Purification and characterization of an α -galactosyltransferase from *Trypanosoma brucei*

Sabine PINGEL¹, Uta RHEINWEILER, Volker KOLB and Michael DUSZENKO²

Physiologisch-chemisches Institut der Universität Tübingen, Hoppe-Seyler-Str. 4, 72076 Tübingen, Germany

A membrane-associated galactosyltransferase from *Trypanosoma brucei* was purified 34000-fold by affinity chromatography on UDP-hexanolamine–SephacroseTM. Using SDS/PAGE under reducing conditions, the isolated enzyme ran as a relatively broad band with apparent molecular masses of 53 kDa and 52 kDa, indicative of glycosylation and the existence of two isoforms. *N*-Glycosylation of the enzyme was subsequently confirmed using Western blotting and either specific binding of concanavalin A or peptide-*N*⁴-(*N*-acetylglucosaminyl)asparagine amidase digestion. The de-*N*-glycosylated enzyme ran with apparent molecular masses of 51 kDa and 50 kDa, indicative of a single *N*-glycosylation site. The galactosyltransferase exhibited a pH optimum at 7.2 and had a pronounced requirement for Mn²⁺

ions ($K_M = 2.5$ mM) for its action. The transferase activity was independent of the concentration of Triton X-100. The enzyme was capable of transferring galactose from UDP-galactose to a variety of galactose-based acceptors in α -glycosidic linkages. The apparent K_M values for UDP-galactose and for the preferred acceptor substrate *N*-acetyl-lactosamine are 46 μ M and 4.5 mM respectively. From these results we would like to suggest that the galactosyltransferase functions in the processing of terminal *N*-acetyl-lactosamine structures of trypanosomal glycoproteins.

Key words: affinity chromatography, enzyme purification, sleeping sickness, trypanosomes

INTRODUCTION

African trypanosomes cause severe diseases in animals and man. These protozoan parasites grow extracellularly in the bloodstream and body fluids. They are covered by a dense coat of a variant surface glycoprotein (VSG) which protects each parasite from lytic serum factors and permits a sustained infection by means of antigenic variation [1]. All VSGs contain at least one Asn-linked oligosaccharide [2,3]. Structural analysis revealed a classical oligomannose-type, biantennary complex and branched poly-*N*-acetyl-lactosamine oligosaccharides [4,5]. Interestingly, certain common constituents of mammalian Asn-linked oligosaccharides, such as fucose, sialic acid and bisecting or core-linked GlcNAc residues are absent, as are certain classes of oligosaccharides, such as the hybrid or the multiantennary-complex type. Instead of these, large amounts of non-typical oligosaccharides containing only four and three mannose residues were identified [5]. Furthermore, complex-type oligosaccharides showed very rapid kinetics of endo- β -*N*-acetylglucosaminidase H resistance [6]. Thus the existence of an alternative glycosylation pathway in African trypanosomes may exist.

An interesting feature of Asn-linked oligosaccharides from VSG was the detection of Gal α 1-3Gal moieties, which occupy the terminal non-reducing position in *N*-acetyl-lactosamine-type carbohydrates [5]. This Gal α 1-3Gal determinant has been shown to be highly immunogenic in man [7,8]. While the Gal α 1-3galactosyltransferase is expressed in virtually all organisms, the respective gene is deleted in humans and Old World monkeys due to a frameshift mutation [9]. Thus about 1% of the total IgG content in humans is directed against the Gal α 1-3Gal epitope. Although this represents an effective tool against the invasion of most infective agents and was thus a major advantage during evolution, these days it causes severe complications in xeno-

transplantation: it has been shown that hyperacute rejection could be inhibited by α -galactosyl oligosaccharides conjugated with poly(ethylene glycol) [10].

The Gal α 1-3Gal epitope has been found on the surface of several trypanosomatid species and the production of anti-(Gal α 1-3Gal) antibodies may be triggered in leishmaniasis and in infections with *T. cruzi* and *T. brucei rhodesiense* [11,12]. For the malaria parasite *Plasmodium falciparum*, it was shown recently that growth in culture could be inhibited by anti-(Gal α 1-3Gal) antibodies, probably by binding to Gal α 1-3Gal epitopes on merozoite surface molecules, causing complement mediated lysis [13].

