Biosynthesis of O-N-Acetylglucosamine-linked Glycans in Trypanosoma cruzi

CHARACTERIZATION OF THE NOVEL URIDINE DIPHOSPHO-N-ACETYLGluCOSAMINE-POLYPEPTIDE N-ACETYLGluCOSAMINYLTRANSFERASE-CATALYZING FORMATION OF N-ACETYLGluCOSAMINE α1→O-THREONINE*

(Received for publication, December 1, 1997, and in revised form, April 6, 1998)

Jose O. Previato‡, Mauro Sola-Penna§, Orlando A. Agrellos‡, Christopher Jones†, Thomas Oeltmann§, Luiz R. Travassos**, and Lucia Mendonças-Previato††‡

From the ‡Instituto de Microbiologia and §Faculdade de Farmacia, CCS-Bloco I, Universidade Federal do Rio de Janeiro, 21944 970 Cidade Universitária, Rio de Janeiro-RJ, Brazil, †Laboratory for Molecular Structure, National Institute for Biological Standard and Control, Potters Bar, Herts EN6 3QG, United Kingdom, †Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235, and ‡Disciplina de Biologia Celular, Universidade Federal de Sío Paulo, 04023 062, São Paulo-SP, Brazil

In this study, we have characterized the activity of a uridine diphospho-N-acetylglucosamine-polypeptide-α-N-acetylglucosaminyltransferase (O-α-GlcNAc-transferase) from Trypanosoma cruzi. The activity is present in microsomal membranes and is responsible for the addition of O-linked α-N-acetylglucosamine to cell surface proteins. This preparation adds N-acetylglucosamine to a synthetic peptide KPTTTTTTTTKTP containing the consensus threonine-rich dodecapeptide encoded by T. cruzi MUC gene (Di Noia, J. M., Sánchez D. O., and Frasch, A. C. C. (1995) J. Biol. Chem. 270, 24146–24149). Incorporation of N-[3H]acetyleglucosamine is linearly dependent on incubation time and concentration of enzyme and substrate. The transferase activity has an optimal pH of 7.5–8.5, requires Mn²⁺, is unaffected by tunicamycin or amphotycin, and is strongly inhibited by UDP. The optimized synthetic peptide acceptor for the cytosolic O-GlcNAc-transferase (YSDSPSTST) (Haltiwanger, R. S., Holt, G. D., and Hart, G. W. (1990) J. Biol. Chem. 265, 2563–2568) is not a substrate for this enzyme. The glycosylated KPTTTTTTTTKPKP product is susceptible to base-catalyzed β-elimination, and the presence of N-acetylglucosamine α-linked to threonine is supported by enzymatic digestion and nuclear magnetic resonance data. These results describe a unique biosynthetic pathway for T. cruzi surface mucin-like molecules, with potential chemotherapeutic implications.

Trypanosoma cruzi is the causative agent of Chagas’ disease, a multisystemic disorder endemic in much of Latin America. This protozoan has a complex life cycle involving morphologically distinct stages in mammalian and insect hosts. Bloodsucking triatomine bugs transmit infective trypomastigotes to the mammalian host, which multiply intracellularly as amastigotes prior to differentiation into trypomastigotes, which, after rupture of the host cell, enter the blood stream, enabling infection of fresh cells or ingestion by a feeding triatomine bug, thus completing the biological cycle of transmission (1). Antigenic glycoconjugates, including the highly O-glycosylated sialglycoproteins, known as mucin-like molecules, have been strongly implicated in the molecular mechanism of attachment to and invasion of mammalian host cells (2). The sialic acid residues present in these molecules are derived from host sialglycoconjugates (3) and are transferred to the T. cruzi surface glycoproteins by a unique trans-sialidase (4). The first evidence for O-glycosylation of serine and/or threonine in trypanosomal glycoproteins came from studies of T. cruzi GP-25 (5), a glycoprotein corresponding to the C-terminal domain of cruzipain (6). More recently, O-glycosylated mucin-like proteins were demonstrated in metacyclic (7) and cell-derived trypomastigotes (8) and in epimastigotes (9, 10). Structural analyses have shown that these O-glycans vary between strains and developmental stages (9–11).

