Structural Analysis of N-Linked Oligosaccharides from Glycoproteins Secreted by Dictyostelium discoideum

IDENTIFICATION OF MANNOSE 6-SULFATE*

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The N-linked oligosaccharides found on the lysosomal enzymes from Dictyostelium discoideum are highly sulfated and contain methylphosphomannosyl residues (Gabel, C. A., Costello, C. E., Reinholds, V. N., Kurtz, L., and Kornfeld, S. (1984) J. Biol. Chem. 259, 13762-13769). Here we report studies done on the structure of N-linked oligosaccharides found on proteins secreted during growth, a major portion of which are lysosomal enzymes. Cells were metabolically labeled with [2-3H]Man and 35S, and a portion of the oligosaccharides were released by a sequential digestion with endoglycosidase H followed by endoglycosidase/N-glycosidase F preparations. The oligosaccharides were separated by anion exchange high performance liquid chromatography into fractions containing from one to six negative charges. Some of the oligosaccharides contained only sulfate esters or phosphodiester, but most contained both. Less than 2% of the oligosaccharides contained a phosphomonoester or an acid-sensitive phosphodiester typical of the mammalian lysosomal enzymes. A combination of acid and base hydrolysis suggested that most of the sulfate esters were linked to primary hydroxyl groups. The presence of Man-6-SO4 was demonstrated by the appearance of 3,6-anhydromannose in acid hydrolysates of base-treated, reduced oligosaccharides. These residues were not detected in acid hydrolysates without prior base treatment or in oligosaccharides first treated by solvolysis to remove sulfate esters. Based on high performance liquid chromatography quantitation of percentage of 3H label found in 3,6-anhydroman- nose, it is likely that Man-6-SO4 accounts for the majority of the sulfated sugars in the oligosaccharides released from the secreted glycoproteins.

The lysosomal enzymes of Dictyostelium discoideum display a diverse series of N-linked oligosaccharides (1), which appear to be derived from the usual lipid-linked oligosaccharide precursor (2, 3). Many of the oligosaccharides found on the slime mold enzymes contain a variable number of sulfate esters and Man-6-P in a methylphosphodiester linkage (Man-6-P-OCH3) (4). Certain sulfate esters block the release of the majority of the oligosaccharides by Endo H (1); however, solvolysis which removes the sulfate residues without destroying any glycosidic linkages, renders the oligosaccharides sensitive to Endo H.

In our previous studies on the structure of the [3H]Man-labeled oligosaccharides we were not able to identify the sulfated sugar (1, 2). There were several reasons for this, but the most difficult problem was the lack of quantitative methods which could be readily applied to metabolically labeled molecules.

In this report we present evidence for the release of a large portion of the N-linked oligosaccharides from an enriched source of lysosomal enzymes. We also identify the presence of a novel sulfated sugar, Man-6-SO4, in these oligosaccharides using approaches which can be applied to the analysis of other biosynthetically labeled sulfated oligosaccharides.

EXPERIMENTAL PROCEDURES*

RESULTS

Labeling and Release of N-Linked Oligosaccharides from Secreted Macromolecules—The array of N-linked oligosaccharides found on each of these different purified lysosomal enzymes from the growth medium of Dictyostelium were very similar to each other, in terms of size, degree of sulfation, the amount and state of esterification of the Man-6-P (1). Furthermore, all of the lysosomal enzymes and a large portion of the secreted proteins are known to contain an antigenic determinant which requires a sulfated oligosaccharide for its recognition (5, 6). Therefore, it seemed likely that the secreted proteins would be a rich source of material for characterization of the lysosomal enzyme oligosaccharides. To successfully analyze the oligosaccharides, they must first be cleaved from the proteins. To accomplish this we used a combination of digestions of Endo H (7) and the newly described Endo/PNGase F to release the oligosaccharides (8). The notation is used because it has recently been reported that this latter preparation contains at least two enzymatic activities (9). One of these is a true endoglycosidase and cleaves between the 2 GlcNAc residues in the di-N-acetyl chitobiosyl core, and the other activity is a peptide N-glycosidase which cleaves the amide linkage to Asn. Thus, there is uncertainty as to which activity is responsible for the cleavage seen. This, however, does not change the overall interpretation of the results.

