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Novel self-associative and multiphasic nanostructured soft carriers based on amphiphilic hyaluronic acid derivatives

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ABSTRACT

The purpose of the present study was to investigate the physicochemical properties in aqueous media of amphiphilic hyaluronic acid (HA) derivatives obtained by reaction of HA's hydroxyl groups with octenyl succinic anhydride (OSA). The self-associative properties of the resulting octenyl succinic anhydride-modified hyaluronic acid (OSA-HA) derivatives were studied by fluorescence spectroscopy using Nile Red as fluorophore. The morphology, size and surface charge of the OSA-HA assemblies were determined by transmission electron microscopy, dynamic light scattering and by measuring their electrophoretic mobility, respectively. OSA-HA was shown to spontaneously self-associate in aqueous media into negatively charged spherical and multiphasic nanostructures with a hydrodynamic diameter between 170 and 230 nm and to solubilize hydrophobic compounds such as Nile Red. This was a good indication that OSA-HA could be used as building block for the formulation of soft nanocarriers towards the encapsulation and controlled delivery of hydrophobic active ingredients or drugs.

Abbreviations: C_{10}-HA, hyaluronic acid modified with alkyl chains containing 10 carbon atoms; C_{12}-HA, hyaluronic acid modified with alkyl chains containing 12 carbon atoms; C_{18}-HA, hyaluronic acid modified with alkyl chains containing 18 carbon atoms; CAC, critical aggregation concentration; C(domain), concentration of hydrophobic domains inside the polymeric micelles; d_H, average hydrodynamic diameter of the polymeric micelles; d_i, diameter of the polymeric micelle class *i*; DLS, dynamic light scattering; DS, degree of substitution; DVS, divinyl sulfone; f_i, probability density of the polymeric micelle class *i*; HA, hyaluronic acid; *l(between.domains)*, distance between vicinal hydrophobic domains in the polymeric micelles; *l(HAu)*, length of the HA unit; *l(OS)*, length of the octenyl succinate chain; MW, molecular weight; *MW(HAu)*, molecular weight of the HA unit; *MW(OSA)*, molecular weight of octenyl succinic anhydride; *n*, refractive index; N_A, Avogadro number; *N(domains)/micelle*, number of hydrophobic domains per polymeric micelle; *N(OSA-HAu)/domain*, number of modified units per hydrophobic domain; OS, octenyl succinate; OSA, octenyl succinic anhydride; OSA-HA, octenyl succinic anhydride-modified hyaluronic acid; OSA6-HA, octenyl succinic anhydride-modified hyaluronic acid with a degree of substitution equal to 6% per disaccharide unit; OSA18-HA, octenyl succinic anhydride-modified hyaluronic acid with a degree of substitution equal to 18% per disaccharide unit; OSA43-HA, octenyl succinic anhydride-modified hyaluronic acid with a degree of substitution equal to 43% per disaccharide unit; *P*, function defined in Eq. (2); PBS, phosphate buffered saline; *q*, scattering vector; RGD, Rayleigh–Gans–Debye; RHAMM, receptor for HA-mediated motility; *R(micelle)*, average radius of the most occurring polymeric micelle; Rt, room temperature; TEM, transmission electron microscopy; THF, tetrahydrofurane; *w(HAu)*, width of the HA unit; α, swelling coefficient; λ, wavelength of light in vacuum; $\rho(HA)$, density of HA; $\rho(OSA\text{-}HA)$, density of OSA-HA; θ, scattering angle.

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1. Introduction

Hyaluronic acid (HA) is a natural linear polysaccharide consisting of D-glucuronic acid and *N*-acetyl-D-glucosamine linked through β-1,3 glycosidic bonds while consecutive disaccharide units are linked through β-1,4 bonds (Weissman & Meyer, 1954). HA is found in all mammalian tissues and is particularly abundant in the vitreous humor of the eye, the synovial fluid of the knee and the skin (Baier Leach & Schmidt, 2004). As biomacromolecule, HA possesses a large number of biological functions. It is for example known to mediate fundamental processes such as cell proliferation, differentiation and migration by binding with cells through specific interactions with hyaladherins such as the cellular receptor CD44 and the receptor for HA-mediated motility (RHAMM) (Baier Leach & Schmidt, 2004).

Due to its native unmatched biocompatibility and resorbability, physicochemical and biological properties as well as to the ease of its chemical functionalization, HA constitutes one of the most promising building blocks for applications in drug delivery (Gustafson, 1998). The use of native HA has already been widely studied towards ophthalmic, nasal, pulmonary and parenteral drug delivery (Liao, Jones, Forbes, Martin, & Brown, 2005). Indeed, when simply co-formulated together with a drug, HA has been shown to act as a mucoadhesive compound retaining the drug at its site of action and modifying the drug's in vivo absorption rate. Due to their relative poor complexity, these formulations can be classified as first generation HA-based drug delivery systems.

However, in cases of more challenging and demanding therapeutic situations, such as when drugs need to be protected and targeted to specific tissues, more advanced systems than co-formulations are required. In this regards, the interest of pharmacy in nanotechnology is explained by classical drug-associated issues such as poor drug solubility and instability in biological milieu (short drug half-life), poor drug bioavailability and unspecific targeting, which commonly result in the use of high drug dosages to achieve a therapeutic effect with the associated toxicity in patients and high health costs (Rawat, Singh, Saraf, & Saraf, 2006). As a consequence, the design of advanced drug delivery systems addressing these specific challenges has become more and more crucial and in this perspective, nanovehicles have often been put forward as a means to provide improved systems for the targeted and sustained/controlled delivery of drugs (Rawat et al., 2006). Second generation HA-based systems taking up a role as nanocarrier rather than simple excipient are therefore expected to be of tremendous significance for future pharmaceutical technologies.

