Effects of manno-1-deoxynojirimycin and 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine on *N*-linked oligosaccharide processing in intestinal epithelial cells

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The effects of manno-1-deoxynojirimycin (ManDJN) and 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) were compared in IEC-6 intestinal epithelial cells in culture. ManDJN caused complete inhibition of *N*-linked complex oligosaccharide synthesis whereas a maximum of 80% inhibition was obtained with DMDP. HPLC showed similar endo H-sensitive oligosaccharides for control and treated cells. ManDJN caused a large increase in the levels of labeled Man_{7-9} GlcNAc and a decrease in $Man_5GlcNAc$. DMDP produced similar changes except that the increase in $Man_{7-9}GlcNAc$ was less pronounced and some increase in glucosylated oligosaccharides was observed. Since the major oligosaccharides found in DMDP-treated cells were non-glucosylated, its primary effect on complex oligosaccharide synthesis is not due to inhibition of glucosidases, in contrast to what has been reported for influenza virus-infected MDCK cells [(1984) J. Biol. Chem. 259, 12409–12413].

Manno-1-deoxynojirimycin *2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine* *Processing inhibitor* *Glycoprotein synthesis*

1. INTRODUCTION

Compounds which can modify the structure of carbohydrates are useful to elucidate their function on glycoproteins. The *N*-linked oligosaccharides consist of high mannose, complex and hybrid structures which all arise from processing of the common precursor, $Glc_3Man_9GlcNAc_2$. Both α-glucosidases and α-mannosidases are involved in the first steps of processing [1]. Inhibitors of these enzymes have been shown to prevent the biosynthesis of *N*-linked complex oligosaccharides [2–6].

Recently, two novel inhibitors of complex oligosaccharide biosynthesis were described: the mannose analog of DJN, ManDJN, was found to inhibit α-mannosidase I [7,14]; and the pyrrolidine alkaloid, DMDP, was reported to prevent the action of glucosidase I [8].

In previous work we demonstrated that DJN and MDJN inhibit processing glucosidases and prevent the synthesis of *N*-linked complex oligosaccharides in intestinal epithelial cells in culture [3,9,10]. Fractionation by HPLC demonstrated that a mixture of glucosylated high mannose oligosaccharides containing 1–3 glucose residues and 7–9 mannose residues accumulate in the presence of these inhibitors, and that a much larger proportion of these oligosaccharides contained 3 glucose residues in the presence of MDJN than with DJN [10].

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Abbreviations: DJN, 1-deoxynojirimycin; DMDP, 2[*R*],5[*R*]-dihydroxymethyl-3[*R*],4[*R*]-dihydroxypyrrolidine; ManDJN, 1,5-dideoxy-1,5-imino-D-mannitol (manno-1-deoxynojirimycin); MDJN, *N*-methyl-1-deoxynojirimycin; endo H, endo-β-*N*-acetylglucosaminidase H

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Here, we compare the effects of ManDJN and DMDP on glycosylation in intestinal epithelial cells with those obtained previously with DJN and MDJN. Of these 4 compounds, ManDJN is the only one which causes complete inhibition of complex chain synthesis; in its presence only high mannose oligosaccharides are formed, and there is a large increase in $Man_{7-9}GlcNAc$ species. DMDP inhibits complex chain formation to about the same extent as DJN and MDJN, but the major oligosaccharides formed in its presence are non-glucosylated high mannose oligosaccharides, in contrast to the results reported in MDCK cells infected with influenza virus [8].

2. MATERIALS AND METHODS

The source of chemicals was described previously [10]. ManDJN was obtained from Dr G. Kinast, Bayer AG, Wuppertal, FRG. DMDP was isolated from the seeds of *Lonchocarpus sericeus* [11], and was shown by ^{1}H-NMR to have the reported structure [12].

