

Mucin structure, aggregation, physiological functions and biomedical applications

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Abstract

An understanding of the basic structure, viscoelastic properties and interactions of mucin glycoproteins is of considerable interest to food science because of the important protective role that these macromolecules play in gastric physiology. The polymeric/colloidal behavior of mucins is complicated due to their large size (2–50 MDa) and complex structure with domains involving hydrophilic/hydrophobic, hydrogen bonds and electrostatic interactions, and their propensity to aggregate and form complexes with other polymers. These properties are also of direct relevance to the numerous diseases involving mucins, and to the problems of uptake of nutrients and delivering drugs through the mucus barrier. In this review we describe the current state of understanding of the relevant properties, with an emphasis on recent research. The findings described in this review are of direct relevance to the uptake of nutrients in digestion.

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1. Introduction

Mucus is a complex viscous adherent secretion synthesized by specialized goblet cells in the columnar epithelium that lines all of the organs that are exposed to the external environment. This includes the respiratory tract, the gastrointestinal tract, the reproductive tract, and the oculo-rhino-otolaryngeal tracts. It serves many functions in those locations, among which are lubrication for the passage of objects, maintenance of a hydrated layer over the epithelium, a barrier to pathogens and noxious substances and as a permeable gel layer for the exchange of gases and nutrients with the underlying epithelium [1,2].

In addition to its protective functions, mucus is also involved in many disease processes. Mucus also is the first barrier with which nutrients and enteric drugs must interact and diffuse through, in order to be absorbed and gain access to the circulatory system and their target end organs.

2. Molecular biology and biochemical structure of mucins

2.1. Composition of mucus

Mucus is composed primarily of water (~95%), but also contains salts, lipids such as fatty acids, phospholipids and cholesterol [1], proteins which serve a defensive purpose such as lysozyme, immunoglobulins, defensins, growth factors and trefoil factors. However, the main component that is responsible for its viscous and elastic gel-like properties is the glycoprotein mucin.

2.2. Mucins

Mucins are large, extracellular glycoproteins with molecular weights ranging from 0.5 to 20 MDa. Both membrane bound mucins, and secreted mucins [3*] share many common features. They are both highly glycosylated consisting of ~80% carbohydrates primarily *N*-acetylgalactosamine, *N*-acetylglucosamine, fucose, galactose, and sialic acid (*N*-acetylneuraminic acid) and traces of mannose and sulfate. The oligosaccharide chains consisting of 5–15 monomers, exhibit moderate branching and are attached to the protein core by

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O-glycosidic bonds to the hydroxyl side chains of serine and threonines and arranged in a “bottle brush” configuration about the protein core.

The protein core, making up the remaining 20% of the molecular mass (~200–500 kDa), is arranged into distinct regions. First, a central glycosylated region, which is comprised of a large number of tandem repeats that are rich in serine, threonine and proline (STP repeats), which can make up greater than 60% of the amino acids. Second, located at the amino and carboxy terminals, and sometimes interspersed between the STP-repeats, are regions with an amino acid composition more representative of globular proteins, relatively little *O*-glycosylation and a few *N*-glycosylation sites [4] and a high proportion of cysteine (>10%). These cysteine rich regions contain domains that possess sequence similarity to von Willebrand factor (vWF) C and D domains, and C-terminal cystine knot domains [5,6,7], and have been shown to be involved in dimerization via disulfide bond formation, and subsequent polymerization of the dimers to form multimers [8] (Fig. 1).

2.3. Mucin genes

Currently, approximately 19 mucin (designated MUC) genes have been identified cloned and partially sequenced in the human, and homologs to many of them have been identified in the mouse and rat [5]. Only three MUC (MUC1, MUC2 and MUC5B) genes have been totally sequenced due to the large size of the central tandem repeats, which are difficult to accurately assemble. Many of the smaller membrane bound mucins are not considered “true” mucins since they only share the STP repeats and glycosylation with other mucins [3]. Of the remaining “true” mucins (MUC1–7), the secreted mucins (MUC 2, 5AC, 5B and 6) are located in a

cluster within ~500 kb on the short arm of chromosome 11 (11p15) [9]. It is thought that they represent homologs that diverged from a common ancestor gene on chromosome 11 [10]. The STP-repeat sequences for each MUC gene are unique to each species, whereas the cys-rich regions share a large degree of similarity [5]. Different collections of mucin genes are expressed in different tissues. Such repeats and super repeats are relatively uncommon in normal proteins.

