and water. Aliquots (20 μl) of the butan-1-ol phase were dried, redissolved in 20 μl of 50% acetonitrile and analysed by electrospray mass spectrometry in positive- and negative-ion mode.

Periodate oxidation

A sample of the final butan-1-ol phase (1 ml) of the HPTLC-purified Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3 product was dried and oxidized with 0.025 M sodium periodate in 50 mM sodium acetate buffer, pH 4.5, for 16 h or 40 h at room temperature in the dark. The oxidation was terminated by the addition of 3 μl of ethylene glycol and incubation for 30 min at room temperature. The products were reduced by the addition of 90 μl of 1 M NH_3, followed by 180 μl of 1 M NaB^2H_4 (1 h at room temperature). Excess reductant was destroyed by adding 20 μl aliquots of 1 M acetic acid until effervescence stopped; the mixture was made up to 5% acetonitrile and subjected to reverse-phase HPLC.

Reverse-phase HPLC

The reaction mixture of periodate-oxidized Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3 products (490 μl) was separated by HPLC on a C_{18} reverse phase column (Hichrom KR100-5C18). The column was equilibrated in 100 mM ammonium acetate/5% acetonitrile (buffer A). The gradient elution programme was as follows: 100% buffer A up to 5 min after injection, then a change to 100% buffer B (5% acetonitrile) between 5 min and 10 min. A linear gradient was then run from 5 to 100% acetonitrile over a period of 50 min and 100% acetonitrile was maintained for a further 10 min. The flow rate was 1 ml/min, and 70 fractions (1 ml) were collected and 100 μl aliquots were taken for liquid-scintillation counting. Aliquots (20 μl) of fractions containing radioactivity were analysed by electrospray mass spectrometry in negative-ion mode. To obtain collision-induced-dissociation (CID) daughter-ion spectra, fractions were concentrated tenfold before analysis.

Electrospray mass spectrometry

Mass-spectrometric data were acquired using a VG-Quattro triple-quadrupole instrument (Fisons Instruments, VG Biotech, Altrincham, Cheshire, U.K.). Samples (20 μl) were introduced into the electrospray ion source at 10 μl/min in 50% acetonitrile using a Michrom microbore HPLC system (Jones Chromatography). The source conditions were optimized for each analysis using 20 pmol/μl Manα1-6Manα1-S-$(CH_2)_7$-CH_3 as a standard. Typical values for negative-ion spectrometry were: capillary voltage 3.0 kV; high-voltage lens 0.35 kV; focus voltage 40 V; skimmer voltage 45 V. Negative-ion CID daughter-ion spectra were recorded after acceleration of the $(M-1)^-$ pseudomolecular parent ion through a potential difference of 25 V into a hexapole collision cell containing argon at 250 mPa. In all cases several scans were averaged, background-subtracted and smoothed using MassLynx software (Fisons Instruments, VG Biotech, Altrincham, Cheshire, U.K.).

RESULTS

Hydrophobic mannosides act as galactose acceptors

Trypanosome membranes in 0.025% Triton X-100 were found to be capable of transferring [^{3}H]Gal from UDP-[^{3}H]Gal to Manα1-S-$(CH_2)_7$-CH_3, Manβ1-S-$(CH_2)_7$-CH_3 and Manα1-6Manα1-S-$(CH_2)_7$-CH_3. The relative rates of transfer were 23, 126 and 34 pmol of Gal/h respectively using membranes derived from 10^8 cells. In all cases the products contained single [^{3}H]Gal residues in both α- and β-linkage. The proportion of αGal products for the Manα1-S-$(CH_2)_7$-CH_3, Manβ1-S-$(CH_2)_7$-CH_3 and Manα1-6Manα1-S-$(CH_2)_7$-CH_3 acceptors was approximately 40, 10 and 90% respectively (results not shown). As

