

Deoxynojirimycin inhibits the formation of Glc₃Man₉GlcNAc₂-PP-dolichol in intestinal epithelial cells in culture

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The lipid-linked oligosaccharides synthesized in the presence of the α -glucosidase inhibitors, 1-deoxynojirimycin (DJN) and *N*-methyl-1-deoxynojirimycin (MDJN), were compared in IEC-6 intestinal epithelial cells in culture. HPLC analysis of the oligosaccharides obtained before and after exhaustive jack bean α -mannosidase digestion indicates that control and MDJN-treated cells synthesize similar amounts of Glc₃Man₉GlcNAc₂-PP-dolichol. In contrast, the formation of this compound is greatly reduced in DJN-treated cells, the major lipid-linked oligosaccharide found being Man₉GlcNAc₂-PP-dolichol. The decreased availability of the preferred donor for protein glycosylation may account for the impaired glycosylation and secretion of certain glycoproteins in the presence of DJN.

<i>1-Deoxynojirimycin</i>	<i>N-Methyl-1-deoxynojirimycin</i>	<i>Glucosidase inhibitor</i>	<i>Dolichol-linked oligosaccharide</i>
		<i>Processing</i>	

1. INTRODUCTION

Several compounds that inhibit the biosynthesis of *N*-linked complex oligosaccharides by preventing the action of glucosidases and mannosidases involved in processing of the oligosaccharides have recently been described [1–7]. These inhibitors are very useful for studying the role of oligosaccharides in glycoproteins, provided that their mode of action is clearly understood.

In previous work, we showed that DJN and MDJN prevent the synthesis of complex oligosaccharides in IEC-6 intestinal epithelial cells, and cause the accumulation of a mixture of oligosac-

charides containing 1–3 glucose residues and 7–9 mannose residues. A much larger proportion of the oligosaccharides formed in the presence of MDJN contained 3 glucose residues compared to those formed in the presence of DJN [8].

Here we report the effects of DJN and MDJN on the synthesis of lipid-linked oligosaccharides in IEC-6 cells, and demonstrate that DJN greatly reduces the synthesis of glucosylated lipid-linked oligosaccharides, particularly of Glc₃Man₉GlcNAc₂-PP-dolichol, whereas normal amounts of the latter are formed in MDJN-treated cells.

2. MATERIALS AND METHODS

The source of chemicals and of D-[2-³H]mannose was described previously [8]. Confluent IEC-6 rat intestinal epithelial cells were incubated in 100-mm dishes for different periods of time as described in [8]. After labeling, the medium was removed, 3 ml methanol was added to the dishes,

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Abbreviations: DJN, 1-deoxynojirimycin; MDJN, *N*-methyl-1-deoxynojirimycin; endo H, endo- β -*N*-acetylglucosaminidase H; R, retention time of each oligosaccharide divided by the retention time of standard Glc₃Man₉GlcNAc

the cells were scraped, transferred with 3 ml methanol into tubes, and chloroform was added to yield chloroform/methanol (2:1, v/v). The pellet was extracted successively with 2 ml chloroform/methanol (2:1, v/v), 0.5 ml methanol, 3 times 3 ml water, 0.5 ml methanol, and finally 3 times 3 ml chloroform/methanol/water (10:10:3, by vol.). The chloroform/methanol/water fraction was dried with air and hydrolyzed in 1 ml of 0.1 M HCl for 30 min at 90°C. It was then neutralized with NaOH, desalted through coupled columns of AG 50W-X8 (H⁺ form, 200–400 mesh) and AG1-X8 (formate form, 200–400 mesh), and lyophilized. The hydrolysate was treated with endo H and chromatographed on a column of Bio-Gel P-6. The fractions containing labeled oligosaccharides were pooled, subjected to HPLC with ¹⁴C-labeled standards, before and after exhaustive digestion with jack bean α -mannosidase, as described [8].