*"Experimental Procedures" are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 85M-2151, cite the authors, and include a check or money order for $2.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
In preliminary experiments (data not shown) at least 90% of the [\(^{3}H\)]Man-labeled oligosaccharides were released from purified \(\alpha\)-mannosidase or from a mixture of three purified lysosomal enzymes by digestion with this preparation. These results suggested that the great majority of the lysosomal enzyme oligosaccharides could be successfully cleaved by the enzyme. Due to the limited amount of labeled oligosaccharides available from the purified enzymes, we decided to first analyze the oligosaccharides released from total cellular secretions.

Macromolecules secreted by cells labeled with \(^{35}S\) and [\(^{3}H\)]Man were denatured in sodium dodecyl sulfate/2-mercaptoethanol and chromatographed on Sephadex G-50. The fractions from the void volume were pooled, digested with Endo H, and rechromatographed on the Sephadex G-50 column (Fig. 1, Panel A). The released oligosaccharides ran within the column while the noncleaved glycoconjugates remained in the \(V_0\) region of the column. About 8% of the \(^{3}H\) and 7% of the \(^{35}S\) were released by this treatment. Re-digestion of the material in the \(V_0\) region of the column with Endo H did not release any more oligosaccharides (not shown). The material which still remained in the \(V_0\) was re-digested with Endo/PNGase F (Endo H \(\rightarrow\) Endo/PNGase F) and again rerun on the same column (Fig. 1). The results shown in Panel C indicate that all of the molecules cleaved by Endo H were also cleaved by Endo/PNGase F.

The material remaining in the \(V_0\) region of the column was composed of about equal amounts of [\(^{3}H\)]Man and [\(^{3}H\)]Fuc, and appears to contain a collection of polysaccharides, \(O\)-linked oligosaccharides, and non-releasable \(N\)-linked oligosaccharides. Extracellular polysaccharides and \(O\)-linked sugars have been reported previously (10, 11) in Dictyostelium macromolecules of size 35-80 kDa (12). Mild base treatment and \(^{3}H\)Fuc is incorporated into a heterodisperse group of linked oligosaccharides, and non-releasable \(N\)-linked oligosaccharides have been reported previously (10, 11) in Dictyostelium.

**Fig. 1.** Sephadex G-50 analysis of released oligosaccharides. The macromolecules secreted by cells grown in the presence of [\(^{3}H\)]Man and \(^{35}S\) were isolated as described under "Experimental Procedures" and treated with 2 milliunits of Endo H for 48 h and then chromatographed on Sephadex G-50 (Panel A). The macromolecules remaining in the \(V_0\) fractions were pooled, precipitated with acetone, resolubilized in sodium dodecyl sulfate, and digested with 2 milliunits of Endo/PNGase F for 48 h. The digest was chromatographed on the same column (Panel B). A separate aliquot which had not been treated with Endo H was digested directly with Endo/PNGase F and chromatographed on the same column (Panel C). O, \(^{3}H\); \(\bullet\), \(^{35}S\).
Anion Exchange HPLC Separation of the Released Oligosaccharides—Anion exchange HPLC analysis was used to more effectively separate the most highly charged oligosaccharides (14). The results of the fractionation are shown in Fig. 3. Each of the pools marked by the brackets on Fig. 3 was pooled, desalted, and analyzed as described below.

Characterization of the Various Species—Each fraction was analyzed for amount and state of esterification of Man-6-P, sulfate ester content, and relative percentages of molecules with various combinations of phosphate and sulfate esters. The results of these analyses are shown in Table I. It is important to give the rationale for these analyses here. The relative amounts of molecules with multiple sulfate esters and mono- or di-esterified Man-6-P residues are based on the fact that all of the sulfate esters are sensitive to solvolysis which does not destroy any glycosidic linkages (13). Although the phosphodiester found on mammalian lysosomal enzymes (GlcNAc) is cleaved by solvolysis (4), the -OCH$_3$ group of the phosphodiester is totally stable to the procedure. Cleavage of the mammalian-type phosphodiester yields a phosphomonoester. Thus, phosphodiester sensitivity of an oligosaccharide following solvolysis, but not before solvolysis, could be due either to the loss of an acid-labile phosphodiester or to the failure of the phosphatase to cleave a phosphomonoester. Since we cannot distinguish between these possibilities we have grouped these two possibilities together in Table I. In addition, each fraction was treated with alkaline phosphatase and rechromatographed on QAE-Sephadex, both before and after solvolysis. This was important because the presence of the sulfate esters could inhibit the action of the phosphatase on the few molecules which are sensitive (15). Several fractions which had no sulfate esters were also treated with phosphatase without solvolysis as shown in Table I. Each of the fractions shown in Table I was chromatographed on ConA-Sepharose (16). Most of the species were bound, but the proportion elution at 10 mM methylglucoside and 100 mM methylmannoside varied with each fraction.

