Controlled Synthesis and Inclusion Ability of a Hyaluronic Acid Derivative Bearing β-Cyclodextrin Molecules

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A new synthetic route to β-cyclodextrin-linked hyaluronic acid (HA-CD) was developed. This was based on the preparation of a HA derivative selectively modified with adipic dihydrazide (HA-ADH) and a β-cyclodextrin derivative possessing an aldehyde function on the primary face, followed by their coupling by a reductive amination-type reaction. The CD−polysaccharide was fully characterized in terms of chemical integrity and purity by high-resolution NMR spectroscopy. The complexation ability of the grafted CD was further demonstrated by isothermal titration calorimetry using sodium adamantane acetate (ADAc) and Ibuprofen as model guest molecules. The thermodynamic parameters for the complexation of these negatively charged guest molecules by the β-CD grafted on negatively charged HA were shown to be largely influenced by the ionic strength of the aqueous medium.

Introduction

Hyaluronic acid (HA), a linear polysaccharide composed of repeating disaccharide units of *N*-acetyl-D-glucosamine and D-glucuronic acid (Figure 1), is the only nonsulfated glycosaminoglycan found in the extracellular matrix. Because of its unique viscoelastic properties and biological performances, HA has become an attractive building block for the development of new biocompatible materials with many applications in viscosupplementation, tissue engineering, and drug delivery.[1,2] As a consequence thereof, this biopolymer has been the subject of various chemical modifications leading for example to chemically cross-linked materials with successful application in the treatment of osteoarthritis[3,4] and HA−drug conjugates having controlled-release and cell-targeted properties.[5] Indeed, HA would be an excellent candidate as a drug-delivery agent primarily due to its biocompatibility and its specific binding to some cell-surface receptors.

On the basis of these studies, we have prepared a new HA derivative by grafting β-cyclodextrin (β-CD) molecules on this polymer. Cyclodextrins are water-soluble cyclic oligosaccharides which can include various guest molecules into their hydrophobic cavity allowing the solubilization, stabilization, and transport of hydrophobic drugs.[6,7] The β-CD-conjugated HA may exhibit promising properties such as high water solubility, biodegradability, and functionality leading to many potential applications. Conversion of HA to its β-CD derivative has already been described.[8] However, it was based on a Mitsunobu reaction performed under heterogeneous conditions and with no control of the number and the exact grafting position of the CD molecule.

We describe here a new route to β-CD-conjugated HA which relies on the efficient selective functionalization of both HA and CD with reactive groups prior to their coupling.

Experimental Section

Materials. Hyaluronic acid under the sodium salt form, having a weight-average molecular weight M_w of 320 000, was a gift from ARD (Pomacle, France). The β-cyclodextrin (β-CD) was kindly supplied by Roquette Frères (Lestrem, France). All other chemical products and reagents were purchased from Fluka (Buchs, Switzerland).

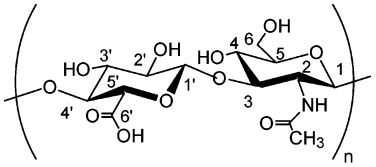

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Figure 1. Chemical structure of hyaluronic acid.

NMR Spectroscopy. ^{1}H NMR experiments were performed using Bruker DRX400 and AC300 spectrometers operating at 400 and 300 MHz, respectively. ^{13}C NMR spectra were recorded with Bruker DRX400 and AC300 spectrometers operating at 100 and 75 MHz, respectively. 1D NMR spectra were collected using 16K data points. All 2D experiments were acquired using 2K data points and 256 time increments. The 2D T-ROESY was acquired using the phase-sensitive time proportional phase incrementation method (TPPI method). Chemical shifts are given relative to external tetramethylsilane (TMS = 0 ppm), and calibration was performed using the signal of the residual protons of the solvent as a secondary reference. Deuteriun oxide was obtained from SDS (Vitry, France). Details concerning experimental conditions are given in the figure captions.

Mass Spectrometry. Electrospray mass spectra were measured in the positive mode on a ZabSpec TOF (Micromass, U.K.) mass spectrometer. The CD derivatives **6′** and **8** were dissolved in methanol/water (1:1 v/v) at a concentration of 0.1 mg/mL and infused into the electrospray ion source. The capillary voltage was set to 4 kV. Poly(ethylene glycol) was used for external calibration.

Titration Calorimetry. Isothermal titration microcalorimetry (ITC) was performed using a model 4200 microcalorimeter from Calorimetry Sciences Corp. (CSC, UT) or a Microcal VP-ITC titration microcalorimeter (Northampton, MA). In individual titrations, injections of 10 μL of sodium adamantane acetate were added from the computer-controlled 250-μL microsyringe at an interval of 5 min into the β-CD monocarboxylic acid **6′**, β-CD acetal **8**, or HA-CD **10** solution (cell volume = 1.3 or 1.4478 mL) containing the same solvent as sodium adamantane acetate (pure water, 0.025 or 0.1 M NaCl), while stirring at 297 rpm at 25 °C. The observed heat effects under identical injections of sodium adamantane acetate into a cell containing only the solvent