3. RESULTS AND DISCUSSION

3.1. Effect of the inhibitors on the incorporation of D-[2-³H]mannose into lipid-linked oligosaccharides

The effects of DJN (5 mM) and MDJN (2 mM) on the labeled lipid-linked oligosaccharides were examined at different times, varying from 15 min to 2 h, after incubation of IEC-6 cells with D-[2-³H]mannose, under the conditions used previously to study the protein-bound oligosaccharides [8]. The incorporation of mannose into these oligosaccharides was slightly higher in the presence of MDJN than in the control, whereas it was either the same or slightly inhibited in the presence of DJN.

When the labeled oligosaccharides were fractionated by HPLC, the major product found in untreated cells and in cells incubated with MDJN was eluted with the ¹⁴C-labeled Glc₃Man₉GlcNAc standard (fig.1A,B). In DJN-treated cells, however, little radioactivity corresponded to Glc₃Man₉GlcNAc, and the major labeled fraction was eluted earlier than the standard glucose-containing oligosaccharides ($R = 0.8$) (fig.1C). Since this elution position corresponds to that of Man₉GlcNAc [8], these results indicate that non-glucosylated lipid-bound oligosaccharide is found in DJN-treated cells.

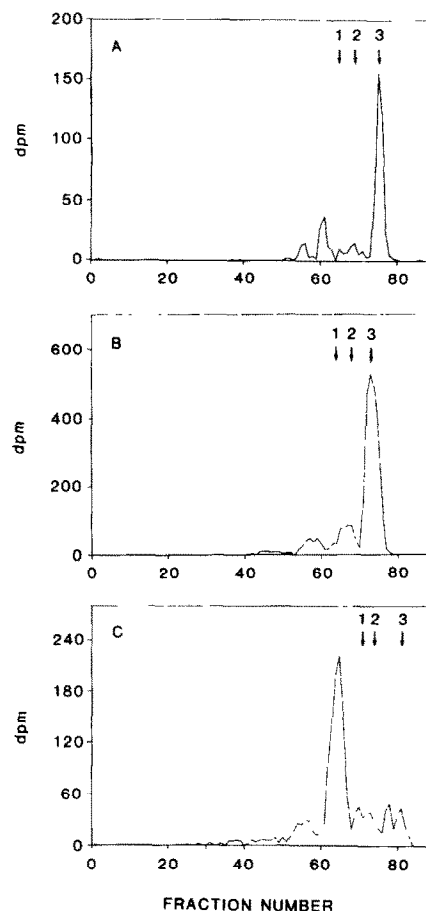


Fig.1. HPLC of oligosaccharides released from lipid-linked oligosaccharides. IEC-6 cells were incubated for 15, 30 and 120 min with 10 μ Ci/ml D-[2-³H]mannose. The chloroform/methanol/water (10:10:3, by vol.) fractions were obtained and pooled. The oligosaccharides were released by mild acid treatment, treated with endo H and fractionated by HPLC as indicated in section 2. A, control cells; B, cells treated with 2 mM MDJN; C, cells treated with 5 mM DJN. The inhibitors were added 5–6 h before D-[2-³H]mannose. Arrows indicate the elution positions of the ¹⁴C-labeled standards: 1, Glc₁Man₉GlcNAc; 2, Glc₂Man₉GlcNAc and 3, Glc₃Man₉GlcNAc. Different aliquots were applied to the HPLC column in A, B and C. Similar inhibition of Glc₃Man₉GlcNAc labeling by DJN was observed after 5 min incubation (unpublished).

