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Synthesis and characterization of a β-CD-alginate conjugate

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Abstract

Selective chemical modification of both β-cyclodextrin (β-CD) and sodium alginate (alg) was performed in order to produce an alginate derivative possessing pendant β-CD cavities along the chain. The latter was then fully characterized in terms of chemical integrity and purity, complexation properties and ability to form hydrogels. Thus, a sodium alginate derivative modified with adipic dihydrazide (alg-ADH) and a β-cyclodextrin derivative possessing an aldehyde function on the primary face were synthesized, and both were selectively coupled by a reductive amination-type reaction. Comparison of the complexation properties of the grafted and natural β-CDs by isothermal titration calorimetry using sodium adamantane acetate as a model guest gave similar enthalpy values suggesting similar mechanisms of binding. However, the association constant for the grafted CD is slightly lower as a result of a less favorable change in the binding entropy. Investigation of the gelation ability in the presence of calcium ions led to the following order: alg > alg-ADH > alg-CD. The decrease of gelation efficiency for the modified alginate samples reflected the reduction of ionic interchain bonds as a result of the unavailability or non-accessibility of G units on the alginate backbone, due to the chemical modification and steric effect of the CD molecule for alg-CD.

Keywords: Alginate; Cyclodextrin; Hydrogel

1. Introduction

Hydrogels have been widely used in many biomedical applications including contact lenses, wound dressings, artificial organs, and delivery carriers for bioactive substances due to their high degree of biocompatibility [1–3]. Biopolymers and several synthetic degradable polymers have been used for the preparation of such materials. Among these compounds, alginates which are naturally occurring polysaccharides are very attractive. These water-soluble linear polymers are composed of (1 → 4)-β-D-mannuronic acid (M) and α-L-guluronic acid (G) units which vary in amount and sequential distribution along the polymer chain depending on the source of alginate [4]. In the presence of divalent cations, such as calcium, alginates form gels spontaneously as a result of cooperative binding of the cations between the G-blocks of adjacent alginate chains, leading to ionic interchain bridges. Thus, due to their gelling ability, stabilizing properties and high viscosity, alginates and its derivatives are widely used in the food, cosmetics and pharmaceutical industries [5–8]. Several chemical modifications of alginates have been performed either to improve adhesion or interaction between cells and alginate-based matrix materials [9,10], or to produce tissue-specific drug delivery systems by grafting targeting molecules (lectins) onto alginates [11]. In the present work, we introduced β-cyclodextrin (β-CD) molecules, onto alginate in order to obtain a molecular carrier exhibiting promising properties owing to the cumulative effects of size specificity and transport properties of cyclodextrins and polymer matrix. Cyclodextrins are water-soluble cyclic oligosaccharides which can include various guest molecules into their hydrophobic cavity allowing the solubilisation, stabilisation and transport of hydrophobic

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drugs [12]. The synthesis of such CD derivatives of alginate has been recently reported [13]. The synthetic route was based on the activation of the hydroxyl groups of alginate with cyanogen bromide followed by coupling with 6-amino-α-cyclodextrin. We report here another strategy that was recently proposed for the synthesis of β-CD-linked hyaluronic acid [14]. This was based on the preparation of an alginate derivative selectively modified with adipic dihydrazide (alg-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 complexation properties of the resulting β-CD-grafted alginate and the effect of CD grafting onto the gelation ability were next investigated.

2. Experimental section

2.1. Materials

β-Cyclodextrin was kindly supplied by Roquette Frères (Lestrem, France). The β-CD acetal derivative **4** was synthesized in our laboratory as described in detail elsewhere [14]. All other chemical products and reagents were purchased from Fluka (Buchs, Switzerland). The commercial sodium alginate **1** (Ref 71238 from Fluka) was purified by solubilisation in distilled water at a concentration of 10 g/L, reprecipitation by successive addition of NaCl to reach a salt concentration of 1 M, and ethanol. The precipitate was successively washed with different mixtures of EtOH/H_2O (7/3, 4/1, 9/1), filtered, and then dried. The weight-average molecular weight of the purified alginate sample was determined to be 2.5×10^5 g/mol by size exclusion chromatography using a Waters GPCV Alliance 2000 chromatograph (USA) equipped with three on-line detectors: a differential refractometer, a viscometer and a light scattering detector (MALLS) from Wyatt (USA); the solutions were injected at a concentration of 1.4×10^{-4} g/mL in 0.1 M $NaNO_3$. The ratio between the mannuronic acids and guluronic acids (M/G) of alginate derived from ^{1}H NMR spectroscopy after partial depolymerization using alginate lyase from *Flavobacterium* sp. (EC 4.2.2.3 from Sigma) was found to be 0.8. Polymer concentrations are expressed in g/L or monomol/L (number of moles of modified or non-modified uronic acid units/L).

