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Controlling the complexation of polysaccharides into multi-functional colloidal assemblies for nanomedicine

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ABSTRACT

The controlled assembly of oppositely charged polysaccharides led to colloids stable in physiological media, capable of encapsulating a molecular drug and of sorbing proteins at their interface. Two types of particles were obtained: both chitosan–dextran sulfate (CS–DS) and chitosan–heparin (CS–HP) stable over 30 days in PBS at 25 and 37 °C. At gastric pH 1.2, these particles remained stable over 3 days, enough for a stomach transit. The structural analysis by small angle X-ray scattering (SAXS) showed that CS–DS surface was semi-rough and chains inside particle exhibited rod-like conformation. Moreover, the particle interfaces could efficiently be functionalized with anti-OVA or anti-α4β7 antibodies, in PBS, with the conservation of the antibody bioactivity over at least 8 days. Finally, during the assembly process, a molecular model drug, AMP, could be encapsulated with a loading efficiency up to 72% for CS–DS particles and 66% for CS–HP. All these data establish that the controlled assembly process under equilibrium conditions lead to colloids well suited for the targeted nanodelivery of drugs.

1. Introduction

Nanoparticles have been under intense development as tools for nanomedicine [1,2] and they now cover a wide range of potential applications from cancer therapy [3] and imaging [4], to vaccine adjuvantation [5,6]. These tools tend to be more and more powerful as they can be multi-functional or response to external stimuli [7]. In the context of nanomedicine, the carriers should be safe for the patients, which require the use of non-toxic materials and also the implementation of manufacturing processes that do not involve any potentially toxic chemicals. Taking these specifications into account, colloids obtained from the complexation of oppositely charged polysaccharides are quite promising because they can be obtained from naturally occurring polymers via an all-in water process, excluding the use of chemical cross-linkers and organic solvents [8]. Chitosan, a β(1 → 4)-linked copolymer of glucosamine and N-acetyl glucosamine, obtained from partial deacetylation of chitin, has been widely used in pharmaceutical applications [1,9,10]. Many authors have used this polysaccharide as nanodelivery system [11] obtained by ionic gelation [12] or via the formation of polyelectrolyte complexes with various polyanions [13]. Over the years, our group intensively investigated the formation of colloidal carriers from the complexation between dextran sulfate as polyanion and chitosan as polycation (i) from the methodological and mechanism standpoint and (ii) for the delivery of antigens in a vaccine adjuvantation prospect [14]. The particle formation procedure, very simple to implement, consisted in mixing two aqueous solutions each containing one of the polymers. Instantaneously, a colloidal complex formed visually characterized by a milky homogeneous dispersion [15,16]. This procedure has been widely used to obtain polysaccharide-based nanocomplexes with a great variety of counterparts [8] but, to remain in the colloidal domain, a high dilution, around 0.1% w/v in polymers, was used to limit the chain interpenetration, leading to macroscopic objects [15,16]. To increase the polymer concentration in the media, and remain in the colloidal domain, one possibility was to move from a kinetically controlled process, to one in which the macromolecular assembly would be close to equilibrium. In a recent work [17], we established the feasibility of such strategy by adding to the polyelectrolyte solutions a critical amount of sodium chloride to screen the attractive electrostatic interactions and prevent assembly on mixing. Then, the salt was dialyzed from the polysaccharide mixture allowing a slow assembly of the oppositely charged polymers. Indeed, this approach allowed us to increase up to 30-folds the polymer concentration in the mixture. Moreover, the obtained particles featured a long term colloidal stability over 40 days on storing at 37 °C in water.

Abbreviations: SSC, screening salt concentration; PSC, particle solid content.

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Our objective in the present work is double. First demonstrates that this strategy can be extended to another naturally occurring polyanion such as Heparin and second to prove that this methodology is compatible with the development of highly performing drug delivery systems by investigating the colloidal stability in physiological media, the encapsulation of a model drug and the surface sorption of various proteins for targeting. Our long-term goal is to develop a safe and efficient anti retroviral drug nanodelivery system targeting the natural reservoirs of HIV. AIDS is now considered as a mucosal disease, with most of the critical events (e.g., transmission, viral amplification, CD4+ *T*-cell destruction) occurring in the gastrointestinal tract, thus leading to the establishment of reservoirs [18]. Among them, a subpopulation of lymphoid *T* cells bear the α4β7 integrin receptor at their surface [19] which constitute a target for clearing the virus from infected individuals [20].

2. Materials and methods

2.1. Materials

The initial chitosan produced from chitin squid pens, with indexes 113, with Mw 532,000 g mol^{-1} and degree of acetylation of 1.5% was purchased from Mahtani chitosan Pvt. Ltd. Before all experiments, the sample was purified by a successively filtration through Millipore membranes of porosity: 3, 1.2, 0.8 and 0.45 μm. Purified chitosan was then N-acetylated with acetic anhydride in a hydroalcoholic mixture according to the procedure described by Vachoud et al. [21]. After N-acetylation duration reaction, the polymer was precipitated, rinsed with de-ionized water and then freeze dried.

Hydrolysis by controlled nitrous deamination was performed to produce low molar weight polymers [16]. Chitosan was dissolved at 0.5% (w/v) in a 0.2 M acetic acid/0.15 M sodium acetate buffer. A 1 g L^{-1} sodium nitrite was added to chitosan solution to obtain a nitrite/glucosamine units molar ratio of 0.1. The reaction was performed under high mechanical stirring for 1 h to obtain Mw close to 150,000 g mol^{-1}. Likewise, in the end of the hydrolysis reaction, chitosan was recovered by precipitation with ammonia until pH 9–10, followed by repeated washings with deionized water until neutrality and finally lyophilized.

