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Identification and characterization of the Sd^a β1,4,*N*-acetylgalactosaminyltransferase from pig large intestine

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The high occurrence in large intestine epithelial cells from pig of a β-*N*-acetylgalactosaminyltransferase with a substrate specificity very similar to that of the Sd^a β1,4-*N*-acetylgalactosaminyltransferase from other tissues is reported. The enzyme strictly recognized the NeuAcα2,3Galβ terminal sequence of *N*- and *O*-linked oligosaccharides bound to glycoproteins. The transferase activity required Mn^{2+} and an optimum pH of 7.4. In contrast to the kidney Sd^a-enzyme from humans and other mammals, the microsomal fraction of pig colonic cells expressed a very high activity even in the absence of Triton X-100. A rapid procedure is presented for the large scale preparation of GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc from NeuAcα2,3Galβ1,4Glc. The biosynthesized tetrasaccharide was completely resistant to the action of neuraminidase from *Vibrio cholerae*, whereas about 60% of *N*-acetylneuramic acid was cleaved by neuraminidase from Newcastle disease virus. HPLC separation of different compounds is reported.

Keywords: β1,4-*N*-acetylgalactosaminyltransferase, Sd^a blood-group antigen, *Vibrio cholerae* neuraminidase; Newcastle disease virus neuraminidase; pig large-intestine

Introduction

The Sd^a antigen was originally described as a human blood-group determinant recognized by antibodies naturally occurring in 2% of the Caucasian population [1, 2]. Over 90% of individuals have the Sd^a antigen on erythrocytes [Sd(a+) individuals], but among persons lacking this antigen [Sd(a−) individuals] only half have anti-Sd^a antibodies in their serum [3]. Sd^a antigen also occurs in tissues and body fluids, particularly in urine, of humans and other mammals [4]. In adult humans, the Sd^a antigen appears to be restricted to the kidney and large intestine, and in the latter tissue is sited both on the brush border of the epithelial cells and in the goblet cells [5]. As is the case of many other histo-blood groups, the Sd^a antigen is carbohydrate in nature and *N*-acetylgalactosamine (GalNAc) in β-anomeric configuration is the immunodominant sugar [6–8]. This finding was originally discovered by carbohydrate analysis of Tamm-Horsfall glycoprotein, isolated from the urine of Sd(a+) and Sd(a−) individuals; in the former the GalNAc content is 1–2% whereas in the latter is 0.2% or less [6]. Donald *et al.* [7, 8] isolated, as the non-reducing terminal portion of Tamm-Horsfall glycoprotein oligosaccharides from Sd(a+) individuals, a GalNAcβ1,4(NeuAcα2,3)Galβ1,4GlcNAcβ1,4Gal structure very active in inhibiting the agglutination of Sd(a+) erythrocytes by anti-Sd^a antiserum. A recent study on the carbohydrate structure of Tamm-Horsfall glycoprotein confirmed that many NeuAcα2,3Galβ-terminal units of tri- and tetrantennary glycans are substituted with GalNAc in β1,4-linkage to subterminal galactose [9].

Our group undertook the study of the biosynthesis of Sd^a determinant and a β1,4-*N*-acetylgalactosaminyltransferase capable of transferring GalNAc preferentially to Tamm-Horsfall glycoprotein from Sd(a−) individuals was first identified in the kidney of guinea-pig [10], a mammal expressing a higher level of Sd^a specificity in the kidney [4]. This β1,4GalNAc-transferase adds the immunodominant sugar of Sd^a character to NeuAcα2,3Gal-R sequences of many glycoproteins as well as to NeuAcα2,3Galβ1,4Glc and NeuAcα2,3Galβ1,4GlcNAc. Its activity requires the substitution of *O*-3 of the subterminal galactose residue with *N*-acetylneuraminic acid, but not with *N*-glycolylneuraminic acid [10–13]. A β1,4GalNAc-transferase with a very similar substrate specificity was found in some subsets of mouse