The neutral oligosaccharides released by both of the enzyme treatments were analyzed by HPLC to determine their size following reduction with sodium borohydride (17). The oligosaccharides released by Endo H have a size equivalent to a standard of Man$_n$GlcNAc while the Endo H → Endo/PNGase F released species are somewhat larger, which is probably the result of the PNGase F activity cleaving the oligosaccharide at the linkage to asparagine (Fig. 4). It should be noted again that the neutral oligosaccharides released by Endo/PNGase F are resistant to Endo H since they could not be released by re-digestion with Endo H. Mild acid hydrolysis followed by paper chromatography showed the presence of about 10% fucose in both. This may account for some of the size heterogeneity of these samples, and variable locations of Fuc on the oligosaccharide may account for the resistance of a portion of these molecules to Endo H digestion (18).

The results presented in Table I show that the oligosaccharides can be quite complex. Thus, none were suitable for complete structural characterization.

Nature of the Sulfate Esters—The identification and quantitation of radioiodinated sulfated sugars of glycoproteins has been seriously hampered by the lack of suitable methods. The variable stability of different types of sulfate esters makes the use of any single analytical method unsatisfactory. We have used several methods including differential sensitivity to acid and base hydrolysis to characterize the sulfate residues. Sulfate esters differ in their sensitivity to acid hydrolysis (19-23). The $t_{1/2}$ of sulfate esters in 0.25 N HCl at 100°C is: equatorial, 6-25 min; axial, 60-84 min; and primary, 90-120 min. Base hydrolysis has only been rarely used, in part, because it will occur in only two instances: 1) if a sugar has primary (6-OH) linked ester together with a free 3-OH group, or 2) if there is a free adjacent -OH group located trans to the sulfate ester (24, 25). Table II shows the behavior expected for each of the sugars found in a complete (unprocessed) and unsubstituted high mannose-high oligosaccharide (3). It is evident that a combination of acid and base hydrolysis treatments can be used as a preliminary characterization of the type of sulfate esters found on an oligosaccharide. If more than one type is present, the relative amount of each can be estimated graphically from the kinetics of $^{35}$SO$_4$ loss (19).

Each of the unfractionated pools released by sequential Endo H and Endo H → Endo/PNGase F digestion was subjected to acid hydrolysis (Fig. 5). All Endo H-released sulfate appears to exist as primary esters. While about 80% of $^{35}$SO$_4$ released from the Endo H → Endo/PNGase F is found in primary linkage and the remainder is in an equatorial location. When the same pools were subjected to base hydrolysis after reduction of the oligosaccharides with NaBH$_4$, about 70% $^{35}$SO$_4$ from the Endo H (Fig. 6, Panel A), and 90% of the $^{35}$SO$_4$ from the Endo H → Endo/PNGase F pool were sensitive to base (Fig. 6, Panel B). These results are also consistent with the presence of the majority of the $^{35}$SO$_4$, being in primary linked ester (Man-6-SO$_4$ or GlcNAc-6-SO$_4$). We can eliminate mannose residues A and C (Table II) from further consideration since the 6-OH is already in glycosidic linkage. Those oligosaccharides which contain Man-6-P residues, also do not have an available 6-OH position.

To determine whether the acid-labile component found in

**FIG. 3.** Anion exchange HPLC analysis of released oligosaccharides. The oligosaccharides released by the digestions shown in Fig. 1, Panels A and B were desalted and applied to a 300 x 4-mm column of Ax-5 in a total of 50 µl. The column was eluted at a flow rate of 1 ml/min for 2 min with water, followed by a gradient of 15-30 mM NaPO$_4$ for 20 min, followed by a gradient of 30-375 mM NaPO$_4$ for 22 min. Fractions of 0.5 min were taken and 10 µl of each sample were counted for $^3H$ (C) and $^{35}$SO$_4$ (G). The fractions were pooled as indicated in each panel. Panel A, Endo H-released oligosaccharides; Panel B, Endo H → Endo/PNGase F-related oligosaccharides. The standards were: A, Glc-6-SO$_4$; B, C, and D were derived from a partial hydrolysate of dermatan sulfate (IdUA-GalNAc-4-SO$_4$), and are di-, tetra-, and hexasaccharides with two, four, and six negative charges, respectively.
Table I