The striking feature of these O-glycans is that they are linked to the peptide backbone through an N-acetylglucosamine (GlcNAc) unit, with threonine (Thr) rather than serine (Ser) being the usual site of attachment (10, 12). The GlcNAc-Thr core can be extended by addition of Galp, Galf, and sialic acid residues. These were the first O-GlcNAc-linked oligosaccharides reported in surface glycoproteins. Previously, single O-GlcNAc units linked to Ser and/or Thr have been described on nuclear and cytosolic glycoproteins (13). This post-translational modification on the nuclear and cytoplasmic proteins is catalyzed by a cytosolic O-GlcNAc-transferase (14).

The unusual addition of O-GlcNAc to T. cruzi surface glycoproteins prompted us to investigate this post-translational modification in more detail. In this paper, we describe a novel UDP-GlcNAc:polypeptide α-N-acetylglucosaminyltransferase from T. cruzi and specify the optimal conditions for its activity. We have also characterized the in vitro glycosylation products and have established the anomic configuration of GlcNAc O-linked to Thr.

EXPERIMENTAL PROCEDURES

Materials—Radioactively labeled UDP-[6-3H]GlcNAc (40–60 Ci/mmol), UDP-[6-3H]GalNAc (5–15 Ci/mmol), NaB[3H]₄ (100–500 mCi/mmol), and [1-3H]glucose (10–30 Ci/mmol) were purchased from American Radiolabeled Chemicals, Inc. The radioactive sugar alcohols...
tissue culture-derived trypomastigotes for 6 days, maintained at 37 °C. 25–120) equilibrated in 50 mM formic acid. The column was washed
min at 12,000

Overhauser enhancement.

ROESY, rotating frame nuclear Overhauser enhancement spectroscopy; RP, reverse phase; TOCSY, total correlation spectroscopy; NOE, nuclear Overhauser enhancement.

1 The abbreviations used are: [3H]GlcNAcO, N-1-[3H]acetamido-2-acetamido-2-deoxy-\beta-D-glucopyranose; [3H]GalNAcO, N-1-[3H]acetamido-2-acetamido-2-deoxy-\beta-D-galactopyranose; MES, 2-(N-morpholino)ethanesulfonic acid; GalF, galactofuranose; Gal, galactopyranose; HPLC, high performance liquid chromatography; ROESY, rotating frame nuclear Overhauser enhancement spectroscopy; RP, reverse phase; TOCSY, total correlation spectroscopy; NOE, nuclear Overhauser enhancement.

Glycopeptide Analysis by HPLC—Incorporation of [\(^{3}H\)]GlcNAc into peptide was determined on aliquots of the eluate by liquid scintillation counting. Unlabeled peptide was monitored by absorbance at 206 nm.

Analysis of the Reaction Product—[\(^{3}H\)]GlcNAc-labeled peptide fraction obtained by RP-HPLC was recrchromatographed on Bio-Gel P-4 column (25–120) equilibrated in 50 mM phosphate-citrate buffer, pH 5.0, and 0.8 units of enzyme. The eluate was monitored by absorbance at 206 nm.

25 mM Tris/HCl buffer (pH 7.4) and centrifuged for 10 min at 12,000 x g. The supernatant was then ultracentrifuged for 1 h at 120,000 x g, and the resulting pellet was resuspended in Tris/sucrose buffer with a glass-Teflon homogenizer and ultracentrifuged as above.

The Y strain of T. cruzi was used in all enzymatic experiments. Native sialoglycoproteins were purified from epimastigotes of the Y, CL-Brener, and Dm28c strains. Epimastigotes were cultured at 28 °C in brain-heart infusion medium supplemented with hemin and 5% of fetal calf serum, and harvested in the exponential phase of growth (15). Trypomastigotes were obtained from LLC-MK2 cells infected with tissue culture-derived trypomastigotes for 6 days, maintained at 37 °C in RPMI medium containing 10% fetal calf serum, under 5% CO\(_2\) (8).

