Aspergillus niger van Tieghem mannosylation: polyprenylphosphate mannosyltransferase specificity

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Abstract *Aspergillus niger* van Tieghem microsomes contain an enzyme that catalyzes mannose transfer from GDP-mannose to polyprenylphosphate. The studies of the specificity of this enzyme for both the sugar donor (nucleoside diphosphate sugar) and the acceptor (polyprenylphosphates that were made available to the enzyme by means of the fusion of acceptor-loaded liposomes with the microsomal membranes) gave the following results. *i*) All the polyprenylphosphates from C_{15} to C_{120} were acceptors except retinylphosphate. *ii*) The specificity of the enzyme for both the sugar and the base is very strict.—**Létoublon, R., B. Mayet, J. Frot-Coutaz, and R. Got.** *Aspergillus niger* van Tieghem mannosylation: polyprenylphosphate mannosyltransferase specificity. *J. Lipid Res.* 1982. **23:** 1053–1057.

Supplementary key words mannosyltransferase • polyprenylphosphate • retinylphosphate • nucleoside diphosphate sugars

There has been and there is still considerable interest in the role of polyprenylphosphates as lipid intermediates in glycoconjugate biosynthesis. The reaction which results in the formation of the lipid intermediate is a two-substrate reaction; one substrate, the acceptor, is a polyprenylphosphate; the other substrate, the sugar donor, is a nucleoside diphosphate sugar.

Depending on the source of the material, the structure of the lipid moiety of the intermediate varies. With bacterial glycosyltransferases, a demand for the unsaturated α-isoprene residue and a short polyprenol chain was observed (1). The lipid intermediates found in animal tissues derive from a series of isoprenologues containing from 17 to 21 isoprene units and the α-isoprene unit which carries the hydroxyl group is saturated (dolichols) (2). Moreover, in some animal tissues like rat liver, two types of polyprenols are involved: dolichols (3–5) and retinol (6, 7), and we have now good evidence that they correspond to two distinct pathways (8). For yeasts, fungi, and plants the number of isoprene residues may vary from 14 to 24 (2). This point raises the question of the specificity for the lipid moiety of the GDP-mannose: lipid phosphate mannosyltransferase(s): enzyme which has been particularly well studied in *Aspergillus niger* van Tieghem (9–11). A recent review (2) shows that some sugars do not form this polyprenylphosphoryl derivative or do so rarely; this fact has been explained by the position of the sugars in the oligosaccharide chain as well as by the processing of glycoproteins during their biosynthesis.

What we know about the sugar contents (12) of the oligosaccharide chains of *Aspergillus niger* glycoproteins led us to carry out some studies that deal with the specificity for different nucleoside diphosphate sugars of the lipid intermediate-forming enzyme.

MATERIALS AND METHODS

Chemicals

3-*sn*-Phosphatidylethanolamine, phosphatidic acid, egg yolk lecithin, (C_{80-105}) dolichylmonophosphate (purity 80–90%, grade III), farnesol, and all-*trans* retinol were purchased from Sigma; undecaprenylphosphate was a generous gift from Polskie Odczynniki Chemiczne, Gliwice, Poland; GDP-[^{14}C]mannose, GDP-[1-^{14}C]fucose, UDP-[^{14}C]glucose, GDP-[^{14}C]glucose, UDP-[^{14}C]galactose, UDP-[^{14}C]-N-acetylglucosamine, and UDP-[^{14}C]glucuronic acid were from the Radiochemical Centre (Amersham, UK); UDP-[^{14}C]xylose was from New England Nuclear (Romainville, France); and DMSO was from Riedel de Haën. RP and farnesylphosphate were prepared according to Frot-Coutaz and De Luca (13).

Materials

Microsomes from *Aspergillus niger* van Tieghem were obtained as previously described (9) and microsomal proteins were determined by the biuret procedure (14) with human serum albumin as standard.

Abbreviations: ADP, adenosine diphosphate; GDP, guanosine diphosphate; UDP, uridine diphosphate; dolP, dolichylphosphate; RP, retinylphosphate; MRP, mannosylretinylphosphate; DMSO, dimethylsulfoxide; TLC, thin-layer chromatography.