Analysis of oligosaccharides released by Endo H and Endo H → Endo/PNGase F digests

| Fraction | % of total<sup>5</sup> | Charge<sup>6</sup> | Man-6-P<sup>7</sup> | % of each fraction<sup>6</sup> |
|----------|------------------------|------------------|--------------------|--------------------------------|
|          | H<sub>2</sub>          | S<sub>H</sub>     |                    | PDE<sup>8</sup> SO<sub>4</sub><sup>−</sup> PME or solvolysis labile PDE<sup>8</sup> |
| Endo H   | %                     |                  |                    | 0                             |
| Ha       | 9                     | 0                | 0                  | 100                           |
| Hb       | 5                     | <1               | 1                  | 37 63                         |
| Hc<sub>1</sub> | 1                  | <1               | 1.0               | 38 45 18                      |
| Hc<sub>2</sub> | 9                  | 0                | 0.15              | 5 15 60 26                    |
| H<sub>d</sub> | 1                  | 0                | 0.10              | 5 17 73 3                     |
| H<sub>d</sub> | 3                  | 4                | 0.80              | 0 7 58 20                     |
| He       | 3                     | 5                | 1.5               | 11                            |
| Hf       | <1                   | 2                | 1.5               | 11                            |
| % in H fractions | % | 31               | 11                | 39 69                         |

| Endo H → Endo/PNGase F | % of total | Charge | Man-6-P | % of each fraction |
|------------------------|------------|--------|---------|--------------------|
| Fa                     | 9          | 0      | 0       | 100                |
| Fb                     | 6          | 2      | 0.60    | 6                  |
| F<sub>c</sub>         | 3          | 2      | 1.3     | 1                  |
| F<sub>c</sub>         | 6          | <1     | 0.27    | 17                 |
| Fd                     | 9          | 7      | 1.0     | 20 28 50           |
| Fe                     | 7          | 11     | 4.0     | 19                 |
| Ff                     | 17         | 39     | 3.0     | 19                 |
| Fg                     | 10         | 29     | 3.8     | 20                 |

<sup>4</sup>Fraction pool designation as shown in Fig. 3.
<sup>5</sup>Calculated as a percentage of the total of all oligosaccharides released by both enzymatic digestions.
<sup>6</sup>Determined from the profile shown in Fig. 3 using the indicated standards.
<sup>7</sup>Content is determined as described under "Experimental Procedures."
<sup>8</sup>Number of phosphodiester (PDE) or sulfate (SO<sub>4</sub>) residues/oligosaccharide.

Determined by a comparison of the pre- and postsolvolysis patterns on QAE-Sephadex. The percentage of each fraction which no longer binds to the column after solvolysis is assumed to have had all charge due to sulfate; the percentage with one, two, and three charges postsolvolysis is assumed to have one, two, and three phosphodiester, with the remainder of the charge being due to sulfate.

Phosphomonoester (PME) is defined as percentage of fraction with two charges which no longer bound to QAE-Sephadex following phosphatase digestion of the indicated oligosaccharides.

Phosphomonoester (PME) is converted to a phosphomonoester by solvolysis, this procedure cannot distinguish between an original phosphomonoester or a solvolysis-sensitive phosphodiester.
the Endo H pool was also base stable, an aliquot was hydrolyzed in 0.25 N HCl for 1 h at 100 °C. These conditions would be sufficient to hydrolyze 75% of the acid-labile component, but only reduce the amount of the more acid-stable type by 25%. If the acid-labile component is also base-stable, then there should be a large (>75%) decrease in the amount of the base-stable component. The results shown in Fig. 6, Panel B, indicate that the base-stable component is reduced by about 80% by the short acid treatment, and suggest that the acid-labile component is also base-stable. The base-stability could be due to its location in the standard oligosaccharide shown in the diagram in Table II (Man-4-SO4 residue A only or GlcNAc-3-SO4 residues A or B) or to the presence of another acid-labile substituent, which blocks base hydrolysis.

