Glycoprotein Biosynthesis in *Phytophthora megasperma* f. sp. *glycinea*. An *in vitro* Study

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Extracellular glycoproteins have been claimed to represent the specificity determinants within different races of *Phytophthora megasperma* f. sp. *glycinea* (formerly *P. m.* var. *sojae*) specifically infective in different soybean cultivars. Microsomal preparations of three different races of Pmg are capable of synthesizing both N-glycosidically linked oligosaccharides and short O-glycosidically linked oligomannoses *in vitro* from corresponding sugar nucleotides. In addition, lipidic glycosidic intermediates (dolichol type derivatives) serve as specific glycosyl donors. In general, the results demonstrate that the well-known ubiquitous biosynthetic pathway of asparagine-di-N-acetyl-chitobiose type glycoproteins is established in that fungus. The significance of the results regarding specific elicitors, suppressors, protectants, and extracellular glycoenzymes is discussed.

Introduction

Surface glycoproteins play a crucial role in many specific cellular recognition systems [1–6]. In the case of plant host-parasite interactions glycoproteins have been demonstrated to represent important regulatory molecules, namely by eliciting [7] or suppressing phytoalexin production [8] or acting as protectants against infections [9].

Phytophthora megasperma f. sp. *glycinea* (formerly *P. m.* var. *sojae*) is the causal agent of root and stem rot of soybeans. At least nine races and several differential host plant cultivars have been described. This host-parasite system is unique in that most of the presently discussed biochemical mechanisms (regarding glycoproteins) involved in host-parasite interactions have been demonstrated or claimed, *i.e.* unspecific elicitation of phytoalexin production [10], specific elicitors [7], and specific suppressors [8] of phytoalexin production, race-specific protectants against infections [9, 11] endogenous elicitors [12, 13], and race-specific extracellular glycoproteins [8, 14, 15].

Many fungi are known to synthesize and secrete extracellular glycoproteins and glycoenzymes [16]. The biochemical mechanism of glycoprotein biosynthesis is well-known and has been worked out in numerous biological systems, including fungi [17]. Mycelial cell walls of *Phytophthora* sp. contain approximately 6% protein, 3% mannose, and less than 1% hexosamine [10]. Although glycoproteins may represent important factors in Pmg-soybean interactions, biosynthesis of relevant glycoproteins has not yet been studied. It is the aim of this report to demonstrate the biosynthetic pathway leading to glycoproteins in different races of Pmg.

Materials and Methods

Materials

GDP-[^{14}C]mannose (192 Ci/mol) and UDP-[^{14}C]-glucose (229 Ci/mol) were obtained from New England Nuclear, and UDP-N-acetyl-[^{14}C]glucosamine (300 Ci/mol) was purchased from Amersham. Endo-N-acetylglucosaminidase H (endo H) was from Miles Laboratories, and pronase from Sigma.

Tunicamycin was kindly supplied by Dr. R. L. Hamill (Eli Lilly, Indianapolis, IN, USA), and diumycin (Squibb SQ 20117) was a generous gift from Mr. S. J. Lucania (Squibb Institute, Princeton, NJ, USA).

Abbreviations: Dol, dolichol; Dol-P, dolichyl monophosphate; Dol-PP, dolichyl diphosphate; GDP, guanosine diphosphate; Glc, glucose; GlcNAc, N-acetylglucosamine; $(GlcNA)_2$, di-N-acetylchitobiose; Man, mannose; Pmg, *Phytophthora megasperma* f. sp. *glycinea* (formerly *Phytophthora megasperma* var. *sojae*); Pmg 1, 2, and 3, physiological races 1, 2, and 3 of Pmg; UDP, uridine diphosphate.

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Culture of Phytophthora megasperma f. sp. glycinea

Stock cultures of races 1, 2, and 3 (kindly supplied by Dr. E. Ziegler, Biol. III, RWTH Aachen, FRG), were maintained on V-8 juice agar plates at room temperature. A cork borer was used to obtain culture discs for inoculation (every 4 wk). Precultures were grown in 1 l flasks containing 300 ml of sucrose-asparagine medium [18] by inoculation with culture discs. Intermittently, liquid cultures were vigorously swirled by hand. Cultures were grown in petri plates (20 cm in diameter) containing 150 ml of sucrose-asparagine medium. Inoculation was performed by adding 5 ml of mechanically disrupted preculture mycelium (Omnimix, Sorvall, 5 s). Cultures were grown for 8 days at 28 °C with intermittent shaking by hand.

