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Macromolecular Structure and Morphology of Native Glycogen Particles Isolated from *Candida albicans*

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A polysaccharide-rich particulate fraction was isolated from cytoplasmic extracts of *Candida albicans* by a procedure using differential centrifugation. The polysaccharide particles obtained after purification with deoxycholate treatment were essentially free of nitrogen and were identified chemically as polyglucosan, in which the glucosidic links were of alpha type. Both the response to amylolytic enzymes and the spectral characteristics of the iodine complexes of the polysaccharide particles were similar to those of rabbit liver glycogen. They also precipitated with concanavalin A, the glycogen value being assessed at 1.04. These data strongly indicated that the polysaccharide particles have the macromolecular structure characteristic of glycogen. The sedimentation analysis revealed that they were polydisperse, with a weight average sedimentation coefficient of 340*S*. In negatively stained specimens, the glycogen particles were seen to form rosette-like structures consisting of a complex unit 40 to 150 nm in diameter. Such complex particles were composed of smaller globules that were fairly uniform in size with an average diameter of 32 nm.

Glycogen is one of the major reserve carbohydrates widely distributed throughout a variety of microorganisms as well as higher organisms. Since the recognition of glycogen-like polysaccharide in bakers' yeast by G. Errera in 1885 (10), a number of papers have been published on the chemical nature of yeast glycogen, which was usually obtained after extraction with dilute acetic acid from alkali-treated cells (18, 29) or with boiling water from mechanically disintegrated cells (11). Later, the topographic localization and morphology of glycogen or glycogen-like granules in the cytoplasm of yeast cells was shown more clearly by the use of electron microscopy with ultrathin sections (22, 24) and freeze-etched specimens (21), although such granules are not chemically identified.

The study of the macromolecular structure, molecular size, and sedimentation characteristics of glycogen requires that the preparation be preserved in a highly native state. For this purpose, the extraction procedure with acetic acid or boiling water in the earlier studies seems inadequate, because it degrades the native glycogen into units of much smaller size. On the other hand, Drochmans (9) reported a mild isolation procedure using differential centrifugation that has been successfully applied for mammalian tissues (33) and protozoa (4).

With the use of this mild procedure, the present study was designed to isolate native fungal glycogen from a strain of *Candida albicans* which contains a large amount of glycogen. Working on the purified polysaccharide particles that were identified to be native glycogen, experiments were also conducted to obtain information on the macromolecular structure as well as morphology of this glycogen of fungal origin, particularly in comparison with glycogens from other sources, such as animal tissues.

MATERIALS AND METHODS

Strains and culture. *Candida albicans* strain 6713, some characteristics of which were described in our previous paper (35), was chosen as a source of glycogen preparation, because this strain accumulates the largest amount of glycogen of all strains of *Saccharomyces* and *Candida* yeasts tested when grown in a convenient medium. The organism was inoculated into a nutrient broth composed of: peptone(Difco), 5 g; yeast extract (Difco), 10 g; KH_2PO_4, 3.6 g; $Na_2HPO_4 \cdot 12H_2O$, 1.2 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; NaCl, 2 g; glucose, 40 g; and water to 1,000 ml (pH 6.0). It was grown at 29 C for 18 h in a jar fermenter with vigorous aeration. Cultures were harvested by centrifugation, washed twice with saline, and then stored at −20 C until analysis.

Isolation and purification of polysaccharide (glycogen) particles. The frozen-stored cells were suspended in 2 volumes of 0.2 M sorbitol solution containing 0.1 mM disodium ethylene-diaminetetra-

