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B. JAN-WILLEM VAN KLINKEN,1 JAN DEKKER,1 HANS A. BÜLLER,2 CARME DE BOLÒS,3 AND ALEXANDRA W. C. EINERHAND1

1Pediatric Gastroenterology and Nutrition, Academic Medical Center, Emma Children’s Hospital AMC, Department of Pediatrics, University of Amsterdam, 1105 AZ Amsterdam; 2Department of Pediatrics, University Hospital Rotterdam, Sophia’s Children Hospital, 3015 GJ Rotterdam, The Netherlands; and 3Unitat de Biologia Cellular i Molecular, Institut Municipal d’Investigació Médica, Barcelona 08003, Spain

MUCOSAE OF THE INTESTINE and stomach are covered by a mucus gel layer that protects the epithelium against mechanical damage and chemical irritants (35). Mucus glycoproteins, often referred to as mucins, are synthesized by the mucosal epithelium and form the most important structural component of mucus (12). The class of epithelial mucins can be divided into either secretory or membrane-bound mucins (reviewed in Refs. 29, 40). The central part of the polypeptide backbones of these molecules consists of many tandemly repeated amino acid sequences that are rich in serine and threonine residues; these tandem repeats are unique for each mucin, may vary in number between individuals, and are densely O-glycosylated. The NH2 and COOH terminals of the mucin polypeptides protrude from the heavily O-glycosylated central part, do not contain tandem repeats, and, for secretory mucins, contain many cysteine residues forming intramolecular disulfide bonds. Usually the terminals of the polypeptides have some N-glycosylation (29).

Due to diverse O-glycosylation, each mucin gene product forms a heterogeneous set of molecules. Several different human epithelial mucin genes have been identified, named MUC1–8. MUC1, which has been fully sequenced, codes for a membrane-bound mucin (14, 23, 24); MUC2 and MUC7 cDNAs have been completely sequenced and code for secretory mucins (2, 15, 17). Only partial cDNA sequences are known for MUC3, MUC4, MUC5AC, MUC5B, MUC6, and MUC8 (11, 16, 18, 26–28, 32). Of these, MUC5AC codes for a secretory mucin and is expressed at a high level in the human stomach (4, 21, 22). The question of whether the other cDNAs encode secretory mucins has to await their full sequence determination and cell biological assessment of the secretory status of each of these mucins.

Mucin expression is tissue as well as cell type specific. For instance, in the small intestine, MUC2 and MUC3 mRNAs are prominently expressed; MUC2 is confined to goblet cells, whereas MUC3 is largely confined to enterocytes (6). In the human large intestine MUC2 is the prominent mucin (34), whereas in the stomach, which also produces a copious mucin layer, MUC2 is hardly detectable (1, 19). In addition to MUC2 and MUC3, several other mucins, namely MUC1, MUC4, MUC5AC, MUC5B, and MUC6, are expressed to some extent in the intestine (1, 19, 41; see Refs. 40 and 42 for review). Interestingly, in the glands of the human stomach, MUC6 protein is also detectable (8), whereas MUC5AC protein seems restricted to the superficial epithelium of the stomach (5, 21, 22). Of the other known mucins MUC8 is not expressed in the small intestine and MUC7 expression seems restricted to the salivary glands (2, 28).

Alterations in intestinal mucins, especially in glycosylation patterns and expression levels, have been associated with diseases such as inflammatory bowel disease and carcinoma (3, 7, 13, 19, 35, 37, 39). Whether these alterations are the result or the cause of disease is presently unclear.

So far, studies of gastrointestinal mucins have focused on steady-state levels of mRNA and protein by means of in situ hybridization, Northern blotting, or immunohistochemistry. Little is known about the biosynthesis of gastrointestinal mucins: only the predominant human colonic and gastric mucins, which were
found to be identical to MUC2 and MUC5AC, respectively, have been studied (21, 22, 34). Our aim was therefore to study mucin biosynthesis along the longitudinal axis of the healthy human gastrointestinal tract by means of metabolic labeling of biopsies, immunoprecipitation, and polyacrylamide gel electrophoresis (PAGE). This information will be valuable in determining which mucin precursors are synthesized in different human gastrointestinal tissues and thus assigning functions to each of the mucins.