The methods used were all described previously [3,10]. Confluent IEC-6 rat intestinal epithelial cells were incubated for 24 h with 10 μCi/ml of D-[2-^{3}H]mannose, and labeled glycopeptides obtained by exhaustive pronase digestion were fractionated on Bio-Gel P-6, before and after endo H treatment to separate glycopeptides containing complex oligosaccharides from high mannose oligosaccharides. The high mannose oligosaccharides were further fractionated by HPLC with [^{14}C]glucose-labeled standards, before and after exhaustive jack bean α-mannosidase digestion.

3. RESULTS

3.1. *Effect of the inhibitors on glycosylation*

The effects of ManDJN and DMDP on glycoprotein biosynthesis in IEC-6 cells were examined after labeling with D-[2-^{3}H]mannose under the conditions used previously with DJN and MDJN [10]. ManDJN completely inhibited the incorporation of labeled mannose into glycopeptides containing complex oligosaccharides and greatly increased (2.4 fold) the labeling of the high mannose oligosaccharides (fig.1A,B). Similarly, the incorporation of labeled mannose into complex oligosaccharides was greatly inhibited by 5 mM

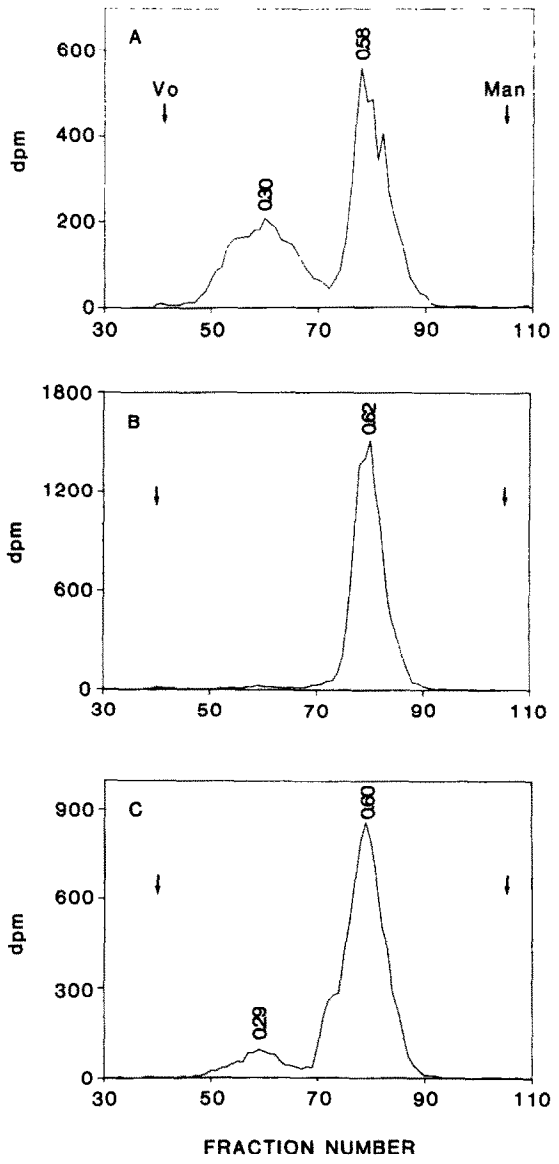

Fig.1. Effects of ManDJN and DMDP on glycopeptides IEC-6 cells labeled with D-[2-^{3}H]mannose. Labeled glycopeptides obtained by exhaustive pronase digestion were first fractionated on a column of Bio-Gel P-6 (not shown). The labeled glycopeptides were then incubated with endo H as described under section 2, and the products chromatographed on the same column of Bio-Gel P-6. Endo H treatment did not affect the elution of glycopeptides with K_{av} = 0.3 and caused a change in elution of glycopeptides (K_{av} = 0.45) to oligosaccharides (K_{av} = 0.6) found in fractions 70–90. (A) Control cells, (B) cells treated with 2.0 mM ManDJN, (C) cells treated with 5.0 mM DMDP.

