Journal of Chromatography, 262 (1983) 299–304
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,718

SEPARATION OF DOLICHOL MONOPHOSPHATE MANNOSE AND DOLICHOL MONOPHOSPHATE GLUCOSE BY THIN-LAYER CHROMATOGRAPHY

GERARDO Z. LEDERKREMER and ARMANDO J. PARODI*
Instituto de Investigaciones Bioquímicas "Fundación Campomar", Obligado 2490, 1428 Buenos Aires (Argentina)
(Received January 18th, 1983)

SUMMARY

Dolichol monophosphate mannose and dolichol monophosphate glucose from mammalian or mold cells were separated by thin-layer chromatography. This separation was not due to a difference in the size of the lipid moieties. It was found that the lipid moieties of dolichol monophosphate mannose and dolichol monophosphate glucose are of the same size when synthesized by the same cell. The thin-layer chromatographic separation appeared to be due, therefore, to the saccharide part of the molecules.

INTRODUCTION

The major pathway of protein N-glycosylation in most eucaryotic cells involves the transfer of an oligosaccharide containing three glucose (Glc), nine mannose (Man) and two N-acetylglucosamine (GlcNAc) residues from a dolichol-P-P derivative to an asparagine residue followed by removal of the three glucose units[1,2]. In some instances a few or none of the mannose residues are also excised, thus forming the "high mannose" type glycoproteins. Alternatively, removal of six mannose units and addition of N-acetylglucosamine, galactose, sialic acid and fucose residues directly from their respective sugar nucleotides lead to the formation of "complex" type glycoproteins. The donors of four of the mannose and of the three glucose residues in the synthesis of $Glc_3Man_9GlcNAc_2$-P-P-dolichol are the respective dolichol-P derivatives, namely dolichol-P-Man and dolichol-P-Glc[3–5]. Dolichol is a generic name for polyprenols of eucaryotic cells containing 17–21 and 14–18 isoprene units in mammalian and yeast cells, respectively. In energy-depleted cells or in certain mutant cells the synthesis of dolichol-P-Man but not that of dolichol-P-Glc is diminished or absent[6–9]. In these instances the oligosaccharide transferred to protein is $Glc_3Man_5GlcNAc_2$ and not $Glc_3Man_9GlcNAc_2$. This fact produces profound changes in the structure of protein-bound oligosaccharides. On the other hand, certain parasitic protozoa are defective in the synthesis of dolichol-P-Glc. In these micro-

0021-9673/83/$03.00 © 1983 Elsevier Science Publishers B.V.

organisms unglucosylated dolichol-P-P derivatives are the saccharide donors in the glycosylation of proteins[10–12].

Evaluation of dolichol-P-Man and dolichol-P-Glc synthesis *in vivo* can be best performed by incubating cells with uniformly labeled glucose or mannose[11]. As no method for separating both dolichol derivatives has so far been reported, discrimination between them has been performed by a destructive method, namely mild acid hydrolysis followed by chromatography of the monosaccharide residues.

In this paper we report a simple, non-destructive thin-layer chromatographic (TLC) method for the separation of dolichol-P-Man and dolichol-P-Glc. The compounds can then be easily eluted from TLC plates, thus allowing further structural analysis of the substances. In addition, the lipid moieties of dolichol-P-Glc and dolichol-P-Man were found to have the same size when synthesized by the same cell.

EXPERIMENTAL

Polyprenol derivatives

Dolichol-P-[^{14}C]Glc, dolichol-P-[^{3}H]Glc and dolichol-P-[^{14}C]Man were prepared as described by Behrens and Tábora[13] using rat liver microsomes, pig liver dolichol-P and the pertinent sugar nucleotides. Undecaprenol-P-[^{14}C]Gal from *Acetobacter xylinum* was prepared according to Garcia *et al.*[14]. Dolichol-P-[^{14}C]Gal was obtained using an enzymatic preparation from *A. xylinum*, UDP-[^{14}C]Gal and pig liver dolichol-P as described by Iñón de Iannino *et al.*[15].

A detailed description of the preparation of *Mucor rouxii* dolichol-P-[^{14}C]Glc and dolichol-P-[^{14}C]Man will be described elsewhere. Briefly, yeast- or mycelium-form cells of *M. rouxii* were incubated with [^{14}C]glucose (283 Ci/mole), broken with a Bio-X press and submitted to a chloroform–methanol–water (3:2:1) partition. The lower phase was then subjected to mild saponification and the alkali-resistant substances were applied to a DEAE-cellulose (acetate form) column equilibrated with chloroform–methanol (2:1). The dolichol-P derivatives were eluted with an ammonium formate gradient in the same solvent.

