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Glycosyltransferase Activities in Golgi Complex and Endoplasmic Reticulum Fractions Isolated from African Trypanosomes

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ABSTRACT Highly enriched Golgi complex and endoplasmic reticulum fractions were isolated from total microsomes obtained from *Trypanosoma brucei*, *Trypanosoma congolense*, and *Trypanosoma vivax*, and tested for glycosyltransferase activity. Purity of the fractions was assessed by electron microscopy as well as by biochemical analysis. The relative distribution of all the glycosyltransferases was remarkably similar for the three species of African trypanosomes studied. The Golgi complex fraction contained most of the galactosyltransferase activity followed by the smooth and rough endoplasmic reticulum fractions. The dolichol-dependent mannosyltransferase activities were highest for the rough endoplasmic reticulum, lower for the smooth endoplasmic reticulum, and lowest for the Golgi complex. Although the dolichol-independent form of *N*-acetylglucosaminyltransferase was essentially similar in all the fractions, the dolichol-dependent form of this enzyme was much higher in the endoplasmic reticulum fractions than in the Golgi complex fraction. Inhibition of this latter activity in the smooth endoplasmic reticulum fraction by tunicamycin A_1 suggests that core glycosylation of the variable surface glycoprotein may occur in this organelle and not in the rough endoplasmic reticulum as previously assumed.

African trypanosomes causing both sleeping sickness in man and nagana in cattle appear in relapsing waves in the course of infection. During each of these waves of parasitemia a different variable surface glycoprotein (VSG),[1] a clone-specific macromolecule constituting the trypanosome surface coat, is expressed. Antigenic variation has thus frustrated many attempts to develop vaccines against trypanosomes. To progress in this field, it seems essential to acquire a thorough understanding of the nature of the variable surface glycoproteins that form a continuous 12–15-nm surface coat on the plasma membrane of these parasites (47). This class of macromolecules (M_r = 65,000 ± 5,000) has been shown to vary both immunochemically (10, 18, 19, 46) and biochemically (9, 11, 24, 46, 47) during different stages of host infection. It has become increasingly clear that this remarkable ability of the trypanosomes to switch frequently from one VSG type to another may preclude the development of a conventional vaccine. However, inasmuch as the integrity of the surface coat is essential for survival of the parasite in its mammalian host, a study of how VSGs are synthesized, processed, and exported to the cell surface may suggest new approaches to chemotherapy.

It is known from previous investigations of the composition and mode of assembly of the constituent glycoproteins that the VSGs of both *Trypanosoma brucei* and *T. congolense* contain at least two classes of carbohydrate side chains (21). One, referred to as the internal side chain, is located in the C-terminal third of the glycoprotein, contains mannose and *N*-acetylglucosamine, and can be removed by endo-β-*N*-acetylglucosaminidase (Endo H) (29). Its incorporation into VSG, the major glycoprotein in trypanosomes, is mediated by a dolichol-monophosphate intermediate, which recognizes the Asn-X-Ser/Thr glycosylation triplet (21) and is sensitive to the antibiotic tunicamycin (31, 38, 39, 43). Some of the enzymes in this biosynthetic pathway have also been characterized and show similarities to those in several other eucaryotes (7, 39, 43). Of more potential pharmacological interest

[1] *Abbreviations used in this paper:* CRD, cross-reacting determinant; ER, endoplasmic reticulum; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; TCA, trichloroacetic acid; VSG, variable surface glycoprotein.

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is the second class of carbohydrate, which contains mannose, galactose, and *N*-acetylglucosamine (21), and which is located at or near the C-terminal end of the protein conjugated through an ethanolamine linkage to the α-carboxyl group (20) possibly via phosphatidylethanolamine (13). The addition of this carbohydrate side chain is not inhibited by tunicamycin (38). The importance of this carbohydrate side chain is evident from the observations that it remains constant on all the variable antigens of *T. brucei* and *T. congolense* and is, therefore, probably responsible for cross-reactivity between VSGs; this is the cross-reacting determinant (CRD) of VSG (3, 4, 5, 12, 21). Little or no information is available for *T. vivax*, and this area obviously needs more attention in view of the economic importance of this species.

It is well established that plasma membrane proteins, secretory proteins, and some lysosomal proteins are synthesized on the rough endoplasmic reticulum (RER) and later transported through the Golgi complex (1, 15, 42) before reaching their ultimate cellular destinations. Protein transport is often accompanied by modifications to bound oligosaccharide as the product passes through the various membrane bounded compartments of the endoplasmic reticulum (ER) and Golgi complex (1, 15). McConnell et al. (30) have already demonstrated that VSGs are assembled on membrane-bound polysomes and contain an N-terminal signal peptide that is 30–40 amino acids long which is subsequently cleaved in the ER. However, little is known about the pathway of glycosylation of proteins in African trypanosomes. The subcellular localization of carbohydrate transfer in these parasites has been difficult to establish because of the heterogeneous nature of the trypanosomal membrane preparations used in published studies. It is assumed that CRD transfer occurs after the synthesis of the internal carbohydrate side chain which presumably occurs in the ER (29, 31, 39). One might postulate that the transfer of the CRD carbohydrate occurs in Golgi complex because of the high galactose content. Alternatively, it has been suggested the CRD is put on at the level of the plasma membrane (38, 43). To identify the site of major VSG glycosylation reactions, we have isolated morphologically enriched RER, smooth endoplasmic (SER), and Golgi complex fractions from all three species of African trypanosomes and have characterized the associated glycosyltransferase activities.

