Type analysis of the oligosaccharide chains on microheterogeneous components of bovine pancreatic DNAase by the lectin–nitrocellulose sheet method

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The oligosaccharide chains of microheterogeneous bovine pancreatic DNAases were characterized by the lectin–nitrocellulose sheet method. The active fractions of the DNAases from column chromatography showed four major and several minor spots on a two-dimensional polyacrylamide gel. They were transferred on to nitrocellulose sheets and treated with glycosidases (neuraminidase, endo- β -*N*-acetyl glucosaminidase H or F, or peptide *N*-glycosidase F) and treated with peroxidase-coupled lectins (concanavalin A, *Ricinus communis* agglutinin or wheat-germ agglutinin). From the results, the most probable oligosaccharide types were proposed to be as follows: the four major spots contained components which had high-mannose type or hybrid-type oligosaccharides, such as those susceptible to endo- β -*N*-acetylglucosaminidase H. In addition, spot 1 contained a complex-type biantennary oligosaccharide without sialic acid and spot 3 contained a tri- or tetra-antennary complex-type oligosaccharide with sialic acid. The component corresponding to spot 2 had a hybrid-type oligosaccharide chain with a 'bisecting' acetylglucosamine, linked 1–4 to the β -mannose residue of the trimannosyl core, and the component corresponding to spot 4 had a high-mannose-type oligosaccharide chain.

INTRODUCTION

Many glycoproteins, even highly purified or crystallized materials, show microheterogeneity. This is due not only to heterogeneity among the protein moieties, such as the substitution of amino acids or other post-translational modifications by phosphorylation or sulphation, but also to heterogeneity among carbohydrate chains, including structural and charge differences [1]. The heterogeneity of carbohydrate groups may reflect the process of biosynthesis of glycoproteins, as for lysosomal enzymes [2], and secretory or membrane glycoproteins, although the final product of glycoproteins may still have carbohydrate heterogeneity [3]. Studies on the processing of the oligosaccharides in lysosomal enzymes have greatly increased our understanding of the heterogeneity of carbohydrates on glycoproteins [2]. It would be desirable and helpful in any study of biosynthesis and processing of glycoproteins to have an easy method of analysis of the type of oligosaccharide chain on each component of microheterogeneous glycoproteins.

In this paper, using bovine pancreatic DNAase as an example, we tried to separate microheterogeneous components by two-dimensional polyacrylamide-gel electrophoresis (2D-PAGE) and to detect the carbohydrate groups by the lectin–nitrocellulose sheet method reported previously [4]. By this method we could distinguish glycoprotein subclasses, i.e. high-mannose, hybrid and complex types. Bovine pancreatic DNAases A, B and C and minor component D are glycoproteins obtained by chromatography [5] and have M_r of about 31000.

DNAases C and D differ from DNAases A and B by the substitution of one proline for one histidine residue in their amino acid sequence. In all enzymes the single polypeptide chain carries only one carbohydrate chain at the Asn-18 residue, and their carbohydrate compositions have been analysed [5]. All contain *N*-acetylglucosamine (GlcNAc) and mannose (Man); DNAases B and D also contain galactose (Gal) and sialic acid. There is also microheterogeneity within the DNAase forms; seven and two different glycopeptides were obtained from DNAases A and B, respectively [6]. We have shown four typical, but different, types of sugar chains on the microheterogeneous DNAases; that is, the high-mannose type, the hybrid type, and the complex type with and without sialic acid. This is the first demonstration of the oligosaccharide types present in microheterogeneous DNAases on a nitrocellulose sheet after 2D-PAGE without further purification.

MATERIALS AND METHODS

Materials

The following materials were obtained commercially: bovine pancreatic DNAase (type III), DNA (salmon testis, type III), ovalbumin (type VII), fetuin (type III) and transferrin (human) from Sigma, nitrocellulose sheets (Bio Rad), silver reagent Ag-STAIN DAI-ICHI (Dai-ichi Pure Chemical Co., Tokyo, Japan), colloidal-gold reagent Aurodye (Janssen Pharmaceutica, Belgium), low-molecular-mass calibration kit (Pharmacia Fine Chemicals) and Ampholines (LKB). Endo- β -*N*-acetyl

Abbreviations used: 2D-PAGE, two-dimensional polyacrylamide-gel electrophoresis; Con A, concanavalin A; WGA, wheat-germ agglutinin; RCA, *Ricinus communis* agglutinin; Endo H or F, endo- β -*N*-acetylglucosaminidase H or F; PNGase F, peptide *N*-glycosidase F; Man, mannose; Gal, galactose; GlcNAc, *N*-acetylglucosamine; FPLC, fast protein liquid chromatography.

