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Size Exclusion Chromatography as a Tool for Evaluation of Fragmentation Pattern of Gastric Mucins under Non-degrading Conditions

Kučerová Z.[1], Muselová H.[1], Miarková E.[1], Tichá M.[1,2]
[1]Charles University in Prague, First Faculty of Medicine, Institute of Pathological Physiology and Centrum for Experimental Haematology, Prague, Czech Republic; [2]Charles University in Prague, Faculty of Science, Department of Biochemistry, Prague, Czech Republic

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Abstract: Gastric mucins are high molecular weight extracellular glycoproteins that play a major role in the protection of the gastrointestinal tract and besides that they are also involved in many disease processes. In the present study, size exclusion chromatography under non-degrading conditions was used to study the fragmentation pattern of native gastric mucins. The samples of gastric mucins of different origin obtained by an extraction of gastric mucosa with Tris-HCl buffer, pH 7.3 were separated using size exclusion chromatography on Sephadex G-100. While samples of rat gastric mucins are characterized by the presence of only high-molecular weight fraction of glycoproteins, fragmented mucin components in non-denaturated samples were observed in canine and human gastric mucins. Differences in the fragmentation pattern were observed in patients with ulcer diseases and gastric cancer. Degradation products of mucins were also detected using polyacrylamide gel electrophoresis in the presence of SDS.

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Mailing Address: Ing. Zdenka Kučerová, PhD., Charles University in Prague, First Faculty of Medicine, Institute of Pathological Physiology and Centrum for Experimental Haematology, U Nemocnice 5, 128 53 Prague 2, Czech Republic; Phone: +420 224 965 929; Fax: +420 224 912 834; e-mail: zkuce@lf1.cuni.cz

Introduction

Gastric mucins are high molecular weight extracellular proteins that are present in the mucosal secretions covering epithelial cell surface. Mucin glycoproteins present in the mucosal secretions covering epithelial cell surfaces are primarily responsible for the protective properties of mucous barrier (Bansil et al., 1995). They are supposed to play a major role in the protection of the gastrointestinal tract from e.g. acid, proteases, pathogenic microorganisms, or mechanical trauma. The key to the protective function of gastric mucin lies in its ability to change from a viscous solution at neutral pH to a gel when in an acidic environment (below a pH of about 4), thus effectively providing a barrier.

Gastric mucins are highly glycosylated containing up to 85% saccharides (mostly O-glycosidically linked). These substances are characterized not only by a high degree of glycosylation and a high molecular weight, but also by their polydispersity, by the ability to form aggregates also with other substances and by conformational changes induced by different factors, such as pH, ionic strength, their concentration in the solution, etc. Mechanism of pH dependent conformational changes of gastric mucins leading to sol-gel transitions and their gelation (at pH below pH 4.0) is not fully understood, even though this phenomenon was investigated in detail (Jumel et al., 1996; Cao et al., 1999; Bansil and Turner, 2006; Maleki et al., 2008). The physical state of the gastric mucus, change in the concentration of secreted mucin, and the strong dependence of its physicochemical properties on environmental factors such as ionic strength and pH play an important role in many diseases (Baldus et al., 2004; Baldus and Hanisch, 2008; Mall, 2008; Yonezawa et al., 2008). The presence of fragmented components of gastric mucin was observed in the case of gastric juice of patients with ulceration, carcinoma and Menetrier disease (Mall et al., 1999, 2000, 2002). In these cases, samples purified under denaturing conditions were analyzed (after extraction in a medium containing 6 M guanidinium chloride and fractionation by caesium chloride density gradient ultracentrifugation).

Similarly, structural studies on gastric mucins were mostly performed using separated glycoprotein subunits or glycoproteins isolated after demodulation or degradation of extracted material.

