Biochimica et Biophysica Acta, 391 (1975) 39—50

BBA 67473

REGIONAL AND CELLULAR LOCALIZATION OF GLYCOSYLTRANSFERASES IN RAT SMALL INTESTINE

CHANGES IN ENZYMES WITH DIFFERENTIATION OF INTESTINAL EPITHELIAL CELLS

YOUNG S. KIM, JOSE PERDOMO, PAMELA OCHOA and RICHARD A. ISAACS

Gastrointestinal Research Laboratory, Veterans Administration Hospital, San Francisco, Calif. 94121 and
Department of Medicine, University of California School of Medicine, San Francisco, Calif. 94143 (U.S.A.)

(Received September 18th, 1974)
(Revised manuscript received December 30th, 1974)

Summary

Optimal assay conditions were determined for four glycosyltransferases in rat small intestinal mucosal homogenates and the regional distribution and cellular localization of these enzymes was studied. For each glycosyltransferase, similar levels of activity were found in duodenal, proximal jejunal and distal ileal segments; activities of the galactosyltransferases were lower in the distal jejunal-proximal ileal segment. Planar section studies indicated that the undifferentiated crypt cells had significantly higher levels of sialyltransferase activities in the jejunum and ileum than the mature villus cells. A similar crypt to villus gradient was found for a galactosyltransferase in the ileum. These data suggest that glycoprotein synthesis may be active in the undifferentiated crypt cells and that certain glycosyltransferases may serve as marker enzymes for cellular differentiation in the intestine.

Introduction

Gastrointestinal mucosal cells are rich in both secretory and structural glycoproteins. These glycoproteins are synthesized by the action of a series of enzymes which are part of a multiglycosyltransferase system [1]. This group of enzymes is present in various tissues such as liver [2], gastrointestinal mucosa [3,4], brain [5] and thyroid glands [6] and in many body fluids such as cerebrospinal fluid, serum [7] and amniotic fluid [8]. In the mucosa of the small intestine, these enzymes have been shown to be primarily associated with

the smooth-membrane fraction which consists of Golgi membranes and the smooth endoplasmic reticulum [3].

Many of the cellular phenomena associated with the process of malignant transformation are shared by embryonic cells such as antigens [9,10] and fetal isoenzymes which have been found in adult tumors [11,12]. Considerable alterations in the activities of glycosyltransferases involved in the synthesis of glycoproteins and glycolipids have been reported in virally transformed cells [13,14] and in solid tumors including those of the gastrointestinal mucosa [15,16]. Therefore, it is of interest to examine the activities of these enzymes in the epithelial cells of intestinal mucosa at different stages of differentiation. In the intestine proliferative cells are localized in the crypt area where they migrate upward into the villi. During the course of this migration, these cells undergo differentiation resulting in the development of mature absorptive epithelial cells. In the present study, the activities of four glycoprotein glycosyltransferases were studied in differentiating cells of the jejunum and ileum of rat small intestine.

Materials and Methods

Preparation of tissue samples

Sprague-Dawley derived Wistar male rats weighing 225—275 g were fed with standard Purina chow. Water was supplied ad libitum at all times. Food was restricted 18 h before sacrifice. The rats were killed by a blow on the head followed by decapitation. All subsequent steps were performed at 4° C. The abdomen was opened immediately after sacrifice and the small intestine was removed in one piece. After the fat and mesentery had been dissected, the lumenal wall was rinsed with 100 ml of ice-cold 0.9% NaCl, suspended vertically in front of a meter stick with a 10-g weight attached to the ileocecal end and its length measured. Keeping the tissue at constant tension, standardized 7 cm long segments were marked at four areas along its length and excised as follows: the duodenal segment was the first 7 cm below the pylorus; the proximal jejunal segment was excised 3 cm below the ligament of Trietz; a third segment (distal jejunum-proximal ileum) was taken from midpoint between the ligament of Trietz and the ileocecal valve; and the distal ileal segment consisted of the terminal 7 cm before the ileocecal valve. Segments were rinsed again with ice-cold saline and were either used for the planar sectioning study or for the regional distribution study of the enzymes. The mucosa was scraped from each segment with a spatula. The resulting scrapings were weighed and homogenized in 4 vol. (v/w) of 0.02 M Tris/maleate/0.15 M NaCl buffer (pH 7.4) with a Potter-Elvehjem tissue homogenizer with a Teflon pestle. This homogenate was aliquoted for enzyme assays and analysis. The enzyme assays were performed within 5 h after sacrifice.

