Purification, Morphometric Analysis, and Characterization of the Glycosomes (Microbodies) of the Protozoan hemoflagellate *Trypanosoma brucei*

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ABSTRACT *Trypanosoma brucei* glycosomes (microbodies containing nine enzymes involved in glycolysis) have been purified to near homogeneity from bloodstream-form trypomastigotes for the purpose of morphologic and biochemical analysis. Differential centrifugation followed by two isopycnic centrifugations in an isotonic Percoll and in a sucrose gradient, respectively, resulted in 12- to 13-fold purified glycosomes with an overall yield of 31%. These glycosomes appeared to be highly pure and contained <1% mitochondrial contamination as judged by morphometric and biochemical analyses. In intact cells, glycosomes displayed a remarkably homogeneous size distribution centered on an average diameter of 0.27 μm with a standard deviation of 0.03 μm. The size distribution of isolated glycosomes differed only slightly from that measured in intact cells. One *T. brucei* cell contained on average 230 glycosomes, representing 4.3% of the total cell volume. The glycosomes were surrounded by a single membrane and contained as phospholipids only phosphatidyl choline and phosphatidyl ethanolamine in a ratio of 2:1. The purified glycosomal fraction had a very low DNA content of 0.18 μg/mg protein. No DNA molecules were observed that could not have been derived from contaminating mitochondrial or nuclear debris.

In contrast to the localization of glycolytic enzymes in mammalian and other eukaryotic cells, those of the Trypanosomatidae are found mainly in a microbody-like organelle, the "glycosome" (1). Since the discovery of these organelles in *Trypanosoma brucei* (1), they have been found in all major representatives of this family (i.e., *T. cruzi* (2), *Leishmania mexicana* (3) and several *Crithidia* spp. (2, 4–6)). The glycosomes in *T. brucei* contain at least nine enzymes involved in or related to glycolysis, which can account for the conversion of glucose to 3-phosphoglycerate plus glycerol. They are uniquely located inside the glycosomes and therefore exhibit high latency in cell-free extracts (1, 7). These organelles also contain part of the adenylate kinase activity (8), two enzymes of pyrimidine biosynthesis (9), and, in insect forms (cultured procyclics), malate dehydrogenase (8, 10) and phosphoenolpyruvate carboxykinase (10). Glycosomes must therefore perform other important metabolic functions in addition to glycolysis.

Morphologically, glycosomes resemble the microbodies present in other eukaryotic cells. They have in general a diameter between 0.2 and 0.8 μm, appear in sections as round or oval-shaped, and are surrounded by a single membrane. The presence of a crystalline nucleoid has frequently been reported (11, 12). Apart from the demonstration of catalase in two representatives of the family of the Trypanosomatidae, i.e., *Crithidia* spp. (13) and *Leptomonas samueli* (14), no other enzymes typical of peroxisomes or the glyoxysomes of plants have been found in the microbody fraction of these protozoan hemoflagellates (12, 15). This has raised two questions: First, what is the relation between the peroxisomes found in the animal and plant kingdoms on the one hand and the glycosomes of the protozoan Trypanosomatidae on

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the other hand (16)? Second, if there is no such relation, do Trypanosomatidae contain more than one class of microbodies (e.g., glycosomes and peroxisomes)?

In this paper we describe the relative contribution of the glycosomes of *T. brucei* to total protein content and cell volume and we show that they constitute a single homogeneous population of organelles. We further describe a procedure for the isolation of highly purified glycosomes and some characteristics of such an organelle preparation.

MATERIALS AND METHODS

Growth and Isolation of the Organism: Bloodstream forms of *Trypanosoma brucei* stock 427 were grown in 300-g Wistar rats (17, 18). Blood was collected from animals showing high parasitaemias (usually 3–4 d after infection) by cardiac puncture under ether anaesthesia. Trypanosomes were removed from erythrocytes and other blood constituents by passage through a DEAE-cellulose column according to the method of Lanham (19). Cells were washed three times in a buffer containing 0.25 M sucrose, 25 mM Tris-HCl, 1 mM EDTA (pH 7.8) by centrifugation at 3,000 rpm (1,000 *g*) for 5 min in the SS-34 rotor of a Sorvall RC-5 centrifuge (E. I. DuPont de Nemours & Co., Inc., Sorvall Instruments Div., Newtown, CT) at 4°C.