Size exclusion chromatography (SEC) represents one of the primary analytical tools used for characterization of the content and size distribution of protein aggregates in proteomic research. An availability of various porous materials differing in the fractionation range as well as the possibility of the application of different separation conditions and simplicity of the separation process belong to advantageous features of this method. In the present study, we have compared the presence of fragmented components of gastric mucins of different origin obtained from gastric mucosa without a use of denaturating or degrading conditions. The following gastric mucins were tested: dog, rat, and human samples from patients with different gastric diseases. The size exclusion chromatography on Sephadex

G-100 column of native samples of gastric mucins represents a simple method to indicate the presence of fragmented components of gastric mucins.

Material and Methods

Samples of human gastric mucosa were obtained from resected stomachs of patients with gastric diseases (11 patients with carcinoma and 11 patients with ulceration) from Charles University in Prague, First Faculty of Medicine, First Department of Surgery. Rat (10 animals) and canine (12 dogs) mucosa were obtained from resected stomach of animals at Charles University in Prague, First Faculty of Medicine, Institute of Pathological Physiology. The obtained material was long-term stored at –18 °C.

Samples of dissected mucosa were homogenized in 0.1 M Tris-HCl buffer, pH 7.3 (1 g/4 ml) and centrifuged at 13,000 rpm for 1 h at 4 °C. The obtained supernatants were used for further analyses.

Size exclusion chromatography

Samples of extracted mucins (2 ml) were applied to the Sephadex G-100 column (21 × 1.5 cm) equilibrated with 0.02 M Tris-HCl buffer, pH 7.3 and separated at the flow rate of 0.5 ml/min. In obtained fractions (1.5 ml), the content of proteins (absorbance at 280 nm) and saccharides (using the orcinol reagent (Ashwell, 1957), absorbance measured at 562 nm) was determined. Total time of the analysis was 120 min. For the electrophoretic separation, eluted glycoprotein peaks were pooled and concentrated (50 times) using Minicon CS15 (Millipore, US); molecular weight cut-off of the Minicon unit is 15,000. Reproducibility of the used method was examined in the case of canine mucin (10 times the same sample).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out on 15% slab gels (Laemmli, 1970). Non-reduced samples of mucins obtained by an extraction of gastric mucosa and reduced molecular weight protein standards were applied. The relative molecular weights of the separated glycoprotein fractions were estimated using molecular weight standards (STD DualColor, BioRad) run in parallel. Separated glycoproteins were detected by the periodic acid-Schiff base method using acidic fuchsine dye (Zacharius et al., 1969).

Results and Discussion

Preparation of mucin samples

Samples of studied human mucins were obtained from resected stomachs of patients with carcinoma and ulceration. In the first case, histological examination confirmed the presence of infiltrative adenocarcinoma of the stomach partially of a solid structure; in the second one, histological investigation of duodenal ulcer showed the chronic atrophic gastritis.

Non-denaturating medium was used for the mucosa extraction to obtain intact samples of mucins. Samples of gastric mucins were prepared by an extraction of gastric mucosa of different origin (human, rat, dog) at neutral pH under conditions that prevent an endogenous proteolytic cleavage of the studied material.

Size exclusion chromatography of gastric mucins

Size exclusion chromatography (SEC) on Sephadex G-100 was used to study the aggregation state of glycoproteins obtained by an extraction of gastric mucosa of different origin. The course of size exclusion chromatography was followed up by the determination of protein and saccharide content in eluted fractions and is presented in Figures 1 and 2. The comparison of the saccharide and protein content in fractions obtained in the separation of mucins of different origin showed