acetate and disrupted by passing through a French-pressure cell at 400 kg/cm². Disrupted cells were subjected to two successive runs of centrifugation (15-min) at 10,000 × *g* for removal of cell debris, and the final supernatant fluid (cytoplasmic extracts) was centrifuged again at 66,000 × *g* for 30 min. The sedimented materials were composed of two parts: a colorless, translucent, hard bottom part and a fluffy, reddish, superficial part. The latter consisted largely of mitochondrial particles, as determined by electron-microscopic and spectrophotometric analyses. After shaking the centrifuge tube gently, the mitochondrial fraction was carefully aspirated off by a pipette. The residual pellet (bottom part) was resuspended in 5 volumes of water and spun down at 49,000 × *g* for 30 min. This procedure was repeated three times, and finally a preparation of "crude polysaccharide(glycogen) particles" was obtained as the 10,000 × *g* supernatant fluid. Where further purification was attempted, 1 volume of this preparation was mixed with one-fourth volume of *n*-amyl alcohol and shaken vigorously at room temperature for 15 h according to the method of Sevag (26). The mixture was sedimented at 4,500 × *g* for 10 min, and the aqueous layer was transferred into a celophane bag (Visking) and dialyzed against water (500 volumes, changed twice) for 3 h with stirring. To the dialyzed sample was added 3 volumes of ethanol, and it was centrifuged at 4,500 × *g* for 10 min. The pellet was resuspended in the original volume of water and precipitated again with ethanol according to the same procedure. Then the particulate fraction obtained after ethanol precipitation was suspended in 5 to 10 volumes of 0.1 M glycine buffer, pH 9.0, containing 1% (wt/vol) sodium deoxycholate. The suspension was kept standing for 10 min in an ice bath and sedimented at 66,000 × *g* for 30 min. The resulting pellet was resuspended in 3 volumes of water and subjected to two cycles of 30-min and 15-min centrifugation at 136,000 × *g* and 10,000 × *g*, respectively. After the final run at 10,000 × *g*, a purified sample of polysaccharide(glycogen) particles free from protein (see Results) was isolated as an opalescent solution in a yield of 70% of starting materials (cytoplasmic extracts). It was stored at −20 C and, unless otherwise noted, used for all analyses. Portions were lyophilized to give snow-white powder and kept in a desiccator.

Quantitative determination of total carbohydrate with anthrone and of reducing sugars by a copper-reducing method. The method for preparation of the anthrone reagent and assay conditions for the amount of total carbohydrate were essentially the same as those reported by Chung and Nickerson (6). The total amount of reducing sugars, such as glucose and maltose, was determined according to Somogyi (27).

Determination of reducing end groups by hypoiodite method. Minimal molecular weight or degree of polymerization of glycogen was estimated from the amount of reducing end groups relative to the total amount of glucose residues, both of which were determined according to the method of Willstatter and Schudel (34).

Quantitative determination of protein. Samples were heated with 1 N NaOH at 90 C for 10 min, and the resultant extract was assayed for protein content by the method of Lowry et al. (17), using bovine serum albumin as a standard.

Complete acid hydrolysis of polysaccharide (glycogen). The procedure employed here essentially followed that of Adams (1). To the sample suspended in 2.0 ml of water was added 0.2 ml of concentrated HCl (specific gravity, 1.125), and the mixture was heated in a flask equipped with a reflux condenser for 2.5 h. After cooling the flask, the solution was nearly neutralized with 10 N NaOH, samples of which were then assayed for the total amount of reducing sugars or analyzed on paper chromatograms, or both.

α- Amylolysis of polysaccharide (glycogen). A digest was prepared to contain 1 to 20 mg of the sample, 0.5 ml of 0.1 M NaCl, 0.25 ml of 0.2 M phosphate-citrate buffer (pH 7.0), 1 to 5 mg of crystalline α-amylase (from *Aspergillus oryzae*) free from maltase and water to a final volume of 5.0 ml. Incubation was carried out at 37 C. Samples were removed at intervals and determined for the reducing sugar (maltose). α-Amylolysis limit was calculated on the basis of the percentage production of maltose after 24 h of incubation.

β- Amylolysis of polysaccharide (glycogen) and subsequent α-amylolysis of β-limit dextrin. Polysaccharide sample (1 to 5 ml), 0.5 ml of 0.2 M acetate buffer (pH 4.6), 2 mg of barley β-amylase (30 U/mg), and water, in a total volume of 5.0 ml, were incubated at 37 C for 24 h. Samples were removed for determination of maltose produced. β-Amylolysis limit was estimated on the basis of the percentage production of maltose during 24 h of incubation. Where indicated, β-limit dextrin obtained after β-amylolysis was further digested with α-amylase under the same condition as that for α-amylolysis of intact polysaccharide.

α-Amylase and β-amylase were generously supplied by T. Sawazaki, Institute of Physical and Chemical Research, Tokyo.

