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Isolation of Mannan-Protein Complexes from Viable Cells of *Saccharomyces cerevisiae* X2180-1A Wild Type and *Saccharomyces cerevisiae* X2180-1A-5 Mutant Strains by the Action of Zymolyase-60,000

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The viable whole cells of *Saccharomyces cerevisiae* X2180-1A wild type and its mannan mutant strain *S. cerevisiae* X2180-1A-5, were treated with an *Arthrobacter* sp. β-1,3-glucanase in the presence of a serine protease inhibitor, phenylmethylsulfonyl fluoride. Fractionation of the solubilized materials of each strain with Cetavlon (cetyltrimethylammonium bromide) yielded one mannan-protein complex. Molecular weights of these complexes were almost the same as that of the mannoprotein of the mutant strain prepared by Nakajima and Ballou, which had a molecular weight of 133,000 and were approximately three times larger than those of the mannans isolated from the same cells by hot-water extraction. Each mannan-protein complex contained up to 2% glucose residue, which was not removed by specific precipitation with anti-mannan sera or by affinity chromatography on a column of concanavalin A-Sepharose. Treatment of these complexes with alkaline $NaBH_4$ produced peptide-free mannan containing small amounts of glucose nearly identical to those of the parent complexes. The above findings provide evidence that the glucose residues exist in a covalently linked form to the mannan moiety. Fractionation of the mannan-protein complex of the *S. cerevisiae* wild-type strain by DEAE-Sephadex chromatography yielded five subfractions of different phosphate content, indicating that these highly intact mannan-protein complexes were of heterogeneous material consisting of many molecular species of different phosphate content.

It is well accepted that the cell walls of many species of yeasts consist of three kinds of polysaccharides, β-glucan, chitin, and mannan, and that the mannan of yeasts exists as a phosphomannan-protein complex (18). To elucidate the nature of linkage between mannan and β-glucan units in the cell wall of yeasts, many workers have conducted degradation studies with β-glucanase with or without sulfhydryl reagent (8), phosphomannanase (16), and protease (6). However, no paper has been published on the isolation by enzymolysis of the intact mannan-protein complex of viable yeast cells.

In the present study, we attempted an enzymatic degradation of the viable cells of *Saccharomyces cerevisiae* X2180-1A wild-type strain and *S. cerevisiae* X2180-1A-5 mannan mutant strain (M-strain), using an *Arthrobacter* sp. β-1,3-glucanase, Zymolyase-60,000, in the presence of a protease inhibitor, to give intact mannan-protein complexes.

Use of the M-strain together with the corresponding W-strain seemed to be advantageous because of the relative simplicity of the mannan moiety in the mannan-protein complex of the M-strain used in analysis of the chemical nature of linkages between the mannan-protein moiety and the other cell wall components.

MATERIALS AND METHODS

S. cerevisiae strains. The W- and M-strains were kindly supplied by C. E. Ballou, Department of Biochemistry, University of California, Berkeley, via T. Nakajima, Tohoku University, Sendai, Japan.

Cultivation of *S. cerevisiae* strains. The *S. cerevisiae* strains were grown in 500-ml flasks in a liquid medium containing 1% yeast extract, 2% peptone, and 5% D-glucose on a reciprocal shaker at 28°C for 24 h. The cells were harvested at the end of logarithmic phase by centrifugation, washed thoroughly with saline (0.9%), and used immediately.

Enzymes. Zymolyase-60,000, used as the source of β-1,3-glucanase, was obtained from Kirin Brewery, Tokyo, Japan. According to the manufacturer's manual, Zymolyase-60,000 contains mannanase as an impurity. Assay of mannanase was conducted with an incubation mixture containing either 0.1% mannan fraction from W-strain (Fr. W-h) or 0.1% mannan fraction from M-strain (Fr. M-h) instead of the viable

cells of W- or M-strain. The amounts of released mannose were quantitated by the Somogyi method (23). The results indicated that the amounts of mannose released from Frs. W-h and M-h after a 3-h incubation at 28°C were up to 0.7 and 0.3%, respectively, on weight basis either in the presence or absence of phenylmethylsulfonyl fluoride (PMSF). It is therefore evident that the decrease in molecular weights of mannan-protein complexes isolated by the action of Zymolyase-60,000 can be disregarded. The other glucosidases were purchased from the following sources: α-glucosidase (EC 3.2.1.20), Sigma, St. Louis, Mo.; β-glucosidase (EC 3.2.1.21), P-L Biochemicals, Inc., Milwaukee, Wis.; glucoamylase (EC 3.2.1.3), Toyobo Co., Tokyo, Japan.