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T-cells [14], human kidney [15], serum [16] and urine of Sd(a+) individuals, but not in urine of Sd(a−) persons [17, 18]. The lack of the β1,4GalNAc-transferase in the urine of the Sd(a−) individuals supports the notion that the genetic base for the Sd(a−) phenotype is the presence of a mutant allele at the locus encoding the β1,4GalNAc-transferase, so that the active enzyme is not produced [17]. This enzyme may for brevity be shortly named Sd^a-βGalNAc-transferase. A very high level of Sd^a-βGalNAc-transferase has been detected in mucosa-cells from the large intestine of all mammals studied to date: humans, mice, rats and guinea pigs [19–22]. In both colon and kidney this enzyme appears to be onco-developmentally regulated. In rat colon and guinea-pig kidney it is practically absent at birth and it is dramatically reduced in human colon carcinomas [19–21]. Colon adenocarcinoma cell lines lack the Sd^aβGalNAc-transferase, but one line (CaCo-2) shows some activity after differentiation [23].

In the present report the high occurrence and some properties of a β1,4GalNAc-transferase from mucosal cells of pig large intestine are described. The substrate specificity of this enzyme is very similar to the Sd^a-βGalNAc-transferase expressed in other tissues. Utilizing an enriched membrane fraction of pig colonic cells a rapid procedure was carried out for a large scale preparation of GalNAcβ1, 4(NeuAcα2,3)Galβ1,4Glc.

Materials and methods

Materials

UDP-[6^3H]GalNAc (8.7 Ci mmol^{-1}) was from New England Nuclear. Unlabelled UDP-GalNAc, fetuin, glycophorin, human chorionic gonadotropin (HCG) and 6′-sialyllactose were from Sigma. 3′-Sialyllactose was purified from a commercial mixture of 3′- and 6′-isomers (Sigma) by HPLC [11]. Asialo glycoproteins were prepared by hydrolysis in 0.1 N H_2SO_4 at 80 °C for 1 h. [^{3}H]GalNAcβ1,4(NeuAcα2,3)-Galβ1,4Glc was prepared as previously described [11]. DEAE-Sephacel was from Pharmacia (Sweden); Bio-Gel P-4 (400 mesh) was from Bio-Rad. β-*N*-acetyl-hexosaminidase (Jack beans) was from Sigma and α-*N*-acetylgalactosaminidase (*Charonia lampas*) from Seikagaku Kogyo, Tokyo. *Vibrio cholerae* neuraminidase was from Behringwerk AG, Marburg, Germany and Newcastle disease virus (NDV) neuraminidase from Genzyme Corporation, Cambridge, MA, USA.

Isolation of epithelial cells from large intestine and preparation of microsomes

Large intestine from an adult pig was obtained from a local farm immediately after killing. Caecum and proximal colon (about 30 cm) were separately excised and flushed with ice-cold 150 mM NaCl containing 1 mM dithiothreitol until the faeces were entirely removed. The distal end of the intestinal portion was tied with a firm thread and filled with 150 mM NaCl containing 1 mM dithiothreitol. The gut was immersed in 0.15 M NaCl and incubated at 37 °C for 15 min. This washing solution was discarded and the gut was filled with about 300 ml of a buffer containing 137 mM NaCl, 3 mM KCl, 1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 1.5 mM EDTA, 0.5 mM dithiothreitol, pH 7.3 (buffer A); the proximal end of the intestine was then also tied. The gut was totally immersed in 0.15 M NaCl and incubated at 37 °C for 1 h with gentle agitation. At the end of the incubation period the fluid containing the released cells was removed. The gut was washed twice with 50 ml of buffer A. The incubation fluid and two washing samples were pooled and the cells were collected by centrifugation at 5000 × **g** for 25 min. The pellet was washed twice with PBS (20 mM sodium phosphate buffer pH 7.4, containing 140 mM NaCl) and homogenized in ice-cold 0.25 M sucrose by a Potter homogenizer. The homogenate was centrifuged at 1500 × **g** for 10 min and the supernatant centrifuged at 100 000 × **g** for 1 h. The resulting pellet, referred to as the microsomal fraction, was suspended in six volumes of 0.25 M sucrose; the protein concentration of this suspension was of about 10 mg ml^{-1}.

