Colloidal gold and colloidal gold labelled wheat germ agglutinin as molecular probes for identification in mucin/chitosan complexes

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Polyelectrolyte complex formation between pig gastric mucin and chitosan and its implications to mucoadhesion have recently been studied using techniques such as analytical ultracentrifugation, static light scattering and turbidimetry (Fiebrig, I., Harding, S.E. and Davis, S.S. (1994) Prog. Coll. Polym. Sci. 93, 66–73; Fiebrig, I., Harding, S.E., Rowe, A.J., Hyman, S.C. and Davies, S.S. (1995) Carbohydr. Polym. 28, 239–44). The findings suggested the formation of large complexes, which were then visualised using electron microscopy. The present investigation employed electron microscopy combined with colloidal gold conjugates in order to identify and localize chitosan within the mucin/chitosan complex. Images of apparently dispersed chitosan molecules could be obtained using an anionic dye molecule, Orange II, which is known to interact stoichiometrically with the cationic sites on chitosan. Once chitosan was complexes with mucin, the colloidal gold labelled techniques revealed that chitosan is concentrated in the centre of the complex, surrounded by a possibly more hydrophilic layer of mucin.

INTRODUCTION

Analytical ultracentrifugation as well as static light scattering and turbidimetry have shown a pronounced interaction between pig gastric mucin and chitosan at low concentrations (Fiebrig et al., 1994a; Fiebrig, 1995). The motivation for these studies was to assess the possibility of using these physical techniques to determine the complexation between oppositely charged polyelectrolytes. Further studies have shown (Fiebrig et al., 1995a) that the resulting complexes could be imaged via electron microscopy, appearing as spherical agglomerates with diameters between 200 and 500 nm. While mucins usually appear as characteristic linear threads in the micrographs, and can be easily identified within the complex, chitosan is difficult to image altogether. The object of the present study is both to confirm this previous study and to pinpoint the molecular species in the complexes using specific labelling techniques. The selected approach is based on the use as conjugated, presumably specific, molecular probes of small colloidal gold particles, or of wheat germ agglutinin conjugated to gold particles.

Lectins, polysaccharide modifying or depolymerizing enzymes, and specifically raised antibodies all possess potential for molecular recognition that can be utilized for localization studies. Recognition of given polysaccharides based on antibodies specifically generated against them represents one viable route for molecular recognition that can be be utilized in electron microscopic studies. Practical difficulties in obtaining monoclonals that are specific to a given polysaccharide are associated both with the fact that polysaccharides in general are not particularly immunogenic and that most polysaccharide samples are heterogeneous. In the present study, we have

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utilized the specificity of a lectin in trying to label specifically only one of the components, and thereby obtain more detailed information of association modes in the chitosan/mucin complexes.

Chitosans are linear, bermoullian co-polysaccharides of 2-acetamido-2-deoxy-β-D-glucopyranose (GlcNAc) and 2-amino-2-deoxy-β-D-glucopyranose (GlcN) connected by (1→4) linkages (Värum et al., 1991a, b). Water-soluble chitosans, commercially prepared by N-deacetylation of chitin, can be obtained with a fraction of N-acetylation (F_A) from 0 to 0.6 (Anthonsen et al., 1993; Nordtveit et al., 1994). Selection of lectins that can be used to label chitosan are thus limited to those showing affinity for the GlcNAc and/or GlcN residues. The selected lectin, wheat germ agglutinin (WGA), is a protein with molecular weight 36 000 g mol⁻¹, which consists of two identical subunits, and with an isoelectric point of 8 (Rice and Etzler, 1975). Inhibition of agglutination reactions of WGA is about 3000 times more potent using the GlcNAc trimer rather than the GlcNAc monosaccharide (Allen et al., 1973; Nagata and Burger, 1974). WGA has also shown binding to sialic acid (Greenaway and Le Vine, 1973).

The sugar composition of the mucin reveals a significant amount of GlcNAc (Allen, 1989), indicating that identification of the polymeric species based on interaction with WGA conjugated to colloidal gold particles cannot be unique. An additional set of experiments was therefore carried out in which the order of complexation between chitosan and mucin and subsequent identification of the species in the complexed products was reversed. First, either chitosan alone was conjugated to colloidal gold according to Horisberger and Clerc (1988) (Fig. 1), or was allowed to interact with the WGA conjugated to the gold colloids (Fig. 1). Secondly, these chitosan coated particles were incubated with mucin to provide the complexation reaction between the polyanion and the polycation. Comparison of the morphology, including localization of the gold particles (and hence the chitosan chains), with that of the other type of experiments allowed us to identify the localization of the two species in the complexes. Chitosan appears to accumulate in the core of the complex surrounded by a layer of mucin.