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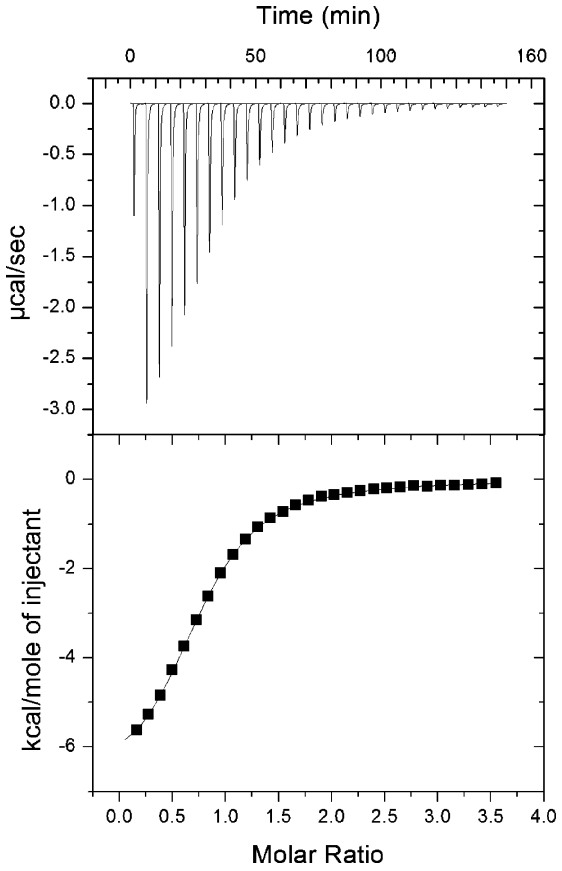


Figure 2. Calorimetric titration of HA-CD with sodium adamantane acetate in 0.025 NaCl at 25 °C: (a) raw data for 30 sequential injections (10 μL/injection) of ADAc (0.117 mM) injected into HA-CD solution ([CD] = 1.851 mM); (b) integrated curve showing experimental points and the best fit for titration of HA-CD with ADAc.

were identical with the heat signals at the end of titration, after the saturation was reached. The raw experimental data were presented as the amount of heat produced by following each injection of sodium adamantane acetate as a function of time. The amount of heat produced/injection was calculated by integration of the area under individual peaks by the instrument software, after taking into account heat of dilution. The experimental data were fitted to a theoretical titration curve using the instrument software (Bindworks program (CSC) or ORIGIN software (Microcal)), with $\Delta H°$ (the enthalpy change in kJ/mol), K_a (the association constant in L/mol), and n (complex stoichiometry) as adjustable parameters. In all cases, calculations were performed using the "one set of binding sites" model. A representative titration curve is shown in Figure 2.

Viscosity Measurements. A low shear viscometer (LS30 from Contraves) was used for the measurement of the viscosity of polymer solutions in the range of concentrations from 0.2 to 10 g/L in 0.025 and 0.1 M NaCl at 25 °C.

Synthesis. *HA-ADH* ***(3)***. Sodium hyaluronate (4 g, 9.97 mmol) was dissolved in water to a concentration of 4 g/L. Adipic dihydrazide (17.3 g, 99.7 mmol) was added to this solution. The pH of the reaction was then adjusted to 4.75 using 0.1 N HCl. Next, an aqueous solution of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) (0.287 g, 1.49 mmol) was added slowly to the mixture. The pH of the reaction mixture was maintained at 4.75 by addition of 0.1 N HCl. The reaction was allowed to proceed at room temperature until no further change in pH was observed (i.e. 4 h). The pH of the reaction was then adjusted to 7.0 with 0.1 N NaOH. After addition of NaCl at a concentration of 0.5 M, the modified HA was precipitated with EtOH in the proportion EtOH/H_2O 3/2 (v/v). The precipitate was successively washed with different mixtures of EtOH/H_2O (7/3, 7.5/2.5, 8/2, 9/1) and then was filtered to give HA-ADH (3.48 g, 85%). The chemical integrity and purity of the final product were checked by ^{1}H NMR. Digital integration of the NMR signals arising from the anomeric protons of HA and methylene protons of ADH gave a substitution degree of approximately 0.08.

β-CD Monocarboxylic Acid ***6′***. β-cyclodextrin (4 g, 3.52 mmol) was preliminary dried under reduced pressure and was dissolved in DMSO (105 mL). Dess−Martin periodinane (DMP) (1.73 g, 3.52 mmol) dissolved in DMSO (5 mL) was added slowly, and the reaction mixture was stirred for 45 min at room temperature. After evaporation of most of the solvent, the residual syrup was poured into acetone (400 mL). The crude product was isolated by filtration and dissolved again in DMSO (15 mL) to remove soluble impurities by reprecipitation with acetone. The oxidized β-CD obtained after filtration was dried under reduced pressure at 25 °C for 24 h and then dissolved in water to be recovered by freeze-drying (4.62 g). To an aqueous solution (300 mL) of oxidized β-CD (4.3 g, 3.79 mmol) at 40 °C *O*-(carboxymethyl)-hydroxylamine hemihydrochloride (415 mg, 3.79 mmol) was added. The pH was adjusted to 4.8 using 0.5 N NaOH. The reaction mixture was stirred for 48 h at room temperature and then was neutralized to pH 7 by addition of 0.5 N NaOH. After evaporation of water, the mixture of initial cyclodextrin and the mono-, di-, and tricarboxylic acid derivatives was fractionated by size exclusion chromatography on a Biogel (Bio-Rad) P-4 column (4 × 100 cm) with $NaNO_3$ (0.05 M) as eluant, at room temperature. The flow rate was 80 mL/h. Fractions were monitored by a differential refractometer. Desalting was performed by diafiltration with deionized water through an ultramembrane Amicon YC05. The diafiltration was stopped when the filtrate conductivity was lower than 10 μS, and the monofunctionalized cyclodextrin was recovered by freeze-drying (0.835 g, 18%).

