Carbohydrate Polymers 143 (2016) 223–230

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Tragacanth as an oral peptide and protein delivery carrier: Characterization and mucoadhesion

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ARTICLE INFO

Article history:
Received 30 October 2015
Received in revised form 20 January 2016
Accepted 31 January 2016
Available online 2 February 2016

Keywords:
Tragacanth
Flow behaviour
Acid gelation
Carrier
Oral administration
Protein/peptides drug delivery
Mucoadhesive
Alginate
Chitosan
PVP
Insulin

ABSTRACT

Biopolymers such as tragacanth, an anionic polysaccharide gum, can be alternative polymeric carrier for physiologically important peptides and proteins. Characterization of tragacanth is thus essential for providing a foundation for possible applications. Rheological studies colloidal solution of tragacanth at pH 3, 5 or 7 were carried out by means of steady shear and small amplitude oscillatory measurements. Tragacanth mucoadhesivity was also analyzed using an applicable rheological method and compared to chitosan, alginate and PVP. The particle size and zeta potential were measured by a zetasizer. Thermal properties of solutions were obtained using a differential scanning calorimetry. The solution exhibited shear-thinning characteristics. The value of the storage modulus (G') and the loss modulus (G'') increased with an increase in angular frequency (Ω). In all cases, loss modulus values were higher than storage values ($G'' > G'$) and viscous character was, therefore, dominant. Tragacanth and alginate showed a good mucoadhesion. Tragacanth upon dispersion created particles of a submicron size with a negative zeta potential (−7.98 to −11.92 mV). These properties were pH dependant resulting in acid gel formation at pH 3.5. Tragacanth has thus a potential to be used as an excipient for peptide/protein delivery.

1. Introduction

An alternative approach of parenteral delivery to administer proteins and peptides orally has encouraged various efforts at delivery development. Development of high bioavailability of oral protein and peptides delivery systems can be achieved through three practical ways: (1) modification of delivery carrier; (2) physicochemical properties change of macromolecules or (3) addition of new function to macromolecules. Obviously, it is important that these methods can retain the biological activity of the protein and peptides (Mathiowitz et al., 1997; Morishita & Peppas, 2006).

The first peptide to be used as a drug was insulin (for treatment of diabetes) and since then numerous proteins and peptides drug have been reported in almost every field of medicine. There are more than 130 currently used protein therapeutics and over 1000 proteins/peptides are being tested in human clinical trials (Yadav, Kumari, & Yadav, 2011). Oral administration of drug is the most widely used route of administration, even though it is generally not practicable for protein and peptide based drugs. Due to enzymatic degradation and poor penetration of the intestinal membrane, oral bioavailability of biologicals is usually very low. Much study has been done in recent years about macromolecular drug absorption from the gastrointestinal (GI) tract, such as the barriers that limit GI absorption. Several approaches have been proposed to overcome such barriers and to create effective oral delivery systems for proteins and peptides (Morishita & Peppas, 2006).

To improve the efficiency of oral delivery of peptides and protein and overcome the gastrointestinal barriers, various carriers have been assessed and developed. Much research in recent years has developed hydrogels and carriers based on biodegradable polymers, such as polypeptides and natural biopolymers. Polysaccharides, such as chitosan, alginate, cellulose and starch, are widely applied in biomedical and pharmaceutical fields because of biocompatibility, low toxicity, and economic benefits (Gao et al., 2014).

Mucoadhesive polymers can be used to improve bioavailability of peptides and protein during oral administration. These materials can preserve contact with intestinal epithelium for extended periods, promoting penetration of active drug through and between cells due to the concentration gradient between nanoparticles and intestinal membrane. Consequently, bioavailability of the peptides and protein is increased leading to improved patient compliance (George & Abraham, 2006). Chitosan and alginate are most popular

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http://dx.doi.org/10.1016/j.carbpol.2016.01.074
0144-8617/

among mucoadhesive polymers used for protein/peptides delivery (Sonia & Sharma, 2012). Tragacanth, a polysaccharide, can potentially become an excipient during delivery system as it has demonstrated high mucoadhesive properties previously (Jackson & Perkins, 2001).

Tragacanth is an anionic polysaccharide gum (molecular weight of 850 kD) obtained from the stems and branches of different species of Astragalus. It is highly acid-resistant and comprises of two major elements: (1) tragacantic acid and arabinogalactan and (2) bassorin (Firooz, Mohammadifar, & Haratian, 2012; Kaffashi, Zandieh, & Khadiv-Parsi, 2006; Mohammadifar, Musavi, Kiumarsi, & Williams, 2006). The peptide/protein delivery mechanism of tragacanth maybe achieved through two types of mechanisms: polyelectrolyte complexes (PECs) and entrapment through hydrogel. Polyelectrolyte complexes are made up of oppositely charged (cationic and anionic) biopolymers formulated under mild conditions to transport peptides/proteins and colloidal carriers. This complexation shows potential as a vehicle to encapsulate proteins and provide protection and sustained release of protein/peptides (McClements, 2014; Sarmento et al., 2006b). Hydrogel is an insoluble semi permeable matrix that can be used to entrap protein/peptide. It can be produced from biopolymers from animal- or plant-based derivatives (Lim, Tey, & Chan, 2014).