MATERIALS AND METHODS

Biochemicals: All reagents were analytical grade or better. Enzymes and biochemicals were purchased from Sigma Chemical Co. (Dorset, England), Boehringer GmbH (Mannheim, Federal Republic of Germany [FRG]), or SERVA Feinbiochemica GmbH & Co. (Heidelberg, FRG). Tunicamycin was a gift of R. L. Hamill (Lot No. 361-91J-165-A, Eli Lilly Co., Indianapolis, IN) and tunicamycin A_1 was purified from this crude preparation by reverse-phase high-performance liquid chromatography using an Ultrasphere-ODS column

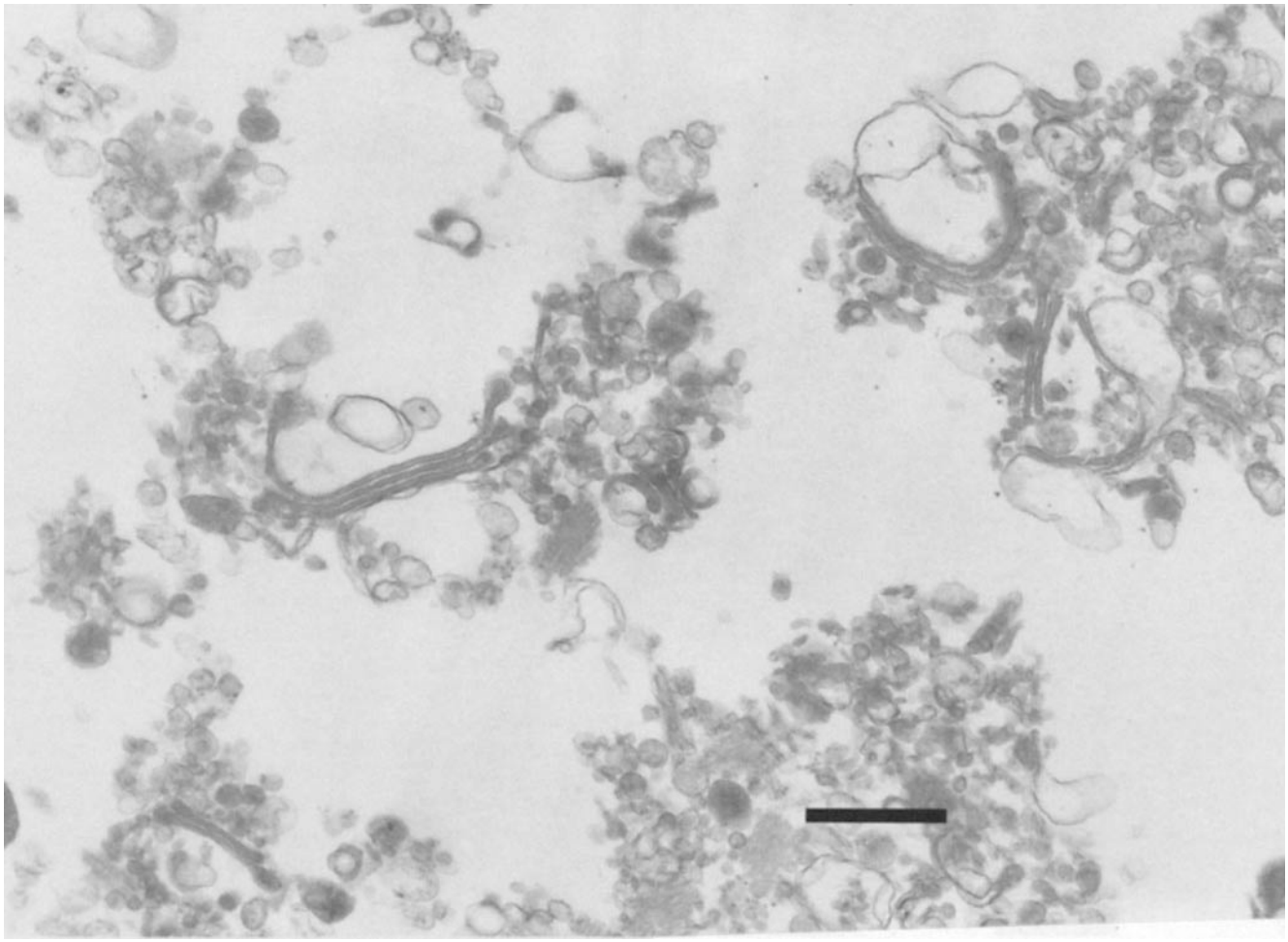

FIGURE 1 Electron micrograph of a rat liver Golgi complex fraction (GF-3). Livers were homogenized in 0.25 M sucrose-HKMM and a Golgi fraction was prepared as described in the text. Bar, 0.5 μm. × 40,000.

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(Rainin Instruments, Co., Inc., Woburn, MA) according to the method of Mahoney and Duksin (27, 28). Tunicamycin A_1 in 80% CH_3OH/H_2O was dried under nitrogen gas before use. UDP-*N*-acetyl-D-(U-^{14}C) glucosamine (7.7 GBq/mmol), UDP-D-(U-^{14}U) galactose (11.3 GBq/mmol), GDP-(U-^{14}C) mannose (7.7 GBq/mmol), and cyclic AMP assay kit (TRK. 432) were obtained from Radiochemical Centre Limited (Amersham, U.K.). Aquasol was obtained from New England Nuclear, (Boston, MA).

Organisms: *Trypanosoma brucei* clones MITat 1.2, 1.52, and 1.7 were derived from stock 427 (21). *Trypanosoma congolense* ILNat clone 2.1 was derived from the Transmara strain supplied by the Kenya Veterinary Laboratories (Nairobi) and *Trypanosoma vivax* ILDat clone 1.2 was derived from strain, Zaria Y 486, which was isolated from a Zebu cow in Nigeria in 1973. Parasites from cryopreserved stabilates were grown in lethally irradiated (600–900 rad) rats and isolated from infected blood isopycnically on Percoll gradients according to the method of Grab and Bwayo (16) followed by DEAE-cellulose chromatography (26). On some occasions, trypanosomes were isolated by DEAE-cellulose chromatography alone (26); however, use of Percoll greatly increased the yield of organisms obtained.

