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Characterization of Rat Gastric Mucins Using a Monoclonal Antibody, RGM23, Recognizing Surface Mucous Cell–Type Mucins

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A novel anti-mucin monoclonal antibody (mAb), designated RGM23, was developed against mucin purified from rat gastric mucosa. RGM23 reacted with the mucin attached to the ELISA well. The reactivity was lost by trypsin treatment, but not by periodate oxidation, indicating that RGM23 recognizes the peptide moiety of the mucin molecule. Histochemical study showed that RGM23 stained the corpus and antral surface mucosa of rat stomach, but not their glandular mucosa, nor duodenal, small intestinal or large intestinal mucosa. The area stained with RGM23 was coincident with that stained with 45M1, a mAb reacting with MUC5AC mucin. Examination of the mucin subunits extracted from rat stomach by Sepharose CL-4B and Q-Sepharose chromatography and CsTFA equilibrium centrifugation showed that RGM23 reacted with the surface mucous cell-type mucins that were stained with periodate-Schiff (PAS) and reacted with mAb RGM21. The gastric gland-type mucin, which reacted with mAb HIK1083, did not react with RGM23. On Q-Sepharose chromatography, a part of the RGM21-reactive mucins was only faintly stained with PAS and did not react with RGM23. The results together indicated that RGM23 probably reacted with the rat MUC5AC (rMuc5AC) mucin present in the surface mucosa of the stomach, and that the surface mucosal cells in rat stomach may contain mucin bearing non-rMuc5AC core protein in addition to rMuc5AC mucins.

Key words: gastric mucin, glandular mucosa, monoclonal antibody, rMuc5AC, surface mucosa.

Abbreviations: CsTFA, cesium trifluoroacetate; DTT, dithiothreitol; GuHCl, guanidinium hydrochloride; HRP, horseradish peroxidase; mAb, monoclonal antibody; PAS, periodate-Schiff; PMSF, phenylmethylsulphonyl fluoride; rMuc5AC, rat MUC5AC mucin.

Recent studies have shown that different types of mucin, differing in their carbohydrates and core protein structure, are expressed in different regions of the gastrointestinal tract. In the stomach, the corpus mucin differs from the antral mucin, and in each region the surface-type mucins (surface mucous cell-type mucins) differ from the gland-type mucins, synthesized in deeper layers of the gastric mucosa (*1*). Histochemical studies revealed that surface-type mucins have different carbohydrate chains from gland-type mucins in the stomach. For instance, surface-type mucins were stained by galactose oxidase-cold thionine Schiff (GOCTS) staining, while glandular mucins were stained by paradoxical concanavalin A staining (PCS) (*2*, *3*). On the other hand, studies using gene technology revealed that, in the stomach, the mucin bearing MUC5AC core protein was expressed in the surface mucosa, while MUC6 was expressed in the glandular mucosa (*4*–*7*).

Because gastric mucin has an important role in protecting the mucosa from gastric acid, pepsin and pathogens (*1*), the biochemical characterization of individual mucin molecules is important to understand their functions, and specific tools to recognize particular mucin species are essential. For these purposes, many monoclonal antibodies (mAbs) against mucins have been developed and used. In our previous report, mAbs RGM21 and HIK1083, which recognize a specific carbohydrate portion of rat gastric surface- and gland-type mucins, respectively (*8*, *9*), were used to characterize the different mucin molecular structures (*10*). Although these mAbs were highly useful for recognizing specific types of mucin, they do not provide full information on the core protein of the mucins: mAbs discriminating the specific site of mucin core peptides are still needed. Therefore, we tried to develop a new mAb recognizing the core protein of the surface-type mucin in rat stomach. The present paper describes the characterization of a novel mAb, RGM23, which probably reacts with the core protein of rat MUC5AC mucin (rMuc5AC). Use of this mAb indicated that the surface-type mucins in the corpus of the rat stomach consists of at least two molecular species: rMuc5AC, and another with a seemingly different core.

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MATERIALS AND METHODS

Materials—The PD-10 column, Q-Sepharose FF and Sepharose CL-2B and CL-4B were purchased from Pharmacia Biotech (Tokyo). Guanidinium hydrochloride (GuHCl, biochemical grade) and cesium trifluoroacetate (CsTFA) were purchased from Nacalai Tesque (Kyoto). The Centriprep 30 centrifugal concentrator was a product of Millipore (Bedford, USA). Monoclonal antibodies, RGM21 and HIK1083, were prepared as previously described (*11*). Monoclonal antibody, 45M1, was the product of Biogenesis (Poole, UK). Horseradish peroxidase (HRP)–conjugated goat anti-mouse antibodies were purchased from DAKO Japan (Kyoto). Microtiter plates (96-well) were products of Sumitomo Bakelite (Tokyo).

