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In vitro evaluation of calcium binding capacity of chitosan and thiolated chitosan poly(isobutyl cyanoacrylate) core–shell nanoparticles

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Abstract

The ability of chitosan and its derivatives to bind cations is well known. Chitosan and thiolated chitosan were recently associated with poly(isobutyl cyanoacrylate) (PIBCA) nanoparticles leading to very promising results in terms of bioadhesion and permeation enhancement properties. Taking into account the influence that cations concentration have in the maintenance of both the permeation and the enzymatic barrier of the oral route, the possible cation binding capacity of these colloidal systems might be interesting in the use of these nanocarriers for the oral administration of pharmacologically active peptides. The aim of the present work was to in vitro evaluate the capacity of these colloidal systems to bind calcium, a model cation of physiological interest in the intestinal tract. The presence of chitosan on the nanoparticle surface importantly increased the calcium binding ability, in comparison to non-coated PIBCA nanoparticles. In addition, its presentation in the gel layer surrounding the nanoparticles, also beneficiated its binding capacity, obtaining 2–3 folds higher values when the polymer coated the nanoparticles than when it was in solution. The cross-linked structure observed for thiolated chitosan, due to the formation of inter- and intra-chain disulphide bonds, diminished the accessibility of cation to active sites of the polymer, decreasing the binding capacity of the calcium ion. However, when the amount of free thiol groups on the nanoparticle surface was high enough, the binding behaviour observed was higher than for nanoparticles elaborated with non-modified polymer.

Keywords: Chitosan; Thiolated chitosan; Poly(isobutyl cyanoacrylate); Core–shell nanoparticles; Calcium binding capacity; Cross-linking

1. Introduction

Chitosan has been extensively studied for its cation binding capacity in neutral or weakly acid media (Guibal et al., 2002). The free electronic doublet of nitrogen, which is present in the polymer in more than 7% (w/w) (Guibal et al., 2002; Chassary et al., 2004), is responsible for the sorption of many cations, especially divalent cations (Lima and Airoldi, 2003; Monteiro and Airoldi, 1999), According to Lima and Airoldi (2003), the high hydrophilicity of chitosan owing to the large number of hydroxyl groups, and the flexible polymeric chain structure which favours the adjustment of the cations dispersed in solution for complex formation, play also a role in the cation binding capacity of this polymer. In addition, chemically grafting of active groups, typically occurring on $–CH_2OH$ and $–NH_2$ groups (Guibal et al., 2002), can improve the cation binding capacity of the polymer (Becker et al., 2000; Jeon and Holl, 2003; Lima and Airoldi, 2003).

In the biopharmaceutical field, the cation binding ability of chitosan can be very useful. For example, the intactness of the drug absorption barrier in the intestine is linked to the presence of Ca^{2+} and Mg^{2+} cations. It is well-known that the reduction of extracellular Ca^{2+} concentration, for example by chelant agents such as EDTA or EGTA, can result in the opening of the tight junctions that interconnect the epithelial cells (Thanou et al., 2001; Schulzke et al., 2005), promoting the paracellular transport of active molecules (Ameye et al., 2001; Roumi et al., 2001). Thus, polymers able to bind extracellular Ca^{2+} might influence the absorption by paracellular route (Borchard et al., 1996; Kriwet and Kissel, 1996). In addition, most proteolytic enzymes present in the gastrointestinal tract are metallopeptidases that require cations such as Mg^{2+} or Zn^{2+} as cofactors at their active sites (Carboxypeptidase A, Leucine aminopeptidases, etc.). Furthermore, other peptidases, such as trypsin, need cations (Ca^{2+}) to maintain their active structure (Woodley, 1994; Ameye et al., 2001; Madsen and Peppas, 1999). The depletion of

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such as cations provokes the inhibition of proteolysis (Ameye et al., 2001), which might be also very beneficial for the efficient administration of peptidic active molecules by the oral route (Bernkop-Schnürch et al., 2004).

Chitosan and thiolated chitosan core–shell poly(isobutyl cyanoacrylates) nanoparticles were recently developed and characterised by our group (Bravo-Osuna et al., 2006, 2007a). The elaboration of those nanoparticles was based on emulsion radical polymerisation of alkyl cyanoacrylate monomers from carbohydrate chains. The block copolymer created is able to spontaneously self-associate forming particles with a hydrophobic core coated by hydrophilic polysaccharide (Chauvierre et al., 2003a,b,c). The presence of chitosan and thiolated chitosan onto the surface improved the mucoadhesive characteristics to the system (Bravo-Osuna et al., 2007b). Those colloidal systems constitute a novel approach in oral peptide delivery.

