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Properties of a Penicillium GDP-Mannose: Glycopeptide Mannosyltransferase Solubilized With Triton X-100

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Membranes from Penicillium charlesii were separated into 6 fractions by sucrose density gradient ultracentrifugation. The least dense fraction (ρ = 1.1 g cm^{-3}) contained GDP-mannose:glycopeptide mannosyltransferases that transferred [^{14}C] mannose onto mannopyranosyl-(seryl/threonyl)-polypeptide and phosphogalactomannan regions of peptidophosphogalactomannan. Approximately 90% of the [^{14}C] mannose incorporated was isolated as mannobiose following treatment of peptidophosphogalactomannan with 0.5 N NaOH. The remainder was located in phosphogalactomannan. About 10% of the membrane-bound mannosyltransferase activity was solubilized with 1% Triton X-100. The soluble mannosyltransferase activity was purified by affinity chromatography on peptidophosphogalactomannan-Sepharose 4B and ammonium sulfate fractionation. Mannose incorporation was shown to be a function of the concentration of added acceptor. No incorporation occurred in the absence of added acceptor or when $MgCl_2$ was substituted for $MnCl_2$. Peptidophosphogalactomannan, phosphogalactomannan, phosphomannan, and mannan, each obtained by appropriate treatment of peptidophosphogalactomannan from P. charlesii, served as mannosyl acceptors. In contrast, α-mannosidase treated peptidophosphogalactomannan did not serve an acceptor of mannosyl residues. Up to 70% of the mannose from GDP-mannose was transferred to added acceptor. Treatment of [^{14}C] mannosyl-labeled peptidophosphogalactomannan with 0.5 N NaOH released 90% of the [^{14}C] mannose as phosphogalactomannan and the remainder was released as mannobiose. [^{14}C] Mannose-labeled phosphogalactomannan was subjected to acetolysis. Mannobiose was the major [^{14}C]-labeled product isolated. Significant quantities of [^{14}C] mannose were isolated also. These results show that soluble mannosyltransferase catalyzes the formation of (1–6)-linked mannosyl residues as well as the transfer of a mannosyl residue to a (1–6)-linked mannosyl residue in the phosphogalactomannan. The specificity of the enzyme is shown by its inability to catalyze mannosyl transfer to α-mannosidase treated peptidophosphogalactomannan, or to incorporate more than 2 mannosyl residues onto the phosphogalactomannan region. Presumably the second mannosyl residue is attached by a (1–2) linkage as the mannan contains only (1–6)- and (1–2)-linked mannosyl residues (Gander et al: J Biol Chem 249:2063, 1974). No evidence was obtained for the participation of a lipid-linked mannosyl-containing intermediate in this system.

Key words: **mannosyltransferase, glycopeptide, GDP-mannose, Penicillium, phosphomannan, galactofuranosyl**

Abbreviations: GDP-mannose – guanosine-5′-(α-D-mannopyranosyl pyrophosphate)

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Penicillium charlesii secretes a novel glycopeptide (peptidophosphogalactomannan) which contains a phosphogalactomannan region and 10–12 mannose-containing low-molecular-weight saccharides each attached through seryl/threonyl residues to a polypeptide of approximately 30 amino acyl residues (1, 2). The phosphogalactomannan contains approximately 90 mannopyranosyl residues, 10 phosphodiester residues, and variable quantities of 5-O-β-D-galactofuranosyl residues located in 10 galactan chains attached by (1→3) glycosidic linkage to the mannan (1). Ethanolamine (2) and N,N′-dimethylethanolamine (3) are attached to the phosphogalactomannan presumably through the phosphodiester residues. The glycopeptide is likely derived from a membrane-bound lipopeptidophosphogalactomannan (4) and not from the cell walls of the Penicillium (5). The function of the glycopeptide or its precursor(s) is unknown.

We have shown previously that a membrane fraction (1.1 g cm^{-3}) from P. charlesii contains GDP-D-mannose:glycopeptide mannosyltransferase which catalyzes the incorporation of D-mannose from guanosine-5′-(α-D-mannopyranosyl pyrophosphate), (GDP-mannose), onto both the phosphogalactomannan and mannosyl but not mannobiosyl or mannotriosyl residues of the glycopeptide (6). This preparation requires Mn^{2+}; Mg^{2+}, Ca^{2+}, Co^{2+}, or Ni^{2+} will not substitute for Mn^{2+}. Following treatment of the glycopeptide with 0.5 N NaOH, approximately 90% of the mannose incorporated was located in mannobiose, and the remainder was located in phosphogalactomannan.

