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Are chitosan–mucin interactions specific to different regions of the stomach? Velocity ultracentrifugation offers a clue

M.P. Deacon[a,*], S.S. Davis[c], R.J. White[d], H. Nordman[e], I. Carlstedt[e], N. Errington[b], A.J. Rowe[b], S.E. Harding[a]

[a]*National Centre for Macromolecular Hydrodynamics, School of Biological Sciences, University of Nottingham, Sutton Bonington LE12 5RD, UK*
[b]*Department of Biochemistry, University of Leicester, Leicester LE1 7RH, UK*
[c]*School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, UK*
[d]*Optokem Ltd., Mold CH7 4EW, UK*
[e]*Department. Cell and Molecular Biology, Section for Molecular Pathogenesis, Lund University, PO Box 94, S-221 00Lund, Sweden.*

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Abstract

Previous work has shown strong interactions between pig gastric mucins and a highly deacetylated chitosan. Recently, mucins purified from different areas of the porcine stomach have been shown to differ in terms of their oligosaccharide substitution and net charge. How this regional variation may affect the properties of these mucins is of great interest in terms of the specificity of mucoadhesion with chitosan. We have investigated the interaction of a chitosan of degree of acetylation $F_A \approx 0.11$ with three different mucins purified from different areas of the porcine stomach (cardia region, corpus and antrum) at two different ionic strengths. Using sedimentation velocity in the analytical ultracentrifuge equipped with a schlieren optical system coupled on line to a CCD camera, the amount of chitosan interacting with mucin was determined. The degree of interaction varies between the three mucins with those from the cardiac region displaying the highest degree of interaction; in the case of the corpus and antrum, however, the interaction increases with increase in ionic strength, implying that the interaction between mucins and chitosans may have a hydrophobic as well as an electrostatic basis.

Keywords: Chitosan; Mucin; Degree of acetylation

1. Introduction

Mucus glycoproteins (mucins) are the major macromolecular constituent of mucus, the secretion that lines the gastrointestinal, respiratory and reproductive tracts, constituting 0.5%–5% by weight (Harding, 1989; Fogg et al., 1996). These mucins are chiefly responsible for the characteristic viscoelastic and gel forming properties of the mucus, essential to its protective role. Although the molecular architecture of mucins from a wide variety of sources appear to follow the same structural pattern (Sheehan et al., 1986, Sheehan and Carlstedt, 1989) of heavily glycosylated (~80%) polypeptide subunits of molecular weight 2–3 million Da linked by disulphide bridges into a linear random coil array, the composition of the mucin molecules themselves varies according to their location in the body. Recently, mucins from different regions of the porcine stomach have been shown to differ in terms of their oligosaccharide substitution and net charge (Nordman et al., 1997; Karlsson et al., 1997). How this may affect the properties of these mucins may be of considerable relevance in terms of mucoadhesion for the oral administration of drugs (Fiebrig et al., 1995a). There is, for example, considerable interest in chitosan, a deacetylated form of chitin, as a possible mucoadhesive and previous work has shown a strong interaction between pig gastric mucins and the chitosan SC210 + a highly characterised chitosan with a degree of acetylation (F_A) ≈ 0.11 (Fiebrig et al., 1994a; Fiebrig et al., 1994b, Fiebrig et al., 1997). This interaction appears to be chiefly electrostatic in origin.

In this paper we describe a study of the interaction of the SC210 + chitosan with four different pig gastric mucin populations: one ''general'' pig gastric mucin, not particular to any specific region and three others purified from three different areas of the porcine stomach (cardia region, corpus and antrum) at two ionic strengths in acetate buffer. Using sedimentation velocity analytical ultracentrifugation with sedimentation diagrams recorded directly on-line using a

* Corresponding author.

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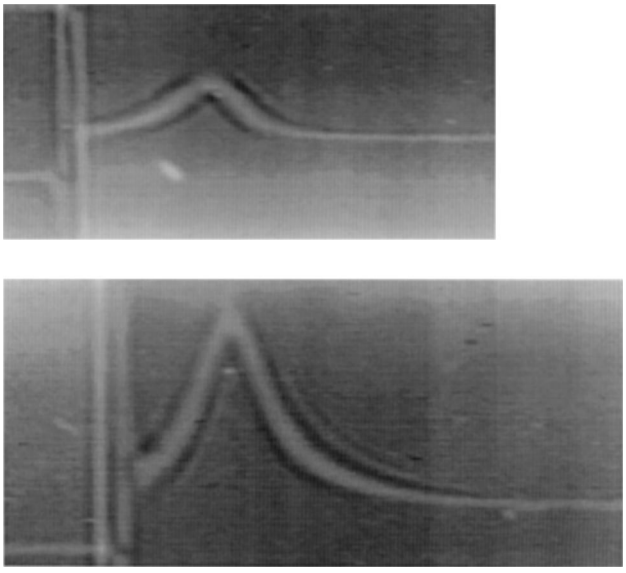