Preparation of Rat Gastric Mucins—Rat gastric mucins were extracted from the corpus mucosa of the stomach and purified as described previously (*10*). After the gel layer was removed (*12*), the corpus mucosa was scraped off with a plastic plate and homogenized in a glass homogenizer very gently by hand at 0°C in ice-cold 6 M GuHCl, pH 7.4, containing 2% (v/v) Triton X-100, 50 mM Tris, 10 mM EDTA, 0.1 M dithiothreitol (DTT), 2 mM benzamidine hydrochloride, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.15 mM pepstatin A. The homogenates were stirred for 15 h at 4°C and centrifuged at 8,000 ×*g* for 60 min. The supernatants, after alkylation with iodoacetamide, were loaded on a column (2.6 × 58 cm) of Sepharose CL-4B using 4 M GuHCl, pH 7.4, containing 0.5% Triton X-100, 50 mM Tris, 10 mM EDTA, 2 mM benzamidine hydrochloride and 1 mM PMSF as an eluent. Each fraction collected was assayed for periodate-Schiff (PAS)-stained materials as described previously (*13*) and for mAb-reactivity as described below. The fractions eluted at nearly void volume were pooled and applied to CsTFA equilibrium centrifugation. The centrifugation was performed at 152,000 × g for 120 h at 10°C with a starting density of 1.40 g/ml in CsTFA/0.4 M GuHCl.

Anion Exchange Chromatography of the Mucins—Q-Sepharose chromatography was carried out on a column (1.5 × 5 cm) equilibrated with 8 M urea containing 30 mM Tris-HCl, pH 7.4, and 0.5% Triton X-100. The samples in GuHCl solution were concentrated by Centriprep 30, then GuHCl was replaced with urea by PD-10 column chromatography. The samples were loaded on a column, washed with starting buffer, then eluted with a linear gradient of 28 mM to 130 mM NaCl in 8 M urea containing 30 mM Tris-HCl, pH 7.4, 0.5% Triton X-100. Fractions were collected and urea was removed by precipitating the mucins with 71% ethanol containing 1.0 g potassium acetate/liter at 4°C overnight. The precipitates were re-dissolved in 4 M GuHCl, pH 7.4, containing 0.01% Triton X-100 and 50 mM Tris, then assayed for mAbs-reactive mucins.

Antibody Production—Monoclonal antibody RGM23 was prepared using rat gastric mucins as antigen and typed as IgG1 as described previously (*11*). The medium of the cultured hybridoma was collected and used.

Enzyme Linked Immunosorbent Assay (ELISA)—Each well of a microtiter plate was coated with the samples in 0.05 M carbonate-bicarbonate buffer, pH 9.6, and kept for 2 days at 4°C. The wells were washed three times with 20 mM Tris-HCl, pH 7.4, containing 0.15 M NaCl and 0.02% Tween 20 (TBS-T), then blocked with 2% skimmed milk in TBS for 1 h at room temperature. After washing the wells, a solution of mAb was added and incubated for an additional 1 h. HRP-conjugated goat anti-mouse immunoglobulin was used as a second antibody, and ABTS-H_2O_2 solution was used for color development. The absorbancy at 405 nm was measured, and the tentative mucin concentration was calculated from a standard curve prepared using purified mucin.

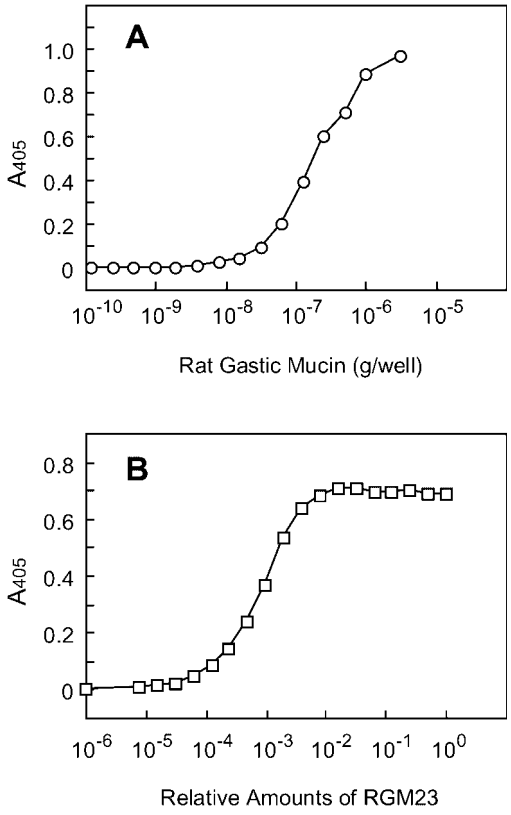

Fig. 1. **Reactivity of RGM23 with rat gastric mucin subunits.** The mucin subunits were extracted from the corpus of the rat gastric mucosa and purified by Sepharose CL-4B chromatography followed by CsTFA equilibrium centrifugation as described in Materials and Methods. The reactivity of RGM23 toward the mucin subunits was assayed by ELISA. (A) The reactivity of RGM23 to various amounts of the mucins. (B) Effects of antibody dilution from the original medium of RGM23-producing cells.