Guanosine-5′-(α-D-mannopyranosyl pyrophosphate) is the mannosyl donor in the biosynthesis of mannans and phosphomannans located in cell-membrane and cell wall glycoproteins of several species of yeasts and fungi (7–13). Sharma et al. (14) showed that in Saccharomyces cerevisiae the mannosylation of seryl/threonyl residues of endogenous membrane bound protein was accomplished with mannosyl-1-phosphoryl dolichol as the mannosyl donor and that GDP-D-mannose served as the direct mannosyl donor to endogenous membrane bound mannosyl-(seryl/threonyl-protein) residues forming a mannobiosyl and larger oligosaccharide. Beyond this, the role of the lipid-mannosyl carriers have not been well delineated in fungal systems.

This paper reports the solubilization and partial purification of membrane-bound mannosyltransferase which catalyzes the incorporation of mannose from GDP-mannose into the phosphogalactomannan region of peptidophosphogalactomannan.

METHODS

Materials and Methods

Stock cultures of Penicillium charlesii G. Smith ATCC 1887 were maintained as described previously (15). GDP-D-[^{14}C] mannose was purchased from Amersham/Searle Corporation. GDP-mannose was obtained from Calbiochem. Other chemicals and reagents used were reagent grade.

Purified peptidophosphogalactomannan was obtained from cultures of P. charlesii as was described previously (1), and phosphogalactomannan, phosphomannan, and mannan were obtained from peptidophosphogalactomannan by treatment with 0.5 N NaOH, and treatment with 0.01 N HCl for 1.5 or 4 h, respectively (6).

Acetolysis of phosphogalactomannan was carried out for 18 h at 37°C as described by Stewart et al. (16). Total carbohydrate was determined by the phenol sulfuric acid procedure (17). Protein was determined by the procedure of Lowry et al. (18) after precipitation of the protein with trichloroacetic acid.

^{14}C was determined by liquid scintillation counting of 0.5-cm paper strips cut from chromatograms or of 0.5 ml of aqueous samples as described previously (6).

Paper chromatography was used to separate the reaction products (6).

Lipid-soluble [^{14}C] mannose-containing substances were determined by extracting the reaction mixture with $CHCl_3$:methanol (2:1, vol/vol) followed by removal of the solvent and chromatography of the residue on Whatman 3MM paper with 95% ethanol:1 M ammonium acetate pH 7.5 (7.5:3, vol/vol) as the developing solvent.

Preparation of Membrane Bound and Soluble Mannosyltransferases

Membrane-bound mannosyltransferase from P. charlesii mycelia was obtained from membranes (ρ = 1.1 g cm^{-3}) as described previously (6). The mannosyltransferase(s) activity was solubilized by treating the membranes in 0.05 M Tris-HCl 1% Triton X-100, pH 7.5/5 mM 2-mercaptoethanol for 30 min at 0–4°C, followed by centrifuging the mixture at 120,000 × g for 30 min in a Beckman L2-65B ultracentrifuge. The supernatant solution obtained from this treatment was made 25 mM in $MnCl_2$ and the solution passed onto a peptidophosphogalactomannan-Sepharose 4B affinity adsorbant (19). The adsorbant was washed with buffers as described in the text. Protein in the active fraction was concentrated by adding solid ammonium sulfate to 30% saturation and the protein was dissolved in Tris-HCl, pH 7.5 containing 1% Triton X-100 and 5 mM 2-mercaptoethanol.

RESULTS

Distribution of [^{14}C] Mannose in Peptidophosphogalactomannan

We reported previously that membranes (ρ = 1.1 g cm^{-3}) from P. charlesii incorporate mannose from GDP-mannose into added peptidophosphogalactomannan as well as into endogenous acceptors (6). Membrane-bound mannosyltransferase was incubated with GDP-D-[^{14}C] mannose, $MnCl_2$, and peptidophosphogalactomannan for 2 h, and the peptidophosphogalactomannan was isolated (1), was treated with 0.5 N NaOH to β-eliminate the saccharides from the polypeptide, and was chromatographed to separate the phosphogalactomannan (fractions 1–4) from mannobiose (fractions 56–62) (Fig. 1a). Approximately 90% of the ^{14}C was located in the mannobiosyl fraction. The experiment was repeated with soluble mannosyltransferase substituted for the membrane-bound enzyme (Fig. 1b). About 10% of the mannosyltransferase was solubilized in 1% Triton X-100 and most of this activity was directed toward mannosylation of phosphogalactomannan. Most of the mannosyltransferase activity remained in the residue following treatment with Triton X-100 (Tonn and Gander, unpublished).

