GALACTOSE TRANSFER FROM DOLICHYL MONOPHOSPHATE GALACTOSE IN ANIMAL TISSUES

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1. Introduction

The involvement of the dolichyl phosphate derivatives of *N*-acetylglucosamine, mannose and glucose in the biosynthesis of glycoproteins has been well established [1–3]. A similar role for galactose derivatives is not clear. The formation of an acid-labile galactolipid has been reported [4,5] and an increase in galactose incorporation into protein in cell cultures by the addition of dolichyl phosphate has been described [6]. Studies have also been carried out on the biosynthesis of retinol phosphate galactose [7].

This communication describes experiments carried out using Dol-P-Gal prepared with a bacterial system. Incubation of this compound with liver microsomes was found to lead to galactose transfer to an endogenous acceptor having the properties of a dolichyl diphosphate oligosaccharide.

2. Materials and methods

Dol-P-[^{14}C]Gal was prepared by incubation of UDP-[^{14}C]Gal and Dol-P with an enzyme from *Acetobacter xylinum* [8]. The Fic-P derivative was prepared similarly.

Rat-liver microsomes were prepared as in [9]. The standard reaction mixture contained ~10 000 cpm (20 pmol) of Dol-P-[^{14}C]Gal (the chloroform/methanol solution was evaporated under nitrogen), 0.1 M Tris–HCl buffer (pH 7.6), 1.2% Triton X-100, 8 mM $MnCl_2$ and enzyme (~1 mg protein) in 50 μl total vol. After a 30 min incubation at 30°C the reaction mixture was extracted with chloroform/methanol (2/1) or butanol, and finally with chloroform/methanol/water (1/2/0.3) (1203 solvent) or (1/1/0.3) (1103 solvent) as in [10], leaving an insoluble residue. Jack-bean α-mannosidase was purified up to the DEAE–Sephadex step [11]. It was used (0.3 unit) in 0.05 M triethanolamine acetate buffer (pH 4.5) at 37°C for 48 h under a toluene atmosphere.

The labeled Dol-P-P-Glc-oligosaccharide was prepared with liver enzymes [12]. This compound has been isolated from several tissues. Its oligosaccharide moiety has been assigned the following composition: Glc_{2-3} Man_{9-11} $GlcNAc_2$ [13–17]. UDP-[^{14}C]Glc (268 Ci/mol), UDP-[^{3}H]Glc (2.1 Ci/mmol) and UDP-[^{14}C]Gal (268 Ci/mol) were obtained as in [18]. Protein was determined by the Lowry method [19] with bovine serum albumin as standard. Paper chromatography was done with Whatman no. 1 paper, either with butanol/pyridine/water (6/4/3) (solvent A) or butanol/pyridine/water (4/3/4) (solvent B).

Abbreviations: Dol, dolichyl; Fic, ficaprenol; Gal, galactose; Glc, glucose; P, phosphate; UDP, uridine diphosphate

3. Results

3.1. *Transfer of galactose from Dol-P-Gal*

Incubation of Dol-P-Gal with liver microsomes led to a transfer of radioactivity from the chloroform/methanol (2/1) soluble fraction to the 1203 solvent-soluble fraction (see table 1). The results are similar to those reported for the transfer of glucose from Dol-P-Glc to the endogenous acceptor yielding

Table 1
Transfer of galactose from different donors[a]

Substrate	[^{14}C]Galactoseincorporation (pmol/mg protein)	
	1203 solvent	residue
Dol-P-[^{14}C]Gal	0.78	0.61
Dol-P-[^{14}C]Gal[b]	5.25	2.41
Dol-P-[^{14}C]Gal[c]	0.31	0.21
Fic-P-[^{14}C]Gal	0.16	0.02
Dol-P-[^{14}C]Gal + UDP-Gal (500 pmol)	0.85	0.62
UDP-[^{14}C]Gal	0.01	0.27
UDP-[^{14}C]Gal + Dol-P (2 μl)	0.01	0.38
UDP-[^{14}C]Gal + Fic-P (1.8 nmol)	0.01	0.37

[a] In the standard incubation mixture Fic-P-[^{14}C]Gal (10 000 cpm, ~20 pmol) or UDP-[^{14}C]Gal (140 000 cpm) were substituted for Dol-P-[^{14}C]Gal, where indicated
[b] Carried out with brain enzyme
[c] Carried out with thymus enzyme

Dol-P-P-Glc-oligosaccharide [10]. It may be observed that microsomes from liver, brain and thymus catalyze the transfer from Dol-P-Gal and that Fic-P-Gal also serves as galactose donor although less efficiently. Some radioactivity was recovered in the 1203 solvent-insoluble residue which was presumably due to transfer to glycoprotein, but this fraction was not studied in detail. An excess of unlabeled UDP-Gal did not interfere with the transfer from Dol-P-Gal and UDP-Gal was not active as donor even in the presence of Dol-P or Fic-P. This latter result indicates that in rat-liver microsomes the Dol-P-Gal-synthesizing system is either absent or not operating under the conditions used.