Purification of Glycosomes: Washed cell pellets were disrupted by being ground with silicon carbide (20) in a disruption buffer containing 0.25 M sucrose, 25 mM Tris-HCl, 1 mM EDTA (pH 7.8). After centrifugation at 3,000 rpm (1,000 *g*) for 10 min, the supernatant was mixed with an equal volume of 80% Percoll containing 0.25 M sucrose, 25 mM Tris-HCl, and 1 mM EDTA, pH 7.2, to give a final Percoll concentration of 40%. After centrifugation at 27,000 rpm for 30 min in a vertical VTi-50 rotor of a Beckman L5-50 centrifuge (Beckman Instruments, Inc., Palo Alto, CA), the lower band enriched in glycosomes, equilibrating at 1.09 g/cm^3, was removed after the side of the tube was punctured with a syringe (21). In some experiments this was used as a glycosome-enriched fraction. A further purification was obtained by layering this suspension on a linear 0.4–2.7 M sucrose gradient containing 25 mM Tris-HCl, pH 7.2, and 1 mM EDTA followed by centrifugation at 49,000 rpm for 90 min in the vertical VTi-50 rotor (8). The highly purified glycosomes, which equilibrated at a density of 1.23 g/cm^3, were removed after the side of the tube was punctured with a syringe.

Separation of Organelle Membrane and Content: Membrane and content of organelles were separated from each other as described by Fujiki and co-workers (22, 23). Glycosomal fractions were diluted 100-fold with ice-cold 100 mM sodium carbonate, pH 11.5, kept on ice for 30 min, and centrifuged for 60 min in a Beckman 50 Ti rotor at 50,000 rpm to separate the matrix from the membrane.

Extraction of Phospholipids: A highly purified glycosomal preparation (prepared after sequential Percoll and sucrose density-gradient centrifugation), was extracted with a chloroform/methanol (2:1) mixture essentially as described previously (24). To 1 vol of suspension, 19 vol of solvent mixture was added and vigorously mixed. 1 vol of 0.05 M KCl was added to 5 vol of mixture, and the solution was thoroughly mixed and then allowed to separate. The upper aqueous layer and the proteinaceous interphase were discarded. An aliquot of the lower layer was evaporated to dryness under vacuum at 30°C and the residue was resuspended to a known volume in chloroform-methanol (2:1).

Phospholipid Analysis: Separation of phospholipids was performed by two dimensional thin-layer chromatography using precoated silica sheets (Merck 5375, Merck AG, Darmstadt, Federal Republic of Germany). The solvent system for the first dimension was chloroform/methanol/acetic acid/H_2O (55:45:10:2) and for the second dimension chloroform/methanol/acetone/acetic acid/H_2O (45:15:16:15:6). After separation and drying, phospholipids were visualized by being sprayed with bromothymol blue (0.05% plus 0.2% citric acid) or an iodine solution before quantification by phosphate analysis. Individual phospholipids were identified by running standards (mixed with trypanosomal extracts). Phospholipids were scraped off (together with equal areas of silica that did not contain phospholipid for blanks), eluted from the silica in 0.4 ml, 60% perchloric acid, and heated to 210°C for 30 min in an oil bath. After mineralization, inorganic phosphate was estimated by the method of Fiske and Subbarow as described in reference 25.

Extraction, Quantification, and Length Determination of DNA: Purified glycosomes were lysed by mixing 2.5 vol of glycosomal suspension in 50% sucrose with 1 vol of lysis mix containing 0.55 M NaCl, 366 mM EDTA, pH 8.0, 3.6% Sarkosyl and pronase (Sigma Chemical Co., St. Louis, MO) at 0.7 mg/ml. The lysate was incubated at 37°C for 2 h under slow stirring, followed by chloroform/phenol extraction and alcohol precipitation. The DNA pellet was resuspended in 10 mM NaCl, 1 mM EDTA, pH 7.5, before further analysis. The amount of DNA in this deproteinized extract was determined by the DAPI (4′,6-diamidino-2-phenylindole) method as described by Brunk et al. (26) using a Perkin-Elmer fluorescence spectrophotometer model 1000 (Perkin-Elmer Corp., Norwalk, CT) and T4 DNA as internal standard.