Experiments were conducted to determine if [^{14}C] mannose was incorporated into substances soluble in $CHCl_3$:methanol (2:1, vol/vol). Reaction mixtures containing soluble mannosyltransferase and approximately 6×10^4 cpm in GDP-[^{14}C] mannose were incubated for varying intervals with, or without, added peptidophosphogalactomannan and with either $MnCl_2$ or $MgCl_2$. The reaction mixtures were extracted with $CHCl_3$:methanol, the solvent was removed, and the residues were solubilized and chromatographed on paper. The radioactivity in the $CHCl_3$:methanol phase was variable and never exceeded 300 cpm. The distribution of radioactivity on a chromatogram showed 2 ^{14}C-containing $CHCl_3$:methanol-soluble substances which migrate like GDP-mannose and mannose (not shown). They likely represent GDP-mannose and mannose carried over from the aqueous phase. The quantity in the $CHCl_3$:methanol phase is insignificant. Significant quantities of mannose and mannose-1-phosphate were found in the aqueous phase along with unreacted

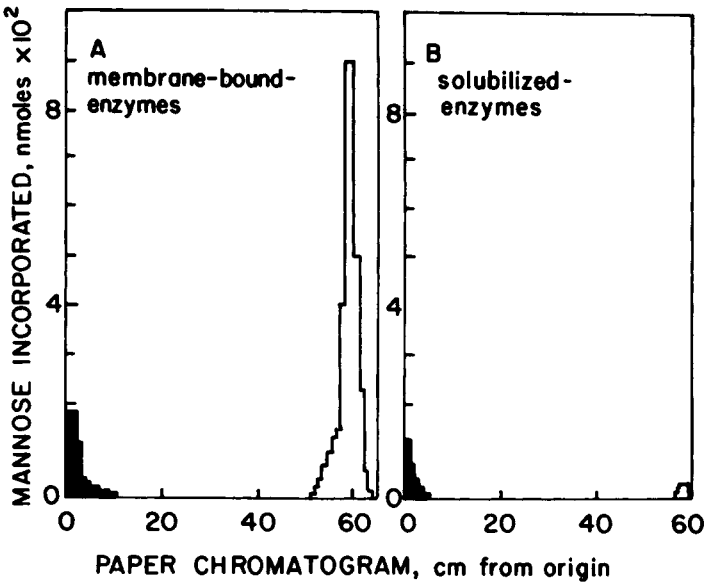

Fig. 1. Distribution of [^{14}C] mannose-containing saccharides on paper chromatograms after treating the peptidophosphogalactomannan with 0.5 N NaOH. The complete system, in a final volume of 60 μl, contained Tris-maleate, pH 7.0, in 5 mM 2-mercaptoethanol; GDP-D-[^{14}C] mannose, 5 nmole; $MnCl_2$, 1.5 μmole; 2-mercaptoethanol, 0.3 μmole; peptidophosphogalactomannan, 3 mg; and membrane preparation 150 μg of protein (A) or 49 μg of protein from membranes treated with 1% Triton X-100 (B). The reaction mixture was incubated for 2 h, the reaction was stopped by heating at 100°C for 2 min, and it was dialyzed against distilled deionized water to remove low-molecular-weight substances. The dialyzed reaction mixture was treated with 0.5 N NaOH (1) and was concentrated and applied to Whatman 3MM paper and chromatographed for approximately 16 h in 95% ethanol, 1 M ammonium acetate, pH 7.5 (7.5:3, vol/vol). The chromatograms were sectioned and ^{14}C determined as described in Materials and Methods.

GDP-mannose and immobile [^{14}C] polymer. We have shown previously that more than 70% of ^{14}C from the polymer is recovered as peptidophosphogalactomannan (6) in a system known to incorporate mannose into endogenous acceptors as well as into peptidophosphogalactomannan.

