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Short communication

Phase transfer-catalyzed synthesis of highly acrylated hyaluronan

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ABSTRACT

Glycosaminoglycans and its derivatives have gained increasing attention for the fabrication of hydrogels usable in biomedicine. Starting from high molecular weight hyaluronan, a natural glycosaminoglycan that forms a mayor component of the native extracellular matrix, we present an efficient phase-transfer-based synthesis for low molecular weight hyaluronan acrylates with a tailored degree of substitution (DS) ranging up to DS values of 1.7. The ability of these compounds to form dimensionally stable hydrogels was proven using different photo-initiator systems.

1. Introduction

Hyaluronan (HA) is a major component of the extracellular matrix widely distributed throughout mammalian tissues. Its intrinsic biocompatibility in combination with its easy availability makes this glycosaminoglycan highly attractive as starting material for the fabrication of hydrogels usable in various biomedical applications (Lee & Mooney, 2001; Prestwich & Kuo, 2008; Van Vlierberghe, Dubruel, & Schacht, 2011). Due to the solubility of HA in aqueous media and its rapid hydrolytic and enzymatic degradation different approaches have been reported to introduce cross-linkable groups including (meth)acrylate or vinyl residues into HA enabling the formation of temporarily stable hydrogels (Schanté, Zuber, Herlin, & Vandamme, 2011; Schiller et al., 2010; Smeds et al., 2001). HA acrylates have attracted interest as hydrogel precursors due to their comparably high reactivity and their ability to undergo not only radical polymerizations but also Michael type reactions with thiols and other deprotonable nucleophilic compounds (Hamcerencu, Desbrieres, Popa, Khoukh, & Riess, 2007; Kim, Won, & Chu, 1999; Sashiwa, Yamamori, Ichinose, Sunamoto, & Aiba, 2003). A few papers about the acrylation of HA have been published (Khetan, Katz, & Burdick, 2009; Kim et al., 2007, 2009; Lei, Gojgini, Lam, & Segura, 2011; Wang, Messman, Mays, & Baskaran, 2010), however, in most cases the acrylate group is not introduced directly into the carbohydrate backbone but via a spacers (e.g. adipic dihydrazide or succinic diester) at the carboxylic group of HA. When bound directly the procedure requires several reaction steps and the degrees of acrylation obtained are relatively low (Wang et al., 2010).

In this communication we present a very simple and effective method for the direct acrylation of low molecular weight hyaluronan with an adjustable degree of substitution using phase transfer conditions.

2. Materials and methods

2.1. Materials

HA (from *Streptococcus*, M_w = 1,175,000 g/mol, polydispersity index (PD) = 4.8) was purchased from Aqua Biochem (Dessau, Germany). Lithium phenyl-2,4,6-trimethyl-benzoylphosphinate (LAP) was prepared according to literature (Fairbanks, Schwartz, Bowman, & Anseth, 2009). I2959 (4-(2-hydroxyethoxy)phenyl-(2-hydroxy-2-propyl)ketone) was provided from Sigma–Aldrich (Taufkirchen, Germany). All other chemicals and solvents were obtained from Fluka (Buchs, Switzerland) in analytical reagent grade and used without further purification.

2.2. Methods

IR spectra were obtained on a FT-IR-Spectrometer *Nicolet Impact 410* (Thermo Scientific) applying ATR technique. NMR spectra were recorded in D_2O on a Bruker *Advance* 300 MHz spectrometer at room temperature. The degree of acrylation (DS_{AC}) giving the average number of acryloyl substituents per disaccharide repeating unit was calculated from the ratio of the proton signals at 5.9-6.4 ppm

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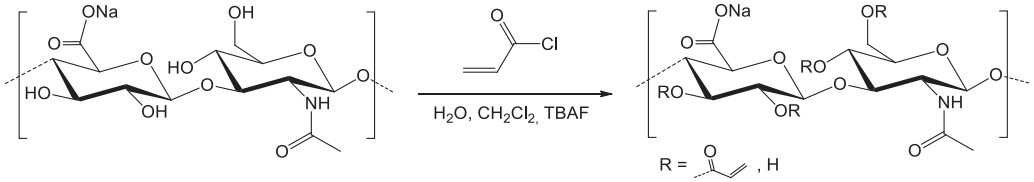

Fig. 1. Reaction scheme for the acrylation of hyaluronan.

(3 acrylic protons) and 1.9 ppm (—CH_3 of HA backbone) in the ^{1}H NMR spectra of the derivatives. Molecular weight and polydispersity determination were performed by gel permeation chromatography (GPC) analysis as described in detail in Möller et al. (2012).