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glucosaminidase H (Endo H) from *Streptomyces griseus* was purchased from Seikagaku Kogyo Co. (Tokyo, Japan). Peptide *N*-glycosidase F (PNGase F) and endo- β -*N*-acetylglucosaminidase F (Endo F) from *Flavobacterium meningosepticum*, the latter containing PNGase F also, and peroxidase (Grade I) were obtained from Boehringer Mannheim. Neuraminidase from *Arthrobacter ureafaciens* was purchased from Nakarai Chemicals (Kyoto, Japan). Peroxidase-concanavalin A (Con A) was obtained from Hohnen Oil (Tokyo, Japan). Other lectins, *Ricinus communis* agglutinin (RCA) and wheat-germ agglutinin (WGA), were obtained from E.Y. Laboratories (San Mateo, CA, U.S.A.) and were coupled with peroxidase in this laboratory according to the method of Nakane [7]. Peroxidase-RCA and -WGA from Hohnen Oil were also used. Purified Taka amylase A was a kind gift from Dr. H. Toda (Osaka University, Japan).

2D-PAGE and blotting on to nitrocellulose sheets

Multiple forms of DNAase (type III preparation without purification, except in Fig. 1c) were analysed by 2D-PAGE [8]. Isoelectric focusing was carried out with a mixture of Ampholines, pH 4–6, 6–8, 3.5–10 in a ratio of 2:2:1, at 300 V for 19 h and at 400 V for 1 h in the first dimension. SDS/PAGE [9] on 10% acrylamide gels was performed in the second dimension. The proteins on the gel were electro-transferred on to nitrocellulose sheets at 40 V for 1 h and then at 60 V for 2 h [4] in 25 mM-Tris/192 mM-glycine/20% (v/v) methanol (pH 8.3) [10]. Proteins on gels were pretreated with 0.1% glutaraldehyde for 10 min and stained with silver reagents [11].

Glycosidase digestion and weak-acid treatment on nitrocellulose sheets

Glycosidase digestions of glycoproteins on nitrocellulose sheets were carried out under the following conditions: for Endo H [12,13], 35 munits of Endo H were incubated in 700 μ l of citric acid/phosphate (0.05 M–0.1 M) buffer, pH 5.1, at 37 °C for 4 h in a moist chamber; for Endo F [14], 1 unit of Endo F in 800 μ l of 0.1 M-sodium acetate buffer, pH 4.0, containing 10 mM-EDTA, 0.8% 2-mercaptoethanol and 0.15 M-NaCl for 46 h at 37 °C in a moist chamber. At this pH PNGase in the Endo F preparation is inactive [14]. For PNGase F [15], 1 unit of pure PNGase and 1 unit of Endo F [also containing an unquantified PNGase activity (see above)] in 200 μ l of 0.1 M-Tris, pH 8.5, containing 40 mM-EDTA were incubated with the nitrocellulose-blotted protein at 37 °C in a moist chamber for 20 h. At this pH PNGase F is predominantly active in the Endo F preparation [14]. Weak-acid treatment was performed by soaking a nitrocellulose blot in 400 ml of 0.025 M-H₂SO₄ in a glass dish on a water bath at 80 °C for 60 min [4] to remove sialic acid.

Neuraminidase treatment before electrophoresis

DNAase (10 μ g) was incubated with 60 munits of neuraminidase in 100 μ l of 0.1 M-acetate buffer, pH 4.8, at 37 °C for 1 h in the presence of phenylmethanesulphonyl fluoride (200 μ g/ml) and solubilized membrane proteins (400 μ g) [16,17] from C3H spleen cells to prevent the degradation of DNAase by the proteinase activity in the DNAase preparation. For a 'mock' incubation, neuraminidase was eliminated from the in-

cubation mixture. After the incubation, 2 μ g of DNAase was analysed on an isoelectric-focusing tube.