DMDP (fig.1C), while the labeling of the high mannose oligosaccharides was increased (about 75%). The maximum inhibition of complex oligosaccharide synthesis was 80% and was never complete even if the concentration of DMDP was increased to 10 mM. Neither compound inhibited the formation of $Glc_3Man_9GlcNAc_2$-PP-dolichol pyrophosphate, in marked contrast to the inhibition observed with DJN [13].

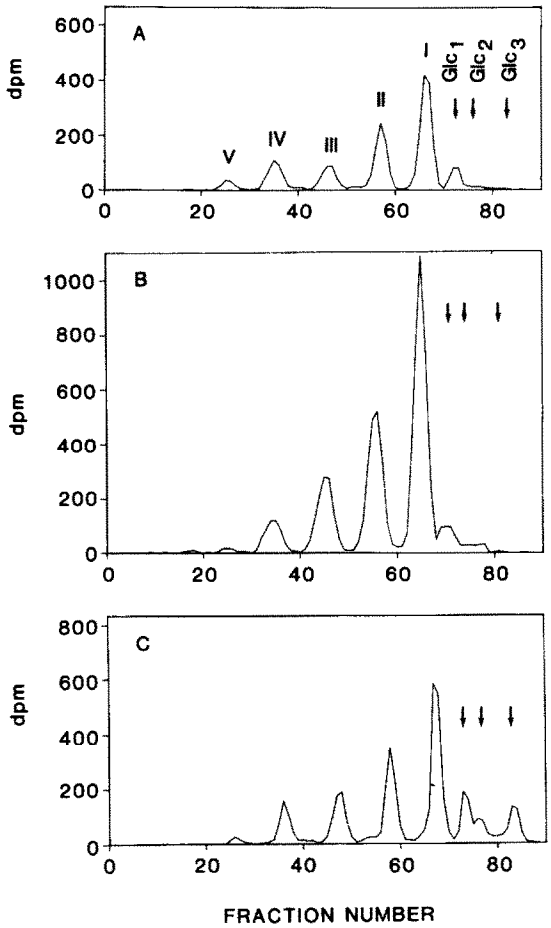

Fig.2. HPLC of endo H-sensitive oligosaccharides. The labeled oligosaccharides (K_{av} = 0.6) released by endo H obtained from Bio-Gel P-6 (fig.1) were fractionated by HPLC as described under section 2. (A) Control cells, (B) cells treated with 2.0 mM ManDJN, (C) cells treated with 5 mM DMDP. The arrows indicate the elution position of ^{14}C-labeled standards: Glc_1, $Glc_1Man_9GlcNAc$; Glc_2, $Glc_2Man_9GlcNAc$; Glc_3, $Glc_3Man_9GlcNAc$. I, $Man_9GlcNAc$; II, $Man_8GlcNac$; III, $Man_7GlcNAc$; IV, $Man_6GlcNac$; V, $Man_5GlcNAc$.

3.2. *HPLC of the oligosaccharides*

The endo H released oligosaccharides were fractionated by HPLC. The patterns of oligosaccharides obtained from control and treated cells were similar; the major oligosaccharides corresponded in elution to $Man_{5-9}GlcNAc$ with only a small proportion eluting with the glucosylated standards (fig.2). The presence of ManDJN caused a large increase in labeled $Man_7GlcNAc$ (3.6 fold), $Man_8GlcNAc$ (2.7 fold) and $Man_9GlcNAc$ (2.6 fold), little change in $Man_6GlcNAc$ and a decrease (50%) in $Man_5GlcNAc$. Similar results were obtained for ManDJN concentrations between 2.0 and 4.0 mM. With DMDP similar changes in the oligosaccharides were observed, except that the increase in $Man_7GlcNAc$, $Man_8GlcNAc$ and $Man_9GlcNAc$ was less pronounced, and some increase in oligosaccharides eluting with the glucosylated standards was observed. When the concentration of DMDP was increased from 5 to 10 mM, there was a further increase in the glucosylated oligosaccharide species, but the inhibition of complex oligosaccharide synthesis remained about the same.

