Comparison between 1-deoxynojirimycin and *N*-methyl-1-deoxynojirimycin as inhibitors of oligosaccharide processing in intestinal epithelial cells

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The α -glucosidase inhibitor *N*-methyl-1-deoxynojirimycin (MDJN) inhibits the synthesis of *N*-linked complex oligosaccharides in rat intestinal epithelial cells to the same extent as reported previously for 1-deoxynojirimycin (DJN) [Saunier, Kilker, Tkacz, Quaroni & Herscovics (1982) *J. Biol. Chem.* **257**, 14155–14161]. Analysis of each of the endo- β -*N*-acetylglucosaminidase H (endo H)-sensitive oligosaccharides separated by h.p.l.c. with yeast glucosidase I, which specifically removes the terminal glucose residue from oligosaccharides containing three glucose residues, and with jack-bean (*Canavalia ensiformis*) α -mannosidase, indicates that both inhibitors cause the accumulation of a mixture of glucosylated oligosaccharides containing one to three glucose residues and seven to nine, and even possibly six, mannose residues. About 70% of the endo H-sensitive oligosaccharides formed in the presence of MDJN contain three glucose residues, compared with only about 20% of the corresponding oligosaccharides of the DJN treated cells. It is concluded that both compounds inhibit the formation of *N*-linked complex oligosaccharides by interfering with the processing glucosidases. These compounds are valuable in the study of the role of oligosaccharides in glycoproteins.

The major pathway for the biosynthesis of *N*-linked oligosaccharides begins with the transfer of Glc₃Man₉GlcNAc₂ from dolichyl pyrophosphate to protein. This oligosaccharide precursor is then processed to yield complex, high-mannose or hybrid structures (Hubbard & Ivatt, 1981; Stanelloni & Leloir, 1982). The first stage in processing is the removal of the glucose residues under the influence of two specific glucosidases (Grinna & Robbins, 1979; Spiro *et al.*, 1979; Elting *et al.*, 1980; Michael & Kornfeld, 1980; Ugalde *et al.*, 1980; Kilker *et al.*, 1981; Saunier *et al.*, 1982). In previous work we showed that the glucose analogue DJN is a potent inhibitor of both glucosidases and that it greatly inhibits the formation of

complex *N*-linked oligosaccharides in intact cells (Herscovics *et al.*, 1982; Saunier *et al.*, 1982). More recently it was found that MDJN inhibits processing of oligosaccharides and the formation of complex chains of viral glycoproteins (Romero *et al.*, 1983). In the present work we compare the effects of DJN and MDJN on glycoprotein biosynthesis in intestinal epithelial cells in culture, since neither the action of these two inhibitors nor the detailed structure of the products synthesized in their presence has yet been studied under identical conditions. Both compounds inhibit processing of *N*-linked oligosaccharides and cause the accumulation of a mixture of high-mannose oligosaccharides containing a variable number of glucose and mannose residues. A larger proportion of the oligosaccharides formed in the presence of MDJN contain three glucose residues.

Abbreviations used: DJN, 1-deoxynojirimycin; MDJN, *N*-methyl-1-deoxynojirimycin; all sugars are of the D configuration; *R* is the retention time of each oligosaccharide divided by the retention time of standard Glc₃Man₉GlcNAc₂; endo H, endo- β -*N*-acetylglucosaminidase H.

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Experimental

Materials

The source of chemicals was described previously (Sasak *et al.*, 1982; Saunier *et al.*, 1982). D-[2-³H]Mannose (sp. radioactivity 24–27 Ci/mmol) and D-[6-³H]galactose (sp. radioactivity 26–

35Ci/mmol) were purchased from New England Nuclear Corp., Boston, MA, U.S.A., and from Amersham, Arlington Heights, IL, U.S.A., respectively. DJN was obtained from Dr. E. Trusheit and Dr. D. Schmidt, Bayer Research Laboratories, Wuppertal, Germany. MDJN was a gift from Dr. R. T. Schwarz, Justus-Liebig-Universität, Gießen, Germany.

