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Synthesis and characterisation of mucoadhesive thiolated polyallylamine

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ABSTRACT

The thiolation of polyallylamine (PAAm) for use in mucoadhesive drug delivery has been achieved. PAAm was reacted with different ratios of Traut's reagent, yielding products with thiol contents ranging from 134–487 μmol/g. Full mucoadhesive characterisation of the thiolated PAAm samples was conducted using swelling studies, mucoadhesive testing on porcine intestinal tissue and rheology. Both swelling and cohesive properties of the thiolated PAAm products were vastly improved in comparison to an unmodified PAAm control. The swelling abilities of the thiolated samples were high and the degree of thiolation of the products affected the initial rate of swelling. High levels of mucoadhesion were demonstrated by the thiolated PAAm samples, with adhesion times of greater than 24 h measured for all three samples and, thus, thiol content did not appear to influence mucoadhesion. Rheological studies of the thiolated PAAm samples showed an increase in G' and G'' values upon the addition of a mucin solution which was not observed in the unmodified control, again highlighting the mucoadhesive interactions between these thiolated polymers and mucin. The synthesis of thiolated PAAm by reaction with Traut's reagent and resulting mucoadhesive properties demonstrates its potential for use a mucoadhesive drug delivery device.

1. Introduction

Mucoadhesion is the binding of a material to a mucosal surface. The mucosal surface of the body can have a rate of absorption of up to four times that of the skin, depending on the site of interest (Madhav et al., 2009) and has great potential as a route of drug administration. Mucoadhesive polymeric drug delivery devices allow for the slow and controlled release of a drug to a specific site, with fewer side effects and greater drug bioavailability in comparison to other methods of drug delivery such as oral delivery. Improved bioavailability may be due to the intimate contact and increased contact time between the drug-loaded mucoadhesive polymer and the target area, reducing the time of action for the drug at the target site.

Polyallylamine has a simple structure with repeating primary amine moieties attached to the backbone. It is available in solution in its free base form, PAAm, or in a salt form as polyallylamine hydrochloride, PAH, or polyallylamine carbonate. The thiolation of polymers has been shown to improve mucoadhesive abilities by up to 140-fold (Bernkop-Schnürch, 2005) by the formation of disulphide bonds between the thiol groups on the polymer to the cysteine rich domains of mucosal glycoproteins, mucins. Thus far, limited work has been published on the thiolation of polyallyalmine or its potential use as a mucoadhesive drug delivery system. As the polymer backbone comprises entirely of primary amines, the potential levels of thiolation could be extremely high. Modification of just 5% of these primary amines using a 15 kDa polymer may result in approximately 900 μmol/g thiol content once thiolated. Thiolation of polyallylamine has previously been conducted using two different methods: thiolation with *N*-acetyl cysteine and EDC (Vigl et al., 2009) or thiolation with Traut's reagent (Bacalocostantis et al., 2012); however, neither paper explored the mucoadhesive properties of the resulting thiolated polyallylamine. Both methods of thiolation, reacting with *N*-acetyl cysteine/EDC and with Traut's reagent, were used by Ibie et al. (2015b) and the resulting thiol content measured 60 μmol/g and 490 μmol/g, respectively. The mucoadhesive properties of the resulting thiolated polyallylamine products were tested by determining the levels of adsorption of a porcine mucin solution to both the thiolated polymers and unmodified

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polyallylamine by UV/vis spectrometry. Thiolation of the polymer displayed improved mucin interactions, increasing the percentage of mucin adsorption from approximately 40% in the unmodified PAAm sample to 65–70% in the thiolated PAAm samples. However, no swelling tests or mucoadhesive testing on mucosal tissue were conducted on either of the thiolated samples.

Due to the cationic nature of polyallylamine, the polymer has been shown to be toxic to both cells and microbes due to its ability to interact with and disrupt the cell wall (Andrews et al., 2011; Boussif et al., 1999; Iarikov et al., 2013; Vigl et al., 2009). In using PAAm as a mucoadhesive drug delivery system, it is vital that the polymeric matrix is not toxic to cells and a decrease in the cytotoxic properties of PAAm was observed upon thiolation (Ibie et al., 2015a; Vigl et al., 2009). Vigl et al. (2009) thiolated a 15 kDa PAH with *N*-acetyl cysteine and EDC, resulting in a thiol content of 77.6 μmol/. Cytotoxicity assays were conducted on both the unmodified PAH and thiolated PAH samples; the unmodified sample displayed 100% cell death but, once thiolated, the 15 kDa thiolated PAH sample displayed lower levels of cytotoxicity at approximately 92% cell death. It was, however, noted that thiol content of the samples was low and that an increase in thiol content may decrease the cytotoxicity. Using an MTT assay, Ibie et al. (2015a) investigated the cell viability of Caco-2 cells having treated them with unmodified and thiolated PAAm samples. The thiolated samples were thiolated with either Traut's reagent or *N*-acetyl cysteine/EDC, resulting in a total thiol content (free thiol and disulphide bond contents) of 1080 μmol/g and 340 μmol/g, respectively. The unmodified PAAm sample showed the highest level of cell toxicity, due to its cationic backbone. Upon thiolation, cell toxicity reduced. The samples thiolated with Traut's reagent were observed to have lower levels of toxicity towards cells and it was thought that this was due to the higher levels of thiolation and, therefore, lower free amine groups along the polymer backbone. In thiolating with Traut's reagent, the proposed reaction schematic is shown in Fig. 1, the nature of the polymer may change due to the decrease in free amine groups along the polymer backbone and introduction of the Traut's reagent pendant chain. With the addition of a charged imine group, and chain with has a free thiol group at its end, this may influence the orientation of the polymer and the overall possible intramolecular which may occur naturally within PAAm.