Electron Microscopy and Morphometry: Fixation of intact trypanosomes was performed in 1% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, and 4% sucrose. After 30 min, 0.5 ml of suspension (0.7×10^9 cells) was filtered on a Millipore® filter (Millipore Co., Bedford, MA) with 0.1-μm pores, according to Baudhuin et al. (27). Postfixation was performed in a solution containing 1% OsO_4 and 1% ferrocyanide in cacodylate buffer. Pellicles were stained with 1% uranyl acetate in 0.03 M veronal acetate/HCl buffer, pH 6, containing 4% sucrose. Samples were then dehydrated and, after dissolution of the filter in propylene oxide, embedded in the epoxy resin mixture described by Spurr (28). Preparation of isolated glycosomes followed the same procedure, except that fixation was performed as described by Leighton et al. (29), in order to prevent osmotic lysis of the particles. Sections were cut with an LKB ultratome III or a Reichert Om U2 ultramicrotome, equipped with a diamond knife. Sections were stained first with 3% uranyl acetate and then with lead citrate (30) and examined with a Philips EM 301 microscope.

For morphometry, each section photographed was taken from a different ribbon, separated from the preceding one by at least 10 μm to avoid analysis of several sections through the same particles. The final magnification was measured accurately by using a grating replica (E. F. Fullam Inc., Schenectady, NY), which was photographed and processed under the same conditions as the micrographs of the sections. Size-distribution analysis was performed according to Wicksell (31), using a Quantimet 720 (Cambridge Instruments, Cambridge, UK) on line with a PDP 11/10 computer (Digital Equipment, Galway, Ireland), as described by Baudhuin et al. (32). A 40-nm thickness of sections was assumed in the calculations.

The DNA extracted from a glycosomal preparation was spread for electron microscopy by the protein monolayer technique of Kleinschmidt (33). The kinetoplast DNA minicircles, present on the same grid as the molecules measured, were used as an internal length standard of 0.32 μm (34).

Other Procedures: Cell volume was determined by [^{3}H]inulin exclusion in buffer used for fixing the cells for electron microscopy, but without glutaraldehyde, essentially as described by Damper and Patton (35).

Activities of *sn*-glycerol-3-phosphate oxidase (36) and glycolytic enzymes (1) were determined as described previously. Protein was measured by the fluorescamine method (37) using BSA as standard.

Materials: Percoll was obtained from Pharmacia Inc. (Uppsala, Sweden). Glycolytic enzymes, substrates, and co-factors were from Boehringer GmbH (Mannheim, Federal Republic of Germany). All other chemicals were of analytical grade.

RESULTS

Glycosomes In Situ

The general appearance of the glycosomes of the bloodstream form of *T. brucei* is illustrated in Fig. 1. The experimental size distribution of profiles and the particle-size distribution derived by Wicksell's (31) transformation are presented in Fig. 2*A*. Although the calculation shows that ~30% of the profiles corresponding to the smaller elements of the distribution were not recognized, the particle-size distribution is rather symmetrical, suggesting that particles with a radius <0.114 μm are scarce. Hence, unidentified profiles most likely represent polar sections through particles with a larger diameter and can be corrected for by computation. The particle-size distribution is fairly homogeneous, with ~75% of the particles having a radius between 0.120 and 0.156 μm.

The average parameters of the glycosome population of *T. brucei* are summarized in Table I. The contribution of glycosomes to the total cell volume is noteworthy; together they represent 4.29% ± 0.33 (mean ± SD) of the volume of the cell. Very few particles exhibited a dense core; when present this core displayed a multilamellar appearance with a spacing of ~10 nm (Fig. 1*B*). The glycosomes were bounded by a single membrane with a thickness similar to that of the membranes of endoplasmic reticulum or mitochondrion. We