**Figure 1.** Schematic drawing of colloidal gold particles stabilized with (a) chitosan and (b) chitosan interacting with wheat germ agglutinin colloidal gold conjugates.

**Materials and Methods**

**Pig gastric mucin**

Highly purified pig gastric mucin (PGM) was prepared according to a modified method of Hutton et al. (1990) from the mucus of stomachs from freshly slaughtered pigs. High shear homogenization was used to solubilize the mucus gel in the presence of enzyme inhibitors, followed by preparative density gradient ultracentrifugation in CsCl to separate glycoproteins from other macromolecules such as proteins, and finally by preparative gel permeation chromatography to obtain high molecular weight material free from degraded mucins (Fiebrig et al., 1994b). The aqueous mucin solution (≈0.4 mg/ml) was stored in 1 ml aliquots at -20°C, gently defrosted before use and dialysed into 0.1 M acetate buffer pH 4.5.

**Chitosans**

Chitosans used were SeaCure 210 (SC210) (Pronova Biopolymers, Drammen, Norway) glutamate salt, $F_A = 0.11$, and KN50, chloride salt, $F_A = 0.58$ (Errington et al., 1993). KN50 was dissolved in distilled water and diluted using a stock solution of tris buffer at pH 8, and SC210 was dissolved in acetate buffer pH 4.5 or water to a polymer concentration of 2 mg/ml.

**All other polymers and chemicals**

Hydroxyethylcellulose (HEC) as well as Carbowax 20M {Poly(ethylene glycol)}, compound with 2,2′-[(1-methylethylidene) bis (4,1 phenyleneoxymethylene)] bisoxirane), were purchased from Aldrich. An alginate sample prepared from *Macrocystis pyrifera* (medium viscosity) as previously described (Stokke et al., 1993) was obtained from the Kelco Division of Merck (San Diego). Orange II (Acid orange 7, CI 15510), 10 nm colloidal gold labeled lectin from *Triticum vulgaris* (WGA-Au10), chloroauric acid, and all other chemicals were purchased from Sigma. Aqueous 60% glycerol at pH 4.5 was prepared by dilution of glycerol with water and addition of ammonium acetate to give a salt concentration of 0.1 M. HEC and the alginate sample were dissolved in acetate buffer pH 4.5 or water to give polymer concentrations of 2 mg/ml and diluted where appropriate. These were then used for conjugation with the colloidal gold preparation (Table 1).

**Colloidal gold**

Colloidal gold (Au₄) was prepared according to the citrate gold method of DeMey (1984) as follows. 65 g of distilled water were boiled with 4.0 ml of a 1% sodium citrate solution for 5 min under reflux. 0.6 ml of a 1% solution of chloroauric acid was added as
Table 1. Preparation of labelled colloidal gold probes.

<table>
<thead>
<tr>
<th>Designation^a</th>
<th>Start solution</th>
<th>First addition</th>
<th>Second addition</th>
<th>Centrifugation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solution</td>
<td>t\textsubscript{incubation}</td>
<td>Solution</td>
<td>t\textsubscript{incubation}</td>
</tr>
<tr>
<td>SC210-WGA-Au\textsubscript{10} (P1)</td>
<td>50 μl WGA-Au\textsubscript{10} SC210</td>
<td>100 μl, 1 mg/ml SC210</td>
<td>30 min</td>
<td>0.2 ml, 1% Carbowax 20M</td>
</tr>
<tr>
<td>SC210-Au\textsubscript{4} (P2)</td>
<td>0.25 ml, 0.1 mg/ml SC210</td>
<td>50 ml Au\textsubscript{4}, pH 4</td>
<td>5 min</td>
<td>0.2 ml, 1% Carbowax 20M</td>
</tr>
<tr>
<td>KN50-Au\textsubscript{4} (P3)</td>
<td>0.5 ml, 0.1 mg/ml KN50</td>
<td>20 ml Au\textsubscript{4}, pH 8</td>
<td>5 min</td>
<td>0.2 ml, 1% Carbowax 20M</td>
</tr>
<tr>
<td>HEC-Au\textsubscript{4} (P4)</td>
<td>0.05 ml, 0.1 mg/ml HEC</td>
<td>20 ml Au\textsubscript{4}, pH 4</td>
<td>5 min</td>
<td>0.2 ml, 1% Carbowax 20M</td>
</tr>
</tbody>
</table>

