Effects of Dolichol Monophosphate on Galactose Incorporation into Glycoconjugates of Cell Cultures

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Cultured-cell homogenates catalysed the incorporation of galactose from UDP-galactose into protein and sphingolipid acceptors. Dolichol monophosphate stimulated the incorporation of galactose into glycoproteins, but it did not affect the rate of glycosylation of either exogenous or endogenous glycosphingolipids. It is proposed that, under certain conditions, galactose may be incorporated into glycoproteins via polyisoprenol intermediates, as is the case with *N*-acetylglucosamine and mannose.

It is now well established that dolichol phosphates are involved in the biosynthesis of mammalian glycoproteins (Hemming, 1973; Behrens, 1974; Heath *et al.*, 1974). Although it is clear that transfer of *N*-acetylglucosamine and mannose from their nucleotide sugars to protein occurs via lipid intermediates, it is not yet certain whether the incorporation of other sugars, such as galactose, into glycoproteins requires an intermediate lipid carrier. Behrens *et al.* (1971) using liver microsomal fractions did not find any effect of dolichol monophosphate on the incorporation of galactose into glycoprotein. However, it has been shown that galactose is transferred from UDP-galactose to a lipid with the acid-lability characteristics of a polyisoprenol phosphate sugar; ATP was required, presumably to protect the UDP-galactose from enzymic hydrolysis (Zatta *et al.*, 1975). There has also been a report of the formation of retinol phosphate galactose in mastocytoma homogenates (Helting & Peterson, 1972). Behrens *et al.* (1971) showed that dolichol monophosphate did not have any significant effect on the transfer of sugars to ceramide, but, in an assay *in vitro*, it has been shown that retinol phosphate galactose can transfer its sugars to the glycosphingolipid, lactosylceramide (Yogeeswaran *et al.*, 1975).

In this laboratory the transfer of galactose to the glycosphingolipids has been investigated in cell-culture systems, and it has been shown that this enzyme activity has a particular dependence on phospholipids and detergents for activity (McEvoy *et al.*, 1976). It was desirable to determine whether isoprenol intermediates were involved in this system, and accordingly the effect of dolichol monophosphate was tested on the incorporation of galactose into glycolipids and glycoproteins in cultured-cell homogenates.

Materials and Methods

NIL 2E cells (clone 8) were cultured and homogenized as previously described (McEvoy *et al.*, 1976). The assay medium consisted of 0.02 μ mol of UDP-[1-¹⁴C]galactose (specific radioactivity 10 μ Ci/ μ mol), 2 μ mol of MnCl₂, 2.5 μ mol of sodium phosphate buffer, pH 6.1, approx. 1 mg of homogenate protein [measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard] in a final volume of 200 μ l. For measurement of radioactivity incorporation into glycolipid, the reaction was terminated by the addition of 5 ml of chloroform/methanol (2:1, v/v) and 1 ml of water. The lower phase was washed twice with Folch upper phase (Radin, 1969) and chromatographed on silica-gel G plates (Baker-Flex, Phillipsburg, NJ, U.S.A.) with chloroform/methanol/water (65:25:4, by vol.) as solvent. The radioactive glycolipid spots were located by radioautography; they were cut out, placed in vials containing 3 ml of scintillant (0.5% 2,5-diphenyloxazole in toluene) and quantified by scintillation spectrometry.

UDP-galactose and its water-soluble products galactose and galactose 1-phosphate were determined by subjecting a 50 μ l sample of the Folch upper phase to ion-exchange t.l.c. on polyethyleneimine-cellulose plates (Baker-Flex) with aq. 0.3 M-LiCl as solvent. For measurement of galactose incorporation into protein, the same incubation medium was used, but the protein was extracted and analysed for radioactivity by the method of Patt & Grimes (1975).

Dolichol monophosphate was a gift from Dr. F. W. Hemming, University of Nottingham. Lactosylceramide was obtained from Miles Yeda Ltd. (Rehovot, Israel) and was purified by t.l.c. before use (McEvoy *et al.*, 1976).