Isolation of Microsomal Membranes: Approximately 5×10^{11} to 1×10^{12} parasites were disrupted in 35 ml 0.25 M sucrose-HKMM buffer (50 mM HEPES, 25 mM KCl, 5 mM $MgSO_4$, 10 μM $MnCl_2$, pH 7.4 at 5°C) containing 50 trypsin inhibitor units of aprotinin/ml by passage through a French press under a chamber pressure of 2,000 psi. The homogenate was centrifuged at 15,000 g_{av} for 10 min in a Beckman JA-20 rotor (Beckman Instruments, Inc., Fullerton, CA) and the pellet which contained ~45% at the total protein content was washed once with 0.25 M sucrose-HKMM buffer. The pooled supernatant fraction was then recentrifuged at 125,000 g_{av} for 90 min in a Beckman 42.1 rotor. A Golgi complex fraction was isolated from the total microsomal fraction (~12.5% of the total homogenate protein) exactly as described by Howell et al. (22). Both SER and RER fractions were obtained from either the residual microsomal fraction obtained during the Golgi complex isolation or from total microsomes essentially after the method of Ragland et al. (36) after adjusting the sucrose concentration to 1.15 M. All procedures were done at 0 to 4°C. All fractions were immediately frozen and stored at −80°C in sucrose-HKMM buffer containing 10 trypsin inhibitor units of aprotinin/ml.

Morphology: For electron microscopy ~0.5–1-mg membrane proteins of the Golgi complex, RER, and SER fractions in the sucrose-HKMM solutions were fixed in an equal volume of fixative containing 1.25% formaldehyde, 2.5% glutaraldehyde, and 0.015% picric acid in 50 mM phosphate buffer pH 7.4 (23). Fixation of the suspension was for 1–2 h at room temperature. The fractions were centrifuged for 30 min at 100,000 *g* to obtain a visible pellet and the supernatant fixative was removed. During the following buffer washes, osmication with 1% OsO_4 in phosphate buffer, uranyl acetate staining, ethanol dehydration, and embedding in Epon-Araldite, the pellet was not disturbed or detached from the centrifuge tube. Thin sections were cut across the entire thickness of the pellet and very little evidence of centrifugal stratification was found within each fraction.

Biochemical Analysis: Membrane protein concentrations were determined according to either the method of Polacheck and Cabib (35) or to the method of Whitaker and Granum (49). RNA content was determined colorimetrically by the method of Mejbaum (32). DNA content was determined as described by Burton (8). Acid phosphatase, was assayed by the method of Tulkens et al. (45) using β-glycerophosphate as substrate. Acid proteinase using ^{14}C-protein standards from Amersham as substrate, and inosine disphosphatase were assayed according to the method of Steiger et al. (42). Oligomycin-sensitive ATPase, and glycerol-3-phosphate dehydrogenase were measured as described by Rovis and Baekkeskov (37). Adenyl cyclase activity was assayed as described by Salomon et al. (40) using cold ATP as substrate. After a 10-min incubation, the reaction was stopped by boiling the samples for 3 min. The cyclic AMP levels in the supernatant was determined using the competitive protein binding assay described by Tovey et al. (44) (provided in kit form from Amersham). In all assays 20–100 μg of membrane protein was used. Inorganic phosphate, when determined, was measured according to the method of Ames and Dubin (2).