However, the development of HA-based carriers is impeded by the high hydrophilicity and the poor biomechanical properties of the native molecule (Prestwich, Marecek, Marecek, Vercruysse, & Ziebell, 1998). The physicochemical properties of HA are indeed incompatible with the spontaneous and stable formation of segregated structures in aqueous media and the durable encapsulation of hydrophobic active ingredients or drugs. In addition, native HA displays a poor in vivo biological stability and residence time due to its relatively high turnover rate (Fraser, Laurent, & Laurent, 1997). A variety of chemical modifications have therefore been devised to provide HA with improved physicochemical properties and prolonged half life (Prestwich et al., 1998). In particular, the introduction of hydrophobic groups, for instance, alkyl chains onto the HA backbone has shown that the resulting HA derivatives exhibit significantly different physicochemical properties compared to the native polymer and that new associative systems can be created (Creuzet, Kadi, Rinaudo, & Auzley-Velty, 2006; Pelletier, Hubert, Lapicque, Payan, & Dellacherie, 2000). In a previous study, we developed a novel, simple and easily upscalable modification method for the preparation of amphiphilic HA derivatives based on the reaction between HA and octenyl succinic anhydride (OSA) in mild alkaline aqueous media (Tømmeraas & Eenschooten, 2007). The objective of this study was to investigate the physicochemical properties of a selection of OSA-HA derivatives. The self-associative properties of these derivatives were studied by fluorescence spectroscopy using Nile Red as fluorophore. The morphology, size and surface charge of the OSA-HA assemblies were determined by transmission electron microscopy, dynamic light scattering and by measuring their electrophoretic mobility, respectively. The results demonstrated that OSA-HA derivatives constitute attractive candidates for the formulation of soft nanocarriers towards the encapsulation and controlled and sustained delivery of hydrophobic active ingredients or drugs.

2. Materials and methods

2.1. Materials

The OSA-HA derivatives with a degree of substitution (DS) of 6, 18 and 43% per disaccharide unit (OSA6-HA, OSA18-HA and OSA43-HA) were prepared as described elsewhere (Eenschooten, Guillaumie, Kontogeorgis, Stenby, & Schwach-Abdellaoui, 2010). Briefly, HA (*Bacillus subtilis*-derived, Novozymes Biopharma DK A/S, Bagsværd, Denmark, with a weight average molecular weight of 21,000 Da) was first dissolved in milli-Q water at room temperature (Rt) for 6 h. $NaHCO_3$ was then added to the HA solution and mixed at Rt for 1 h. The pH of the resulting alkaline solution was measured and adjusted to 8.5 with NaOH (0.5 M). OSA was then added dropwise under vigorous stirring. The reaction medium was mixed at Rt for 16 h (overnight). The resulting crude product was collected and dialyzed against milli-Q water. The purified OSA-HA product was finally freeze-dried. Sodium chloride (NaCl), potassium chloride (KCl), disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), tetrahydrofurane (THF), acetone, Nile Red and uranyl acetate were used as purchased without further purification. The water used for sample preparation or analysis was distilled and purified to a resistivity of 18.2 MΩ cm in a milli-Q apparatus. The physicochemical properties of the OSA-HA derivatives were investigated in a phosphate buffered saline (PBS, pH 7.4) which was prepared as followed: NaCl (8.0 g), KCl (0.2 g), Na_2HPO_4 (1.4 g) and KH_2PO_4 (0.2 g) were first dissolved in milli-Q water (1 L) for 1 h, at Rt. The pH of the resulting mixture was then measured and, if necessary, adjusted to 7.4 with HCl (0.2 M) or NaOH (0.2 M). The resulting buffer was finally filtered through glass microfiber filters (GF/F 1825 110, porosity 0.7 μm; Whatman, Maidstone, United Kingdom). The uranyl acetate solution used to stain the OSA-HA polymeric micelles for the transmission electron microscopy observations was a distilled water/saturated uranyl acetate mixture (60:40, v/v).

2.2. Methods

2.2.1. Critical aggregation concentration of the OSA-HA derivatives

The critical aggregation concentration (CAC) of the OSA-HA derivatives was determined in PBS (pH 7.4) at 25 °C by fluorescence spectroscopy (FluoroMax; HORIBA Jobin Yvon Inc, Edison, New Jersey, United States) using Nile Red as fluorophore. OSA-HA was dissolved in PBS at the following concentrations: 0.001, 0.002, 0.004, 0.006, 0.008, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 g/L. The 0.2, 0.4, 0.6 and 0.8 g/L OSA-HA solutions were prepared from the 1-g/L OSA-HA solution by mixing 2, 4, 6 and 8 g of the latter into 8, 6, 4 and 2 g of PBS. Each of the remaining OSA-HA solutions was prepared from the 10-fold more concentrated OSA-HA solution by mixing 1 g of the solution into 9 g of PBS. Nile Red (3.184 mg) was dissolved in a THF/acetone mixture (50:50, v/v,