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Short communication

Comb-like ionic complexes of hyaluronic acid with alkyltrimethylammonium surfactants

Ainhoa Tolentino, Abdelilah Alla, Antxon Martínez de Ilarduya, Sebastián Muñoz-Guerra*

Department d'Enginyeria Química, Universitat Politècnica de Catalunya, ETSEIB, Diagonal 647, Barcelona 08028, Spain

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ABSTRACT

Stoichiometric complexes of hyaluronic acid with alkyltrimethylammonium surfactants bearing octadecyl, eicosyl and docosyl groups were prepared by ionic coupling in aqueous solution. The complexes were non soluble in water but soluble in organic solvents. In the solid state they self-assembled in a biphasic layered structure with the alkyl side chains forming a separate phase that melted in the 50–60 °C range. They were stable to heating up to above 200 °C.

1. Introduction

Hyaluronic acid (HyalA) is a linear polysaccharide ubiquitous in the human body. It is composed of repeating disaccharide units of β-1,3-N-acetyl glucosamine and β-1,4-glucuronic acid with a molecular weight up to 6 million Da. With excellent viscoelasticity, high moisture retention capacity, high biocompatibility and non-immunogenicity, HyalA finds a wide-range of applications in surgery, cosmetology, veterinary science, and hygiene medicine, where it has been used for over 30 years (Kogan, Soltes, Stern, & Gemeiner, 2007; Necas, Bartosikova, Brauner, & Kolar, 2008). When chemically modified by reaction of the pendant reactive groups, HyalA can be transformed into a variety of new biomaterials with properties suitable for tissue repair and regeneration (Allison & Grande-Allen, 2006; Burdick & Prestwich, 2011; Schante, Zuber, Herlin, & Vandamme, 2011). HyalA has been recently explored for its use in novel drug delivery systems with increasing enthusiasm because HyalA-binding receptors are believed to be involved in cancer metastasis (Yadav, Mishra, & Agrawal, 2008). Traditionally HyalA was extracted from rooster combs but now it is increasingly produced through microbial fermentation, which has enhanced its application interest and commercial value (Izawa et al., 2009; Liu, Liu, Li, Du, & Chen, 2011).

In these last years it has been revealed that coupling of polyelectrolytes with ionic surfactants is a convenient method for the preparation of ionic complexes with remarkable structure and properties (Macknight, Ponomarenko, & Tirrell, 1998; Ponomarenko, Waddon, Tirrell, & Macknight, 1996). Specifically, coupling of polyacids with tetraalkylammonium surfactants bearing long alkyl chains is known to lead to amphiphilic comb-like systems displaying a layered biphasic structure (Fig. 1a) able to lodge agents with chemical or biomedical activity (Portilla-Arias, García-Alvarez, Martínez de Ilarduya, Holler, & Muñoz-Guerra, 2006a, 2006b; Pérez-Camero et al., 2004; Portilla-Arias, García-Alvarez, Martínez de Ilarduya, & Muñoz-Guerra, 2007a). In this communication we wish to report on the preparation, structure and thermal behavior of complexes made of hyaluronic acid with alkyltrimethylammonium surfactants, abbreviated as *n*ATMA·HyalA, with alkyl chains containing 18, 20 and 22 carbon atoms (Fig. 1b). Recently we have reported on similar complexes made of polyuronic acids (*n*ATMA·PUR), specifically, polygalacturonic and alginic acids (Tolentino, Alla, Martínez de Ilarduya, & Muñoz-Guerra, 2011; Tolentino, Martínez de Ilarduya, Alla, & Muñoz-Guerra, 2010) and the binding of certain amphiphilic drugs to HyalA was investigated with regards to the flexibility of the polyanion (Caram-Lelham, Hed, & Sundelöf, 1997). The choice of HyalA as polyacid made in this work obeys to two reasons, to extend the ionic coupling method to this polysaccharide in order to broaden its potential as biomaterial, and to appraise how the alternating ionic structure of Hyal may affect the formation of these comb-like ionic complexes.

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* Corresponding author. Tel.: +34 934016680; fax: +34 934017150.
E-mail address: sebastian.munoz@upc.edu (S. Muñoz-Guerra).

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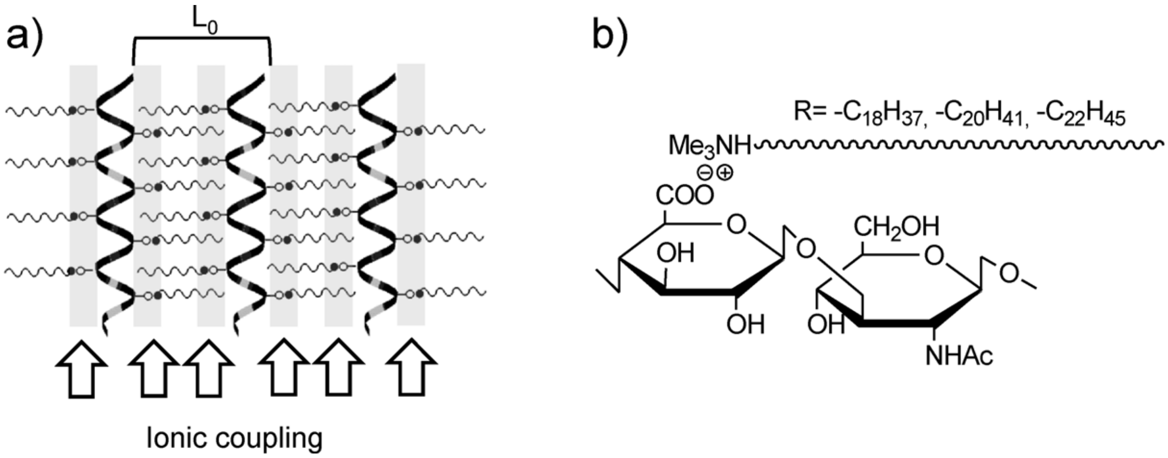

Fig. 1. (a) Side view of the layered structure usually adopted by comb-like ionic polymer complexes and (b) chemical structure of *n*ATMA·HyalA ionic complexes.

