ON THE STRUCTURE OF A PEPTIDO-PHOSPHOGALACTOMANNAN COMPLEX FROM A BLACK YEAST, *CLADOSPORIUM WERNECKII*

Kenneth O. LLOYD

Departments of Dermatology and Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032, USA

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1. Introduction

Mannan-protein complexes have been isolated from a number of yeasts and fungi [1–3]. These fungal mannans often contain diesterified phosphate [4–6]. Sentandreu and Northcote [7] showed that a peptido-mannan from *Saccharomyces cerevisiae* has carbohydrate linked to the peptide moiety by two types of linkages: (a) alkali-labile linkages to serine and threonine (mainly mannose residues) and (b) alkali-stable linkages to asparagine (high molecular weight mannan). Recently Cawley and Letters [8] showed that phosphodiester linkages are present in this mannan.

Extraction of the yeast form of *Cladosporium werneckii* with phosphate buffer at 100° and fractionation of the product with Cetavlon in borate buffer yields a peptido-galactomannan containing about 3% phosphate [9]. Fragmentation of this complex by mild acid treatment and by alkali at room temperature has given some insight into the manner in which the three components are linked together in a high molecular weight complex.

2. Experiments and results

The isolation of the peptido-galactomannan (fraction B) from *C. werneckii* has been described [9]. The product is completely excluded from Sephadex G-100. Analytical methods have also been described [9, 10].

Titration of the acid form of the polysaccharide gave a titration curve typical of phosphodiesters [cf. 5, 6]; after hydrolysis in 0.1 N HCl at 100° for 15 min the titration curve of the product showed a second inflection indicating that hydrolysis to phosphomonoester had occurred. To isolate the products, peptido-galactomannan (500 mg) was hydrolyzed in 25 ml HCl and chromatographed on a Sephadex G-100 column (fig. 1a). Two peaks, one in the excluded volume (fraction 1a; 150 mg), were obtained. Retreatment of fraction 1a under the same acid conditions did not lead to further hydrolysis. The low molecular peak was chromatographed on a Sephadex G-25 column (60 × 1.8 cm) and yielded a single broad peak (V_e/V_0 = 1.24; fraction 1b; 147 mg). Analytical data on the fractions are given in table 1. Fraction 1b contained galactose, mannose and phosphate; it was shown to be negatively charged by paper electrophoresis in acetic acid–pyridine (pH 5.3) buffer (the fraction also contained a small proportion of a neutral component). Gel filtration in 0.15 M NaCl on calibrated Sephadex G-25 column indicated material with molecular weights in the 1600–3500 range. The reducing residue was shown to be mannose by reduction with sodium borohydride.

Fraction 1a (75 mg) was then treated with 0.33 N NaOH and 0.33 M $NaBH_4$ (7.5 ml) at room temperature for 15 hr. The product was chromatographed on a Sephadex G-100 (fig. 1B) and the excluded fraction was rechromatographed to give fraction 2a (6 mg). The low molecular peak was chromatographed on a Sephadex G-25 column (60 × 1.8 cm) and yielded a single broad peak (V_e/V_0 = 1.50; fraction 2b; 50 mg). This fraction contained only mannose and mannitol (5 : 1, table 1), it did not migrate in paper electrophoresis and was shown by gel filtration to have a molecular weight of 1000–1200.

Treatment of the original galactomannan complex with 0.2 N NaOH at room temperature lead to a 3.5-fold rise in the absorbance at 241 nm, indicating the