**MATERIALS AND METHODS**

\[^{35}S\]-labeled amino acids, \(^{35}S\)labeled sulfate, \(^{3}H\)galactose and Amplify were obtained from Amersham International (Buckinghamshire, UK). Eagle’s minimum essential medium was from Gibco-Life Technologies (Breda, The Netherlands); aprotinin, phenylmethylsulfonyl fluoride (PMSF), sodium dodecyl sulfate (SDS), pepstatin A, leupeptin, and inosacetylamide were all obtained from Sigma (St. Louis, MO). Igg molecular weight markers were obtained from Bio-Rad (Richmond, CA), protein A-Sepharose from Pharmacia (Uppsala, Sweden), Triton X-100 from BDH (Poole, UK), and mouse laminin from Becton Dickinson (Bedford, MA). Films were obtained from Kodak. Chemicals were also obtained from New England Biolabs (Beverly, MA), Merck (Darmstadt, Germany), and Boehringer Mannheim (Mannheim, Germany).

**Antibodies.** Rabbit polyclonal antisera were used in all experiments, except for one monoclonal antibody, WE9, which recognizes a peptide epitope in the unique terminals of human MUC2 (36). Anti-human colonic mucin was raised against purified human colonic mucin and recognizes the non-O-glycosylated terminals of the polypeptide of human MUC2 (34, 36). Anti-MRP and anti-M3P were raised against synthetic peptides representing the tandemly repeated amino acid sequences of MUC2 and MUC3, respectively (19). Anti-gastric mucin was raised against purified rat gastric mucin and recognizes the unique non-O-glycosylated terminal of the polypeptides of both rat and human gastric mucin (10, 22). By peptide and cDNA sequencing this human gastric mucin was identified as MUC5AC (21). An independent anti-MUC5AC antibody was raised against a synthetic peptide representing a non-O-glycosylated amino acid sequence of MUC5AC (21). The method used for MUC4 antibody production was previously described (37) and confirmed for the MUC61 antibody (C. de Bolós, A. Lopez-Ferrer, M. Garrido, and F. X. Real, unpublished observations). For a summary of the antibodies see Table 1.

**Tissues.** All samples were taken from healthy tissues: biopsies from stomach antrum, human duodenum (10–20 cm distal from the pylorus), ascending colon, transverse colon, and sigmoid colon (30 cm proximal to the anus) were obtained by endoscopy. Samples of healthy proximal jejunum (9 individuals) were obtained from patients undergoing a Whipple operation procedure for carcinoma of the pancreas or carcinoma of the papilla Vateri. Biopsies of the ascending colon (4 individuals), colon transversum (1 individual), and sigmoid colon (19 individuals) were taken from healthy tissue after polypectomy for non-polypoid colli, control for colon carcinoma, diverticulosis, irritable bowel syndrome, or constipation. Biopsies of the stomach antrum (3 individuals) and duodenum (17 individuals) were taken from otherwise healthy individuals with unexplained anemia, reflux esophagitis, or Barrett’s esophagitis or from individuals after successful eradication of Helicobacter pylori. All patients gave informed consent, and use of tissue was approved by the Medical Ethics Committee of the Academic Medical Center.

**Metabolic labeling and immunoprecipitation.** Mucin biosynthesis was studied by metabolic labeling with radiolabeled amino acids, sulfate, or galactose. Intracellular methionine/cysteine, sulfate, or galactose was depleted by preincubation for 30 min in Eagle’s minimum essential medium containing 1 g/l glucose (for methionine/cysteine and sulfate labeling) or 50 mg/l glucose (for galactose labeling), nonessential amino acids, penicillin, and streptomycin, but without methionine/cysteine, cysteine, or galactose, respectively. Biopsies were cultured at 5% CO\(_2\)/95% O\(_2\) at 37°C.