R_f = 0.47 (1:1:2 DMF−H_2O−*n*-BuOH). ^{1}H NMR (400 MHz, D_2O): δ 7.62 (d, 1H, J = 7.41 Hz, −C*H*═), 5.01 (d, 1H, H-1′), 4.97−4.88 (m, 6H, H-1), 4.35 (s, 2H, CH_2COOH), 4.29 (dd, 1H, H-5′), 3.90−3.84 (m, 6H, H-3), 3.87 (m, 1H, H-3′), 3.86−3.65 (m, 12H, H-6), 3.83−3.73 (m, 6H, H-5), 3.65−3.45 (m, 12H, H-2, H-4), 3.62 (m, 2H, H-2′, H-4′).

^{13}C NMR (100 MHz, D_2O): δ 177.9 (C═O), 150.2 (CH═), 103.1, 102.9, 102.8 (C-1, C-1′), 83.7 (C-4′), 82.3, 82.2, 81.6 (C-4), 73.6 (*C*H_2–COOH), 74.4, 74.3, 74.2,74.1 (C-3, C-3′), 73.6, 73.3, 73,72.8, 72.6 (C-5, C-2, C-2′), 70 (C-5′), 61.5, 61.3, 60.7 (C-6).

ESI-HRMS ([M + Na]$^+$): calcd for $C_{44}H_{71}N_2O_{37}Na$, 1228.3603; found *m*/*z* 1228.3597.

β-CD Acetal ***8***. To a solution of β-CD monocarboxylic acid **6′** (0.5 g, 0.42 mmol) in dry DMF (40 mL) were successively added hydroxybenzotriazole (HOBt) (0.113 g, 0.84 mmol), diisopropylcarbodiimide (DIC) (0.212 g, 1.68 mmol), and aminoacetaldehyde dimethyl acetal (0.057 g, 0.55 mmol). The resulting mixture was stirred under nitrogen at room temperature for 36 h. After evaporation of most of the solvent, the residual syrup was poured into acetone (400 mL). The white precipitate was collected by filtration, washed three times with acetone, and dried to give pure acetal **8** (0.835 g, 84%).

R_f = 0.575 (1:1:2 DMF−H_2O−*n*-BuOH).

^{1}H NMR (400 MHz, D_2O): δ 7.69 (d, 1H, J 7.18 Hz, −C*H*═), 5.02 (d, 1H, H-1′), 5.01−4.97 (m, 6H, H-1), 4.41 (d, 1H, J 5.18 Hz, C*H*$(OCH_3)_2$), 4.36 (s, 2H, CH_2CO−), 4.32 (dd, 1H, H-5′), 3.92−3.81 (m, 6H, H-3), 3.87 (m, 1H, H-3′), 3.81−3.69 (m, 6H, H-5), 3.84−3.70 (m, 12H, H-6), 3.66 (m, 1H, H-4′), 3.63 (m, 1H, H-2′), 3.60−3.55 (m, 6H, H-2), 3.55−3.49 (m, 6H, H-4), 3.37 (s, 6H, CH_3) 3.36 (m, 2H, CH_2CH$(OCH_3)_2$).

^{13}C NMR (100 MHz, D_2O): δ 172.9 (C═O), 151.6 (CH═), 103.6 (*C*H$((OCH_3)_2)$), 103.1−102.9−102.5 (C-1, C-1′), 83.6 (C-4′), 82.4−82.3−82.2−82.1−81.9 (C-4), 74.4−74.3−74.2−74.1−73.9 (C-3, C-3′), 73.6−73.3−73.02−72.8 (C-5, C-2, C-2′), 73.6 (*C*H_2CO−), 69.7 (C-5′), 61.5−61.3 (C-6), 56.1−55.7 (−O$(CH_3)_2$), 42 (CH_2NH−).

ESI-HRMS ([M+Na]$^+$): calcd for $C_{48}H_{80}N_2O_{38}Na$, *m*/*z* 1315.42868; found 1315.4285.

HA-CD ***(10)***. The first step consisted in deprotecting the aldehyde function of **8**. Thus, the modified cyclodextrin **8** (0.43 g) was dissolved