Microsomal Membrane Preparation—Pellets rich in \(10^{-11}\) epimastigotes or 1.12–1.25 mg of protein. Other conditions and the method for the implementation of the method of Wider and Wuthrich (24). The mixing curve was constructed using D-GlcNAc similarly chromatographed on Sephadex G25 SF (10 cm × 10 cm) column, using water as eluent. The oxidized glycoproteins were reduced with glycerol. The oxidized products were recovered by gel filtration on Sephadex G25 SF. The oxidized glycoproteins were digested under identical conditions. The reaction was terminated by addition of 900 Ci of [\(^3\)H]GlcNAc, 3.4 nmol of acceptor peptide, and 25

Preparation of GlcNAc-rich Glycopeptides from Native Sialoglycoproteins for NMR Spectroscopy—Sialoglycoproteins from epimastigote forms were purified as described by Previto et al. (4). The sialoglycoproteins from T. cruzi CL-Brener strain were subjected to partial acid hydrolysis (0.2 M trifluoroacetic acid for 2 h at 100 °C). The GlcNAc-rich glycopeptides were recovered by gel filtration on a column of Sephadex G25 SF (1 × 10 cm).

\[\text{column (5 \mu m, 4 × 250 mm, Amersham Pharmacia Biotech) eluted with an H}_2\text{Oacetoni trileufluoroc acid gradient. Solvent A was 0.1% aqueous trifluoroacetic acid, and solvent B was 0.089% trifluoroacetic acid in acetonitrile. The linear gradient was started 5 min after injection from 0 to 15% solvent B over 40 min (held until 50 min). The flow rate was 0.5 ml/min. Solvent B was produced from 100% acetonitrile and 0.1% trifluoroacetic acid. The eluent was mixed with 5 ml of Bray solution, and the radioactivity was determined by liquid scintillation counting. Unlabeled peptide was monitored by absorbance at 206 nm. Analysis of the Reaction Product—[\(^{3}H\)]GlcNAc-labeled peptide fraction obtained by RP-HPLC was recrchromatographed on Bio-Gel P-4 column (25–120) equilibrated in 50 mM phosphate-citrate buffer, pH 5.0, and 0.8 units of enzyme. The eluate was monitored by absorbance at 206 nm. The solution was neutralized with 2 M acetic acid and passed through Dowex 50W-X8 (25–50 mesh H\(^+\) form). Boric acid was removed by repeated evaporation with methanol. The residue was dissolved in distilled water and analyzed by gel filtration on Bio-Gel P-4 column (as above). A standard curve was constructed using D-GlcNAc similarly chromatographed on Sephadex G25 SF. Other conditions were as described above. The reaction mixture contained, in a final volume of 50 ml, 25 mM Tris/ClH buffer (pH 7.4), 5 mM MgCl\(_2\), 5 mM MnCl\(_2\), 0.1% Triton X-100, 1.5 \mu Ci of UDP-[\(^{3}H\)]GlcNAc (40–60 Ci/mMol), and 6.8 nmol of synthetic peptide acceptor (0.1 pmol of 5KTTTTKPKP). The reaction was initiated by addition of microsomal membranes (250 \mu g of protein). Control assays without the acceptor peptide were used to correct for endogenous activity. The mixture was incubated at 28 °C for 30 min, and the reaction terminated by addition of 950 \mu l of 50 mM formic acid. The reaction mixture was loaded onto a 1 ml sulfoethyl-Sephadex column (SP-C 25–120) equilibrated in 50 mM formic acid. The column was washed with 1 ml of the same buffer, and the eluted peptide was collected and lyophilized, and the radioactivity was determined by liquid scintillation counting. p-Nitrophenyl \(\beta\)-N-acetylglucosaminidase was used as substrate control. As a control for the purity of the UDP-[\(^3\)H]GlcNAc-labeled peptide, the oxidized synthetic glycopeptide YSPTSPSK, with the \(\beta\)-GlcNAc on the serine at position 5 (kindly provided by G. W. Hart) was digested under identical conditions. The reaction was terminated by addition of 50 mM formic acid, and the liberated GlcNAc was recovered on SP-Sephadex as above and quantified using the Morgan-Elsdon reaction (22). A standard curve was constructed using b-GlcNAc similarly chromatographed on Sephadex B. Other conditions were as described above. The optimization of the O-GlcNAc transferase Assay—Incorporation of [\(^{3}H\)]GlcNAc into synthetic peptide was confirmed by RP-HPLC on a C18
**T. cruzi** KPPTTTTTTTTP proved to be successful as a substrate for incorporation of [3H]GlcNAc into the peptide was time dependent—activity was slightly less than that observed at 4 °C (Fig. 1). Increasing amounts of protein (using a microsomal membrane preparation; concentration, reported as the amount of protein in the microsomal Tris-MES buffer. The assays were performed as described under “Experimental Procedures.”