Planar sectioning

A planing apparatus described previously [17] was used. It consisted of a razor blade mounted at a 45° angle on a vertically oriented micrometer. The calibrated micrometer blade was mounted on sliding rods allowing the blade to travel freely on a fixed block along its entire length (5 cm) on which the tissue

was stretched. The proximal jejunal and distal ileal segments described above were carefully cut longitudinally in order to expose the mucosal side and the tissue was mounted serosal side down on the block under a 10-g tension by means of a spring loaded holder. After the tissue was mounted the apparatus was transferred to a cryostat (Harris Lo Temp Freezer, Mass.) set at −10° C where the tissue was allowed to freeze for 20 min. The frozen tissue was sectioned in 50-μm layers and shavings were collected from the blade by using a pair of fine forceps. This procedure was continued until the blade began to cut off the serosa from the mounting block. An average of 12 and 7 sections were obtained from the proximal jejunal and distal ileal segments, respectively. The sections were homogenized in 1 ml of 0.02 M Tris/maleate/0.15 M NaCl buffer (pH 7.4). Aliquots from these homogenates were used within 5 h for enzyme assays and analysis. For differential cell counting, segments of intestine were cut cross-sectionally in 5-μm layers from the villus tip into the sub-mucosal serosa. The slices were stained with hematoxylin and eosin.

Preparation of sugar acceptors

The ovine submaxillary mucin acceptor for both a sialyltransferase and a galactosyltransferase was prepared from purified ovine submaxillary mucin [18]. Sialic acid (*N*-acetylneuraminic acid) was removed by treatment with a neuraminidase purified from *Clostridium perfringens* as described previously [19].

Another acceptor for a sialyltransferase was prepared from calf fetuin [20] by treatment with neuraminidase in the same fashion as was performed on the ovine submaxillary mucin. This acceptor is called Fetuin I. As an acceptor for another galactosyltransferase, Fetuin I was further treated by Smith degradation as described previously [3,21] yielding Fetuin II. This treatment reduced the galactose content by 96% exposing *N*-acetylglucosamine residues as terminal sugars.

Preparation of radioactive glycoproteins

A radioactive glycoprotein (Fetuin III) was prepared from Fetuin II by using a highly purified galactosyltransferase from human serum [22] and UDP[U-^{14}C]galactose (200 Ci/mol, New England Nuclear). This radioactive glycoprotein was rendered free of galactosyltransferase activity by heating at 80° C for 20 min and salts and unreacted substrate were removed by exhaustive dialysis. Another radioactive glycoprotein (Fetuin IV) was made from Fetuin I by using a crude sialyltransferase from human serum [7] and CMP-*N*-acetyl-[4,5,6,7,8,9-^{14}C]neuraminic acid (229 Ci/mol, New England Nuclear). After incubation, the mixture was heated and dialysed in the same manner as in the preparation of Fetuin III. Both glycoproteins were lyophilized and were kept at −20° C until used.

Glycosyltransferase assays

The incubation mixture for the assay of a galactosyltransferase that catalyzes the transfer of galactose to Fetuin II contained the following reagents: 0.5 mg of Fetuin II acceptor; 33 μmol of cacodylate/acetate buffer, pH 6.5; 1.65 μmol of $MnCl_2$; 0.17 μmol of ATP; 0.17 μmol of UDPglucose;

100 000 dpm of UDP[U-^{14}C]galactose (6 Ci/mol), Triton X-100 to a final concentration of 0.1% and 10 to 40 μg protein of enzyme in a final volume of 165 μl. The reaction mixtures were incubated at 37° C for 1 h. The assay mixture for a galactosyltransferase that transfers galactose to the ovine submaxillary mucin acceptor was the same as the assay mixture described above except that 0.5 mg of desialized ovine submaxillary mucin was used, the pH of the cacodylate/acetate buffer was 7.5 and the concentration of $MnCl_2$ was 4 times higher. The incubating mixture and condition for the assay of a sialyltransferase that catalyzes the transfer of sialic acid to Fetuin I was as follows: 1.0 mg of Fetuin I; 24 μmol of cacodylate/acetate buffer, pH 6.4; 50 000 dpm of CMP-*N*-acetyl[4,5,6,7,8,9,-^{14}C]neuraminic acid (80 Ci/mol); Triton X-100 to a final concentration of 0.1% and 20 to 300 μg of enzyme in a final volume of 110 μl. The reaction mixtures were incubated for 1 h at 30° C. For the assay of another sialyltransferase that transfers sialic acid to the ovine submaxillary mucin acceptor, the only change in the incubation mixture was the acceptor. The same amount of the ovine submaxillary mucin acceptor was added. A control for the endogenous activity, i.e. in the absence of exogenous acceptor, was included for each enzyme assayed and its value was subtracted from the complete mixture. After incubation the enzyme reaction was stopped by addition of 3 ml of 1% phosphotungstic acid in 0.5 M HCl followed by 0.1 ml of a 10 mg/ml serum albumin solution as a protein carrier. The tubes were kept in ice/water for 20 min at 4° C before the precipitate was separated by centrifugation. The precipitate was washed three times with the same acid solution, dissolved in 0.5 ml of NCS solubilizer (Amersham-Searle) at 37° C and protein-bound radioactivity was counted in 10 ml of a toluene/phosphor scintillization solution using a Packard Tri-Carb liquid scintillation spectrometer.