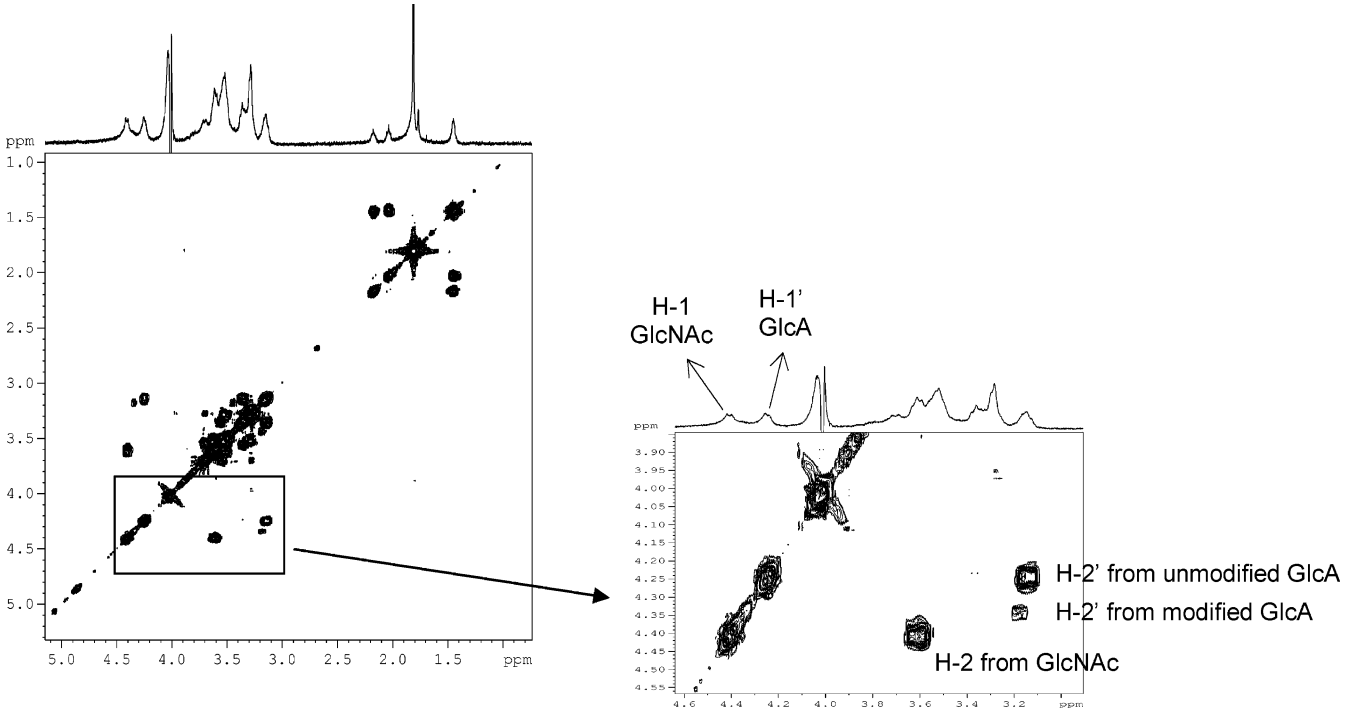


Figure 3. Complete and partial contour plots of a COSY experiment (400 MHz, 80 °C) performed on HA-ADH **3** (6 mg/mL) in D_2O.

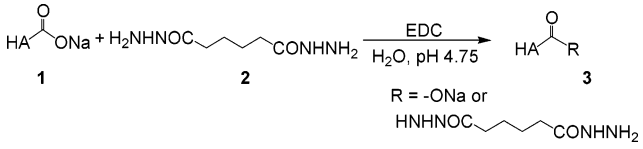
in water (80 mL) and 0.2 M HCl (13.4 mL) was added. The resulting mixture was stirred for 3 h at 55 °C and then was neutralized to pH 7 to give the desired aldehyde. The latter (429 mg, 0.356 mmol) was added to a solution of HA-ADH (**3**) (0.488 g, 1.18 mmol) in water (50 mL). The pH was adjusted to 5.1 using 0.1 N HCl and was maintained at this value during all the reaction by addition of 0.1 N HCl. A solution of $NaCNBH_3$ (0.66 g, 10.62 mmol) in water (5 mL) was added, and the mixture was stirred overnight. The pH of the reaction was then adjusted at 7.0 using 0.1 N NaOH. The modified HA was purified by diafiltration through an ultramembrane Amicon YM 10. The diafiltration was stopped when the filtrate conductivity was lower than 10 μS, and the HA-CD derivative was recovered by freeze-drying (0.427 g, 77%).

^{1}H NMR (400 MHz, D_2O): δ 5−4.8 anomeric protons of β-CD, 4.55 (H-1 from *N*-actylglucosamine unit), 4.25 (H-1 from glucuronic acid), 3.85−3.1 (protons of HA and β-CDs (H-2, H-3, H-4, H-5, H-6, H-1′, H-2′, H-3′, H-4′, H-5′, H-6′), 2.2 (CH_2 from ADH), 2.05 (CH_2 from ADH), 1.85 (CH_3 from acetamide of HA), 1.45 (4H, $2CH_2$ from ADH).