Other materials. Mannoprotein from the M-strain, isolated by 90-min autoclaving of the corresponding whole cells with neutral citrate buffer, possessing a molecular weight of 133,000 (17), and designated Fr. M-90, was kindly donated by T. Nakajima. A portion of Fr. M-90 was treated with 0.1 M NaOH, in accordance with the description of Nakajima and Ballou (17), to yield the corresponding alkali-degradation product, Fr. M-90-b, possessing a molecular weight of 41,000. Concanavalin A (ConA)-Sepharose and Dextran T2,000 were purchased from Pharmacia, Uppsala, Sweden. Antisera to W- and M-strains were prepared as described previously (15). Before use, these antisera were checked for the absence of antibody against β-glucan or α-glucan by using a partially acid-hydrolyzed β-glucan of an *S. cerevisiae* strain and soluble starch on agar gel double-diffusion analysis (data not shown).

Mannans of W- and M-strains prepared by hot water extraction. Isolation of the mannan fractions Fr. W-h and Fr. M-h from the crude polysaccharide extracted at 135°C for 3 h from the acetone-dried whole cells of each strain was conducted by a slightly modified method of Lloyd (13) by Cetavlon (cetyltrimethylammonium bromide) as described previously (20).

Treatment of viable cells of W- and M-strains with Zymolyase solution. A 100-g (wet weight) amount of the cell paste of W- or M-strain was shaken gently with 500 ml of 1:15 M sodium phosphate buffer (pH 7.5) containing 0.1 M 2-mercaptoethanol at 28°C for 1 h, and the suspension was centrifuged at 1,500 × *g* for 10 min to remove the supernatant containing solubilized external invertase (10). The β-1,3-glucanase solution used in the present study was prepared as follows. A 750-mg amount of PMSF in 50 ml of isopropyl alcohol was added to a solution of 450 ml of 1:15 M sodium phosphate buffer (pH 7.5) containing 0.8 M D-mannitol, 0.1 M 2-mercaptoethanol, and 150 mg of Zymolyase-60,000 and incubated at 28°C for 1 h. The 2-mercaptoethanol-treated cells were added to the enzyme solution, and the suspension was shaken gently at 28°C for 3 h. The remaining *S. cerevisiae* cells were removed by centrifugation at 500 × *g* for 10 min, and the supernatant was heated at 100°C for 5 min. After centrifugation at 1,500 × *g* for 10 min, the supernatant was dialyzed against deionized water for 48 h and lyophilized to yield crude mannan-protein complexes, Frs. W and M.

Purification of the solubilized mannan-protein complex. The crude mannan-protein complex of each strain was fractionated by the Cetavlon method, as described by Lloyd (13), to yield three fractions each, designated Frs. W-I, W-II, W-III, M-I, M-II, and M-III, respectively, as reported previously (20). Fr. W-II and M-II were further fractionated by repetition of precipitation with Cetavlon. These fractions were then separately applied to a column (2.5 by 100 cm) of Toyopearl HW-60 (superfine) equilibrated with 1:15 M sodium phosphate buffer (pH 7.5), and elution was made with the same buffer. Samples of eluates, each 4.0 ml, were collected and assayed for their carbohydrate content. Eluates containing polysaccharide were combined, dialyzed against water, and lyophilized. The purified mannan-protein complexes which were not contaminated with Coomassie brilliant blue staining material in polyacrylamide gel electrophoresis were designated Frs. W-II′ and M-II′.