Periodate Treatment—Periodate treatment was performed by exposing the mucin antigen coated on the microtiter wells to 0.1 to 2.5 mM $NaIO_4$ in 50 mM sodium acetate, pH 4.5, for 1 h at room temperature.

Trypsin Digestion—Trypsin digestion was performed by exposing the mucin antigen coated on the microtiter wells to trypsin for 1 h at 37°C. 2.5 mg/ml trypsin in 10 mM Tris-HCl, pH 8.0, containing 2 mM $CaCl_2$ was used with two-fold serial dilution.

Histochemical Study—Tissues were fixed with 100% ethanol for 2 h at 0°C and embedded in paraffin. Thin sections on slide glass were treated in a microwave three times for 5 min each in 50 mM sodium citrate buffer, pH 6.0, in the case of RGM23 and 45M1 staining. Immunostaining of mucins was performed using DAKO LSAB2

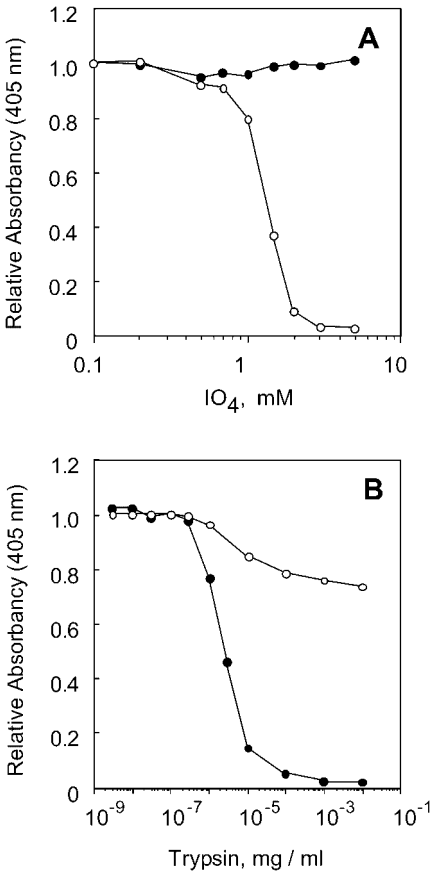

Fig. 2. **Effects of periodate and trypsin treatment of rat gastric mucins on the reactivity of RGM23 and RGM21.** The mucins coated on the ELISA plates were treated with various concentrations of periodate (A) or trypsin (B), and the reactivities of RGM23 (solid circles) or RGM21 (open circles) were assayed.