Although structural features of the glycans on glycoproteins in trypanosomes have been thoroughly studied, more information is required about the enzymes involved in their biosynthesis. In previous studies we identified galactosyltransferases acting on Asn-linked oligosaccharides of *T. brucei* glycoproteins and demonstrated the galactosylation of a VSG membrane anchor *in vitro* [14]. Furthermore, we developed an *in vitro* assay for the trypanosome-specific UDP-gal:glycosylphosphatidylinositol (GPI) anchor α 1,3-galactosyltransferase [15]. In the present study, we present a detailed characterization of an α -galactosyltransferase from *T. brucei* with specificity for *N*-acetyl-lactosamine and report the purification of the enzyme to electrophoretic homogeneity by affinity chromatography on UDP-hexanolamine–SephacroseTM.

EXPERIMENTAL

Materials

UDP-D-[U-¹⁴C]galactose (150–330 Ci/mol) was purchased from Amersham–Buchler (Braunschweig). Coffee-bean α -galactosid-

Abbreviations used: GPI, glycosylphosphatidylinositol; PNGaseF, peptide-*N*⁴-(*N*-acetylglucosaminyl)asparagine amidase; VSG, variant surface glycoprotein. All sugars are of the α -configuration.

¹ Present address: Department of Immunology, The Babraham Institute, Cambridge CB2 4AT, U.K.

² To whom correspondence should be addressed (e-mail michael.duszenko@uni-tuebingen.de).

ase (EC 3.2.1.22), β -galactosidase from bovine testes (EC 3.2.1.23) and peptide- N^4 -(N -acetylglucosaminyl)asparagine amidase (PNGaseF; EC 3.5.1.52) were from Boehringer (Mannheim). UDP-hexanolamine-Sepharose[™] and concanavalin A were from Sigma Chemie (Deisenhofen). All other reagents used were of analytical grade.

Trypanosomes

Bloodstream forms of monomorphic *Trypanosoma brucei brucei* strain 427 from clone MITat 1.4 were isolated from infected rats as described previously [16].

Galactosyltransferase assays

Galactosyltransferase activity was routinely assayed in a volume of 100 μ l containing between 10 μ l and 50 μ l enzyme preparation, 50 mM Pipes buffer (pH 7.0), 15 mM MnCl₂, 1 mM dithiothreitol, 10 mM ATP, protease inhibitors (1 μ M each of chymostatin, leupeptin and pepstatin), 20 mM of a sugar acceptor, 0.15 mM UDP-galactose and 0.05 μ Ci of UDP-[¹⁴C]galactose. Incubation mixtures contained up to 1% (v/v) Triton X-100, depending on the detergent concentration in the original lysate. The mixtures were incubated for 20–60 min at 37 °C, subsequently diluted to 500 μ l with distilled water and loaded on to 1 ml Dowex 1-X8 (Cl⁻-form, 100–200 mesh) columns packed in Bio-Rad poly-prep chromatography columns. The eluate and three washes of 0.5 ml distilled water were combined and assayed for radioactivity using 3 ml of Ultimagold^{XR} liquid scintillation cocktail (Packard, Frankfurt). The columns were regenerated using 2.5 ml of 5 M NaCl and 2.5 ml of 0.1 M HCl followed by 5 ml of distilled water. In all galactosyltransferase assays the values obtained were corrected using the results of the respective control assays without acceptor.