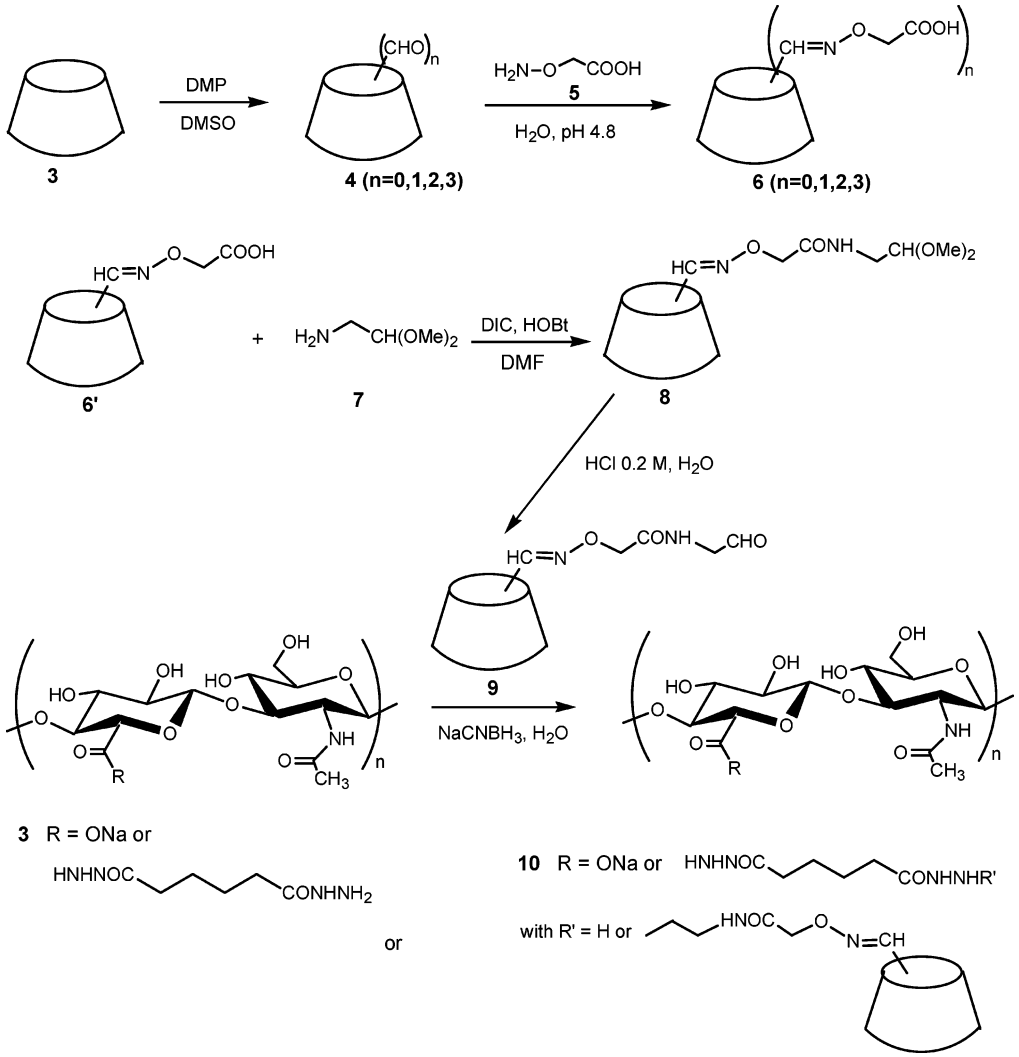
Results and Discusssion

1. Selective Functionalization of HA by Hydrazide Groups. The chemical structure of HA should offer several possibilities for its selective chemical modification. However, they are limited due to problems of reactivity of its functional groups. The amino function at the C-2 position of the glucosamine moiety resulting from alkaline N-deacetylation might be a possible site for specific chemical reaction, but the experimental conditions required for N-deacetylation lead to extensive degradation of HA.[9,10] Moreover, although carboxylate−amine coupling reactions affording amides can be performed under mild aqueous conditions using 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide (EDC) as a water-soluble carbodiimide, such reactions result in negligible coupling with HA. In fact, the *O*-acylurea intermediate was shown to be relatively unreactive and rearranges to the *N*-acylurea in preference to trapping by amine compounds.[11] To overcome this problem, a modified method was developed in which HA is reacted with a large excess of bifunctional amines (30-fold molar excess) in the presence of excess EDC (4-fold excess) and HOBt or sulfo-NHS (4-fold excess) at pH ≈ 7, affording HA derivatives with pendant reactive amines along the chain.[12] This methodology relies on the formation of an active ester intermediate, avoiding rearrangement to the stable *N*-acylurea. It was also shown that HA can be reacted with dihydrazide compounds, such as adipic dihydrazide (ADH), when present in sufficient excess in the presence of EDC at pH 4.75, with no formation of the *N*-acylurea.[13,14] This reaction, resulting in a HA derivative with pendant reactive hydrazide functionalities along the polymer backbone (HA-ADH), could be achieved thanks to the high nucleophilicity of dihydrazides even at the coupling pH value of 4.75 owing to the α-effect. This approach appeared to us to compete favorably with the other one, which requires the use of excess HOBt or sulfo-NHS as a coreagent, to specifically introduce cyclodextrin molecules to HA under mild aqueous conditions. Contrary to the literature method[13,14] which leads to HA-ADH derivatives with high degrees of substitution ($0.1 < DS < 0.6$), our aim was to obtain samples with lower DS ($0.05 < DS < 0.1$). Indeed, the targeted DS for the final HA-CD compound was no more than 0.1 because of the large size of the CD molecule which might generate sterical constraints. Furthermore, low DS values for HA-ADH should allow it to maintain the unique viscoelastic properties observed with the native polymer without decreasing too much the charge density of the HA backbone, which is at the origin of the high water solubility of the polymer. Hence, for this purpose, optimization of the grafting conditions of ADH on HA was required. The use of a large excess of ADH appeared to be primordial to avoid cross-linking and rearrangement to *N*-acylurea side reactions. Thus, reaction of HA with 10 molar equiv of ADH and 0.15 molar equiv of EDC allowed us to obtain a highly pure HA-

Scheme 1. Synthesis of HA-ADH



Scheme 2. Synthesis of HA-CD



ADH derivative with a DS of 0.08, as expected (Scheme 1). The value of the DS was determined by digital integration of the NMR signals arising from the anomeric protons of HA and the CH_2 protons of ADH. ^{1}H NMR spectroscopy also provided evidence of the conjugation of ADH on the carboxylic acid group of HA. Indeed, the COSY spectrum of HA-ADH shows two cross-peaks for the H1′−H2′ correlations corresponding to the nonmodified and modified glucuronic moieties, whereas only one cross-peak can be seen for the glucosamine unit (Figure 3). After we functionalized HA with reactive groups, the next step involved its coupling with selectively modified cyclodextrins.