Assay for β1,4GalNAc-transferase

The standard assay mixture had the following final concentrations in a total volume of 25 µl: 80 mM Tris-HCl buffer pH 7.4, 10 mM $MnCl_2$, 0.8 mM UDP-[^{3}H]GalNAc (27.5 dpm pmol^{-1}). Usually 50 µg of proteins from the cell homogenate or microsomal fraction were added as the enzyme source and the reaction mixture was incubated at 37 °C for 1 h. The concentrations of various acceptors are given in the Figure or Table legends. When glycoproteins were used as acceptors the incubation was stopped by the addition of 1 ml of 1% phosphotungstic acid in 0.5 M HCl and the precipitates were washed and counted as previously described [21]. When 3′- and 6′-sialyllactose were used as acceptors the incubation was stopped by the addition of 1 ml of 50 mM pyridine-acetic acid buffer, pH 5, and the reaction product was separated from the residual UDP-GalNAc and the derived metabolites (free GalNAc and GalNAc-1-P) by DEAE-Sephacel chromatography as detailed elsewhere [24].

Bulk preparation of GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc

One mg of 3′-sialyllactose was incubated in 1 ml of 150 mM Tris-HCl, pH 7.4, containing 10 mM $MnCl_2$, 20 mM UDP-GalNAc, 100 mM NaCl and 4 mg of microsomal proteins from colonic cells. One µCi of UDP-[^{3}H]GalNAc was added to the reaction mixture in order to facilitate the identification of the reaction product through the isolation procedure. The reaction mixture was incubated overnight at 37 °C, then centrifuged at 100 000 × **g** for 1 h in order to discard microsomal membranes. The supernatant was applied

to a DEAE-Sephacel column equilibrated with 0.05 M pyridine-acetic acid buffer, pH 5, and eluted with a gradient of pyridine-acetic acid buffer, pH 5 [24]. Fractions eluted in the elution position of GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc were pooled, lyophilized and applied to a Bio-Gel P-4 (400 mesh) column (1 × 75 cm) equilibrated with 0.1 M pyridine-acetate buffer, pH 5, in order to separate the GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc from the residual NeuAcα2,3Galβ1,4Glc.

TLC and HPLC

TLC was performed on plates of silica gel, twice developed with ethanol:pyridine:1 butanol:acetic acid:water (100:10:10:3:30) solvent [20]. A Merck NH_2-Lichrosorb (12.5 × 0.4 cm) column was used for HPLC with 78:22 acetonitrile:15 mM KH_2PO_4 as mobile phase at a speed of 2 ml min^{-1} and the optical density detected at 200 nm.

Glycosidase treatments

Digestion with *Vibrio cholerae* neuraminidase (0.1 U ml^{-1}) was in 0.1 M sodium acetate buffer, pH 5, containing 10 mM $CaCl_2$ at 37 °C for 24 h, and with NDV neuraminidase (20 mU ml^{-1}) in 0.1 M sodium cacodylate buffer, pH 6.5, at 37 °C for 24 h [25]. β-*N*-acetylhexosaminidase treatment was performed as previously described [10].

Analytical methods

Proteins were determined by the Lowry method with BSA as standard [26], sugar by the phenol/sulphuric acid method [27] and neuraminic acid by Warren's method [28].