The transfer from Dol-P-Gal was maximal at 30°C and reached a plateau after about 15 min. The addition of detergent was indispensable. The optimal concentration for Triton X-100 was 1%.

3.2. *The galactose-containing lipid*

On treatment with 0.1 N HCl for 150 min at 100°C the compound formed by transfer from Dol-P-Gal was found to release galactose as judged by paper chromatography with Solvent A.

The galactose-labeled compound was compared with Dol-P-P-Glc-oligosaccharide as regards to its elution from DEAE-cellulose columns with 1103 solvent and increasing concentrations of ammonium formate in the same solvent [20]. Both compounds were eluted with 10 mM ammonium formate, whereas Dol-P-Gal appeared with the void volume. On paper chromatography with solvent A, both the glucose- and galactose-containing compounds remained at the origin while in solvent B they migrated slightly more than glucose (R_{Glc} 1.15) (see fig.1A).

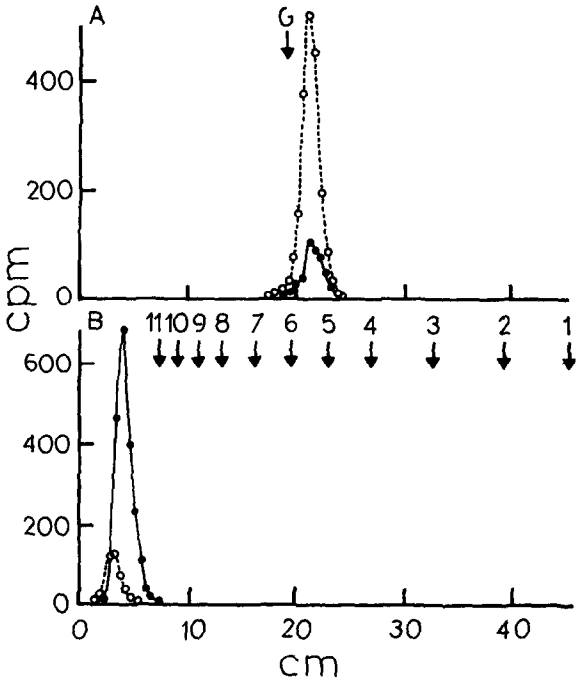

Fig.1 (A). Paper chromatography of [^{14}C]galactose- and [^{3}H]glucose-labeled material extractable with 1203 solvent. Both substances were spotted together on paper and then chromatographed with solvent B for 24 h. The paper strip was cut in pieces 0.5 cm long and counted for ^{3}H and ^{14}C. (●——●) [^{14}C]Galactose-labeled material; (○- - -○) Dol-P-P-[^{3}H]Glc-oligosaccharide. G = glucose.

Fig.1 (B). Paper chromatography of the water-soluble [^{14}C]galactose-labeled material obtained after pH 2 hydrolysis of the 1203 solvent extract. Dol-P-P-[^{3}H]Glc-oligosaccharide was added to the extract before the acid treatment. The chromatogram was developed in solvent B for 3 days and counted as above. (●——●) ^{14}C; (○- - -○) ^{3}H. Numbers on the arrows indicate the position of oligosaccharides of the maltose series.

3.3. *Properties of the galactose-containing oligosaccharide*

After mild acid hydrolysis (pH 2, at 100°C) of the galactose-containing lipid, the radioactivity became water-soluble in < 10 min. Paper chromatography of the product in solvent B gave a single radioactive spot (R_{Glc} 0.09). Under the same conditions the Glc-oligosaccharide gave R_{Glc} 0.07 (fig.1B).

Incubation of the oligosaccharides with α-mannosidase led to an increase in their chromatographic mobility, estimated to correspond to the loss of 3–5 sugar residues (fig.2A,B). The same treatment on the Glc-oligosaccharide produced a similar decrease in size (fig.2D,E). In no case was radioactive glucose or galactose liberated.