Staining of the glycoprotein on nitrocellulose sheets with lectin reagents

Peroxidase-coupled lectins were dissolved in 50% glycerol and stored at –20 °C. Before staining, the reagents were diluted appropriately [4] with a buffer containing 10 mM-Tris/HCl, pH 7.4, 0.05% Tween 20 and 0.15 M-NaCl. The diluted lectin solution (1 ml for 10 cm $\times$ 10 cm nitrocellulose sheet) was transferred into a clean, flat plastic dish and covered with a nitrocellulose sheet whose underside had been blotted with glycoproteins, and kept in a cold room at 4 °C for 1 h. The conditions of washing and detection were described previously [4].

RESULTS

Multiple forms of DNAase and detection of oligosaccharide chains by Con A

Fig. 1(a) shows multiple forms of bovine pancreatic DNAase separated by 2D-PAGE and detected by silver staining. The DNAase preparation contained many unrelated proteins. The group of proteins indicated by arrows (numbered 1–5 and the four unnumbered arrows) were confirmed to be the active DNAase components when analysed on a Mono S (cation-exchange) column by the fast protein liquid chromatography (FPLC) system of Pharmacia Fine Chemicals (Fig. 1b). The active fractions showed one main peak [insert Fig. 1(b), fraction e–d], two middle peaks (fractions d–c and c–b) and a minor shoulder (fraction a). An aliquot of each fraction was analysed by 2D-PAGE [Fig. 1(c), left-hand side]. To confirm their relative positions, fraction e was added to the others and analysed [Fig. 1(c), right-hand side]. At least 12 components were detected in the active fractions.

The DNAase preparation (3 μ g), without FPLC purification, was separated on 2D-PAGE, transferred on to nitrocellulose sheet and stained with peroxidase-Con A. The four major components of 1–4 in Fig. 1(a) were stained heavily by Con A (Fig. 2a). A nitrocellulose sheet, identical with Fig. 2(a), was treated with Endo H, and the sheet was treated with Con A. Components 1 and 2 stained very faintly (Fig. 2b), while those in the 'mock' incubation without enzyme were clearly detectable (Fig. 2c). Endo H cleaves the di-*N*-acetylchitobiose linkage in high-mannose-type and hybrid-type oligosaccharide chains (see Table 1). Taka amylase A [18] and transferrin [19] were treated with Endo H as positive and negative controls (Fig. 2d). The above results showed that all four major components of DNAase must have high-mannose- and/or hybrid-type oligosaccharide chains on their molecules.

Detection of components having sialic acid

RCA can detect terminal galactose moieties, particularly in a Gal-GlcNAc structure, of complex-type oligosaccharide chains after the removal of sialic acid [20]. When a nitrocellulose sheet was treated with RCA reagent without removal of sialic acid, only the position of component 1 was stained faintly (Fig. 3a). Subsequently, the sheet was subjected to mild acid hydrolysis to remove sialic acid and again treated with RCA;

Fig. 1. Multiple forms of bovine pancreatic DNAase detected by FPLC and 2D-PAGE analysis

(a) Bovine pancreatic DNAase (4 μ g) was analysed by 2D-PAGE and a gel was stained by silver reagent. The size markers on the right-hand side (arrow heads) are 43 kDa (ovalbumin) and 30 kDa (carbonic anhydrase). IEF, isoelectric focusing. (b) Chromatography of DNAase on a Mono S column. Column 0.5 cm $\times$ 5 cm; flow rate 1 ml/min; 0.5 ml fractions were collected. Chart speed, 1 cm/min. Column was equilibrated with 0.05 M-acetate buffer, pH 4.7. The initial and the final eluents were 0.05 M- and 0.67 M-acetate buffer, pH 4.7, respectively. DNAase (475 μ g) in 500 μ l of 0.05 M-acetate buffer, pH 3.5, was applied to a column. An aliquot of each fraction was assayed for DNAase activity [22]. The active fractions were designated a-f as indicated in the inserted Figure. ---, Activity and —, A_{280} . (c) 2D-PAGE analysis of the active fractions by silver staining. Each fraction from the Mono S column was dialysed against 10 mM-Tris, pH 7.4, condensed and an aliquot (1/10 for fraction e and 3/20 for other fractions) was analysed by 2D-PAGE (left-hand side). Aliquots of the fractions (e and others) were added and analysed (right-hand side). Arrows indicate the position of component 1 and a bar indicates 1 cm of the original gels. The magnification of all photographs is the same.

component 3 was then stained strongly and component 5 was also detectable (Fig. 3b). Above the position of components 3 and 5, some new spots appeared (Fig. 3b). When sheet (b) was stained with Con A, components 2 and 4 appeared and their presence was confirmed (Fig. 3c). Thus it was concluded that of the four major components, components 1 and 3 had oligosaccharide chains that could be stained with RCA, while components 2 and 4 did not.