Determination of molecular weights by gel chromatography on Bio-Gel A-5m. For molecular-weight determinations, the mannan-protein complexes were passed through a column (1 by 200 cm) of Bio-Gel A-5m (200 to 400 mesh) equilibrated with 3.5×10^{-4} M sodium phosphate buffer (pH 7.0) containing 0.05 M NaCl. Elution was performed with the same buffer in a flow rate of 9 ml/h, and 2.0-ml fractions of each complex were collected. Dextran T2,000, Fr. M-90, and its alkali degradation product, Fr. M-90-b, were used as the standard materials.

DEAE-Sephadex chromatography of mannan-protein complex. An aqueous solution of Fr. W-II′ (500 mg in 4 ml) was applied on a column of DEAE-Sephadex A-50 (acetate, 2.5 by 35 cm). Elution was performed with water and 0.025, 0.05, 0.1, and 0.25 M of NaCl, subsequently. Samples of eluates, 20 μl, were assayed for their carbohydrate content with phenol-sulfuric acid reagent (5). Eluates corresponding to each peak were combined, concentrated in vacuo to a small volume, dialyzed against water, and lyophilized.

Alkaline sodium borohydride treatment of Frs. W-II′ and M-II′. Alkaline sodium borohydride treatment was conducted by the method of Matsumoto et al. (15), using 50 mg of each specimen. Each fraction was dissolved in 5.0 ml of 1 M NaOH containing 1 M $NaBH_4$ and heated at 100°C for 6 h.

Quantitative precipitin reaction of Frs. W-I, W-II, W-III, M-I, M-II, and M-III with homologous antisera. The quantitative precipitin reaction was performed according to the description of Okubo et al. (19).

Affinity chromatography of Frs. W-II′ and M-II′ with a ConA-Sepharose column. Fraction W-II′ or M-II′ (each 40 mg) was dissolved in 1 ml of 0.1 M sodium phosphate buffer (pH 7.0), and the solution was applied to a column (1.8 by 15 cm) of ConA-Sepharose equilibrated with 0.1 M sodium phosphate buffer (pH 7.0). The unabsorbed material was removed by washing with 300 ml of the same buffer. Elution was performed by the stepwise additions (300 ml each) of 0.5 M D-glucose and 0.1 M methyl α-D-mannopyranoside in the same buffer. Eluates corresponding to each haptenic solution were separately concentrated in vacuo to small volumes and dialyzed against water for 24 h. After concentration in vacuo to a small volume, each nondialyzable material was fractionated on a column of Toyopearl HW-60 (2.5 by 100 cm) and elution was made with 1:15 M sodium phosphate buffer (pH 7.5) at 60 ml/h to remove small amounts of retained hapten. Eluates corresponding to the polysaccharide were combined, dialyzed against water, and lyophilized to yield two mannan-protein complex subfractions each, designated Frs. W-II′-G, W-II′-M, M-

TABLE 1. Chemical composition of the fractions obtained by fractional precipitation of the crude polysaccharides of W- and M-strains, using Cetavlon

Fraction	Total carbohydrate[a] (%)	Molar ratio of sugar components[b]			Total protein[c] (%)	Yield[d] (%)
		Ribose	Mannose	Glucose		
W-I	91	74.2	25.5	0.3	3.7	0.1
W-II	95	0.1	98.4	1.5	2.6	1.1
W-III	87	0.6	50.5	48.9	10.9	0.1
M-I	89	85.7	13.4	0.9	2.9	0.1
M-II	94	Trace	98.6	1.4	4.1	1.1
M-III	88	Trace	67.4	32.6	8.7	0.2

[a] Determined by the phenol-sulfuric acid method (5).
[b] Determined by gas-liquid chromatography by converting the component sugars of each acid-hydrolyzed specimen into corresponding alditol acetates (12).
[c] Determined by the Folin method of Lowry et al. (14).
[d] Weight basis of the wet-packed whole cells.

II'-G, and M-II'-M, respectively, with yields of 6, 30, 5, and 30 mg, respectively.