Identification of Man-6-SO4 in the Released Oligosaccharides—Base treatment of Man-6-SO4 in glycosidic linkage leads to the formation of 3,6-anhydromannose, provided that the 3 position is free (in the standard oligosaccharide shown in Table II, residues A and C do not have a free 6 position). Since this derivative is stable to strong acid hydrolysis, it can be recovered and quantitated by HPLC. The [3H]Man serves as an internal standard to allow the calculation of the percentage of the total label which is present as [3H]3,6-anhydromannose. Fig. 7 shows that analysis of the neutral oligosaccharides or those treated by solvolysis prior to base treatment show no evidence of any 3,6-anhydromannose. In contrast, when the Endo H or Endo H → Endo/PNGase F pools are first treated with base, the amount of the 3,6-anhydromannose is proportional to the amount of sulfate ester. Total acid hydrolyses of non-base-treated pooled fractions also showed no evidence of 3,6-anhydromannose.

Each of the fractions shown in Table I was assayed for Man-6-SO4 content. Table III shows these values along with the calculated maximum possible Man-6-SO4 content assuming that all of the sulfate is present in this sugar. From these calculations, it is clear that many of the fractions could be solely composed of Man-6-SO4 while others, notably those released by Endo H → Endo/PNGase F digests which have 5 and 6 charges must contain other sulfated sugars.

Search for GlcNAc-6-SO4 Residues—Similar base treatments of [3H]GlcN-labeled released oligosaccharides did not show the appearance of any 3,6-anhydroGlcNAc. Partial acid hydrolysis (0.25 N HCl, 100 °C, 2 h) of 106 cpm of [3H]GlcN-labeled material failed to show any evidence of GlcNAc-6-SO4 by paper chromatography (26). It is possible that this method was not sensitive enough to detect or quantify it.

**DISCUSSION**

The structure and biosynthesis of sulfated N-linked oligosaccharides has previously received little attention. More recently, a large number of glycoproteins with important physiological functions have been shown to contain sulfated N-linked oligosaccharides. These include a variety of pituitary polypeptide hormones (27–29), cell adhesion, and other developmentally regulated molecules in both mammals and lower eukaryotes (1, 30–32), and several proteins including the low density lipoprotein receptor (33) and the basement membrane proteins (34–36). Although these findings have generated more enthusiasm, a serious limitation in studying these oligosaccharides is that there are few reliable analytical methods of structural characterization. For instance, there is

| Residue | Designation | Position | Base sensitivity | Kinetics of acid hydrolysis |
|---------|-------------|----------|-----------------|---------------------------|
| Man     | A, C        | 2        | No              | Axial                     |
|         | B, D, E, F, G, H, I | 3       | Yes             | Equatorial                |
|         | G, H, I     | 2        | No              | Axial                     |
| GlcNAc  | A, B        | 3        | No              | Equatorial                |
|         |             | 6        | Yes             | Primary                   |
Sulfated N-Linked Oligosaccharides

**FIG. 5.** Kinetics of acid hydrolysis of $^{35}$SO$_4$ from released oligosaccharides. The pools of oligosaccharides released by Endo H and Endo H $\rightarrow$ Endo/PNGase F digestions (Panels B and A, respectively) were hydrolyzed in 0.25 N HCl at 100 °C for various periods of time and the free $^{35}$SO$_4$ was precipitated with barium as described under "Experimental Procedures." The results are calculated as the percentage of $^{35}$SO$_4$ which is not precipitable as normalized to the $^3$H remaining in solution. Nonspecific precipitation of the $^3$H is generally <10%.

only a single known sulfatase which acts on a oligosaccharide bound sulfate ester (27). This is a chondro-4-sulfatase from Proteus vulgaris which acts on GalNAc-4-SO$_4$ in leutropin. This is probably a coincidence due to their similarity to the structure of the natural substrate. Hydrolytic conditions which remove the sulfate are accompanied by destruction of many glycosidic linkages (19). Also, exo-glycosidases which have been invaluable in the structural analysis of N-linked oligosaccharides can be blocked by sulfate esters even when they are present on neighboring sugars (38), and the presence of certain sulfate esters blocks cleavage by various endoglycosidases (1). It would be misleading to say that no methods are available to study the sulfated glycoconjugates, but many of the methods developed in the past were designed to analyze large quantities of polysaccharides (23-25) or proteoglycans (26). For our purposes, it was necessary to develop methods which could be adapted to the analysis of the sulfate esters found on rare proteins, and their biosynthetic intermediates.

