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New insights into the mucoadhesion of pectins by AFM roughness parameters in combination with SPR

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ABSTRACT

The object of this study was to assess the mucoadhesion of the three main commercially available types of pectin by atomic force microscopy (AFM) and surface Plasmon resonance (SPR). Polyacrylic acid and polyvinyl pyrrolidone were used as positive and negative control, respectively.

Image analysis of the AFM scans revealed a significant change of roughness parameters when low-ester pectin was introduced to mica supported bovine submaxillarymucin, indicating a high mucoadhesion for this type of pectin. Only minor changes were observed with high-ester and amidated pectin. The same ranking order of adhesion affinity was confirmed by SPR.

In conclusion, a high specific mucin interaction of pectin with a high charge density was demonstrated directly on a molecular scale without interference from the viscoelastic properties or the intra-molecular interactions between the polymer chains themselves, using two independent methods.

1. Introduction

Mucoadhesive drug formulations are generally considered advantageous, both to prolong the treatment time and the effect of locally acting drugs, but also as a strategy for increasing the bioavailability of elsehow poorly absorbed drugs, for example proteins and other peptides. In the last case, especially the buccal cavity has emerged as a promising alternative to peroral systemic administration, due to a lower enzymatic activity, a better accessibility and its robustness (Bruschi and de Freitas, 2005). Another advantage of the buccal cavity in the context of exploitation of mucoadhesion is the lower amount of fluid present as compared to for example the gastrointestinal tract. In fact, already in 2005, Rossi et al. published a paper suggesting that buccal drug delivery was a challenge already won. However, even under conditions when the amount of water is rather low, the hydration of a mucoadhesive formulation will increase with time, which will eventually lead to reduced adhesive properties (Surapaneni et al., 2006). When developing a formulation intended to stay on the mucosa for prolonged periods of time, it is therefore of importance to have knowledge about the mucoadhesive properties of polymers in an aqueous environment.

Many different polymers have been synthesized and tested with regard to mucoadhesion (Grabovac et al., 2005). Pectins are cheap, safe and abundant polymers that have been used extensively by the pharmaceutical and food industries (Bengmark, 1998). Commercial pectins consist of a galacturonic acid backbone (Fig. 1). The acid groups can be esterified (methoxylated) or amidated, as indicated by R in the figure. Based on the relative amount of ester groups, pectins are classified into high- and low-ester pectin (HM and LM pectin, respectively). Additionally, LM pectin can be amidated (NH pectin). Due to the manufacturing process, the average molecular weight (Mw) will typically vary between the types. Both the Mw and chemical structure are expected to influence on the mucoadhesion. The mucoadhesive properties of pectins have been investigated by many groups in the past. However, controversial results were reported, and still there is no consensus as to which type of pectin is optimal in view of mucoadhesiveness. LM pectin was reported to be more mucoadhesive than HM pectin for solutions (Schmidgall and Hensel, 2002) and gels (Liu et al., 2005), visualized on porcine colonic tissue and demonstrated by rheological synergism. In contrast, Thirawong et al. found, using a texture analyzer, that HM pectin discs were more mucoadhesive towards a porcine GI mucosa. Amidation had a positive effect on the mucoadhesion of LM pectin (Thirawong et al., 2007). Later they confirmed these findings by rheological synergism studies of pectin solutions and commercially available mucin (Thirawong et al., 2008).

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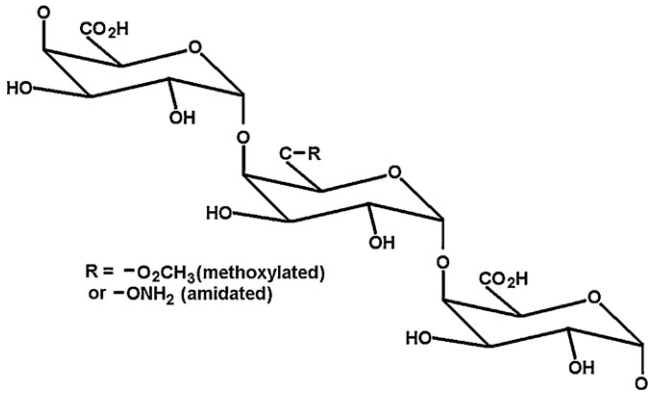

Fig. 1. A schematic illustration showing a part of the galacturonic backbone of pectin, as well as the substitution at the carboxylic acid.

One reason for these conflicting results may be the use of different formulations and methods to test mucoadhesion. Unfortunately, no standardized method exists, and the testing was done in many different ways, ranging from un-physiological *in vitro* situations to *in vivo* testing in animals and humans. Moreover, mucoadhesive interactions have been tested from nanoscale to bulk-level. Advantages and disadvantages exist for all methods. Generally speaking, there seems to be a conflict between gaining information about the important factors and mechanisms involved, and at the same time simulating the relevant *in vivo* conditions.