Specific precipitation of Frs. W-II' and M-II' with corresponding antiserum. Corresponding homologous antiserum diluted 9 to 20 ml with saline was added to a solution of each fraction (7 mg in 100 ml of saline). After incubation at 37°C for 1 h, the mixture was allowed to stand at 4°C for 16 h. The precipitate was collected by centrifugation at 1,500 rpm for 10 min, washed carefully with chilled saline, and dissolved in a minimum amount of 2 M NaOH. A 20% aqueous solution of trichloroacetic acid was added to this solution until precipitation ceased. The suspension was centrifuged at 1,500 × *g* for 10 min, and the clear supernatant solution was dialyzed against water and lyophilized to yield the mannan-protein complex of each strain.

Treatment of Frs. W-II' and M-II' with α- and β-glucosidases. Fr. W-II' or M-II' (10 mg each) was dissolved in a solution of either 2 mg of α-glucosidase in 10 ml of 0.1 M Tris-hydrochloride buffer (pH 6.8) or 2 mg of β-glucosidase in 10 ml of 0.2 M acetate buffer (pH 4.7). Each solution was then incubated at 37°C for 24 h. After heating at 100°C for 5 min, the reaction mixture was dialyzed against water and centrifuged. The supernatant was concentrated in vacuo and lyophilized. Treatment of Frs. W-II' and M-II' with glucoamylase in 2 M acetate buffer, pH 4.7, was also conducted in a manner similar to that for treatment with α- and β-glucosidase.

Other methods. Total carbohydrate was determined by the phenol-sulfuric acid method (5) with D-mannose as the standard. Total phosphate was determined by the method of Ames and Dubin (1), using KH_2PO_4 as the standard. Total protein was determined by the Folin method of Lowry et al. (14), using bovine serum albumin as the standard. Molar ratios of the sugar components were determined by the method of Lindberg (12). Alditol acetates were chromatographed on a glass column (5 mm by 150 cm) containing column packing, 3% ECNSS-M on Gas-Chrom Q (80 to 100 mesh), at 190°C with N_2 at a flow rate of 60 ml/min. Amino acid analysis was conducted with a Hitachi 835-50 automatic amino acid analyzer. Samples were hydrolyzed with 6 M HCl at 110°C for 24 h in sealed evacuated tubes. Polyacrylamide gel electrophoresis was carried out as described by Davis et al. (4).

RESULTS

Preparation of mannan-protein complex. For inhibition of contaminating protease activity, the Zymolyase solution was treated with PMSF, a serine protease inhibitor, before use. The results of a preliminary experiment indicate that protease activity was completely inhibited by the addition of 5 mg of PMSF per mg of Zymolyase-60,000 without any decrease in β-1,3-glucanase activity. The action of the PMSF-containing Zymolyase solution on either suspension of the viable whole cells of W- or M-strain caused a decrease of turbidity. However, the extent of decrease in turbidity was about one-fourth of those of the *S. cerevisiae* suspensions incubated with Zymolyase contaminated with protease. The isolation of solubilized crude mannan-protein complexes Frs. W and M from the supernatant of each incubation mixture by the action of Zymolyase was conducted after centrifugation

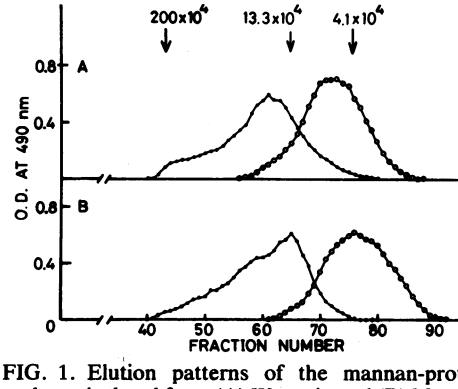

FIG. 1. Elution patterns of the mannan-protein complexes isolated from (A) W-strain and (B) M-strain on a column of Bio-Gel A-5m. Frs. W-II' and M-II' (●). Frs. W-h and M-h (○). The arrows indicate the elution positions of Dextran T2,000, Fr. M-90, and Fr. M-90-b, and the numbers indicate the subsequent molecular weights.