3. Physical properties of mucin in dilute solution

Mucin has been difficult to characterize, owing to its large molecular weight, polydispersity and high degree of glycosylation. Earlier biophysical studies, reviewed by Bansil et al. [11] and Harding [12] primarily using light scattering methods showed that mucin was a somewhat stiffened random coil with a radius of gyration around 100 nm. NMR studies of MUC1 [13,14] revealed very little alpha helix, a small amount of beta and mostly random coil. The large size of mucin has, on the other hand, turned out to be of great advantage in imaging the molecule directly. Earlier transmission electron micrographic studies by Fiebrig et al. [15] revealed long fibers approximately 400 nm long in pig gastric mucin (PGM). More recent Atomic Force Microscopy (AFM) studies of ocular mucin [16] show individual fibers with a broad distribution of contour lengths. While most of the fibers are between 200–600 nm long, the tail of the distribution extends to 1500 nm. They also estimated a persistence length of about 36 nm from these images, which confirms the extended nature of the polymer. In another AFM study of ocular mucin, Brayshaw et al. [17] demonstrated the multimeric nature of mucin, by observing in situ depolymerization on treatment with DTT (dithiothreitol). Longer fibers, up to 2 μm in length, were observed in PGM by Deacon et al. [18]. McMaster et al. [19] examined ocular mucin using tapping

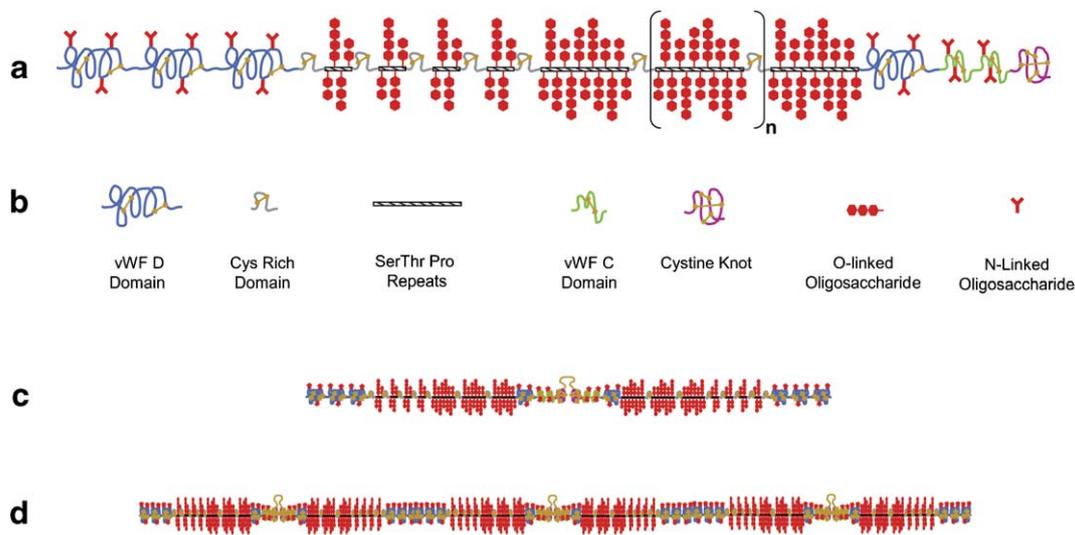


Fig. 1. (A) A schematic drawing of the pig gastric mucin (PGM) monomer consisting of glycosylated regions flanked by regions with relatively little glycosylation. (b) The symbols indicate the different domains in the sketch in (a). (This representation is based in part on Figs. 1 and 2 of Dekker et al. [3]). At the bottom of the figure we show (c) a dimer formed by two monomeric subunits linked via disulfide bonds in the non-glycosylated regions and in (d) dimers that are further disulfide linked to form higher multimers. This gives rise to the high molecular weight and polydispersity of secretory mucins. Polymers >16-mers have been described in MUC5AC from human airway secretions by Sheehan et al. [8]. (The bottom part of the figure is adapted from Fig. 8 of Ref. [8].) Reprinted with permission from [21].

mode AFM under a buffer and observed regular variations in height along the length of the fiber which they interpret as glycosylated regions of the mucin molecules. Round et al. [20] correlated the conformations with differing amounts of glycosylation by imaging different fractions obtained on a CsCl gradient. However the sugar chains are too mobile in their solvated state to be seen fully extended in an AFM image, which requires a high degree of immobilization on the substrate, the AFM tip might have penetrated the brush without sensing it. AFM images of PGM which was about 50% deglycosylated showed that the deglycosylated portions re-folded forming compact globular structures [21]. Thus the sugars are important for maintaining the extended conformation of mucin.