Pulse labeling was performed by adding either \[^{35}S\]-labeled amino acids (Cell Labeling Mix; sp act 1,000 Ci/mmol, containing 65% L-[\(^{35}S\)]methionine and 25% L-[\(^{35}S\)]cysteine) to label the polypeptides or by adding \[^{35}S\]sulfate (sp act 1,050 Ci/mmol) or \(^{3}H\)galactose (sp act 1–20 Ci/mmol) to label mature glycoproteins (9, 10). In experiments with intestinal biopsies, pulse labeling was performed for 30 min (\[^{35}S\]methionine/cysteine or \[^{35}S\]sulfate) or \(^{3}H\)galactose (sp act 1–20 Ci/mmol) to label mature glycoproteins (9, 10). In experiments with intestinal biopsies, pulse labeling was performed for 30 min (\[^{35}S\]methionine/cysteine or \[^{35}S\]sulfate) or \(^{3}H\)galactose (sp act 1–20 Ci/mmol) to label mature glycoproteins (9, 10). In experiments with intestinal biopsies, pulse labeling was performed for 30 min (\[^{35}S\]methionine/cysteine or \[^{35}S\]sulfate) or \(^{3}H\)galactose (sp act 1–20 Ci/mmol) to label mature glycoproteins (9, 10). In experiments with intestinal biopsies, pulse labeling was performed for 30 min (\[^{35}S\]methionine/cysteine or \[^{35}S\]sulfate) or \(^{3}H\)galactose (sp act 1–20 Ci/mmol) to label mature glycoproteins (9, 10). In experiments with intestinal biopsies, pulse labeling was performed for 30 min (\[^{35}S\]methionine/cysteine or \[^{35}S\]sulfate) or \(^{3}H\)galactose (sp act 1–20 Ci/mmol) to label mature glycoproteins (9, 10).

Pulse labeling was performed for 30 min in Eagle’s minimum essential medium containing 1 g/l glucose (for methionine/cysteine and sulfate labeling) or 50 mg/l glucose (for galactose labeling), nonessential amino acids, penicillin, and streptomycin, but without methionine/cysteine, sulfate, or galactose, respectively. Biopsies were cultured at 5% CO\(_2\)/95% O\(_2\) at 37°C.

Pulse labeling was performed for 30 min in Eagle’s minimum essential medium containing 1 g/l glucose (for methionine/cysteine and sulfate labeling) or 50 mg/l glucose (for galactose labeling), nonessential amino acids, penicillin, and streptomycin, but without methionine/cysteine, sulfate, or galactose, respectively. Biopsies were cultured at 5% CO\(_2\)/95% O\(_2\) at 37°C.

**Table 1. Antibodies used to immunoprecipitate gastrointestinal mucins**

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Name in Study</th>
<th>Original Name</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-O-glycosylated region of MUC2</td>
<td>Anti-MUC2(1)</td>
<td>WE9</td>
<td>36</td>
</tr>
<tr>
<td>Non-O-glycosylated region of MUC2</td>
<td>Anti-MUC2(2)</td>
<td>anti-HCM</td>
<td>34, 36</td>
</tr>
<tr>
<td>Tandem repeat region of MUC2</td>
<td>Anti-MUC2(3)</td>
<td>anti-MRP</td>
<td>19</td>
</tr>
<tr>
<td>Tandem repeat region of MUC2</td>
<td>Anti-MUC3</td>
<td>anti-M3P</td>
<td>19</td>
</tr>
<tr>
<td>Tandem repeat region of MUC2</td>
<td>Anti-MUC4</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>Non-O-glycosylated region of MUC5AC</td>
<td>Anti-MUC5AC(1)</td>
<td>anti-MUC5AC</td>
<td>4</td>
</tr>
<tr>
<td>Non-O-glycosylated region of MUC5AC</td>
<td>Anti-MUC5AC(2)</td>
<td>anti-RGM</td>
<td>10, 21, 22</td>
</tr>
<tr>
<td>Tandem repeat region of MUC6</td>
<td>Anti-MUC6</td>
<td>anti-MUC6.1</td>
<td>8</td>
</tr>
</tbody>
</table>