Time in the TOCSY spectra was 80 and 150 ms in the ROESY spectra. Presaturation of the residual water signal was achieved using a low power pulse from the transmitter. In the WHSQC spectra, obtained at 500 MHz, heteronuclear decoupling was achieved using the GARP sequence. 1H and 13C chemical shifts were referenced to internal 3-(trimethylsilyl)tetra-deuteropropionic acid at zero ppm (1H) and –1.8 ppm (13C, to tetramethylsilane at zero) (25).

### RESULTS

**Development of an Assay for T. cruzi O-α-GlcNAc-transferase:** Optimization of Assay Conditions—An assay for O-GlcNAc-transferase activity was developed using microsomal membranes prepared from epimastigotes of the Y strain of *T. cruzi*. To measure the transfer of GlcNAc in *vivo*, UDP-[6-3H]GlcNAc was used as the GlcNAc donor and the synthetic peptide KPPTTTTTTTTTKPP as acceptor. This peptide was chosen due to its similarity with a common motif in the peptide sequence. 1H and 13C chemical shifts were referenced to internal 3-(trimethylsilyl)tetra-deuteropropionic acid on the P-4 column (Fig. 2).

**Characterization of the Glycosylated Peptide Product**—The radiolabeled material recovered from the SP-Sephadex column was characterized by several techniques. A single peak of radiolabeled material indicated that its apparent molecular mass was greater than that of the unlabeled peptide acceptor (Fig. 2B). After base-catalyzed β-elimination and reduction of the purified radiolabeled glycopeptide, radioactive material eluted at the same volume as authentic N-acetylhexosaminitol on the P-4 column (Fig. 2C), and its identity was confirmed as [3H]GlcNAco by descending paper chromatography (Fig. 2D). In assays using larger amounts of UDP-[6-3H]GlcNAc, two radiolabeled fractions were observed in RP-HPLC (Fig. 3A) and on the P-4 column (Fig. 3B). One fraction showed a chromatographic profile identical with that of the glycopeptide obtained under standard conditions (Fig. 2A, A and B). The other fraction was assumed to be a glycopeptide substituted with more than one GlcNAc residue (Fig. 3A and B), as both fractions liberated [3H]GlcNAcO (identified by descending paper chromatography) on reductive β-elimination (Fig. 3C).

**Effect of Inhibitors**—Addition of 5 mM UDP reduced incorporation of [3H]GlcNAc into the synthetic peptide by 90%. Transferase activity was not reduced in the presence of tunicamycin or amphomycin, even when added at 10 μM.

**Ion Dependence and Optimum pH**—The transferase was active in the absence of metal ions, but activity was regained by adding Mn2+. Table I shows that among seven divalent cations tested, Mn2+ was the most effective in restoring activity. Co2+ and Ca2+ were able to restore 20 and 15% of the activity observed in the presence of Mn2+. The O-GlcNAc-transferase activity had a pH optimum between 7.5 and 8.5, with maximum activity at pH 8.5. Activity decreased gradually below pH 7.0 and above pH 8.5, with only 50% of the activity at pH 8.5 present at pH 9.0 (Fig. 1D).

**Effect of divalent cations on the activity of T. cruzi O-α-GlcNAc transferase**

| Metal cation  | Transferred to peptide cpm |
|--------------|---------------------------|
| No added metal cations | 150          |
| Mn2+         | 16,925                  |
| Co2+         | 3033                    |
| Ca2+         | 2618                    |
| Mg2+         | 1308                    |
| Cu2+         | 308                     |
| Ni2+         | 308                     |
| Zn2+         | 175                     |

All metal ions were added as the chloride salt at 5 mM concentration in the conditions used for a standard assay.