Purification of Mannosyltransferase(s)

The requirement for Mn^{2+} as a cofactor in the peptidophosphogalactomannan: mannosyltransferase catalyzed reaction suggested that the enzyme might require Mn^{2+} as a cofactor in binding the glycopeptide. The Triton X-100 solubilized mannosyltransferase preparation containing 25 mM $MnCl_2$ was passed onto a peptidophosphogalactomannan-Sepharose 4B affinity column and the adsorbant washed successively with 1) 0.05 M Tris-maleate, pH 7.5, buffer containing 25 mM $MnCl_2$ and 2-mercaptoethanol; 2) 0.05 M Tris-maleate, pH 7.5, buffer containing 0.1% Triton X-100 and 5 mM 2-mercaptoethanol; and 3) 0.05 M Tris-maleate buffer, pH 7.5, containing 0.1% Triton X-100, 5 mM 2-mercaptoethanol and 0.5 M NaCl (Fig. 2). Mannosyltransferase was released from the adsorbant with an eluent containing no $MnCl_2$. The protein in fractions 32–42 was concentrated by

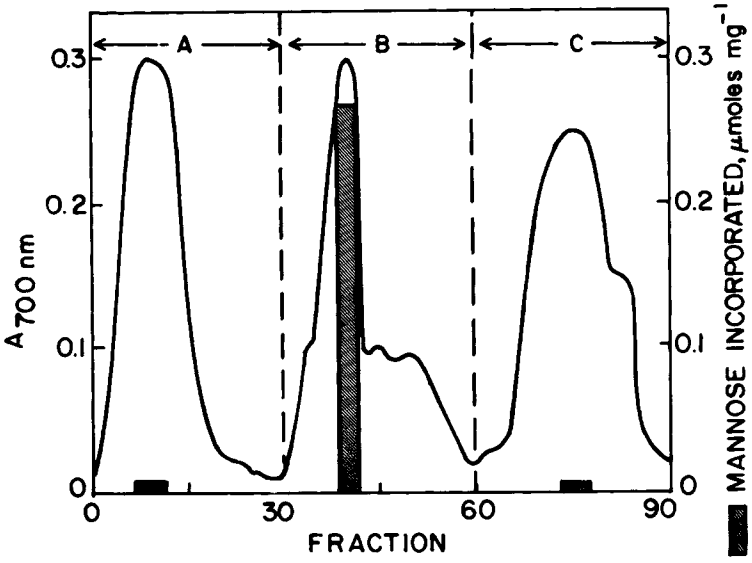

Fig. 2. Partial purification of soluble-mannosyltransferase by affinity chromatography on peptidophosphogalactomannan-Sepharose 4B. Membranes were prepared as described in Materials and Methods section and were extracted with 1% Triton X-100. The supernatant obtained following centrifuging the mixture at 120,000 × g for 30 min was made 25 mM in $MnCl_2$ and applied to a column containing peptidophosphogalactomannan-Sepharose 4B. The adsorbent was washed with 0.05 M Tris-maleate buffer, pH 7.5, containing 5 mM 2-mercaptoethanol and 25 mM $MnCl_2$ (A), 0.05 M Tris-maleate buffer, pH 7.5, containing 5 mM 2-mercaptoethanol (B), and 0.05 M Tris-maleate buffer, pH 7.5, containing 0.5 M NaCl and 5 mM 2-mercaptoethanol (C). Each fraction contained approximately 1 ml. An aliquot was removed and the protein was precipitated with trichloroacetic acid and the protein was determined by the Lowry procedure (18) (——). An aliquot was removed from the fraction containing the greatest quantity of protein from each of the 3 solvents and mannosyltransferase activity was determined (▒) as described in Fig. 1 except the reaction mixture was not dialyzed or treated with NaOH. The nmole of mannose incorporated was calculated from ^{14}C remaining at the origin of the chromatogram.

the addition of solid ammonium sulfate to 30% saturation and the residue was redissolved in Tris-maleate buffer, pH 7.5, containing 1% Triton X-100 and 5 mM 2-mercaptoethanol.

Influence of the Concentration of Mannosyltransferase, GDP-mannose, and $MnCl_2$ on Mannosyltransferase Activity

The activity of the partially purified mannosyltransferase is linear over a range of protein concentrations (not shown). Optimum mannosyltransferase activity is observed over a range of 25–32 mM $MnCl_2$ (Fig. 3). Negligible mannosyltransferase activity was observed if $MnCl_2$ is omitted or if $MnCl_2$ was replaced with $MgCl_2$ (Fig. 4). The requirement for Mn^{2+} was not decreased by the addition of 5, 10, or 20 mM $MgCl_2$. Some inhibition of mannose incorporation was observed upon the addition of $MgCl_2$ to reaction mixtures containing 10 or 20 mM $MnCl_2$ (Table I).