Results and discussion

Characterization of Sd^a-βGalNAc-transferase from pig colon

The Sd^a β1,4GalNAc-transferase, independently of the tissue and species source, strictly requires the presence of sialic acid α2,3-linked to the subterminal galactose of the acceptor for its activity. Thus a good index of the Sd^a-enzyme activity is the transfer of [^{3}H]GalNAc from UDP-[^{3}H]GalNAc to fetuin, a glycoprotein carrying several NeuAcα2,3Gal terminal units, minus that transferred to asialofetuin. By this method a high Sd^a β1,4GalNAc-transferase activity was found in the homogenate of mucosal cells of the proximal portion of pig large intestine. The proximal portion of pig large intestine was selected because in other mammals a proximal-distal gradient of the Sd^a-enzyme was observed [20, 21]. As Table 1 shows, the activity measured in cells detached from pig proximal colon was significantly higher than that found in cells from the corresponding tissue of human and rats. About 30% of the total activity present in the cell homogenate was recovered in the pellet collected by the centrifugation at 1500 × **g**, and close to 50% in the microsomal fraction collected at 100000 × **g**. The specific activity of the latter fraction was about twice as high as that of the total homogenate, therefore the microsomal fraction was used as the enzyme source in all the subsequent experiments. The transferase activity was dependent on Mn^{2+} (optimal concentration, 10 mM). The enzyme had a broad, flattened pH curve, with a pH optimum of 7.4. In contrast to the enzyme from guinea-pig and human kidney Triton X-100 was not required for the activity. We previously reported [20, 23] that the Sd^a-enzyme from human colon and CaCo-2 cells exhibits a relatively high activity in the absence of a detergent, but the presence of 0.5% Triton X-100 in both cases results in about a two-fold increment in activity. At the same concentration Triton X-100 inhibited the activity of pig colon enzyme, whereas the addition of *n*-octyl glucoside resulted in a moderate enhancement of the activity only at 40 mM (Table 2). The explanation that in pig colonic cells the enzyme was mainly in soluble form is unlikely, because the microsomal fraction was used as the enzyme source and the 100000 × **g** supernatant contained less than 20% of the total activity. This high activity in the absence of detergents could be related to a peculiar anchorage of this glycosyltransferase in the Golgi apparatus of pig colonic cells.

Table 1. β1,4-*N*-Acetylgalactosaminyltransferase activity in cell homogenates of caecum and proximal colon of pig, rat and human.

	GalNAc-transferase activity (nmol h^{-1} per mg protein)	
Animals	*Caecum*	*Proximal Colon*
Pig	8.5	12.1
Rat	9.5	6.4
Human	5.3	9.8

Cells were released from each segment of large intestine by 1 h incubation at 37 °C with a buffer containing EDTA as described in the Methods section. The transferase reaction was performed as described in the text with 50 μg of cell homogenate protein and 250 μg of fetuin and asialofetuin as acceptors in a final volume of 25 μl. The values of transferase activity correspond to the incorporation into fetuin minus that in asialofetuin. The results are means of duplicate experiments.

Under standard assay conditions the activity was proportional to the protein concentration and incubation time up to 3 mg ml^{-1} and 4 h (Fig. 1a and b). The microsomal fraction could be stored at −20 °C for 1 year without significant loss of activity.

Previously, we showed that Sd^a-βGalNAc-transferase from different sources exhibits fine differences towards various α2,3-sialylated glycoproteins. In particular glycophorin, in which all NeuAcα2,3Galβ-units are carried by *O*-linked chains [29], is not a good acceptor for the Sd^a-enzyme present in human kidney and urine [15, 18]. In Table 3 the capability of pig colon enzyme to transfer [^{3}H]GalNAc to glycophorin, fetuin, α1-acid glycoprotein, and HCG is shown. The latter three glycoproteins, which

Table 2. Effect of detergents on the β1,4-*N*-acetylgalactosaminyltransferase activity of the microsomal fraction from pig colonic cells.

Detergent	*GalNAc-transferase activity (nmol h^{-1} per mg protein)*
None	21.4
0.5% Triton X100	19.4
20 mM *n*-Octyl glucoside	20.7
40 mM *n*-Octyl glucoside	31.3

The reaction mixture was as described in the text. The values of activity correspond to the transfer of [^{3}H]GalNAc incorporated into 250 μg of fetuin minus that into 250 μg of asialofetuin. Means of two experiments.