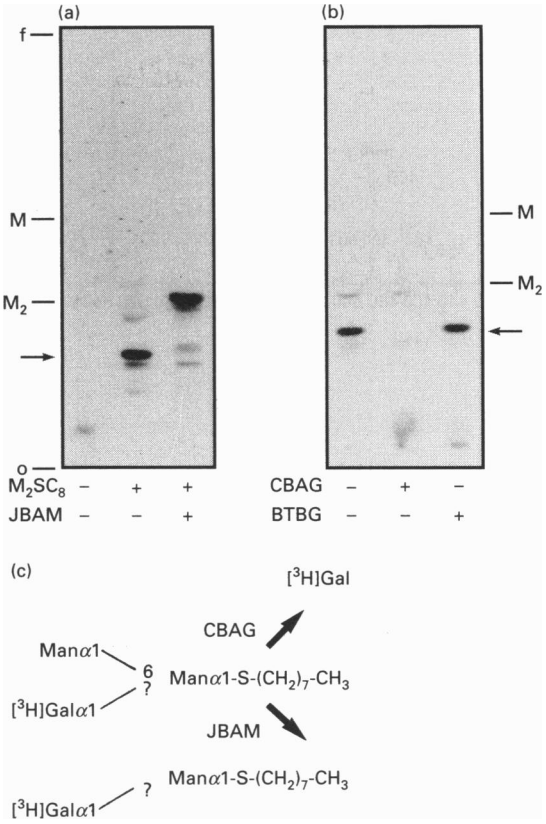

Figure 1 Transfer of [^{3}H]Gal to the Manα1-6Manα1-S-$(CH_2)_7$-CH_3 acceptor

Trypanosome membranes were incubated with UDP-[^{3}H]Gal (**a**) in the presence (+) or absence (−) of the acceptor Manα1-6Manα1-S-$(CH_2)_7$-CH_3 (M_2SC_8) as indicated, or (**b**) in the presence of the acceptor. The extracted [^{3}H]galactosylated products were treated with (+) or without (−) jack-bean α-mannosidase (JBAM), coffee-bean α-galactosidase (CBAG) or bovine testes β-galactosidase (BTBG) as indicated. Radiolabelled products were re-extracted and analysed by HPTLC and fluorography. The migration positions of the [^{3}H]Gal-[Manα1-6Manα1-S-$(CH_2)_7$-CH_3] product (arrow) and of the standard compounds Manα1-S-$(CH_2)_7$-CH_3 (M) and Manα1-6Manα1-S-$(CH_2)_7$-CH_3 (M_2) are indicated. o, Origin; f, solvent front. The products of the exoglycosidase digestions are summarized in (**c**).

the aim of this project was to identify α-galactosyltransferase substrates, only the products of the dimannoside acceptor were studied in full (see below).

In the presence of Manα1-6Manα1-S-$(CH_2)_7$-CH_3 acceptor and UDP-[^{3}H]Gal donor, the trypanosome membranes generated major [^{3}H]galactosylated products which ran as a doublet on HPTLC with mobilities consistent with the transfer of one [^{3}H]Gal residue to the dimannoside acceptor (Figure 1a, lane 2). Digestion of this material with jack-bean α-mannosidase converted the majority of these products into [^{3}H]galactosylated structures, with similar mobilities to the dimannoside acceptor (Figure 1a, lane 3). These data suggest that the major [^{3}H]galactosylated products contain the transferred [^{3}H]Gal residue on the inner Man residue of the acceptor (see Figure 1c). The [^{3}H]galactosylated products were digested with coffee-bean α-galactosidase and bovine testes β-galactosidase (Figure 1b). The results indicate that the upper band of the doublet (the major product) contains α[^{3}H]Gal [i.e. Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3] (see Figure 1c), whereas the lower band (a minor product) contains β[^{3}H]Gal [i.e. Manα1-6(Galβ1-?)Manα1-S-$(CH_2)_7$-CH_3]. The ratio of the upper to the lower band was found to vary between experiments, but the upper band was always the major product.