[^{14}C]Glucose-labelled $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2$, $\text{Glc}_2\text{Man}_9\text{GlcNAc}_2$ and $\text{Glc}_1\text{Man}_9\text{GlcNAc}_2$ were prepared from UDP-[^{14}C]glucose in chicken liver or calf pancreas microsomal fractions as described previously (Kilker *et al.*, 1981), and were treated with endo H.

Labelling of cells

Confluent IEC-6 rat intestinal-epithelial cells were labelled for 24 h with D-[2- ^3H]mannose (5–10 $\mu\text{Ci/ml}$) or with D-[6- ^3H]galactose (10–20 $\mu\text{Ci/ml}$) in 10 ml of low glucose (1 g/l) Dulbecco's modified essential medium supplemented with dialysed 5% (v/v) fetal-calf serum, 50 units of penicillin/ml, 50 μg of streptomycin/ml, 10 μg of insulin/ml and 4 mM-L-glutamine as described previously (Sasak *et al.*, 1982; Saunier *et al.*, 1982). After 24 h of labelling the cells were washed three times with 10 ml of Dulbecco's phosphate-buffered saline, pH 7.4. The cells were scraped from the dishes and extracted successively with chloroform/methanol (2:1, v/v), three times with water, twice with methanol, and finally with chloroform/methanol/water (10:10:3, by vol.).

Fractionation of oligosaccharides

The delipidated residue was exhaustively digested with Pronase and the resulting glycopeptides were chromatographed on a column of Bio-Gel P-6, before and after treatment with endo H as described previously (Sasak *et al.*, 1982). The endo H-sensitive oligosaccharides were pooled, concentrated, and chromatographed with ^{14}C -labelled standards on a column (25 cm $\times$ 0.46 cm) of 5 μm -particle size Aminospherisorb (Phase Separations; packed by Chromatography Sciences Co., Ville Mont Royal, Québec, Canada) with a Varian model 5000 liquid chromatograph. Filtered samples (100 μl) dissolved in acetonitrile/water (1:1, v/v) were applied to the column previously equilibrated with the starting solvent. Elution was performed isocratically at 1 ml/min for 20 min with acetonitrile/water (3:2, v/v) followed by a linear gradient to acetonitrile/water (21:29, v/v) for 95 min. The water contained 15 mM-potassium phosphate and 5 mM- NaN_3 adjusted to pH 5.0. Fractions (1 ml) were collected and assayed for radioactivity using 4 ml of Aquassure (New England Nuclear) as scintillation fluid and an LKB

model 1218 Rackbeta liquid-scintillation counter equipped with d.p.m. calculation program.

α -Mannosidase treatment

Oligosaccharides released by endo H obtained from h.p.l.c. or Bio-Gel P-6 columns were freeze-dried and then incubated for 72 h at 37°C in 100 μl of 50 mM-sodium acetate buffer, pH 5.0, containing 100 mM- NaCl , 0.1 mM- ZnSO_4 , 25 μg of cycloheximide and 25 μg of chloramphenicol. Jack-bean α -mannosidase (2 units) was added at the beginning, and after 24 h and 48 h of incubation. The samples were boiled for 3 min, centrifuged, and the supernatant was desalted through coupled columns of AG 50W (X8, H^+ form, 200–400 mesh) and AG1 (X8, formate form, 200–400 mesh) resin. The α -mannosidase treatment was repeated and the samples were subjected to h.p.l.c. as described above.

Separation of mannose and disaccharide

To resolve mannose and disaccharide formed after α -mannosidase treatment, descending paper chromatography was performed on Whatman no. 3MM paper in propan-2-ol/acetic acid/water (27:4:9, by vol.) for 30 h.