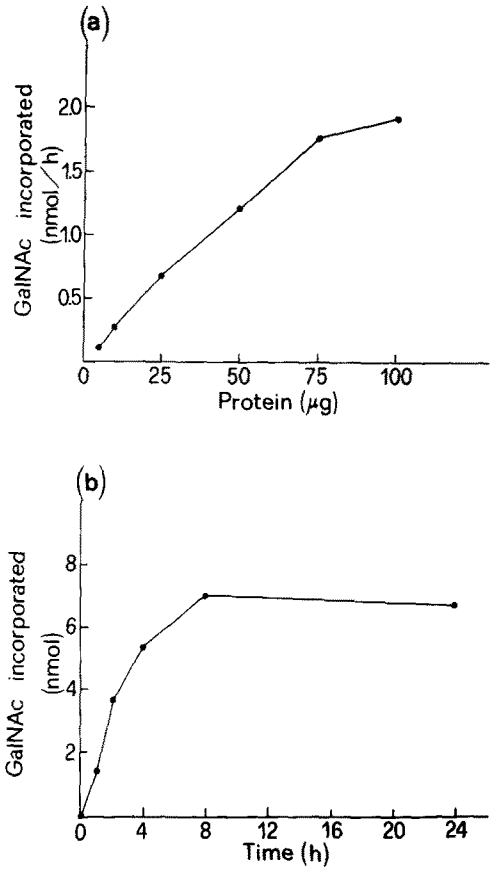

Figure 1. Dependence of pig colon β1,4GalNAc-transferase activity on the amount of microsomal protein and time of incubation. (a) The reaction mixture (25 μl of total volume) was as described in the text and the incubation time was of 1 h at 37 °C. The values of activity correspond to the transfer of [^{3}H]GalNAc incorporated into 250 μg of fetuin minus that into 250 μg of asialofetuin. Means of two experiments. (b) The reaction mixture was as in (a) and contained 50 μg of protein microsomal-fraction in a total volume of 25 μl. The incubation was at 37 °C. The values of activity were calculated as described in (a).

have the majority of the NeuAcα2,3Galβ-unit as the terminal portion of *N*-linked chains, behaved as good acceptors in the absence of detergent and, as occurs for

Table 3. β1,4-*N*-Acetylgalactosaminyltransferase activity of microsomal fraction from pig colonic cells towards various sialylated glycoproteins in the presence and absence of detergent.

	β1,4GalNAc-transferase (nmol per mg protein)	
Acceptor (200 μg)	*No detergent*	n-*Octyl glucoside (40 mM)*
Fetuin	21.6	30.3
α1-acid-glycoprotein	21.8	25.4
HCG	27.8	32.3
Glycophorin	7.6	24.9

The transferase activity was assayed in duplicate as described in the Methods section. The value of activity was the amount of [^{3}H]GalNAc transferred to each glycoprotein minus that found in the corresponding desialylated form.

fetuin, the addition of *n*-octyl glucoside only enhanced by 20–30% the incorporation of [^{3}H]GalNAc. On the contrary, in the absence of detergent the glycophorin behaved as a poor acceptor, but the addition of *n*-octyl glucoside enhanced three-fold the incorporation of [^{3}H]GalNAc. Since glycophorin carried short *O*-linked chains closely spaced on the peptide backbone [30] it can be suggested that in the native configuration the terminal acceptor sequences are hardly accessible to the catalytic site of the enzyme, and that the detergent positively affects the accessibility by changing the folding of the protein. This explanation is supported by previous evidence that the Sd^a-βGalNAc-transferase from human kidney and urine exhibits higher activity towards Pronase-digested and heat denatured glycophorin [15, 18].