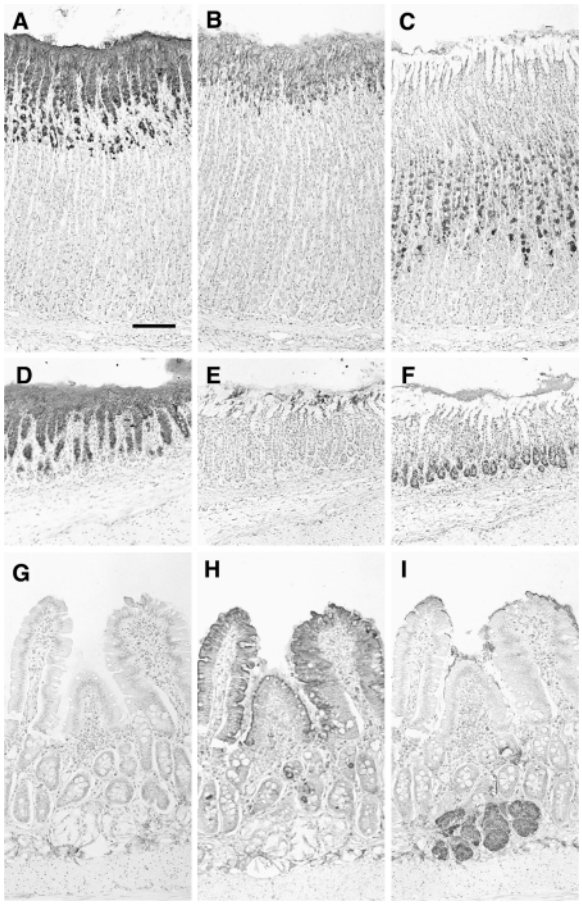

Fig. 3. **Histochemical staining of rat gasrointestinal tract.** The tissues were fixed with ethanol and then embedded in paraffin. Thin sections of the corpus of the stomach (A–C), the antrum of the stomach (D–F) and the duodenum (G–I) were stained immunohistochemically with RGM23 (A, D, G), RGM21 (B, E, H), or HIK1083 (C, F, I). The SAB system was used for staining, and counterstaining was performed with haematoxylin. The bar indicates 100 μm.

(HRP) kits according to the manufacturer's protocols with counterstaining using Meyer's Haematoxylin.

RESULTS

Epitope Specificity of RGM23—Monoclonal antibody RGM23 was prepared using rat gastric mucins as antigen. RGM23 reacted with the mucin subunits attached to the microtiter plate dose-dependently (Fig. 1). RGM23 also reacted with the whole mucin extracted from the rat corpus mucosa with 6 M GuHCl – 2% Triton X-100 not containing reducing agent (data not shown). To characterize the epitope of RGM23, the peptide and carbohydrate moieties of the purified mucin attached to the microtiter wells were degraded by trypsin digestion and periodate oxidation, respectively, and the residual antigenic activity was then tested by ELISA. Periodate treatment did not affect the reactivity of RGM23 in the range to 5 mM (Fig. 2A). In contrast, trypsin digestion destroyed the antigenic activity (Fig. 2B). The decrease in antigenicity was not exclusively due to the release of mucin molecules from the microtiter plate, because most of the antigenic activity against RGM21 still remained after the trypsin treatment. The results indicated that RGM23 recognized the peptide moiety of the mucin rather than the carbohydrate moiety.

Identification of the Target Molecules for RGM23 by Histochemical Examination—To survey the antigenic mucin distribution in the rat gastrointestinal mucosa, an immunohistochemical technique was applied to the ethanol-fixed sections of the rat gastrointestinal mucosa. As already established (*8*, *9*, *14*), RGM21 stained the corpus surface epithelium and duodenal villous epithelium (Fig. 3, B and H), and HIK1083 stained the corpus and antral gland epithelium and the duodenal Brunner's gland (Fig. 3, C, F, and I).

RGM23 stained the corpus and antral surface mucosa, but not their glandular mucosa nor duodenal mucosa (Fig. 3, A, D and G). RGM23 also showed a distinct pattern of immunohistochemical staining from RGM21: (a) RGM23 stained the lower part of the corpus surface mucosa, while RGM21 did not (Fig. 3, A and B); (b) RGM23 reacted strongly with the antral surface mucosa, while RGM21 did not (Fig. 3, D and E); (c) RGM23 did not react with any duodenal portion, while RGM21 reacted with the villus epithelial mucosa (Fig. 3, G and H). The

Table 1. **Immunohistochemical reactivity of RGM23 with normal rat gastrointestinal tissues.**

Tissue	Reactivity with RGM23
Esophagus	–
Stomach	
Corpus	
Surface epithelium	+
Mucous neck cell	–
Antrum	
Surface epithelium	+
Pyloric gland cell	–
Small intestine	
Duodenum	–
Jejunum	–
Ileum	–
Cecum	–
Large intestine	–

The ethanol-fixed paraffin-embedded sections were processed by the streptoavidin-biotin peroxidase method.

immunohistochemical reactivity of RGM23 was tested throughout the gastrointestinal tract of the rat. As summarized in Table 1, the antigen to RGM23 could be detected only in the surface epithelial layer of the corpus and antrum of the stomach.

These results suggested that RGM23 might recognize the core protein of rMuc5AC, because of the distribution of its gene expression (*5*). To test this idea, the staining patterns of RGM23 were compared with those of 45M1 antibody, which is known to recognize rMuc5AC in addition to human MUC5AC mucin (*15*, *16*). Figure 4 shows that the staining patterns of 45M1 and RGM23 were almost the same in both the corpus and antral mucosa, indicating that RGM23 probably recognizes rMuc5AC.

Identification of the Target Molecules for RGM23 in the Mucin Subunits Extracted from Rat Corpus—The mucins were extracted from the corpus of the rat stomach with buffered 6 M GuHCl containing 2% Triton X-100 and proteinase inhibitors in the presence of DTT and were chromatographed on a Sepharose CL-4B column. RGM23 reacted with macromolecules detected in the fractions included slightly in the column, which were co-eluted with PAS-positive materials, but did not react with any other included fractions such as the retarded PAS-positive peak, putatively small glycoproteins (Fig. 5A). Figure 5B shows that the elution profile of RGM23 was almost the same as that of RGM21 except in the relatively smaller fractions. RGM23 did not react with the glandular mucin detected by HIK1083, which was excluded from the column.