Thin-layer chromatography

TLC was performed on silica gel 60 glass plates purchased from Merck (Darmstadt, G.F.R.) with the following solvents: A, 1-propanol–water (7:3); B, chloroform–methanol–water (65:25:4), and C, chloroform–2-propanol–95% ethanol–glacial acetic acid (2:2:3:1).

Radioautography was carried out using Kodak X-omat R films.

Periodate treatment of dolichol derivatives

The dolichol derivatives were treated in the dark with 0.5 ml of 0.1 *M* sodium periodate containing 0.1% Triton X-100. After 48 h at 4°C, 20 μl of ethylene glycol were added and the samples were left at room temperature for 30 min. This was followed by a chloroform–methanol–water (3:2:1) partition. The lower phases were spotted on the TLC plates.

Gel filtration

The procedure described previously was employed[11]. The column, containing

Sephadex G-75 equilibrated with 0.05 *M* sodium phosphate buffer (pH 7.6)–0.5% sodium deoxycholate, was eluted with the same solution.

RESULTS AND DISCUSSION

Separation of dolichol-P derivatives

As shown in Table I and Fig. 1,, no separation between dolichol-P-Man and dolichol-P-Glc was obtained on TLC plates developed with two widely used solvents (A and B). In contrast, TLC with solvent C resulted in a clear separation. It should be noted that a solvent similar to C but containing 1 *N* acetic acid instead of the water-free acid failed to separate the dolichol-P derivatives[16,17]. After autoradiography the substances can be eluted from the silica gel with chloroform–methanol (2:1). Solvent C also separates dolichol-P-Glc from dolichol-P-Gal (Table I). The latter substance had been prepared using an enzyme from *Acetobacter xylinum* and liver dolichol-P, as eucaryotic cells are unable to synthesize the galactosylated derivative.

Periodate treatment of dolichol derivatives

As mentioned above, dolichol is a generic name for a family of compounds having different numbers of isoprene residues in the same tissue. As the length of the polyprenol moiety influences the migration of dolichol-P derivatives in TLC, the larger compounds migrating faster, two types of experiments were performed in order to investigate the possibility that separation between dolichol-P-Man and dolichol-P-Glc could be due to a difference in the chain lengths of the lipid parts of the molecules. Dolichol-P-Man and dolichol-P-Glc, both from liver, were treated with sodium periodate, thus destroying the difference between the hydrophylic moieties of the compounds, and subjected to TLC with solvent C. As shown in Table II, periodate-treated dolichol-P-Man and dolichol-P-Glc migrated with the same R_F value. The same occurred with dolichol-P-Man and dolichol-P-Glc, both from *Mucor rouxii* (Table II). On the other hand, periodate-treated liver dolichol-P-Glc and un-

TABLE I

R_F VALUES OF POLYPRENOL DERIVATIVES

Experiments 1 and 2 correspond to different runs. The samples in each run were spotted on the same plate.

Experiment No.	*Substance*	*Solvent*		
		A	*B*	*C*
1	Liver dolichol-P-[^{14}C]Glc	0.71	0.29	0.52
	Liver dolichol-P-[^{14}C]Man	—	—	0.43
	M. rouxii dolichol-P-[^{14}C]Glc	0.71	0.26	0.48
	M. rouxii dolichol-P-[^{14}C]Man	0.71	0.26	0.39
	A. xylinum undecaprenol-P-[^{14}C]Gal	—	0.23	0.35
2	Liver dolichol-P-[^{14}C]Glc			0.50
	Liver dolichol-P-[^{14}C]Gal			0.45