**Transferred**

| Metal cation | Transferred to peptide |
|--------------|------------------------|
| No added metal cations | 150          |
| Mn2+         | 16,925                  |
| Co2+         | 3033                    |
| Ca2+         | 2618                    |
| Mg2+         | 1308                    |
| Cu2+         | 308                     |
| Ni2+         | 308                     |
| Zn2+         | 175                     |

All metal ions were added as the chloride salt at 5 mM concentration in the conditions used for a standard assay.
hydrolysis (CL-Brener strain) or Smith degradation (Y, CL-Brener, and Dm28c strains) was determined by high field NMR spectroscopy. The spectra from all these samples were effectively identical. Fig. 5 shows expansions of the TOCSY (Fig. 5A) and ROESY (Fig. 5B) of the GlcNAc-rich glycopeptide from Y strain. The resonances between 4.4 and 5.4 ppm (Fig. 5A) were assigned to saccharide anomic protons. The most intense of these originate from the O-linked glycanics. Those between 4.44 and 4.52 ppm displayed a pattern of cross-peaks in the TOCSY spectrum typical of β-Galp spin systems, (3JH3,H4 small and H-4 at low field) (9). The anomeric protons from the GlcNAc O-linked to Thr resonated between 4.75 and 4.95 ppm, with the most intense at 4.85 ppm. This chemical shift dispersity is presumably attributable to heterogeneous substitution by GlcNAc on the peptide backbone and to the presence of galactosylation of some GlcNAc residues.

Because glycopeptide from strain Dm28c was available in greatest quantity, WHSQC spectra with and without heteronuclear decoupling were recorded from this material (Fig. 6), which enabled partial assignment of the 13C spectrum and determination of the value of one bond proton carbon-coupling constants (1J,H1). N-Acetyl amino sugars are easily recognized by the high field positions of the C-2 resonance in their 13C spectra, which are observed at approximately 51.4 and 56.1 ppm for the methyl glycosides of GlcNAc and GalNAc, respectively (27). In the WHSQC spectrum of the GlcNAc-rich glycopeptide from Dm28c, the resonance at 54.6 ppm was assigned to C-2 of GlcNAc and was correlated to an anomeric proton from the GlcNAc residues. The corresponding anomic carbons resonated between 99.5 and 99.9 ppm (from the same WHSQC spectrum) (Fig. 6, A and B).

In the ROESY spectrum (Fig. 5B), NOEs were observed from the most intense GlcNAc H-1 resonance at 4.75 ppm and the N-acetyl methyl resonances to Hβ and methyl resonances of Thr, indicating linkage of the GlcNAc to Thr in the peptide backbone.

Some minor resonances, not attributable to the O-linked glycans, were also present. One set had chemical shifts for the H-1, C-1, and other structural reporter groups consistent with those reported for high mannose chains (28, 29), although only a Manα2GlcNAc2 stub was present in the Smith-degraded samples (Fig. 5A).

The anomeric configuration of the GlcNAc residue was assigned from the values of the 3JH2,H3 and 1JH1,C1 coupling constants, the pattern of intra-residue NOEs, and the proton and the carbon shifts of the GlcNAc residues. The value of 3JH2,H3 in the GlcNAc residue was estimated as 3–4 Hz from resolved cross-peaks observed in the ROESY spectrum (Fig. 7C). This is consistent with the α-configuration (H-1 and H-2 gauche) (30), as a value of 8 Hz would be expected for GlcNAcβ1→OThr (in which H-1 and H-2 have a trans diaxial orientation), as was observed for the GlcNAcβ1→Asn present in the stub of the high mannose oligosaccharide chain (Fig. 7A). The value of 1JH1,C1 for the GlcNAc1→OThr was determined from the WHSQC spectrum, obtained at 600 MHz without heteronuclear decoupling (Fig. 6B). The magnitude of this coupling constant depends principally on the orientation of the anomic proton and is typically 160 and 170 Hz for axial and equatorial H-1 values, respectively (i.e., for β and α linkages in the case of a β-hexopyranose in the C1 conformation) (31, 32). A value of 171 Hz was observed for 1JH1,C1 of GlcNAc H-1 (Fig. 6A), confirming an α linkage whereas for the β-Galp resonances the measured value was 161 Hz.

The only significant NOE observed between the GlcNAc H-1 to other protons on the saccharide ring was to the H-2, which is characteristic of the α-glyco configuration. In contrast, β-glyco
systems typically show strong intra-residue NOEs to H-3 and H-5.