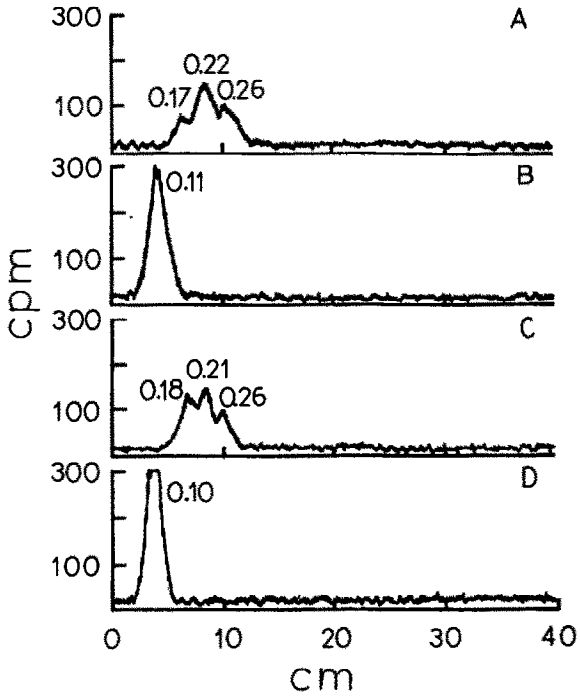

Fig.2. Effects of α-mannosidase treatment on the oligosaccharides obtained after mild acid hydrolysis. Frames A, B, [^{14}C]galactose-labeled oligosaccharides; frames C, D, [^{14}C]glucose-labeled oligosaccharides. The samples in A and in C were incubated with α-mannosidase as in section 2. Frames B, D show untreated samples. After the incubation, a chloroform/methanol/water (3/2/1) partition was performed and the upper phase was spotted on Whatman no. 1 paper and run with solvent B. The paper strips were scanned for radioactivity. The numbers of the peaks correspond to their R_{Glc}.

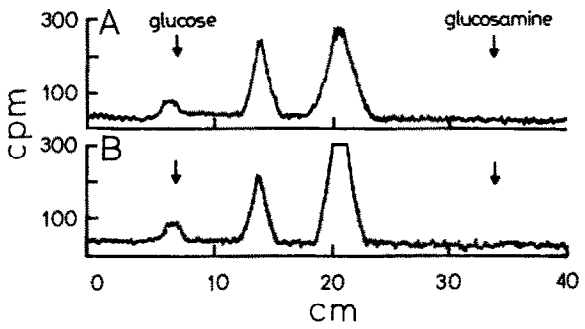

Fig.3. Electrophoresis of partially deacetylated oligosaccharides. The water-soluble products obtained after pH 2 hydrolysis of 1203 solvent extracts were treated with 2 M NaOH/1 M $NaBH_4$, at 100°C, for 6 h and processed as in [12]. The samples were run in Whatman no. 1 paper with 5% formic acid at 20 V/cm for 3 h [26], and the paper strips were scanned as in fig.2. Frame A, [^{14}C]Gal-oligosaccharide; frame B, [^{14}C]Glc-oligosaccharide.

Further information on the similarity of the Glc-oligosaccharide and the galactose-containing compound was obtained by treatment with borohydride and KOH. Under these conditions the hemiacetal group was reduced and the acetyl groups joined to hexosamines were removed [20]. When the products were separated by electrophoresis three peaks of radioactivity could be observed (fig.3): neutral, slow and fast, which corresponded to the removal of 0, 1 or 2 acetyl groups. The Glc-oligosaccharide and the galactose-containing oligosaccharide behaved identically. *N*-Acetylation of either slow or fast compounds produced the neutral one (experiment not shown).

4. Discussion

The evidence presented shows that Dol-P-Gal can donate its galactose moiety to an endogenous acceptor resulting in the labeling of an *N*-acetylglucosamine- and mannose-containing compound, very similar to Dol-P-P-Glc-oligosaccharide studied in [10,13–17]. The probable structure was inferred from experiments carried out in parallel on both compounds.

The similarity between these two products is so great that one wonders if the formation of the Dol-P-P-galactose-oligosaccharide might not be attributed to a lack of specificity of the glucosyl transferase. This possibility however seems unlikely because in

experiments carried out in the presence of a 2000-fold excess of unlabeled UDP-Glc, the incorporation of [^{14}C]galactose was reduced only to 40%.

The slight difference observed in the sizes of the Glc-oligosaccharide and the galactose-oligosaccharide (fig.1B) can be attributed to the different procedures used to prepare them. The Glc-oligosaccharide was obtained in the absence of detergent [12]. In its presence, the Glc-oligosaccharide formed was of the same size as the galactose-oligosaccharide. Furthermore, in incubations containing simultaneously UDP-[^{3}H]Glc and Dol-P-[^{14}C]Gal, both sugars were incorporated into a product of about the same size (experiments not shown), although it is not clear if they are linked to the same or different oligosaccharides.

The similarity of the galactose-containing compound with Dol-P-P-Glc-oligosaccharide suggests that they may have a similar metabolic role. Dol-P-P-Glc-oligosaccharide is now known to be an important intermediate in protein glycosylation. Its oligosaccharide moiety is transferred en bloc to an asparagine residue of the acceptor protein. Some of the external sugars (glucose and mannose) are subsequently removed, and in some cases, other sugars (*N*-acetylglucosamine, galactose, *N*-acetylneuraminic acid) are then incorporated [1,16,21–25]. The galactosylated compound might also participate, either before or after its transfer to a protein, in some recognition process, after which removal of some hexose units, galactose among them, would follow.

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