Figure 6 shows the results of CsTFA equilibrium centrifugation. RGM23-reactive mucin subunits showed a profile quite similar to those detected by PAS-staining and RGM21 but different from those detected by HIK1083.

On ion-exchange chromatography using a Q-Sepharose column, the mucins were divided into at least three fractions, UB, B1 and B2, depending on the reactivity with

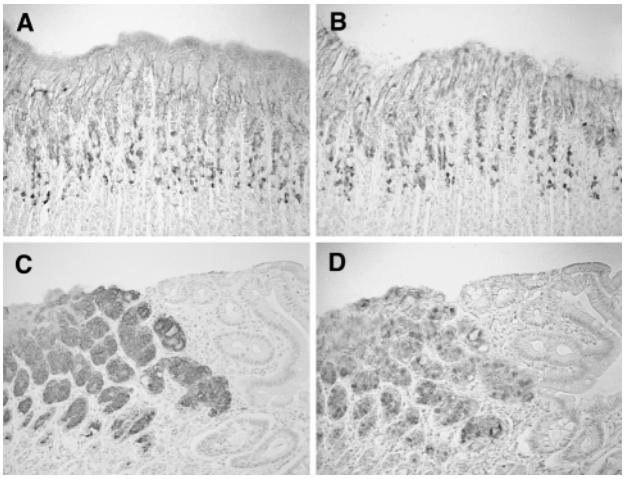

Fig. 4. **Comparison of the staining patterns of RGM23 and 45M1.** Semi-serial sections of ethanol-fixed tissues obtained from the corpus mucosa (A, B) or the border area of the antrum and duodenum (C, D) were immunostained using RGM23 (A and C) or 45M1 (B and D).

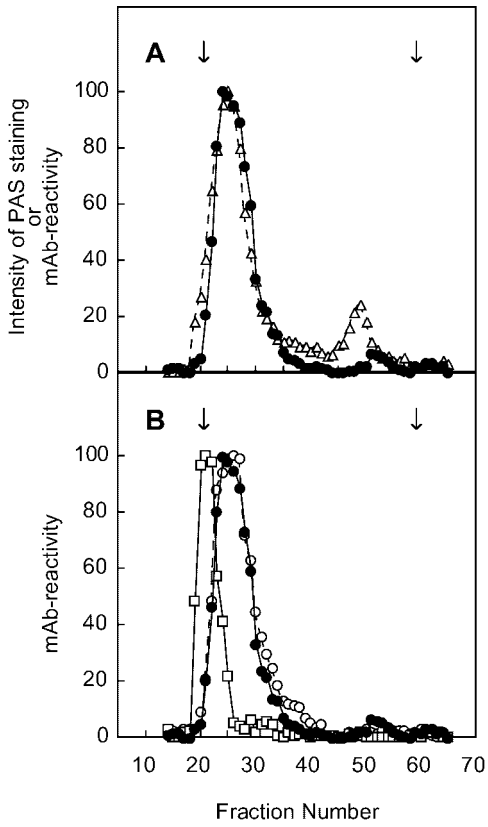

Fig. 5. **Sepharose CL-4B chromatography of the corpus mucin subunits of the rat stomach.** The corpus mucosa of rat stomach was extracted with buffered 6 M GuHCl, pH 7.4, containing 2% Triton X-100 and 0.1 M DTT as described in Materials and Methods. The extracts were loaded onto a column of Sepharose CL-4B, and each fraction was assayed for PAS-stained materials (open triangles with dashed line in A); RGM23- (solid circles in A and B), RGM21- (open circles with dashed line in B), and HIK1083- (open squares in B) reactive materials. Maximal reactivity of each mAb and maximal intensity of PAS staining were set as 100. The arrows indicate the voids and total volume of the column.