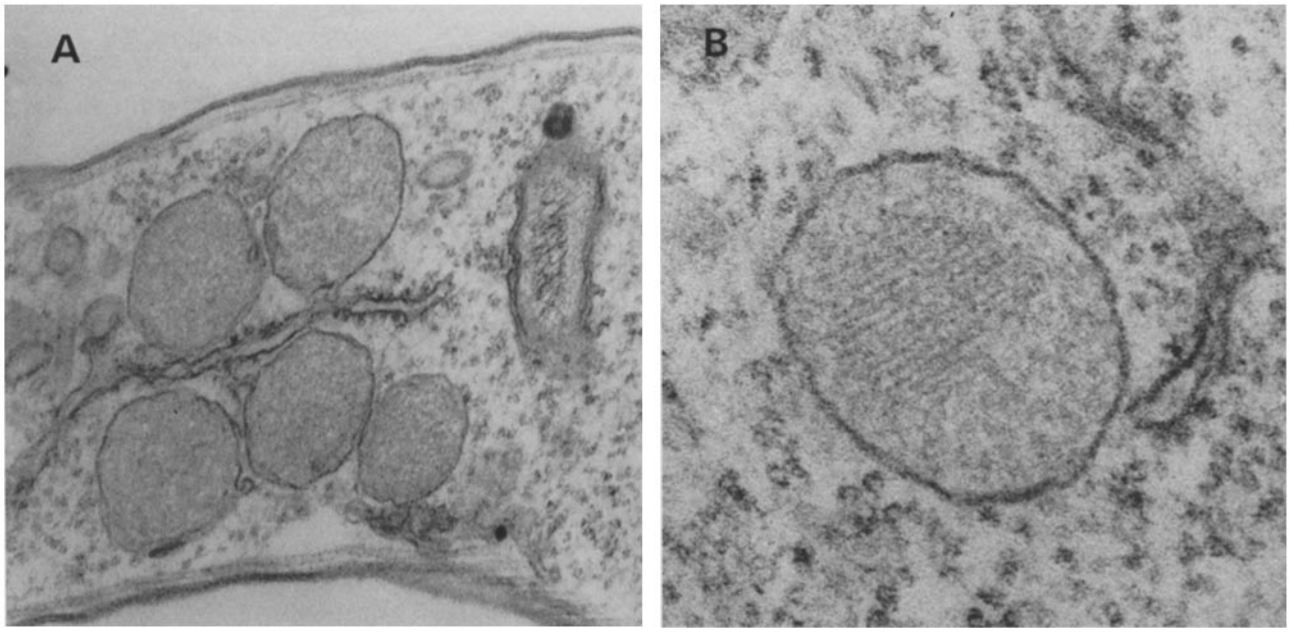

FIGURE 1 Glycosomes in situ (*A*) Cluster of five glycosomes in the vicinity of the rough endoplasmic reticulum and of the kinetoplast region of the mitochondrion containing DNA fibers. (*B*) Example of glycosome in situ with a dense core displaying a lamellar structure. (*A*) × 59,000; (*B*) × 172,000.

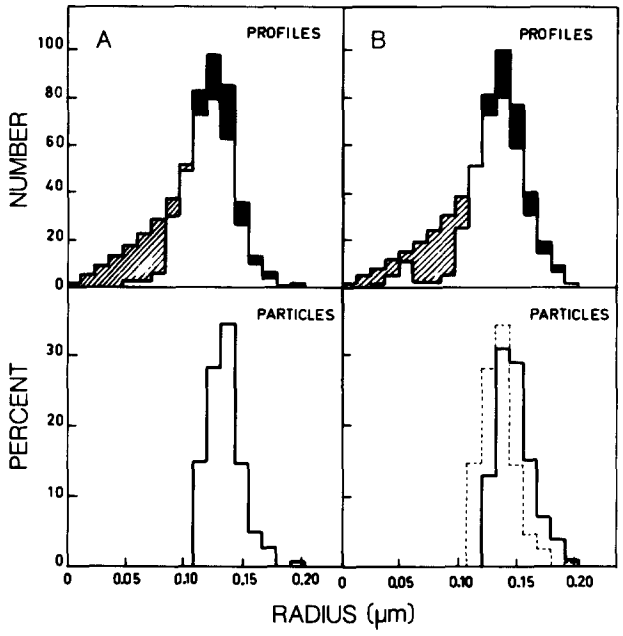

FIGURE 2 Size distribution of glycosomes in intact *T. brucei* (*A*) and of isolated glycosomes (*B*). In the upper graph the black area represents a correction for section thickness (0.04 μm) which is substracted from the experimental profile size distribution. The hatched area represents a correction for unidentified polar profiles. This correction is a minimal evaluation, since it assumes that no equatorial sections through particles were missed. The lower graph gives the corresponding computed particle-size distribution. In *B*, the size distribution of the particles as measured in intact cells has been superimposed (broken line in lower graph) for comparison.

have measured a cell volume of 58 μm^3 for our *T. brucei* stock (see Materials and Methods) which is smaller than the 81 μm^3 found by Damper and Patton (35) for another *T. brucei* stock. Using our value of 58 μm^3 it can be calculated from the data of Table I that each *T. brucei* cell contains on average 230 glycosomes.