Degradation of nucleotide sugar substrates

To determine the extent and nature of degradation of nucleotide sugar substrates during incubation, aliquots of the incubation mixtures before addition of phosphotungstic acid, were applied onto Whatman 3 MM paper for galactosyltransferase assays and onto Whatman No. 1 paper for sialyltransferase assays. The samples applied on Whatman 3 MM paper were run in a Savant high-voltage tank electrophoresis apparatus at 2800 V for 2.5 h in 0.15 M formic acid, 1.60 M acetic acid. The samples applied to Whatman No. 1 paper were subjected to descending chromatography for 21 h using ethanol/1 M ammonium acetate, pH 7.5 (75 : 30, v/v). After electrophoresis or chromatography the papers were dried in a 50° C convection oven, cut at 0.5-inch intervals and the strips counted in a toluene/phosphor scintillation solution. The electrophoresis procedure was capable of separating UDPgalactose, galactose-1-*P*, and galactose. The potential degradation products of CMP-*N*-acetylneuraminic acid were separated adequately by paper chromatography.

Glycosidase activities using glycoprotein substrates

To determine if glycosidase activities capable of hydrolyzing the products of the glycosyltransferases were present in the samples assayed, two glycoproteins, each containing either terminal radioactive galactose (Fetuin III) or sialic acid (Fetuin IV) were used as substrates. The incubation mixtures and

conditions were the same as those described for glycosyltransferase assays except that the acceptor and nucleotide sugar were omitted. Each assay contained a radioactive glycoprotein substrate (8000 cpm). Following incubation, aliquots were applied onto Whatman No. 1 paper and chromatographed in a descending system using ethyl acetate/pyridine/water (12 : 5 : 4, by vol.) for 16 h. Non-radioactive standards were chromatographed simultaneously and were visualized by $AgNO_3$ reagent (for galactose) or Ehrlich reagent (for sialic acid) [U-^{14}C]galactose-1-*P* (New England Nuclear) was also used as a standard.

Other chemicals and enzyme assays

The protein content of the samples were determined by the method of Lowry et al. [23] using crystalized bovine serum albumin as standard. β-Fructofuranosidase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.16, formerly known as sucrase) was determined as described by Dahlqvist [24]. Thymidine kinase actively was assayed by the method of Behki and Morgan [25].

Results

Requirements for glycosyltransferase assay

The requirements of the glycosyltransferases examined in this study are summarized in Table I. The galactosyltransferases required in the presence of Mn^{2+}, Triton X-100, UDPglucose and acceptor for maximal activity. The presence of ATP enhanced the activity by only 5%. UDPglucose nearly doubled the amounts of counts transferred to both acceptors. When UDPglucose was substituted by GDPmannose a greater enhancement was observed. The sialyltransferase activity required the presence of Triton X-100 and the acceptor. None of

TABLE I

REQUIREMENTS OF THE GALACTOSYLITRANSFERASES AND SIALYLTRANSFERASES FROM RAT SMALL INTESTINE

Results are expressed as a percentage of enzyme activity in the complete assay mixtures. The composition of the complete incubation mixtures are described in Materials and Methods. Ovine submaxillary mucin, Fetuin I and Fetuin II are acceptors.

Incubation mixture	Galactosyltransferases (percentage activity)		Sialyltransferases (percentage activity)	
	Ovine submaxillary mucin	FetuinII	Ovine submaxillary mucin	Fetuin II
Complete assay mixture	100	100	100	100
Minus enzyme	1.3	0.8	3.5	2.1
Minus acceptor	2.2	2.2	3.3	3.3
Minus Triton X-100	33.4	40.3	45.6	20.8
Minus $MnCl_2$	4.2	5.7	—	—
Minus ATP	94.7	96.4	—	—
Minus UDP-glucose	56.2	53.4	—	—
Minus UDP glucose plus 1 mM GDPmannose	130.5	143.6	—	—

(A) UDP-Gal + GlcNAc-glycoprotein (Fetuin II) ⟶ Gal-GlcNAc-glycoprotein + UDP.