Preparation of a detergent extract

Trypanosomes (0.5×10^{11} to 1×10^{11} cells) were hypotonically lysed by rapidly vortexing the cells in lysis buffer (10 mM Pipes/1 mM dithiothreitol/1 μ M each of chymostatin, leupeptin and pepstatin, pH 7.0) at a concentration of 5×10^9 cells/ml. The lysate was incubated at 37 °C for 5 min and microscopic analysis revealed cell ghosts, nuclei and flagellae. All subsequent steps were performed on ice or at 4 °C. The crude homogenate was diluted to about 1.5×10^9 cells/ml with lysis buffer and a membrane fraction was prepared by ultracentrifugation at 100 000 *g* for 60 min (r_{av} 6 cm). The membrane pellet was resuspended (1×10^{10} cell equivalents/ml) in buffer A (25 mM Pipes, pH 7.0/5 mM MnCl₂/5 mM MgCl₂/1 mM dithiothreitol/1 μ M each of chymostatin, leupeptin and pepstatin) using a tight fitting Teflon Dounce homogenizer; an equal volume of buffer B [buffer A plus 4% (v/v) Triton X-100] was added to disrupt the membranes. After storage on ice for 10 min the suspension was diluted to about 2×10^9 cell equivalents/ml with buffer C [buffer A plus 2% (v/v) Triton X-100]. The detergent extract was prepared by ultracentrifugation (100 000 *g*, 60 min, r_{av} 2.5 cm).

Enzyme purification

The detergent extract of 5×10^{10} to 1×10^{11} trypanosomes was immediately applied to a column of 0.5 ml UDP-hexanolamine-Sepharose[™], equilibrated in buffer D [buffer A plus 0.1% (v/v) Triton X-100] at a flow rate of 5 ml/h. The column was washed with buffer D at a flow rate of 2 ml/h; fractions of 10 ml each were collected and assayed for protein content [17] and galactosyltransferase activity. Elution was performed with buffer E (buffer

D containing 10 mM UMP and 500 mM NaCl) at a flow rate of 2 ml/h and fractions of 2 ml were collected. The fractions were concentrated and desalted using Centricon-30 (cut-off M_r = 30 000; Amicon, Witten). UMP was removed by three consecutive ultrafiltration steps using buffer A. To avoid protein losses by non-specific adsorption, the ultrafiltration devices were coated overnight with 2 ml of 5% (v/v) Triton X-100 and subsequently thoroughly cleared with water. The fractions were assayed for protein content [18] and galactosyltransferase activity. Fractions containing activity were pooled (approx. 10 ml total volume) and concentrated to about 1 ml by ultrafiltration, and again assayed for galactosyltransferase activity and protein concentration. The purified enzyme was stored in buffer D containing 0.1% (w/v) BSA at –20 °C.

Exoglycosidase digestions and BioGel P2 chromatography

For product analysis, galactosyltransferase assays were performed for 60 min at 37 °C in a total volume of 100 μ l containing 50 μ l of detergent extract, 50 mM Pipes (pH 7.0), 15 mM MnCl₂, 1 mM dithiothreitol, 10 mM ATP, 1% (v/v) Triton X-100, 20 mM LacNAc as acceptor substrate and 0.125 μ Ci UDP-[¹⁴C]galactose as donor substrate. After passing the reaction mixtures over Dowex anion exchange columns to remove excess UDP-[¹⁴C]galactose, the reaction products were dried in a vacuum concentrator. Exoglycosidase digestions were performed for 18 h at 37 °C in 20 μ l of either 0.1 M sodium phosphate buffer (pH 6.5) with coffee-bean α -galactosidase (1.5 units) or 20 μ l of 0.1 M citrate/phosphate buffer (pH 4.3) with bovine testes β -galactosidase (0.043 units). The reactions were terminated by heating (100 °C, 5 min) and the products were diluted to 420 μ l with water and stored at –20 °C.

Radiolabelled reaction products were size-fractionated at 4 °C on a BioGel P2 column (1 cm $\times$ 89 cm, BioGel P2 –400 mesh; Bio-Rad Laboratories, München). The column was eluted with water at a flow rate of 6.4 ml/h. Glucose size standards for calibrating the column were generated by hydrolysing 100 mg dextran (M_r = 72 600; Sigma, Deisenhofen) in 1 ml of 0.1 M HCl for 4 h at 95 °C [19]. The hydrolysed material was freeze dried, resuspended in 1 ml of water and stored at –20 °C. The galactosidase-treated reaction products (420 μ l) and 80 μ l of dextran hydrolysate were applied to the BioGel P2 column. Fractions of 500 μ l were collected and 300 μ l aliquots were used to monitor radioactive products. The elution positions of glucose oligomers of the dextran hydrolysate were determined by incubating 100 μ l aliquots of each fraction with 100 μ l phenol (5% w/v) and 500 μ l of concentrated H₂SO₄. The mixtures were maintained at room temperature for 10 min, carefully mixed and stored for a further 30 min before the absorption at λ = 490 nm was determined. The position of the glucose monomer of the dextran hydrolysate was confirmed in a separate experiment using radiolabelled glucose.