Fig. 1. Image taken from a run on the MSE Mk II ultracentrifuge for PGM and chitosan mixture (top) and chitosan control (bottom) at 0.1 M ionic strength, 35,000 rev/min, 20°C.

schlieren optical system coupled to a CCD camera the amount of chitosan interacting has been determined quantitatively. Whereas the more highly charged mucins appear to behave as expected for an interaction governed by electrostatic phenomena, a rather surprising observation is observed for the two other mucins.

2. Experimental

2.1. Materials

Sea cure + 210 (''SC210 + ''), a glutamate salt of chitosan (Pro-Nova Ltd., Drammen, Norway) was used. This is a preparation with a degree of acetylation of 11% (i.e. of ''F_A'' = 0.11) and which had previously been well characterised in our laboratory (Errington et al., 1993). Four different preparations of pig gastric mucus glycoprotein (PGM) were used. The first PGM (''PGM-MD'') was purified according to the modified procedure of Hutton et al. (1990), the others were purified using isopycnic density-gradient ultracentrifugation as described by Nordman et al. (1997). These latter were from the cardiac region of the stomach (hitherto referred to as ''cardia''), the corpus region, ''corpus-LD'' and antrum, ''antrum-LD''. The purified mucin preparations were gently defrosted and dialysed into buffer overnight at 4°C before use. All mucins had their molecular integrity checked by SEC/MALLS (Jumel et al., 1996). For all sedimentation velocity analyses, an acetate buffer pH 4.5 (Dawson et al., 1986) was used and the ionic strength was adjusted using NaCl. A known amount of chitosan was weighed out and dissolved into the acetate buffer to a concentration of 4 mg/ml; this was then left to dissolve overnight. The mixture was prepared by adding equal volumes of the chitosan and mucin solutions and was left for at least 30 min at room temperature before analysis.

2.2. Sedimentation velocity

Sedimentation velocity experiments were performed using an analytical ultracentrifuge (initially an MSE Mk II (Crawley, Sussex) and then a Beckman Model E (Palo Alto) both equipped with conventional Philpott-Svensson schlieren optical systems and a novel coupling on-line to a CCD camera. Solutions (700 μl) were injected into 20 mm (12 mm with 400 μl for the Model E) optical path length ultracentrifuge cells prior to being loaded into a four place rotor. Sedimentation was measured at rotor speeds of 2000, 10,000 and 35,000 rev/min to monitor the movement of the sedimenting complex, mucin and chitosan, respectively and at a temperature of 20°C. The schlieren method measures the refractive index gradient d*n*/dr as a function of radial position (see Lloyd, 1974). By calculating the area under the schlieren peak for each sedimenting species its (weight) concentration may be determined. The mucin concentration available was too small to allow optical registration using any of the 3 conventional optical systems on the ultracentrifuge; the complexes were also too large to be followed on the analytical ultracentrifuge, even at the lowest stable rotor speed of 1,500 rev/min. This meant that it was impossible to estimate the sedimentation coefficient ratios of the complex to chitosan (Fiebrig et al., 1994a; Harding, 1997). The assay used for complexation was thus a ''fingerprinting'' one i.e. by *quantifying how much of the chitosan had been lost through complexation*. To do this, the chitosan concentration in the mixture and the control was compared by accurately calculating the areas under the schlieren peaks as indicated before. This provides a value in pixel units

Table 1
1. Analytical ultracentrifugation data for mucins interacting with chitosan *SC210* + at 0.1 and 0.2 M ionic strength. Area under schlieren peak for control and mixture

Mucin	Ionic strength = 0.1 M			Ionic strength = 0.2 M		
	Control (pixel area)	Mixture (pixel area)	% Chitosan interacted	Control (pixel area)	Mixture (pixel area)	% Chitosan interacted
PGM –MD	1017	247	75.7	808	266	67.1
Cardia	980	660	32.7	N.D.	N.D.	N.D.
Corpus-LD	895	844	5.8	705	309	56.1
Antrum-LD	574	616	0.0	885	820	7.3