Glycosyltransferase Assays: The transfer of *N*-acetyl-D-(^{14}C) glu-

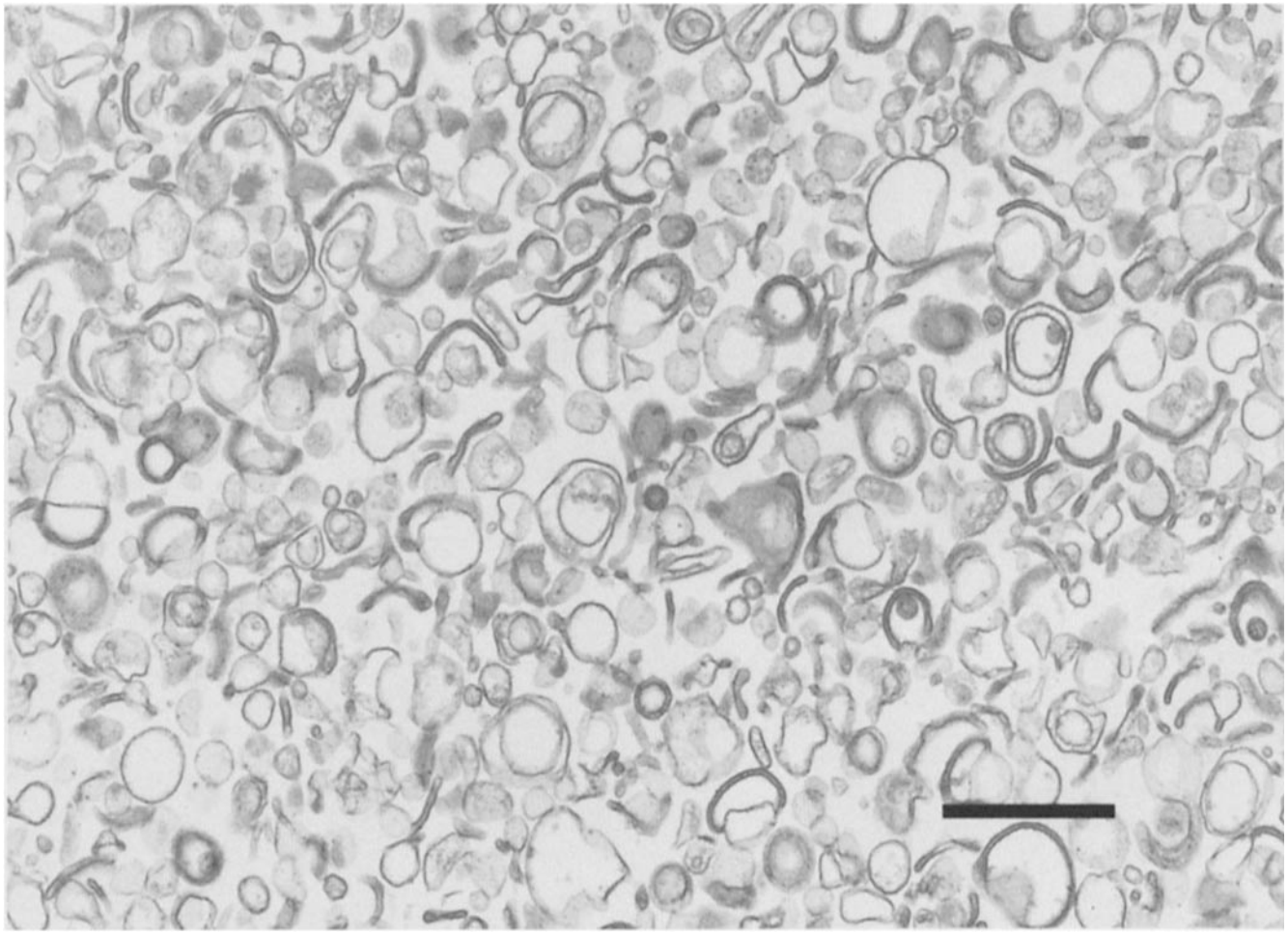

FIGURE 2 Electron micrograph of a trypanosome Golgi complex fraction (GF-3). (*T. vivax* ILDat clone 1.2). Bar, 0.5 μm. × 50,000.

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cosamine from UDP-*N*-acetyl-D-(^{14}C) glucosamine into lipid carrier was assayed in a system (39) that contained 2.8 mmol dolichol monophosphate, 0.5% Triton X-100, 50 mM Tris–HCl buffer pH 7.4, 5 mM $MnCl_2$, 0.25 μCi UDP-*N*-acetyl-D-(^{14}C) glucosamine, and 40–100 of μg membrane protein in a final volume of 200 μl. The reaction was carried out at 30°C. After 20 min, 5 ml $CHCl_3/CH_3OH$ (2:1, vol:vol) was added and the mixture was centrifuged at 500 *g* for 10 min. The extraction was repeated and the combined $CHCl_3/CH_3OH$ layers were washed once with NaCl and once with 0.9% NaCl/CH_3OH (1:0.5, vol:vol). The organic phase was transferred into scintillation vials, evaporated under N_2 gas, and counted in 10 ml of Aquasol.

The standard incubation mixture to study the incorporation of (^{14}C)GlcNAc from UDP-(^{14}C)GlcNAc into trichloroacetic acid (TCA)-insoluble endogenous acceptors (39) contained in a total volume of 200 μl: 50 mM sodium acetate buffer pH 6.5, 0.25 μCi UDP-(^{14}C)GlcNAc, 5 mM $MnCl_2$, and 100–200 μg of microsome protein. The reaction was started by the addition of membranes and carried out at 37°C. 2 ml of 10% (wt:vol) TCA were added to the mixture to terminate the reaction. The precipitates were washed and counted in 10 ml of Aquasol.

The standard assay conditions used to measure the incorporation of D-(^{14}C) mannose from GDP-D-(^{14}C) mannose into lipid carrier contained in a total volume of 200 μl of 50 mM Tris–HCl, buffer pH 7.4, 0.7 mmol dolichol monophosphate, 0.025% Triton X-100, 10 mM $MgCl_2$, 0.25 μCi GDP-D-(^{14}C) mannose, and 40–100 μg of membrane protein. After incubation for 10 min at 18°C the reaction was stopped with 5 ml of $CHCl_3/CH_3OH$ (3:2, vol:vol). The $CHCl_3/CH_3OH$ extraction was repeated and the supernatant discarded. The precipitate was washed twice with 6 ml of $CHCl_3/CH_3OH/H_2O$ (10:10:3, vol:vol:vol) and the pooled supernatants were transferred to a scintillation vial, evaporated under N_2 gas, and counted after addition of 10 ml of Aquasol.

Incorporation of D-(^{14}C) galactose from UDP-D-(^{14}C) galactose into TCA-insoluble endogenous acceptors was assayed in a reaction in volume of 200 μl containing 50 mM sodium acetate buffer pH 6.5, 5 mM $MnCl_2$, 0.25 μCi UDP-D-(^{14}C) galactose, and 40–100 μg of membrane protein. After incubating the mixture for 20 min at 37°C the reaction was terminated by the addition of ice-cold 10% TCA. The precipitates were transferred onto GF/C glass microfiber disks (Whatman Ltd., England) and washed several times with TCA followed by absolute ethanol. The paper disks were transferred into scintillation vials and counted in 10 ml of Aquasol.

RESULTS AND DISCUSSION

Electron microscopy of thin sections of the isolated subcellular fractions from *T. brucei*, *T. congolense*, and *T. vivax* were remarkably homogeneous and essentially similar. Although isolated stacks of Golgi cisternae were not seen as in rat liver fractions (Fig. 1), the Golgi complex fraction (Fig. 2), which banded at the 1.15/0.85 M sucrose interface, was predominantly smooth surface vesicles and flattened cisternae. These thin cisternae were ~20–30 nm wide and 100–500 nm long. The short profiles were relatively flat but the longer profiles were curved, U-, or cup-shaped. Some cisternae had expanded rims at one or both ends. The cisternal contents were of moderate density. The other major component of the fraction consisted of smooth surfaced vesicles 40–250 nm diam containing little or no internal density. Granules and other particles were found only rarely. Because of the nature of the starting material we only occasionally obtained the small light Golgi complex fraction. It is of interest to note that the morphology of the Golgi complex fraction was destroyed if the trypanosomes were disrupted by other methods; e.g., N_2 cavitation or freeze-thawing.