2. Selective Monofunctionalization of β-Cyclodextrin and Coupling with HA Activated with Hydrazide Groups. The pendant hydrazido moieties on HA can be reacted with various derivatives having aldehyde or carboxylate functionalities. However, since aldehydes can be easily reacted with hydrazides to give hydrazones under mild conditions, our strategy was to selectively modify the CD molecule by an aldehyde function. Such a derivative was prepared in four steps from the natural β-CD (Scheme 2). The first step consisted in the oxidation of natural β-CD using Dess−Martin periodinane (DMP), according to a literature procedure.[15] This reaction, performed in DMSO at room temperature for 45 min in the presence of 1 molar equiv of DMP, afforded a mixture of mono-, di-, and trialdehyde derivatives of β-CD together with the nonoxidized β-CD. Indeed, electrospray mass spectrometry revealed the presence of these four derivatives in the molar proportions given in Table 1. It should be noted that although this technique is not quantitative, it was assumed in this case that the presence of one or several aldehyde functions on the CD cavity did not fundamentally modify its ionization properties. The mixture of aldehyde derivatives of β-CD and initial β-CD were reacted in a second step with *O*-(carboxymethyl)hydroxylamine in water at pH 4.8 leading to the formation of carboxylic acid derivatives (see Scheme 2). The presence of charged groups, which significantly increases the hydrodynamic volume of the CD molecule, allowed the efficient separation of the different products on a preparative scale by size exclusion chromatography. The molar proportions of the initial β-CD and the mono-, di-, and tricarboxylic acid derivatives, derived from the areas of the different peaks of the chromatogram, were found to be 40, 29, 22, and 7, respectively. It can be noticed that these values are rather close to those calculated from the mass spectrometry

Table 1. Mole percentage Ratios Derived from Electrospray Mass Spectrometry and Size Exclusion Chromatography of Initial β-CD and Mono-, Di-, and Trifunctionalized Derivatives of β-CD (Mixtures **4** and **6**)

β-CD derivatives	β-CD	monofunctionalized β-CD	difunctionalized β-CD	trifunctionalized β-CD
aldehydes[a] **4**	55	29	11	5
acids[b] **6**	42	29	22	7

[a] Percentage ratios derived from ESI-MS. [b] Percentage ratios derived from SEC.

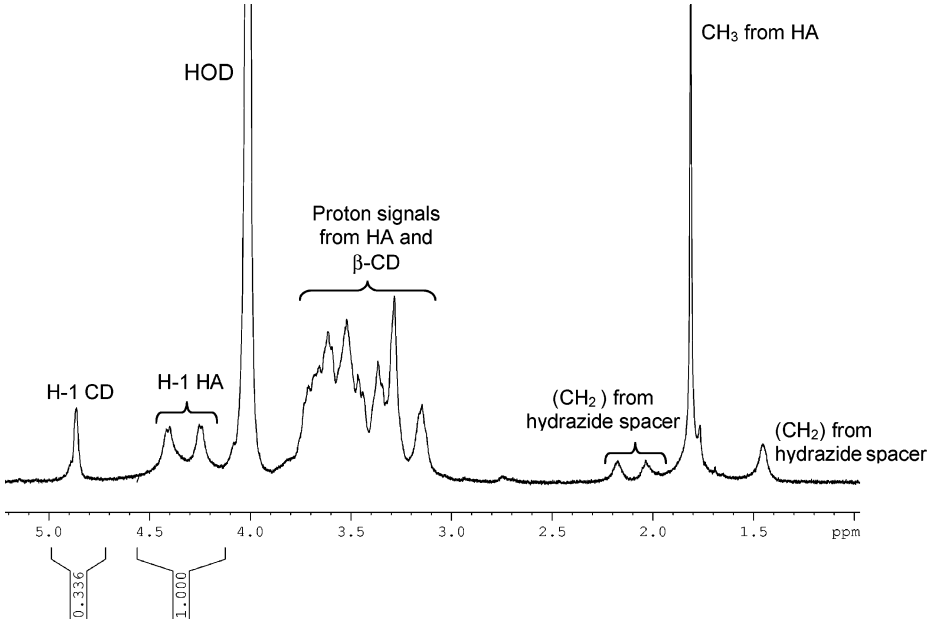


Figure 4. ^{1}H NMR spectrum (400 MHz, 80 °C) of HA-CD (6 mg/mL) in D_2O.

Table 2. Thermodynamic Parameters for Inclusion Complex Formation of Sodium Adamantane Acetate with Natural β-Cd, β-CD Monocarboxylic Acid **6′**, and β-CD Acetal **8**, Derived from Calorimetric Titration Experiments

CD derivative	[NaCl] (M)	[CD cavity] (mM)	[AD] (mM)	$10^{-4}K_a$ (M^{-1})	$\Delta H°$ (kJ/mol)	$T\Delta S°$ (kJ/mol)	*n* (*n*:1 AD:CD derivative)
natural	0	0.798	8.81	7.5 ± 0.9	−25.8 ± 1.2	2.00 ± 1.13	0.90 ± 0.02
β-CD	0.1	0.798	8.78	8.5 ± 1.1	−25.9 ± 0.9	2.21 ± 0.98	0.97 ± 0.08
β-CD monoacid **6′**	0	0.686	6.905	4.6 ± 0.7	−25.5 ± 2.3	1.09 ± 0.26	0.84 ± 0.03
	0.1	0.707	6.81	7.0 ± 0.3	−27.2 ± 0.5	0.43 ± 0.39	0.72 ± 0.03
β-CD acetal	0	0.731	7.44	1.9 ± 0.4	−32.6 ± 0.1	-8.19 ± 0.06	0.95 ± 0.17
β-CD acetal **8**	0.1	0.747	7.47	5.6 ± 0.9	−26.3 ± 0.8	0.78 ± 0.82	0.85 ± 0.04