TABLE I

Characterization of the Glycosome Population In Situ

Parameter	Mean value
Radius (μm)	0.135
Membrane area (μm^2)	0.233
Volume (μm^3)	0.0108
Number of particles per unit cellular volume (μm^{-3})	3.97
Total membrane area per unit cellular volume (μm^{-1})	0.925
Volume fraction	0.0429

344 profiles, occurring in a total area of 346 μm^2 of sections throughout *T. brucei*, were measured.

Purification of Glycosomes

The results of a typical glycosome purification experiment are shown in Table II. The first six enzymes of the glycolytic pathway and two enzymes associated with it all co-purified in an identical fashion. They were only two- to threefold purified in the glycosomal band taken from the Percoll gradient owing to the presence of soluble protein (21). Removal of Percoll particles and soluble protein by sucrose-gradient equilibration resulted in an average purification of ninefold; the overall yield was 31% (average value of the eight above mentioned enzymes). The data for aldolase are not shown since this enzyme was activated during purification owing to the removal of an endogenous inhibitor. In the course of three purification experiments, some variation in specific activities of the enzymes in the starting homogenates was observed. The reason for this is not clear, but such variations in specific activities did not seriously affect the overall purification factor which ranged for hexokinase between 9- and 15-fold with a mean of 12.8 and for phosphoglucose isomerase between 8- and 14-fold with a mean of 11.5. If it is assumed that these preparations were pure (see below), their relative specific activity with respect to the homogenate is the inverse of the protein fraction associated with glycosomes. Hence these or-

TABLE II

Purification of Glycosomes

Component	Homogenate units	Percoll fraction RSA	Purified glycosomal fraction RSA	Purified glycosomal fraction Yield
				%
Protein	283*	—	—	3.5
Hexokinase	0.66	3.21	9.91	35
Phosphoglucose isomerase	1.06	3.00	8.36	29
Phosphofructokinase	0.81	2.37	8.31	29
Triosephosphate isomerase	0.92	3.14	7.71	27
sn-Glycerol-3-P dehydrogenase	0.59	2.91	8.20	29
Glycerol kinase	0.88	3.88	12.2	43
Glyceraldehyde-P dehydrogenase	0.15	1.87	10.2	36
Phosphoglycerate kinase	0.88	2.05	5.42	19
sn-Glycerol-3-P oxidase	0.054	0.22	<0.1	<0.26
DNA	10‡	—	0.018	—

Units are expressed as micromoles of reaction product formed per milligram of cell protein and per minute, except for total protein and DNA content of the homogenate. Relative specific activities (*RSA*) are with respect to the homogenate, taken as unity.

* Total amount of protein in milligrams.

‡ Expressed as μg DNA/mg protein. This value was calculated from Borst et al. (38).

ganelles represent between 7.8 and 8.7% of the cell protein.

sn-Glycerol-3-phosphate oxidase, involved in the cyanide-insensitive respiration of the trypanosome, was included as a marker of mitochondrial contamination (4). Table II indicates that the purified glycosomes were contaminated by <1% with mitochondria.

For all further experiments we have selected two glycosomal preparations that had the highest specific activities in hexokinase and phosphoglucose isomerase and the highest purification factors (13- and 14-fold, respectively).

Morphology and Morphometry of Isolated Glycosomes

The appearance of a glycosomal fraction taken from a sucrose gradient and fixed as described in Materials and Methods is shown in Fig. 3. The fraction contained almost exclusively single membrane-bounded microbody-like organelles of spherical shape with diameters extending from 0.25–0.50 μm. Very few kinetoplasts (one for every 400 glycosomes) and occasional membrane fragments were observed. Numerous dense particles (~25 nm diam) were also present; these were identified as Percoll particles by direct examination of a suspension of 10% Percoll (not shown). Apparently the Percoll particles stemming from the Percoll gradient are not entirely removed during density equilibration in the sucrose gradient.