The SER (Fig. 3) fraction consisted, for the the most part,

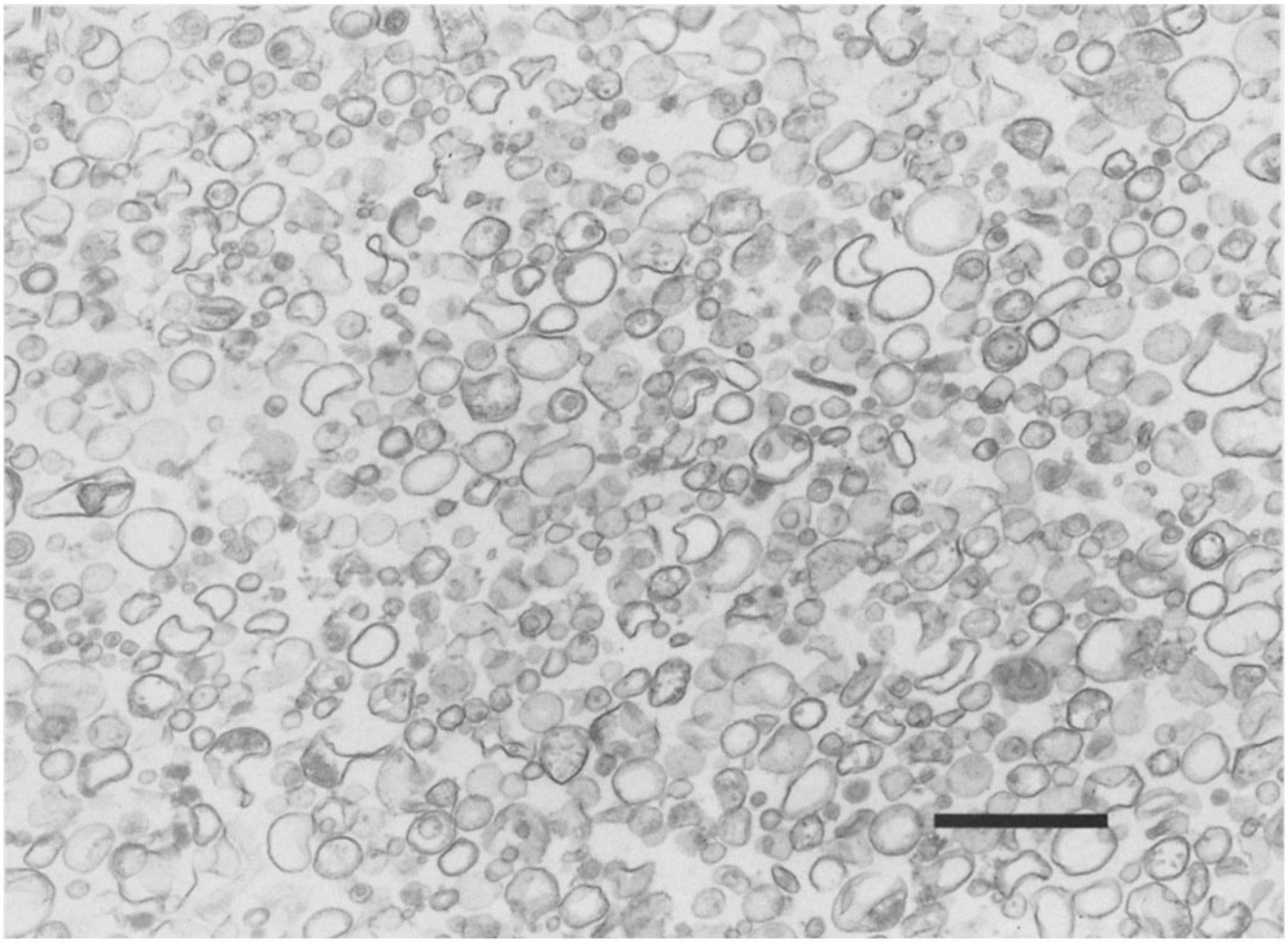

FIGURE 3 Electron micrograph of a trypanosome SER fraction (*T. vivax* ILDnat clone 1.2). Bar, 0.5 μm. × 50,000.

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of clear agranular vesicles 30–200 nm diam. Some vesicles contained flocculent material of moderate density. Vesicles with attached ribosomes and flattened Golgi saccules were very rare. As with any other centrifugally prepared SER fraction, it is difficult to ascertain the ratio of ER to other vesiculated fragments of other membranous organelles. The RER fraction (Fig. 4) was classic in its morphology in that it contained spherical vesicles and cisternal profiles with attached ribosomes. Clusters of free ribosomes were also present, as well as fragmented microtubules and small amounts of other nonmembrane material. This fraction also contained ~4.2 and 12.8 times more RNA than either the SER or Golgi complex fractions, respectively (Table I). All the fractions were free of any detectable DNA demonstrating no nuclear contamination (Table I).