spectrum (see Table 1). The β-CD monocarboxylic acid derivative **6′** was further reacted with excess aminoacetaldehyde dimethyl acetal **7** under peptide-like coupling conditions (*N*,*N*′-diisopropylcarbodiimide (DIC), hydroxybenzotriazole (HOBt)) in DMF resulting in the β-CD acetal derivative **8**. The latter was purified by precipitation in acetone allowing us to remove excess aldehyde and other reactants. The chemical purity and integrity of the β-CD acetal derivative **8** was confirmed by ^{1}H NMR spectroscopy and electrospray mass spectrometry. Compound **8** was easily activated to the reactive aldehyde by mild acid treatment. However, when it was mixed with HA-ADH in water at neutral pH, this led to negligible coupling. On the other hand, the coupling yield could be improved by the addition of sodium cyanoborohydride as a reducing agent (Scheme 2). The reductive amination-like reaction was performed in aqueous solution at an optimal pH value of 5.1. A pH increase of the mixture since the beginning of the reaction was observed, which could be explained by the concomitant reduction of the oxime bond.[16] The reaction was thus allowed to proceed at room temperature while maintaining the pH at constant value by the dropwise addition of 0.1 N HCl. The expected HA-CD derivative **10** was isolated by a diafiltration process followed by freeze-drying. Indeed, attempts to recover HA-CD by precipitation in an ethanol/0.5 M NaCl mixture followed by washing steps using ethanol/water mixtures lead to samples exhibiting nonreproductible properties in aqueous solution. This was attributed to irreversible interchain interactions promoted by enhanced hydrogen bonding between the grafted cyclodextrins.

The NMR analysis performed in D_2O demonstrated that **10** was free of any byproduct (see Figure 4). Digital integration of the NMR signals arising from the anomeric protons of HA and cyclodextrin gave a substitution degree of 0.05 ± 0.01 for a HA-CD sample prepared from 0.3 molar equiv of CD aldehyde **9** with respect to the repeating unit of HA-ADH. Although the reaction is not quantitative, the DS could be precisely controlled by varying the feed ratio between HA-ADH and **9**.

3. Complexation Properties of the free β-CD Derivatives and HA-CD. The inclusion ability of the monofunctionalized CD derivatives, i.e., β-CD monocarboxylic acid **6′** and β-CD acetal **8**, and HA-CD (**10**) was investigated by isothermal titration calorimetry using sodium adamantane acetate (ADAc) (**11**) as a model guest and compared with that of natural β-CD. It is well-known that deep and snug-fitting complexes with a 1:1 stoichiometry are formed between adamantane derivatives and natural β-CD, leading to very high association constants ($K_a \sim 80\,000\ M^{-1}$). In this study, experiments were performed by varying the ionic strength as the presence of electrostatic interactions between negatively charged ADAc and CD **6′** on one hand and ADAc and HA-CD (**10**) on the other may influence the complexation thermodynamics. Indeed, it has been reported that recognition between charged guest molecules and cyclodextrin polymers can be altered by the presence of interacting groups on the polymer backbone.[17]

Table 2 gives the thermodynamic parameters obtained for the complexes between the free CD derivatives and ADAc in water and 0.1 M NaCl. As can be seen from Table 2, addition of salt has an important effect on the complexation thermodynamics for the **6′**/ADAc complex. Indeed, the presence of NaCl at a 0.1 M concentration allows the increase of the association constant, K_a, to a value close to that of the natural β-CD/ADAc complex. This can be attributed to the screening of electrostatic

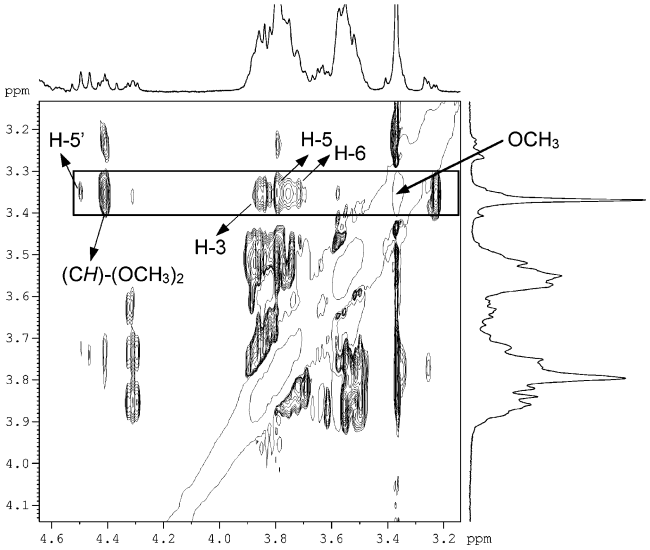


Figure 5. Partial contour plot of a 2D T-ROESY experiment (400 MHz, 25 °C, 300 ms spin-lock time at 16 dB attenuation) performed on β-CD acetal **8** (5 mM) in D_2O.

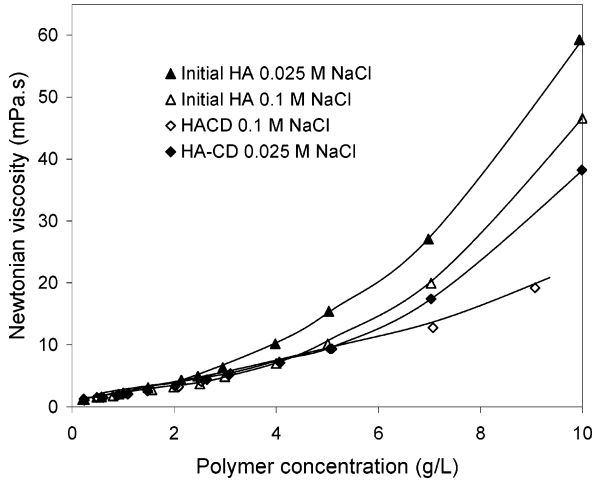


Figure 6. Variation of the viscosity of solutions of initial HA and HA-CD with the concentration in 0.025 and 0.1 M NaCl at 25 °C.