A few profiles displayed a denser core typical of the one

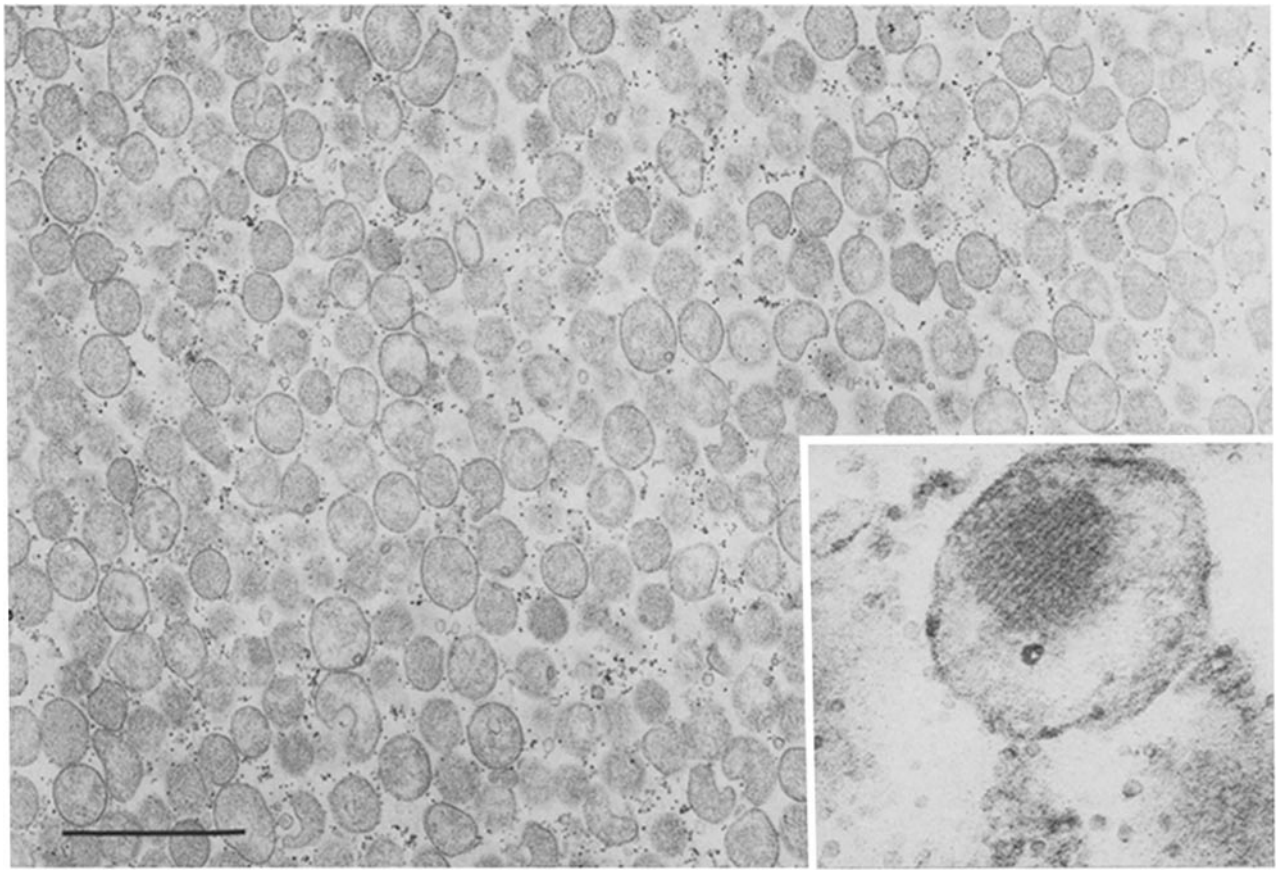

FIGURE 3 Electron micrograph of purified glycosomes from a sucrose gradient. *Inset* shows an isolated glycosome with a crystalloid core. Bar, 0.5 μm. × 48,000.

observed in peroxisomes, and a tubular or lamellar component could sometimes be seen in the core. The frequency of cores in the purified fractions, where glycosomes were kept as intact as possible, was extremely low (<1%), but appeared to be higher in fractions in which glycosomes were deliberately damaged by freezing and thawing before fixation (not shown). As mentioned above, the frequency of cores in the glycosomes of intact cells was also low and probably not significantly different from fractions not frozen and thawed.