HCM, human colonic mucin; RGM, rat gastric mucin.
MUCIN BIOSYNTHESIS IN HUMAN STOMACH AND INTESTINE

Results

Gastrointestinal mucin precursors are synthesized region specifically and can be distinguished by SDS-PAGE. By pulse labeling with [35S]methionine/cysteine of human gastrointestinal biopsies and immunoprecipitations, we studied the biosynthesis of mucin precursors along the human gastrointestinal tract. For the purpose of this study, we define mucin precursors as mucin proteins that are N-glycosylated but not yet O-glycosylated and that are present in the rough endoplasmic reticulum, whereas mature mucins are the fully O-glycosylated end products of biosynthesis (29). Molecular masses of the mucin precursors that contain N-glycans presented in this study are represented as apparent molecular masses. Actual molecular masses may differ from apparent molecular masses on SDS-PAGE, since it is difficult to accurately estimate the molecular masses of these very large proteins on SDS-PAGE. Moreover, in very high molecular weight range there are very few molecular weight markers.

The antisera directed against a synthetic peptide of MUC5AC [anti-MUC5AC(1)] or against rat gastric mucin [anti-MUC5AC(2)] both detect a mucin precursor of similar apparent molecular mass in the stomach of the same individual, visible as a band of ~500 kDa on SDS-PAGE (Fig. 1A). Moreover, in the gastric homogenate of the same individual this mucin precursor is easily detected, suggesting that it is a predominant mucin biosynthesized in the stomach (Fig. 1A). In 3 of 3 individuals we detected MUC5AC precursors in the stomach (Table 2). MUC6 precursors were detected in the stomach in 3 of 3 individuals, with apparent molecular mass of 400 kDa (Fig. 1A). In the LS 174T cell line, a colon adenocarcinoma cell line, MUC6 precursors have similar apparent molecular mass as determined by SDS-PAGE (41). No MUC5AC or MUC6 precursors could be detected in the small or large intestine of different individuals (Fig. 1B and Table 2).

MUC2 precursors (apparent molecular mass of ~600 kDa) were immunoprecipitated by different anti-MUC2 antibodies from homogenates of duodenum, jejunum, and sigmoid colon (Fig. 1B). Similar results were obtained by the anti-MUC2 monoclonal antibody, anti-MUC2(1) (data not shown). In fact, MUC2 precursors were detected in both the small and large intestine of each of the individuals examined (Table 2). In some cases, MUC2 precursors could be detected as double bands on SDS-PAGE, for instance, in the jejunum of...
one individual (Fig. 1B). In 3 of 38 individuals we detected double bands for MUC2 precursors (Table 2).

In the duodenum of one individual and the jejunum of another, MUC3 precursors were detected as a double and a single band, respectively, at ~550 kDa (Fig. 1B). In the Caco-2 cell line, a colon adenocarcinoma cell line, MUC3 precursors displayed similar apparent molecular mass on SDS-PAGE (41). In the stomach and colon no MUC3 precursors were detected (Table 2). Interestingly, in one individual we found double bands of MUC3 precursors in two organs, the jejunum and the gallbladder (not shown). In 5 of 17 individuals we could show double bands of MUC3 precursors (Table 2). One individual displayed double bands for both MUC2 and MUC3 precursors (not shown).