Consistent with the substrate specificity of the Sd^a-βGalNAc-transferase from other sources, the pig colon enzyme possesses high activity towards NeuAcα2,3Galβ1,4Glc and the biosynthetic product isolated from the reaction mixture behaves on TLC and HPLC as an authentic sample of GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc tetrasaccharide (see Fig. 2c). The NeuAcα2,6Galβ1,4Glc isomer and lactose were completely devoid of the capability to serve as acceptors.

Larger scale preparation of GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc

Considering the high expression of Sd^a-enzyme in the microsomal fraction of pig colonic cells a rapid procedure was carried out to prepare larger amounts of GalNAcβ1,4-(NeuAcα2,3)Galβ1.4Glc using NeuAcα2,3Galβ1,4Glc as an acceptor. When 1 mg of NeuAcα2,3Galβ1,4Glc was incubated overnight with an excess of UDP-GalNAc and the pig-colon microsomal fraction (see Materials and methods), about 70% of the trisaccharide was transformed into the tetrasaccharide. The procedure for the isolation

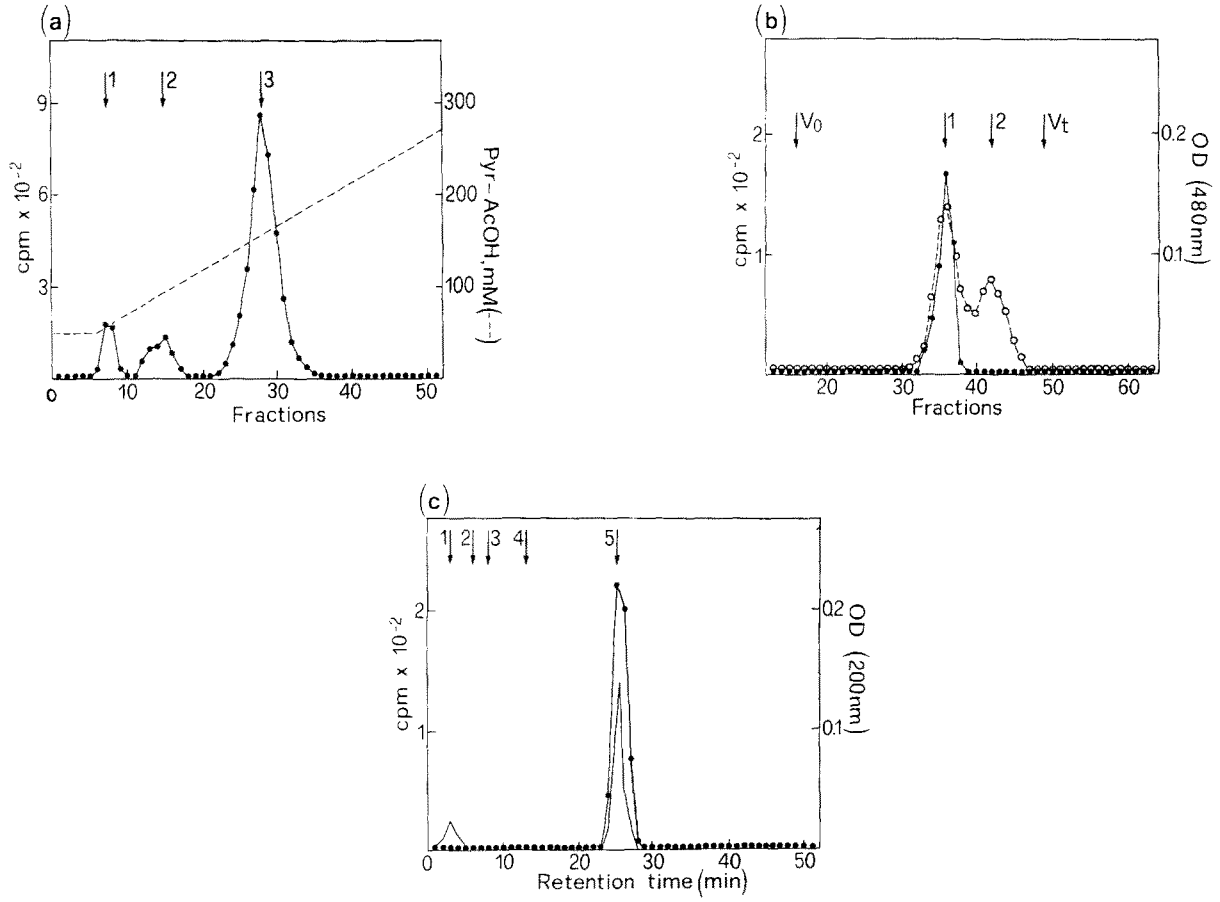

Figure 2. Separation and HPLC profile of [^{3}H]GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc synthesized via pig colon Sd^a βGalNAc-transferase. (a) DEAE-Sephacel chromatography: the supernatant solution from the incubation reaction performed as described in the Methods section (Bulk preparation of GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc) was loaded on a DEAE-Sephacel column (1 × 10 cm) and eluted as described in the text. Fractions of 1 ml were collected and an aliquot was counted for radioactivity. The elution positions of standard compounds are indicated: arrow 1, GalNAc; arrow 2, NeuAcα2,3Galβ1,4Glc or GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc; arrow 3, UDP-GalNAc. (b) Bio-Gel P-4 filtration: fractions 12–17 from Fig. 2(a) were pooled, lyophilized and applied to the Bio-Gel P-4 column. Fractions were collected and monitored for radioactivity (•) and for sugar content by phenol/sulphuric acid method (○). Arrows 1 and 2 indicate the elution of GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc and NeuAcα2,3Galβ1,4Glc, respectively. (c) HPLC separation: fractions 32–38 from Fig. 2(b) were pooled, lyophilized and subjected to HPLC as described in the text. Fractions were monitored for radioactivity (•). The continuous line gives the absorbance at 200 nm. The retention times of markers are indicated: arrow 1, GalNAc; arrow 2, Galβ1,4Glc; arrow 3, NeuAc; arrow 4, NeuAcα2,3Galβ1,4Glc; arrow 5, GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc.

of GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc from the reaction mixture comprised a DEAE-Sephacel chromatography to remove the excess UDP-GalNAc and its degradation products (Fig. 2a), and a Bio-Gel P-4 filtration which allowed the separation of GalNAcβ1,4(NeuAc2,3)Galβ1, 4GlcNAc from the residual NeuAcα2,3Galβ1,4Glc (Fig. 2b). By this procedure about 500 μg of the tetrasaccharide as obtained, which in HPLC migrated as a single component with a retention time of 25 min *versus* a retention time of 13 min for NeuAcα2,3Galβ1,4Glc (Fig. 2c).

Using the same procedure even the GalNAcβ1, 4(NeuAcα2,3)Galβ1,4GlcNAc tetrasaccharide could be prepared by using 3′-sialyl-*N*-acetyllactosamine instead of 3′-sialyllacrose as an acceptor. The activity of the pig colon enzyme towards the two acceptors is very similar (results not shown). This point is relevant, because Soh *et al.* [12] have shown that the GalNAcβ1,4(NeuAcα2,3)-Galβ1,4GlcNAc tetrasaccharide is a powerful inhibitor of the haemagglutination induced by anti-Sd^a antiserum whereas GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc is a poor inhibitor.

Susceptibility of GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc to glycosidase digestion

We utilized a preparation of GalNAcβ1,4(NeuAcα2,3)-Galβ1,4Glc biosynthesized in the presence of 1 μCi of UDP-[^{3}H]GalNAc in order to investigate its susceptibility to glycosidase digestion. We confirmed that the

Table 4. Resistance of [^{3}H]GalNAcβ1,4(NeuAcα2,3)Galβ1, 4Glc to various neuraminidases and to desialylation by mild acid hydrolysis.