Fig. 2*B* shows the size distribution of the glycosomal profiles in a purified preparation. The size distribution of the particles was rather homogeneous with an average radius only slightly larger than that of the organelles in situ (0.149 versus 0.135 μm). Since the glycosomes in this specific preparation were purified with an overall recovery of 28%, the size homogeneity of the purified organelles could have resulted from a selection of a specific class of microbodies present in the intact cell during the isolation procedure. However, when the size distribution of the microbodies present in intact cells was compared with that of purified glycosomes (Fig. 2) the distribution differed only by a displacement of 0.012 μm in radius. This could easily be the result of small changes in volume due to the isolation medium and does not suggest a selection of a specific class of microbodies.

Phospholipid Analysis

Bloodstream trypanosomes contained the phospholipids sphingomyelin, phosphatidyl choline, and phosphatidyl ethanolamine, in approximately equal ratios, which together accounted for ~85% of the total phospholipids identified (Table III). Phosphatidyl inositol and phosphatidyl serine were not well separated after bromothymol blue or iodine staining and accounted for the remainder of those recovered from whole organisms. Highly purified glycosomes contained only two major phospholipids: phosphatidyl choline and phosphatidyl ethanolamine in a ratio of approximately 2:1.

Subsequently, carbonate treatment at pH 11.5, as described by Fujiki and co-workers (22, 23), was used to separate the glycosomal content from their membranes. Such a membrane fraction had a phospholipid content of 580 nmol/mg protein, or, if assuming an average molecular weight of 800 for phospholipid, a composition of 32% phospholipid and 68% protein by weight. 30% of the protein and 97% of all the glycosomal phospholipid were recovered in this fraction.

TABLE III

Phospholipids Present in Whole Cells of Bloodstream Trypomastigotes and Their Respective Glycosomes

Phospholipid	Percentage of total	
	Whole cells	Glycosomes
Sphingomyelin	24	ND
Phosphatidyl choline	32	68
Phosphatidyl inositol plus phosphatidyl serine	16	ND
Phosphatidyl ethanolamine	28	32

Phospholipids were extracted and separated by thin-layer chromatography as described in Materials and Methods. The phospholipid content of whole cells was 99.4 ± 12.6 nmol/mg protein and that of the glycosomes 188 ± 8 nmol/mg protein (five determinations).
ND, not detected.

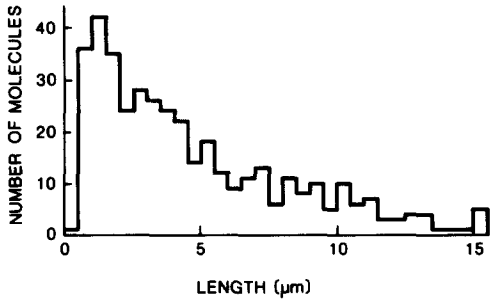

FIGURE 4 Size distribution of linear DNA molecules present in a highly purified glycosomal preparation. Only free molecules longer than minicircle length (0.32 μm) are included. Minicircles were used as an internal length standard.

DNA in Glycosome Preparations

DNA was extracted from a highly purified glycosomal preparation and quantitated by DAPI fluorescence (see Materials and Methods). Although 0.018 μg DNA/mg protein was present (Table II), it was a very low amount compared with that present in the intact cell (10 μg/mg protein, calculated from Borst et al. [38], using a value of 1 mg protein per 10^8 cells). The DNA was spread on grids by the Kleinschmidt method (33) and analyzed by electron microscopy. Three types of molecules were observed: a few typical kinetoplast-DNA networks with long maxicircles extending from the rim of the complex (cf., reference 39); many small circles with a contour length of *T. brucei* minicircles (0.32 μm); and heterogeneous linear DNA. All linear molecules in randomly chosen fields were measured and the resulting histogram of 400 molecules is presented in Fig. 4. The histogram shows no predominant length classes and all molecules present could represent the degradation products of nuclear DNA or kinetoplast DNA.