The conformation of mucin also depends on factors such as pH and ionic strength. For example, dynamic light scattering (DLS) studies [22] have shown that as the pH is lowered in dilute solutions (<5 mg/ml) PGM undergoes a conformational change from an isotropic random coil (with end to end length of 390 nm and persistence length of 8–10 nm) at pH 7 to an anisotropic extended random coil (with end-to-end length of 490 nm, persistence length 43 nm) at pH 2. This is paralleled by an increase in the sedimentation coefficient from 11S at pH 7 to greater than 31S at pH 3 [23]. In a recent paper the conformation of commercially available PGM from Sigma in dilute solution examined by viscosity and circular dichroism measurements reveals similar changes as function of pH which are attributed to the unfolding of hydrophobic domains at low pH [24]. These authors also measured the zeta (ζ) potential of mucin and found that its isoelectric point lies between 2 and 3, suggesting that the charge on the molecule varies as pH is lowered.

4. Colloidal properties of mucin

4.1. Aggregation and gelation

Mucins exhibit a tendency to aggregate and form gels. Taylor et al. [23] used rheological techniques to investigate the structure and formation of the pig gastric mucus gel and showed that both transient and non-transient interactions are responsible in maintaining the gel matrix. Purified mucin also has been shown to form gels. Human tracheobronchial mucin forms a gel below 30 °C at concentrations above 14 mg/mL [25]. Gelation was also observed in canine submaxillary mucin in the chaotropic (denaturing) solvent guanidine HCl [26]. These authors suggest that since non-covalent interactions are destabilized by guanidine HCl, gelation in these high molecular weight fractions involves the interpenetration of the carbohydrate side chains [26].

Our laboratory has focused on gelation of gastric mucin at low pH, which has implications for the physiologically relevant question of how the stomach is prevented from being digested by the acidic gastric juice it secretes. Bhaskar et al. [27] showed a reversible increase in viscosity of aqueous solutions of pig gastric mucin (PGM) at pH 2. DLS studies [22] show that at concentrations above 10 mg/ml gelation occurs below pH 4. At low pHs we also observed an increase in the hydrophobicity of

the protein core of PGM as indicated by its increased binding of the fluorescent hydrophobic dye 1-anilino-naphthyl-8-sulfonic acid (ANS). Atomic force microscopy images confirm that while PGM exists as single molecules at pH 6 (about 400 nm in length), it forms aggregates below pH 4 [21,28]. The viscosity increase and gelation are not observed if PGM is broken into its subunits (by treating with enzymes such as pronase or disulfide reduction with DTT) or salt concentration is increased. Commercial preparations of PGM available from Sigma chemicals (which include protease treatment during purification) also do not gel [28].

4.2. Model for pH induced gelation of PGM

This accumulated data showing a sharp increase in the observed changes at pH 4 and the lack of gelation in PGM which is broken into the subunits has led us to suggest that PGM gelation involves interactions of side chains of amino acids with pKs around 4, such as Asp or Glu from the non-glycosylated regions of the molecule. At neutral pH of 6–7 these non-glycosylated Cys-rich regions of PGM are in conformations with hydrophobic domains hidden in folds of stabilized by salt bridges between negatively charged carboxylates and positively charged amino groups. At acidic pH 2 the carboxylates of the salt bridges are protonated, breaking the salt bridges and allowing the unfolding and exposure of hydrophobic regions of protein (Fig. 2). Circular dichroism studies reported by Lee et al. [24] on pig gastric mucin from Sigma also confirm the conformational transition at pH 4 and below. The hydrophobic domains on adjacent molecules are then able to associate acting as the crosslinks of a gel. Hydrophobic interactions among protein domains were also shown to be involved in aggregation of human tracheobronchial mucin [25]. The gelation of mucin is a complex problem, involving the interplay of electrostatic and hydrophobic interactions, somewhat reminiscent of the association of multiblock copolymers in solvents that have differential solubility to the component blocks. The entanglement of the sugar side chains further contributes to the high viscosity of mucin solutions (Fig. 2).