Mannosyltransferase activity increased with increasing GDP-mannose concentration (not shown). Half maximal activity was observed at about 0.6 mM GDP-mannose.

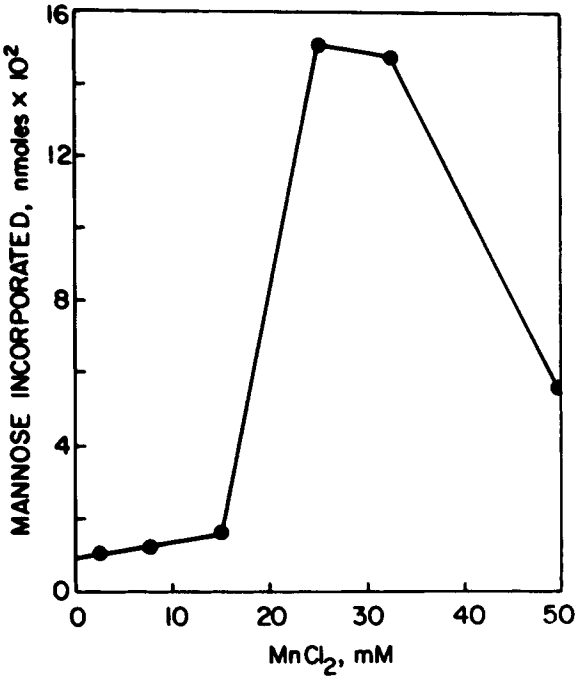

Fig. 3. Influence of $MnCl_2$ concentration on mannosyltransferase activity of the partially purified enzyme preparation. Mannosyltransferase was obtained from affinity chromatography as described in Fig. 3. The protein was concentrated by precipitation with 30% saturated ammonium sulfate and 18 μg of protein was used in each assay which contained all reaction components described in Fig. 1, except the final $MnCl_2$ concentration was as indicated on the abscissa. The mannosyltransferase was assayed as described in Fig. 3.

TABLE I. Influence of $MgCl_2$ on Mannosyltransferase Activity in the Presence of $MnCl_2$

	$MgCl_2$ concentration, mM			
$MnCl_2$ concentration, mM	0	5	10	20
	mannosyltransferase activity, nmol/mg protein			
0	0.4	0.1	0.1	0.3
5	2.1	1.3	1.8	1.4
10	3.1	1.8	2.1	2.1
20	2.7	2.9	1.8	2.2
25	2.6	–	–	–

The reaction conditions were those described in Fig. 1B except the concentration of divalent metal ion(s) were as shown above. Each reaction mixture contained 77 μg of protein.

Influence of Mannosyl Acceptor Concentration of Mannosyltransferase Activity

Peptidophosphogalactomannan was degraded stepwise to its various constituents: phosphogalactomannan, phosphomannan, and mannan. In addition, peptidophosphogalactomannan was treated with jack bean exo-α-mannosidase which degrades the oligosaccharides