PNGaseF digestion

PNGaseF digestion was performed for 12–15 h at 37 °C. Purified enzyme preparations (15 μ l) were de-*N*-glycosylated in 10 μ l of 1% (w/v) SDS, 90 μ l de-*N*-glycosylation buffer (14 mM Na₂HPO₄/6 mM NaH₂PO₄/10 mM NaN₃/50 mM EDTA, pH 7.2 without HCl), 5 μ l of 20% (w/v) *n*-octylglucoside and 0.4 units of PNGaseF. The reaction was terminated by heating (100 °C). The sample was dialysed and concentrated to approx. 40 μ l before SDS/PAGE was performed.

Analytical methods

Protein concentrations were determined by the method of Bradford [17], using 0.1 % Triton X-100 to solubilize membrane proteins, except for highly purified samples of α -galactosyltransferase containing more than 0.2 % Triton X-100. In this case proteins were adsorbed on to a cellulose acetate membrane and the protein concentrations were determined according to the procedure of Guttenberger et al. [18], exploiting the binding of proteins to a fluorescent dye (benzoxanthene yellow). Interfering substances (e.g. Triton X-100 or Pipes) were removed by saturated ammonium sulphate as described [18]. BSA was used as a standard. SDS/PAGE (10 % running gel) was performed [20] and protein bands were visualized by silver staining [21]. Western blotting of the fractions containing activity was performed using a semi-dry electroblotting apparatus (Phero-multiblott; Biotech-Fischer). Glycoproteins were detected by *N*-glycan-specific staining with concanavalin A [22]. The de-*N*-glycosylated enzyme was detected by silver staining [21].

RESULTS

Characteristics of *T. brucei* α -galactosyltransferase

N-acetyl-lactosamine has been shown to be a preferred acceptor substrate for several galactose α 1,3-galactosyltransferases from mammalian sources [23–25]. In order to detect this enzyme activity in the protozoan parasite, lysates of bloodstream form trypanosomes were incubated with the activated sugar donor UDP-galactose and *N*-acetyl-lactosamine as acceptor. The cells were found to contain considerable transferase activity (7.5 pmol/min per mg of protein). The UDP-galactose:*N*-acetyl-lactosaminide galactosyltransferase appeared to be membrane-bound, since activity was almost completely (≥ 80 %) sedimented

upon ultracentrifugation of the homogenate following osmotic lysis of trypanosomes. Triton X-100 has been shown to stimulate the enzymic activity of several glycosyltransferases [26], however, the addition of Triton X-100 had no effect on *T. brucei* galactosyltransferase activity, indicating that a specific membrane topology is not required for the catalytic activity of the enzyme. The transfer of [14 C]galactose to *N*-acetyl-lactosamine occurred in the absence of detergent or in a concentration range between 0.01 % and 2 % (v/v) Triton X-100 with similar activities (1327 d.p.m. $\pm$ 165; $n = 10$). However, galactosyltransferase was solubilized from trypanosomal membranes in the presence of 2 % (v/v) Triton X-100 and was found quantitatively within the supernatant following ultracentrifugation. Using standard conditions, transfer of [14 C]galactose from UDP[14 C]galactose to *N*-acetyl-lactosamine was linear in the detergent extract for an incubation time of up to 60 min and for protein content up to 1.7 mg of protein.