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TABLE 1. Transfer of [^{14}C]mannose from GDP-[^{14}C]mannose to endogenous or exogenous polyprenylphosphates

	dpm × mg^{-1} prot × min^{-1}
Control	550
+ DMSO (0.5 M)	550
+ dolP (60 μM) in DMSO (0.5 M)	620
+ dolP (100 μM) in DMSO (0.5 M)	980
+ RP (175 μM) in DMSO (0.5 M)	300
+ Triton X-100 1.25‰	300
+ dolP (12 μM) in TX-100 1.25‰	300
+ dolP (25 μM) in TX-100 1.25‰	330
+ dolP (60 μM) in TX-100 1.25‰	900
+ dolP (85 μM) in TX-100 1.25‰	950
+ dolP (150 μM) in TX-100 1.25‰	950
+ RP (175 μM) in TX-100 1.25‰	50

One hundred μl of the microsomal suspension (1.5 mg of protein) in 50 mM Tris–HCl buffer, pH 8.5, Mg^{2+} 5 mM, were incubated at 30°C with 0.05 μCi of GDP-[^{14}C]mannose (sp act 166 mCi/mmol).

Liposomes preparation

Lipid vesicles were routinely prepared according to the following method (15) using egg yolk lecithin (10 mg), 3-*sn*-phosphatidylethanolamine (5 mg), and the same concentration of the following negatively charged lipids: phosphatidic acid (0.140 mg) or ($C_{80\text{-}105}$) dolP (0.280 mg) or farnesylphosphate (0.06 mg) or undecaprenylphosphate (0.170 mg) or RP (0.075 mg). After drying under a nitrogen stream, the lipids were swollen in 1 ml of 50 mM Tris–HCl buffer, pH 8.5. The suspension was ultrasonically irradiated under nitrogen at 20°C for 15 min at power level 4 with a Branson Sonifier Model B-12 equipped with a microtip. The sonicate was then centrifuged for 1 hr at 100,000 *g* at room temperature. The clear supernatant containing small unilamellar vesicles was referred to as liposomes.

Fusion of microsomes and liposomes was obtained by incubating, for 20 min (15) at room temperature, 0.5 ml of liposomes with 10 mg (as protein) of microsomes resuspended in 0.6 ml of the buffer used for the preparation of liposomes; the resulting mixture is referred to as "activated microsomes."

Standard assay

Assays for [^{14}C]mannose incorporation into polyprenylphosphate mannose and endogenous glycoproteins were carried out according to the general procedure described by Létoublon, Comte, and Got (9) with the following modifications: *1*) "activated microsomes" were used in place of microsomes (unless otherwise specified), and *2*) the conditions for extracting short-chain polyprenylphosphate mannose are described in reference 7.

Characterization of labeled products

Thin-layer chromatography was carried out on silica gel precoated plates (60F254, 0.25-mm thickness, Merck) developed with chloroform–methanol–water 60:25:4 (v/v) (solvent A in ref. 16) and 60:35:6 (solvent B). Radioactive zones were determined either by scanning with a radiochromatogram scanner (Packard model 7201) and/or by scraping off transversal strip from the TLC plates and counting directly in liquid scintillation system (PPO/POPOP/toluene, 3 g:0.1 g:1000 ml). RP was detected under UV light.

Farnesylphosphate [^{14}C]mannose was synthesized by incubating GDP-[^{14}C]mannose and farnesylphosphate-"activated microsomes" and then extracted with chloroform–methanol 2:1 from the lyophilized upper phase (about 90% of farnesylphosphate [^{14}C]mannose goes in the upper phase when the incubation mixture is extracted according to the Folch extraction procedure); the extraction procedure described in reference 7 was also used.

RESULTS AND DISCUSSION

The phosphorylated derivatives of different polyprenols have been tested for their ability to accept mannose from GDP-mannose, the transfer reaction being catalyzed by *Aspergillus niger* microsomes. The reaction has been carried out under different conditions that concern the way these phosphorylated derivatives are added to the incubation medium.