Estimation of values relating to the branching structure of polysaccharide (glycogen). The average chain length ($\overline{CL}$) of polysaccharide was determined according to the following equation proposed by Manners and Wright (19): $P_{1,6} = 100/\overline{CL} = 23.3 - 0.21\,(Pm)$, where Pm represents the apparent percentage conversion into maltose and, hence, $P_{1,6}$ represents the percentage of interchain (α-1,6-glucosidic) linkages in polysaccharide.

The exterior chain length ($\overline{ECL}$) and the interior chain length ($\overline{ICL}$) of polysaccharide were estimated by the use of the equations proposed by Archibald et al. (3): $\overline{ECL}$ = number of glucose residues removed by β-amylase + 2.5; $\overline{ICL} = \overline{CL} - \overline{ECL} - 1$.

Paper chromatography of acid hydrolysates and enzymatic digest. Products after complete hydrolysis with concentrated HCl and the supernatant fluid of 48-h digests with α- or β-amylase obtained after precipitating protein and residual polysaccharide with 2 volumes of ethanol were deionized by successive treatments with IR-4B (OH^-) and IR-120 (H^+) ion-exchange resins and then concentrated. Chroma-

tography of these samples was effected on Whatman no. 1 filter paper with the two solvent systems as indicated in Results. Spots were detected with the alkaline $AgNO_3$ reagent of Trevelyan et al. (32). For chromatographic identification of sugars, the sample was directly compared with appropriate reference compounds on the same chromatogram.

Gas-liquid chromatographic analysis of sugars. After hydrolyzing the materials tested, the products were reduced, acetylated, and analyzed according to the method of Albersheim et al. (2).

Preparation of concanavalin A solution and determination of GV. A solution of concanavalin A was prepared from the extracts of Jack Bean meal with 2% (wt/vol) saline, stabilized by the addition of polyvinyl alcohol, and stored at 4 C as described by Cifonelli and Smith (8). Glycogen value (GV) of the polysaccharide sample was turbidimetrically determined according to Cifonelli et al. (7), using rabbit liver glycogen that was prepared by the method of Stetten et al. (28) as a standard. Oyster glycogen and wheat amylopectin were the products of Tokyo Kasei Kogyo Co., Tokyo.

Difference spectrophotometry of iodine-polysaccharide complexes. Complex of the polysaccharide sample with iodine was formed in the presence of the half-saturated concentration of $(NH_4)_2SO_4$ and of the saturated concentration of $CaCl_2$ by the methods of Schlamowitz (25) and Krisman (15), respectively. Immediately after mixing all components together in the cuvette, the absorption spectra were measured on a Shimadzu MPS-50L spectrophotometer against respective iodine-iodide reference solution.

Sedimentation analysis. All work was carried out using a Spinco model E analytical ultracentrifuge. Centrifugation was done at 20 C at various speeds ranging from 10,490 to 37,290 rpm, and photographs were taken at intervals of 2 to 4 min. The sedimentation coefficient corrected to water at 20 C ($s_{20,w}$) was calculated by extrapolating the values obtained for finite concentrations (1 to 10 mg/ml) at infinite dilution.

Electron microscopy. The negative-staining method of Horne and Greville (12) was used for examination of the fine structure of glycogen particles. The specimens were examined at an instrumental magnification of ×50,000 with one or more of the following Japan Electron Optics Laboratory electron microscopes: JEM 7B, JEM 100U, and JEM 120.

RESULTS

Isolation and purification of polysaccharide particles. When cytoplasmic extracts from *C. albicans* cells were subjected to three cycles of high-speed centrifugation (49,000 × *g*) after the removal of the mitochondrial fraction, 80% (wt/wt) carbohydrates present in the cytoplasmic extracts was recovered in the colorless, translucent sediment that was provisionally termed crude polysaccharide particles (Table 1). They still contained a small amount of protein (⅑ of total weight), although the ratio of carbohydrate to protein was increased by about 400-fold as compared with the starting material (cytoplasmic extracts). Subsequent treatment with *n*-amyl alcohol or ethanol precipitation was not effective in deproteinization from the crude particles, whereas deoxycholate treatment markedly diminished the protein content to a level as low as 0.2% in weight. Such a deproteinized preparation was designated "purified polysaccharide particles" and employed for further analyses.