^a The shorthand notation in the parentheses is used in the Tables 2 and 3.
^b First centrifugation step to remove large aggregates, supernatant used further.
^c Second centrifugation step to concentrate the colloidal gold particles.
quickly as possible under vigorous stirring. The preparation was kept boiling for a further 15 min under reflux, cooled to room temperature and kept in the refrigerator (4°C) until use.

Colloidal gold probes

The flocculation test with sodium chloride following different amounts of polymer added to the uncoated colloidal gold (DeMey, 1984; Horisberger and Rosset, 1977) was employed to determine the optimum amount of polymer needed for coating the surface of the gold particles. The minimum amount of KN50, SC210 or HEC needed to prevent flocculation was taken as the appropriate concentration, plus a 10% excess, for the preparation of a larger amount of Polymer-Au₄ (Horisberger and Clerc, 1988). The addition of Carbowa 20M is further for stabilization of the gold probes, especially to facilitate redispersion following a centrifugation step (DeMey, 1984). Conjugated WGA to 10 nm gold probes was obtained from Sigma. Other colloidal gold probes were prepared with the solutions of different polymers and stabilizing solutions as specified in Table 1. Complexation of chitosan and mucins and blending of labelled gold probes with the different samples were carried out as specified in Table 2. Labelling experiments were conducted as in Table 3. All incubation steps were carried out on ice or in a cold room (4°C). A Desktop Eppendorf Centrifuge 5415 C was used for all centrifugation procedures <10 000 rpm. All other centrifugations were performed on an MSE Ultracentrifuge (Beckman K-21B, fixed angle rotor JA 21).

Sample preparation for TEM

The samples, appropriately diluted to a concentration of 5–20 µg/ml in approximately 55% glycerol, 0.1 M ammonium acetate, pH 4.5 (Tables 2 and 3), were sprayed onto freshly cleaved mica disks with a diameter of 5 mm employing a modified painter’s airbrush and dried in vacuum at room temperature as described (Tyler and Branton, 1980). This was followed by rotary shadowing with platinum from an angle of 5° and carbon supporting film deposition from an angle of 90° in a freeze-etch unit described by Elgsæter (1978). The resulting replicas were floated off on distilled water and picked up on 400 mesh copper grids. Electron micrographs were obtained using a Philips EM 400T electron microscope operated at 80 keV.

RESULTS AND DISCUSSION

Vacuum dried samples of the pig gastric mucin show linear threads of varying length with kinks and bends (Fig. 2 (a)) and occasionally more complex structures with mucin strands emanating from and returning to a core structure consistent with the observations of others (Rose et al., 1984; Harding, 1989; Sheehan and

<table>
<thead>
<tr>
<th>Designation</th>
<th>Start solution</th>
<th>First addition</th>
<th>Second addition</th>
<th>Third addition</th>
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</thead>
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<tr>
<td>SC210-Orange II</td>
<td>0.2 ml 0.1 mg/ml SC210</td>
<td>0.1 ml 0.175 mg/ml Orange II</td>
<td>&gt; 30 min</td>
<td>60% glycerol, pH 4.5 ( a ), 0.02 mg/ml SC210</td>
</tr>
<tr>
<td>PGM-SC210</td>
<td>0.05 ml 0.4 mg/ml PGM</td>
<td>0.05 ml 2.0 mg/ml SC210</td>
<td>30 min</td>
<td>0.5 ml 60% glycerol, pH 4.5</td>
</tr>
<tr>
<td>PGM-Au₄</td>
<td>0.5 ml 0.4 mg/ml PGM, pH 4.5</td>
<td>0.5 ml Au₄, pH 4</td>
<td>30 min</td>
<td>60% glycerol, pH 4.5 ( a ) in ratio 8:1 (v/v)</td>
</tr>
<tr>
<td>PGM-WGA-Au₁₀</td>
<td>15 ml 0.4 mg/ml PGM, Tris buffer, pH 8</td>
<td>30 µl WGA-Au₁₀</td>
<td>30 min</td>
<td>0.5 ml 60% glycerol, pH 4.5 ( a )</td>
</tr>
<tr>
<td>PGM-SC210-(P3)</td>
<td>30 µl 0.4 mg/ml PGM</td>
<td>0.05 ml 2.0 mg/ml SC210</td>
<td>15 min</td>
<td>0.02 ml KN50-Au₄ (P3)</td>
</tr>
<tr>
<td>PGM-HEC</td>
<td>15 µl 0.4 mg/ml PGM</td>
<td>15 ml 2.0 mg/ml HEC</td>
<td>30 min</td>
<td>0.5 ml 60% glycerol, pH 4.5</td>
</tr>
<tr>
<td>PGM-SC210-(P4)</td>
<td>15 µl 0.4 mg/ml PGM</td>
<td>0.05 µl 2.0 mg/ml SC210</td>
<td>15 min</td>
<td>0.02 ml HEC-Au₄ (P4)</td>
</tr>
</tbody>
</table>