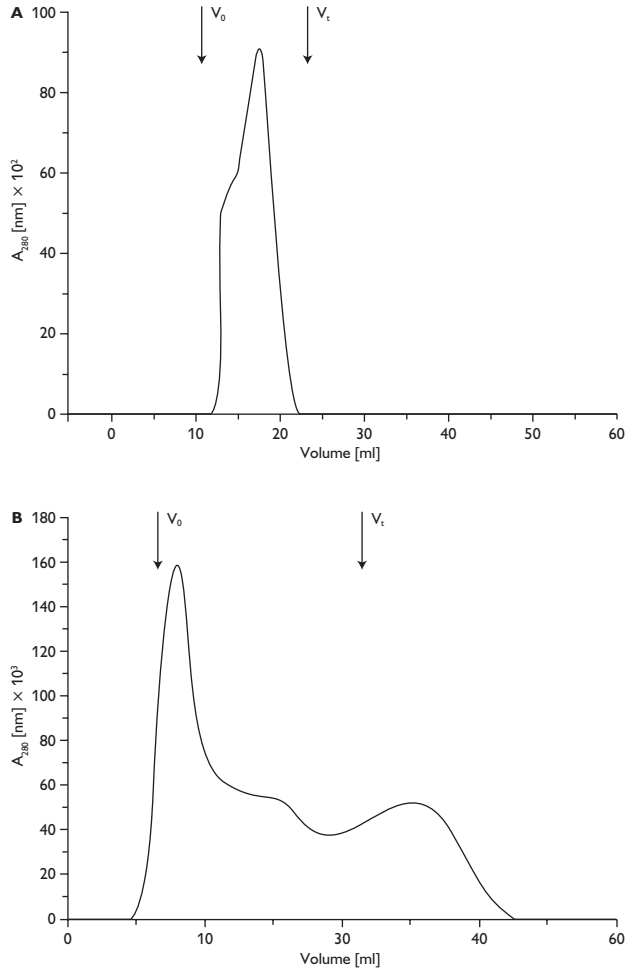

Figure 1 – Size exclusion chromatography of canine (A) and rat (B) gastric mucin on Sephadex G-100 column. Conditions of chromatography: Sephadex G-100 column (21 × 1.5 cm) equilibrated with 0.02 M Tris-HCl buffer, pH 7.3; flow rate: 0.5 ml/min; protein content: absorbance at 280 nm (—); saccharide content determined using the orcinol reagent (Ashwell, 1957): absorbance at 562 nm (…).

that a distribution of saccharide and peptide components in dependence on their rel. molecular weights did not differ (Figures 1 and 2). However, the studied gastric mucins differ in the presence of low molecular weight components in native samples of non-treated mucins.

Size exclusion chromatographic profiles obtained in experiment with mucins of different origin indicated that a distribution of glycoprotein components of gastric mucins depending on their size was different in samples of the examined species. Samples of rat gastric mucins are characterized by the presence of only high-molecular weight fraction of glycoproteins that were eluted in the void volume of the used Sephadex G-100 column (Figure 1). On the other hand, canine and esp. human mucin samples contained a portion of components being eluted in the in a volume close to V_t (total volume of the used sorbent column) (Figure 2). In