Results and Discussion

Galactose can be transferred from UDP-galactose to form either the monohexoside galactosylceramide or a series of neutral glycosphingolipids (Brady & Fishman, 1974). In NIL-cell homogenates, however, the main radioactive lipid product after incubation with UDP-[^{14}C]galactose was ceramide trihexoside, with no significant radioactivity being incorporated into either the monohexoside or the dihexoside. This reaction, which is presumably a galactosyltransferase activity towards endogenous ceramide dihexoside, is very sensitive to detergent inhibition. Considerable activity towards the exogenous substrate, however, is obtained by addition of a suitable detergent, such as Triton X-100 (McEvoy *et al.*, 1976). Neither of these activities was affected by the addition of dolichol monophosphate under the standard assay conditions (Table 1). No effect of dolichol monophosphate on glycolipid glycosylation could be observed under other conditions, such as lower temperatures and shorter incubation times. Also, there was no appearance of a new radioactive spot which might have indicated that the dolichol monophosphate was acting as an acceptor for galactose.

There was considerable transfer of galactose to protein acceptors and this activity was easily saturated. Activity appeared to be linear only for short times, less than 1 h, compared with the activity of the glycosphingolipid glycosylation which remained linear up to 2 h. Incorporation of galactose into protein was stimulated by dolichol monophosphate between 2- and 4-fold in four different experiments. Control experiments showed that this was a specific effect; other phospholipids (phosphatidylinositol and phosphatidylserine) added at this concentration had no effect on the enzyme activity.

Other workers have shown that UDP-galactose is

Table 1. *Effect of dolichol monophosphate on the incorporation of galactose into glycolipids and glycoproteins of NIL-cell homogenates*

For measurement of exogenous glycosylation of lactosylceramide, 20 μg of the lipid substrate and 600 μg of Triton X-100 were added to the incubation. Endogenous glycosylation was measured in the absence of detergent (McEvoy *et al.*, 1976).

Acceptor	Dolichol monophosphate (μg)	Conditions	Activity (pmol/h per μg)
Protein	0	15 min (20°C)	705
	10	15 min (20°C)	2300
Lactosylceramide	0	1 h (37°C)	19.0
(exogenous)	10	1 h (37°C)	19.4
Lactosylceramide	0	1 h (37°C)	12.4
(endogenous)	10	1 h (37°C)	12.8

Table 2. *UDP-galactose hydrolysis during incubation*

Dolichol monophosphate (μg)	Conditions	Radioactivity remaining as UDP-galactose (%)
0	0	99
10	15 min (20°C)	92
0	15 min (20°C)	89
0	2 h (37°C)	84
10	2 h (37°C)	82

very susceptible to enzymic hydrolysis (Zatta *et al.*, 1975; Patt & Grimes, 1975). In the experimental conditions used here, UDP-[^{14}C]galactose was present at a significant molarity (0.1 mM), and a considerable proportion of the nucleotide still remained after incubation, and this was little affected by the presence of dolichol monophosphate. These findings are consistent with the fact that we could not find any stimulation of galactose incorporation into glycolipids by the nucleotides ATP or AMP; these nucleotides have been reported to protect UDP-galactose against enzymic hydrolysis (Zatta *et al.*, 1975; Patt & Grimes, 1975). The breakdown product from UDP-galactose was mainly galactose 1-phosphate, with a very minor proportion (less than 2%) as the free galactose.

These results show that dolichol monophosphate gave a definite stimulation of galactose incorporation into protein, and thus, in agreement with the data of Zatta *et al.* (1975), it seems likely that dolichols are to some extent involved in the transfer of galactose, as well as mannose and *N*-acetylglucosamine, into protein. However, the difficulty in demonstrating the presence of isoprenol phosphate galactose intermediate cannot simply be due to the lability of the UDP-galactose (Zatta *et al.*, 1975), since in our experiments the concentration of this nucleotide was not greatly diminished during the incubation (Table 2). It is possible, however, that a dolichol phosphate galactose compound is formed *in vivo*, but cannot be detected *in vitro* owing to its extreme lability.

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