Glucosidase treatment

Yeast glucosidase I was prepared as described previously (Kilker *et al.*, 1981; Saunier *et al.*, 1982). Oligosaccharides obtained from h.p.l.c. were dried, resuspended in water and were desalted through coupled columns (see above). The oligosaccharides were then incubated for 8 h at 37°C in a total volume of 40 μl containing 1 mg of bovine serum albumin/ml, 25 μg of cycloheximide, 25 μg of chloramphenicol and 25 mM-sodium EDTA previously adjusted to pH 6.8. Glucosidase I, which was eluted from DEAE-Sephadex with potassium phosphate buffer, pH 6.8, was used.

Two units of enzyme as defined by Kilker *et al.* (1981) were added at the beginning of incubation. At the end of the incubation, the samples were boiled for 3 min, filtered and subjected to h.p.l.c. as described above. Although the enzyme preparation still contained a trace of α -mannosidase activity, the addition of EDTA completely prevented the removal of mannose residues from labelled $\text{Man}_9\text{GlcNAc}$.

Acid hydrolysis

Samples were hydrolysed with 0.5 M- H_2SO_4 for 8 h at 100°C. The hydrolysates were desalted as above and were chromatographed with galactose, mannose and glucose standards on Whatman no. 3MM paper in ethyl acetate/propan-2-ol/water (65:23:12, by vol.). Guide strips with standards were stained with AgNO_3 (Trevelyan *et al.*, 1950).

Radioactivity on the chromatograms was determined by cutting the paper into 1 cm strips, which were then placed into radioactivity-counting vials to which 0.5 ml of water and 4 ml of Aquassure scintillant were added.

Results and discussion

Effect of MDJN on glycopeptides

The effect of MDJN on glycoprotein biosynthesis was examined in IEC-6 cells under conditions similar to those used previously with DJN (Saunier *et al.*, 1982). The glycopeptides obtained by exhaustive Pronase digestion were fractionated on Bio-Gel P-6 before and after treatment with endo H to distinguish between complex and high-mannose oligosaccharides. MDJN greatly de-

creased the incorporation of D-[2-³H]mannose into the higher-*M_r* glycopeptide fractions (Figs. 1*a* and 1*b*). These glycopeptides were not affected by endo H, whereas the elution of the lower-*M_r* fraction was retarded (Figs. 1*c* and 1*d*). These results demonstrate that the synthesis of complex oligosaccharides is greatly inhibited by MDJN. Similar inhibition was observed with concentrations of this compound between 1 and 4 mM, and with cell-surface and cell-pellet components separated by mild trypsin treatment of the cells (Saunier *et al.*, 1982). MDJN inhibits complex-oligosaccharide formation to the same extent as DJN, but at a lower concentration, since it was shown previously that 1 mM-DJN was much less effective (Saunier *et al.*, 1982). In none of the experiments with either inhibitor was protein synthesis inhibited by

Fig. 1. Effect of MDJN on glycopeptides of IEC-6 cells

Bio-Gel P-6 chromatography of total cell glycopeptides obtained after a 24 h incubation with D-[2-³H]mannose. (a) and (b), glycopeptides obtained before treatment with endo H; (c) and (d), fractions 45–80 from (a) and (b) were pooled, freeze-dried, digested with endo H, and then rechromatographed on the same column of Bio-Gel P-6. (a) and (c) were obtained from untreated cells, whereas (b) and (d) were from cells incubated with 2 mM-MDJN. *K_{av}* values are given against the peaks.

more than 10% during a 24h labelling with [^{35}S]methionine.

H.p.l.c. of oligosaccharides

The endo H-sensitive oligosaccharides were fractionated by h.p.l.c. with ^{14}C -labelled oligosaccharide standards. In control cells, six labelled oligosaccharides were obtained from total glycopeptides; five of these were eluted earlier than the standard glucose-containing oligosaccharides (Fig. 2a).