(B) UDP-Gal + GalNAc-glycoprotein (mucin) ⟶ Gal-GalNAc-glycoprotein + UDP.

(C) CMP-NANA + Gal-glycoprotein (Fetuin I) ⟶ NANA-Gal-glycoprotein + CMP.

(D) CMP-NANA + GalNAc-glycoprotein (mucin) ⟶ NANA-GalNac-glycoprotein + CMP.

Fig. 1. Summary of glycosyltransferase reactions. The following acceptors were used for each reaction: Fetuin II for Reaction A; desialyzed ovine submaxillary mucin for Reactions B and D; and Fetuin I for Reaction C. NANA, *N*-acetylneuraminic acid.

the following chemicals, when added to the incubation mixtures for the sialyltransferase assays, increased the counts obtained with the complete mixture: 1 mM ATP, 1 mM AMP, 1 mM GMP, 10 mM EDTA, 1.5 and 10 mM KF and 0.1 and 1% β-mercaptoethanol. The optimal conditions for assay of the two galactosyltransferases were as follows: when Fetuin II was used as an acceptor, the optimal pH was 6.0, temperature was 45° C and Mn^{2+} concentration was 10 mM; when ovine submaxillary mucin was the acceptor, the maximal enzyme activity was obtained at pH 7.0, 37° C and at 40 mM $MnCl_2$. For the sialyltransferase assays, a pH of 6.1 and an incubation temperature of 30° C were found to be optimal for both of the acceptors. All of the enzyme assays were carried out under the conditions in which the assays were linear with respect to time of incubation and enzyme concentration. A summary of the reactions catalyzed by the enzymes examined in this study is illustrated in Fig. 1.

Regional distribution of glycosyltransferases

The regional distribution of glycosyltransferases in the mucosa of the rat small intestine is shown in Fig. 2. The similar profile was obtained whether the enzyme activity was expresses as enzyme activity per g of tissue wet weight or per mg of protein. The levels of the galactosyltransferases in the duodenum and proximal jejunum were similar. In contrast, the next segment (distal jejunum-proximal ileum) had a lower level of activity. The last segment, distal ileum,

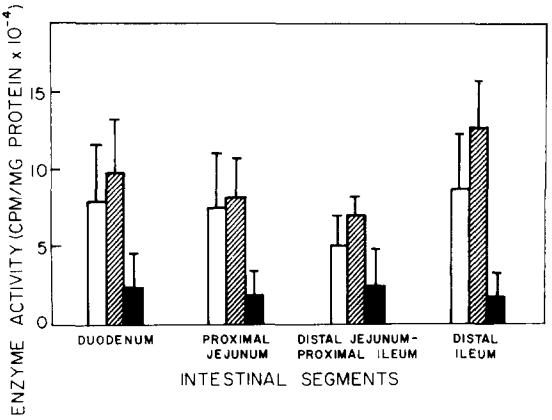

Fig. 2. Regional distribution of glycosyltransferases in rat small intestinal mucosa. The values presented are the means of five experiments (mean ± S.D.). □, galactosyltransferase using Fetuin II as an acceptor; ▨, galactosyltransferase using desialyzed ovine submaxillary mucin as an acceptor; and ■, sialyltransferase using Fetuin I as an acceptor.

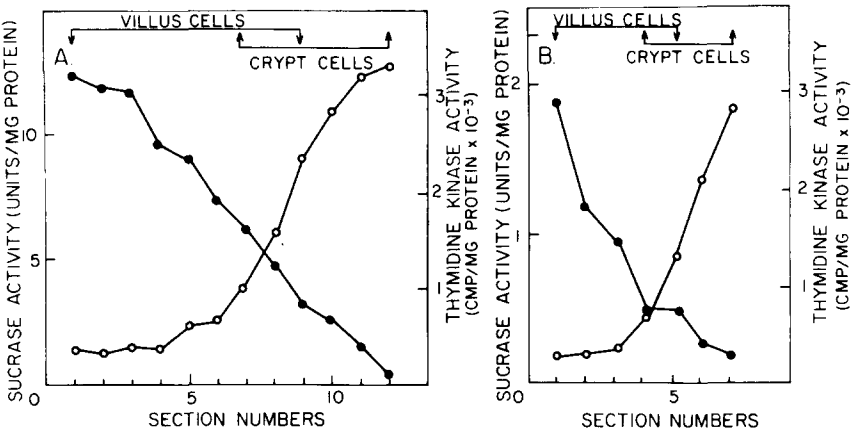

Fig. 3. Sucrase activity (●) and thymidine kinase activity (○) in planar sections taken from successive levels of the villi and crypts from the proximal jejunum (A) and the distal ileum (B). The values presented are the means of five experiments.