Microsomal membrane preparation

Fungal mycelia were harvested by filtration and washed with 50 mM Tris-HCl, pH 7.4 containing 0.15 mM $MgCl_2$, and broken in a glass bead homogenizer (Braun-Melsungen) at 4 °C, using 20 g of wet mycelium, 20 g of glass beads (0.45–0.5 mm in diameter), and 20 ml of 50 mM Tris-HCl, pH 7.4 containing 0.15 mM $MgCl_2$. Cell disruption was achieved in 1 min at highest intensity. Glass beads were filtered off, and the homogenate was centrifuged at $5000 \times g$ (10 min), and then at $50{,}000 \times g$ (20 min). The membrane pellet was washed once with the same buffer and suspended in 50 mM Tris-HCl, pH 7.4 containing 3.5 mM $MgCl_2$. This microsomal membrane preparation was adjusted to appropriate protein concentrations. Typically, absorbance measurement ($\lambda = 578$ nm) of membrane suspension gave a value of $\Delta E = 0.275$ corresponding to 150 µg protein/10 µl of 50 mM Tris-HCl, pH 7.4 (3.5 mM $MgCl_2$). A calibration curve with bovine serum albumin as protein standard was used.

Glycosyl transferase assays

The well-established methods for the yeast system were used throughout this study, *i.e.* mannosyltransfer from GDP-[^{14}C]mannose (0.1 µCi) into glycoprotein and lipid [19], mannosyltransfer from Dol-P-[^{14}C]mannose into polymer [19], N-acetylglucosaminyltransfer from UDP-N-acetyl-[^{14}C]glucosamine (0.1 µCi) into lipid, lipid-oligosaccharide and glycoprotein [20, 21], glycosyltransfer from UDP-N-acetyl-glucosamine and GDP-mannose into Dol-PP-N-acetyl-[^{14}C]glucosamine [22], glucosyltransfer from UDP-[^{14}C]glucose (0.1 µCi) into lipid, lipid-oligosaccharide, and polymer [23].

Radioactive labelled dolichol derivatives were prepared according to published methods, *i.e.* Dol-P-[^{14}C]mannose [24], Dol-PP-N-acetyl-[^{14}C]glucosamine, and Dol-PP-di-N-acetyl-[^{14}C]chitobiose [20]. Samples of pure Dol-PP-N-acetyl-[^{14}C]glucosamine, Dol-PP-di-N-acetyl-[^{14}C]chitobiose, and Dol-P-[^{14}C]glucose were kindly provided by Dr. L. Lehle.

Other procedures

Pronase digestion [24], β-elimination [24], and endo-N-acetylglucosaminidase H treatment [23] were performed as described. Lipid hydrolysis was conducted as in [20]. Antibiotics, *i.e.* tunicamycin [22] and diumycin [25] were assayed by published procedures.

Thin layer chromatography was performed on Silica gel G plates (Merck) in chloroform-methanol-water = 65:25:4 (v/v, unless stated otherwise), and paper chromatograms (Whatman No. 1) were run in ethylacetate-butane-1-ol-acetic acid-water = 30:40:25:40 (v/v) unless stated otherwise. Carbohydrates were located with alkaline silver nitrate. Protein was measured according to Lowry *et al.* [26].

Radioactivity was measured by liquid scintillation counting in a dioxane cocktail or by radioscanning of paper chromatograms and thin layer plates.

Results

1. Mannosyltransfer from GDP-[^{14}C]mannose

Microsomal preparations from Pmg incorporate radioactivity from GDP-[^{14}C]mannose into methanol-insoluble polymer and into a chloroform/methanol-soluble lipid fraction. The latter's synthesis precedes polymer formation (Fig. 1) and is rapidly saturated at low levels of radioactivity incorporated. These properties of the lipid fraction indicate that it might be an intermediate in polymer biosynthesis as previously demonstrated in yeast [27].

Incorporation of mannose into polymer is linear with time (up to 10 min, 100 µg of protein, Fig. 1) and amount of microsomal protein in the incubation mixture (up to 100 µg of protein (10 min incubation), Fig. 2). The latter also holds for the

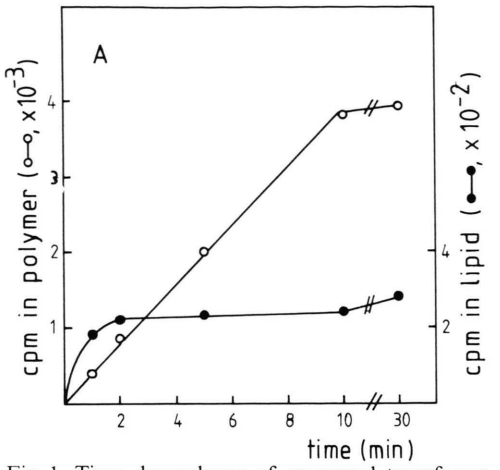

Fig. 1. Time dependence of mannosyl transferase activity from GDP-[^{14}C]mannose (A); microsomal membranes from Pmg 1 (100 µg of protein) were incubated as described under Materials and Methods.