Table 1 shows that only a little part of ($C_{80\text{-}105}$) dolP "solubilized" in DMSO is available for mannosylation and that RP acts as an inhibitor of ($C_{90\text{-}120}$) dolichylphosphate [^{14}C]mannose biosynthesis. ($C_{90\text{-}120}$) dolP refers to endogenous dolP (see reference 17). When Triton X-100 is used, the observed inhibition due to this detergent is overcome by the addition of high concentrations of exogenous ($C_{80\text{-}105}$) dolP; if exogenously added ($C_{80\text{-}105}$) dolP is replaced by RP, an even greater inhibition of the [^{14}C]mannolipid (extracted as described in reference 7) is observed. Thin-layer chromatography of this material showed, in all cases, that only ($C_{90\text{-}120}$) dolichylphosphate [^{14}C]mannose was formed and no MRP could be detected (**Fig. 1**).

The lack of MRP synthesis (as well as a lower level of ($C_{90\text{-}120}$) dolichylphosphate [^{14}C]mannose) cannot be explained by an increased transfer to glycoproteins since [^{14}C]mannose incorporation into the insoluble material is inhibited.

This result is very much in contrast with what is observed in rat hepatocytes; indeed, microsomes prepared from these cells do transfer mannose from GDP-mannose to either dolP or RP in a manner that strongly suggests the existence of two different pathways (8).

Neither DMSO nor Triton X-100 are good polyprenylphosphate solubilizing agents; indeed, while Triton X-100 has by itself a strong inhibitory effect on the

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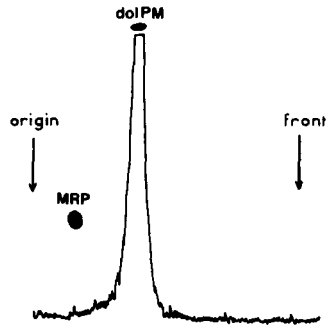

Fig. 1. Thin-layer chromatography on silica gel precoated plates, developed in solvent A, of the polyprenylphosphate [^{14}C]mannose obtained under the conditions described in Table 1 when RP (175 μM in DMSO) was added to the standard incubation medium. MRP, mannosylretinylphosphate; dolPM, (C_{90-120}) dolichylphosphate mannose.

mannosyltransferase, DMSO dissolves long-chain polyprenylphosphates very poorly. Recently, Shidoji and De Luca (18) have used bovine serum albumin instead of DMSO or detergent to study the mannosylation of RP by rat liver microsomes; but again, in our system, the formation of MRP in presence of serum albumin could not be detected. For these reasons, in a third experiment we used liposomes to obtain, as described under "Methods," microsomes activated with (C_{80-105}) dolichylphosphate, farnesylphosphate, undecaprenylphosphate, phosphatidic acid, and retinylphosphate at a final concentration which was three times the K_m value (15). **Fig. 2A** shows that the initial velocity for the formation of C_{15}, C_{55}, and (C_{80-105}) dolichylphosphate [^{14}C]mannose decreases when the chain length gets shorter; still an increased synthesis of (C_{15}, C_{55}, and (C_{80-105}) polyprenylphosphate [^{14}C]mannose) results in an activation of [^{14}C]mannose transfer to glycoproteins (Fig. 2B). This is confirmed by the fact that a direct transfer can be obtained from (C_{90-120} dolichylphosphate [^{14}C]mannose (15) as well as from farnesylphosphate [^{14}C] mannose (**Fig. 3**).

Rössler et al. (19) found that *Dictyostelium discoideum* glycosyltransferases reach maximum activity for α-saturated C_{55} polyprenylphosphate. Our results show that the enzyme responsible for lipid intermediate synthesis, as well as for glycoprotein mannosylation via the lipid intermediate, easily mistakes short-chain polyprenylphosphate for the endogenous lipid phosphate which has a long chain (19). However, the presence of five conjugated double bonds and/or the presence of a hexenyl ring makes retinylphosphate inadequate for mannosylation and even results in an inhibition of (C_{90-120}) dolichylphosphate [^{14}C]mannose synthesis. It is noteworthy that under the same conditions, phosphatidic acid, which is not a mannose acceptor, does not cause any inhibition.