2.3. Synthesis of low molecular weight hyaluronan (HA-low)

7 g HA are dissolved in 700 ml double distilled water in a screw cap bottle and the pH of the solution is adjusted to 7. The bottle is placed in an autoclave *HV-50L* (HMC-Europe GmbH) at 130 °C for 120 min. Afterwards the solution is cooled down quickly to room temperature and dialyzed against water. The product is isolated by lyophilization and dried at high vacuum. Yield: 95%. M_w (LLS) = 48,000 g/mol; PD(RI) = 1.7. FT-IR (ATR): 3416, 2920, 1616, 1552, 1413, 1373, 1321, 1230, 1202, 1147, 1074, 944, 895, 790, 746, 613 cm^{-1}. ^{1}H NMR (D_2O, 298 K): δ = 4.8–3.2 (m, hyaluronan backbone), 2.1 (s, 3H, —CH_3) ppm. ^{13}C NMR (D_2O, 298 K): δ = 175.5 (C=O), 174.7 (C=O), 103.7 (C_1), 101.1 (C_1'), 83.3 (C_3'), 80.6 (C_4), 77.0 (C_5), 76.1 (C_5'), 74.3 (C_3), 73.2 (C_2), 69.2 (C_4'), 61.3 (C_6'), 54.9 (C_2'), 23.2 (CH_3) ppm.

2.4. Acrylation of low molecular weight hyaluronan (HA-AC)

1 g HA-low is dissolved in 100 ml water. 10 mg of tetra-*n*-butyl ammonium fluoride (TBAF) is added and the solution is cooled down to 0 °C. After adding 20 ml CH_2Cl_2 and the required amount of acryloyl chloride (10-, 15- and 30-fold molar excess, respectively, per disaccharide repeating unit) the reaction is continued at 0 °C under vigorous stirring for 4 h. The aqueous phase is precipitated in acetone. The precipitate is dissolved in 200 ml water and dialyzed against water. The product was isolated by lyophilization and subsequently dried at high vacuum. With the mentioned excess of acryloyl chloride derivatives with DS_{AC} = 0.7, 0.9 and 1.7, respectively, were obtained. Yield: 98%. FT-IR (ATR): 3434, 2896, 1725, 1618, 1562, 1407, 1375, 1303, 1201, 1165, 1041, 989, 945, 894, 807, 610 cm^{-1}. ^{1}H NMR (D_2O, 289 K): δ = 6.4 (d, 1H, J = 14.7 Hz, —CH=CH_2), 6.1 (m, 1H, —C*H*=CH_2), 5.9 (d, 1H, J = 9.6 Hz, —CH=CH_2), 4.9-3.1 (m, hyaluronan backbone), 1.9 (s, 3H, —CH_3) ppm. ^{13}C NMR (D_2O, 289 K): δ = 175.6 (C=O), 174.6 (C=O), 168.8 (C=O, acrylate), 133.7 (CH=CH_2), 127.9 (*C*H=CH_2), 103.6 (C_1), 101.5 (C_1'), 83.4 (C_3'), 80.6 (C_4), 77.0 (C_5), 76.1 (C_5'), 74.3 (C_3), 73.2 (C_2), 69.2 (C_4'), 61.2 (C_6'), 54.9 (C_2'), 23.2 (CH_3) ppm.

2.5. Hydrogel formation

250 μl of a 2 wt% aqueous solution of HA-AC were mixed with (A) 15 μl 5 M triethanolamine in water and 3 μl Eosin Y (EY, 0.3 wt% in *N*-vinyl-2-pyrrolidone), (B) 40 μl of a 1 wt% solution of Irgacure 2959 (I2959) in 20 vol% ethanol, and (C) 25 μl of 1 wt% aqueous solution of LAP. The respective mixture was poured into cylindrical silicon casting moulds (*Ø* = 7 mm, *h* = 4 mm) and irradiated for 10 min with visible light (514 nm, 16 W, mixture A) and UV light (365 nm, 36 W, mixtures B and C), respectively.

2.6. Compression tests

Freshly prepared cylindrical hydrogels (prepared according to method A) were used to perform compression testing and determine E. Measurements were conducted on a Texture Analyser *TA-XT2i* (Stable Microsystems, Godalming, UK) at 23 °C. Experiments were run in triplicate. The applied experimental conditions and the calculation of elastic modulus (E) are described in detail in Berg et al. (2011).

2.7. Swelling properties

Hydrogels prepared according to method A were stored in water for 24 h, weighed, dried at high vacuum overnight and weighed again. The swelling index was calculated as follows:

$$\text{Swelling index}\,(\%) = \left[\frac{(m_s - m_d)}{m_d}\right] \times 100$$

where m_s is the weight of the swollen hydrogel and m_d its dry weight.