4.3. Liquid crystalline properties of mucin

Mucin glycoprotein has also been shown to exhibit liquid crystalline order. Viney, et al. [30] had shown that slug mucin forms nematic liquid crystals. A detailed study of the temperature and concentration dependence of the liquid crystalline behavior of commercially available mucin from Sigma was reported by Davis et al. [31] who suggest that the interactions of interdigitated sugar side chains are responsible for the nematic behavior. Waigh et al. [32] confirmed these observations by neutron scattering, comparing Sigma mucin at high concentrations in the presence and absence of an external magnetic field to produce orientation. Purified PGM, on the other hand, did not show this liquid crystalline orientation, suggesting that the Sigma mucin, which consists of a single mucin monomer, is more rigid and easier to orient than the multimeric form of native PGM in which the non-glycosylated

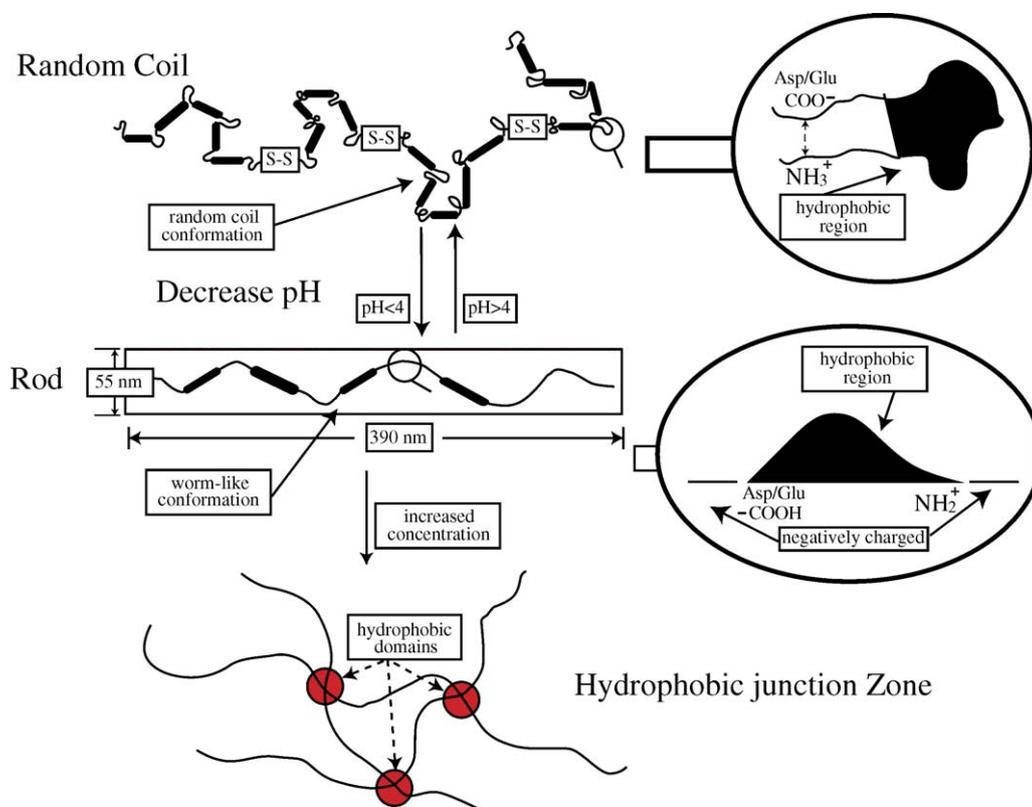


Fig. 2. A model showing how the breaking of electrostatic interactions around pH 4 and below can produce a conformational change in PGM from a random coil to a rod (or stiff worm-like chain) and lead to gelation at high concentrations at low pH by exposing hydrophobic regions which were folded and thus sequestered in the interior at neutral pH. [Reproduced from Ref. [29], PhD dissertation of Xingxiang Cao, Boston University, 1997, with permission of the author].

portions serve as flexible links between the rigid, glycosylated, monomeric domains.

4.4. Adhesive properties of mucin

The well known tendency of other substances to adhere to mucin, known as mucoadhesivity, is not surprising given that this glycoprotein exhibits electrostatic, hydrophobic, and H-bonding interactions [33]. By the same token the molecule can be anti-adhesive, for example to negatively charged species. Thus mucin coated AFM tips adhered to mica in presence of divalent cations [34] but did not adhere to mica coated with mucin, presumably due to the polyelectrolytic charge repulsion. Bovine submaxillary mucin from Sigma could be adsorbed to hydrophobic polystyrene surface rendering it hydrophilic [35]. Mucin lipid interactions are well known, reflecting the presence of hydrophobic domains [36]. In this context, it is worth noting that since mucin is negatively charged it binds to positive ions. For example, Chromium III binding has been shown to cause significant conformational changes and it can lead to aggregation of mucin [37].