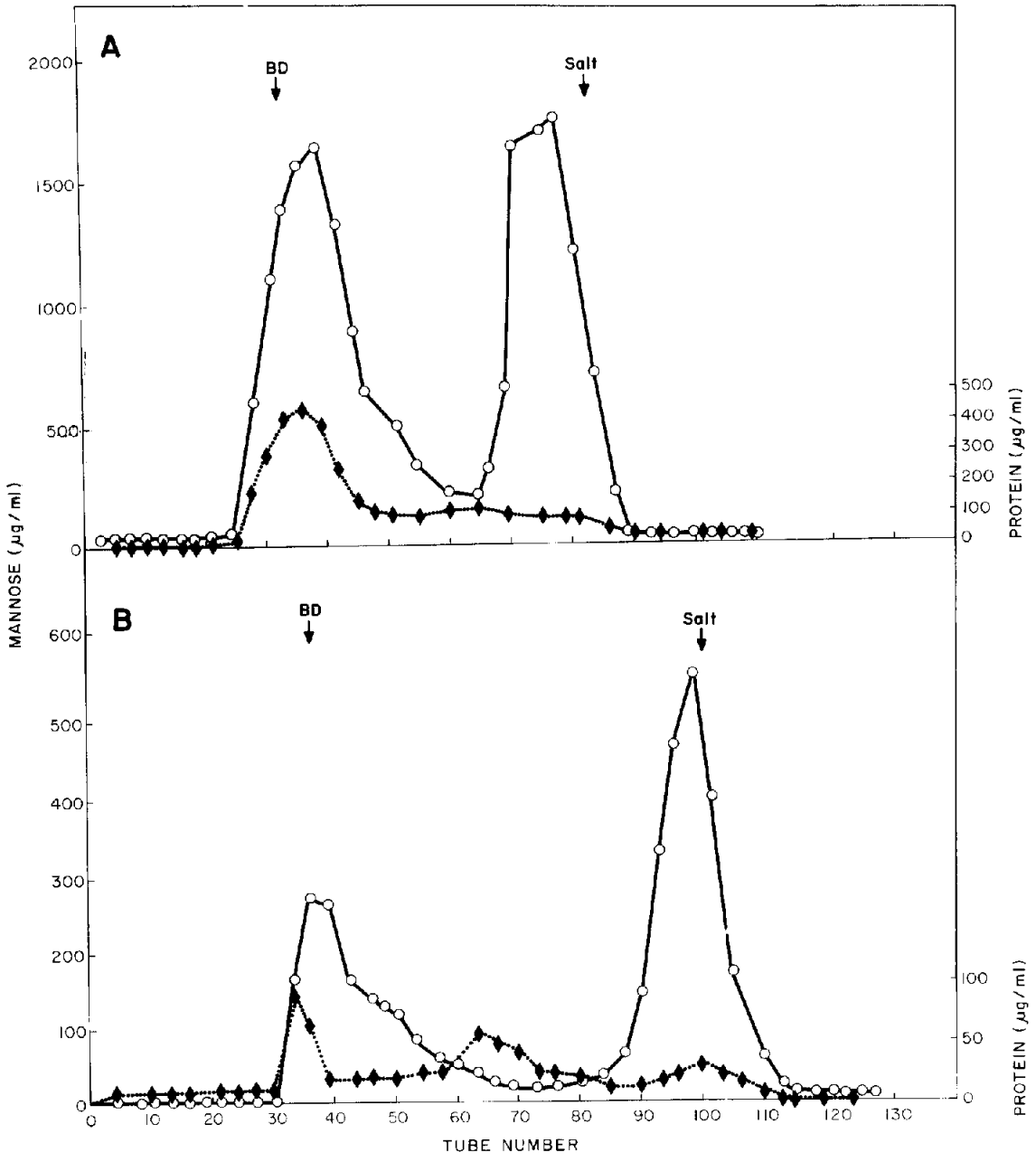

Fig. 1(A). Gel filtration on Sephadex G-100 of peptido-phosphogalactomannan after mild acid hydrolysis. Column size = 60 X 2.9 cm. Fraction volume = 4.2 ml. (B). Gel filtration on Sephadex G-100 of high molecular weight peak from column 1A (fraction 1a) after treatment with $NaOH-NaBH_4$. Column size = 60 X 2.9 cm. Fraction size = 3.2 ml. BD = Elution position of blue dextran. o—o mannose. ♦.....♦ protein.

formation of unsaturated amino acids by β-elimination reactions from serine or threonine [11].

Table 1
Analyses of peptido-galactomannan and fractions from acidic and alkaline degradations.

Analysis	Original (fraction B) (%)	Fractions (%) 1a	2a	2b	1b
N	1.8	3.7	2.7	0.5	0.2
N as protein	11.5	23.1	17.0	3.0	1.2
Protein (Lowry)	9.6	19.0	23.0	2.0	2.0
Mannose*	78	78	51	87	78
Galactose	13	8	20	0	14
Mannitol	0	0	0	17	0
Total carbohydrate	91	86	71	104	92
PO_4	3.2	0.4	0.5	0.2	5.0

* Monosaccharides are calculated as the free sugar. The theoretical "percentage" therefore exceeds 100.