In jejunum, MUC4 precursors were detectable, migrating halfway in the stacking gel on SDS-PAGE (Fig. 1B). MUC4 precursors were also immunoprecipitated from homogenates of the sigmad colon but not from the duodenum (Fig. 1B). Electrophoresis was performed for twice the period of time for analysis of MUC4 precursors in the sigmoid colon compared with analysis of MUC4 precursors in the jejunum (Fig. 1B), thus enabling MUC4 precursors to enter the running gel. The apparent molecular mass of MUC4 precursors was estimated at >900 kDa, because the M, appeared higher than that of mouse laminin (Mr, 900,000) on SDS-PAGE. We detected MUC4 precursor in the jejunum of one individual and in the sigmoid colon in 11 of 12 individuals, but not in the stomach or duodenum of different individuals (Table 2). In the ascending colon, however, only 1 of 3 individuals expressed MUC4, and in the transverse colon no MUC4 precursors were detected (Table 2).

Strikingly, no double bands were detected on SDS-PAGE for MUC4, MUC5AC, and MUC6 (Table 2).

Mobility on SDS-PAGE suggests that MUC2 is glycosylated differently in the small and large intestine. Intestinal biopsies were pulse labeled with radiola-beled amino acids, sulfate, or galactose and chase incubated. The biosynthesis of mature MUC3, MUC4, and MUC6 could not be studied, because the available antibodies against the respective mucins were directed against the tandemly repeated amino acid sequences, which become masked upon O-glycosylation. The biosynthesis of mature MUC5AC in the stomach has been documented previously (22). After pulse labeling with [35S]methionine/cysteine and immunoprecipitation with anti-MUC2 (2), MUC2 precursors were immunoprecipitated from the homogenate of the duodenum and detected at ~600 kDa on SDS-PAGE (Fig. 2A). This band became fainter after a 4-h chase incubation compared with the band after no chase incubation, due to conversion into mature fully O-glycosylated mucin. Mature MUC2 was secreted into the medium, visible as a smear just entering the stacking gel (Fig. 2A, lane m4). This indicates that the mature MUC2 synthesized in the duodenum has a very low mobility on SDS-PAGE. Similar results were obtained for the jejunum (not shown). After pulse labeling with [35S]methionine/cysteine, MUC2 precursor was immunoprecipitated from the sigmoid colon homogenate, visible as a band at 600 kDa on SDS-PAGE (Fig. 2B). After a 4-h chase incubation, a short smear was detectable just below the MUC2 precursor with a mobility of 550 kDa, which represents the mature fully glycosylated MUC2 that was immunoprecipitated from the tissue homogenate. This mature MUC2 was also secreted into the medium, whereas the MUC2 precursor was not secreted (Fig. 2B, lane m4).

To further study the biosynthesis of mature MUC2, we performed metabolic labeling experiments with [35S]sulfate or [3H]galactose, which are incorporated in the last steps of mucin biosynthesis, enabling the detection of mature mucins (9). Mature MUC2 was immunoprecipitated from the tissue homogenates of

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Table 2. Biosynthesis of MUC2–6 mucin precursors in the human gastrointestinal tract

<table>
<thead>
<tr>
<th>MUC2</th>
<th>MUC3</th>
<th>MUC4</th>
<th>MUC5AC</th>
<th>MUC6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach antrum</td>
<td>ND</td>
<td>0/3</td>
<td>0/1</td>
<td>3/3</td>
</tr>
<tr>
<td>Duodenum</td>
<td>12/12</td>
<td>1/9</td>
<td>0/4</td>
<td>0/6</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1/8</td>
<td>8/8</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Ascending colon</td>
<td>1/4</td>
<td>1/3</td>
<td>1/3</td>
<td>0/2</td>
</tr>
<tr>
<td>Transverse colon</td>
<td>1/3</td>
<td>1/3</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Sigmoid colon</td>
<td>13/13</td>
<td>1/3</td>
<td>0/1</td>
<td>0/1</td>
</tr>
</tbody>
</table>