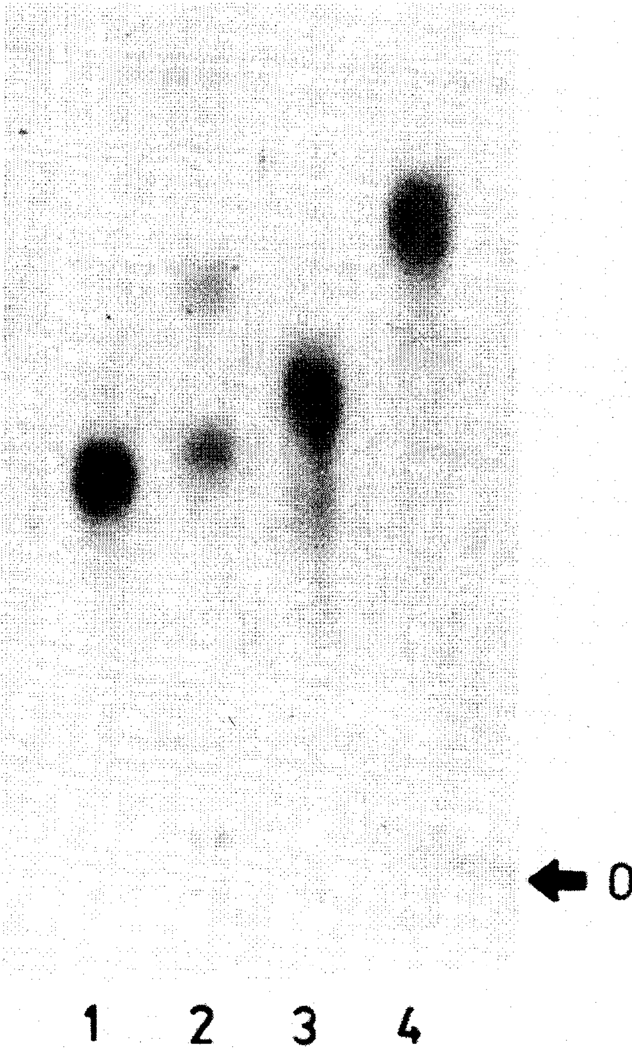

Fig. 1. TLC of polyprenol derivatives. Lane 1, *A. xylinum* undecaprenol-P-[^{14}C]Gal; lane 2, *M. rouxii* dolichol-P-[^{14}C]Man and dolichol-P-[^{14}C]Glc; lane 3, liver dolichol-P-[^{14}C]Man; lane 4, liver dolichol-P-[^{14}C]Glc. Solvent C was used. The front was 14.7 cm from the origin.

decaprenol-P-Gal from *A. xylinum* migrated differently, thus indicating dissimilar sizes of the lipid moieties. These results show that separation of dolichol-P-Glc and dolichol-P-Man was caused by the saccharide and not the lipid moieties.

Gel filtration of dolichol-P derivatives

Sodium deoxycholate forms inclusion compounds with lipids and the number of deoxycholate molecules combined depends on the chain length of the lipids. This property has been used to measure the size of polyprenol derivatives[11,18]. As shown in Fig. 2A, dolichol-P-[^{14}C]Man and dolichol-P-[^{3}H]Glc, both from liver, were not

TABLE II

R_F VALUES OF SODIUM PERIODATE-TREATED AND UNTREATED POLYPRENOL DERIVATIVES

Experiments 1, 2 and 3 correspond to different runs. The samples in each run were spotted on the same plate. Solvent C was employed.

Experiment No.	*Substance*	R_F
1	Liver dolichol-P-[^{14}C]Glc	0.48
	Liver dolichol-P-[^{14}C]Man	0.38
	Sodium periodate-treated:	
	Liver dolichol-P-[^{14}C]Glc	0.72
	Liver dolichol-P-[^{14}C]Man	0.72
2	Liver dolichol-P-[^{14}C]Glc	0.54
	A. xylinum undecaprenol-P-[^{14}C]Gal	0.32
	Sodium periodate-treated:	
	Liver dolichol-P-[^{14}C]Glc	0.76
	A. xylinum undecaprenol-P-[^{14}C]Gal	0.69
3	Sodium periodate-treated:	
	Liver dolichol-P-[^{14}C]Glc	0.66
	M. rouxii dolichol-P-[^{14}C]Glc	0.63
	M. rouxii dolichol-P-[^{14}C]Man	0.63

separated when submitted to column chromatography through Sephadex G-75 containing 0.5% sodium deoxycholate. Liver dolichol-P-[^{3}H]Glc and bacterial undecaprenol-P-[^{14}C]Gal, on the other hand, eluted in different fractions (Fig. 2B). A sample containing approximately equal amounts of dolichol-P-[^{14}C]Glc and dolichol-P-[^{14}C]Man, both from *Mucor rouxii*, eluted as a single peak, differently from liver