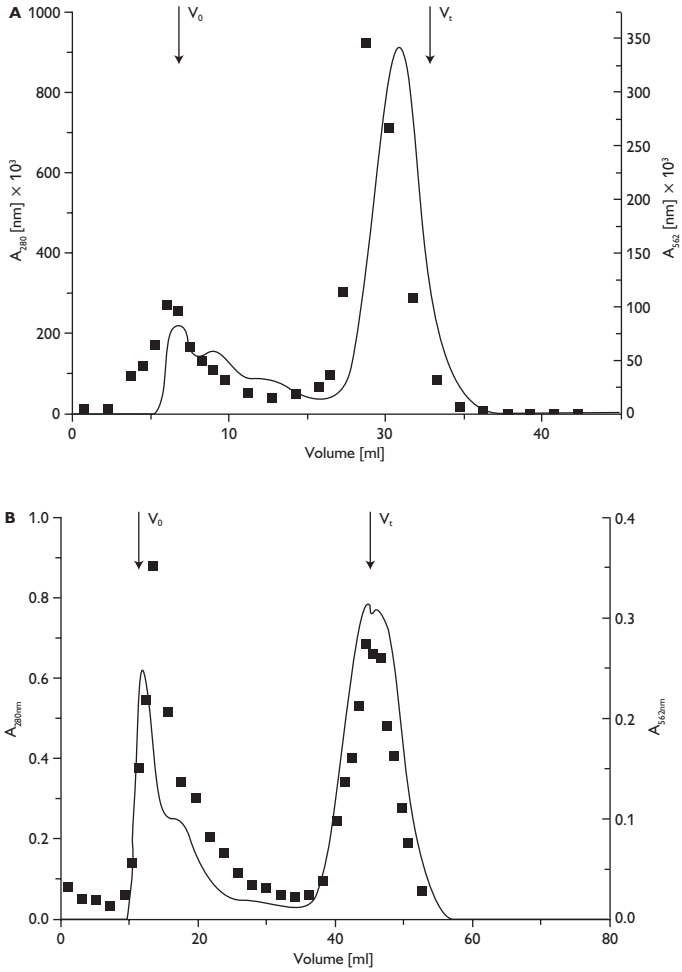

Figure 2 – Size exclusion chromatography of human gastric mucin on Sephadex G-100: (A) a patient with gastric cancer, (B) a patient with ulcer disease. Conditions of chromatography: Sephadex G-100 column (21 × 1.5 cm) equilibrated with 0.02 M Tris-HCl buffer, pH 7.3; flow rate: 0.5 ml/min; protein content: absorbance at 280 nm (—); saccharide content determined using the orcinol reagent (Ashwell, 1957): absorbance at 562 nm (…).

the rat and canine samples of gastric mucins, the high molecular weight fraction predominated, but in the tested human samples, it formed only about 20–40% of all proteins (as approximately evaluated from the area of peaks of the size exclusion chromatography profile). In the case of human gastric mucins, a portion of the glycoprotein fraction with lower rel. molecular weight significantly differed in samples obtained from patients with ulcer diseases and gastric cancer (Figure 2). Reproducibility of results of size exclusion chromatographic separation was tested in the case of canine mucin: the elution profile did not significantly change in 10 repeated analyses.

Results of size exclusion chromatography of extracts of non-denaturated gastric mucins without preliminary fractionation have shown that this approach represents a simple tool for an evaluation of the presence of fragmented mucin components. The obtained results are in an agreement with data described by Mall et al. (2000, 2002) who tested gastric mucins after their extraction under denaturating conditions and fractionation in a CsCl gradient.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of gastric mucins

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was used to analyze non-reduced mucin samples of different origin. Glycoprotein components of tested samples were detected using periodic acid-Schiff base method and acidic fuchsine dye (Zacharius et al., 1969). Similarly as in the case of size exclusion chromatography experiments described above, low molecular weight fragments were also detected in samples of gastric mucins obtained from patients with gastric diseases after electrophoretic separation in the presence of SDS (Figure 3). Degradation products of gastric mucin of patients with gastric carcinoma revealed using SDS polyacrylamide gel electrophoresis were also shown by Mall et al. (1999). However, no such fragments were observed in the case of electrophoretic separation of rat and canine mucins (Figure 3).

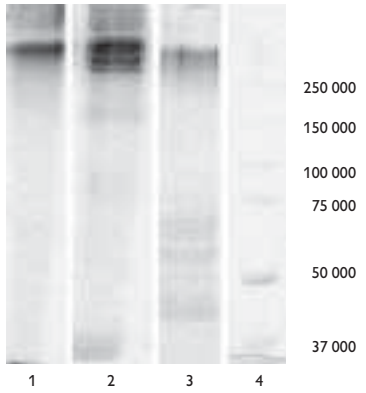

Figure 3 – SDS polyacrylamide gel electrophoresis of gastric mucins of different origin: 1 rat; 2 canine; 3 human; 4 molecular weight standards (STD DualColor, BioRad); non-reducing conditions of electrophoresis; detection: periodic acid-Schiff base method using acidic fuchsine dye (Zacharius et al., 1969).

Conclusion

Size exclusion chromatography of extracts of native samples of gastric mucosa obtained under non-degrading conditions at neutral pH represents a simple method for an evaluation of the fragmentation pattern of gastric mucins.

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