3. Discussion

The experiments demonstrate that the peptido-phosphogalactomannan from *C. werneckii* is a complex which contains both acid-sensitive and alkali-sensitive linkages. The former would appear to be phosphodiester linkages (although it is difficult to exclude a few internal glycofuranosyl linkages). The alkali-sensitive linkages occur between the carbohydrate and the peptide moieties and are probably glycosyl linkages to serine and threonine [12]. These two amino acids make up 42% of the peptide [9].

The fragments (1b) released by acid hydrolysis, with concomitant scission of phosphate diesters, contained mannose and most of the phosphate and much of the galactose of the original. These fragments, representing about half of the weight of the original complex, are galactomannan phosphates of relatively low molecular weight (ca. 2500) with galactose:mannose:phosphate ratios of 1.5 : 8.2 : 1.0. Treatment of the peptide-rich, acid-stable fraction (1a) with $NaOH–NaBH_4$ released small, uncharged, oligosaccharides containing only mannose and mannitol. A small portion of the carbohydrate (about 10% of the original) is not susceptible to mild acid hydrolysis and is linked to the peptide by alkali-stable linkages (fraction 2a).

The structure shown in fig. 2 would explain the above results. The degradation experiments are explained as follows: mild acid hydrolysis cleaves bonds A releasing the phosphogalactomannan units. Remaining is a peptide-rich moiety consisting mainly of short mannosyl oligosaccharides linked to peptide by alkali-labile bonds. These are released as mannitol-terminated oligosaccharides by scission of linkages B with $NaOH–NaBH_4$. The structure is consistent with preliminary experiments on reversing the sequence of the

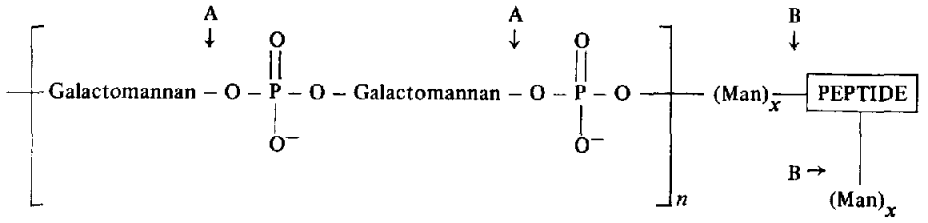

Fig. 2. Proposed structure of peptido-phosphogalactomannan complex. The number of phosphogalactomannan units in the chains is not known. The $(mannosyl)_x$ side-chains are more numerous than the phosphogalactomannan chains. Not shown in this structure is the small proportion of high molecular weight galactomannan which is linked to the peptide by alkali-stable bonds. A, acid-labile linkages. B, alkali-labile linkages.

degradations. Thus alkaline degradation gives low molecular weight, mannose-containing oligosaccharides together with a high molecular weight, nitrogen-free, poly-(phosphogalactomannan). This eliminates the possibility that the low molecular weight phosphogalactomannan fragments are linked individually by acid-labile bonds to the peptide or to the alkali-stable mannan moieties. Since galactose is absent from the fraction (2b) eliminated by alkali, it would seem that the poly-(phosphogalactomannan) chains are not linked directly to the peptide but through mannose-containing linkage regions.

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References

[1] D.H. Northcote and R.W. Horne, Biochem. J. 51 (1952) 232.
[2] A.A. Eddy, Proc. Roy. Soc. London Ser. B 149 (1958) 425.
[3] S.A. Barker, O. Basarab and C.N.D. Cruickshank, Carbohydrate Res. 3 (1967) 325.
[4] M.E. Slodki, Biochim. Biophys. Acta 57 (1962) 525.
[5] T.S. Stewart and C.E. Ballou, Biochemistry 7 (1968) 1855.
[6] W.L. McLellan and J.O. Lampen, J. Bacteriol. 95 (1968) 967.
[7] R. Sentandreu and D.H. Northcote, Biochem. J. 109 (1968) 419.
[8] T.N. Cawley and R. Letters, Biochem. J. 110 (1968) 9P.
[9] K.O. Lloyd, Biochemistry 9 (1970) 3446.
[10] K.O. Lloyd, Arch. Biochem. Biophys. 137 (1970) 460.
[11] J.P. Greenstein and M. Winitz, in: Chemistry of the Amino Acids (Wiley, New York, 1961) p. 856.
[12] B. Anderson, P. Hoffman and K. Meyer, J. Biol. Chem. 240 (1965) 156.