3.2. α -Mannosidase treatment of the oligosaccharides

To distinguish between glucosylated and non-glucosylated oligosaccharides, exhaustive α -

mannosidase treatment was performed, followed by HPLC of the products. We showed in [8] that this procedure separates $\text{Glc}_3\text{Man}_4\text{GlcNAc}$, $\text{Glc}_2\text{Man}_4\text{GlcNAc}$ and $\text{Glc}_1\text{Man}_4\text{GlcNAc}$, and provides an estimate of the number of glucose residues originally present in the oligosaccharides. In control cells, 60% of the radioactivity present in the oligosaccharides was released as mannose; this value is close to the 56% expected from standard $\text{Glc}_3\text{Man}_9\text{GlcNAc}$. Furthermore, the major oligosaccharide product of α -mannosidase treatment corresponded to $\text{Glc}_3\text{Man}_4\text{GlcNAc}$ (table 1). Similar results were obtained for MDJN-treated cells, demonstrating that the major lipid-linked oligosaccharide formed in these two cases is $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$. In DJN-treated cells, however, an increased proportion of labeled mannose was released by α -mannosidase, indicating the presence of non-glucosylated oligosaccharides. In addition, there was a large decrease in the proportion of oligosaccharides containing 3 glucose residues (table 1). These results indicate that the major lipid-linked oligosaccharide fraction ($R = 0.8$) found in DJN-treated cells contains primarily $\text{Man}_9\text{GlcNAc}$, and some $\text{Glc}_1\text{Man}_8\text{GlcNAc}$, $\text{Glc}_2\text{Man}_8\text{GlcNAc}$ and $\text{Glc}_3\text{Man}_7\text{GlcNAc}$ [8].

3.3. Conclusions

The above results demonstrate that MDJN-treated cells synthesize normal amounts of $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ -PP-dolichol which is the major oligosaccharide donor required for protein glycosylation [9]. DJN, on the other hand, greatly reduces the amount of glucosylated lipid-linked

oligosaccharide formed, and the proportion of $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ -PP-dolichol. In previous work, an inhibitory effect of DJN on the incorporation of D-[2- ^3H]mannose into lipid-linked oligosaccharides in medium containing pyruvate was observed [10], but the present results demonstrate that even with little inhibition of total lipid-oligosaccharide synthesis, a major effect of DJN is the greatly reduced synthesis of $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ -PP-dolichol precursor. The mechanism whereby this inhibition occurs in vivo is unclear since DJN does not inhibit the formation of dolichol-P-glucose and dolichol-PP-oligosaccharide from UDP-[^{14}C]glucose in calf pancreas microsomes (unpublished).

It is evident therefore that the effects of DJN on glycoproteins in vivo may result not only from its action on processing glucosidases, but also from its inhibitory effects on glucosylation of lipid oligosaccharides. Since $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ is the preferred substrate for transfer to protein [9], the decrease in $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$ -PP-dolichol donor in DJN-treated cells may account for the incomplete glycosylation of certain proteins which has recently been reported [11–13]. It is also possible that the decreased transport from rough endoplasmic reticulum to Golgi and the decreased secretion of certain glycoproteins which has been observed [11–14] are caused by conformational changes resulting from this incomplete glycosylation. It may also be responsible for the lower proportion of protein-bound oligosaccharides containing 3 glucose residues obtained in the presence of DJN [8]. Conclusions derived from studies with

Table 1
Products of α -mannosidase treatment of oligosaccharides

Additions	Concentration (mM)	dpm (%)			
		Glc_1^a	Glc_2^a	Glc_3^a	Mannose ^b
None	—	130 (4)	80 (3)	960 (33)	1760 (60)
MDJN	2.0	30 (1)	180 (4)	1980 (43)	2430 (53)
DJN	5.0	270 (9)	160 (5)	360 (11)	2400 (75)

^a Glc_1 , $\text{Glc}_1\text{Man}_4\text{GlcNAc}$; Glc_2 , $\text{Glc}_2\text{Man}_4\text{GlcNAc}$; $\text{Glc}_3\text{Man}_4\text{GlcNAc}$

^b Under these conditions mannose and $\text{Man}\beta 1,4\text{GlcNAc}$ disaccharide are not separated

The endo H-treated oligosaccharides were exhaustively digested with α -mannosidase and the products were fractionated by HPLC (see section 2)

DJN regarding the role of carbohydrate in glycoproteins should therefore be assessed in light of the above findings.

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