* The 60% glycerol, pH 4.5 solution also contains 0.1 M ammonium acetate.
Table 3. Labelling experiments

<table>
<thead>
<tr>
<th>Designation</th>
<th>Start solution</th>
<th>First solution</th>
<th>( t_{\text{incubation}} )</th>
<th>Second solution</th>
<th>( t_{\text{incubation}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGM-(SC-WGA-Au(_4))</td>
<td>0.03 ml P1</td>
<td>0.03 ml 0.4 mg/ml PGM, pH 4.5</td>
<td>30 min</td>
<td>0.5 ml 60% glycerol, pH 4.5</td>
<td>&gt; 15 min</td>
</tr>
<tr>
<td>PGM-(SC-Au(_4))</td>
<td>0.05 ml P2</td>
<td>0.05 ml 0.4 mg/ml PGM, pH 4.5</td>
<td>30 min</td>
<td>0.5 ml 60% glycerol, pH 4.5</td>
<td>&gt; 15 min</td>
</tr>
<tr>
<td>PGM-(KN 50-Au(_4))</td>
<td>0.04 ml P3</td>
<td>0.015 ml 0.4 mg/ml PGM, pH 4.5</td>
<td>30 min</td>
<td>0.5 ml 60% glycerol, pH 4.5</td>
<td>&gt; 15 min</td>
</tr>
<tr>
<td>PGM-(HEC-Au(_4))</td>
<td>0.06 ml P4</td>
<td>0.015 ml 0.4 mg/ml PGM, pH 4.5</td>
<td>30 min</td>
<td>0.5 ml 60% glycerol, pH 4.5</td>
<td>&gt; 15 min</td>
</tr>
</tbody>
</table>

\(^a\) The 60% glycerol solution, pH 4.5, also contains 0.1 M ammonium acetate.

Fig. 2. Electron micrographs of (a) pig gastric mucin and (b) chitosan complexed with Orange II (SC210-Orange II, Table 2). The scale bar is 200 nm.

Carlstedt, 1990; Fiebrig et al., 1995a). The employed technique for preparation of dilute solutions of biopolymers has previously been extensively used for the study of polysaccharide conformations and conformational transitions (Stokke and Brant, 1990; Stokke and Elgsæter, 1994) and it was shown that preparation-induced artefacts can be recognized and excluded as dominant to the apparent shape of the polymer molecules. Although it is not possible to carry out the same test for the mucin samples because of difficulties in unique assignments of the chain trajectory, it is assumed that artefacts do not significantly contribute to the present case either.