Chemical analyses—identification of glucose as a sole constituent. The purified polysaccharide particles were easily soluble in water or dilute alkali, giving a slightly opalescent solution. No ultraviolet absorbance was noted. When a solution of 10 mg of this material (lyophilized and dried in vacuo at 80 C to a constant weight) in 5 ml of 1 N HCl was hydrolyzed completely, the neutralized hydrolysates were found to contain a total of 9.58 mg of reducing sugar(s) calculated as glucose. The unhydrolyzed material, on the contrary, scarcely had reducing activity. Paper chromatograms of the hydrolysates developed by each of the two solvent systems, *n*-butanol-pyridine-water (6:4:3, vol/vol/vol) and phenol-water (5:1, vol/vol), coincidentally gave a single spot with R_f values of 0.42 and 0.36, respectively, indicating the presence of D-glucose but no other sugar in comparison with reference sugars. The same result was also obtained by means of gas-liquid chromatography. The elemental analysis of the purified particles (dried in vacuo at 75 C) yielded the theoretical value for polyhexosan as follows: Analysis calculated for $(C_6H_{10}O_5)_n$: C, 44.44; H, 6.22; O, 49.34; Found: C, 44.39; H, 6.34. Neither nitrogen nor phosphorus was detected. The value of specific rotation ($[\alpha]_D^{20}$) was + 176° in water (*c*, 0.5%; 2 cm). The degree of polymerization (number of glucose residue per reducing end group) was determined to be 1,900, from which the minimal molecular weight was calculated to be 3.6×10^5.

Summarizing these analytical data, it follows that chemical characteristics of the purified polysaccharide particles are those of highly polymerized polyhexosan, in which D-glucose is a sole consituent, that is, polyglucosan.

Spectrophotometric analyses. The absorption spectra of iodine complexes of the polysaccharide (polyglucosan) particles were examined in comparison with those of several reference polysaccharides. They were measured upon addition of $(NH_4)_2SO_4$ to a half-saturation (Fig. 1A). It was noted that λ_{max} for *Candida* polyglucosan was 500 nm. The value was closely

TABLE 1. *Carbohydrate and protein content of different fractions of cell extracts of C. albicans obtained by the differential centrifugation method with or without subsequent treatments*

Fraction	Carbohydrate content (mg/g of cell)	Protein content (mg/g of cell)	Carbohydrate: protein ratio in weight
Cytoplasmic extracts	6.86 (100[a])	48.9 (100[a])	0.14
Crude polysaccharide particles	5.90 (86.0)	0.11 (0.23)	53.2
Polysaccharide particles after treatment with:			
n-Amyl alcohol	5.77 (84.2)	0.10 (0.20)	57.7
Ethanol precipitation	5.50 (80.1)	0.09 (0.18)	61.2
Deoxycholate	4.89 (71.3)	<0.01 (<0.02)	>490.0

[a] Numbers in parentheses represent the yield (%).