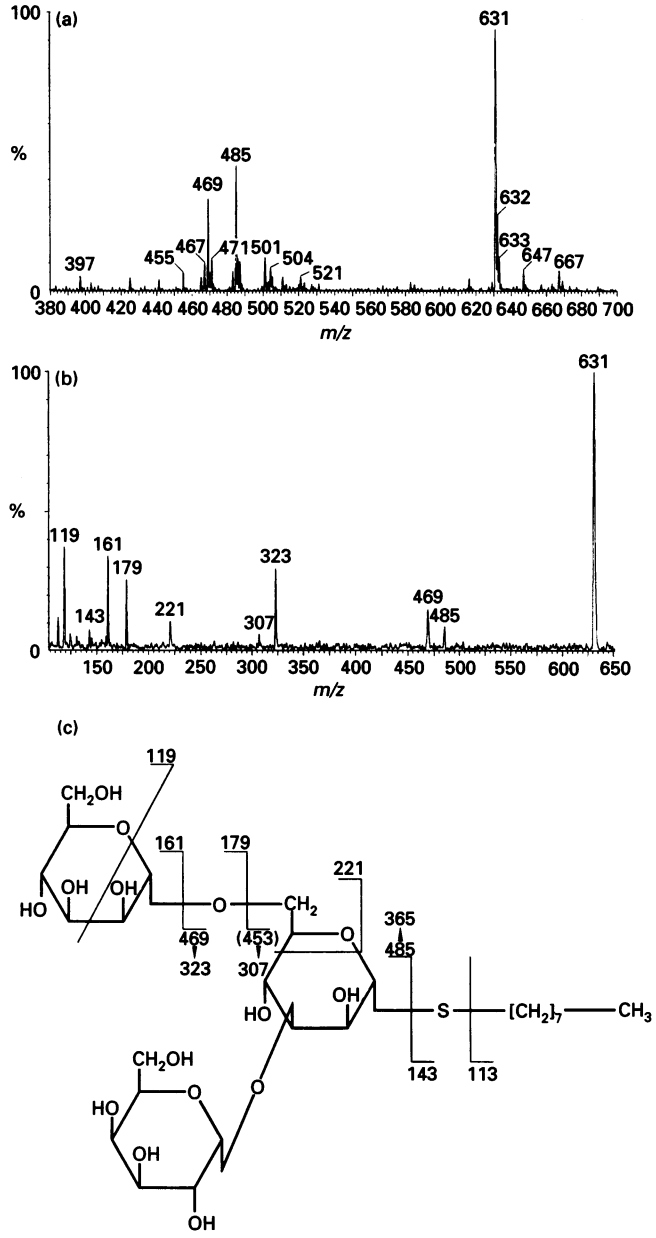

Figure 2 Electrospray mass-spectrometric analysis of the Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3 product

Trypanosome membranes were incubated with UDP-Gal and the acceptor Manα1-6Manα1-S-$(CH_2)_7$-CH_3. The Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3 reaction product was purified by preparative HPTLC and partitioned between water and butan-1-ol as described in the Materials and methods section. (**a**) Negative-ion electrospray mass spectrum of the butan-1-ol phase. (**b**) CID daughter-ion spectrum of the pseudomolecular ion at *m/z* 631 and interpretation of this spectrum (**c**). Numbers in (**c**) indicate the major primary and secondary fragmentation ions; numbers in parentheses are predicted intermediate products.