Chitosans, however, are difficult molecules to image using EM, especially as unfolded single molecules. They have been found to appear as aggregates of sizes between 1 and 5 \( \mu m \), employing solutions of an initial concentration of 30–300 \( \mu g/ml \) (Anthonisen et al., 1994). Images from single molecules at lower initial solution concentrations have so far not been obtained, possibly due to the fact that a resolution limit for these molecules is reached. A new approach was tried, employing an anionic dye, Orange II, used for metachromatic titrations of chitosan (Gummow and Roberts, 1985; Maghami and Roberts, 1988). The dye is known to interact stoichiometrically with the cationic sites of chitosan. At the equivalence point the maximum amount of dye molecules are expected to interact with the cationic sites on the chitosan molecule. The micrographs obtained for the SC210-Orange II sample (Table 2, Fig. 2 (b)) show two kinds of structures. The apparent structures of the assumed chitosan structures observed using this method are unbranched and with lengths between 10 nm and 100 nm and thicknesses between 5 nm and 10 nm (the most slender structures in Fig. 2 (b)). The additional, much thicker, and branched structures more evident in the micrographs most likely reflect aggregated structures of chitosan. Their appearance is quite different from that of other polysaccharides not complexed with any dye (Stokke and Elgsæter, 1994). The contour length bears no relationship to the molecular weight and it can be assumed that the molecule is somehow folded up or is an aggregate of more than one molecule. The larger Pt grains yielding a more coarse apparent background appearance in Fig. 2 (b) compared to the other micrographs are most likely due to the presence of Orange II in the SC210-Orange II preparation (Table 2).

Mixtures of pig gastric mucin and chitosan SC210, essentially the same as those used in previous analytical ultracentrifugation experiments (Fiebrig et al., 1994a), show globular structures with a dense core of diameter 100–500 nm (Fig. 3 (a)) surrounded by mucin filaments. No structures of this kind were observed in either the mucin or the chitosan control. Usually, these complexes were surrounded by mucin material of a similar appearance as uncomplexed mucin. Although chitosan is present in an excess by weight and experimental data have shown that at these concentration ratios there is free chitosan left, it was difficult to localize the excess of chitosan due to the
problems discussed above. Figure 3 (b) shows a globular structure surrounded by mucin in a background of rigid structures that are comparable to the chitosan structures observed previously (Anthonsen et al., 1994).

Proteins are reported to be excellent stabilisers for cAu, suggesting that a glycoprotein such as PGM would have similar properties deriving from the ‘naked’ protein region which can readily be adsorbed to the cAu surface. Colloidal gold has been employed previously as a marker for these naked protein regions in degraded mucins (Sheehan et al., 1986). Figure 4 (a) shows representative cAu marked PGM, cAu appearing as attached to the mucin molecules in random fashion either as linear or as more tangled structures. More condensed structures, presumably due to complexation with cAu were also observed (data not shown). This suggests that complexation with cAu alone may lead to partial condensation of the mucin structures. A morphologically similar interaction of the PGM was observed upon complexation with WGA-Au₁₀ (Fig. 4 (b)). This finding suggests that WGA may interact with the sugar residues of the glycoprotein, as can be expected from the sugar composition. There was, however, close morphological resemblance of the WGA-Au₁₀ complexed PGM and the uncomplexed one, and in particular, no globular agglomerates comparable to those for complexation with chitosan could be observed.

Preparation of the gold–chitosan conjugates using the SC210 chitosan resulted in a large fraction of structures that could not be resuspended after the centrifugation step using a sedimentation force comparable to that used for protein labelling of colloidal gold particles of similar size (Stokke et al., 1985). In trying to increase the yield of the gold–chitosan conjugates, the pH during conjugation was increased above the pKₐ value of chitosan using the soluble KNS0 chitosan at that pH. After removal of the largest aggregates also in this case, this yielded a larger fraction of resuspendable gold–chitosan conjugates following the second sedimentation step (Table 1).

The micrograph in Fig. 5 shows spherical complexes of PGM-SC210 having a diameter between 150 and 200 nm; the KNS0-Au₄ probe is not taken up into the core of the spheres but appears bound to the mucin filaments extending from the complex. The lack of binding of the KNS0-Au₄ probes to the cores of the complexes suggests that the cores have a net positive charge due to the complexation between the mucin and the unconjugated chitosan. Thus, the dense cores seen in many of the mucin/chitosan complexes appear to consist of a fraction of chitosan that yields charge reversal of the complexed mucins.

Mucoadhesive properties have been attributed to cellulose derivatives such as sodium carboxymethyl
cellulose, methyl cellulose, hydroxypropylmethyl cellulose, methylethyl cellulose and hydroxypropyl cellulose (Fiebrig et al., 1995b; Hunt et al., 1987). Junginger and Lehr (1990) argue however, that cellulose derivatives have no intrinsic adhesive properties once they are in their fully hydrated state. Anderson (1991) used ethylcellulose as an excipient for a non-biodegradable control formulation in a gastrointestinal endoscopy study in humans. Turbidimetry shows no interaction between HEC and PGM in dilute solution (Fiebrig et al., 1994b). This is supported by the micrographs of PGM and HEC, with Fig. 6 (a) showing a representative area. Mucin appears normal, while HEC can be identified as less irregular structures of 50-200 nm length.