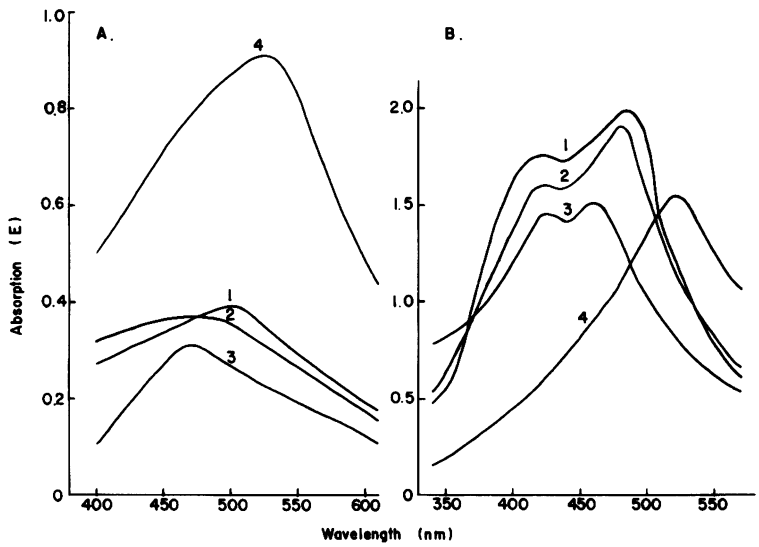

FIG. 1. *Absorption spectra of iodine complexes of Candida polysaccharide(glycogen) particles and several other polysaccharides. In experiment A, 0.05 ml of 0.125% (wt/vol) I_2 solution in 0.25% (wt/vol) KI and 2 ml of a saturated solution (at room temperature) of $(NH_4)_2SO_4$ were added to 2 ml of a 0.1% (wt/vol) aqueous solution of polysaccharide samples. In experiment B, 2.6 ml of the iodine reagent (0.5 ml of 2.6% [wt/vol] I_2 solution in 2.6% [wt/vol] KI plus 130 ml of saturated $CaCl_2$ solution) was added to 0.4 ml of a 0.1% (wt/vol) polysaccharide solution. Curve 1, purified Candida polysaccharide particles; curve 2, rabbit liver glycogen; curve 3, oyster glycogen; curve 4, wheat amylopectin.*

similar to that for rabbit liver glycogen (485 nm), but slightly greater and much lower than those for oyster glycogen (465 nm) and wheat amylopectin (530 nm), respectively. In the presence of saturated $CaCl_2$, a major peak of *Candida* polyglucosan was located at 485 nm, and similar λ_{max} values were obtained for rabbit liver glycogen (480 nm) and oyster glycogen (460 nm) (Fig. 1B). A second intensive peak at the region of 410 to 420 nm was also shown with all three samples. On the contrary, wheat amylopectin did not show any peak throughout the visible region but showed a major peak at 520 nm.

It seems likely, therefore, that the branching structure of *Candida* polyglucosan is more closely related to that of animal glycogen than plant amylopectin.

Enzymatic analyses. In order to clarify the branching structure of the polyglucosan particles from *Candida* yeast after analysis of glucosidic intra- and inter-chain linkages, experiments were conducted to examine their susceptibility to hydrolytic effects of α- and β-amylases. The degree of hydrolysis was estimated by an increase in the reducing activity (14). Both of the hydrolytic reactions proceeded rapidly at 37 C and were almost completed within 12 h,

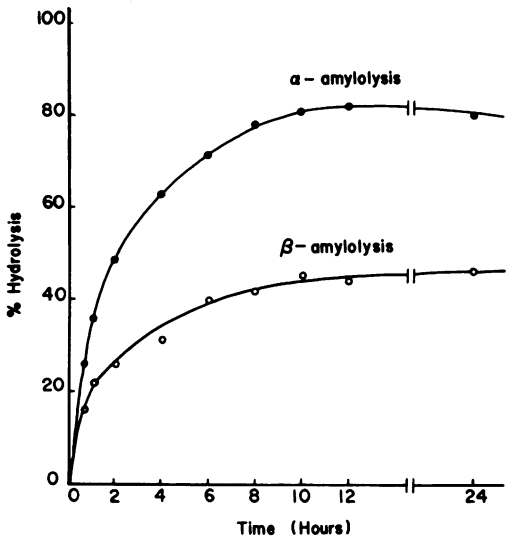

FIG. 2. *Time course of digestion of Candida polysaccharide(glycogen) particles with α- and β-amylases. A digest for α-amylolysis contained: 2.5 mg of purified Candida polysaccharide particles, 2.5 ml of 0.1 M NaCl, 1.25 ml of 0.2 M phosphate-citrate buffer (pH 7.0), 3 mg of crystalline α-amylase, and distilled water to a total volume of 25 ml. A digest for β-amylolysis was prepared containing 3 mg of the sample, 4 ml of 0.2 M acetate buffer (pH 4.6), 450 U of β-amylase, and distilled water to 20 ml. Both digests were incubated at 37 C, and 4-ml portions were removed at intervals for estimation of maltose liberated.*

giving α- and β-amylolysis limit values of 80 to 82% and 44 to 46%, respectively (Fig. 2). The paper chromatographic analysis showed the presence of glucose and maltose in α-amylase digests and of maltose alone in β-amylase digests. The results supported the possibility that a majority of glucose residues in the *Candida* polyglucosan particles are linked with α-D-1,4-linkages and a minor portion with amylase-resistant ones, presumably α-D-1,6-linkages at branching points of the chain. On the basis of the α- and β-amylolysis limit obtained here, the following values referring to the branching structure were calculated according to empirical equations (see Materials and Methods): $\overline{CL}$, 15.9; % interchain linkages, 6.3; $\overline{ECL}$, 9.8; and $\overline{ICL}$, 5.1. These values were favorably consistent with the corresponding values reported for animal glycogens (3, 20).