DISCUSSION

Several methods have been described for the purification of glycosomes from *Trypanosoma brucei* (4, 21, 40). One utilized isopycnic centrifugation of a large-particle preparation in a linear sucrose gradient, where glycosomes equilibrate at a density of 1.23 g/cm^3. The organelles, however, were still contaminated with flagella, kinetoplasts, and cell debris (4). Another method consisted of centrifugation of cell-free extracts in a Percoll gradient resulting in an equilibration of glycosomes at 1.09 g/cm^3 with good separation of the glycosomal markers from other cell constituents (21). In this paper we have combined both methods which resulted in glycosomal preparations that were almost devoid of contamination by other organelles.

Average purification factors of 12.8 for hexokinase and 11.5 for phosphoglucose isomerase indicate that glycosomes represent 8–9% of the total trypanosomal protein. Morphometric analysis, however, indicated a volume density of only 4.3%. One trypanosome represents 1.01×10^{-11} g protein (F. R. Opperdoes, unpublished observation) and a volume of 58 μm^3. Hence the protein concentration of a *T. brucei* cell amounts to 175 mg/ml. By combining the above data we calculate that the protein concentration in the glycosome is between 320 and 360 mg/ml. This value is higher than the range that can be calculated from the literature data on

mammalian peroxisomes. Leighton et al. (41) have reported that they represent 1.79 ± 0.95 mg protein per gram liver, while in hepatocytes their volume fraction is 1.05% (42). This corresponds to a protein content for liver peroxisomes of 188 ± 100 mg/ml.

From our morphometric data on the total membrane area of glycosomes (Table I), using a specific area of 0.6 nm^2 per phospholipid polar head group (43) (3.61 cm^2/nmol), and assuming a membrane consisting of pure phospholipid, an estimation of the maximal amount of glycosomal phospholipid per milliliter of cell can be obtained. This value is $2(0.925 \times 10^4)/3.61 = 0.512 \times 10^4$ nmol/ml. Taking into account that glycosomal protein corresponds to 14.7 mg/ml (8.4% of 175 mg/ml), the phospholipid content of the glycosome would be 349 nmol/mg protein. Experimentally, we find a value of 188 nmol/mg, suggesting that approximately half the surface area of the membrane is occupied by phospholipid. This is in reasonable agreement with our observation that the glycosomal membrane contains 68% protein by weight.

The glycosomes of the Trypanosomatidae strongly resemble the microbodies of other eukaryotes and morphologically they cannot be distinguished from each other. Both are spherical or ellipsoid, bounded by a single membrane, and both contain as phospholipids phosphatidyl choline and phosphatidyl ethanolamine and no other detectable phospholipid components (22). Like other microbodies, glycosomes have an electron-dense matrix, and occasionally crystalloid cores can be seen. No evidence was found for the presence of DNA in glycosomes. No DNA was found in the peroxisomes of either rat liver (29) or the yeast *Candida tropicalis* (44). In *T. brucei* the microbodies constitute a single population of organelles, of extremely homogeneous size, with an average diameter of 0.27 μm. Their diameter is only slightly affected by the purification procedure. This makes it unlikely that *T. brucei* would contain more than one class of microbody-like organelles, e.g., peroxisomes and glycosomes.

Peroxisomes are generally defined as microbody-like organelles involved in peroxide metabolism (45); i.e., they contain hydrogen peroxide–producing oxidases in combination with catalase. For some Trypanosomatid species the presence of typical peroxisomal enzymes like D-aminoacid oxidase, alpha-hydroxyacid oxidase (13), and glyoxylate cycle enzymes (46) has been reported, but none of these was ever localized in microbodies. In the case of other species, however, peroxisomal marker enzymes have never been detected (10, 36). *T. brucei* is a typical example of such an organism, which lacks catalase and H_2O_2-producing oxidases (36, 47). Nevertheless all Trypanosomatids contain cytoplasmic structures bounded by a single membrane and morphologically resembling the microbodies or peroxisomes of other eukaryotic organisms. In *T. brucei* these microbodies contain a number of enzymes involved in the conversion of glucose into phosphoglycerate and glycerol (1), adenylate kinase (8, 40), enzymes involved in pyrimidine biosynthesis (9), and enzymes involved in CO_2 fixation (10). The glycosome apparently is a microbody highly specialized in glucose degradation rather than in hydrogen-peroxide metabolism.

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