had the highest specific activity of all of the segments. The differences between the enzyme activities of the most distal segment and those of the distal jejunum-proximal ileum was significant with p values of less than 0.01 with either acceptor. When the enzyme activities of the distal jejunum-proximal ileum were compared to those of the duodenum, p values of less than 0.02 and 0.05 were obtained for the enzymes utilizing Fetuin II and ovine submaxillary mucin acceptors, respectively. The enzyme activities of the proximal jejunum when compared to those of the distal jejunum-proximal ileum segment showed p values of less than 0.04 for the Fetuin II acceptor. No significant difference was observed for the ovine submaxillary mucin acceptor. No statistically significant differences in sialyltransferase activity was found among the four segments studied.

Glycosyltransferase activities in planar sections

The distribution of various enzymes in crypt cells and villus cells is shown in Fig. 3. Thymidine kinase is confined primarily to crypt cells and disappears rapidly as the cell reaches the crypt-villus cell junction. The converse is true for sucrase, an enzyme associated with brush-border membranes, indicating that the planar sectioning technique used in this study is effective in separating villus cells from crypt cells. The galactosyltransferases in the proximal jejunal segment had an even distribution in all of the planes with both acceptors (Fig. 4A). The distal ileal segment (Fig. 4B) shows an increase in the specific activity of a galactosyltransferase assayed with ovine submaxillary mucin acceptor in the crypt cell region. The p value of the difference between Section 1 and 6 is less than 0.025. In contrast, when Fetuin II was the acceptor galactosyltransferase activity did not show any significant difference between sections. The specific activity of sialyltransferases in planar sections of jejunum and ileum is depicted in Fig. 5A and B. The activities of the sialyltransferases were considerably higher in crypt cells than in the villus cells with either acceptor. The difference in enzyme activities between Sections 1 and 11 in the proximal

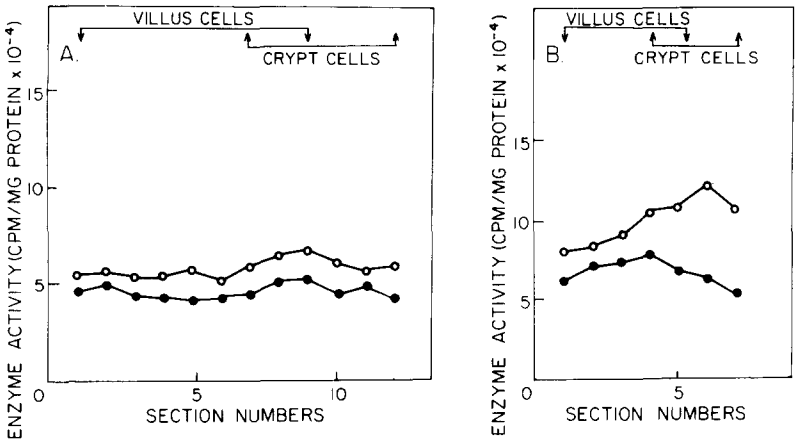

Fig. 4. Specific activities of galactosyltransferases in planar sections taken from successive levels of the villi and crypts from the proximal jejunum (A) and the distal ileum (B). Galactosyltransferase activity was measured using desialyzed ovine submaxillary mucin (○) and Fetuin II (●), as acceptors. The values presented are the means of five experiments.

jejunum was highly significant with p values of less than 0.005 with either acceptor. In the distal ileum, the difference in enzyme activities between Sections 1 and 7 were also highly significant with p values of less than 0.005 with either acceptor.