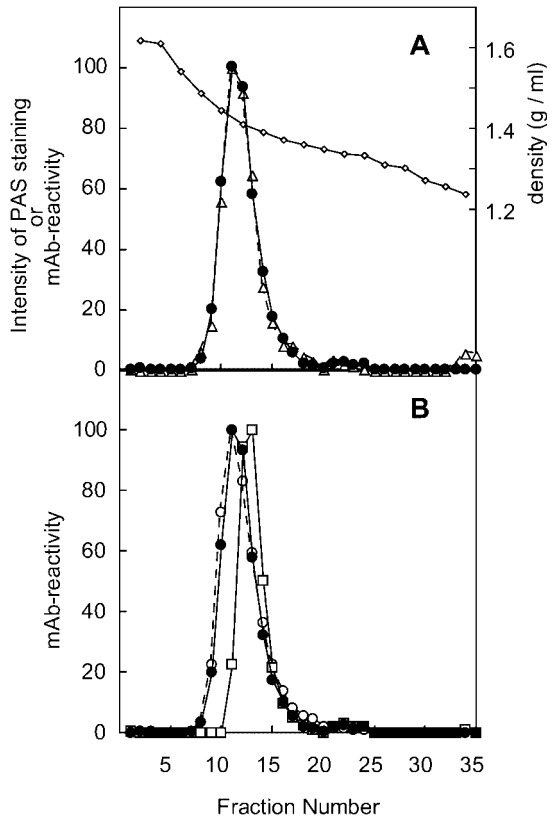

Fig. 6. **CsTFA equilibrium centrifugation of the corpus mucin subunits of the rat stomach.** The corpus mucin subunits eluted from a Sepharose CL-4B column were subjected to CsTFA equilibrium centrifugation. The initial density was adjusted to 1.40 g/ml in CsTFA/0.4 M GuHCl, and centrifugation was performed at 152,000 × *g* for 120 h at 10°C. Each fraction was assayed for density (diamonds), PAS-stained materials (open triangles with dashed line in A); RGM23- (solid circles in A and B), RGM21- (open circles with dashed line in B), and HIK1083- (open squares in B) reactive materials. Maximal reactivity of each mAb and maximal intensity of PAS staining were set as 100.

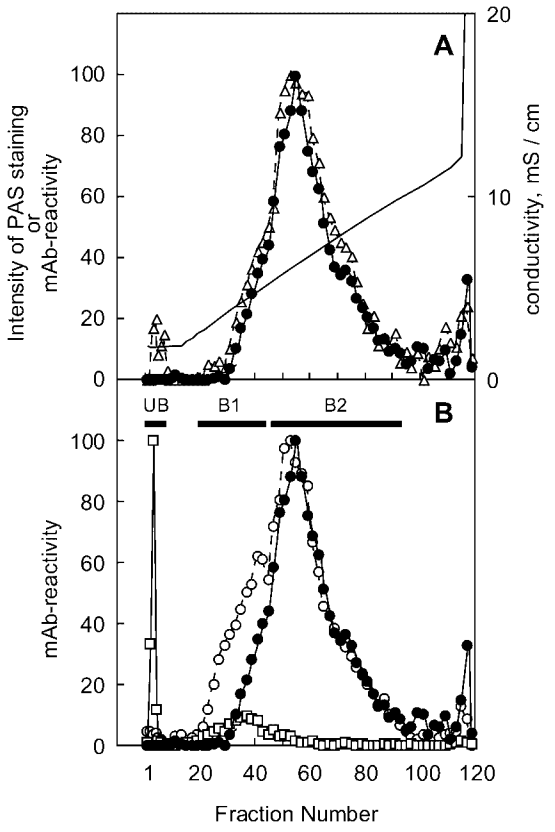

Fig. 7. **Q-Sepharose chromatography of the mucin subunits.** The mucin subunits were loaded onto a Q-Sepharose column equilibrated with 8 M urea containing 30 mM Tris-HCl, pH 7.4, and 0.5% Triton X-100 and eluted with the same solution followed by a salt-gradient. Each fraction was assayed for: conductivity (straight line in A), and PAS-stained materials (open triangles with dashed line in A); RGM23- (solid circles in A and B), RGM21- (open circles with dashed line in B), and HIK1083- (open squares in B) reactive materials. Maximal reactivity of each mAb and maximal intensity of PAS staining were set as 100.

mAbs (Fig. 7). The fraction that passed through the column, UB, reacted with HIK1083 but not with RGM21 or RGM23 and was therefore identified as a mucin derived from the glandular mucosa as described (*10*). The weakly bound fraction, B1, reacted mainly with RGM21, although this fraction also contained substances reacting with HIK1083 or RGM23. The strongly bound fraction, B2, reacted with RGM21 and RGM23 but not with HIK1083; therefore, this fraction seemed to comprise components of the surface-type mucins. This fraction was stained by PAS and might consist of two components with different degrees of sulfation as described (*10*).