Precipitation reaction with concanavalin A. Concanavalin A has been reported to give a precipitate with glycogen, the extent of which is usually expressed as GV, but not to react with amylopectin (8, 30). Later studies also showed that GV linearly relates to the degree of branching of glycogen molecules (20).

TABLE 2. *GV of Candida glycogen particles and several other preparations of polysaccharides before and after digestion with β-amylase*[a]

Polysaccharide	GV	
	Before	After
Candida glycogen particles(purified)	1.04	1.33
Rabbit liver glycogen	1.00	1.36
Oyster glycogen	1.15	1.97
Wheat amylopectin	0	0

[a] Digestion with β-amylase (β-amylolysis) was performed at 37 C for 24 h under the conditions described in Materials and Methods.

As given in Table 2, the *Candida* polyglucosan particles and their β-dextrin had GV essentially comparable to the corresponding values for rabbit liver glycogen and its dextrin, but distinctly different from wheat amylopectin. This and all other experimental data led us to the conclusion that the *Candida* polysaccharide particles have the macromolecular structure peculiar to glycogen.

Sedimentation analyses. Typical patterns on the analytical ultracentrifuge displayed with the *Candida* glycogen particles before and after deoxycholate treatment were reproduced in Fig. 3. Particles sedimented so inhomogenously as to make it difficult to locate the maxima of the peak accurately, unless sufficiently high concentrations of the material were applied. At least five runs were performed with each sample at different concentrations to minimize the experimental error for estimation of the sedimentation coefficient as well as for analysis of the shape of profiles. The sedimentation profiles of both crude and purified glycogen particles gave a single broad peak, from which inhomogeneity of particle size was suggested. Such a tendency of polydispersity was more conspicuous with crude particles than with purified particles. The average values of $s_{20,w}$ were assessed to be 338 for the former and 345 for the latter, both values being virtually identical. Irrespective of the variation of the concentration of the sample applied, $s_{20,w}$ values obtained were fairly constant, suggesting that the particles would be of globular shape rather than fibrous. The solvent was varied from distilled water to a 1 M NaCl solution without producing any significant change in the sedimentation pattern. It seems likely, therefore, that the

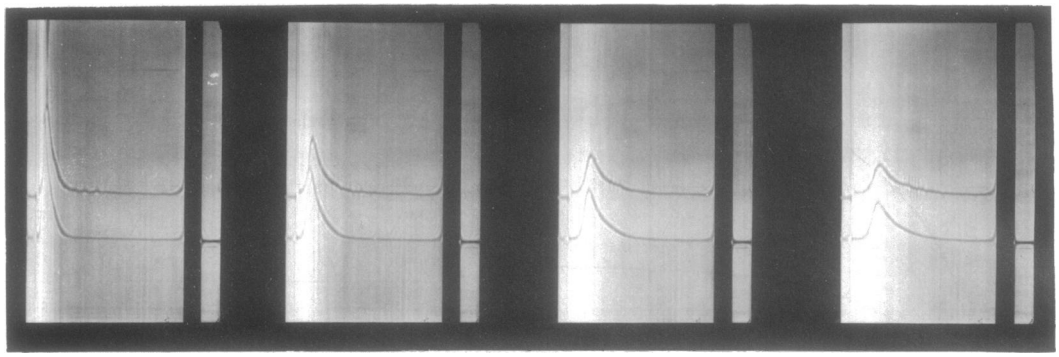

FIG. 3. *Sedimentation profiles of crude and purified Candida glycogen particles. Aqueous solutions (1% [wt/vol]) were centrifuged at 10,490 rpm. Photographs were taken at 4-min intervals. Upper: crude particles,* $s_{20,w} = 338$. *Lower: purified particles,* $s_{20,w} = 345$.

presence of associated proteins bears no relationship to the particle size, and that neither agglutination nor dissociation of the particles takes place at a wide range of ionic strength of the solvent.