Activities of nucleotide sugar pyrophosphatases and glycosidases in planar sections

A preliminary experiment showed that addition of UDPglucose or GDP-mannose to the galactosyltransferase assay resulted in an increase of enzyme activity by almost 2- to 3-fold, respectively. Thus, there was a possibility that a

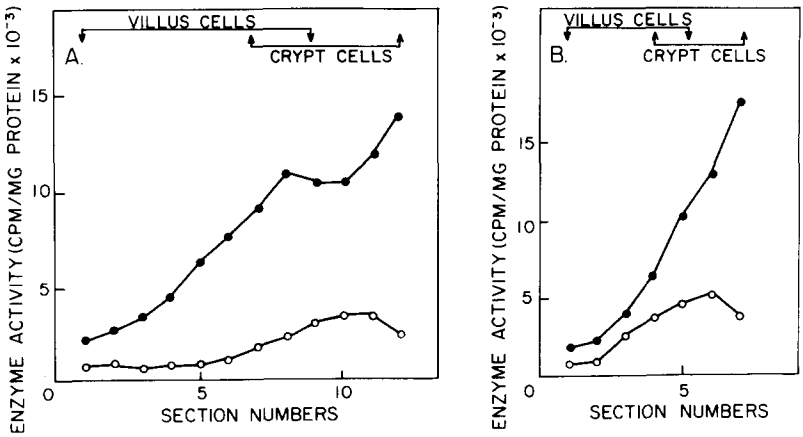

Fig. 5. Specific activities of sialyltransferases in planar sections taken from successive levels of the villi and the crypts from the proximal jejunum (A) and the distal ileum (B). Sialyltransferase was measured using an ovine submaxillary mucin acceptor (○) and the Fetuin I acceptor (●). The values presented are the means of five experiments.

substrate-degrading enzyme such as a nucleotide sugar pyrophosphatase might be present in the intestinal mucosa. Table II summarizes the results of this study. In the absence of UDPglucose a considerable degradation of the substrate to galactose 1-phosphate and galactose occurred in all sections. The addition of 1 mM UDPglucose resulted in a considerable restoration of the level of substrate during incubation time, about 90% of UDPgalactose remained unaltered at the end of incubation. Incubation mixtures containing CMP-*N*-acetylneuraminic acid were also examined for possible substrate degradation during incubation. Only one radioactive spot corresponding to CMP-*N*-acetylneuraminic acid was found irrespective of the segment or section used indicating that this substrate was resistant to degradation.

The observed distribution of glycosyltransferase activities may be a net result of glycosyltransferase activity and endogenous glycosidase activities in the incubation mixtures. As shown in Table III the ability of planar sections of proximal jejunum and distal ileum to hydrolyze the glycosidic bond of the terminal radioactive sugar of two glycoproteins studied is minimal. In all sections about 90% of the radioactivity remained at the origin of the paper chromatogram while negligible amounts were detected in the areas corresponding to galactose when radioactive Fetuin III was used and to *N*-acetylneuraminic acid when Fetuin IV was the substrate.

Mixing experiments

The possibility was considered that the presence of inhibitors of glycosyltransferases which may yield erroneous conclusion on the levels of the enzyme in different planes of the mucosa. The results of the mixing experiments,

TABLE II

DEGRADATION OF UDPGALACTOSE BY PLANAR SECTION HOMOGENATES OF JEJUNAL AND ILEAL MUCOSA

Results are expressed as percent of total radioactivity recovered. Numbers represent mean values obtained from three experiments. The details of the experimental procedure are described in Materials and Methods.

Mucosal section numbers	1mM UDP-Glucose	Percentage distribution of radioactivity		
		UDPGalactose	Galactose-1-*P*	Galactose
Jejunum				
2	None	20	44	36
2	Present	91	8	1
5	None	50	36	14
5	Present	92	6	2
7	None	38	45	17
7	Present	89	10	1
9	None	23	46	31
9	Present	95	5	0
Ileum				
2	None	19	50	31
2	Present	91	8	1
5	None	30	46	24
5	Present	89	10	1
7	None	28	49	23
7	Present	92	7	1

TABLE III

GLYCOSIDASE ACTIVITIES IN THE PLANAR SECTIONS OF JEJUNAL AND ILEAL MUCOSA ON GLYCOPROTEIN

Results are expressed as cpm associated with the substrate or the liberated monosaccharide. Experimental details are described in Materials and Methods.

Mucosal section numbers	Radioactivity released after incubation of Fetuin III with planar section homogenates		Radioactivity released after incubation of Fetuin IV with planar section homogenates	
	Fetuin III (cpm)	Galactose (cpm)	Fetuin (IV) (cpm)	Sialic acid (cpm)
Jejunum				
2	2094	6	2312	16
5	2081	5	2250	12
7	1933	10	2215	10
9	1973	58	2253	0
Ileum				
2	2078	0	2263	15
4	1990	5	2317	0
6	2058	20	2333	0

although not shown, demonstrated that the theoretical values and those obtained by experimental means were similar, thus ruling out the presence of inhibitors or activators.