Many "classic" eucaryotic marker enzymes are known to be totally absent or represented in extremely low levels in African trypanosomes (37, 42). It has also been found that

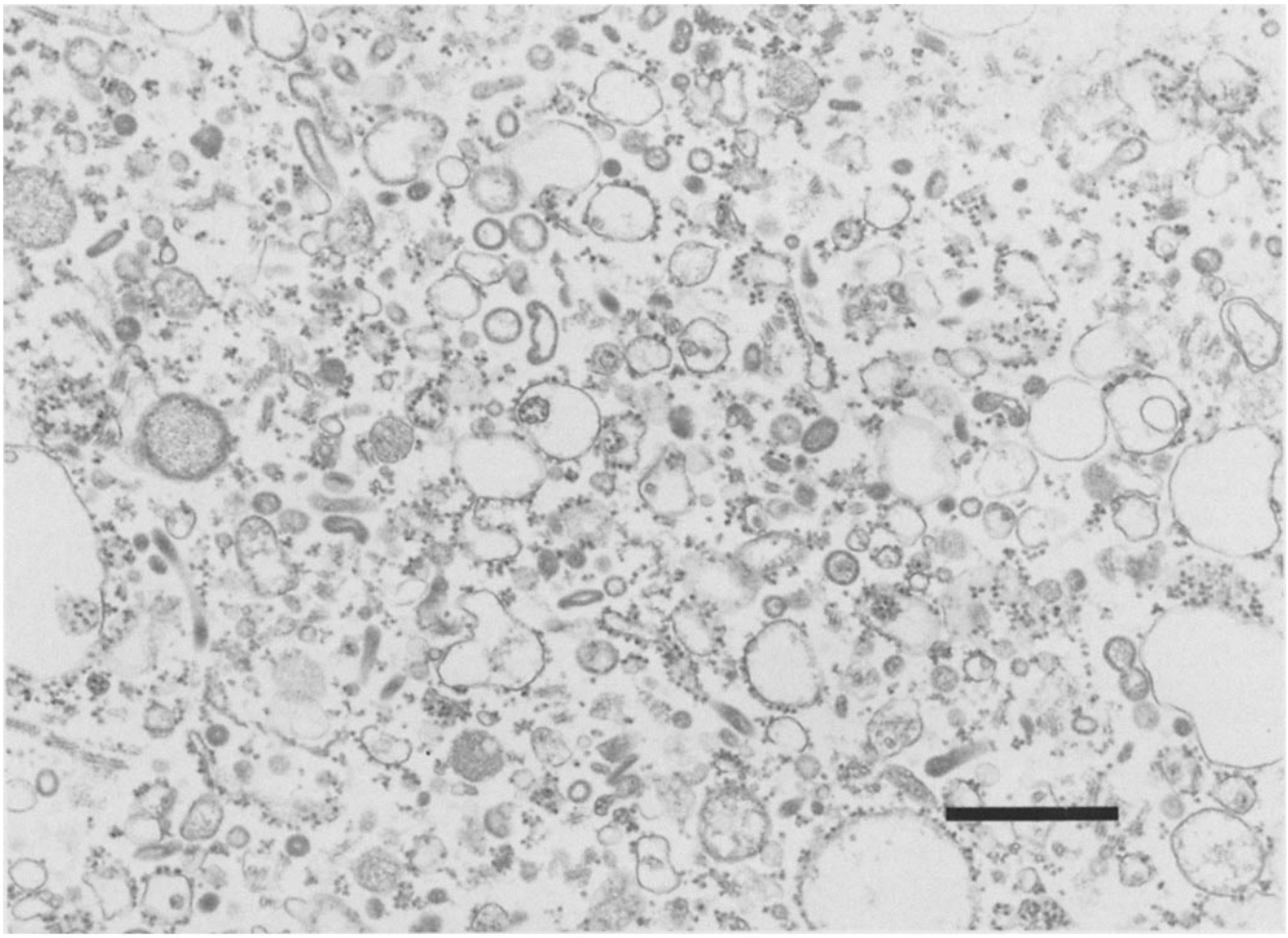

FIGURE 4 Electron micrograph of a trypanosome RER fraction (*T. brucei* MITat clone 1.2). Bar, 0.5 μm. × 50,000.

TABLE I

Biochemical Analysis at MITaT 1.2 Membrane Fractions

Composition	*n*	Fraction				
		Homogenate‡	15,000 *g* pellet	RER	SER	Golgi
Enzyme*						
Acid phosphatase	4	1.0 (27)	3.1	0.5	1.6	6.0
Inosine diphosphatase	2	1.0 (13)	1.6	2.0	2.0	1.4
Glycerol-3-phosphate dehydrogenase	2	1.0 (121)	2.3	1.4	0.6	0.2
Oligomycin-sensitive ATPase	2	1.0 (11.4)	1.0	1.9	1.2	1.0
Adenylate cyclase	4	1.0 (0.2)	3.3	4.2	8.2	7.8
Acid proteinase	4	1.0	1.6	0.2	0.2	0.1
Nucleic acid content§						
RNA	4	57	38	230	55	18
DNA	3	28	120	0	3	0

* Specific activities are expressed as relative to total homogenate. Mean of duplicate determinations from two or more different membrane preparations.
‡ Numbers in parentheses indicate actual specific activity expressed as nanomole of product formed or P_i released per minute per milligram of proten. Acid proteinase activity is expressed as increase in TCA-soluble radiolabeled product relative to homogenate.
§ Microgram of nucleic acid per milligram protein.

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the activities and subcellular distribution of several glycolytic enzymes in trypanosomes can be altered depending on the mode of disruption (33). Although there are virtually no data in the literature describing what the distribution of certain enzymes should be in isolated RER, SER, and Golgi complex subfractions we have tested some potential marker enzymes which have been described for other trypanosome subcellular fractions (34, 37, 40, 42, 45) including crude microsomes (33, 37, 42). We have looked at the distribution of acid proteinase, inosine diphosphatase, oligomycin-sensitive ATPase, acid phosphatase, glycerol-3-phosphate dehydrogenase, and adenylate cyclase (Table I). Acid phosphatase, an enzyme activity which in *T. brucei* has been localized in lysosomelike structures, in the flagellar pocket, as well as in cisternae and vesicles at the Golgi apparatus (25), was also higher in the Golgi complex fraction then in either ER fraction (Table I). High activity was also found in the 15,000 *g* pellet fraction. The specific activity of inosine diphosphatase, a putative ER marker in trypanosomes (42), was highest in both ER fractions followed by the Golgi, whereas the levels of adenylate cyclase, a trypanosomal membrane marker (37), was eightfold higher in both the SER and Golgi complex fractions. The RER fraction displayed a fourfold increase in activity over the homogenate value. Insofar as the data can be compared, our findings are in agreement with those described by Rovis and Baekkeskov (37). They found that the specific activity for adenylate cyclase was some fourfold higher in a total microsome fraction isolated from *T. brucei* than in total cells, however, this activity is dramatically lower than the 20–26-fold increase in specific activity observed when a highly purified plasma membrane preparation is used as the source of enzymatic activity (37). Walter and Opperdoes (48) recently described an adenylate cyclase activity in the flagellar pocket; however, they could not exclude the possibility that the enzyme is associated with either the endoplasmic reticulum or Golgi. The specific activity of oligomycin-sensitive ATPase was highest in the RER fraction, whereas the activity of glycerol-3-phosphate dehydrogenase, was highest in the 15,000 *g* pellet fraction followed by the RER. Data for both enzymes are again in agreement with those published by Rovis and Baekkeskov (37). Low levels of acid proteinase in all our microsome fractions indicate that these fractions were relatively free of lysosomal elements.