To analyse the anomeric configuration generated by the galactosyltransferase, the reaction product was size fractionated by BioGel P2 filtration. Figure 1 shows the profile of the BioGel P2 run, indicating that the reaction product behaves in size as a tetrasaccharide (Figure 1a). However, the reaction product was expected to be a trisaccharide and the increase in size was due to the *N*-acetylglucosamine residue, which behaves like a glucose dimer in these gel filtration systems [19]. A small amount of the radioactive monosaccharide (≤ 20 %) was detectable in the reaction mixture, which was also seen in assays without exogenously added acceptors, and represents the hydrolysis product of UDP[14 C]galactose. Accordingly, for the determination of all galactosyltransferase activities the values obtained were corrected using the results of the corresponding control experiments without acceptor substrate. Fractions containing the galactosyltransferase reaction product were pooled, treated

Figure 1 BioGel P2 gel filtration of the galactosyltransferase reaction product

The radiolabelled product of the galactosyltransferase reaction was prepared using *N*-acetyl-lactosamine as acceptor. The [14 C]galactose-labelled reaction product was either untreated (a), treated with α -galactosidase (b) or with β -galactosidase (c) and subsequently analysed by BioGel P2 chromatography.

Table 1 Purification of the α -galactosyltransferase from *T. brucei*

The purification shown is from 7×10^{10} trypanosomes. For details of the procedure see the Experimental section.

Step	Volume (ml)	Protein (mg/ml)	Specific activity (nmol $\cdot$ min $^{-1}$ $\cdot$ mg $^{-1}$)	Total activity (nmol $\cdot$ min $^{-1}$)	Yield (%)	Purification (fold)
1. Cell homogenate	16.02	24.15	7.5×10^{-3}	2.917	100	1
2. Membrane fraction	15.40	5.95	0.051	4.713	162	7
3. Detergent fraction	33.91	0.74	0.203	5.103	175	27
4. UDP-Sephadex eluate	0.73	0.008	254.74	1.488	51	34 000

with coffee-bean α -galactosidase or bovine testicular β -galactosidase and re-applied to BioGel P2. As judged from these results, the terminal non-reducing sugar appeared to be α -linked D-galactose because of its sensitivity to coffee-bean α -galactosidase (Figure 1b) and its resistance to bovine testicular β -galactosidase (Figure 1c). Thus the enzyme transfers galactose from UDP-galactose to *N*-acetyl-lactosamine, generating an α -linkage. This specificity is consistent with the α -galactose linkage present on Asn-linked oligosaccharides of VSG molecules. Structural analysis of VSG *N*-glycans revealed biantennary complex and branched poly-*N*-acetyl-lactosamine oligosaccharides which were terminally substituted with α 1,3-linked galactose residues. We therefore propose that the enzyme detected is the UDP-galactose:*N*-acetyl-lactosaminide α 1,3-galactosyltransferase, which is involved in the processing of terminal *N*-acetyl-lactosamine structures of trypanosomal glycoproteins.

Enzyme purification

The galactosyltransferase activity detected in the trypanosomal membrane extract generated α -linked terminal galactose residues on *N*-acetyl-lactosamine. To characterize this enzyme activity further, the galactosyltransferase was purified from isolated trypanosomal membranes. The results of the purification procedure are summarized in Table 1. Following osmotic cell lysis and ultracentrifugation, the α -galactosyltransferase was found in the membrane pellet, resulting in a 7-fold purification step. The galactosyltransferase was readily extracted from the sedimented material by addition of 2% (v/v) Triton X-100. A second ultracentrifugation step yielded a detergent extract containing a 27-fold-enriched transferase activity. For further purification the extract was applied immediately to a UDP-hexanolamine-Sephadex[®] column. The elution profile is shown in Figure 2. The galactosyltransferase bound quantitatively to a small amount of UDP-Sephadex (0.5 ml) at pH 7.0 in the presence of MnCl₂. Only 12% of the applied activity was found in the flow through during the washing procedure with the same buffer. Specific elution of the bound enzyme was achieved by adjusting the buffer to concentrations of 10 mM UMP and 0.5 M NaCl. This single affinity-column step resulted in approx. 1200-fold enrichment. As shown in Table 1, the galactosyltransferase was isolated with an overall 51% yield and 34000-fold purification. The purification of the galactosyltransferase was monitored by SDS/PAGE and silver staining of the gel (Figure 3). The purified enzyme appeared a broad, single band at an apparent molecular mass of 53 kDa, which, upon Western blotting, was apparent as two individual bands, consistent with the occurrence of isoforms as stated previously [27]. Western blotting followed by specific staining using concanavalin A revealed *N*-glycosyl residues. These bands disappeared after *N*-glycan-specific digestion with PNGaseF and could only be visualized by silver staining. As the