Electron microscopic studies. Electronmicrographs of negatively stained specimens of the purified glycogen particles revealed particulate structures forming rosettes of varying size (40 to 150 nm in diameter) (Fig. 4). Such a particulate structure was visibly divided into one of 15 spherical subunits. They were quite uniform in size and contour with diameters of 32 ± 4 nm. It was above the resolving power of the instruments used to distinguish any further fine structure in the subunit globules from the granularity produced by the electron beam. On the basis of examining size and ultrastructure of 200 particles on electronmicrographs, the average values of the diameter of the purified particles was approximately 70 nm and the number of associated subunits was 6.5. Virtually the same morphological characteristics were also shown with crude glycogen particles.

DISCUSSION

As compared with isolation techniques for glycogens from animal sources, preparation of yeast glycogen has usually been made after more drastic extraction procedures, using dilute acid or boiling water, which were preceded by mechanical disintegration of cells or alkaline cytolysis (11, 13, 18, 29) because of the presence of tenacious cell wall. Such procedures degrade glycogen to a considerable extent, so that the isolated preparation is not good for analysis of macromolecular structure or morphology. The present paper describes a new method for isolating native glycogen from yeasts or fungal cells, in which cytoplasmic extracts obtained from mechanically disrupted cells were fractionated by differential centrifugation. *Candida* glycogen particles obtained after this mild procedure has a $s_{20,w}$ value of 340, from which the minimal average molecular weight is assessed to be 6×10^7. It suggests that the glycogen molecule is preserved in a highly polymerized form and, therefore, presumably in a native state as is reported for "native" glycogens carefully isolated from mammalian tissues (9, 23, 33) or protozoa (4).

As far as we know, this paper first presents certain information on the macromolecular structure and morphology of native glycogen particles of a fungal origin. However, it remains to be determined whether those enzymes involved in glycogen metabolisms, e.g., glycogen synthetase(s) and phosphorylase(s), are bound to crude *Candida* glycogen particles as reported for preparations from liver homogenates (5, 16, 31).

Archbald et al. (3) reported that several different preparations of rabbit liver glycogen share $\overline{CL}$ values of 15 to 18 and λ_{max} of complexes with the iodine-iodite reagent at 480 to 500 nm. Manners and Wright (19) demonstrated that α-amylolysis limit (%) 51 and 46 are obtained for rabbit liver glycogen and rabbit muscle glycogen, respectively. Compared with these glycogen preparations, *Candida* glycogen particles show virtually the same glycogen value (1.04), λ_{max} of iodine complexes (500 nm), and α-amylolysis limit (45%). We also found that $\overline{CL}$ 15–16, $\overline{ICL}$ 9–10, and $\overline{ECL}$ 5–6, as determined enzymatically with *Candida* glycogen particles, fall within the range of the corresponding values reported for glycogens from various sources, including vertebrates and in-