3. Results and discussion

3.1. Synthesis and characterization of acrylated low molecular weight HA

Native hyaluronan (HA) with a molecular weight (M_w) of about 1×10^6 g/mol forms extremely viscous aqueous solutions even at concentrations of 1 wt%. At slightly higher concentrations HA solutions start to jell preventing derivatization under homogeneous reaction conditions. To overcome these problems and to improve

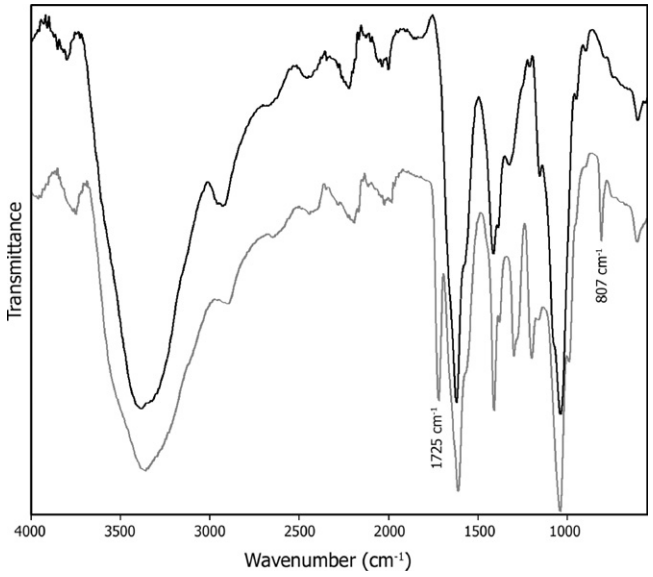

Fig. 2. IR-spectra of HA-low (black) and HA-AC (gray).

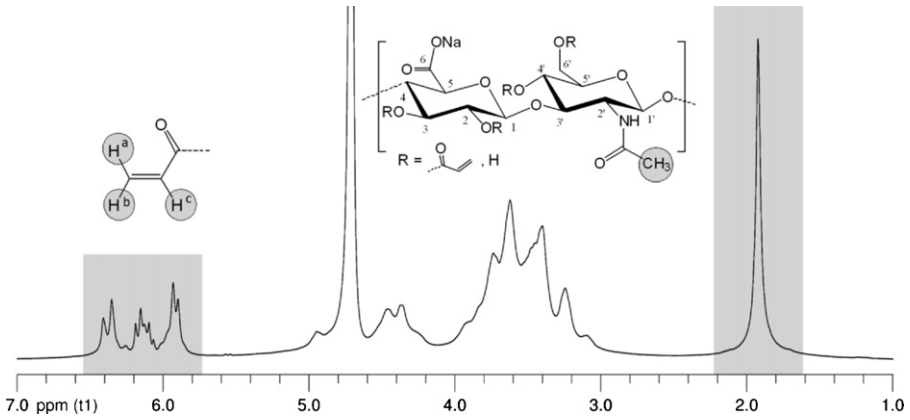

Fig. 3. ^{1}H NMR spectrum of HA-AC.

the handling of the material the molecular weight of HA was firstly reduced by thermal degradation of HA in an autoclave at 130 °C yielded a derivative with M_w = 48,000 g/mol as proven by GPC analysis. The PD of the degraded HA (HA-low) was determined to be 1.7 which proves that the molar mass distribution is considerably closer than in the starting high molecular weight HA. The IR and NMR data of the degraded HA are consistent with those of the starting HA which means that the thermal degradation did not cause any structural changes. For all subsequent chemical modifications degraded HA was used.

The synthesis of the acrylated HA (HA-AC, Fig. 1) was performed in a classic 2-phase reaction with acryloyl chloride and TBAF in water/CH_2Cl_2 starting from HA-low. TBAF acts a phase transfer catalyst.

The presence of the new carbonyl ester group is proven by a strong signal at 1725 cm^{-1} in the FT-IR spectra of the acrylates that was absent in the starting HA-low (Fig. 2). Besides a new signal for a=C—H-vibration can be found at 807 cm^{-1} in the HA-AC spectrum. In the ^{1}H NMR spectra (Fig. 3) the acrylic protons H^{a} and H^{b} appear as dubletts at 5.9 and 6.4 ppm, at which the signal at 6.4 ppm shows with 14.7 Hz the higher coupling constant and therefore can be assigned to the trans-proton H^{a}. The H^{c} proton is found as a multiplett at 6.1 ppm. The newly appearing signals in the ^{13}C NMR can be assigned to the carboxylic group of the acrylate substituent (168.8 ppm) and to the carbon atoms at the acrylic double bond (133.7 and 127.9 ppm).