No data for GlcNAc-a13O-Thr containing model compounds have been reported, but in glycopeptides containing GlcNAc-b13O-linked to Ser (33, 34) or Thr (34, 35), the chemical shift of the GlcNAc H-1 is in the range 4.40 to 4.57 ppm, clearly different from the values observed in the T. cruzi glycopeptides. The shifts for the a-GlcNAc H-1 resonances are unusually high field compared with a-GlcNAc in oligosaccharides (where the value is typically >5 ppm) but are consistent with the high field location of the GalNAc-a13O-Thr anomeric proton in model systems for mammalian mucin, where published values are in the range 4.87–4.92 ppm (36, 37). The observed values for the GlcNAc residue in the T. cruzi mucin are thus in better agreement with an α- rather than a β-linkage. Other proton chemical shifts were in agreement with this assignment. The carbon
The first step in the biosynthesis of these O-glycan chains is attachment of GlcNAc to the peptide backbone. In the present study, we show that microsomal membrane preparations from epimastigotes and trypomastigotes of *T. cruzi Y* strain have an O-α-GlcNAc-transferase that attaches GlcNAc to threonine in a suitable acceptor peptide. We used several approaches to define the activity of this enzyme and to assess its relationship to the cytosolic O-α-GlcNAc-transferase (14, 38).

We demonstrate that the synthetic peptide KPPTTTTTTTTTT-KPP can function as acceptor for this novel transferase. This peptide incorporates a threonine-rich sequence, originally reported by DiNoia et al. (26), who showed that TTTTTTTTTT-KPP is a common repeating motif in *T. cruzi* MUC gene products. The locations of the glycosylated threonines were not identified, but the peptide was a good substrate for *in vitro* glycosylation, at least two GlcNAc residues being incorporated in heavily labeled experiments. The nonapeptide YSDPSTST, described by Haltiwanger et al. (38) as an optimum substrate for the cytosolic O-α-GlcNAc-transferase, was not glycosylated by the *T. cruzi* enzyme. UDP-[3H]GlcNAc was the activated GlcNAc donor in the *T. cruzi* system. Potentially, the mechanism of the reaction could be either direct transfer from UDP-GlcNAc or via formation of activated dolichol donors. To distinguish between these possibilities, the transferase activity was assayed in the presence of excess UDP and with the antibiotics tunicamycin or amphotycin, which are potent inhibitors of Dol-P-dependent glycosylation (39, 40). Only UDP abolished incorporation of [3H]GlcNAc into the acceptor peptide, indicating that UDP-GlcNAc acts directly as the GlcNAc donor. The cytosolic O-α-GlcNAc-transferase (38) also uses sugar nucleotides directly as sugar donors.

The *T. cruzi* enzyme, in common with most glycosyltransferases (41), requires divalent metal cations for activity, with Mn²⁺ being the most effective. This is in contrast to the cytosolic O-α-GlcNAc-transferase, which shows no metal ion dependence (38). Other differences between the *T. cruzi* microsomal enzyme and the ubiquitous cytosolic O-α-GlcNAc-transferase are that the former has an optimal pH range of 7.5–8.5, remains active at 37 °C, and has increased activity when treated with Triton X-100. Most strikingly, the *T. cruzi* enzyme attaches GlcNAc to the hydroxylated amino acid via an α-linkage, whereas the anomeric specificity of the cytosolic enzyme is β. In support of this, the [3H]GlcNAc peptide (produced *in vitro*) was not susceptible to digestion of with β-N-acetylglucosaminidase from jack beans, although under identical digestion conditions GlcNAc was readily liberated from unlabeled synthetic YSPSSPSK (with the O-β-GlcNAc on the serine at position 5), corresponding to a sequence from the C-terminal repeat domain of the large subunit of RNA polymerase II (42), which *in vivo* is glycosylated by the cytosolic transferase. It is unlikely that the lack of susceptibility of the [3H]GlcNAc-K₆P₅T₅ peptide to β-N-acetylglucosaminidase is attributable to its amino acid sequence, as the jack bean enzyme is able to deglycosylate a diverse range of structurally distinct glycoproteins and glycopeptides, including sequences from nuclear pore protein, human erythrocyte band 4.1 protein, and the 65-kDa erythrocyte glycoprotein cytosolic protein (38, 42). More compellingly, NMR analysis of GlcNAc-rich glycopeptides from the native sialoglycoconjugates of *T. cruzi* showed unambiguously that the GlcNAc residue linked to Thr has the α-anomeric configuration, the U₁C, H, NOE, and chemical shift data all being consistent with the α-rather than the β-anomer.