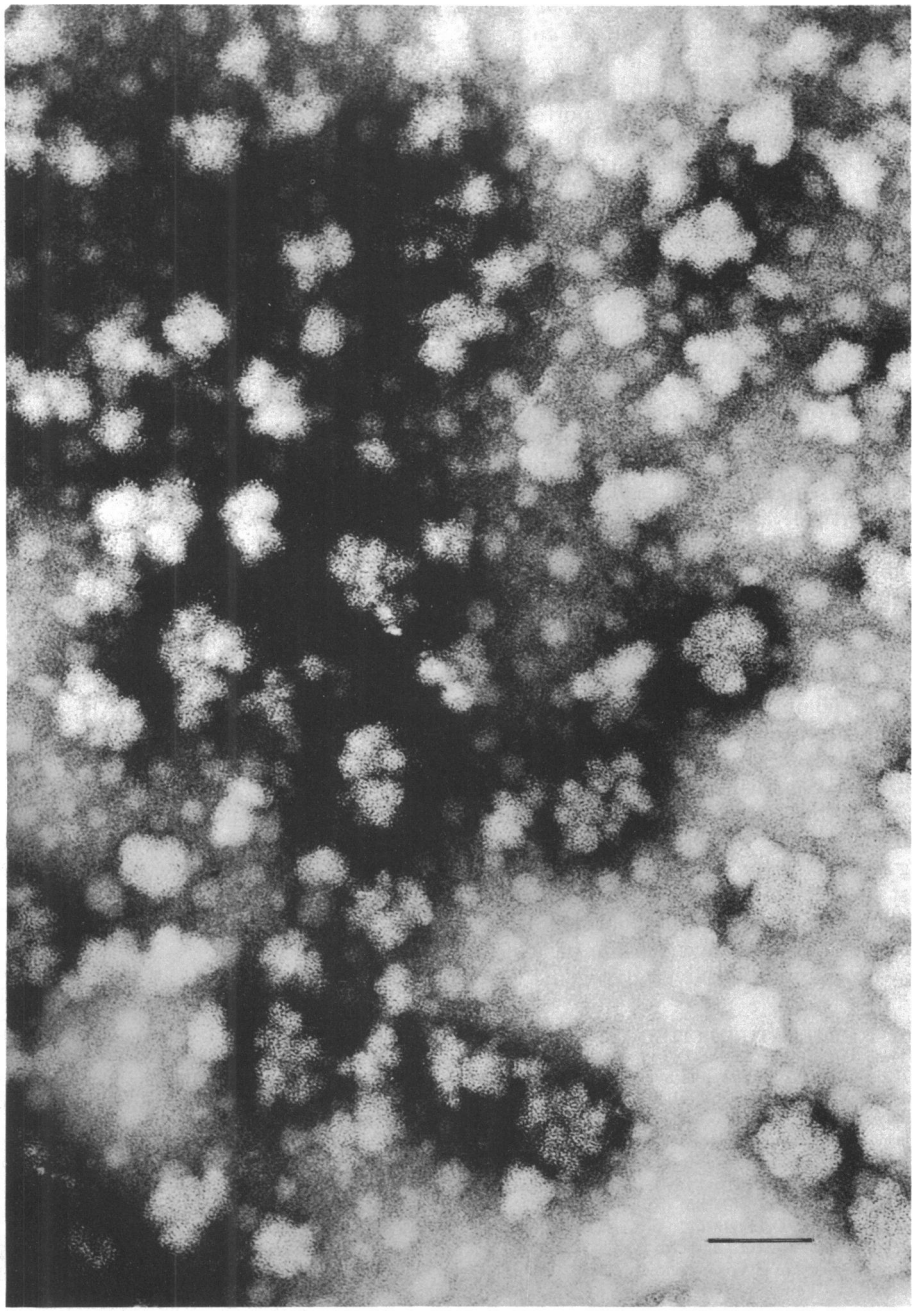

FIG. 4. *Electron micrograph of negatively stained specimens of purified Candida glycogen particles. Note polyparticulate or rosette-like forms and constituent smaller particles. Bar represents 0.1 μm.*

vertebrates (3). It appears, therefore, that the macromolecular structure of native *Candida* glycogen is closely related to that of animal glycogens, in particular rabbit liver glycogen. On the other hand, unusually high GVs, e.g., 1.36 (20) and 1.80 to 2.85 (7), were reported for glycogen preparations from bakers' yeast which were isolated from pressed cells with NaOH or boiled water. Degradation of glycogen during such a drastic isolation procedure might be responsible.

Drochmans' intensive studies with negatively stained specimens on the ultrastructure of rat liver glycogen revealed that normal glycogen particles appear as a complex unit, 60 to 200 nm in diameter (α-particle), which represents the highest level of organization found in the liver glycogen (9). He also demonstrated that within this α-particle can be recognized smaller units, 20 to 30 nm in diameter. Particles of a comparable size (β-particle) have been noted in glycogens isolated from *Tetrahymena pyriformis* (4) or from rabbit skeletal muscle (33) as well. Like rat liver glycogen, *Candida* glycogen examined in the electron microscope, with negative contrast, forms rosettes which are heterogenous in size and contour with diameters ranging between 40 and 150 nm. Furthermore, these particles are found to form complexes consisting of smaller particles, 32 nm in diameter. On the basis of size and constitution, these two types of particles of *Candida* glycogen can be favorably compared with α- and β-particles, respectively, according to Drochmans' nomenclature (9). It is of interest to note that not only the macromolecular structure but also the morphological constitution of *Candida* glycogen are basically identical with mammalian liver glycogen.

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