Dextran sulfate and heparin sodium salt from porcine intestinal mucosa were purchased from Sigma Aldrich and were used without further purification. The average molar masses of these polyanions were determined by gel permeation chromatography, according to our previous work [16]. The weight average molar mass of dextran sulfate used was 1.28×10^6 g mol^{-1} ± 5000 with a polydispersity index *Ip* of 1.9 ± 0.1. Heparin weight average molar mass was 1.8×10^4 g mol^{-1} ± 5000 with a polydispersity index *Ip* of 1.0 ± 0.1. The water content was determined by thermogravimetric analysis (TA Instrument TGA Q500). The degree of sulfation, corresponding to the number of sulfate moieties per glucosidic unit was determined by colloidal titration using toluidine blue [16]. The degree of sulfation was 2.1 for dextran sulfate and 3.18 for heparin.

The monoclonal Immunoglobulin A (IgA), a chimeric anti-Ovalbumin (anti-OVA) clone A1 and a chimeric anti-α4β7 Integrin clone 7G3 were provided by B Cell Design (Limoges, France). Before using, their concentrations were confirmed by BCA Assay according to the procedure provided by Pierce, Thermo Fischer Scientific. Phosphate buffered solution (PBS) was from Invitrogen and the drug model adenosine 5′-monophosphate monohydrate (AMP) with 97% of purity was from Sigma Aldrich.

2.2. Methods

2.2.1. Characterization of chitosan

Degree of acetylation was determined by ^{1}H NMR spectroscopy (Brucker Avance III 400 MHz) at 25 °C according to the method developed by Hiraï et al. [22]. The water content was determined by thermogravimetric analysis (TA Instrument TGA Q500). The weight average molar mass Mw and the polydispersity indexes (*Ip*) were measured by size exclusion chromatography (2500 and 6000 PW TSK gel columns from Tosohaas) coupled on-line with a differential refractometer (Wyatt Optilab T-rEx) and a multiangle laser light scattering detector (Wyatt Dawn EOS) operating at λ = 633 nm. A degassed 0.2 M acetic acid/0.15 M ammonium acetate buffer with a pH 4.5 was used as the eluent. The flow rate was maintained at 0.5 mL/min. The refractive index increments (dn/dc) for each degree of acetylation of chitosan were determined according to a previous work [23]. The chitosan used in this study had a DA of 49% and a Mw of 133,000 g mol^{-1}.

2.2.2. Preparation of chitosan based nanoparticles by controlled polyelectrolytes association

Chitosan was dispersed at 1.5% (w/v), 3% (w/v) and 4% (w/v) in deionized water taking into account the residual water. The dissolution was achieved under mechanical stirring by adding a stoichiometric amount of acetic acid with respect to the free amine groups corresponding to the degree of acetylation used. The pH was adjusted to 4.0 with 0.1 M acetic acid. Then, sodium chloride was added to reach 2 mol L^{-1} or higher (3; 4; 5 and 6 mol L^{-1}) to assess the impact of the ionic strength of the starting solution on the structural aspect of the colloids (see Section 2.2.6.5). Dextran sulfate solutions at 1.5% (w/v) and 3% (w/v) and heparin solutions at 1.5% (w/v), 3% (w/v) and 4% (w/v) were prepared directly in deionized water, the pH was adjusted and the concentration of salt was 2 mol L^{-1}.

The polyelectrolyte complex formation by controlled association was performed at room temperature. Dextran sulfate or heparin solutions were mixed with the chitosan solution at a molar charge ratio $R(n^+/n^-) = 2$, under a constant magnetic stirring of 800 rpm. This solution was then poured into a dialysis bag (Spectra/Por®) with a molecular weight cut-off (MWCO) of 3500 to progressively remove the salt and, consequently, induce the complexation of the two natural polyelectrolytes. Dialysis was carried out against deionized water and the bath was changed every hour during the three first hours. It was then changed every 2 h until the end of the dialysis process (8 h).

2.2.3. Nanoparticle colloidal stability investigation

After formation, the CS–DS and CS–HP particles were let to stabilize 10 days at 37 °C. They were centrifuged 1 h at 23 000 g and re-dispersed at different solid contents in PBS, hydrochloride acid at pH 1.2. The particle stability monitoring was achieved by measuring the sizes and zeta potentials at various time intervals of samples stored at RT or 37 °C.

2.2.4. Entrapment of adenosine 5′-monophosphate monohydrate (AMP) into nanoparticles

To prepare AMP loaded CH–DS or CS–HP nanoparticles, 40 mg of AMP was dissolved in 6 mL of 1 M NaOH. One volume was then diluted into 1 M acetic acid, to obtain an AMP concentration of *ca* 20 mg mL^{-1}. Then, the pH of this solution was adjusted to 7–8 using a few microliters of pure acetic acid, to prevent the precipitation of chitosan in the following step. A volume of AMP solution corresponding to 10%, 20%, 30% and 50% mass ratios (m_{AMP} (mg)/$m_{Chitosan}$ (mg)) was then added to polyanions and salt solution required for complexation. This mixture was then added in one shot into the chitosan/salt solution maintained under a constant magnetic stirring of 800 rpm. This solution was put to dialysis using a Spectra/Por® membrane with MWCO of 100, to prevent the loss of drug into water bath. To avoid the loss of drug in the dialysis solution, the water bath remained unchanged throughout the dialysis step (5 h).