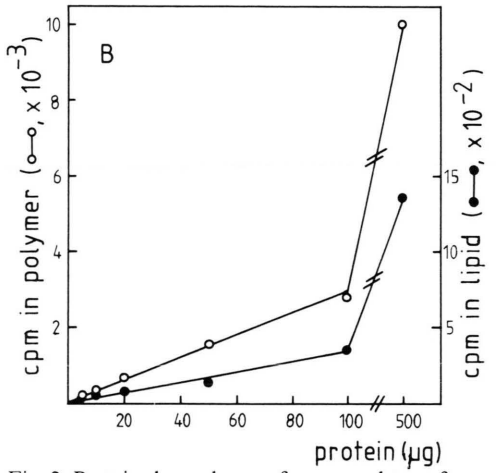

Fig. 2. Protein dependence of mannosyl transferase activity from GDP-[^{14}C]mannose (B). Microsomal membranes from Pmg 1 were incubated for 10 min.

formation of labelled lipid. Longer incubation periods lead to the formation of significant amounts of [^{14}C]mannose-1-P probably generated by a phosphodiesterase present in the incubation mixture.

As compared with the yeast system [27] the Pmg microsomal system is much less active. This might be explained by the extremely low amount of glycoproteins present in the oomycete cell wall as compared to yeast (Table I).

Incorporation of radioactivity into polymer and lipid is dependent on the age of the fungal mycelial culture (data not shown). A linear increase of incorporation into both fractions can be observed after 8 to 10 days growth; within that period the fungal mycelia show logarithmic radial growth. A slight increase in incorporation can be achieved by storing microsomal preparations at −20 °C for 24 h.

On the basis of gross incorporation of radioactive mannose into lipid and polymer no significant differences are observed among membrane preparations from three different races of Pmg (Table II).

2. *Characterization of [^{14}C]mannose-labelled polymer*

During all incubations a high background "incorporation" of radioactivity into polymer is observed which is probably due to non specific adsorption of labelled substrate to endogenous (glucan?) acceptors. Therefore, all results were corrected by the respective control values.

In order to characterize the polymer fraction synthesized, the labelled material was purified by boiling in SDS (7%, 30 min, 10 mM NaP_i, pH 7.0), chromatography (Sephadex G 25, 7% SDS/H_2O), dialysis, and lyophilization.

Table I. Cell wall composition of *Phytophthora megasperma* f. sp. *glycinea* and *Saccharomyces* sp., respectively.

	Pmg, mycelial wall [a] [%]	*Saccharomyces* sp., cell wall [b] [%]
Total carbohydrate	60	60
Total protein	6.7	13
Cell wall polysaccharides:		
Glucose	95	47
Mannose	3	50
Hexosamine	1	3

[a] Ref. 10.
[b] Ref. 16.

Table II. Mannosyl transfer from GDP-[^{14}C]mannose. Microsomes from three different Pmg races. Two separate microsomal membrane preparations were incubated as described in Materials and Methods. 500 µg of protein was present in the assay, and incubations were run for 30 min.

	Experiment 1		Experiment 2	
	Protein [cpm]	Lipid [cpm]	Protein [cpm]	Lipid [cpm]
Pmg 1	17 280	1210	31 810	1960
Pmg 2	24 980	1210	28 880	2360
Pmg 3	22 250	1370	27 080	2520

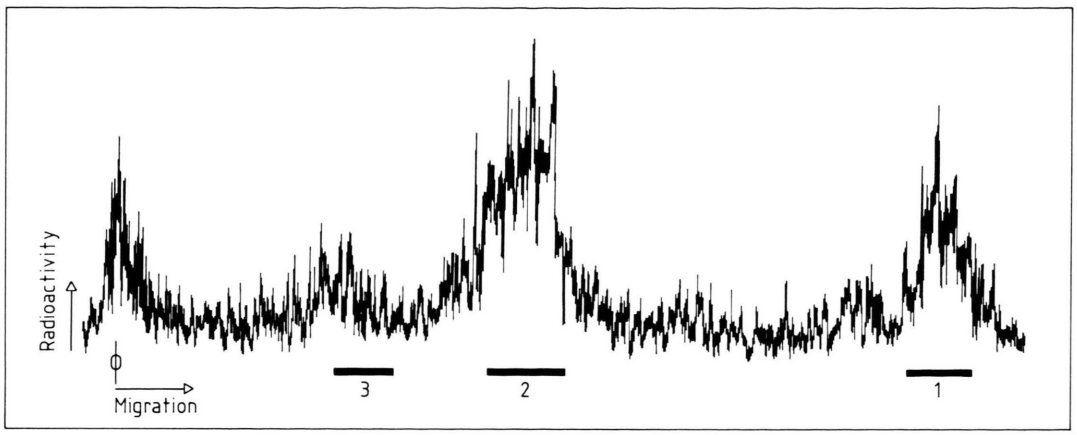

Fig. 3. Chromatographic separation of β-elimination products after incubation of Pmg 1 microsomes with GDP-[^{14}C]mannose. Purified methanol-insoluble polymer was treated with 0.1 N NaOH for 24 hr (20 C). Dialyzable products were separated on paper in ethyl acetate/butane-1-ol/acetic acid/water = 30:40:25:40 (v/v). Bars indicate standard sugars (1, mannose; 2, maltose; 3, raffinose).