Mankowski, Sasak, and Chojnacki (20) and Palamarczyk et al. (21) have shown that the phosphorylated derivatives of partially hydrogenated plant undecaprenol and C_{45}-solanesol were better acceptors of glucose from UDP-glucose (rat liver microsomes as the source of enzyme) than the phosphates of fully unsaturated and undecaprenol and solanesol.

Between *Aspergillus niger* van Tieghem (9) and, for instance, rat hepatocytes (10), evolution has made vi-

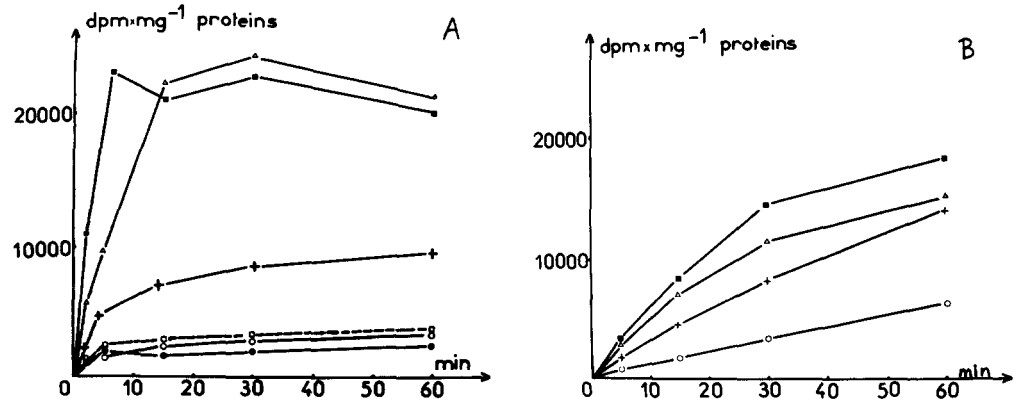

Fig. 2. Time course of [^{14}C]mannose transfer to lipid phosphates and to proteins. [^{14}C] Mannose transfer to lipid phosphates (Fig. 2A) and to proteins (Fig. 2B) were studied; the conditions were the same as for Fig. 1 except that liposomes were used (see Methods). A: ○ —— ○, microsomes + buffer; ● —— ●, microsomes + liposomes containing PA (100 μM final concentration); □ —— □, microsomes + liposomes containing RP (100 μM); + —— +, microsomes + liposomes containing farnesylphosphate (100 μM); △ —— △; microsomes + liposomes containing undecaprenylphosphate (100 μM); ■—— ■, microsomes + liposomes containing (C_{80-105}) dolP (100 μM). B: Kinetic studies of the influence of an excess of polyprenylphosphate on [^{14}C]mannose labeling of glycoproteins; the incubation conditions were the same as for Fig. 2A. ○ —— ○, microsomes + buffer; + —— +, microsomes + liposomes containing farnesylphosphate (100 μM); △ —— △, microsomes + liposomes containing undecaprenylphosphate (100 μM); ■—— ■, microsomes + liposomes containing (C_{80-105}) dolP (100 μM).