Atomic Force Microscopy (AFM) is an *in vitro* method that explores the topology and, occasionally, elasticity of a sample in a non-invasive way. A minute tip on a cantilever serves as a nanoscopic finger that probes a sample by scanning it for surface modulations. Essentially, an AFM generates an image that is a 3D map of constant interaction potential between the tip and the surface. The tip-sample interaction is, in a sense, translated into small changes in the bending of the cantilever as it touches or comes close to the sample. The reflection from a laser beam that is incident on the cantilever face can be read on a detector, electronically assembling the image proper from the cantilever deflection. Depending on the AFM settings and the sample details, the resolution for wet samples may go down to the Ångström range, and the images are then depicted on the cm-scale, i.e. in a magnification of $\sim 10^8$.

This setup can allow mucoadhesion to be directly tested on a molecular scale by measuring the force–distance curve between a mucus surface and a polymer microshere that is attached to an AFM cantilever as a colloidal tip. This approach was successfully used by Cleary et al. to study the molecular interaction between glass beads coated with a co-polymer and bovine submaxillary mucin on a planar carrier, in aqueous surroundings under different pH and salt concentrations (Cleary et al., 2004). Later Catron et al. (2006) did a similar study, but used a sharp AFM tip that was covalently functionalized with polymer molecules to test their affinity for adhering to a mucous interface. However, in Cleary et al.ĭs self-assessment of their method they point to its complexity regarding functionalization of both the colloidal probe and the planar surface, as well as the effect on reproducibility. This is also indicated by Li et al. (2010) who mostly focused on achieving a smooth and uniform polymer coating of the AFM tip.

The alternative is to focus on topological images that refrain from detailed force measurements, but give an overview of the coverage of a surface with mucin, and on its modification upon interaction with another polymer (Dedinaite et al., 2005). Scans of polymers premixed with mucin have also been used (Deacon et al., 2000). Even this may be a challenge, as getting good and informative pictures of soft matter, especially of swelling polymers in an aqueous environment, is difficult, and there is a risk that the assessment of the pictures will become somewhat subjective unless a detailed method for the image analysis can be defined.

In this paper we report on a study about the mucoadhesion of pectins, performed by AFM in an aqueous environment cell. We propose to compare and quantify the different interaction behavior found by use of statistical roughness parameters that can be extracted by image analysis from the topographical pictures taken by AFM. Bovine submaxillary mucin (BSM), earlier used to simulate the buccal mucosa, was deposited onto mica and then scanned in air and immersed in water. The pre-coated mica surface was then incubated with different pectin polymers that were introduced into the bulk of the water cell. After washing to remove excessive polymer, AFM images were taken under full hydration. The images obtained were assessed by extracting surface roughness parameters. Their changes depending on the sample details were taken as quantifiers of mucosal adhesion of the polymers under regard. The three main commercially available types of pectin (LM, NH and HM pectin, Table 1) were tested by this method and the outcome was compared to the one obtained for polyacrylic acid (PAA) as a positive control and to the one for polyvinyl pyrrolidone (PVP) as a negative control. PAA and PVP are known to possess high and low mucoadhesion, respectively, e.g. (Thongborisute and Takeuchi, 2008). The results for pectin were validated by an independent method based on surface plasmon resonance (SPR). In short, the change of refractive index upon adhesion of polymer to a test chip is optically explored by the shift of the Plasmon resonance angle in total reflection conditions, which is converted to SPR response measured in resonance units (RU). The SPR response correlates linearly with mass bound to the sensorchip plus the refractive index of the bulk medium (Mol and Fischer, 2010). The test chip consisted of an Au surface that was pre-coated with mucin and mounted into the flow cell of a Biacore instrument. Upon injection of the different polymers into the flow cell, their mucoadhesion was directly monitored. If the polymer permanently binds to the mucinated surface, the refractive index along the sensor chip surface is modified, leading to a quantitative SPR signal in so called response units (RU, in effect proportional to the interfacial refractive index).

2. Materials and methods

2.1. Materials

Mucin from bovine submaxillary glands (BSM), type I–S, batch 068K7001, was purchased from Sigma–Aldrich and used as received.

5 different test polymers were used, all dissolved in pure water. The LM, NH and HM pectin were derived from citrus and kindly provided by the manufacturer (CP Kelco, Denmark) and used as received. Details are listed in Table 1. The pectins differed in the degree of and functionalization (methoxylation and amidation) of the acid groups, as well as Mw. Polyacrylic acid (PAA; Carbopol 980, batch 80035089) and polyvinyl pyrrolidone (PVP; Povidone 25, batch 81430488) were used as positive and negative control, respectively. They were both of Ph. Eur. quality and purchased from Caelo, Hilden, Germany. Both were used as received.

Milli-Q water from a QTUM000EX obtained from Millipore A/S, with a 0.22 μm filter unit on the outlet was used as solvent.

All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Atomic force microscopy (AFM)

AFM was performed with a PicoScan – AFM (PicoSPM from Molecular Imaging Inc. (now Agilent Technologies Inc.)) using a scanner that has a maximal range of 30 μm × 30 μm. Sam-