Upon treatment of the resulting material with 0.1 N NaOH for 24 h at room temperature, which is fairly specific for cleavage of O-glycosidic linkages between sugars and serine/threonine, 7.5% of the total radioactivity is released as small molecular weight material (Pmg 2), whereas 16.7% is released from Pmg 1. At this low level, this difference probably lies within the experimental error range.

Upon paper chromatography, β-eliminated material in both cases appears to migrate as a series of oligosaccharides, presumably comprising mannoses as sugar components (Fig. 3). This situation would be identical to other fungal systems where O-glycosidically bound mannose and oligomannoses are well-known components of membrane-bound glycoproteins [19, 24, 28].

Upon treatment of the purified polymeric material of any race with pronase, 87 to 90% of the total radioactivity is liberated as low molecular weight glycopeptides. This indicates that, with all three races, glycoprotein material is synthesized when GDP-[^{14}C]mannose is used as sugar donor. Approximately one half of the [^{14}C]labelled glycopeptide fraction is bound to Sepharose-ConA and can be eluted with 50 mM α-methylglucoside.

Upon treatment of [^{14}C]mannose-labelled polymer with endo-N-acetylglucosaminidase H most of the radioactive material appears, upon chromatography (Bio-Gel P30), as two or three more or less well separated distinct oligosaccharides. This material has not been characterized further. Significant differences between the three Pmg races were not observed. Nevertheless, these results clearly show that most of the radioactivity incorporated from GDP-[^{14}C]mannose may be bound to protein via asparagine-chitobiose linkages.

3. Characterization of [^{14}C]mannose-labelled lipid

Upon thin layer chromatography of the lipophilic fraction (Figs. 1 and 2) two radioactive peaks are evident, one of which migrates at the R_f of mannosylphosphoryl-dolichol (Dol-P-man, Fig. 4). The minor second peak has not been investigated

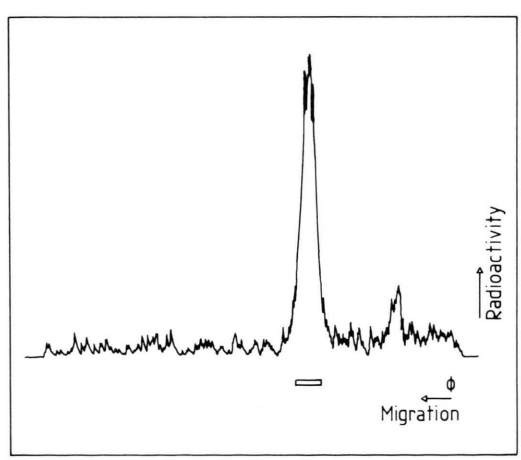

Fig. 4. Endogenous [^{14}C]-lipid fraction after incubation of Pmg 1 membranes with GDP-[^{14}C]mannose. 500 µg of protein was incubated for 30 min. Purification of lipid fraction and thin-layer chromatography as described in Materials and Methods. Bar indicates position of yeast Dol-P-mannose.

Table III. Dol-P-mannose formation from yeast Dol-P and GDP-[^{14}C]mannose by Pmg 1 microsomal membranes. Purified lipid fractions from the incubation mixtures (100 µg of protein) were separated by thin layer chromatography. Areas with R_f value corresponding to Dol-P-mannose were scraped out and counted in scintillation cocktail (corrected against background).

	Dol-P-[^{14}C]mannose formation [cpm]		
Incubation time	15 min	30 min	60 min
Control			290
5 µg Dol-P added	780	1390	1960
10 µg Dol-P added			3620
5 µg Dol-P and (after 30 min) 7 mM GDP-mannose added			390
5 µg Dol-P and diumycin (80 µg/ml) added		190	

further. Materials in the main peak were hydrolyzed with 0.1 N HCl (chloroform/methanol = 2:1, room temperature) to yield mannose as the only labelled compound. On the other hand, upon alkaline hydrolysis (propane-1-ol/1 M NaOH = 9:1, 20 min, 65 °C) most of the radioactivity was recovered upon paper chromatography in the mannose phosphate region. Both result are in agreement with the presence of lipid intermediates of the Dol-P-man type.

When yeast phosphoryldolichol (Dol-P) is added (Table III), strong stimulation only of the larger peak is evident. The stimulation is dependent upon time and amount of added Dol-P. Interestingly, addition of non-radioactive GDP-mannose leads to a drastic decrease in this peak indicating that this material shows a high mannosyltransfer potential as expected for its function as a lipid intermediate.

As in the yeast system [27], addition of GDP to the reaction mixture causes a rapid drop in the radioactivity of the lipid fraction and the formation of GDP-[^{14}C]mannose, indicating that the reaction is reversible (Fig. 5). This is evidence for the presence of a phosphodiester link in this lipid material.

Diumycin is known to inhibit Dol-P-man synthesis in microsomal preparations of yeast [25], and similar results were obtained with Pmg membrane preparations. A 86% inhibition was observed with a diumycin concentration of 80 µg/ml (Table III).