For further confirmation of the presence of sialic acid in the sugar chains, DNAase was treated with neuraminidase before electrophoresis (Figs. 3d and e). Neuraminidase-treated DNAase (Fig. 3d) showed a different staining pattern from the pattern of DNAase that was not treated with neuraminidase, but treated with weak acid after blotting (Fig. 3b). The result of 'mock' incubation during neuraminidase treatment (Fig. 3f) was essentially the same as that of control (Fig. 3a). This was

true even after weak-acid treatment and also after Con A staining (results not shown). The staining patterns before and after acid treatment of the neuraminidase-treated DNAase were essentially the same (Figs. 3d and e); no new spots appeared after acid treatment. This indicated that sialic acid was almost removed by the neuraminidase treatment. Even after the neuraminidase treatment, three groups of spots were observed; two had the same pI [region 1 in Fig. 3(e)] but different molecular masses and the other had more acidic pI [region 2 in Fig. 3(e)]. The basic and acidic groups of the components were probably due to differences in amino acid residues: the two basic groups probably have histidine and the acidic one probably has proline [5].

Further characterization of components 1-4

To examine the susceptibility towards Endo H treatment of the oligosaccharide chain on component 3 that

Table 1. Glycoprotein types, their susceptibilities to glycosidases and reactivities with lectins

Abbreviations: GN, GlcNAc; M, mannose; G, galactose; SA, sialic acid; F, fucose; R, R', substituted residual groups; *n*, *n'*, number of mannose molecules; A, (SA)-G-GN; (), means presence or absence of the monosaccharide.

Types and general structure*	Substitution of		Susceptibility to: ‡			Reactivity with: §			Glycoproteins used §
						After removal of SA			
	R	R'	PNGase	Endo F	Endo H	Con A	WGA	RCA	
High mannose:	Mn	Mn'	+	+	+	+	—	—	— Taka amylase A
Hybrid:	Mn	(SA)-(G)-GN							
—GN†			+	+	+	+			
+GN†			+	—	+	+	+		≥ Ovalbumin
Complex									
—biantennary:	A	A	+	+	—	+		+	≥ Transferrin
—multiantennary:	2A	1A or 2A	+	—	—	—		+	≥ Fetuin

* Generalized oligosaccharide structure for *N*-linked glycoproteins:

† 'Bisecting' GN.

‡ From ref. [15].

§ From ref. [4]. For more precise specificity of lectins see ref. [21].

Fig. 2. Detection of DNAase on nitrocellulose sheets by Con A staining and effect of Endo H

(a) Con A staining of a Western-blotted nitrocellulose sheet (3 μg of DNAase). (b) An identical nitrocellulose sheet to (a) was treated with Endo H at pH 4.8, at 37 °C for 4 h and stained with Con A. (c) 'Mock' incubation without Endo H under the same conditions as (b). (d) Confirmation of Endo H activity for the blotted proteins. Standard glycoproteins (run on SDS/PAGE and transferred on to nitrocellulose sheet) were treated with (+) or without (−) Endo H simultaneously with sheet (b) and stained with Con A. Upper arrow head, transferrin and lower one, Taka amylase A. A bar indicates 1 cm of original gel or sheet. The magnification of sheets was the same. Arrows in sheets (b) and (c) indicate the position of the component 1.

Fig. 3. Effects of neuraminidase and weak acid on DNAase

Before electrophoresis, samples were incubated with (d) or without (f) 60 munits of neuraminidase for 1 h or not incubated at all (a). Details were as described in the Materials and methods section. After 2D-PAGE, proteins on gels were transferred on to nitrocellulose sheets. The sheets (a), (d) and (f) (each with 2 μg of DNAase) were stained with RCA; the sheets (a) and (d) were then treated with weak acid (0.025 M-H₂SO₄ at 80 °C, 1 h) and restained with RCA [(b) and (e)]. Finally sheet (b) was stained with Con A (c). The numbers in (e) indicate the two groups having different pI (see the text). The arrows and bar are explained in the legend to Fig. 1.