To confirm that the majority of the labeled oligosaccharides were non-glucosylated high mannose species, they were exhaustively digested with jack bean α-mannosidase, and the products were fractionated by HPLC. For control and ManDJN-treated cells, α-mannosidase released almost all the radioactivity (96–98%) in the mannose-containing fraction (table 1), as expected for non-glucosylated high mannose oligosaccharides. For DMDP-treated cells, a similar treatment released slightly less radioactivity (about 85%), and a small proportion of radioactivity was recovered in $Glc_{1-3}Man_4GlcNAc$ oligosaccharides. For comparison the results obtained previously [10] for a similar α-mannosidase treatment of the oligosaccharides formed in the presence of the glucosidase inhibitor MDJN are shown in table 1. In this case, less than 50% of the radioactivity was released as mannose by α-mannosidase, and a major proportion of the radioactivity was recovered in $Glc_3Man_4GlcNAc$.

4. DISCUSSION

The above results demonstrate that ManDJN causes a much more complete inhibition of *N*-

Table 1

Products of α-mannosidase treatment of oligosaccharides

Additions	Concentration (mM)	Glc_1[a]	Glc_2[a]	Glc_3[a]	Mannose[b]
		(dpm (%))			
None	–	110 (4)	–	–	2880 (96)
ManDJN	2.0	60 (2)	–	–	2950 (98)
DMDP	5.0	65 (4)	75 (4)	100 (5)	1600 (87)
MDJN	2.0	570 (7)	555 (7)	3220 (41)	3490 (45)

[a] Glc_1 = $Glc_1Man_4GlcNAc$, Glc_2 = $Glc_2Man_4GlcNAc$, Glc_3 = $Glc_3Man_4GlcNAc$
[b] Under these conditions mannose and Manβ1⟶4GlcNAc disaccharide are not resolved

Endo H-sensitive oligosaccharides obtained after Bio-Gel P-6 chromatography (fig.1) were treated with jack bean α-mannosidase and fractionated by HPLC

linked complex oligosaccharide synthesis in IEC-6 cells than either DJN, MDJN or DMDP. In its presence, the major oligosaccharides, which account for 86% of the labeled oligosaccharides, are $Man_7GlcNAc$, $Man_8GlcNAc$ and $Man_9GlcNAc$. These results agree with those obtained in other cells [7]. It was reported recently that ManDJN is a specific inhibitor of rat liver Golgi α-mannosidase I, and has no effect on the RER α-mannosidase [14] which can utilize $Man_{6-9}GlcNAc$ oligosaccharides as substrates [15]. It is likely, therefore, that the removal of mannose residues observed in the presence of ManDJN in vivo is due to RER α-mannosidase activity. Studies in other cells have demonstrated that processing to $Man_6GlcNAc$ and even to $Man_5GlcNAc$ can occur in the RER [16–19].

DMDP inhibits the synthesis of complex oligosaccharides in IEC-6 cells to about the same extent as was previously observed for DJN and MDJN [3,10]. In these cells, a mixture of high mannose oligosaccharides is found after DMDP treatment, with an increase in the relative proportions of $Man_{7-9}GlcNAc$ which account for 67% of the labeled oligosaccharides. There is also a small increase in glucosylated oligosaccharides, but this increase cannot account for the observed inhibition of complex oligosaccharide formation. In fact, the pattern of oligosaccharides formed in the presence of DMDP is very different from that obtained in the presence of the glucosidase inhibitors DJN and MDJN [10], and much more similar to that found in control and ManDJN-treated cells. It is concluded that the primary effect of DMDP on complex oligosaccharide synthesis does not result from inhibition of the glucosidases. DMDP may be acting by inhibiting processing α-mannosidases, or may have some other effect. The results described with DMDP in IEC-6 cells differ from those obtained in influenza virus-infected MDCK cells [8] in which the major oligosaccharides were identified as $Glc_3Man_{8-9}GlcNAc$, and the effect of DMDP on glycosylation was ascribed to inhibition of glucosidase I. The reasons for this difference in the action of DMDP in the intestinal epithelial cells and in MDCK cells are unclear, and remain to be elucidated.

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