4. Mannosyltransfer from Dol-P-[^{14}C]mannose

In the yeast system, Dol-P-mannose is a potent donor of mannosyl residues incorporated into serine/threonine positions of endogenous protein acceptors [19, 24]. Negligible radioactivity was transferred from previously prepared yeast Dol-P-[^{14}C]mannose [24] into the polymer fraction by Pmg microsomes. After pre-incubation with non-radioactive UDP-GlcNAc and GDP-Man significant incorporation of radioactivity occurs (Fig. 6). GDP again fully

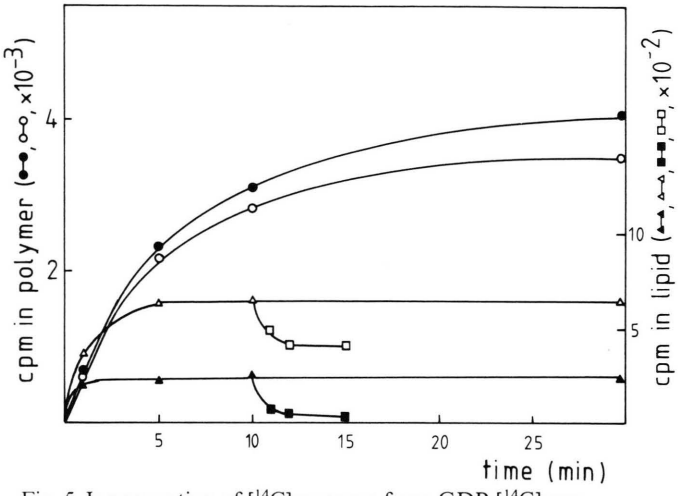

Fig. 5. Incorporation of [^{14}C]mannose from GDP-[^{14}C]mannose into lipid and polymer by Pmg 1 microsomal membranes. Stimulation by exogenous yeast Dol-P. Assay was run in presence of 100 µg of protein. ●, ○, [^{14}C]polymer; ▲, △, [^{14}C]lipid; ●, ▲, ■, without exogenous Dol-P; ○, △, □, in presence of 5 µg of yeast Dol-P; ■, □, addition of 1 mM GDP after 10 min of incubation.

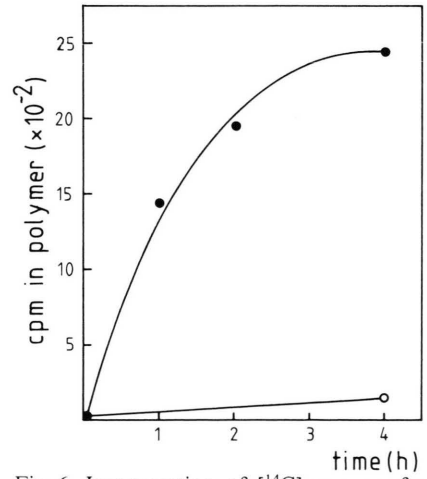

Fig. 6. Incorporation of [^{14}C]mannose from yeast Dol-P-[^{14}C]mannose into polymer (Pmg 1 microsomal membranes). Assay was run in presence of 30,000 cpm of yeast Dol-P-[^{14}C]mannose and 500 µg of protein (●—●). A pre-incubation period of 1 h in presence of 7 mM UDP-GlcNAc and 70 mM GDP-Man was performed. Control experiment was conducted in presence of 70 mM GDP (○—○).

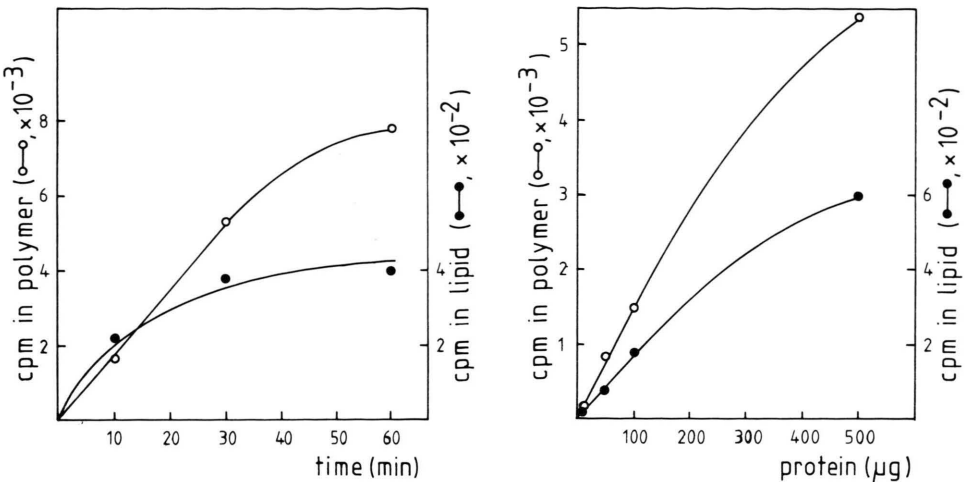

Fig. 7. Incorporation of radioactivity from UDP-[^{14}C]-GlcNAc into lipid and polymer by Pmg 1 microsomal membranes. Time dependence (left), protein dependence (right). Assay was run in presence of 500 µg of protein (left) and for 30 min (right), respectively as described in Materials and Methods.

reverses the reaction. Because of the small amount of material, it was not possible to characterize the material synthesized further.