Figure 2 Affinity chromatography on UDP-hexanolamine-Sephadex[®]

The detergent extract was applied to a column (0.5 ml) of UDP-hexanolamine-Sephadex[®]. Elution of α -galactosyltransferase activity was achieved using 10 mM UMP and 500 mM NaCl as indicated by the arrow. (▲) Protein concentration (μ g/ml); (●) enzyme activity (d.p.m.).

de-*N*-glycosylated enzyme had apparent molecular masses of 51 kDa and 50 kDa, the change in migration after PNGaseF treatment indicated the existence of one *N*-linked carbohydrate chain [28].

Kinetic measurements

In order to further characterize the enzyme activity, the following studies were performed using the isolated enzyme. The pH

Figure 3 Purification of α -galactosyltransferase

Silver-stained SDS/polyacrylamide gel from the purification steps of the α -galactosyltransferase. Lane 1, molecular-mass markers; lane 2, trypanosome homogenate; lane 3, membrane fraction; lane 4, detergent extract; lanes 5 and 6, different amounts of eluted α -galactosyltransferase activity; lane 7, following PNGaseF treatment; lane 8, after PNGaseF treatment. Arrow A, VSG (60 kDa); arrow B, α -galactosyltransferase (53/52 kDa); arrow C, de-N-glycosylated α -galactosyltransferase (51/50 kDa); arrow D, PNGaseF (35.5 kDa).

Figure 4 Mn^{2+} -dependence of α -galactosyltransferase activity

The purified galactosyltransferase activity was assayed using *N*-acetyl-lactosamine as acceptor with the $MnCl_2$ concentrations shown. The enzyme incubation was for 1 h and the radiolabelled product was monitored by scintillation spectroscopy. The enzyme activity is expressed as c.p.m./h. The K_m was 2.46 ± 0.82 mM and the V_{max} was 1298 ± 68 c.p.m./h.

dependence of the galactosyltransferase was studied in the range of pH 5.5–6.5 (Mes buffers) and 6.0–7.5 (Pipes buffers), and a pH optimum of 7.2 was determined (results not shown). The enzyme has a pronounced requirement for Mn^{2+} ions, which is illustrated in Figure 4. Maximum activity was obtained at a concentration of 20 mM Mn^{2+} and the apparent K_m for Mn^{2+} was 2.5 mM.

Figure 5 Hanes plots of the initial rates of the α -galactosyltransferase reaction

Purified galactosyltransferase activity was assayed using standard incubation conditions. (a) Kinetic data for the donor substrate were obtained by varying the UDP-galactose concentration over a range from 10 μ M to 1 mM (the concentration of *N*-acetyl-lactosamine was 20 mM). The K_m was determined as 46 μ M. (b) Kinetic data for the acceptor substrate were obtained by varying the *N*-acetyl-lactosamine concentration over a range from 1 mM to 100 mM (the concentration of UDP-galactose was 0.15 mM). The K_m was determined as 4.5 mM.

Table 2 Acceptor specificity of *T. brucei* α -galactosyltransferase

The activities of the purified α -galactosyltransferase were assayed with various oligosaccharide acceptors at 15 mM. The galactosylation rates are expressed as percentage of that obtained with *N*-acetyl-lactosamine (Gal β 1-4GlcNAc).