O-GlcNAc has previously been reported in surface glycoproteins from *Plasmodium falciparum* (43) and *Giardia lamblia* (44). Subsequent studies, however, showed that O-linked Glc-
N-Acetylglucosamine is the O-linked sugar in *G. lamblia* (46). Because the O-GlcNAc-transferase activity of *T. cruzi* is associated with the microsomal fraction, and because its known natural substrates are GPI-anchored N-linked surface glycoproteins (10), it seems likely that it is associated with some compartment of the secretory pathway, although confirmation of this must await completion of detailed localization studies. Because the enzyme differs in its anomeric specificity, kinetic properties, and possibly in cellular location from the cytosolic enzyme described in higher eukaryotes, it may prove to be unique to *T. cruzi*. If so, it constitutes an exciting potential target for the rational design of novel chemotherapeutic agents. Purification of the transferase is currently in progress and should soon enable us to address these questions.

**Acknowledgments**—We thank Dr. A. C. C. Frasch and Dr. J. M. DiNoia (Instituto de Investigaciones Biotecnologicas, UNSAM, San Martin, Provincia de Buenos Aires, Argentina), Dr. R. Haltiwanger (Dept. of Biochemistry, SUNY, Stony Brook, NY), and Prof. G. W. Hart (Johns Hopkins University School of Medicine, Baltimore) for the synthetic peptides. Also, we thank Dr. R. Wait for critical reading of the manuscript, X. Lemercinier for the 13C-coupled WHSQC spectrum, the MRC Biomedical NMR Center for access to the Unity 600 NMR spectrometer, and the Sir Halley Stewart Trust for funding.