Mucin precursors of MUC2, MUC3, MUC4, MUC5AC, or MUC6 detected in biopsies of stomach antrum, duodenum, jejenum, ascending colon, transverse colon, and sigmoid colon. For each mucin studied in each organ or intestinal region, the number of persons expressing the mucin precursor over the number of persons studied is shown. Values with asterisks indicate number of persons showing double bands for a particular mucin precursor; ND, not determined. For MUC4, MUC5AC, and MUC6, no double bands were found. All analyses were performed on healthy human tissues by metabolic labeling with [35S]-labeled amino acids, immunoprecipitation, and reducing SDS-polyacrylamide gel electrophoresis.
the duodenum: the mature MUC2, labeled with either [35S]sulfate or [3H]gala lactose, just entered the stacking gel and was also detected in the medium, displaying a very similar mobility (Fig. 3A). This indicates that mature secreted MUC2 has the same mobility on SDS-PAGE as was shown for the duodenum in Fig. 2, independent of its mode of labeling. In the ascending colon and sigmoid colon, [35S]sulfate-labeled mature MUC2 is detectable with a mobility of ~600 kDa on SDS-PAGE in both the tissue and media homogenates (Fig. 3, B and C), similar to the mobility of the mature MUC2 in the sigmoid colon after labeling with [35S]methionine/cysteine, as shown in Fig. 2. On SDS-PAGE mobility of mature MUC2 did not vary between ascending colon, transverse colon, and sigmoid colon (not shown). However, between small and large intestine a clear difference in mobility on SDS-PAGE was observed for mature MUC2 (Fig. 3), most likely due to a different glycosylation pattern. In the immunoprecipitated mature MUC2 from duodenum, an additional band can be observed at ~600 kDa (double arrows in Fig. 3A). These could be due to heterogeneity in glycosylation of MUC2 in the duodenum, where the 600-kDa band represents a small subpopulation of mature MUC2 that is similarly glycosylated as the mature MUC2 in the large intestine.

DISCUSSION

In this study we have demonstrated the biosynthesis of mucins in the gastrointestinal tract. With the use of metabolic labeling of gastrointestinal tissue, immunoprecipitation using mucin-specific antibodies, and analysis on SDS-PAGE we were able to discriminate between mucin precursors.

In the stomach, the detection of MUC5AC and MUC6 precursors was likely; high MUC5AC and MUC6 mRNA levels are reported for this organ (1, 5, 8, 20, 21, 32, 41). Klomps et al. (22) showed that antibodies directed against purified rat or human gastric mucin immunoprecipitated mucin precursors with apparent M, 500,000 from human gastric tissue (22), similar to our findings with antibodies directed against a synthetic peptide of MUC5AC. We conclude that these antibodies all recognize the same mucin, namely, MUC5AC. We could not detect MUC6 precursors in the duodenum. Ho et al. (20) showed, by immunohistochemistry, MUC6 protein in the Brunner’s glands, which are exclusively found in the duodenum. However, de Bolos and co-workers (8) did not detect MUC6 protein in the duodenum (8). In the present study most biopsies do not contain functional Brunner’s glands, possibly impeding the detection of MUC6. In the jejunum and colon no MUC6 precursors were detected, in accordance with immunohistochemical studies (20) and mRNA data (41).

We detected MUC3 precursors in small but not large intestine and MUC2 precursors in both small and large intestine. This was expected, because the MUC3 mRNA level is high in small intestine but low in large intestine, whereas the MUC2 mRNA level is high throughout the intestinal tract (1, 6, 19, 34, 41). In the stomach, no immunoprecipitations of MUC2 were performed, but MUC2 mRNA had previously been shown to be virtually absent in this organ (1, 5, 41). MUC4 precursors were detected in the colon, and studies have suggested that MUC4 is also an abundant colonic mucin: MUC4 mRNA levels are high in the colon, but low or nil in the jejunum and stomach (1, 37, 41). The mucin precursor MUC4, with apparent molecular mass >900 kDa, has the highest apparent molecular mass thus far identified. The biosynthesis of MUC1 was not studied; MUC1 has been shown to be expressed at low levels throughout the gastrointestinal tract and does not exhibit region-specific expression (5, 41).