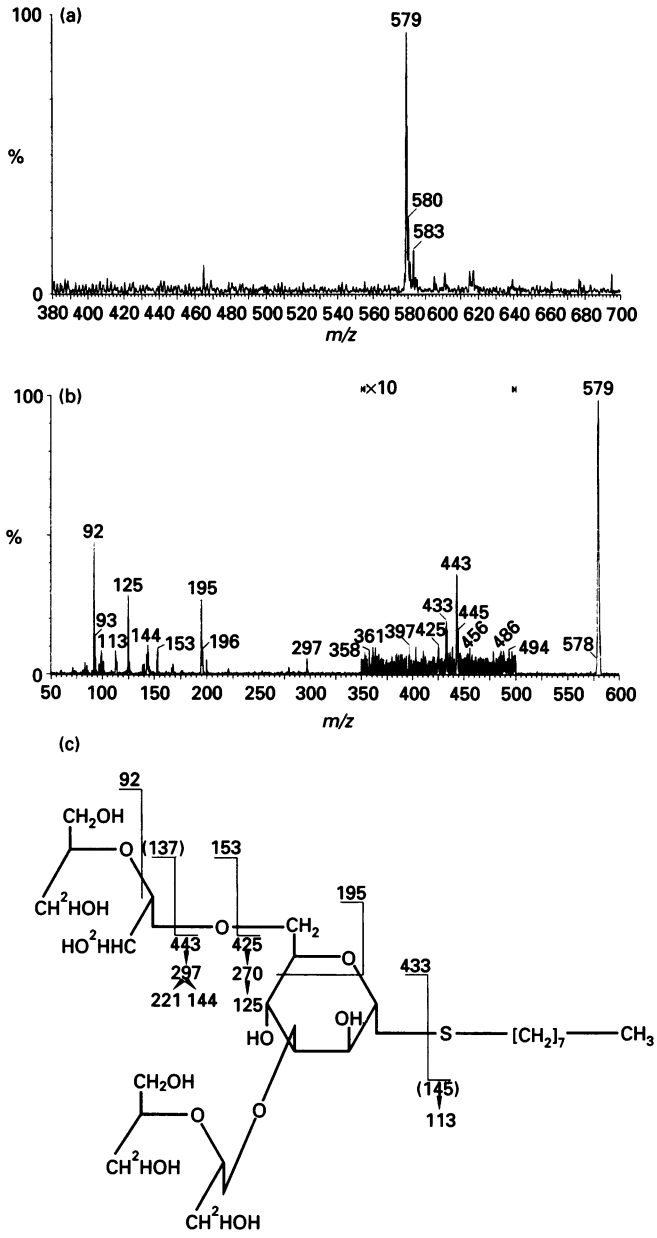

Figure 3 Linkage analysis by periodate oxidation

The Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3 product was subjected to periodate oxidation, NaB^2H_4 reduction and purification by reverse-phase HPLC as described in the text. (**a**) Negative-ion electrospray mass spectrum of the major HPLC peak at 50% acetonitrile. (**b**) CID daughter-ion spectrum of the pseudomolecular ion at *m/z* 579 and interpretation of this spectrum (**c**).

Isolation of the Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3 products

The reaction of UDP-Gal with Manα1-6Manα1-S-$(CH_2)_7$-CH_3, catalysed by trypanosome membranes, was scaled-up as described in the Materials and methods section. The products of the large-scale preparation were mixed with a radioactive tracer of Manα1-6([^{3}H]Gal-)Manα1-S-$(CH_2)_7$-CH_3 and separated by preparative HPTLC. The band corresponding to Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3 was scraped off and extracted with methanol/pyridine/water (2:2:1, by vol.). The products were partitioned between water and butan-1-ol and the majority (85%) of the Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3 was recovered in the butan-1-ol phase. Analysis of this fraction by electrospray mass spectrometry confirmed the presence of hexose$_3$-S-$(CH_2)_7$-CH_3. The pseudomolecular ions recorded were *m/z* 655, corresponding to $(M+Na)^+$ in positive-ion mode (results not shown), and *m/z* 631, corresponding to $(M-1)^-$ in negative-ion mode (Figure 2a). The other major ions in Figure 2(a) at *m/z* 469 and 485 are presumably cone-fragmentation