4.5. Diffusion of macromolecules and particles and fluid flow through mucin

In view of the protective function of mucus studying the diffusion of other molecules through it is of considerable physiological interest. It is also an important factor in the

design of drugs which have to diffuse through the mucus layer, or kept from entering it. While many small molecules diffuse readily through mucus, the diffusion of larger particles depends both on size and muco-adhesive interactions. The diffusion of latex particles and lipid vesicles in purified gall bladder mucin has been examined using DLS [38] and fluorescence recovery after photobleaching [39] methods. Olmsted et al. [40] showed that while many small viruses (20–200 nm) could diffuse through cervical mucus, others such as the Herpes Simplex virus got stuck to the mucus. Celli et al. [41] have exploited the movement of latex particles through mucin solutions to measure its rheological properties as a function of pH using microscope based DLS.

Finally, it is worth noting that the transport of water and other fluids through mucus and purified mucin solutions is a very interesting problem in fluid dynamics. Due to its high viscosity, water and other low viscosity fluids injected under a pressure gradient across an unstirred mucin solution do not simply diffuse, but are transported by a viscous fingering mechanism [42,43]. This transport of HCl through “channels” produced by the viscous fingering mechanism has also been demonstrated in vivo in rats [44]. The mucus in the immediate vicinity of the surface of the acid channel will gel, thereby confining the acid in a “tube”. Similarly the mucus layer on the luminal surface of the stomach will gel as the acid is emptied into the lumen. The negatively charged mucin gel prevents back diffusion of H⁺ via the mechanism of Donnan equilibrium.

5. Biomedical function and applications

5.1. Mucus and disease

The physical state of the 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. For example, besides serving as a barrier to bacteria, many bacteria reside within the mucus and possess specific adhesins that specifically bind to it [45]. This includes pathogenic strains of *Pseudomonas*, *Streptococcus* and *Pneumococcus*. *Helicobacter pylori* particularly resides in the mucus layer of the stomach, and is a common cause of ulcers [46]. Some parasitic organisms also produce their own layers of mucus to evade the immune system [47]. Second, overproduction of mucus is involved in cystic fibrosis [48*], bronchitis, asthma [49] and in middle ear infections, and mucus gels serve as the matrix in which gallstones are nucleated and grow [50]. Thirdly, mucus underproduction is present in dry eye syndromes [51*] and in some forms of ulcer disease. Finally, mucus expression and composition is altered in cancers of epithelial origin [52**].

5.2. Mucus and pharmacology

Mucus is the first barrier with which nutrients and enteric drugs must interact and diffuse through, in order to be absorbed and gain access to the circulatory system and their target end organs. There is great interest in methods to optimize these so-called muco-adhesive interactions for improved drug delivery. Various molecular interactions have been exploited to enhance mucoadhesion, including, polyelectrolytic interactions (chitosans, poly-acrylic acid, etc.) hydrogen bonds (hydrogels), [33] and disulfide binding (thiomers) [53]. Efforts are underway to design pH sensitive drug carriers such as gels which will not release the drug in the acidic environment of the stomach but will do so in the basic environment of the intestine and colon. In this context, we emphasize recent studies [54] which show that the resting stomach has a pH close to 4 which drops to 2 during active acid secretion. Mucin can be used as a high molecular weight additive to improve the adherence of artificial tear drops in treating dry eye syndrome [55]. Efforts to develop nanoparticles for mucosal DNA vaccines and gene therapy are also being considered [56]. The abnormality of the sugars in cell membrane bound mucins from cancerous cells has also been targeted as a potential for cancer vaccine development. Excellent reviews of oral mucosal drug delivery are collected in Ref. [57*].

6. Conclusions

We have reviewed recent, as well as some pertinent older literature describing the biophysical properties of mucin in solution and its properties of interest in colloid science. The picture that emerges is that of a macromolecule with a complex organization into domains with different structures. From the

polymeric viewpoint mucin can be considered as a multiblock copolymer with alternating polyelectrolytic domains having a grafted sugar brush, connected by flexible regions with less glycosylation and the tendency to form multimers. The molecule has both hydrophobic and hydrophilic regions with the ability to form H-bonds, and electrostatic interactions. This wide range of interactions causes it to aggregate gel and form mucoadhesive interactions with other substances. These physical properties are of direct relevance to the physiological functions of mucus in normal and diseased states. An understanding these properties is of considerable current interest because of the many biomedical applications.

[Note: Topics that are not covered in this review such as mucin glycosylation [58], signal transduction [59], and secretion [60], can be found in the indicated references.]

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