TABLE 2. Amino acid composition of Frs. W-II' and M-II'

Amino acid	Amino acid molar ratio in:	
	W-II'	M-II'
Aspartic acid	8.10	8.67
Threonine	23.8	19.3
Serine	16.7	16.3
Glutamic acid	8.32	9.20
Glycine	4.45	4.04
Alanine	10.1	11.9
Half-cystine	0.10	0.11
Valine	8.19	9.15
Methionine	0.03	0.02
Isoleucine	3.28	3.59
Leucine	4.64	4.96
Tyrosine	1.18	1.38
Phenylalanine	1.16	1.16
Lysine	1.66	2.11
Histidine	0.63	0.71
Arginine	0.33	0.27
Proline	7.33	7.10

and heating of the supernatant to remove the remaining cells and heat-aggregated materials. Frs. W and M were further fractionated by the Cetavlon method, described by Lloyd, to yield three fractions each, designated W-I, -II, -III, M-I, -II, and -III, respectively. Fractions W-I and M-I were found to consist largely of ribonucleic acid because of their high ribose content. However, yields of these fractions were very low, accounting for only 0.1% of the packed viable whole cells on weight basis and indicating low ratios of burst of protoplasts during the action of β-1,3-glucanase. Frs. W-II and M-II were found to the major mannan-protein fractions among each of the Cetavlon fractions of the parent yeast cells as judged from their yields and mannose contents (Table 1). The results of quantitative precipitin reaction of each of three Cetavlon fractions against the homologous antisera also indicated that Frs. W-II and M-II exert the strongest antibody-precipitating activity among the corresponding three Cetavlon fractions (data not shown). On the basis of the above results, Frs. W-II and M-II were further purified by repetition of Cetavlon fractionation and then by gel chromatography on a column of Toyopearl HW-60. These purified mannan-proteins were shown to be free of contaminating protein as judged by gel electrophoresis and were designated Frs. W-II' and M-II', respectively.

Molecular weights of Frs. W-II' and M-II'. To determine the molecular weights of these purified mannan-proteins, gel chromatography was performed with a column (1 by 200 cm) of Bio-Gel A-5m. Figure 1 shows the elution patterns of Frs. W-II' and M-II', indicating that the molecular weights of both complexes are ca. 130,000, as judged from the elution volumes of Frs. M-90 and M-90-b, molecular weights of which were shown to be 133,000 and 41,000, respectively, by Nakajima and Ballou (17). Additionally, molecular weights of Frs. W-h and M-h, which were prepared by autoclaving the whole cells of W- and M-strains for 3 h, were shown to be only one-third of those of the corresponding mannan-protein complexes Frs. W-II' and M-II' obtained by means of PMSF-treated Zymolyase.

Chemical analyses of Frs. W-II' and M-II'. As shown in Table 2, both Frs. W-II' and M-II' contained large amounts of serine, threonine, and acidic amino acids as reported by Sentandreu and Northcote for the glycopeptide of *S. cerevisiae* (21), and the amino acid compositions of these complexes are very similar. On the other hand, the glucose contents of Frs. W-II' and M-II' were 2.1 and 2.5%, respectively (Table 3).

Treatment of Frs. W-II' and M-II' with α-, and β-glucosidases. Frs. W-II' and M-II' were further treated with several glucosidases. As shown in Table 3, α-glucosidase did not cleave glucose residues in Frs. W-II' and M-II', whereas a slight decrease of glucose content was observed in each complex by the action of β-glucosidase.

Purification of Frs. W-II' and M-II' by affinity chromatography with a ConA-Sepharose column. To obtain substantiating evidence for the assumption that the glucose residue might be covalently linked to the mannan-protein complex, Frs. W-II' and M-II' were fractionated by affinity chromatography with a ConA-Sepharose column. Glucose content of all polysaccharide fractions, Frs. W-II'-G, M-II'-G, W-II'-M, and M-II'-M, did not differ significantly from those of the parent complexes (Table 3). These results provide strong evidence for the presence of covalently linked glucose residues in these complexes.