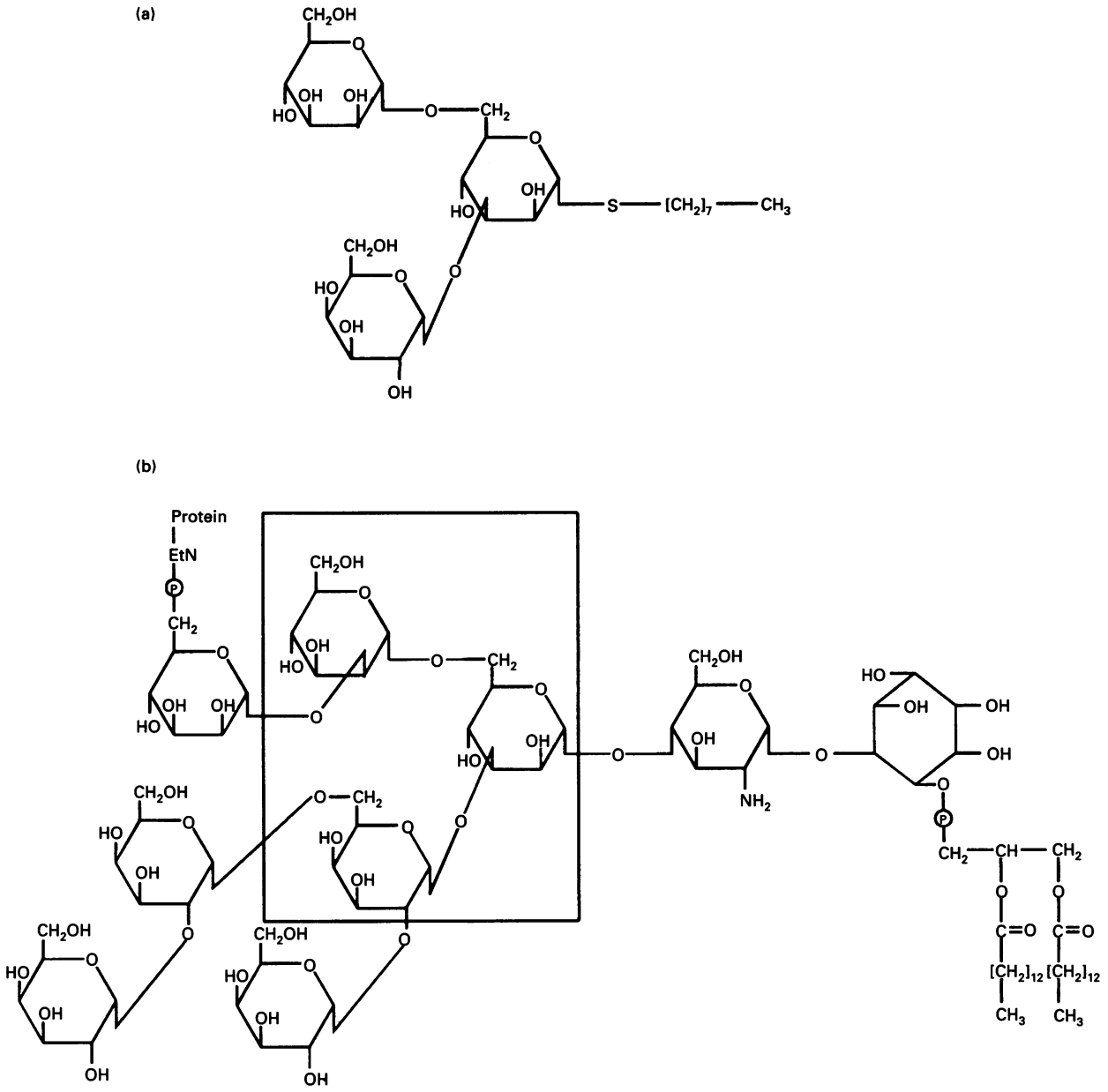

Figure 4 Product of the galactosyltransferase reaction

Trypanosome membranes catalyse the formation of predominantly Manα1-6(Galα1-3)Manα1-S-$(CH_2)_7$-CH_3 (**a**) from UDP-Gal and Manα1-6Manα1-S-$(CH_2)_7$-CH_3. This structure is identical with part of the GPI anchor of VSG variant MITat 1.4 [2] as indicated by the box (**b**). EtN, ethanoline.

ions, because they are also seen in the CID daughter-ion spectrum of the $(M-1)^-$ parent ion (Figures 2b and 2c).

Linkage analysis by periodate oxidation

The Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3 products were subjected to periodate oxidation for 16 h, followed by reduction with NaB^2H_4. The products were purified by reverse-phase HPLC, using the [^{3}H]Gal trace-label to locate the products, and eluted at about 45% acetonitrile. Analysis of this material by electrospray mass spectrometry revealed a heterogeneous set of products that suggested incomplete oxidation (results not shown). The products were therefore reoxidized for a further 24 h, reduced and repurified by reverse-phase HPLC. In this case, two main peaks were observed at about 45 and 50% acetonitrile (in a ratio of 1:2). Analysis of the major peak by negative-ion electrospray mass spectrometry revealed a major pseudomolecular ion at m/z 579 (Figure 3a). The CID daughter-ion spectrum of this ion is shown in Figure 3(b) and the interpretation of this spectrum is shown in Figure 3(c). The mass of the pseudomolecular ion defines the number of oxidation sites in the molecule and defines the substitution position of the Gal on the inner Man residue as the 3-position (see Figures 3c and 4).