In cells incubated with DJN, at least nine labelled oligosaccharides were resolved (Fig. 2b), and about 55% of the radioactivity was recovered in the larger oligosaccharides (I–IV). In cells treated with MDJN, nine labelled oligosaccharides were also obtained (Fig. 2c), but as much as 80% of the radioactivity was recovered in the larger oligosaccharides (I–IV). With both inhibitors, labelled oligosaccharides corresponding in elution to standard $\text{Glc}_{1-3}\text{Man}_9\text{GlcNAc}$ were obtained.

Labelling of oligosaccharides from D-[6- ^3H]galactose

When the cells were labelled for 24h with D-[6- ^3H]galactose, and the glycopeptides were fractionated on Bio-Gel P-6 before and after treatment with endo H, no endo H-sensitive glycopeptides were found in control cells. The glycopeptides of treated cells, however, contained a labelled fraction that was sensitive to endo H. H.p.l.c. of these oligosaccharides showed that the major oligosaccharides obtained from treated cells corresponded in elution to those obtained after D-[2- ^3H]mannose labelling (results not shown). Acid hydrolysis of the endo H-sensitive oligosaccharides labelled from D-[6- ^3H]galactose monitored by paper chromatography demonstrated that at least 60–70% of the radioactivity was recovered as labelled glucose, indicating the presence of glucosylated high-mannose oligosaccharides.

Characterization of the oligosaccharides

Treatment of endo H-sensitive oligosaccharides labelled from D-[2- ^3H]mannose in control cells with jack-bean α -mannosidase released 80% of the radioactivity as mannose and about 10% as disaccharide, which is probably $\text{Man}(\beta 1\rightarrow 4)\text{GlcNAc}$ (Table 1). These values correspond to the results expected from α -mannosidase treatment of non-glucosylated high-mannose oligosaccharides and indicate that the oligosaccharides in Fig. 2(a) are mainly $\text{Man}_{5-9}\text{GlcNAc}$. A similar treatment of oligosaccharides from treated cells released only 43% of the radioactivity as mannose and 1–3% as disaccharide (Table 1), further suggesting that a large proportion of the oligosaccharides formed in the presence of the inhibitors still contain glucose.

Fig. 2. H.p.l.c. of oligosaccharides formed from D-[2- ^3H]mannose

Labelled oligosaccharides ($K_{av} = 0.5\text{--}0.6$) released by endo H treatment of total cell glycopeptides after 24h labelling with D-[2- ^3H]mannose were obtained from Bio-Gel P-6 as described for Fig. 1, and were fractionated by h.p.l.c. as described in the Experimental section. (a) Control cells; (b) cells treated with 5mM-DJN and (c) cells treated with 2mM-MDJN. The arrows indicate the positions of the ^{14}C -labelled standard oligosaccharides: Glc_1 , $\text{Glc}_1\text{Man}_9\text{GlcNAc}$; Glc_2 , $\text{Glc}_2\text{Man}_9\text{GlcNAc}$; Glc_3 , $\text{Glc}_3\text{Man}_9\text{GlcNAc}$.

Table 1. *Products of α -mannosidase treatment of oligosaccharides*

Endo H-sensitive oligosaccharides from total glycopeptides obtained from Bio-Gel P6 were treated with α -mannosidase and subjected to h.p.l.c. as described in the Experimental section. The mannose-containing region was fractionated by paper chromatography to separate mannose from disaccharide.

Additions	Concn.	Radioactivity (c.p.m.) (%)		
		Oligosaccharides	Disaccharide	Mannose
None		675 (9)	750 (10)	6330 (81)
DJN	5 mM	5885 (54)	295 (3)	4755 (43)
MDJN	2 mM	8825 (56)	220 (1)	6830 (43)

Each one of the labelled endo H-sensitive oligosaccharides from treated cells was characterized after preparative h.p.l.c., which gave elution profiles similar to the analytical h.p.l.c. shown in Fig. 2. Yeast glucosidase I was used to identify oligosaccharides containing three glucose residues (Fig. 3). This enzyme is not inhibited by the removal of mannose residues from adjacent branches on the oligosaccharides (Saunier *et al.*, 1982). Exhaustive treatment with jack-bean α -mannosidase, which yielded $\text{Glc}_3\text{Man}_4\text{GlcNAc}$, $\text{Glc}_2\text{Man}_4\text{GlcNAc}$ and $\text{Glc}_1\text{Man}_4\text{GlcNAc}$ from standard $\text{Glc}_3\text{Man}_9\text{GlcNAc}$, $\text{Glc}_2\text{Man}_9\text{GlcNAc}$ and $\text{Glc}_1\text{Man}_9\text{GlcNAc}$ respectively, was also used to distinguish between oligosaccharides containing a different number of glucose residues (Fig. 4).