The B1 fraction was divided into two components on Sepharose CL-2B chromatography (Fig. 8): one was excluded from the column and was recognized by HIK1083, the other was included in the column and reacted with both RGM21 and RGM23. The peak recognized by RGM23 was slightly larger than that recognized by RGM21. The results indicated the B1 fraction to be a mixture of three mucin components: The first peak recognized by HIK1083 was derived from the glandular mucosa but was slightly more acidic than the UB. A small amount of HIK1083-reactive mucin in the second peak might also be derived from the glandular mucosa but be partially degraded. The second peak was recognized by RGM23, and was derived from the surface mucosa. The last peak, recognized by RGM21 but not by RGM23, was derived from the surface mucosa and might have a different core from the mucin recovered in B2 mucin fractions.

DISCUSSION

The results of the present study indicated that the newly developed mAb, RGM23, probably recognized the peptide core of rMuc5AC, for the following reasons. First, the reactivity of RGM23 with rat gastric mucins was lost on trypsin treatment, while the reactivity of RGM21 with rat gastric mucins was only slightly lost under the same conditions. The results indicated that this loss of RGM23 reactivity was not due to the release of mucin from the plate. In contrast, the reactivity of RGM23 was not lost by periodate treatment under the conditions where the reactivity of RGM21 was completely lost. Because RGM21 seems to recognize the carbohydrate chains of mucin (*8*), the results indicated that RGM23 recognized

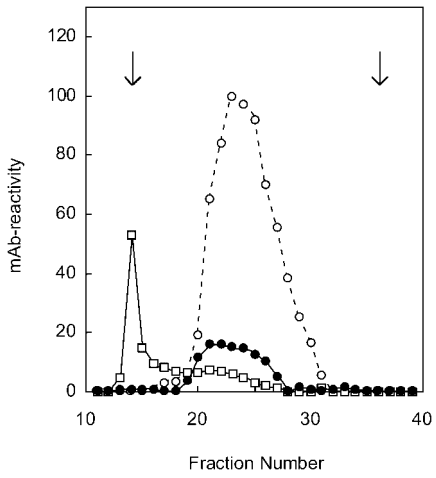

Fig. 8. **Sepharose CL-2B chromatography of the B1 fraction.** The B1 fraction in Fig. 7 was loaded onto a column of Sepharose CL-2B equilibrated with 4 M GuHCl, pH 7.4, containing 0.5% Triton X-100, 50 mM Tris, 10 mM EDTA, 2 mM benzamidine hydrochloride, and 1 mM PMSF. Aliquots of each fraction were assayed for RGM23- (solid circles), RGM21- (open circles with dashed line), and HIK1083- (open squares) reactive materials. Data in RGM21-reactivity are indicated as the percent of the maximal reactivity, while the data of RGM23- and HIK1083-reactivity represent the relative amount of mAb-reactive materials of B1 fraction in Fig. 7. The arrows indicate the voids and total volume of the column.

the protein portion of the mucins rather than the carbohydrate chains. Second, RGM23 stained the surface mucosa but not the glandular mucosa of both the corpus and antrum in an ethanol-fixed paraffin-embedded section of the stomach. It was reported that MUC5AC was expressed in the surface mucosa of the stomach but not in the glandular mucosa, while MUC6 was expressed in the glandular mucosa (*7*). Furthermore, RGM23 did not stain the mucosa of the esophagus, duodenum, jejunum, ileum, cecum or colon. These mucosa do not contain MUC5AC mucin under normal conditions. Third, the immunohistochemical profile of the rat gastric mucosa stained by RGM23 was quite similar to that stained by 45M1. Bara and co-workers reported that 45M1 recognized the core protein of MUC5AC and that it also recognized rMuc5AC (*15*, *16*). More direct evidence that RGM23 recognizes rMuc5AC may be obtained by examining the reactivity of the synthetic core protein of rMuc5AC with RGM23. The determination of the complete amino acid sequence of rMuc5AC may make this possible in the near future.

Both RGM23 and 45M1 reacted with the rat gastric mucins and immunostained the rat gastric mucosa similarly. However, the two mAbs differ significantly in that RGM23 reacts with the mucin subunits as well as whole mucin, while 45M1 does not react with the subunits but with whole mucin (*16*). The reason for this difference is unknown, but RGM23 and 45M1 may recognize respectively the primary and the higher order structure of the peptide moiety. Reduction of disulfide bonds in the core protein of the mucin destroys the higher order structure and therefore may decrease the reactivity of 45M1 toward the mucin subunit (*15*). That RGM23 could react with the mucin subunits in addition to the whole mucin molecule is a great advantage for biochemical characterization of the mucin. Immunochemical analyses of the mucin molecule in the subunit form are essential for chromatographies as well as equilibrium centrifugation or velocity sedimentation to characterize the mucin (*10*). In particular, only the subunits are suitable for the analysis of the electrical properties of the mucin by ion-exchange chromatography, because whole mucin does not produce satisfactory results, due to its very low recovery from the column (*10*).