Differential cell counting of the tissue sections

In order to rule out the possibility that the observed increase in sialyltransferase activity in the crypt area may be due to the possible increase in the proportion of fibroblasts in this region as compared to the villous area, cell counts were made of three main cell types, e.g. epithelial cells, goblet cells and fibroblasts in all sections. The sections from the upper third of villi had the following proportions of cells per high-power field; epithelial cells (78—83%), goblet cells (6—7%), and fibroblasts (10—14%). When the sections from the remaining two-thirds of villi and crypt areas were compared, remarkably similar proportions of cells were observed. These sections contained epithelial cells (67—77%), goblet cells (3—5%) and fibroblasts (28—33%). Thus, although sialyltransferase activity showed 3- to 5-fold increase in the crypt area over that in the lower two-thirds of the villi, the proportion of fibroblasts remained fairly constant in these regions.

Discussion

In the present study, the whole mucosal homogenate was used as a source of glycosyltransferases and, therefore, it is important to examine the effects of other enzymes on the substrates and products of glycosyltransferase reactions. Our data showing an extensive degradation of the substrate, UDPgalactose to galactose 1-phosphate and galactose indicates the presence in the intestinal mucosal cells of at least two enzymes, a sugar nucleotide pyrophosphatase and a phosphatase. The addition of nucleotide sugars such as UDPglucose or GDP-

mannose to the incubation mixture virtually abolished the activities of these degrading enzymes and therefore UDPglucose was included in our assay mixture. No such degradation of CMP-*N*-acetylneuraminic acid occurred under our incubation conditions. In contrast, only minimal glycosidase activities, i.e. galactosidase and sialidase, were observed in our assay mixture.

Having established the optimal conditions for the quantitative assay of each glycosyltransferase, we examined the regional distribution of these enzymes in the small intestine. The levels of all four glycosyltransferase activities were similar in duodenal, proximal jejunal and distal ileal segments but the activities of the galactosyltransferases were lower in the distal jejunal-proximal ileal segment. The reasons for the observed lower level of galactosyltransferases in the midintestinal segment are not clear.

In the intestine, undifferentiated cells undergoing active mitosis are located in the crypt area. These cells migrate upward into the villi where these cells reach full maturity. Several methods have been devised to fractionate intestinal cells in different stages of differentiation [17,26]. The planar sectioning method used in the present study is rapid and enables the measurement of enzyme activities in fresh tissues and has previously been used for the study of various enzymes such as disaccharidases, alkaline phosphatase [27] and thymidine kinase [17] during differentiation of intestinal epithelial cells. Our data revealed distinct changes during the differentiation of intestinal epithelial cells in sialyltransferase activities involved in the biosynthesis of glycoproteins. The undifferentiated crypt cells had elevated levels of sialyltransferases in both the jejunum and ileum and a galactosyltransferase in the ileum. These data are in part consistent with the previous observation made in synchronized mouse lymphoma cell lines that although the activities of glycosyltransferases occurred throughout the cell cycle, the peak activity was in the S-phase [28]. The possibility that the observed increase in sialyltransferase activity in the crypt area was the result of an increased fibroblast content was ruled out by our observation that the proportion of fibroblasts to epithelial cells in the crypt area was similar to that in the villi. It would appear from these data that the synthesis of both structural and secretory glycoproteins may be active in cells that are undergoing DNA synthesis.

Although the majority of cellular glycosyltransferase activities are associated with the membrane of the Golgi apparatus and microsomes [2,3], these enzymes have also been found to be associated with the external surface of plasma membranes, so called ectoglycosyltransferases [29,30]. Although the precise role of these ectoglycosyltransferases is yet to be elucidated, these enzymes are thought to play a role in cell-cell interaction and cellular adhesion. Recently it has been reported that most ectoglycosyltransferase activities were high in crypt cell surfaces except for a sialyltransferase which was markedly elevated in villus cell surfaces [30]. It is of interest that the reported distribution of the ectosialyltransferase activity is different from that of the total cellular sialyltransferases observed in the present study. This difference may in part be due to the need for a constant repair of the oligosaccharide side-chains of the brush-border membrane glycoproteins which may be degraded by glycosidases.