As seen in Tables II–IV, the relative distributions of glycosyltransferase activities in the several subcellular microsomal fractions obtained from *T. brucei*, *T. congolense*, and *T. vivax* were remarkably similar. It should be noted that to ascertain its nature and purity before use in the assays, every membrane preparation was examined by electron microscopy. As seen in Table II, the Golgi complex fraction (consisting of predominantly heavy Golgi elements) contained the majority of the galactosyltransferase activity, which is considered one of the most reliable Golgi markers (1, 15), and this activity was significantly lower in the ER fractions. It is of interest that in rat liver, Bergeron et al. (6) also found that galactose transfer to endogenous acceptors occurred within heavy, intermediate, and intact Golgi complex fractions. The enzymatic reaction as optimized for total MITat 1.52 and 1.2 *T. brucei* microsomes (prepared as described in reference 37), had a pH optimum of 6.5, required Mn^{+2} and this cation could not be replaced by Mg^{+2}. The reaction rate was linear using up to 200 μg of microsomal protein (the highest amount tested). In addition, the K_m was 2.4 μM. The reaction was also not inhibited by the antibiotic tunicamycin and immunoprecipitation with monospecific antisera revealed that VSG was also heavily labeled. The highest activity for dolichol-dependent mannosyltransferase, was consistently found in RER followed

TABLE II

Transfer of UDP-(U-^{14}C) Galactose into Endogenous Acceptors

Clone	Enzyme activity*			
	Microsomes	Golgi	SER	RER
	pmol/mg of protein			
T. brucei MITat 1.2	43	140	68	26
T. brucei MITat 1.7	49	227	34	17
T. brucei MITat 1.52	55	79	39	24
T. congolense ILNat 2.1	36	129	38	23
T. vivax ILDat 1.2	ND	89	28	14
	47	352	97	31
	16	541	52	24
	26	513	129	123

ND, not determined.

* Specific activities shown are the average of at least two separate determinations from different membrane preparations.

TABLE III

Transfer of GDP-(U-^{14}C) Mannose into Lipid Carrier

Clone	Enzyme activity*			
	Microsomes	Golgi	SER	RER
	pmol/mg of protein			
T. brucei MITat 1.2	1,320	610	920	2,760
T. brucei MITat 1.7	1,560	1,260	1,770	3,050
T. brucei MITat 1.52	2,800	550	1,090	1,900
T. congolense ILNat	ND	510	1,080	2,440
T. vivax ILDat 1.2	1,070	530	1,740	5,430
	820	410	870	1,160
	680	430	750	1,100

ND, not determined.

* Specific activities shown are the average of at least two separate determinations from different membrane preparations.

TABLE IV

Dolichol-independent Transfer of N-Acetyl-(U-^{14}C) Glucosamine into Endogenous Acceptors

Clone	Enzyme activity*			
	Microsomes	Golgi	SER	RER
	pmol/mg of protein			
T. brucei MITat 1.2	420	390	460	500
T. brucei MITat 1.7	320	330	370	360
T. vivax ILDat 1.2	ND	315	490	480
	390	240	410	380

ND, not determined.

* Specific activities shown are the average of at least two separate determinations from different membrane preparations.

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by the SER and Golgi complex fractions (Table III).

N-acetylglucosaminyltransferase activities have been described in relatively crude membrane fractions obtained from *T. brucei* organisms. Brett and Voorheis (7) were able to detect a dolichol-independent form of this enzymatic activity in a plasma membrane fraction. However, Rovis and Dube (39) recently characterized a dolichol-dependent as well as a dolichol-independent form of this enzymatic activity in total microsomal membrane preparations. This enzymatic activity requires Mn^{2+}, is time and temperature dependent, and has an optimum pH of 7.4, and exogenous dolichol monophosphate enhances the glycosyltransferase activity (25). The kinetics of incorporation are characterized by a K_m of 1.45 μM for dolichol monophosphate and 2.6 μM for UDP-*N*-acetylglucosamine (39). We find that the dolichol-independent form of the *N*-acetylglucosaminyltransferase in trypanosomes is equally distributed in all our fractions (Table IV). We also find a dolichol-dependent *N*-acetylglucosaminyltransferase activity (Table V) which is higher in the ER fractions than in the Golgi fraction for all three species of African trypanosome.