5. Glycosyltransfer from UDP-N-acetyl-[^{14}C]glucosamine

Microsomal preparations from Pmg incorporate radioactivity into lipophilic as well as methanol-insoluble material upon incubation with UDP-N-acetyl-[^{14}C]glucosamine (Fig. 7). As with GDP-mannose, lipid formation precedes polymer synthesis and is saturated at low levels of radioactivity incorporated. Lipid and polymer formation is dependent on protein concentration (Fig. 7) and requires divalent cations as cofactors, Mg^{2+} being more active in glycolipid formation than Mn^{2+}. However, Mn^{2+} is obligatory for [^{14}C]polymer synthesis (data not shown).

In order to obtain significant incorporation of radioactivity into both fractions the protein concentration used in the incubation mixtures has to be considerably higher (approximately by a factor of ten) as compared to incorporation from GDP-[^{14}C]mannose.

Again, levels of gross incorporation do not differ significantly among the three different races of Pmg (data not shown).

6. Characterization of N-acetyl-[^{14}C]glucosamine-labelled lipid

As shown in Table IV [^{14}C]lipid formation can be stimulated by the addition of exogenous (yeast) Dol-P; simultaneously incorporation into polymer is decreased. Again, there was no difference in preparations from two races of Pmg.

The radioactive lipid fraction obtained from UDP-N-acetyl-[^{14}C]glucosamine migrates upon thin layer chromatography as a single band (Fig. 8, panel A, peak I). Minute amounts of radioactivity can be observed at lower R_f values. The addition of exogenous (yeast) Dol-P (5 µg) effectively stimulates formation of peak II (Table V). Peak III is slightly enhanced, whereas peak I is unaffected. More clearly, when 15µg of Dol-P is added, peaks II

Table IV. Stimulation of GlcNAc-transferase activity by exogenous yeast Dol-P. Microsomal membranes from Pmg 1 and 2, respectively (100 µg of protein) were incubated for 30 min in absence or presence (5 µg) of yeast Dol-P.

	Pmg 1		Pmg 2	
	Lipid [cpm]	Polymer [cpm]	Lipid [cpm]	Polymer [cpm]
−Dol-P	320	1630	240	1310
+Dol-P	1650	530	1130	580

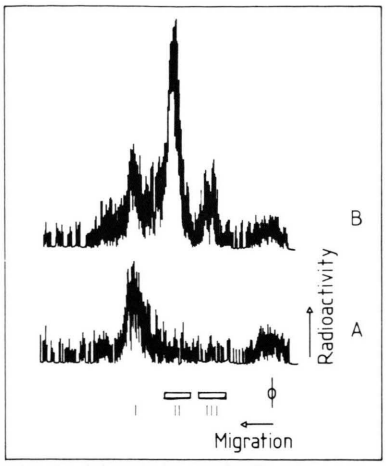

Fig. 8. Stimulation of GlcNAc-transferase activity by exogenous yeast Dol-P. Assay (400 µg of protein, 180 min) was run in absence (panel A) or presence of 15 µg of yeast Dol-P (panel B). Purification of lipid fraction and thin layer chromatography was performed as described under Materials and Methods. Bars indicate positions of yeast Dol-PP-GlcNAc (II) and Dol-PP-$(GlcNAC)_2$, respectively (III).

and III become pronounced, but not peak I (Fig. 8, panel B and Table V). As compared to corresponding standards, peak I has an R_f value close to that of Dol-P-man, whereas peaks II and III migrate at the R_f values of Dol-PP-GlcNAc and Dol-PP-di-N-acetylchitobiose, respectively (standards obtained from yeast microsomal incubations). Formation of all three radioactive peaks is abolished if unlabelled UDP-GlcNAc is present in the incubation mixture.

Peaks II and III were eluted from the plates and hydrolyzed with 1 N HCl (50% propane-1-ol/H_2O, 15 min, 50 °C) yielding N-acetylglucosamine and di-N-acetyl-chitobiose, respectively, as detected by paper chromatography. Alkaline decomposition (10% NH_4OH/H_2O, 1 h, 100 °C) resulted in conversion of lipid-bound radioactivity into material which migrated on paper at expected R_f corresponding to sugar phosphates (data not shown).

The situation is similar to that described for the yeast system [20] although much less lipid material is labelled endogenously when Pmg microsomal preparations are used.