Each mucin studied shows a typical pattern of expression in the gastrointestinal tract: MUC2 is expressed in the small and large intestine, whereas MUC3 expression was confined to the small intestine; MUC4 is biosynthesized typically in the distal colon, whereas MUC5AC and MUC6 proteins are only expressed in the stomach. Moreover, these results were consistent in almost all individuals (Table 2). In some individuals a mucin precursor may be deficient or synthesized at a very low level and may therefore escape detection. For instance, MUC4 precursor was not detected in the sigmoid colon of one individual.

Not much is known about the specific functions of each of the epithelial mucins. Mucins are generally thought to play a role in protecting the underlying epithelium from luminal noxae and mechanical stress (12, 29). However, the regional distribution of MUC2–6 in the gastrointestinal tract indicates specific functions for these mucins. For instance, the gastric mucins

![Fig. 3. Biosynthesis of MUC2 in duodenum (A), ascending colon (B), and sigmoid colon (C). Biopsies were pulse labeled for 1 h with [35S]sulfate ([38S]SO₄₃-) or [3H]galactose ([3H]Gal) and chase incubated with complete medium for 4 h and homogenized after 0- or 4-h chase incubation. In all experiments media were collected and homogenized after 4-h chase incubation, indicated by m4. Mucin was immunoprecipitated using anti-MUC2(2) antiserum. Analysis was on reducing SDS-PAGE, as in Fig. 1. Chase periods (h) are indicated. Double arrows are explained in text.](image-url)
MUC5AC and MUC6 could be particularly beneficial in protecting against the low pH in the stomach. Also, the mucins with the largest polypeptides, MUC4 and MUC2, are typically highly expressed in the distal colon and may form very large hydrated polymers, functioning as a lubricant to enable the passage of stool and/or as a protective layer against pathogenic organisms.

We have shown that at least two intestinal mucins, MUC2 and MUC3, displayed double precursor bands in some individuals, whereas no double bands for MUC4, MUC5AC, and MUC6 precursors were detected. A possible explanation could be that these double bands result from allelic variation of the respective genes. Due to the variable number of repeated amino acid sequences of mucins, allelic variation among mucins is common and was demonstrated at the genomic level for the MUC1 and MUC2 genes (30, 31) and at the protein level for rat gastric mucin and rat and human MUC2 (10, 33, 39). The frequencies of these double bands may be underestimated, because small variations in the sizes of the proteins encoded by the different alleles may not be distinguished by SDS-PAGE. Side-by-side analysis of mucin precursors of different individuals is another experimental alternative. This side-by-side analysis was previously performed for MUC2, showing MUC2 protein products with different apparent molecular mass and occasionally double bands on SDS-PAGE (39).

Using antibodies that are directed against the non-O-glycosylated epitope of MUC2, we were able to detect precursor and mature MUC2, and we were able to show intracellular processing of this mucin. This will also be possible for other mucins, such as MUC3, MUC4, and MUC6, when antibodies against the non-O-glycosylated regions become available. Previous studies have shown that mature MUC2 is synthesized and migrates at a position around 550-600 kDa on SDS-PAGE. Side-by-side analysis of mucin precursors of different individuals is another experimental alternative. This side-by-side analysis was previously performed for MUC2, showing MUC2 protein products with different apparent molecular mass and occasionally double bands on SDS-PAGE (39).

In conclusion, we have shown that mucin precursors of MUC2, MUC3, MUC4, MUC5AC, and MUC6 can be distinguished electrophoretically and exhibit region-specific expression in the gastrointestinal tract. Moreover, it seems likely that some forms of allelic variation of MUC2 and MUC3 can be detected through expression of double bands of precursors in metabolic labeling experiments. Differences in mobility of mature secreted MUC2 on SDS-PAGE between small and large intestine suggest differences in glycosylation of this mucin between these regions of the intestine.

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REFERENCES


MUCIN BIOSYNTHESIS IN HUMAN STOMACH AND INTESTINE