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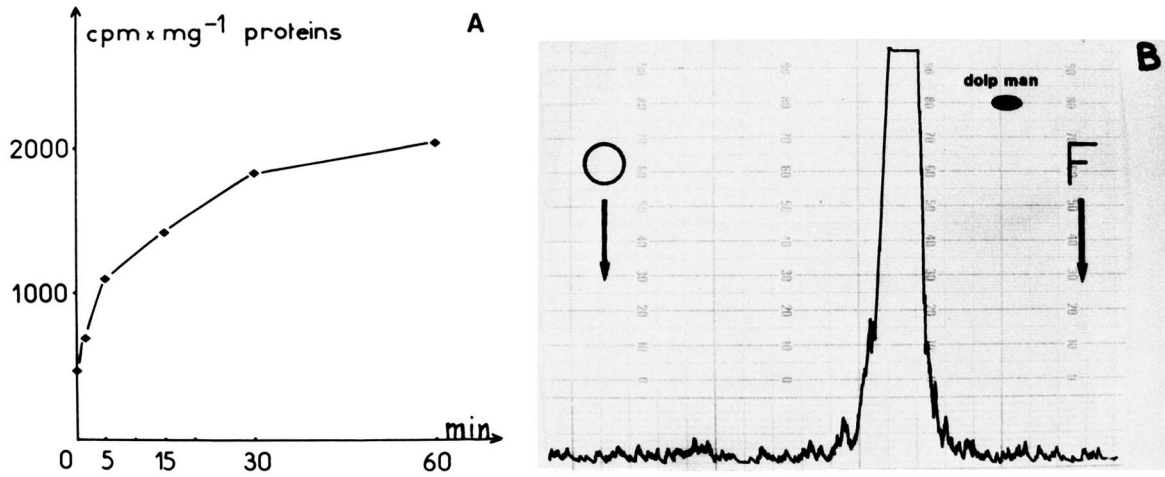

Fig. 3. Time course of [^{14}C]mannose transfer from farnesylphosphate [^{14}C]mannose to endogenous protein acceptors. A. Liposomes (0.5 ml) containing 5 mg of egg yolk lecithin, 2.5 mg of phosphatidylethanolamine, and 70,000 dpm of farnesylphosphate [^{14}C]mannose (prepared and purified as described in Methods) was added to 5 mg of microsomes resuspended in 0.6 ml of the following buffer: 50 mM Tris–HCl, pH 8.5, 5 mM Mg^{2+}. At various times the radioactivity of proteins was measured. B. Thin-layer chromatogram of the farnesylphosphate [^{14}C]mannose (developed in solvent B). O, origin; F, front; dolP man, (C_{90-120}) dolichylphosphate mannose.

tamin A necessary from the point of view of glycoprotein mannosylation, but we still do not know the significance for such a change. One interesting observation deserves to be mentioned: we did not detect in vitro any polyprenylphosphate oligosaccharide-like material in *Aspergillus niger* van Tieghem.

In a last experiment, the specificity of the enzyme for the sugar donor (NDP-hexose) was studied. To be sure that sufficient amounts of acceptor were available for glycosylation, "activated microsomes" containing (C_{80-105}) dolP (100 μM) were used. From **Table 2** it is obvious that GDP-mannose is the best donor; some fucose and very little glucose and N-acetylglucosamine derivatives were detected, but no dolP galactose, dolP glucuronic acid, or dolP xylose. Moreover, in a pulse chase experiment using GDP-mannose, ADP-mannose and UDP-mannose (15 μM), the synthesis of (C_{90-120}) dolichylphosphate [^{14}C]mannose was not affected by the ADP and UDP-mannose showing a remarkable specificity of the enzyme for the base (guanine) as observed in rat liver by Kerr and Hemming (5).

In conclusion, *Aspergillus niger* van Tieghem microsomal polyprenylphosphate NDP-hexose transferase is very specific for GDP-mannose; this agrees with the data obtained by Vessey, Lysenko and Zakim (22) who concluded that dolP-mannose, dolP-glucose, or dolP-N-acetylglucosamine are synthesized by different enzymes with the same pool of endogenous (C_{90-120}) dolichylphosphate.

TABLE 2. Specificity of the dolichylphosphate-NDP-hexosyltransferase for the substrate

Substrate (final concentration in the incubation mixture)	Specific Activity
	pmol × mg⁻¹ prot × min⁻¹
GDP-mannose (3.5 μM)	15
GDP-fucose (15 μM)	1
GDP-glucose (7 μM)	0.04
UDP-glucose (7 μM)	0.01
UDP-galactose (7 μM)	0
UDP-xylose (1 μM)	0
UDP-glucuronic acid (7 μM)	0
UDP-N-acetylglucosamine (7 μM)	0.015

One hundred μl of the (C_{90-120}) dolichylphosphate-loaded microsomes (1.5 mg of protein) in 50 mM Tris–HCl buffer, pH 8.5, Mg^{2+} 5 mM, were incubated at 30°C with various NDP-sugars.

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