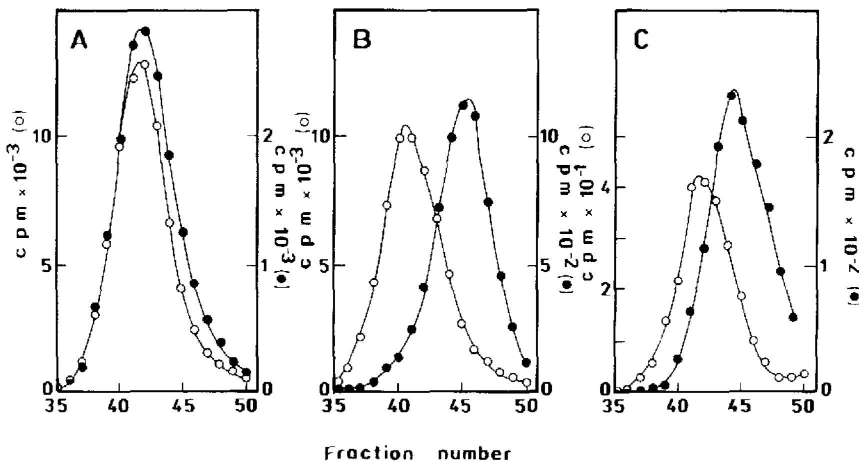

Fig. 2. Gel filtration of polyprenol derivatives. A, Liver dolichol-P-[^{3}H]Glc (○) and liver dolichol-P-[^{14}C]Man (●); B, liver dolichol-P-[^{3}H]Glc (○) and *A. xylinum* undecaprenol-P-[^{14}C]Gal (●); C, liver dolichol-P-[^{3}H]Glc (○) and a mixture of dolichol-P-[^{14}C]Glc and dolichol-P-[^{14}C]Man (●) from *M. rouxii*.

dolichol-P-[^{3}H]Glc (Fig. 2C). These results indicate that the polyprenol moieties of the glucosylated and mannosylated derivatives of dolichol-P have the same size when they originate from the same cell. A previous suggestion by Bergman *et al.*[19] that dolichol-P-Man and dolichol-P-Glc could have polyprenol moieties with different sizes when synthesized by the same cell does not seem to be substantiated by the present findings. Further, dolichol from *M. rouxii*, the same as that from *Saccharomyces cerevisiae*, appeared to be smaller than that from mammalian cells[18].

ACKNOWLEDGEMENTS

G.Z.L. is a Fellow and A.J.P. a Career Investigator of the National Research Council (Argentina).

REFERENCES

1 P. W. Robbins, S. C. Hubbard, S. J. Turco and D. F. Wirth, *Cell*, 12 (1977) 893.
2 I. Tabas, S. Schlesinger and S. Kornfeld, *J. Biol. Chem.*, 253 (1978) 716.
3 A. J. Parodi, *J. Biol. Chem.*, 254 (1979) 8343.
4 R. J. Staneloni, R. A. Ugalde and L. F. Leloir, *Eur. J. Biochem.*, 105 (1980) 275.
5 J. I. Rearick, K. Fujimoto and S. Kornfeld, *J. Biol. Chem.*, 256 (1981) 3762.
6 J. Stoll, A. R. Robbins and S. S. Krag, *Proc. Nat. Acad. Sci. U.S.*, 79 (1982) 2296.
7 A. Chapman, K. Fujimoto and S. Kornfeld, *J. Biol. Chem.*, 255 (1980) 4441.
8 J. I. Rearick, A. Chapman and S. Kornfeld, *J. Biol. Chem.*, 256 (1981) 6255.
9 R. Datema and R. T. Schwarz, *J. Biol. Chem.*, 256 (1981) 11191.
10 A. J. Parodi, L. A. Quesada-Allue and J. J. Cazzulo, *Proc. Nat. Acad. Sci. U.S.*, 78 (1981) 6201.
11 A. J. Parodi and L. A. Quesada-Allue, *J. Biol. Chem.*, 257 (1982) 7637.
12 A. J. Parodi and J. J. Cazzulo, *J. Biol. Chem.*, 257 (1982) 7641.
13 N. H. Behrens and E. Tábora, *Methods Enzymol.*, 50 (1978) 402.
14 R. C. García, E. Recondo and M. Dankert, *Eur. J. Biochem.*, 43 (1974) 93.
15 N. I. Iñón de Iannino, R. J. Staneloni, N. H. Behrens and M. Dankert, *FEBS Lett.*, 99 (1979) 73.
16 A. Mentaberry, V. Idoyaga-Vargas and H. Carminatti, *Exp. Eye Res.*, 33 (1981) 563.
17 E. Belocopitow, L. R. Maréchal and L. A. Quesada-Allue, *Mol. Cell. Biochem.*, 16 (1977) 127.
18 A. J. Parodi, *Eur. J. Biochem.*, 75 (1977) 171.
19 A. Bergman, T. Mankowski, T. Chojnacki, L. M. De Luca, E. Peterson and G. Dallner, *Biochem. J.*, 172 (1978) 123.