2.2. NMR spectroscopy

^{1}H NMR experiments were performed using a Bruker DRX400 spectrometer operating at 400 MHz. 1D NMR spectra were collected using 16K data points. The gradient-selected two-dimensional ^{1}H, ^{1}H-COSY experiments [15] were acquired using 2K data points and 256 time increments. 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. Deuterium oxide was obtained from SDS (Vitry, France). Details concerning experimental conditions are given in the figure captions. The M/G ratio and degree of substitution of ADH groups (average number of mole of ADH per mole of carboxylate) were obtained from digital integration of the c-i proton signals and k-m proton signals (see Fig. 1, as an example) which allowed to calculate the respective peak areas of the anomeric protons of alginate (M and G moieties) and CH_2 protons of ADH. Assignment of the ^{1}H NMR spectrum of alginate was derived from literature data [16].

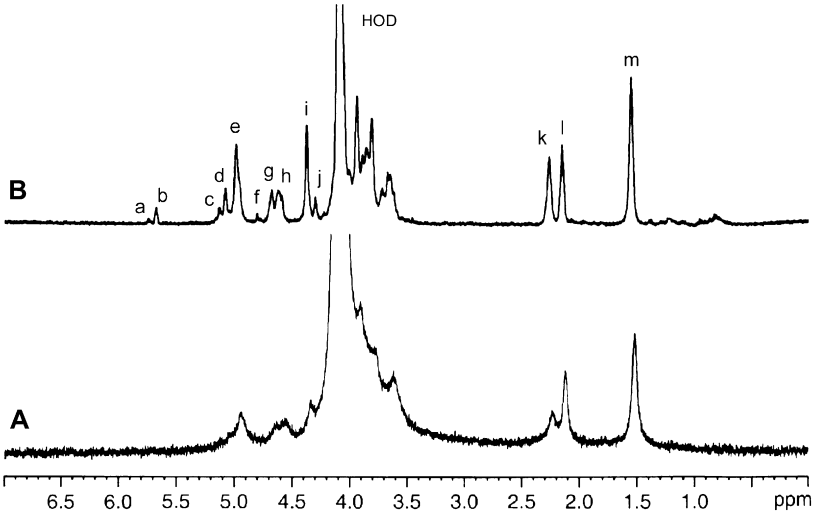


Fig. 1. ^{1}H NMR spectra (400 MHz, 80 °C) of alg-ADH with a DS of 0.12 in D_2O (6 mg/mL): (A) before and (B) after partial enzymatic hydrolysis. (a and b) H-4 protons of unsaturated residues resulting from a β-elimination reaction on a mannuronic unit having neighbouring guluronic residue and mannuronic residue, respectively; (c) H-1 proton of reducing mannuronic acid residue with α-configuration; (d) H-1 proton of the unsaturated residue resulting from a β-elimination reaction on a mannuronic unit having neighbouring mannuronic residue; (e) H-1 proton of guluronic residue; (f) H-1 proton of reducing mannuronic acid residue with β-configuration; (g) H-5 proton of guluronic residue having neighbouring mannuronic acid residue; (h) H-1 proton of mannuronic acid residue; (i) H-5 proton of guluronic residue having neighbouring guluronic acid residue; (j) H-3 proton of mannuronic residue having undergone a β-elimination; (k) CH_2–CONH–NH; (l) CH_2–CONH–NH_2; (m) CH_2–$(CH_2)_2$–CH_2.

2.3. Titration calorimetry

Isothermal titration calorimetry (ITC) was performed using a Microcal VP-ITC titration microcalorimeter (Northampton, USA). 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 alg-CD **6** solution (cell volume = 1.4478 mL) containing the same solvent as sodium adamantane acetate (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 were identical to 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 following each injection of sodium adamantane acetate as a function of time. The amount of heat produced per 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 (Origin software), with ΔH^0 (the enthalpy change in kJ/mol), K_a (the association constant in L/mol), and n (complex stoichiometry) as adjustable parameters. Calculations were performed using the ''one set of binding sites'' model.

2.4. Complete hydrolysis of the β-CD molecules

Quantification of the content of β-CD molecules grafted onto alginate was determined by complete hydrolysis of CD. Thus, alg-CD **6** (2.8 mg, 0.01 mmol) was heated under acidic conditions (trifluoroacetic acid 2 M (0.5 mL), 4 h at 100 °C). Quantitative determination of glucose, as glucitol acetate derivative, was achieved by gas chromatography (Hewlett Packard 5890A) fitted with a flame-ionisation detector and a SP2380 column (30 m × 0.53 mm i.d.) using N_2 as carrier gas. The same procedure of heating under acidic conditions was applied on initial alginate. Analysis by gas chromatography provided evidence of the absence of glucose in the initial alginate sample.