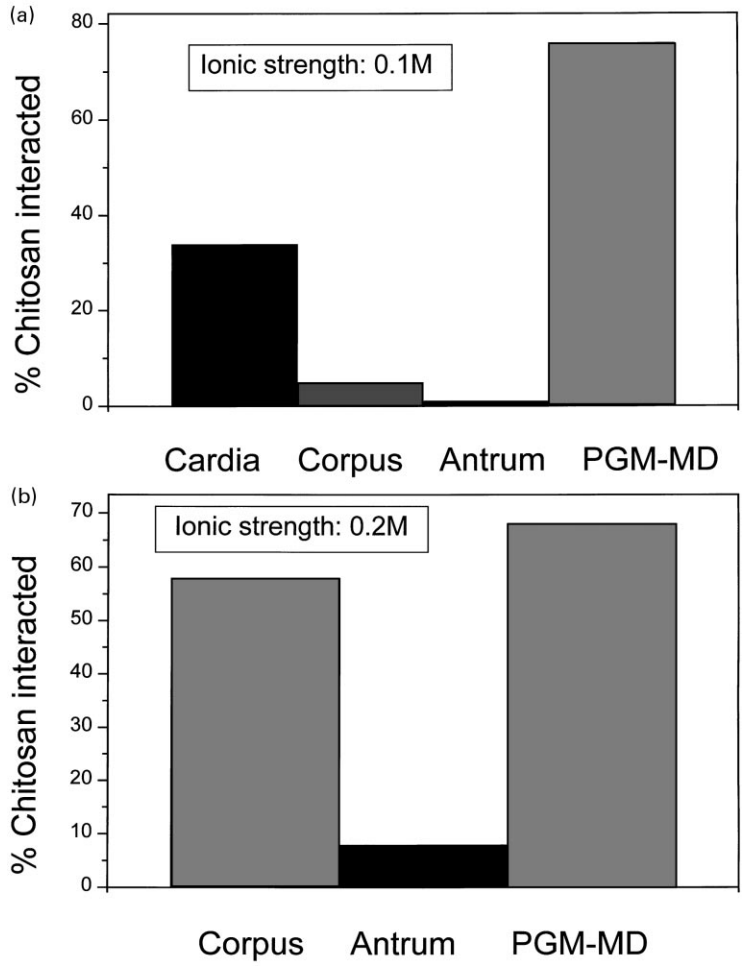

Fig. 2. Comparison of the interaction between ''SC210 + '' chitosan with three mucin populations purified from different regions of the porcine stomach (cardia, corpus-LD and antrum-LD) and one mucin population purified from the whole porcine stomach (PGM-MD). (a) $I = 0.1$ M, (b) $I = 0.2$ M.

which can then be converted, by comparison with a control containing pure chitosan at the same loading concentration into a percentage of chitosan remaining: thus by simple subtraction we can obtain a quantitative measure or index of the amount of chitosan bound to mucin.

3. Results and discussion

Fig. 1 shows a scan taken from the MSE Mk II using the CCD camera, the difference between the area of the peak for the control and for the mixture is clearly apparent, and Table 1 gives the quantitative estimates. The mucin preparation PGM-MD, which is not particular to any region of the stomach shows a strong interaction (~ 70% chitosan taken up) at the lower ionic strength, I of 0.1 M which is reduced upon increase of I to 0.2 M (Fig. 2). This behaviour is entirely as expected for an electrostatic type of interaction as previously indicated (Fiebrig et al., 1994a). For the mucins isolated from particular regions of the stomach a more intriguing observation emerges. Mucins from the cardiac region have previously been shown to be the most ''acidic'' ones with the higher charge density explained by differences in sulphate substitution (Nordman et al., 1997). At 0.1 M ionic strength, the more highly charged cardiac-region mucin shows a considerably stronger interaction (~30% chitosan taken out) compared to the other two (~5% for corpus-region and no measurable interaction for the antrum-region). However, as the ionic strength is increased to 0.2 M the interaction strengths for both corpus-LD and antrum-LD regions increases significantly. This is strongly indicative of an additional hydrophobic interaction which becomes apparent when the electrostatic one becomes suppressed, an observation supported by experiments using chitosans of low charge (i.e. high degree of acetylation, F_A) with the mucin PGM-MD. These observations, together with further investigations using atomic force microscopy will be the subject of a future presentation.

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