Precipitation of Frs. W-II' and M-II' with each corresponding anti-*S. cerevisiae* serum. Frs. W-II' and M-II' were further subjected to specific precipitation with each corresponding homologous antiserum. The proportions of mannan-protein complexes and antisera were the same as those of the equivalence points in the preliminary precipitin reactions carried out on a microscale, as described previously (19). Although a slight change in glucose content of the complexes was observed after the specific precipitation (Table 3), these findings seem to provide one line of evidence against the presence of free glucan in these complexes.

Alkaline sodium borohydride treatment of Frs. W-II' and M-II'. To investigate whether the glucose residues are connected with the peptide moieties of these mannan-protein complexes, Frs. W-II' and M-II' were treated with alkaline

TABLE 3. Effect of glycosidase treatment and fractionation with antisera or ConA-Sepharose on glucose content of Frs. W-II' and M-II'

Treatment or fractionation	Carbohydrate composition[a] (%)			
	Fr. W-II'		Fr. M-II'	
	Mannose	Glucose	Mannose	Glucose
None	97.9	2.1	97.5	2.5
α-Glucosidase	97.9	2.1	97.4	2.6
β-Glucosidase	98.4	1.6	98.0	2.0
Glucoamylase	98.1	1.9	97.6	2.4
$NaBH_4$ and NaOH	99.0	1.0	98.1	1.9
ConA-Sepharose with D-glucose	98.0	2.0	96.9	3.1
ConA-Sepharose with Me-α-D-Man[b]	98.4	1.6	97.9	2.1
Anti-mannan serum	98.4	1.6	98.0	1.8

[a] Determined by gas-liquid chromatography of the alditol acetates (12).

[b] Me-α-D-Man, Methyl-α-D-mannopyranoside.

sodium borohydride. Because the protein-free modification products corresponding to Frs. W-II' and M-II' still contained 1.0 and 1.9% of glucose residues, respectively (Table 3), it is reasonable to state that the glucose residues are located in the mannan moieties of both complexes.

Fractionation of Frs. W-II' on a DEAE-Sephadex column. Figure 2 shows the elution profile of Fr. W-II' on a column of DEAE-Sephadex A-50 by a stepwise elution system. Under the chromatographic conditions, Fr. W-II' was resolved into five subfractions, designated Frs. W-II'-a to W-II'-e. The results of chemical analyses indicated that the mannose to phosphate ratios of these subfractions were proportional to the concentration of NaCl solutions used for elution (Table 4).

DISCUSSION

To clarify the architecture of yeast cell wall, many workers have used the degradation of the cell wall to isolate the polysaccharide constituents containing small fragments of the other sugar polymers, e.g., mannan-protein complex with glucosyl moiety (3, 11), glucan-mannan (9), and glucan-chitin (22) complexes. Concerning the linkage between mannan and glucan, Fleet and Manners (7) reported that these polysaccharide units were connected by β-1,6-linked oligoglucosyl residues because treatment of an alkali-soluble glucan fraction with *Bacillus circulans* β-1,6-glucanase released a mannan fraction containing 3% glucose (2), although the nature of the linkage between the glucose residue(s) and the mannan moiety has yet not been clarified.

In the present study, the viable cells of W- and M-strains were subjected to digestion with an *Arthrobacter* sp. β-1,3-glucanase, Zymolyase-60,000, in the presence of PMSF in the hope of isolating a mannan-protein complex possessing a highly intact chemical composition, especially in its peptide moieties. The mannan-protein complexes of the *S. cerevisiae* wild-type and mutant strains isolated by repetition of fractional precipitation with Cetavlon and subsequent fractionation of a column of Toyopearl HW-60 were free from any proteinous contaminants as judged by polyacrylamide gel electrophoresis. The purified mannan-protein complexes Frs. W-II' and M-II' were strongly stained with the periodic acid-Schiff reagent and did not combine with Coomassie brilliant blue dye, indicating that the mannan moieties in the complexes serve as a protective barrier against attack of various agents on the peptide moieties or on the β-glucan meshwork.