Treatment	*Percentage recovery of [^{3}H]GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc*
Vibrio cholerae neuraminidase	>95
Newcastle disease virus neuraminidase	41
0.01 M HCl 2 h	64
0.02 M HCl 2 h	53
0.05 mM H_2SO_4 2 h	59

Neuraminidase digestions were performed as indicated in the Methods section. After each treatment the sample was subjected to HPLC as described in the text. Fractions of 1 ml were collected and counted for radioactivity. The values represent the percentage of radioactivity recovered in the fractions emerging at the retention time of [^{3}H]GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc relative to the total activity recovered in all fractions of each cromatogram.

tetrasaccharide was completely resistant to neuraminidase from *Vibrio cholerae*. Indeed after prolonged digestion with this enzyme, it was entirely recovered as a single compound with a retention time identical to that of the untreated sample and no peak of absorbance was detectable in the elution position of *N*-acetylneuraminic acid. On the contrary, when the digestion was performed with NDV neuraminidase, specific for α2,3-linked sialic acid [25], a clear peak of absorbance emerged at the retention time of *N*-acetylneuraminic acid. Less that half of the [^{3}H]-GalNAcβ1, 4(NeuAcα2,3)Galβ1,4Glc was recovered, and a new compound with a shorter retention time (14 min) containing 59% of radioactive GalNAc was detected. Different conditions of mild acid hydrolysis resulted in a less efficient desialylation of the tetrasaccharide. Table 4 summarizes the resistance of [^{3}H]GalNAcβ1,4(NeuAcα2,3)-Galβ1,4Glc to various desialylation procedures. When [^{3}H]GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glc was treated with β-hexosaminidase (from jack beans) and then subjected to HPLC, it was almost entirely recovered in the elution position of the tetrasaccharide both in term of optical density and radioactivity. On the contrary the same treatment on the desialylated trisaccharide resulted in the production of 85% of free [^{3}H]GalNAc, emerging in HPLC at the retention time of 3.5 min and of lactose at the retention time of 6 min. The tetrasaccharide and the desialylated trisaccharide were entirely resistant to α-*N*-acetylgalactosaminidase treatment. Altogether these results support the notion that the desialylated trisaccharide has the GalNAcβ1,4Galβ1,1,4Glc structure.

In conclusion, current results indicate that mucosa cells of caecum and proximal colon of adult pig express at a very high level a β1,4GalNAc-transferase with a substrate specificity very similar to that of the Sd^a-βGalNAc-transferase from other tissues. Since it is easy to prepare large amounts of the microsomal fraction from pig colon, and the Sd^a-enzyme from this source also acts with a high efficiency in the absence of detergents, it may be preferentially used to transfer *N*-acetylgalactosamine in β1,4-linkage to α2,3NeuAcGalβ terminal sequences of oligosaccharides carried by soluble and cell-bound glycoproteins. The preparation of soluble glycoproteins with the NeuAcα2,3Galβ-terminal units substituted with *N*-acetylgalactosamine β1,4-linked to galactose, as well as the direct transfer of βGalNAc to cell-bound glycoproteins may be useful to investigate whether this addition changes the capability of glycoproteins to bind lectin-like molecules of both mammal and bacterial origin that specifically recognize α2,3-sialylated glycoproteins. It has been shown that some pathogenic strains of *Escherichia coli* adhere to colonic cells by adhesins that recognize the NeuAcα2,3Galβ terminal unit of glycoproteins located on the surface of colonic cells [31]. We postulated [21, 23] that the addition of βGalNAc by the Sd^a enzyme to glycoproteins of colonic cells may reduce the adhesion of these *Escherichia coli* strains and that the high expression of Sd^a enzyme in colonic cells of adult mammals may be considered a selective event that correlates with a reduced susceptibility to *Escherichia coli* infection. To prove this point Sd^a βGalNAc-transferase from pig colon will be useful.

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