Fig. 4. Characterization of oligosaccharide chains on components 1-4

All sheets have 3 μ g of DNAase. (a) The sheet in Fig. 2 (b) was treated with weak acid and stained with RCA reagent. (b) A blot was treated with Endo F at pH 4.0 for 46 h and stained with Con A. Right-hand lane, standard glycoproteins treated with Endo F and stained with Con A. Upper and lower arrow heads, Taka amylase A and ovalbumin. (c) Another nitrocellulose sheet was treated with weak acid, and then stained with WGA reagent. (d) A sheet having DNAase was treated with PNGase F at pH 8.5 for 20 h, treated with weak acid and stained with RCA reagent. (e) The sheet (d) was stained with a colloidal-gold reagent, 'Aurodyne'. (f) 'Mock' incubation for sheet (d) without PNGase and stained with RCA after weak-acid treatment. (g) A blot was treated with Endo F and stained with RCA reagent. (h) 'Mock' incubation for sheet (g). (i) Another sheet was treated with Endo H and stained with RCA reagent. Conditions for the glycosidases were described in the Materials and methods section. The circles mark the position where DNAase would be found if present. The arrows and bar are explained in the legend to Fig. 1.

was treated with RCA after weak-acid treatment, the nitrocellulose sheet of Fig. 2(b), which had been treated with Endo H and stained with Con A, was treated with weak acid and restained with RCA. Then component 3 appeared clearly (Fig. 4a). Component 5 was also detectable. Therefore, the oligosaccharide chain on component 3 that was not stained with Con A, but was stained with RCA, was not susceptible to Endo H. This showed that the spot corresponding to component 3 contained more than one component even after 2D separation: one was susceptible to Endo H and was Con A-stainable (component 3H; H, high-mannose or hybrid), the other was Endo H-resistant and RCA-stainable after acid treatment (component 3C; C, complex).

A nitrocellulose sheet was treated with Endo F and stained with Con A. One spot was then observed clearly (Fig. 4b). Endo F cannot cleave hybrid-type sugar chains with bisecting GlcNAc (see Table 1), as shown for ovalbumin in Fig. 4(b). Thus the component in Fig. 4(b) must be stained with WGA [21]. WGA interacts with the hybrid-type oligosaccharide chain for which the essential structure for the binding is GlcNAc β 1-4Man β 1-4GlcNAc β 1-4GlcNAc-Asn [21]. WGA can also interact with sialic acid at the terminal position of the sugar chains [21], but sialic acid can be removed from the termini of oligosaccharide chains by weak-acid treatment. Thus the interaction of WGA with sialic acid can be eliminated by this method. When a blot was treated with peroxidase-WGA after the removal of sialic acid, component 2 was predominantly stained, and components 1 and 3 were detectable faintly (Fig. 4c). WGA can also interact with poly(*N*-acetyl-lactosamine)-type glycans [21], but component 2 did not react with RCA, which can probably detect these glycans after weak-acid treatment. Thus component 2 probably has hybrid-type oligosaccharide chains with bisecting GlcNAc.

RCA can react with a Gal-GlcNAc structure, but it has been reported [20] to cross-react with *O*-glycoside-type Gal-GalNAc to some degree. Hence, a nitrocellulose sheet was treated first with PNGase F, which can cleave all *N*-glycosidic linkages on glycoproteins, subsequently treated with weak acid and then stained with RCA. After this, no spot was detectable (Fig. 4d) in spite of the presence of protein molecules detected by colloidal-gold staining (Fig. 4e). These components incubated without PNGase F were stained with RCA (Fig. 4f). Thus it was shown that the oligosaccharide chains on components 1 and 3 that bound RCA were *N*-linked.

Component 1 also seems to have two sub-components, because it was stained faintly with Con A even after treatment with Endo H (Fig. 2b) and stained with RCA (Fig. 3a). To confirm this point, nitrocellulose sheets were treated with Endo F or Endo H and stained with RCA (Figs. 4g-i). Endo F can cleave a biantennary structure but Endo H cannot (Table 1). No spot was detected by RCA-staining after treatment with Endo F (Fig. 4g), while component 1 was detected after treatment with Endo H (Fig. 4i) and after incubation without Endo F (Fig. 4h). Thus it was shown that component 1 contained a sub-component that had a biantennary oligosaccharide chain without sialic acid (component 1C) in addition to the major sub-component that had a high mannose-type or a hybrid-type chain (component 1H).