Evidence about the site of the mucin core protein where RGM23 reacts is limited. However, RGM23 may recognize a non-tandem repeat portion of rMuc5AC, especially the C-terminal domain, because RGM23 cross-reacted with human gastric mucin (Goso, Ikezawa, and Ishihara, unpublished observation); that is, RGM23 stained the surface mucosa of the human stomach histochemically and RGM23 reacted with mucins obtained from the human stomach on ELISA plates. It has been reported that the C-terminal domain was highly conserved between rat and human MUC5AC, although no common sequence was observed in the tandem repeat region (*17*).

The present study indicated that the mucin recognized by RGM23, probably rMuc5AC, differs from that recognized by HIK1083 in its size, density and charge. Because the mucin recognized by HIK1083 is strongly indicated to be MUC6 (*10*, *18*), the results of the present study indicate that rMuc6 is larger and has a lower charge than rMuc5AC, as already suggested (*10*). Similar differences in the size between MUC5AC and MUC6 mucins have also been reported in porcine gastric mucin (*19*). Most recently, Nordman *et al.* reported that MUC5AC and MUC6 in human gastric mucosa have different sizes, similarly to rat gastric mucins (*20*). Although the density and charge profiles of the mucins seem to differ with the species, MUC6 may generally be larger than MUC5AC.

Although both RGM21 and RGM23 are thought to recognize rMuc5AC in the gastric mucosa, they differ in the immunostaining pattern of the gastric mucosa: RGM23 stained the antral surface mucosa but RGM21 did not. This means that rMuc5AC in the antral mucosa has different carbohydrate chains from that in the corpus mucosa. Furthermore, in the corpus surface mucosa, RGM23 stained the pit portion but RGM21 did not. This also means that rMuc5AC in the pit portion has different carbohydrate chains than the rMuc5AC which occupies most of the corpus surface mucosa. It has been reported that the gastric stem cells in the corpus region are present in the pit region. Each rMuc5AC mucin having characteristic carbohydrate chains in the surface mucosa of the stomach may possess a site-specific function.

Our previous study showed that the major component of the B1 mucin on Q-Sepharose chromatography might have a core protein different from that of the B2 fraction (divided into two fractions, B2a and B2b, in our previous report) based on their amino acid composition (*10*). The present study supported this hypothesis, because B1 contained a fraction which was recognized by RGM21 but not by RGM23. Alternatively, the B1 mucin might be a partially degraded form of B2 that had lost its reactivity with RGM23. However, this is not likely, because the carbohydrate chains of B1 and B2 mucin might be different; B2 was stained intensely with PAS, while B1 was stained

only faintly. The loss of the reactivity of B1 with RGM23 might be explained by a modification of the rMuc5AC core protein, such as further glycosylation. However, this idea cannot explain the different amino acid compositions of B1 and B2. If B1 contained a mucin other than rMuc5AC, this poses a question about which mucin was expressed. Although more than 15 mucin genes have been reported, only 4 of them, MUC2, 5AC, 5B, and 6, have been considered to be gel-forming mucins (*1*, *21*). If B1 contains a gel-forming mucin other than rMuc5AC or rMuc6, it is possibly rMuc5B, because MUC2 is reported not to be expressed in the normal stomach. Further study is needed to clarify these points.

The present study revealed that distinct types of mucin molecules having a different carbohydrate chain and/or core protein are distributed in specific regions and layers of the gastric mucosa. Although the meaning of the presence of the particular mucin components in the gastric mucosa is still unclear, evidence which can elucidate these phenomena has been accumulating in recent studies. For example, a gastric pathogen, *Helicobacter pylori*, was located on the surface mucous cells, which produced MUC5AC mucin, and also in the excreted mucus gel-layer composed mainly of MUC5AC (*22*, *23*). This bacterium, however, does not locate in the gel-layer rich in MUC6 type mucin. This phenomenon may indicate that different gastric mucin species provide different environments for the growth and survival of specific pathogens such as *Helicobacter pylori*. Other results suggesting the significance of the carbohydrate moiety of gastric mucins were obtained from our laboratory. During the recovery of rat gastric mucosa from chronic gastric mucosal injury induced by acetic acid ingestion, a specific mucin having intestinal mucin-type carbohydrate chains attached to the rMuc5AC core protein appeared in the regenerating epithelial mucosa (*24*).

The combined use of two types of mAbs, one recognizing carbohydrate chains and the other recognizing the core protein, as mentioned in this paper, will serve to clarify the physiological and pathological meaning of the mucin in the gastric mucosa.

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