Acceptor	Galactosylation rate (%)
1. Galactose	25
2. Gal α -D-methyl	22
3. Gal β -D-methyl	65
4. GalNAc	4
5. Mannose	0
6. GlcNAc	0
7. Gal β 1-4Glc	52
8. Gal β 1-4GlcNAc	100
9. Gal β 1-4Glc β -D-methyl	55
10. Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc	31
11. Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc	12

Manganese ions could be partially replaced by Mg²⁺ or Ca²⁺ ions, since a concentration of 15 mM of each of these divalent cations led to about 50 % of the activity reached with the same amount of Mn²⁺ ions. In contrast, addition of the complexing agent EDTA (10 mM) abolished the transfer reaction completely (results not shown).

Kinetic parameters for the donor and acceptor substrates of the galactosyltransferase reaction were determined. As calculated from Hanes plots, an apparent K_m of 46 μ M was determined for UDP-galactose (Figure 5a), whereas the apparent K_m for *N*-acetyl-lactosamine appeared to be 4.5 mM (Figure 5b).

Acceptor specificity of the α -galactosyltransferase

To study the specificity of the purified α -galactosyltransferase from *T. brucei*, incorporation of [¹⁴C]galactose into low-molecular-mass substrates was determined and the results are presented in Table 2. The disaccharide Gal β 1-4GlcNAc (*N*-acetyl-lactosamine) was the best acceptor substrate and was assigned a galactosylation rate of 100 %. A single galactose residue, on the other hand, was galactosylated at 25 % of the rate of the disaccharide, and a similar value was reached using methylgalactoside containing an α -galactose residue. In contrast, the anomeric β -methylgalactoside served as a potent acceptor, leading to 65 % of galactosylation. Replacement of the *N*-acetylglucosamine residue in *N*-acetyl-lactosamine by glucose resulted in a galactosylation rate of 52 %. Elongation of this Gal β 1-4Glc unit at the reducing end (Table 2, acceptor 9), rendering the glucosaminyl residue to the β -configuration, did not increase the transferase activity. A tetrasaccharide containing the terminal non-reducing galactose residue in β 1,4-glycosidic linkage with *N*-acetylglucosamine was almost three times less efficiently galactosylated than the free disaccharide (31 %). Changing the terminal galactose residue to a β 1,3-linkage led to a very poor acceptor substrate (galactosylation rate, 12 %). Monosaccharides, such as *N*-acetylglucosamine, mannose and *N*-acetylglucosamine, could not serve as acceptors.

DISCUSSION

In the present work, the purification of an α -galactosyltransferase from African trypanosomes using affinity chromatography is presented. The procedure is another demonstration of the utility

of UDP-hexanolamine-Sepharose[®] in the purification of glycosyltransferases which use UDP-sugar as a donor substrate [29]. Regarding its biochemical requirements, the trypanosomal *N*-acetyl-lactosamine-specific galactosyltransferase behaves similarly to *N*-acetyl-lactosamine-specific transferases from mammalian sources, e.g. *N*-acetyl-lactosamine α 1,3-galactosyltransferases from calf thymus [30], rabbit stomach [23] and Ehrlich ascites tumour cells [25]. The *T. brucei* galactosyltransferase required 30 mM Mn²⁺ for optimal activity compared with 5 mM to 60 mM for the mammalian enzymes, and the kinetic properties of the protozoan enzyme (K_m for UDP-galactose = 46 μ M; K_m for *N*-acetyl-lactosamine = 4.5 mM) were equivalent to the mammalian transferases, which show K_m values of 12–200 μ M for UDP-galactose and 1–3 mM for *N*-acetyl-lactosamine. The pH-optimum of 7.2, determined for the trypanosomal enzyme, was shifted towards the alkaline range when compared with the mammalian enzymes, which show optimal activities at pH 6.0 to pH 6.6.