The results of gel-filtration chromatography of Frs. W-II' and M-II' on a column of Bio-Gel A-5m indicate that the molecular weights of these complexes are about 130,000, because the elution volume of a mannoprotein of M-strain, Fr. M-90, isolated by Nakajima and Ballou (17) was nearly identical to those of Frs. W-II' and M-II' and was approximately three times larger than those of mannans Fr. W-h and Fr. M-h extracted from the corresponding whole cells at 135°C for 3 h.

The fact that the mannan-protein complexes were released by the action of β-1,3-glucanase in the absence of protease indicates the presence of covalent linkage between mannan and glucan units or entrapment of the mannan-protein com-

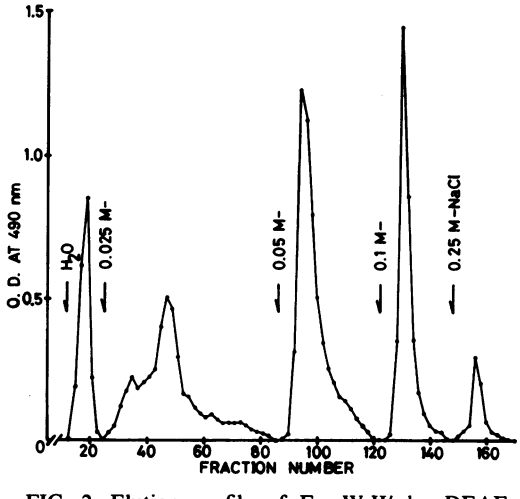

FIG. 2. Elution profile of Fr. W-II' by DEAE-Sephadex chromatography (A-50, acetate, 2.5 by 35 cm) by stepwise elution with water and NaCl solutions.

TABLE 4. Chemical composition of mannan-protein subfractions obtained from Fr. W-II' by DEAE-Sephadex chromatography

Sub-fraction	Composition (%)			Yield[b] (%)
	Carbohydrate	Protein	Phosphate[a]	
W-II'-a	98	1.1	0.11	10
W-II'-b	97	1.2	0.22	20
W-II'-c	97	1.6	0.26	28
W-II'-d	96	2.2	0.42	20
W-II'-e	95	3.5	0.81	6

[a] Determined by the method of Ames and Dubin as $-PO_3H_2$ (1).

[b] Weight basis of the bulk mannan-protein complex.

plex by the glucan meshwork, as speculated earlier by Bacon et al. (2). As shown in the results of chemical analyses, Frs. W-II' and M-II' were found to contain small amounts of glucose. Affinity chromatography of Frs. W-II' and M-II' on a column of ConA-Sepharose provided evidence that both mannan-protein complexes contained small amounts of covalently bound glucose residues. Furthermore, Frs. W-II' and M-II' were investigated for the presence of covalently bound glucose residues by specific precipitation with antisera corresponding to W- and M-strain cells. Because the glucose content of specifically precipitated Frs. W-II' and M-II' with each corresponding anti-whole cell serum was nearly identical to each of the parent complexes, both mannan-protein complexes were believed to contain covalently linked glucose residues. Because the origin of these glucose residues is obscure, i.e., it is not known whether they are derived from the glucan unit of the parent cell wall or some other source, it is necessary to conduct further structural analyses.

In 1978, Okubo et al. (19) reported that the mannan of an *S. cerevisiae* wild-type strain prepared by Fehling's solution method was a heterogeneous material consisting of a variety of molecular species containing different amounts of phosphate. However, these mannan fractions could not be regarded as having a highly intact composition, because the isolation process involved an autoclave extraction at 135°C for 3 h and treatment with the strongly alkaline Fehling's solution. In the present study, Fr. W-II' was investigated for its anionic heterogeneity by means of a chromatographic technique similar to that of a previous study (19). The results show that Fr. W-II' is of heterogeneous material resolved into many subfractions of different phosphate contents and that this heterogeneity might be responsible for the diversity of the phosphomannan moiety-assembling system located in the Golgi apparatus of the parent cells.

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