2.5. Synthesis

2.5.1. alg-ADH *3*

Sodium alginate (4 g, 20 mmol) was dissolved in water to a concentration of 4.5 g/L. Adipic dihydrazide (3.484 g, 20 mmol) was added to this solution. The pH of the reaction was then adjusted to 4.75 using 0.1 M HCl. Next, an aqueous solution of 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide (EDC) (0.230 g, 1.2 mmol) was added slowly to the mixture. The pH of the reaction mixture was maintained at 4.75 by addition of 0.1 M 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 7.5 with 0.2 M NaOH. After addition of NaCl at a concentration of 1 M, the modified alginate 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, 4/1, 9/1) and then, was filtered to give alg-ADH (3.5 g, 87%). The chemical integrity and purity of the final product were checked by ^{1}H NMR (see Fig. 1, as an example). Digital integration of the NMR signals arising from the anomeric protons of alginate and methylene protons of ADH gave a substitution degree of approximately 0.06.

2.5.2. alg-CD *6*

First, the aldehyde function of β-CD acetal derivative **4** was deprotected. Thus, the modified cyclodextrin **4** (0.50 g, 0.39 mmol) was dissolved in water (70 mL) and 0.2 M HCl (15 mL) was added. The resulting mixture was stirred for 3 h at 55 °C and then, was neutralized at pH 7 to give the desired aldehyde. The latter was added to a solution of alg-ADH (0.34 g, 1.7 mmol) in water (110 mL). The pH was adjusted to 5.1 using 0.1 M HCl and was maintained at this value during all the reactions by the addition of 0.1 M HCl. A solution of $NaCNBH_3$ (0.735 g, 11.7 mmol) in water (5 mL) was added and the mixture was stirred overnight. The pH of the reaction was then adjusted to 7.5 using 0.1 M NaOH. The modified alginate was purified by diafiltration through an ultramembrane Amicon YM 10. Diafiltration was stopped when the filtrate conductivity was lower than 10 μS, and the alg-CD derivative was recovered by freeze-drying (0.401 g, 86%). The chemical integrity and purity of the final product were checked by ^{1}H NMR (see Fig. 2). The substitution degree determined by gas chromatography after complete hydrolysis of the CD molecules was found to be 0.05.

2.6. Preparation and characterization of alginate hydrogels

Gelation of initial and modified alginate samples in the presence of calcium ions was obtained by dialysis through a porous cellulosic membrane ''Spectra/Por'' (exclusion limit M_w = 6–8000 g/mol). Solutions of alginates under the sodium form in distilled water at a concentration of 20 g/L were dialyzed for 48 h against 1 M $CaCl_2$. The resulting calcium crosslinked hydrogels were cut into disks (20 mm diameter and approximately 2 mm thickness) and subjected to dynamic rheological measurements. The latter were performed on a controlled-stress AR2000 (TA Instruments) rheometer. A plate–plate system was used (diameter 20 mm); the gap between the plates was 1.6 mm which led to a normal force of 5 N (measuring temperature 25 °C). All the dynamic rheological data were checked as a function of strain amplitude to ensure that the measurements were performed in the linear viscoelastic region. Experiments were carried out with a film of silicone to avoid solvent evaporation. The swelling ratios of the gels at equilibrium (defined as SR (g/g) = $[(W_s - W_d)/W_d]$, in which W_s is the weight of the hydrogel after swelling and W_d is the dry gel weight) were also measured. For this purpose, hydrogels were incubated in water at 4 °C and the surrounding water was exchanged five times in order to remove excess $CaCl_2$ included in the gel. The swollen hydrogel disks were then weighted, dried and weighted again.