In this study, polyallylamine, in its free base form PAAm, was thiolated with varying ratios of Traut's reagent, yielding three thiolated products. Full mucoadhesive characterisation of thiolated PAAm has not been discussed before and, therefore, these products was fully assessed for their mucoadhesive properties by investigating their swelling ability and cohesion, mucoadhesive properties on porcine intestinal tissue and rheological properties. Further characterisation by thermal analysis was conducted. This gives an in-depth insight in the mucoadhesive properties of a more novel thiolated synthetic polymer and investigates its potential as a mucoadhesive drug delivery device. Polyacrylic acid is an anionic synthetic polymer which, once thiolated, is commonly used in mucoadhesive drug delivery (Bernkop-Schnürch and Steininger, 2000; Hornof et al., 2003; Iqbal et al., 2012; Marschütz and Bernkop-Schnürch, 2002; Palmberger et al., 2007). Utilising PAAm, a cationic synthetic polymer, as a mucoadhesive delivery device could allow for a greater range of drugs to be deliverable by mucoadhesive delivery systems as it has the potential to form polyion/counterion complexes and the ability to deliver drugs of opposite charge to that of thiolated polyacrylic acid.

2. Materials and methods

2.1. Thiolation of polyallylamine (PAAm)

PAAm of 15 kDa MW (Polysciences Europe, Germany) was dissolved in deionised water. The pH of the solution was adjusted from pH 9.5 to approximately pH 7.5 with 1 M HCl. To this, 0.375 mg, 0.1875 mg and 0.075 mg of Traut's reagent (Soltec Ventures, USA) per mg of polymer were added to achieve 5%, 2.5% and 1% theoretical thiolation of the polyallylamine backbone, respectively. The solution was left stirring at room temperature for 20 min, before it was readjusted to pH 5 with 1 M HCl. The solution was then left stirring, again at room temperature, for 3 h before being dialysed exhaustively in the dark in a 3.5 kDa cellulose membrane against 5 mM HCl and 1 mM HCl for 5 days. After dialysis, the samples were frozen and freeze dried.

2.2. Thiol content determination

The thiol content of the samples was determined by iodometric titration in accordance to Vigl et al. (2009). 1 mg/mL thiolated polymer solutions were dissolved in deionised water and the pH of the solutions was adjusted to between 1 and 2. The samples were titrated against a 1 mM iodine solution to which 300 μL of a 1% starch indicator was added to aid end point detection. The end point was noted as a colour change from colourless to a permanent light blue colour. Thiol content was determined from a set of standards which were also titrated against a 1 mM iodine solution. Thiolated PAAm samples were also reduced using $NaBH_4$ to analyse

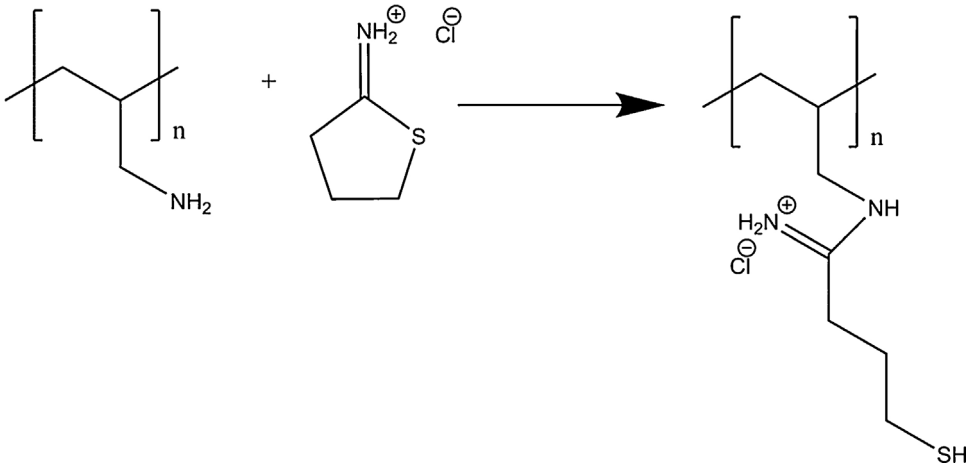

Fig. 1. Thiolation of PAAm with Traut's reagent (Bacalocostantis et al., 2012).