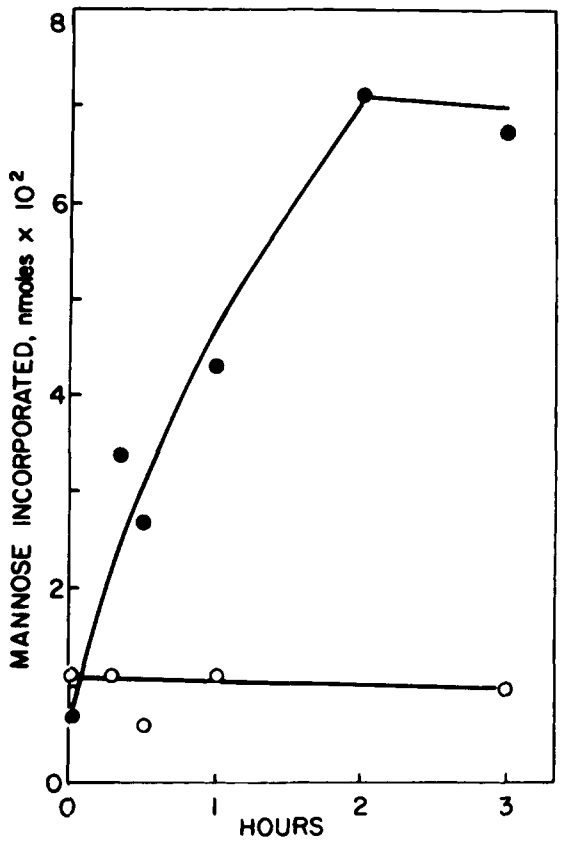

Fig. 4. Time course of mannose incorporation into peptidophosphogalactomannan in the presence of $MnCl_2$ (•) or $MgCl_2$ (○). Partially purified mannosyltransferase (17 μg) was incubated in reaction mixtures as described in Fig. 1. The reactions were stopped by heating for 2 min at 100°C after the times indicated on the abscissa and the remainder of the assay was conducted as described in Fig. 3. A separate series of reactions were conducted in which 25 mM $MgCl_2$ was substituted for $MnCl_2$.

attached to the polypeptide to mannosyl-(seryl/threonyl)-polypeptide and degrades the phosphogalactomannan region from the nonreducing terminal mannosyl residue to the first phosphomannosyl residue. All the mannan-containing polymers, except the exo-α-mannosidase treated polymer, served as mannosyl acceptors (Fig. 5) using the solubilized mannosyltransferase. Removal of the polypeptide from the glycopeptide appears to improve the polymer as a potential mannosyl acceptor from GDP-mannose. Removal of the galactan chains also improves the mannan as an acceptor when used at 10 mg 60 μl^{-1}. The long galactan chains may interfere because of increased polymer-polymer interaction with increasing concentration of mannosyl acceptor.

Acetolysis of [^{14}C] Mannose-containing Phosphogalactomannan

A reaction mixture containing phosphogalactomannan as the mannosyl acceptor was incubated 2h, and after removal of the low-molecular-weight substances by dialysis the phosphogalactomannan was isolated by precipitation as its borate-cetyltrimethylammonium

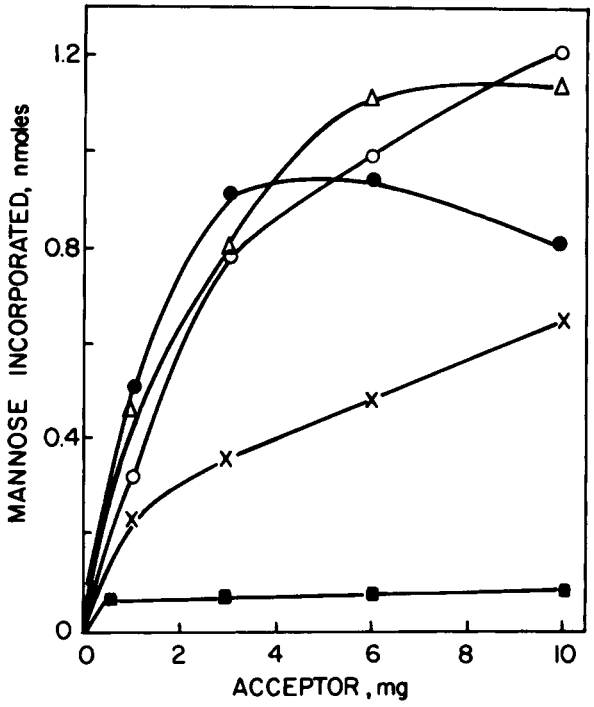

Fig. 5. Influence of acceptor concentration on mannosyltransferase activity. Crude soluble mannosyltransferase (26 μg) was incubated in reaction mixtures as described in Fig. 1, except the concentration of the acceptor was as shown in the abscissa. The reaction was stopped and assayed as described in Fig. 3. Peptidophosphogalactomannan (X), phosphogalactomannan (•), phosphomannan (○), mannan (△), and α-mannosidase-treated peptidophosphogalactomannan (■) were tested as mannosyl acceptors.

complex, followed by chromatography on DEAE-cellulose-borate (1). The fractions containing the polymer were dialyzed extensively and the samples were freeze-dried. The phosphogalactomannan was subjected to acetolysis for 18 h (16), and the products were deacetylated and fractionated on a Bio-Gel P-2 column (1). [^{14}C] Mannose was located primarily in mannobiose with lesser quantities of ^{14}C in mannose (Fig. 6). There were negligible quantities of ^{14}C in other carbohydrate-containing fractions. The large quantity of carbohydrate in the monosaccharide fraction comes from galactose which, being in the furanosyl form, is cleaved to its monomer. This figure shows that mannosyl residues were incorporated into the phosphogalactomannan in a manner to form (1–6)-linked mannosyl and mannobiosyl residues.