The differences in the carbohydrate constituents of the membrane surface

of small intestinal crypt cells and villus cells have recently been demonstrated using various lectins, suggesting an association between changes in surface-membrane carbohydrates and cellular differentiation [31]. However, the detailed carbohydrate analyses of membranes or total cellular glycoproteins or glycolipids of crypt or villus cells are not yet available.

Not all of the glycosyltransferase activities were elevated in crypt cells as shown by the uniform cellular distribution of two galactosyltransferases in jejunum and of one of these in ileal mucosa. The nature of the differences in the activities of sialyltransferases between crypt and villus cells remains unclear but it would appear from our mixing experiments that the presence of inhibitors or activators of the enzymes in these cells is not the cause. Further studies are necessary to clarify factors regulating the changes in glycosyltransferase activities and the possible relationship of glycoprotein metabolism with cellular differentiation.

Acknowledgements

This investigation was supported by Public Health Service Grant CA-14905 from the National Cancer Institute and a Veterans Administration Research Grant.

References

1 Roseman, S. (1970) Chem. Phys. Lipids 5, 270—297
2 Schachter, H., Jabbal, I., Hudgin, R.L., Pinteric, L., McGuire, E.J. and Roseman, S. (1970) J. Biol. Chem. 245, 1090—1100
3 Kim, Y.S., Perdomo, J. and Nordberg, J. (1971) J. Biol. Chem. 246, 5466—5476
4 Kim, Y.S. and Perdomo, J. (1972) J. Clin. Invest. 51, 1135—1145
5 Den, H., Kaufman, B. and Roseman, S. (1970) J. Biol. Chem. 245, 6607—6615
6 Spiro, M.J. and Spiro, R.G. (1968) J. Biol. Chem. 243, 6529—6537
7 Kim, Y.S., Perdomo, J., Bella, A. and Nordberg, J. (1971) Biochim. Biophys. Acta 244, 505—512
8 Nelson, J.D., Jato-Rodriguez, J.J. and Mookerjea, S. (1973) Biochem. Biophys. Res. Commun. 55, 530—537
9 Gold, P. and Freedman, S.O. (1965) J. Exp. Med. 122, 467—481
10 Gold, P. (1971) Prog. Exp. Tumor Res. 14, 43—58
11 Sato, K., Morris, H.P. and Weinhouse, S. (1972) Science 178, 879—881
12 Weinhouse, S. (1974) in Control Processes in Neoplasia (Mehlman, M.A. and Hanson, R.W., eds), pp. 1—30, Academic Press, New York
13 Grimes, W.J. (1970) Biochemistry 9, 5083—5092
14 Bosmann, H.B. (1972) Biochem. Biophys. Res. Commun, 49, 1256—1262
15 Stellner, K. and Hakomori, S. (1973) Biochem. Biophys. Res. Commun. 55, 439—445
16 Kim, Y.S., Perdomo, J. and Isaacs, R. (1974) Proc. Natl. Acad. Sci. U.S. 71, 4869—4873
17 Imondi, A.R., Balis, M.E. and Lipkin, M. (1969) Exp. Cell Res. 58, 323
18 Pigman, W. and Tettamanti, G. (1968) in Biochemistry of Glycoproteins and Related Substances. Proceedings of the Fourth International Conference on Cystic Fibrosis of the Pancreas, pp. 117—140, S. Karger, Basel
19 Bella, Jr, A. and Kim. Y.S. (1971) Arch. Biochem. Biophys. 147, 753—761
20 Spiro, R.G. (1960) J. Biol. Chem. 235, 2860—2869
21 Spiro, R.G. (1964) J. Biol. Chem. 239, 567—573
22 Bella, Jr, A. and Kim, Y.S. (1972) Abstracts of the American Chemical Society Meeting, August, Biol. 54, New York, N.Y.
23 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
24 Dahlqvist, A. (1964) Anal. Biochem. 7, 18—25
25 Behki, R.M. and Morgan, W.S. (1964) Arch. Biochem. Biophys. 107, 427—434
26 Weiser, M.M. (1973) J. Biol. Chem. 248, 2536—2541
27 Nordstrom, C., Dahlqvist, A. and Josefsson, L. (1968) J. Histochem. Cytochem. 15, 713—721
28 Bosman, H.B. (1971) Arch. Biochem. Biophys. 145, 310—318
29 Roth, S. and White, D. (1972) Proc. Natl. Acad. Sci. U.S. 69, 485—489
30 Weiser, M.M. (1973) J. Biol. Chem. 248, 2542—2548
31 Etzler, M. and Branstrator, M.L. (1974) J. Cell Biol., 62, 329—343