**References**

1. Breezer, Z. (1973) *Annu. Rev. Microbiol.* 27, 347–382
2. Burleigh, B. A., and Andrews, N. W. (1985) *Annu. Rev. Microbiol.* 49, 175–200
3. Previti, J. O., Andrade, A. F. B., Des Reis, G. A., and Mendonça-Previti, L. (1991) *Mem. Inst. Oswaldo Cruz* 86, Suppl. 1, 53–54
4. Previti, J. O., Andrade, A. F. B., Pessolani, M. C. V., and Mendonça-Previti, L. (1995) *Mol. Biochem. Parasitol.* 66, 85–96
5. Mendonça-Previti, L., Gorin, P. A. J., Braga, A. F., Scharfstein, J., and Previti, J. O. (1995) *Biochemistry* 34, 4890–4897
6. Cazulo, J. J., Stoka, V., and Turk, V. (1997) *Biochemistry* 36, 1–10
7. Schenken, S., Ferguson, M. A. J., Heise, N., Cardoso de Almeida, M. L., Mortara, R. A., and Yoshida, N. (1995) *Mol. Biochem. Parasitol.* 69, 293–304
8. Almeida, I., Ferguson, M. A. J., Schenken, S., and Travassos, L. R. (1994) *Biochim. J.* 304, 793–802
9. Previti, J. O., Jones, C., Gonzalez, L. P. B., Wait, R., Travassos, L. R., and Mendonça-Previti, L. (1994) *Biochem. J.* 301, 151–159
10. Previti, J. O., Jones, C., Xavier, M. T., Wait, R., Travassos, L. R., Parodi, A. J., and Mendonça-Previti, L. (1995) *J. Biol. Chem.* 270, 27241–27250
11. Serrano, A. A., Schenken, S., Yoshida, N., Meeker, A., Richardson, J. M., and Ferguson, M. A. J. (1995) *J. Biol. Chem.* 270, 27244–27253
12. DiNoia, J. M., Pollevick, G. D., Xavier, M. T., Previti, J. O., Mendonça-Previti, L., Sanches, D. O., and Frasch, A. C. C. (1996) *J. Biol. Chem.* 271, 32078–32083
13. Hart, G. W., Kreppel, L. K., Comer, F. I., Arnold, C. S., Snow, D. M., Ye, Z., Cheng, X., Dellamanna, D., Caine, D. S., Earles, J., Akimoto, Y., Cole, R. N., and Hayes, B. K. (1996) *Glycobiology* 6, 711–716
14. Haltiwanger, R. S., Blomberg, M. A., and Hart, G. W. (1992) *J. Biol. Chem.* 267, 9005–9013
15. Previti, J. O., Gorin, P. A. J., Mazurek, M., Xavier, M. T., Fournet, B., Wieruszewski, J. M., and Mendonça-Previti, L. (1990) *J. Biol. Chem.* 265, 2518–2526
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
17. Yen, P. H., and Ballou, C. E. (1974) *Biochemistry* 13, 4240–4247
18. Do, K.-Y., Do, S.-I., and Cummings, R. D. (1997) *Glycobiology* 7, 183–194
19. Goldstein, I. J., Hay, G. W., Lewis, B. A., and Smith, F. (1965) *Methods Carbohydr. Chem.* 3, 361–370
20. Wider, G., and Wuthrich, K. (1993) *J. Magn. Reson.* 102, 239–241
21. Winkhart, D., Biggam, C. G., Yao, J., Aihigaard, F., Dyson, H. J., Oldfield, E., Markley, J. L., and Sykes, B. D. (1995) *J. Biomol. NMR* 6, 135–140
22. DiNoia, J. M., Sánchez, D. O., and Frasch, A. C. C. (1995) *J. Biol. Chem.* 270, 24146–24149
23. Bock, K., and Pedersen, C. (1983) *Adv. Carbohydr. Chem. Biochem.* 41, 77–86
24. Lu, J., and Van Halbeek, H. (1996) *Carbohydr. Res.* 296, 1–21
25. Vliegenthart, J. F. G., Dorland, L., and Kamerling, J. P. (1983) *Adv. Carbohydr. Chem. Biochem.* 41, 209–233
26. Dahrowski, J. (1994) in *Two Dimensional NMR Spectroscopy Applications for Chemistry and Biochemistry* (Croasmun, W. R., and Carlson, R. M. K., eds), VCH, New York
27. Bock, K., and Pedersen, C. (1974) *J. Chem. Soc. Perkin Trans. II*, 293–297
28. Tvaroska, I., and Taravel, F. R. (1995) *Adv. Carbohydr. Chem. Biochem.* 51, 15–61
29. Pao, Y.-L., Wormald, M. R., Dwek, R. A., and Lelievre, A. C. (1996) *Biochem. Biophys. Res. Commun.* 219, 157–162
30. Saha, U. K., Griffiths, L. S., Rademann, J., Geyer, A., and Schmidt, R. R. (1997) *Carbohydr. Res.* 304, 233–239
31. Seitz, O., and Wang, C.-H. (1997) *J. Am. Chem. Soc.* 119, 8766–8776
32. Davoust, D., Platzier, N., Darpepe, C., Lemonnier, M., Ferrar, B., and Pavis, A. A. (1988) *Carbohydr. Res.* 143, 231–239
33. Klich, G., Paulsen, H., Meyer, B., Mebdal, M., and Bock, K. (1997) *Carbohydr. Res.* 299, 33–48
34. Haltiwanger, R. S., Holt, G. D., and Hart, G. W. (1990) *J. Biol. Chem.* 265, 2563–2568
35. Ericson, M. C., Gafford, J. T., and Elbein, A. D. (1977) *J. Biol. Chem.* 252, 7431–7433
36. Banerjee, D. K., Scher, M. G., and Waechter, C. J. (1981) *Biochemistry* 20, 1561–1568
37. Schachter, H., and Brockhausen, I. (1992) in *Glycoconjugates* (Allen, H. J., and Kisailus, E. C., eds), pp. 263–332, Marcel Dekker, Inc., New York
38. Hart, G. W., Kelly, W. G., Blomberg, M. A., Roque, C., Dong, L.-Y., Kreppel, L., Chou, T.-Y., Snow, D., and Greis, K. (1993) *Colloqium* *Mosbach* 44, 91–103
39. Dietmann-Schuppert, A., Bause, E., and Schwarz, R. T. (1993) *Carbohydr. Res.* 233–239
40. Dieckmann-Schuppert, A., Bause, E., and Schwarz, R. T. (1993) *J. Biomol. NMR* 304, 233–239
41. Ortega-Barria, E., Ward, H. D., Evans, J. E., and Pereira, M. E. A. (1990) *Mol. Biochem. Parasitol.* 43, 151–166
42. Gowda, D. C., Gupta, P., and Davidson, E. A. (1997) *J. Biol. Chem.* 272, 6428–6439
43. Papanastasiou, P., McConville, M. J., Hulsmier, A., Ralton, J., Hiltgold, A., and Kohler, P. (1997) *Glycoconjugate J.* 14, Suppl. 1, S55