Fraction I ($R = 1$). The largest oligosaccharide which corresponded to standard $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ was completely converted by glucosidase I into a smaller oligosaccharide that was eluted with standard $\text{Glc}_2\text{Man}_9\text{GlcNAc}$, thereby demonstrating that removal of the terminal glucose residue from $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ was complete under the conditions of incubation (Fig. 3).

Fraction II ($R = 0.92$). For MDJN-treated cells, about 80–90% of the radioactivity in this fraction, which was eluted with standard $\text{Glc}_2\text{Man}_9\text{GlcNAc}$, was converted by glucosidase I into a smaller oligosaccharide ($R = 0.82$) that was eluted earlier than standard $\text{Glc}_1\text{Man}_9\text{GlcNAc}$ (Fig. 3) and which is likely to be $\text{Glc}_2\text{Man}_8\text{GlcNAc}$. Treatment with α -mannosidase yielded about 85% $\text{Glc}_3\text{Man}_4\text{GlcNAc}$ and 15% $\text{Glc}_2\text{Man}_4\text{GlcNAc}$ (Fig. 4). This fraction therefore contains mainly $\text{Glc}_3\text{Man}_8\text{GlcNAc}$ and only a small amount of $\text{Glc}_2\text{Man}_9\text{GlcNAc}$. For DJN-treated cells, glucosidase I converted only about 30% of the radioactivity into the smaller oligosaccharide ($R = 0.82$) (Fig. 3), and α -mannosidase digestion yielded about 40% $\text{Glc}_3\text{Man}_4\text{GlcNAc}$ and 60% $\text{Glc}_2\text{Man}_4\text{GlcNAc}$ (Fig. 4). These results indicate that this fraction contains about equivalent amounts of $\text{Glc}_3\text{Man}_8\text{GlcNAc}$ and $\text{Glc}_2\text{Man}_9\text{GlcNAc}$. For both inhibitors, 48–50% of the radioactivity in this oligosaccharide fraction was released

as mannose by α -mannosidase. This value is consistent with the presence of glucosylated oligosaccharides.

Fraction III ($R = 0.88$). Only a small amount of this oligosaccharide, corresponding to standard $\text{Glc}_1\text{Man}_9\text{GlcNAc}$, was obtained from MDJN-treated cells. Insufficient material was available from preparative h.p.l.c. for enzyme treatment. For DJN-treated cells, this oligosaccharide was unaffected by glucosidase I (Fig. 3) and yielded only $\text{Glc}_1\text{Man}_4\text{GlcNAc}$ on α -mannosidase treatment (Fig. 4). This fraction therefore contains only $\text{Glc}_1\text{Man}_9\text{GlcNAc}$. About 50% of its radioactivity was released as mannose after the action of α -mannosidase.