2. Experimental part

2.1. Materials

The sodium salt of hyaluronic acid (Na·HyalA), poly(sodium-β-D-glucuronate-[1-3]-β-*N*-acetyl-D-glucosamine-[1–4]), with a weight-average molecular weight of about 50,000 g mol^{-1} used in this work was purchased from Enze Chemicals. Linear alkyltrimethylammonium bromide surfactants of general formula RMe_3NBr were purchased from Sigma–Aldrich (octadecyl, $R = —C_{18}H_{37}$) or synthesized by us according to a procedure described in the literature (eicosyl, $R = —C_{20}H_{41}$ and docosyl, $R = —C_{22}H_{45}$) (Hendrix & Von Rosenberg, 1976).

2.2. Complexes preparation

The complexes were prepared by following the methodology initially reported by Ponomarenko et al. (1996) for poly(α-glutamate) complexes and later applied by us with some minor modifications to the preparation of other complexes (Pérez-Camero et al., 2004; Portilla-Arias et al., 2006a, 2006b; Tolentino et al., 2011). In brief, an aqueous solution of *n*ATMA·Br surfactant was added dropwise to a solution of Na·HyalA in water under stirring at temperatures between 40 and 60 °C depending on the surfactant. Equimolecular amounts of hyaluronic acid and surfactant were used in all cases. The complex precipitated upon standing for a few hours as a white fine powder that was isolated by centrifugation, washed several times in water and dried under vacuum.

2.3. Structure and thermal characterization methods

^{1}H NMR spectra were recorded on a Bruker AMX-300 NMR instrument with samples dissolved in deuterated water (sodium hyaluronate) or methanol-d_4 (complexes) using TMS as internal reference. Calorimetric analysis was performed under a nitrogen atmosphere in the −30 to 120 °C temperature range with a Perkin-Elmer Pyris 1 DSC instrument provided with an Intracooler device and calibrated with indium and zinc. Thermogravimetric analysis was performed at a heating rate of 10 °C min^{-1} within the 30–800 °C interval under inert atmosphere using a Perkin-Elmer TGA6 thermobalance. X-ray diffraction studies were carried out using synchrotron X-ray radiation (WAXS and SAXS at A2 Hasylab beam line of DESY in Hamburg (Germany) with an energy corresponding to a 0.15 nm wavelength.

3. Results and discussion

3.1. Synthesis of complexes

The complexes *n*ATMA·HyalA with $n = 18$, 20 and 22, were synthesized by mixing aqueous solutions of equimolecular amounts of polyacid and surfactant at the minimum temperature required to dissolve the surfactant. As a result of ionic coupling between the polyacid and the surfactant, complexes precipitated from the mixed aqueous solution after several hours of standing with release of sodium bromide. Complexes were readily isolated by centrifugation as white hygroscopic powders that were soluble in methanol but non-soluble either in chloroform or ethyl ether. The ^{1}H NMR spectra of the complexes displayed resolution enough as to ascertain the presence of the two counterparts. The spectra of Na·HyalA and 18ATMA·HyalA complex are comparatively depicted in Fig. 2 with full assignment of their respective signals (Blundell, Reed, & Almond, 2006). The composition of the complexes was accurately determined by ^{1}H NMR on the basis of the area ratio of the hyaluronic acid methyl protons signal (CH_3, $\delta = 2.0$ ppm) to the alkylammonium interior methylenes accumulative signal ($^{3-17}CH_2$, $\delta = 1.2–1.5$ ppm). The results afforded by this analysis revealed that all the *n*ATMA·HyalA complexes had essentially a stoichiometric composition showing an ATMA to HyalA ratio between 1 and 1.1.

3.2. Thermal properties

The thermal behavior of *n*ATMA·HyalA complexes was examined by both TGA and DSC, and data afforded by these analyses are gathered in Table 1. The TGA traces recorded from the complexes in the 25–800 °C range are plotted in Fig. 3 with their respective derivative curves displayed in the inset. These results indicated that *n*ATMA·HyalA complexes have a thermal stability similar to complexes of polygalacturonic and alginic complexes with onset temperatures near below 210 °C (Tolentino et al., 2011). In all cases, decomposition took place along a multi-stage process involving at least three successive stages with maximum rate decomposition temperatures in the 215–225 °C, 265–300 °C and in the proximities of 400 °C, respectively. The weight lost in these three steps was 30–35%, 40–50% and 10–15% of the original mass; no residue was left after heating at 800 °C. The thermal decomposition mechanism of comb-like ionic complexes made from polyglutamic and polymalic acids has been studied by us in some detail (Portilla-Arias et al., 2006a, 2006b; Portilla-Arias, García-Alvarez, Martínez de