In order to select appropriate biopolymer for protein/peptides delivery, comparing the physical and mucoadhesive properties of polymers is essential. This measurement can determine their strength to potentially improve the bioavailability of protein/peptides. The relative mucoadhesion efficiencies of materials are normally reported as ranking orders that are specific to the method of evaluation (Ivarsson & Wahlgren, 2012; Tsibouklis, Middleton, Patel, & Pratten, 2013). For this reason, tragacanth as a new material need to be tested potential candidate of mucoadhesive polymer.

This study, therefore, was aimed to (1) establish physical properties of tragacanth under a range of conditions that would allow for creation of a hydrogel and/or a polyelectrolyte complex with other biopolymer or direct complex with peptide/protein, (2) assess and compare its mucoadhesivity to others biopolymers.

2. Materials and methods

2.1. Materials

The powder form of polymers, i.e. tragacanth, low molecular weight (MW) chitosan, low viscosity alginate and polyvinyl pyrrolidone (PVP), were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Dried mucin from porcine stomach, type III and glucono-δ-lactone powder (GDL) (Sigma-Aldrich) were also used without further purification. The insulin sample containing 100 U/ml of insulin was purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). The water used was of Millipore quality.

2.2. Characterization of tragacanth

2.2.1. Sample preparation

Tragacanth stock solution (2% w/w) was prepared by dissolving appropriate amount of the powder in 0.1 M citric acid phosphate buffer at different pH (3, 5 or 7). Sodium azide was added during the preparation of samples (0.2 g/l) to preserve them. The resulting solution was gently stirred at room temperature and stored overnight at 4 °C. The stock solution was then diluted with the buffer to give concentrations of 0.5% (w/w) which was used in all experiments. To characterize tragacanth after loading with insulin, mixture of tragacanth in milli-Q water and insulin were prepared by mixing insulin (0.2 mg/ml) and 0.5% tragacanth at pH 3.7 by adding GDL and gently stirring overnight to create a colloidal dispersion.

2.2.2. Rheological analysis

The ability of biopolymer to form a gel and its strength under certain condition (ex. different pH level) is crucial in predicting the behaviour of polymeric carrier. These properties can be analyzed rheologically. The flow behaviour measurements of 0.5% (w/w) tragacanth solution at different pH 3, 5, or 7 were performed with a stress-controlled rheometer (MCR 301, Anton Paar GmbH, Ostfildern, Germany) using a double gap geometry (Anton Paar). The temperature was set to 20 °C (Qomarudin et al., 2015). In order to assure that the experiments were carried out inside the linear viscoelasticity region, the samples were previously submitted to stress sweep tests. The applied stress was varied from 0 to 50%, to keep sensibility in the measurements in the frequency range and to avoid noise. Apparent viscosity and dynamic oscillation measurements were performed using the following protocol: Samples were pre-sheared 500/s at 20 °C for 0.5 min and then rested for 30 s, continued frequency sweeps were carried out at angular frequency from 0.1 to 10 rad/s at a constant strain value of 5%, then rested for 30 s. Following this, apparent viscosity was measured by applying shear rate from 0.1 to 1000/s.

To assess the effect of temperature and shearing, tragacanth was also analyzed various temperatures (60, 80 or 120 °C) and shear rates (500, 1000 or 1500/s). Briefly, tragacanth mixtures were sheared and heat treated in a pressure cell (CC 25/Pr 150/A1/SS, Anton Paar, GmbH, Ostfildern, Germany) mounted on the same rheometer using a bob and cup geometry (CC 25/PR-SN, Anton Paar) under a constant shear (500, 1000 or 1500/s) and pressure (~1 bar). All treatments were conducted at a heating rate of 5 °C/min, kept at a final temperature for 60 s, and then cooled at the rate of 5 °C/min down to 20 °C. Tragacanth flow behaviour was documented using Rheoplus 32 v2.81 (Anton Paar) software. The magnetic coupling of the pressure cell was expected to reduce the sensitivity of the rheometer, hence flow data were expressed in a log scale to minimize any influence as a consequence of coupling and log-scale variations (Liyanaarachchi, Ramchandran, & Vasiljevic, 2015).

2.2.3. Particle size and zeta potential measurements

The particle size and zeta potential of the tragacanth solution at different pH (3, 5 or 7) were measured using a zetasizer (ZEN3600, Malvern Instrument Ltd., Worchestershire, UK). Tragacanth solutions were diluted 1:100 with a citric phosphate buffer and stored at room temperature for 24 h prior to particle size analysis (Qomarudin et al., 2015). All particle-size measurements were performed in a He–Ne laser beam at 658 nm. The particle size and zeta potential after loading with the insulin was analyzed at pH 3.7.

2.2.4. Determination of loading efficiency

Loading efficiency was measured indirectly after centrifugation of tragacanth and insulin dispersion after mixing insulin (0.2 mg/ml) and 0.5% tragacanth colloidal solutions at pH 3.7. The mixture was centrifuged at 20,000 × g for 60 min at room temperature by a high-performance centrifuge (Beckman Coulter Inc., Brea, CA). The amount of insulin in supernatant was measured by the Bradford method at 595 nm. The loading efficiency was calculated as:

$$\text{Loading efficiency}\,(\%) = \frac{\text{Total amuunt of insulin} - \text{Free insulin in supernatant}}{\text{Total amount of insulin}} \times 100 \quad (1)$$