DISCUSSION

GDP-mannose:glycopeptide mannosyltransferase(s) that catalyze the transfer of mannosyl residues to the phosphogalactomannan region of peptidophosphogalactomannan is easily solubilized by Triton X-100. This contrasts sharply with the lack of solubility in a number of detergents of the mannosyltransferase that catalyzes the transfer of mannosyl

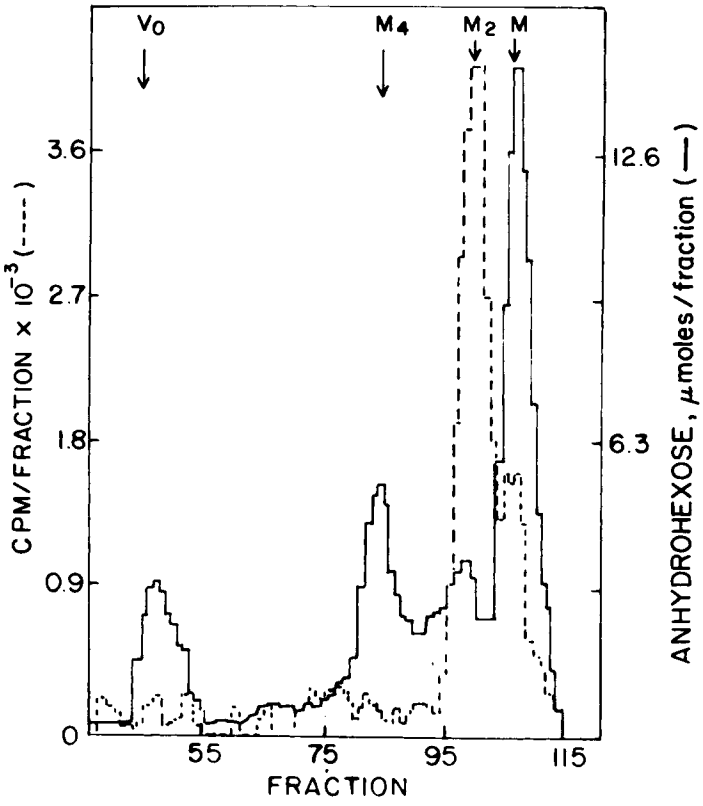

Fig. 6. Location of [^{14}C] mannosyl residues derived from [^{14}C] phosphogalactomannan following acetolysis. A reaction mixture containing 30 mg of phosphogalactomannan, 125 μg of crude mannosyltransferase, and other components at the concentrations given in Fig. 1 was incubated in a total volume of 600 μl for 2 h and the reaction was stopped by heating for 2 min at 100°C. The reaction mixture was dialyzed against distilled H_2O, and the phosphogalactomannan was isolated as described previously (1). Twenty milligrams of phosphogalactomannan was obtained. The polymer was subjected to acetolysis (1, 16), the products were deacetylated and fractionated on Bio-Gel P-2 (minus 400 mesh). The anhydrohexose (——) and ^{14}C (---) were determined in each fraction. The void volume, v_0, and the position of elution of mannose, M; mannobiose, M_2; mannotetraose, M_4 are given on the figure.

residues to mannosyl-(seryl/threonyl)-polypeptide acceptor (Tonn and Gander, unpublished). Thus, the soluble mannosyltransferase(s) are bound to the membranes relatively weakly.

The soluble mannosyltransferase was partially purified by affinity chromatography using peptidophosphogalactomannan as the ligand in a medium containing 25 mM $MnCl_2$. Removal of $MnCl_2$ from the buffered medium released the enzyme (Fig. 2). This shows that the mannosyltransferase has little affinity for peptidophosphogalactomannan in the absence of Mn^{2+}. It is of particular interest to note that the mannan, resulting after removal of phosphoryl and galactofuranosyl residues, was an excellent mannosyl acceptor. This suggests that the role of Mn^{2+} is to modify the enzyme so that it binds substrate rather than the alternative possibility that Mn^{2+} alters the conformation of the mannan

by complexing with the mono-oxy anions of phosphomannan since Mn^{2+} binding to mannan would be weak at best.