Our long-term goal is to understand the roles that the oligosaccharides of lysosomal enzymes play in various developmental and physiological processes in Dictyostelium (39). To accomplish this we need to understand their structure and biosynthesis. The growth medium is an enriched source of the lysosomal enzymes and many other sulfated anionic proteins. In these initial characterizations, we wanted to examine the broadest possible spectrum of proteins with sulfated N-linked oligosaccharides.

The sensitivity of the sulfate esters to acid hydrolysis depends on its orientation on the sugar and is relatively independent of other glycosidic linkages on the same residue (19). The procedure can be applied to monosaccharides, oligosaccharides, and polysaccharides as well (19-22). The lability of a sulfate ester to base hydrolysis, on the other hand, depends upon not only the location of the sulfate, but also on the presence of other glycosidic linkages found on the sugar carrying the sulfate ester (23-25). Thus, if the nature of the underlying structure of the oligosaccharide is known, the general types of sulfate esters can be predicted by acid and base hydrolysis.

The procedure used to identify Man-6-SO$_4$ appears to be
products were analyzed by HPLC chromatography. The average content completeness of the base hydrolysis (85%).

Hydride and treated with 1 oligosaccharides; dried, and the hydrolysate was again reduced with sodium borohydride as described under "Experimental Procedures." The products were analyzed by HPLC chromatography. The average recovery was about 50% of the initial radioactivity prior to base hydrolysis. Panels are: A: standards; B: neutral oligosaccharides (Fraction H2); C: total Endo H-released oligosaccharides; D, solvolyis-treated Endo H-released oligosaccharides; E, Endo H → Endo/PNGase F released total oligosaccharides; F, solvolyis-treated Endo H → Endo/PNGase F released oligosaccharides. Arrow denotes position of 3,6-anhydromannitol.

**TABLE III**

| Fraction* | % [3H]3,6-anMan† | % Predicted if all SO4 is Man-6-SO4‡ |
|-----------|-----------------|------------------------------------|
| Endo H    |                 |                                    |
| A         | 0               | 0                                  |
| B         | 3.2             | 4.5                                |
| C         | 16.2            | 15.1                               |
| C9        | 3.4             | 3.1                                |
| D         | 3.4             | 3.4                                |
| D9        | 12.6            | 15.6                               |
| E         | 19.1            | 19.5                               |
| F         | 0.5             | 0.5                                |
| Endo H → Endo/ PNGase F |                 |                                    |
| A         | 1.3             | 0                                  |
| B         | 3.8             | 9.1                                |
| C         | 14.0            | 18.7                               |
| C9        | 4.0             | 9.6                                |
| D         | 18.1            | 17.6                               |
| E         | 29.4            | 35.0                               |
| F         | 35.8            | 42.0                               |
| G         | 37.2            | 59.0                               |

* Fraction designation is shown in Fig. 3.
† Calculated as a percentage of the total counts recovered on HPLC analysis of base-treated, acid-hyrdolysed fraction. Corrected for the content of Man-6-P, its degradation to manose, and for the completeness of the base hydrolysis (85%).
‡ Determined from the percentage of each fraction containing the variable amounts of sulfate as indicated in Table I and assuming a Man8 structure.

The formation of the anhydrosugar derivative can also be used to assess the location of the sulfated residue in the oligosaccharide, since the resulting ring strain makes the glycosidic linkage extremely acetyable (40). Mild acid hydrolysis of the base-treated oligosaccharide should generate fragments which are characteristic of the number and location of the sulfate esters in the intact oligosaccharide. Such a procedure could be used for oligosaccharide structural analysis much like acetylation which also relies on the selective acetylation of certain glycosidic linkages (41).

The presence of sulfate esters does not account for all of the Endo H resistance of newly synthesized α-mannosidase (42). In addition, some of the neutral oligosaccharides in this study are also resistant to Endo H. Ivatt et al. (18) have previously reported that the presence of Fuc may block Endo H digestion. Since we have detected Fuc in the acid hydrolysates of both pools of released neutral oligosaccharides, resistance could be due to the presence of Fuc residues in different positions on the chain, some of which block the cleavage by Endo H. Clearly, the presence of Fuc does not account for all of this resistance, since the highly charged, Endo H → Endo/PNGase F releasable oligosaccharides have very little Fuc. Furthermore, the carbohydrate composition of purified, solvolyis-treated α-mannosidase and β-glucosidase also shows the presence of very little Fuc (15), and yet the majority of the oligosaccharides are resistant to the release by Endo H. Thus, other modifications besides sulfation may contribute to the Endo H resistance.

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