The acceptor specificity study revealed *N*-acetyl-lactosamine as the preferred acceptor substrate of the purified α -galactosyltransferase. Interestingly, the terminal galactose residue of the acceptor substrate appeared to be a specific recognition site for the enzyme, since both β -methylgalactoside and lactose led to about half of the activity obtained with *N*-acetyl-lactosamine, and even free galactose could serve as an acceptor. When compared with mammalian *N*-acetyl-lactosamine-specific galactosyltransferases, the rather broad specificity of the trypanosomal enzyme is markedly different. The *N*-acetyl-lactosaminide α 1,3-galactosyltransferase of calf thymus, for instance, showed activities of only 0.3 % with free galactose and with the α -anomer, and even very low activity (1.7 %) with the correct β -anomeric galactose [24]. Likewise, the *N*-acetyl-lactosaminide α 1,3-galactosyltransferases of Ehrlich ascites tumour cells and rabbit stomach exhibited almost undetectable rates of galactosylation with galactose as acceptor. Moreover, for the mammalian enzymes, the *N*-acetyl group of *N*-acetyl-lactosamine appeared to be important for enzymic activity, since lactose behaved as a relatively poor acceptor showing only 5 % to 20 % of the galactosylation rates obtained with *N*-acetyl-lactosamine [23–25]. These data suggest that the active site of the protozoan galactosyltransferase may differ from mammalian enzymes, making it possible to create specific inhibitors that may prove suitable for drug design.

According to structural analysis of the predominant *T. brucei* glycoprotein VSG, trypanosomes are expected to be a rich source of different galactosyltransferases. In a previous study [14], galactosyltransferase activities acting on Asn-linked glycans of VSG molecules were demonstrated and, based on the galactosylation of the exogenously added substrate ovalbumin, a UDP-Gal:*N*-acetylglucosaminylglycopeptide β 1,4-galactosyltransferase was detected in a trypanosome lysate [14]. Indeed, in addition to the *N*-acetyl-lactosamine-specific activity that led to the purification of a galactosyltransferase described in the present paper, trypanosome membranes were found to contain a distinct galactosyltransferase activity when *N*-acetylglucosamine was used as an acceptor (S. Pingel and M. Duszenko, unpublished work). Moreover, a unique feature of the VSG GPI anchor is the α -galactose side chain, which is linked by a Gal α 1-3Man linkage to the core structure of the GPI anchor [31]. Using the hydrophobic mannoside Man α 1-6Man α 1-*S*-(CH₂)₇-CH₃, we recently established an enzyme assay for the UDP-Gal:GPI anchor α 1,3-galactosyltransferase [15]. To keep track of these additional enzyme activities during the entire purification procedure of the *N*-acetyl-lactosamine-specific galactosyltransferase, fractions were also monitored for *N*-acetylglucosamine and Man α 1-

6Man α 1-S-(CH₂)₇-CH₃ specific galactosyltransferase activities. The *N*-acetylglucosamine-specific activity was readily solubilized from the trypanosome membrane fraction by the presence of 2 % Triton X-100 (Table 1, step 3) and was found in the detergent extract. However, the activity did not bind to UDP-Sepharese (Table 1, step 4) and was quantitatively recovered in the flow through of the affinity column (S. Pingel and M. Duszenko, unpublished work). In contrast, the Man α 1-6Man α 1-S-(CH₂)₇-CH₃-specific galactosyltransferase(s) appeared to be tightly membrane bound; 50 % of this enzyme activity was insoluble in the presence of 2 % Triton X-100 and was recovered in the pellet fraction of the second ultracentrifugation step. Further efforts to purify the enzyme are currently underway. However, no Man α 1-6Man α 1-S-(CH₂)₇-CH₃-activity could be detected in the fractions of the UDP-Sepharese column (results not shown).

The purified galactosyltransferase shows a high specificity for *N*-acetyl-lactosamine, and catalyses an α -galactosidic linkage. This specificity is consistent with biantennary complex and branched poly-*N*-acetyl-lactosamine oligosaccharides of VSG Asn-linked glycans, which are terminally substituted with α -linked galactose residues [5]. We, therefore, find it likely that the characterized enzyme is involved in the processing of terminal *N*-acetyl-lactosamine structures of trypanosomal glycoproteins.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft. S.P. thanks the Studienstiftung des deutschen Volkes for a Ph.D. studentship.

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Received 23 July 1998/23 November 1998; accepted 17 December 1998