The optimum concentration of $MnCl_2$ was approximately 20 mM which is several-fold greater than that usually found in physiological systems. However, maleate from the buffer most likely complexed a major portion of the divalent cation which resulted in the requirement for an aphysiological concentration of $MnCl_2$. Furthermore, it is apparent from Fig. 3 that $MgCl_2$ will not replace $MnCl_2$, nor will $MgCl_2$ reduce the quantity of $MnCl_2$ required for optimum mannosyltransferase activity (Table I). Other divalent cations (Fe^{2+}, Co^{2+}, Ca^{2+}, and Ni^{2+}) have been shown previously (6) to provide minimal or no activation of mannosyltransferase activity in the absence of Mn^{2+}.

We have no evidence for the participation of a lipid-linked mannose-containing substance as an intermediate in the incorporation of mannosyl residues into either the mannosyl-(seryl/threonyl)-polypeptide (6) or the phosphogalactomannan. The insignificant quantity of ^{14}C in the chloroform:methanol phase, following extraction of the reaction mixture containing crude, soluble mannosyltransferase with $CHCl_3$:methanol (2:1, vol/vol), could be accounted for as GDP-mannose and mannose that were carried through the extraction procedure. ^{14}C was found in only GDP-mannose and the polysaccharide following paper chromatography when the partially purified mannosyltransferase was used (not shown). Thus the solubilized crude enzyme preparation degraded GDP-mannose to mannose-1-phosphate and mannose. The time course of mannose incorporation was linear during the earliest times. However, this evidence is, at best, indirect in excluding a lipid-linked intermediate.

The acetolysis data show that the soluble crude mannosyltransferase(s) catalyze the transfer of mannosyl residues from GDP-mannose to either mannosyl-(1–6)-mannan or to mannan to give mannobiosyl-(1–6)-mannan and mannosyl-(1–6)-mannan, respectively, Currently we do not know if the mannosyl residues are added at the nonreducing terminal end of the mannan or if they branch from some internal mannosyl residue.

The concentration of peptidophosphogalactomannan used was approximately 8-fold larger than the concentration of GDP-mannose in the routine assay. We calculate that only 1 in 40 peptidophosphogalactomannan molecules in the system were mannosylated. If the polymer is released from the enzyme after each transfer of a mannosyl residue then it is improbable that any 1 mannan would have received more than 1 mannosyl residue in this system. We do not have information concerning the heterogeneity of the nonreducing terminal region of the phosphogalactomannan. For instance, the peptidophosphogalactomannan may be composed of molecules containing (1–6)-linked mannosyl, mannobiosyl, mannotriosyl, mannotetraosyl, and phospho-mannotetraosyl residues at the nonreducing terminus.

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REFERENCES

1. Gander JE, Jentoft NH, Drewes LR, Rick PD: J Biol Chem 249:2063, 1974.
2. Rick PD, Drewes LR, Gander JE: J Biol Chem 249:2073, 1974.
3. Gander JE: Exp Mycol 1:1, 1977.
4. Beachy J; Fed Proc Fed Am Soc Exp J Biol 35:1642, 1976.
5. Gander JE, Fang F: Biochem Biophys Res Commun 71:719, 1976.
6. Gander JE, Drewes LR, Fang F, Lui AJ: J Biol Chem 252:2187, 1977.
7. Mayer RM: Biochim Biophys Acta 252:39, 1971.
8. Bretthauer RK, Kozak LP, Irwin WE: Biochem Biophys Res Commun 37:820, 1969.
9. Raizada MK, Kloepfer HG, Schutzbach JS, Ankel H: J Biol Chem 249:6080, 1974.
10. Raizada MK, Schutzbach JS, Ankel H: J Biol Chem 250:3310, 1975.
11. Behrens NH, Cabib E: J Biol Chem 243:502, 1968.
12. Nakajima T, Ballou CE: Proc Natl Acad Sci USA 72:3912, 1975.
13. Samuel O, Nordin JH: Biochem Biophys Res Commun 45:1376, 1971.
14. Sharma CB, Babczinski P, Lehle L, Tanner W: Eur J Biochem 46:35, 1974.
15. Preston JF, Gander JE: Arch Biochem Biophys 124:504, 1968.
16. Stewart TS, Mendershausen PB, Ballou CE: Biochemistry 7:1843, 1968.
17. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F: Anal Chem 28:350, 1956.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: J Biol Chem 193:265, 1951.
19. Rietschel-Berst M, Jentoft NH, Rick PD, Pletcher C, Fang F, Gander JE: J Biol Chem 252:3219, 1977.