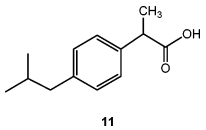


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Figure 7. Chemical structure of Ibuprofen.

repulsions between negatively charged CD **6′** and ADAc. Additionally, the presence of salt appears to slightly enhance the binding constant for the complexation of natural β-CD with ADAc, which can be related to a more pronounced hydrophobic character for ADAc. Thus, the thermodynamic parameters for both complexes, i.e., the natural β-CD/ADAc and monoacid **6′**/ADAc complexes, are similar in 0.1 M NaCl, indicating that the inclusion performances of the CD derivative **6′** are completely retained despite its monofunctionalization. However, although the acetal **8** is derived from **6′**, this compound exhibits a lower binding ability in 0.1 M NaCl. Apparently, this can be attributed to the inclusion of the acetal group in the CD cavity which is also supported by the observation of spatial proximities between protons of the acetal group and the CD cavity using a NMR 2D T-ROESY experiment.[18] Indeed, the presence of strong cross-peaks between the CH_3 protons of the acetal group and the H-3 and H-5 protons of cyclodextrin in the 2D T-ROESY spectrum (see Figure 5) shows evidence of the inclusion of the acetal group in the CD cavity. However, no clear distinction between intra- and intermolecular complexes could found. Nevertheless, this inclusion phenomenon should not be a problem in the present case, since the acetal group is removed for the CD to be grafted to HA.

A profound effect of electrostatic interactions on the complexation between HA-CD and ADAc can also be observed (see Table 3). Indeed, addition of salt appears to improve the complexation ability of the grafted CD cavity by screening electrostatic repulsions. From the data given in Table 3, the optimal NaCl concentration, leading to the highest association constant, is 0.025 M. Although an increase in NaCl concentration to 0.1 M could enhance the inclusion ability of the grafted CD, this in counterpart tends to increase polymer−polymer interaction as shown by Figure 6. Indeed, as can be seen from this figure, solutions of HA-CD exhibit lower viscosity values compared to those of initial HA indicating the presence of additional intra- and/or interchain interactions promoted by enhanced hydrogen bonding between pendant cyclodextrins. Consequently, such interactions may hamper binding process with the grafted CD cavities, slightly lowering the affinity constant.

These results thus highlight potential applications of HA-CD in drug formulations. In this context, complexation of Ibuprofen (**11**), a nonsteroidal antiinflammatory drug, by HA-CD was also investigated (Figure 7). Inclusion of water-soluble Ibuprofen in the pendant CD cavities of HA-CD was clearly demonstrated by isothermal titration calorimetry. As in the case of ADAc, a decrease of the association constant likely due to the presence of the carboxylate group was observed for HA-CD in comparison to the natural β-CD. The K_a value for the Ibuprofen/natural β-CD and the Ibuprofen/HA-CD systems were found to be 10 700 ± 330 and 3500 ± 80 M^{-1} at 25 °C in 0.025 M NaCl, respectively. Such an approach, allowing the proper combination of HA with antiinflammatory drugs in a versatile way, could be advantageously used in osteoarthritis therapy. Indeed, two therapeutic approaches are currently used for the treatment of osteoarthritis. One is based on local injections of high molecular weight linear HA or slightly chemically cross-linked HA,[2] whereas the other relies on the use of intraarticular antiinflammatory drugs. Inclusion of antiinflammatory molecules in CD cavities grafted on HA appears as an interesting alternative to both approaches mentioned above and also to prodrugs on the basis of the covalent attachment of such drugs on HA as reported in the literature.[13,19]

In conclusion, a new monosubstituted derivative of β-cyclodextrin possessing an aldehyde function was prepared, allowing

Table 3. Influence of the Ionic Strength on the Thermodynamic Parameters for Inclusion Complex Formation of Sodium Adamantane Acetate with the CD of HA-CD in Aqueous Solution, Derived from Calorimetric Titration Experiments

[NaCl] (M)	[CD cavity] (mM)	[AD] (mM)	$10^{-4}K_a$ (M^{-1})	$\Delta H°$ (kJ/mol)	$T\Delta S°$ (kJ/mol)	n (n:1 AD:CD derivative)
0	0.112	1.902	2.5 ± 0.1	−32.9 ± 3.8	−7.81 ± 0.23	0.69 ± 0.03
0.025	0.117	1.851	5.9 ± 0.9	−29.4 ± 0.7	−2.19 ± 0.06	0.76 ± 0.02
0.1	0.112	1.747	4.7 ± 0.8	−22.7 ± 1.3	3.95 ± 1.09	1.04 ± 0.04

chemoselective coupling with polymers bearing amine-type groups. In this work, the monoaldehyde derivative was selectively grafted on a hyaluronic acid derivative possessing reactive hydrazide groups along the chain, yielding a new CD polymer. The latter was shown to exhibit interesting binding properties toward sodium adamantane acetate and Ibuprofen as model guest molecules. The presence of salt was necessary to partially screen electrostatic repulsions between these anionic guests and the polymer chain and, thus, improve the inclusion properties of the grafted CD. The HA-CD polymer shows promising properties for encapsulation and delivery of bioactive molecules, in particular antiinflammatory drugs, owing to the cumulative effects of size specificity, transport properties, and biological activity of cyclodextrins and polymer matrix. The formation of chemical and physical hydrogels from this HA-CD is under investigation and will be reported in the near future.

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