Following with the biopharmaceutical evaluation of these colloidal systems as nanocarriers for the oral delivery of peptides, the aim of this work was to evaluate in vitro the capacity of these core–shell nanoparticles to bind divalent cations of physiological interest. Among them, calcium was selected because its modulation can play an important role in the maintenance of the intestinal physiological barriers hence, in the absorption of peptidic drugs by the oral route. The effect of chemical modification of chitosan (changes in molecular weight and inclusion of thiol groups) on the cation binding capacity was evaluated in this work.

2. Materials and methods

2.1. Materials

Isobutyl cyanoacrylate (IBCA) was kindly provided as a gift by Loctite (Dublin, Ireland). Chitosan Mw 400,000 g/mol, and L-cysteine HCl were purchased from Fluka (Saint-Quentin Fallavier, France). Eriochrome® Black T was obtained from Sigma–Aldrich (Saint-Quentin Fallavier, France). 2-Iminothiolane HCl (Traut's reagent) was synthesised in the Department of Organic Chemistry (Biocis UMR CNRS 8076), Faculty of Pharmacy, University Paris-XI (Chatenay-Malabry) France.

All other chemicals were reagent grade and used as received.

2.2. Methods

2.2.1. Chitosan modifications and characterisation

Chitosan was selectively depolymerised following the method developed by Huang et al. (2004). By reaction with sodium nitrite at different concentrations (7.0 and 2.7 g/l), two final molecular weight were obtained: 20,000 g/mol (Chito20) and 50,000 g/mol (Chito50) respectively. ^{1}H NMR analysis (Bruker MSL-400 spectrometer, Bruker Instrument Inc. Wissembourg, France) showed no changes in the percentage of deacetylation after the depolymerisation process, with values around 86–88% in all cases. The capillary viscosity (viscometer AVS400, Schott Gerate) measurements showed molecular weight values according to the predicted ones in agreement with previous works (Bravo-Osuna et al., 2007a).

The inclusion of thiol groups in the different hydrolysed chitosan was carried out by reaction with 2-iminothiolane following the method developed by Bernkop-Schnürch et al. (2003). The resulting polymers were chitosan-4-thiol-butylamidine (chitosan-TBA), named Chito20-TBA and Chito50-TBA according to the original molecular weight of the corresponding non-modified polymers.

Sulphur elemental analysis (Analyzer LECO SC144, Service central d'analyse du CNRS, Vernaison, France) was performed in order to determine the total sulphur content: 4.9%, and 4.7% for Chito20-TBA and Chito50-TBA respectively. These values give the total amount of sulphur present in the sample, but they do not provide any information about the chemical state of the sulphur, whether it exists as a reactive thiol or whether it has been oxidised (unreactive disulphide). The determinations of the reactive thiol groups was performed using the iodine titration method that allows the exclusive determination of the amount of reduced sulphur groups in the polymer, which resulted to be the 35% and 36% of the total amount of sulphur for Chito20-TBA and Chito50-TBA, respectively.

2.2.2. Nanoparticles preparation

PIBCA core–shell nanoparticles were prepared by the redox radical emulsion polymerisation method developed by Chauvierre et al. (2003a,c) and optimised by Bravo-Osuna et al. (2006, 2007a). Mixtures of modified and non-modified hydrolysed chitosan (%chitosan/%chitosan-TBA: 0/100; 25/75; 50/50; 75/25; 100/0) were used as shell component.

Control non-coated PIBCA nanoparticles were elaborated by anionic emulsion polymerisation according to Monza da Silveira et al. (1998) using Poloxamer as stabiliser agent.

2.2.3. Characterisation of the nanoparticles

2.2.3.1. Size measurement. The hydrodynamic mean diameter and the size distribution of the nanoparticles were determined at 20 °C by quasi-elastic light scattering using a Nanosizer® N4 PLUS (Beckman-Couter, Villepinte, France). The scattering angle was fixed at 90°. Samples were diluted in acetic acid solution (16 μmol/l) in MilliQ® water in order to achieve a signal level ranging from 5×10^4 to 5×10^6 counts per second. The results were expressed as the mean hydrodynamic diameter, the standard deviation and the polydisperisity index of the size distribution. This lately parameter is linked with the width of the curve of hydrodynamic radius distributions, small values indicating narrower distributions. Results corresponded to the average of three determinations.

2.2.3.2. Determination of the ζ potential. The ζ potential of the polymer particles was deduced from the electrophoretic mobility of the particles measured by Laser Doppler Electrophoresis (Zetasizer Nano serie. Malvern Instruments Ltd., Worcestershire, UK) in a NaCl 1 mmol/l solution (pH 6.8) after suitable dilutions (1/200, v/v) of the different nanoparticles suspensions.

2.2.3.3. Determination of thiol content. The quantification of reduced thiol groups on the nanoparticle surface was determined using a modified iodine titration method. In brief, 0.25 ml