Several groups have reported the specific inhibition of the first enzyme of the dolichol cycle by the antibiotic tunicamycin (14, 24, 27). Core glycosylation of VSG of *T. brucei* has been shown to be inhibited by crude tunicamycin preparations both in vivo (31, 38, 43) and in vitro (39). However, it has been demonstrated that crude tunicamycin preparations contain at least 10 closely related compounds (27, 28). Some of these are also potent inhibitors of protein synthesis, and the biological activities of most of the others have not been fully determined (27, 28). We have therefore chosen to study the effects of a single high-performance liquid chromatography–purified tunicamycin derivative (tunicamycin A_1) known to have a strong inhibitory effect on *N*-acetylglucosamine-1-phosphate transferase while exhibiting a minimal effect on protein synthesis (27, 28). From the data in Table VI, and from the inhibition curve shown in Fig. 5, one can now state that the tunicamycin A_1 sensitive form of this enzyme, previously detected in crude microsome preparation (39), can now be assigned more specifically to the SER subfraction. It now appears that there are at least two forms of the dolichol-dependent *N*-acetylglucosamine-1-phosphate transferase in the African trypanosomes, one which is tunicamycin resistant residing in the RER and the other enzyme, sensitive to the effects of tunicamycin, residing in the SER. This would also

TABLE V

Transfer of N-Acetyl-(U-^{14}C) Glucosamine into Dolichol Monophosphate

Clone	Enzyme activity*			
	Microsomes	Golgi	SER	RER
	pmol/mg of protein			
T. brucei MITat 1.2	390	230	730	570
T. brucei MITat 1.7	440	380	1,400	620
T. brucei MITat 1.52	460	320	700	680
T. congolense ILNat 2.1	220	280	640	500
T. vivax ILDat 1.2	ND	190	500	310
	410	220	640	390

ND, not determined.

* Specific activities shown are the average of at least two separate determinations from different membrane preparations.

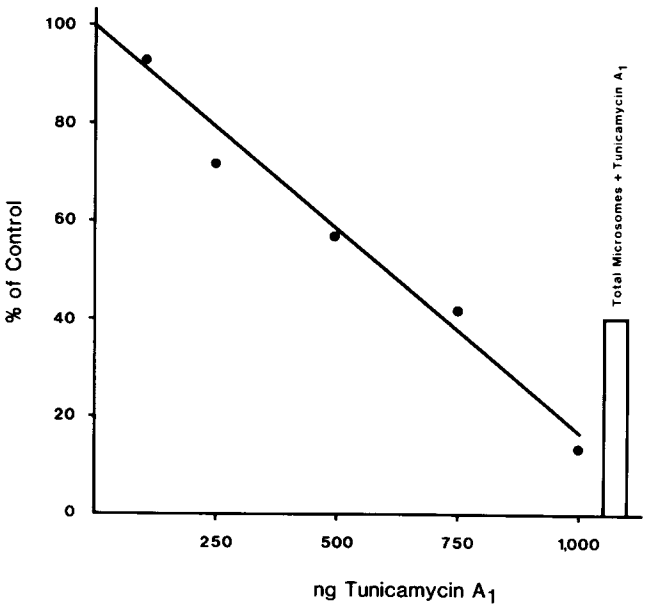

FIGURE 5 Tunicamycin inhibition of dolichol-dependent *N*-acetylglucosaminyltransferase activity in SER membranes isolated from *T. brucei* MITat 1.2. Membranes containing approximately 350 enzyme units of *N*-acetylglucosaminyltransferase activity were incubated with dolichol-phosphate with various concentrations of tunicamycin A_1 (0–1,000 ng). Each point represents the average of two to four determinations. The method of least squares was used to derive the linear regression coefficient (r). For these data $r = 0.9850$. Total microsomes (bar) were incubated with 1,000 ng tunicamycin A_1.

TABLE VI

Inhibition of Transfer of UDP-N-(U-^{14}C) Acetylglucosamine into Lipid Carrier by Tunicamycin

Clone	Enzyme activity*											
	Microsomes			Golgi			SER			RER		
	−TM	+TM	%I‡	−TM	+TM	%I	−TM	+TM	%I	−TM	+TM	%I
	pmol/mg of protein											
T. brucei MITat 1.2	456	382	17	410	428	0	498	358	38	436	424	3
	410	330	20	350	420	0	440	250	43	410	400	2
	308	250	19	272	285	0	345	230	33	293	282	4
T. brucei MITat 1.7				170	170	0	900	580	35	450	440	2
				320	310	3	870	690	21	630	620	1

* Specific activities shown are the average of at least two separate determinations from different membrane preparations.

‡ % Inhibition by tunicamycin (*TM*) was determined after preincubation of 50 μg of membrane protein with 500 ng of tunicamycin A_1.

suggest that VSGs of trypanosomes may have some if not all their internally localized *N*-glyosidically linked carbohydrate side chains assembled at the level of the SER and not the RER as previously assumed (29, 31, 39). This last point has also been verified by immunohistochemistry (L. Jenni, Swiss Tropical Institute; personal communication). Thus, we add yet another enzymatic activity in trypanosomes which does not follow in the pattern of higher eucaryotes.

The data presented in this manuscript provide further insights into the pathway of protein glycosylation in African trypanosomes. The relative distribution of glycosyltransferase activities and certain other enzymatic activities is described here for the first time in electron microscopically identified Golgi complex and ER fractions of these organisms. The findings suggest that the internal core oligosaccharides in VSG may in fact be added at the level of the smooth or transitional ER. In an attempt to clarify these events further we have been conducting pulse-chase experiments to determine the intracellular pathway of newly formed trypanosomal protein especially of VSG. Preliminary results suggest that VSG is synthesized in the RER, a process that takes 8 min, is shuttled to the Golgi complex fraction 8 min later (Grab, D. J., and Y. Verjee, manuscript in preparation). It will be of much interest to determine whether all the CRD is added on the VSG molecule in one organelle or serially in several organelles. For example, is the phosphatidylethanolamine moiety described by Duvillier et al. (13) put on in the ER with the final maturation steps being carried out the level of the Golgi apparatus? However, we have recently found on frozen sections that affinity-purified anti-CRD IgG appears to localize intracellularly in the *trans*-Golgi region (Grab, D. J., and P. Webster, manuscript in preparation). Future studies utilizing these membrane fractions will help clarify further the glycosylation pattern of VSG and, we hope, shed some light onto possible areas for potential chemotherapeutic intervention.

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