The degree of substitution can be controlled by varying the ratio of HA-low and acryloyl chloride. Here, an excess of acryloyl chloride per free OH-group of the disaccharide repeating unit between 2.5 and 7.5 leads to derivatives with DS_{AC} = 0.7–1.7. Advantageously relatively high DS_{AC} values and hence a considerable higher density of cross-linkable substituents per disaccharide repeating unit are accessible by this method compared to previously synthesized HA acrylates (Kim et al., 2007; Wang et al., 2010) and methacrylates (Bencherif et al., 2008; Leach, Bivens, Patrick, & Schmidt, 2003; Möller, Weisser, Bischoff, & Schnabelrauch, 2007; Noh et al., 2006; Smeds et al., 2001), where only DS values < 1.0 were achieved. Obviously the phase transfer conditions substantially enhance the reactivity of acryloyl chloride in relation to HA-low and allow higher conversion rates.

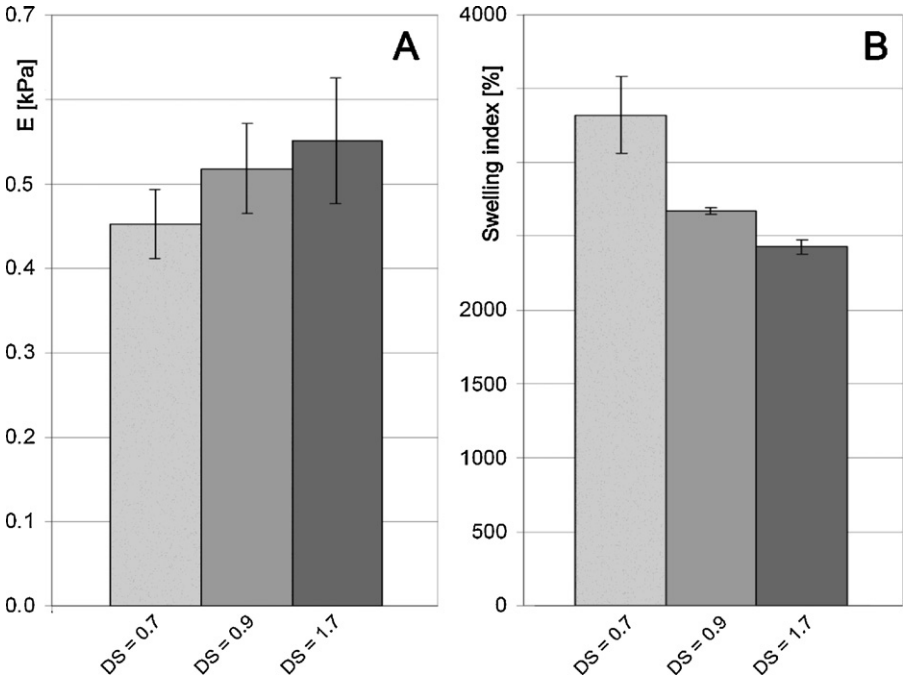

Fig. 4. (A) Elastic moduli of HA-AC hydrogels plotted against the DS_{AC}. (B) Swelling index of HA-AC hydrogels with different DS_{AC} values.

3.2. Hydrogel formation and characterization

To prove the ability of the HA-AC-derivatives to form stable hydrogels aqueous solutions thereof were photo-cross-linked with different water-soluble initiation systems. For cross-linking under visible light (514 nm) EY was used. UV-curing (365 nm) was performed with I2959 and LAP, respectively. With all cross-linking systems dimensionally stable hydrogels were obtained that appear transparent and rubbery.

In preliminary investigations on the properties of the obtained hydrogels the compression stability and swellability of hydrogels prepared by method A was studied. Fig. 4A shows the elastic moduli (E) of hydrogels prepared from HA-AC derivatives with different DS_{AC}-values at constant macromer and initiator concentrations whereas Fig. 4B shows the swelling index of the same hydrogels. As expected E increases with an increasing density of acrylate groups in the starting macromer leading to more rigid hydrogels. At the same time the swellability decreases in the same order. For the hydrogels prepared by UV curing values for the elastic modulus and the swelling index in the same order of magnitude have been found (data not shown).

4. Conclusions

A new method to synthesize cross-linkable HA acrylates was developed based on a reaction of low molecular weight HA with acryloyl chloride under phase transfer conditions. The method allows the preparation of derivatives with controlled acrylation degrees also in a high DS_{AC} range not accessible by other reaction conditions. The ability of these derivatives to form dimensionally stable hydrogels was proven using UV-initiators and an initiator that reacts at visible light. Thus HA acrylates represent cross-linkable GAG derivatives with well adjustable properties in a wide range that might have considerable potential for the fabrication of tailored hydrogels.

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