7. Formation of lipid-bound di-N-acetylchitobiose and mannosyl-di-N-acetylchitobiose

With UPD-[^{14}C]GlcNAc, using endogenous lipid acceptors, labelled material can be recovered, corresponding to peak I in Fig. 8, panel A. Exogenous (yeast) Dol-P results in recovery of labelled Dol-PP-GlcNAc (compare Fig. 8, panel B). Tunicamycin is known to specifically block the glycosyl transfer from UDP-GlcNAc to Dol-P thus inhibiting the formation of Dol-PP-GlcNAc [22]. As expected, tunicamycin effectively inhibited the formation of both peaks II and III (Table V) while peak I was almost unaffected.

To prove that peak II acts as precursor of peak III, previously prepared (yeast) Dol-PP-[^{14}C]GlcNAc was incubated with unlabelled UDP-GlcNAc

Table V. N-Acetylglucosamine transfer reactions into GlcNAc-lipids under various conditions using Pmg 1 microsomal membranes. Purified lipid fractions from the incubation mixtures (400 µg of protein, 180 min) were separated by thin layer chromatography. Areas with R_f values corresponding to the GlcNAc-lipids listed in the table were scraped out and counted in scintillation cocktail (corrected against background). Peak numbers refer to Fig. 8.

Glycosyldonor	[^{14}C]-GlcNAc-lipid formation [cpm]			
	Unknown (Peak I)	Dol-PP-GlcNAc (Peak II)	Dol-PP-$(GlcNAc)_2$ (Peak III)	Dol-PP-$(GlcNAc)_2$Man
a) UDP-[^{14}C]-GlcNAc	190	70	45	–
b) as a), 5 µg yeast Dol-P added	175	1190	140	–
c) as a), 15 µg yeast Dol-P added	185	1980	770	–
d) as c), tunicamycin (7 µg/ml) added	170	90	50	–
e) Dol-PP-[^{14}C]GlcNAc (yeast; 10.000 cpm) plus UDP-GlcNAc (7 mM)		7450	1540	–
f) Dol-PP-[^{14}C]$(GlcNAc)_2$ (yeast; 10.000 cpm) plus GDP-Man (7 mM)			6855	1970

(Table V). As can be seen, peak III is formed, and the reaction is completely insensitive to tunicamycin.

GDP-mannose is known to act as glycosyldonor during the formation of lipid-bound trisaccharide [22]. When previously prepared (yeast) Dol-PP-[^{14}C]-$(GlcNAc)_2$ was incubated with unlabelled GDP-mannose, a new lipid was formed which ran on thin layer as expected for Dol-PP-$(GlcNAc)_2$Man (Table V). Its synthesis was insensitive to tunicamicyn. Furthermore, diumycin did not interfere with lipid-trisaccharide synthesis. Therefore, Dol-P-Man does not seem to act as mannosyldonor in this reaction.

8. Lipid-bound oligosaccharides

With the Pmg microsomal system only minute amounts, even in the presence of exogenous yeast Dol-P in the incubation mixture, of labelled oligosaccharide were detected sticking to the origin on paper chromatograms after hydrolysis of putative lipid-oligosaccharide fractions. This may again be explained by the low amount of glycoprotein material present in the Pmg mycelial wall.

Upon incubation of Pmg microsomal membranes with UDP-[^{14}C]glucose, a labelled lipid was formed which behaved on thin layer as yeast Dol-P-Glc. At very low recoveries in addition a lipid-oligosaccharide fraction (chloroform/methanol/water = 10:10:3) was obtained which migrated into a position which has been described for yeast Dol-PP-oligosaccharide (cellulose plates, butane-1-ol/ethylacetate/acetic acid/water = 40:30:25:40, v/v, [21]). Upon acid hydrolysis, radioactive material was released which stayed at the origin in a paper chromatography system where oligoglucosides (starch hydrolysate) up to a degree of polymerization = 8 still migrated (Whatman No. 1, butane-1-ol/pyridine/water = 4:3:4, v/v). Considering the small amount of material, both lipid species could not be characterized further.

Discussion

Within many host-pathogen systems, for each gene that confers resistance to the host plant there exists a corresponding gene in the fungal pathogen that governs avirulence (gene-for-gene host parasite systems). Albersheim and Anderson-Prouty [29] hypothesized that the avirulence genes of a gene-for-gene pathogen are manifested at the cell surface. Furthermore, the products of the avirulence genes of fungal pathogens were suggested to be glycosyltransferases active during the synthesis of complex carbohydrates of fungal cell surfaces or secreted glycoproteins [30].

Glycoconjugates are of great importance within many plant-pathogen interactions (for a review, see [31]). Glycoproteins secreted by Pmg have been reported to represent the specificity factors governing avirulence in the Pmg-soybean system [9], Pmg surface glycoproteins have been claimed to be race specific phytoalexin elicitors [7], and Pmg extracellular mannan-glycoproteins were described as specific suppressors of phytoalexin accumulation in infected soybean cotyledons [8].