We concluded therefore that the most probable oligosaccharide types of the major components were as follows (Fig. 5): components 1 and 3 consisted of at least two sub-components; 1H + 1C and 3H + 3C. Components 1H and 3H had high mannose-type or hybrid-type oligosaccharide chains that were susceptible to Endo H. In addition, components 1 and 3 had *N*-linked complex-type oligosaccharide chains without and with sialic acids

Fig. 5. Summarized results of oligosaccharide types on multiple forms of DNAase analysed by the lectin-nitrocellulose method

H, high-mannose type or hybrid-type structures sensitive to Endo H; IEF, isoelectric focusing.

(1C and 3C, respectively), and 1C had biantennary, while 3C had tri- or tetra-antennary structures. Component 2 probably had hybrid-type oligosaccharide chains with bisecting GlcNAc. Since component 4 reacted with none of the lectins used in this experiment except Con A, we concluded that, as far as could be analysed by the lectin-nitrocellulose sheet method, component 4 probably had a high-mannose-type oligosaccharide chain on the molecule.

DISCUSSION

Bovine pancreatic DNAase was shown by Salnikow *et al.* [22] to yield four active fractions by chromatography on phosphocellulose, the principal component (DNAase A) accompanied by fractions B, C and, in very minor amount, D. However, they are still heterogeneous, because seven glycopeptides are obtained from DNAase A [6] and two from DNAase B [22]; glycopeptide B₂ has Gal and sialic acid in addition to GlcNAc and Man of glycopeptide B₁. By comparing these results with ours, it was considered that DNAases A, B, C and D corresponded to the Mono S column fractions e-d, d-c, b and a in Fig. 1(b) respectively; components 1, 2 (and 4) corresponded to DNAase A and components 3 (and 4) corresponded to DNAase B; DNAase B₂ was probably component 3C which had tri- or tetra-antennary complex type with sialic acid. DNAases C and D probably corresponded to components 5, more basic spots and their above components, and they seemed to have complex-type oligosaccharide chains.

It is interesting that DNAase has all types of *N*-linked oligosaccharide chains in its multiple forms: high-mannose type, hybrid type, and complex types with and without sialic acid. It seems that these components are involved in the process of glycosylation. It is well known that *N*-linked oligosaccharide chains on glycoproteins are transferred from a dolichol compound to protein in the form of the high-mannose-type chain having glucose on the terminus, and processed and synthesized to a complex-type chain [3,23]. If a complex type with sialic acid is the final form of oligosaccharide chains on DNAase, one would expect pancreatic juice to have

mainly this type. However, it was reported that the juice contained multiple forms of DNAase, although the relative amounts of these differed from those of tissue [22]. Thus, the multiple forms of DNAase arose not only from the process of glycosylation but also from the fact that the final form of DNAase still had micro-heterogeneous sugar chains.

Several practical points should be considered when analysing the types of oligosaccharides. Firstly, it is desirable to detect the spots concerned positively by another method when they were not detected by one method [example 1, Figs. 2(b) and 4(a); example 2, Figs. 4(d) and (e)]. Such confirmations were necessary because isoelectric-focusing or blotting variability was sometimes encountered. Secondly, care should be taken to ensure that glycosidase digestions are complete using suitable standard glycoproteins. Thirdly, loss of proteins from nitrocellulose sheets should be considered in some cases. The proteins on nitrocellulose sheets are extractable with high pH in the presence of detergent [24]. Reported incubation conditions for PNGase [3] were similar to such conditions; the results obtained with PNGase treatment or other glycosidase digestion must always be compared with those obtained without enzyme.

The lectin-nitrocellulose sheet method is not quantitative and the different affinity of many lectins towards the diverse *N*-linked oligosaccharide [21,25] makes it difficult to get accurate oligosaccharide structures by this method. In spite of these limitations, the types of oligosaccharide chains on each component of multiple forms of DNAase were analysed at microgram levels without isolation. The type analysis of oligosaccharide on a glycoprotein by this method was possible even in the presence of many other unrelated proteins, as is the case in Fig. 3, and would be of use in studies of the processing of glycoproteins, such as lysosomal enzymes [2], if the enzyme was identified on a nitrocellulose sheet.

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