The minor pseudomolecular ion seen in Figure 3(a) at m/z 583 is consistent with the predicted oxidation/reduction product of a species bearing the Gal residue on the 2- and/or 4-position of the

inner Man residue. In this case one more oxidation site (in the inner Man residue) would be available, resulting in an increase in 4 mass units after NaB^2H_4 reduction.

The oxidized/reduced molecules found in the minor HPLC fraction contained molecules that had been oxidized on the sulphur atom to sulphoxides, giving rise to pseudomolecular ions at m/z 595 (i.e. the sulphoxide of m/z 579) and m/z 599 (i.e. the sulphoxide of m/z 583) (results not shown). Interestingly, in this fraction the ratio of m/z 595 to m/z 599 was about 2:1. The reason for the discrepancy between the m/z 579: m/z 583 ratio (about 10:1) and the m/z 595:m/z 599 ratio is not clear, but it is possible that the oxidation state of the Man residue attached to the sulphur atom may affect the probability of sulphoxide formation. Taking all of the data into account, the ratio of the products Manα1-6(Galα1-3)Manα1-S-$(CH_2)_7$-CH_3 to Manα1-6(Gal1-2/4)Manα1-S-$(CH_2)_7$-CH_3 is about 5:1. The CID daughter-ion spectrum of the pseudomolecular ion at m/z 599 suggested that both Manα1-6(Gal1-2)Manα1-S-$(CH_2)_7$-CH_3 and Manα1-6(Gal1-4)Manα1-S-$(CH_2)_7$-CH_3 were present in approximately equal amounts as judged by the abundance of fragmentation ions characteristic for the respective products (results not shown). We have not assigned the anomeric configuration of the Gal residues in these two minor products because it is possible that the HPLC-purified Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3 fraction analysed might contain a trace of the adjacent Manα1-6(Galβ1-?)Manα1-S-$(CH_2)_7$-CH_3 band (see Figure 1b).

DISCUSSION

Detergent-lysed trypanosome membranes were able to catalyse the transfer of Gal from UDP-Gal to the hydrophobic glycoside Manα1-6Manα1-S-$(CH_2)_7$-CH_3. The major product of this reaction was sensitive to coffee-bean α-galactosidase and jack-bean α-mannosidase, suggesting a Manα1-6(Galα1-?)Manα1-S-$(CH_2)_7$-CH_3 arrangement. The linkage position of the Gal residue could not be determined by conventional methylation analysis because of the stability of the thioglycosidic bond. This problem was solved using periodate oxidation and NaB^2H_4 reduction, followed by tandem negative-ion electrospray mass spectrometry, which showed that the major product was Manα1-6(Galα1-3)-Manα1-S-$(CH_2)_7$-CH_3. Minor products of Manα1-6(Gal1-2)-Manα1-S-$(CH_2)_7$-CH_3 and Manα1-6(Gal1-4)Manα1-S-$(CH_2)_7$-CH_3 were also identified. An estimation of a K_m value for the acceptor, using the crude membrane preparation as a source of enzymes, was complicated by the fact that the acceptor has detergent-like properties that appear to inhibit the enzyme(s) at high concentrations (> 2 mM). However, 2 mM was clearly the optimum acceptor concentration for detecting enzyme activity under the conditions used.

The sugar portion of the major product is identical in structure with part of the mature VSG GPI anchor (Figure 4), suggesting that Manα1-6Manα1-S-$(CH_2)_7$-CH_3 can act as an acceptor for the trypanosome UDP-Gal:GPI anchor α1,3-galactosyltransferase. The activity of this enzyme has been demonstrated previously in crude trypanosome membranes using endogenous VSG as an acceptor [6]; however, this assay did not function in the presence of detergent. The synthetic glycoside Manα1-6Manα1-S-$(CH_2)_7$-CH_3 greatly simplifies the assay of this enzyme, as the product can be conveniently isolated by solvent (butan-1-ol) extraction or reverse-phase cartridge (results not shown), and it will be used to help purify the α-galactosyltransferase. This trypanosome-specific enzyme, which initiates the biosynthesis of the entire GPI αGal side chain, may play an important role in the defence of the parasite. It has been suggested that the unique αGal side chain of the VSG anchor may be important in maintaining the molecular diffusion-barrier characteristics of the VSG coat [10,11], on which the trypanosome depends for its resistance to complement-mediated lysis in the bloodstream.

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