The micrograph of the mucin complexed with chitosan and subsequently labelled with HEC-Au4 (Fig. 6 (b)) reveals the dense cored globular structures surrounded by mucin molecules. cAu can be seen in the background as apparently unbound material, while some of the markers are bound to rigid elongated structures. Compared to the observation of the gold-chitosan probes (Fig. 5), the HEC-Au4 probes appear to bind to a lesser extent to the mucin chains extending from the central part of the polyelectrolyte complex.

Complexation of PGM with chitosan (SC210) and subsequent labelling with WGA colloidal gold conjugates (WGA-Au10) reveal spheroidal structures where most of the cAu can be seen to accumulate in the centre of the sphere surrounded by mucin (Fig. 7 (a)). These structures share many of the morphological features that were observed when pig gastric mucin was complexed with chitosan conjugated to gold particles. This was observed when chitosan of different F_A was used. Figure 7 (b) shows the result for chitosan SC210 (F_A = 0.11), and Fig. 7 (c) for chitosan designated KN50 (F_A = 0.58). The close morphological resemblance of these aggregates with those seen in the mucin/chitosan complexes and the finding that the gold particles were mainly located in the central core suggest that the complexes are assembled around a core with high concentration of chitosan surrounded by a layer with (mainly) mucin with less chitosan. The close qualitative resemblance between the complexed mucins obtained using the SC210 and KN50 gold-chitosan conjugates (Fig. 7 (b) and (c)) indicate that increasing the F_A to 0.58, and thereby lowering the charge density on the chitosan, is not sufficient to impair the polyelectrolyte complex formation between chitosan and mucin.

CONCLUSIONS

The electron microscopy data have shown that the spherical structures of pig gastric mucin (PGM) and chitosan complexes tend to accumulate chitosan in the core, surrounded by PGM. It would appear reasonable to propose that chitosan, whilst complexing with mucin, becomes insoluble and increasingly hydrophobic (more 'chitin-like' due to shielding of the charged amine groups), excluding water, whilst the water solubility of the interacting mucin molecules is not largely affected. This may be analogous, in some way, to the formation

Fig. 5. Electron micrograph of pig gastric mucin complexed with chitosan SC210 and subsequently labelled with chitosan conjugated to 4 nm gold particles (PGM-SC210-P3, Table 2). The scale bar is 200 nm.

Fig. 6. Electron micrographs of (a) mixtures of pig gastric mucin and hydroxyethyl-cellulose (PGM-HEC, Table 2) and (b) complexes of pig gastric mucin and chitosan (SC210) to which HEC colloidal gold conjugates subsequently was added (PGM-SC210-(P4), Table 2). The scale bar is 200 nm.
Fig. 7. Electron micrographs of (a) pig gastric mucin complexed with chitosan labelled with WGA-Au10 (PGM-(SC-WGA-Au10), Table 3), (b) pig gastric mucin complexed with chitosan–colloidal gold conjugates using chitosan SC210 (PGM-(SC-Au10), Table 3), and (c) chitosan KN50 (PGM-(KN50-Au10), Table 3). The scale bar is 200 nm.

of oil-in-water micelles under addition of a surfactant. The oil-like phase in this model would be represented by the acetylated, and hence hydrophobic, sequences of chitosan, whereas the mucin acts as a surfactant, thus stabilizing the hydrophobic domains from further aggregation. However, these data need to be treated with caution. Numerous negative as well as positive controls are required for these type of experiments due to the fact that WGA can be expected to bind both chitosan and PGM. Furthermore, while chitosans as well as HEC give stable cAu probes, aspects of affinity of cAu to PGM along with displacement of the stabilising polymers by PGM have to be taken into consideration. The technique seems, however, interesting for showing the location of chitosan within chitosan/mucin complexes. Attempts to make a negative control with alginites or dextran sulfate (polyanionic) have thus far failed due to the severe instability of the resulting cAu-alginate or cAu-dextran complexes.

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Molecular probes for mucin/chitosan complexes