Polysaccharide-protein complexes probably occur in all fungal walls [32]. Among the various fungal glycoproteins, mannoproteins have been studied in some detail, but biosynthetic work mainly refers to *Saccharomyces* sp. Other fungal systems investigated comprise *Hansenula* sp., *Kluyveromyces lactis*, *Cryptococcus laurentii*, *Aspergillus* sp., *Penicillium* sp., *Neurospora crassa*, *Fusarium solani* f. *pisi*, and *Candida albicans* (for a review, see [28]).

Although it has not been demonstrated in the present investigations that the glycoprotein material synthesized *in vitro* is identical to any of the distinct Pmg glycoproteins described in the literature it is assumed that they are synthesized *via* the very same steps as demonstrated *in vitro*, if they belong to the same structural type. This has been demonstrated *e.g.* in the case of yeast *in vitro* glycoprotein biosynthesis and formation of yeast external invertase [28, 33, 34].

Pmg microsomal membranes are capable of synthesizing glycoproteins of the common asparagine-di-N-acetylchitobiose type. At least the basic steps (up to lipid-bound trisaccharide) in the "core" assembly of the asparagine-linked carbohydrate component have been demonstrated. In addition, evidence has been obtained for larger Dol-PP-linked oligosaccharides labelled with [^{14}C]glucose. This points to the existence of a lipid-linked oligosaccharide precursor which is common to yeast and animal systems (Dol-PP-$(GlcNAc)_2Man_9Glc_3$); final oligosaccharide transfer to asparagine residues within protein acceptor molecules has been demonstrated in those systems [23, 35].

The fact that Pmg membranes catalyze the transfer of GlcNAc from UDP-GlcNAc into glycoprotein

may be explained by either synthesis of complete lipid-oligosaccharide utilizing endogenous substrates or (more reasonable) by the well known artificial reactions (in yeast) involving GlcNAc- and $(GlcNAc)_2$-transfer from the corresponding Dol-PP-derivatives into protein [28]. Experiments with endo-N-acetylglucosaminidase H, exogenous yeast Dol-P, and tunicamycin, respectively, clearly demonstrate that the dolichol-mediated sequence during GlcNAc transfer reactions is established in Pmg microsomal membranes.

GDP-mannose as well as Dol-P-mannose act as mannosyldonors in glycoprotein biosynthesis in yeast during the assembly of carbohydrate parts linked N-glycosidically to asparagine and O-glycosidically to hydroxyamino acids, respectively [19, 24, 28]. In this system, mannose is effectively incorporated into β-eliminable positions [19, 24]. At least from GDP-mannose, also Pmg microsomes incorporate mannose into O-glycosidically linked mannose (presumably via endogenous Dol-P) and manno-oligosaccharides. O-mannosylation has been demonstrated besides in *Saccharomyces cerevisiae* also in other fungi like *Hansenula*, *Aspergillus*, *Penicillium*, *Neurospora*, and *Fusarium* [28]. The specific site of mannose incorporation from Dol-P-mannose by Pmg microsomes could not be demonstrated. Nevertheless, the pre-incubation experiment with unlabelled UDP-GlcNAc and GDP-Man demonstrates that Dol-P-Man might also serve as mannosyl donor during the lipid-oligosaccharide assembly (Fig. 6). With GDP-Man as glycosyl donor only approximately 10% of the total radioactivity in the glycoprotein part is incorporated as mannose and short manno-oligosaccharides O-glycosidically linked to serine/threonine. Most of it seems to be located within the asparagine-linked carbohydrate part since it can be liberated as a large oligosaccharide after treatment with endo H (enzyme which specifically cleaves glycosidic bonds between two GlcNAc residues linked to asparagine).

The exact chemical nature of the lipid involved in the transfer reactions has not been clarified. From its chromatographic characteristics (as glycosylated lipid-phosphates), its behaviour upon hydrolysis, the stimulation experiments with yeast dolichol derivatives, and experiments with antibiotics (diumycin, tunicamycin) it seems clear that the lipid part of the Pmg glycosylation intermediates is of the dolichol type. The endogenous lipid acceptor in yeast has been identified as dolichylmonophosphate (14 to 18 isoprene units, [36]).

An important question concerning glycoprotein biosynthesis in Pmg relates to specific patterns in Pmg glycoproteins of different races. Besides small differences in β-eliminable radioactivity after glycosylation with GDP-mannose no race-specific synthetic capabilities could be observed. As expected because of the gross methods used no such difference could be detected. As is known from other (non-pathogenic) systems, *e.g.* yeast [37], species-specific determinants often reside in mannan portions of glycoproteins which differ slightly (by less than 5% of the molecule) in the glycosyl linkage and/or sugar composition.

Detailed analysis of N- and O-linked glycosides could well lead to the discovery of structural differences as shown for surface "bulk" mannoproteins as well as external invertases from yeast mutants [34, 37, 38], and for mycelial and extracellular invertases from different Pmg races [8, 14, 15], respectively. If structural variations within glycoprotein molecules are responsible for Pmg race specificity a detailed analytical study of lipid-bound intermediates and of carbohydrate parts within *in vitro*-synthesized glycoproteins could be a valuable tool to detect these determinants.

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