Fraction IV ($R = 0.81$ – 0.82). This fraction had the same elution as the product of glucosidase I treatment of fraction II, i.e. $\text{Glc}_2\text{Man}_8\text{GlcNAc}$. For MDJN-treated cells, between 70 and 75% of the radioactivity was converted into smaller oligosaccharide by glucosidase I (Fig. 3). About 80% of the radioactivity was converted into $\text{Glc}_3\text{Man}_4\text{GlcNAc}$ after α -mannosidase treatment (Fig. 4), thereby confirming that the major component contained three glucose residues. The rest of the radioactivity was recovered in equivalent amounts of $\text{Glc}_2\text{Man}_4\text{GlcNAc}$ and $\text{Glc}_1\text{Man}_4\text{GlcNAc}$. From these results and the fact that this fraction is eluted earlier than the standard $\text{Glc}_1\text{Man}_9\text{GlcNAc}$, it is concluded that the major oligosaccharide is $\text{Glc}_3\text{Man}_7\text{GlcNAc}$ and the minor ones are probably $\text{Glc}_2\text{Man}_8\text{GlcNAc}$ and $\text{Glc}_1\text{Man}_8\text{GlcNAc}$. In contrast, for DJN-treated cells only about 30% of the radioactivity was converted into smaller oligosaccharide with glucosidase I (Fig. 3), and three major oligosaccharides were obtained after α -mannosidase treatment, consisting of 50% $\text{Glc}_3\text{Man}_4\text{GlcNAc}$, 17% $\text{Glc}_2\text{Man}_4\text{GlcNAc}$ and 33% $\text{Glc}_1\text{Man}_4\text{GlcNAc}$ (Fig. 4). In this case, therefore, the oligosaccharide fraction contains a mixture of $\text{Glc}_3\text{Man}_7\text{GlcNAc}$, $\text{Glc}_2\text{Man}_8\text{GlcNAc}$ and $\text{Glc}_1\text{Man}_8\text{GlcNAc}$. About 50% of the radioactivity was released as mannose by α -mannosidase for MDJN-treated cells, showing that only glucosylated oligosaccharides are

Fig. 3. Effect of glucosidase I on elution of oligosaccharides from h.p.l.c.

Individual oligosaccharides (fractions I–IV) from DJN-treated cells obtained from Fig. 2(b) (right panel) and from MDJN-treated cells obtained from Fig. 2(c) (left panel) were subjected to h.p.l.c. before (·····) and after (—) treatment with glucosidase I as described in the Experimental section. For abbreviations, see Fig. 2.

present; this value was increased to 65% in DJN-treated cells, indicating the presence of some non-glucosylated oligosaccharides.

Fraction V ($R = 0.70$ – 0.72) and *VI* ($R = 0.65$ – 0.66). These fractions were pooled, as they were not clearly resolved on preparative h.p.l.c. The oligosaccharides obtained in the presence of both inhibitors were not affected by glucosidase I treatment (results not shown). α -Mannosidase treatment yielded approximately equivalent amounts of $\text{Glc}_2\text{Man}_4\text{GlcNAc}$ and $\text{Glc}_1\text{Man}_4\text{GlcNAc}$ in both cases (Fig. 4). From their relative elution, it is concluded that these oligosaccharides consist mostly of $\text{Glc}_2\text{Man}_7\text{GlcNAc}$ and $\text{Glc}_1\text{Man}_7\text{GlcNAc}$. Furthermore, about 62–65% of the radioactivity in these fractions was released as mannose by α -mannosidase; this increased release indicates that a small amount of non-glucosylated oligosaccharide is also present.

Fractions VII–IX ($R = 0.33$ – 0.58). In both cases, these smaller oligosaccharides yielded a mixture of $\text{Glc}_2\text{Man}_4\text{GlcNAc}$ and $\text{Glc}_1\text{Man}_4\text{GlcNAc}$ after α -mannosidase treatment (Fig. 4), suggesting the presence of glucosylated oligosaccharides containing one and two glucose residues and less than seven mannose residues. For MDJN-treated cells, about 73% of the radioactivity was released as labelled mannose by α -mannosidase, whereas for DJN-treated cells this value was increased to 90%, indicating that, in this case, a much larger proportion of the oligosaccharides in these fractions were non-glycosylated.

From the results obtained on treatment with glucosidase I and jack-bean α -mannosidase it is estimated that about 70% of the radioactivity recovered in the oligosaccharides of MDJN-treated cells contain three glucose residues, compared with only about 20% of the oligosaccharides for DJN-treated cells.

These results demonstrate that DJN and MDJN greatly inhibit *N*-linked complex-oligosaccharide biosynthesis by preventing the removal of glucose residues during processing of the oligosaccharides. The maximum inhibition observed with these compounds is similar, but the *N*-methyl derivative is effective at a lower concentration than the parent compound (1 mM versus 5 mM). With both inhibitors a mixture of endo H-sensitive oligosaccharides containing one to three glucose and seven to nine mannose residues accumulate, accounting for the existence of at least nine different oligosaccharides. A small proportion of these oligosaccharides may contain only six mannose residues. These results indicate that, even in the presence of glucose, two, and possibly three, mannose residues can be removed.

The above results demonstrate that h.p.l.c. provides a resolution not attained with standard

gel-filtration procedures used in other studies (Datema *et al.*, 1982; Gross *et al.*, 1983; Pan *et al.*, 1983; Romero *et al.*, 1983) in which the extent of heterogeneity of the oligosaccharides formed in the presence of processing inhibitors could not be assessed. Furthermore, the use of yeast glucosidase I, which was shown previously to specifically remove the terminal glucose from oligosaccharides containing three glucose residues regardless of the number of mannose residues present (Saunier *et al.*, 1982), combined with that of jack-bean α -mannosidase, allowed the identification of oligosaccharides containing one, two and three glucose residues. It should be pointed out that, even with the resolution afforded by h.p.l.c., enzyme treatment was essential, since some of the fractions still contained a mixture of oligosaccharides.

Although both inhibitors inhibit glucosidases I and II, it appears that MDJN is a more effective inhibitor of glucosidase I *in vivo* than is DJN, since a much larger proportion of the oligosaccharides formed in its presence contain three glucose residues. This difference probably reflects a higher sensitivity of glucosidase I to MDJN, since it was found that yeast glucosidase I was more greatly inhibited by MDJN than by DJN at concentrations between 10 and 50 μ M (B. Saunier, S. Jelinek-Kelly & A. Herscovics, unpublished work). Similarly, Hettkamp *et al.* (1984) found that glucosidase I in calf liver microsomes is more strongly inhibited by MDJN than by DJN.

With both inhibitors there are always residual endo H-resistant glycopeptides remaining, and a small amount of disaccharide is obtained after α -mannosidase treatment, indicating that some normal processing to non-glucosylated oligosaccharides is still occurring. These results may be due to incomplete inhibition of glucosidases and/or to the existence of a pathway that is not dependent on the transfer of glucosylated oligosaccharides to

Fig. 4. Effect of α -mannosidase on elution of oligosaccharides from h.p.l.c.

Individual oligosaccharides from DJN-treated cells obtained from Fig. 2(b) (right panel) and from MDJN-treated cells obtained from Fig. 2(c) (left panel) were subjected to h.p.l.c. with standard $\text{Glc}_{1-3}\text{Man}_9\text{GlcNAc}$ after treatment with α -mannosidase as described in the Experimental section. Under the same conditions, α -mannosidase treatment of standard $\text{Glc}_{1-3}\text{Man}_9\text{GlcNAc}$ yielded $\text{Glc}_3\text{Man}_4\text{GlcNAc}$, $\text{Glc}_2\text{Man}_4\text{GlcNAc}$ and $\text{Glc}_1\text{Man}_4\text{GlcNAc}$ with *R* values of 0.41–0.46, 0.30–0.35 and 0.23–0.28 respectively. The *R* values for the oligosaccharides are noted. Mannose was eluted in the fraction with a peak at tube nos. 6–7.

protein. It is noteworthy that similar observations were made in the mutant described by Reitman *et al.* (1982), in which glucosidase II activity was reduced to less than 0.3% of normal and yet significant synthesis of complex chains still occurred. Similarly, in other studies with glucosidase inhibitors (Datema *et al.*, 1982; Gross *et al.*, 1983; Pan *et al.*, 1983), the inhibition of complex-chain synthesis was not always complete.

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