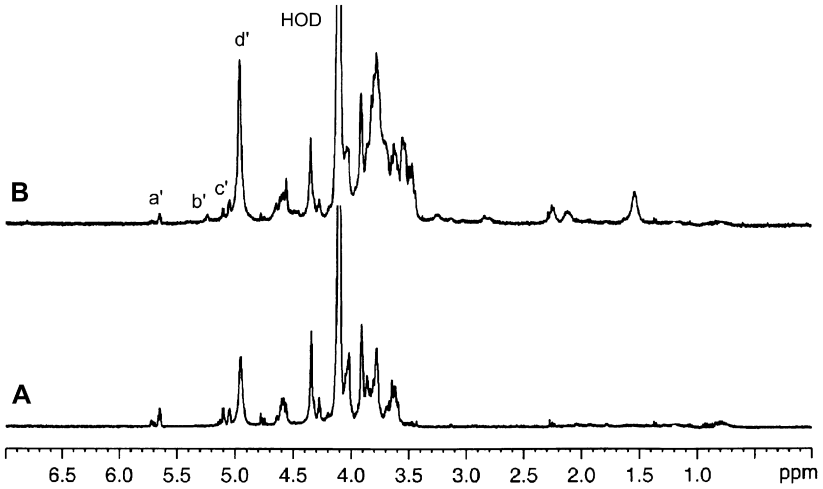
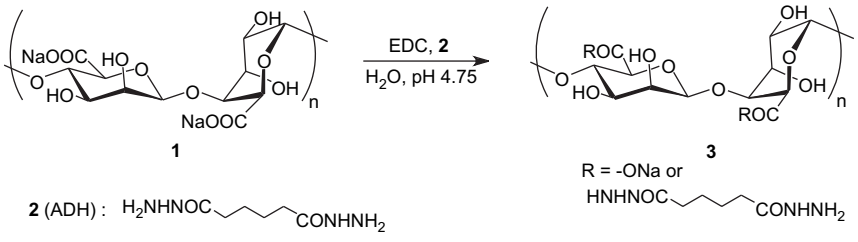


Fig. 2. ^{1}H NMR spectra (400 MHz, 80 °C) of: (A) initial alginate and (B) alg–CD in D_2O (6 mg/mL) after partial enzymatic hydrolysis. (a′) H-4 protons of unsaturated residues resulting from a β-elimination reaction on a mannuronic unit having neighbouring guluronic residue and mannuronic residue; (b′) non-assigned; (c′) H-1 proton of reducing mannuronic acid residue with α-configuration and of the unsaturated residue resulting from a β-elimination reaction on a mannuronic unit having neighbouring mannuronic residue; (d′) H-1 protons of guluronic residue and glucose residues of β-CD.

3. Results and discussion

3.1. Synthesis and characterization of β-cyclodextrin-grafted alginate

Due to the presence of a carboxylic acid group, aqueous carbodiimide chemistry has been shown to be the method of choice for the selective functionalization of alginate [9–11,17–22]. This approach uses a water-soluble carbodiimide, 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide (EDC), which allows the formation of amide linkages by the nucleophilic attack of primary amines onto an *O*-acylisourea intermediate as an activated form of the carboxylic group. However, a common side reaction that occurs when EDC is used is the internal rearrangement of the unstable *O*-acylisourea active ester into a stable *N*-acylurea. The latter not only precludes any nucleophilic attack of a primary amine on the carboxyl, but also is irreversibly grafted onto the polymer which may affect its properties [22]. Additionally, the amount of *N*-acylurea covalently grafted onto alginate increases with increasing EDC concentrations in the reaction medium, which does not allow to easily control the targeted degree of grafting of a molecule, even in the presence of *N*-hydroxysuccinimide (NHS) or its sulfonated derivative (Sulfo-NHS). These co-reagents, developed for peptide synthesis, are normally expected to limit rearrangement to the stable *N*-acylurea by the formation of an active ester intermediate which is more stable than the *O*-acylisourea. On the basis of this observation, our strategy for the grafting of cyclodextrin onto alginate was to apply a mild and versatile methodology developed for chemical modification of hyaluronic acid (HA) and that we recently used for the synthesis of β-CD-linked hyaluronic acid (HA-CD) [14]. This methodology consists in the synthesis of polysaccharide derivatives with pendant reactive hydrazide functionalities along the polymer backbone, by the reaction of the polysaccharide with a dihydrazide compound, such as adipic dihydrazide (ADH), in the presence of EDC at pH 4.75. Indeed, when the dihydrazide compound is present in sufficient excess, no formation of the *N*-acylurea occurs in the case of HA, thanks to the high nucleophilicity of dihydrazides even at the coupling pH value of 4.75, resulting from the α-effect. Our aim was to obtain samples with lower DS ($0.05 < DS < 0.1$). Indeed, the targeted DS for the final CD-grafted alginate (alg-CD) 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 alg-ADH should allow to maintain the gelation properties observed with the native polymer. Hence, for this purpose, optimization of the grafting conditions of ADH onto alginate was requested. Such a work had been already performed for the synthesis of HA-CD. We had found that reaction of HA with 10 M equiv of ADH and 0.15 M equiv of EDC leads to a HA-ADH derivative with a DS of 0.08. Under such conditions (Scheme 1), alginate



Scheme 1. Synthesis of alg-ADH.

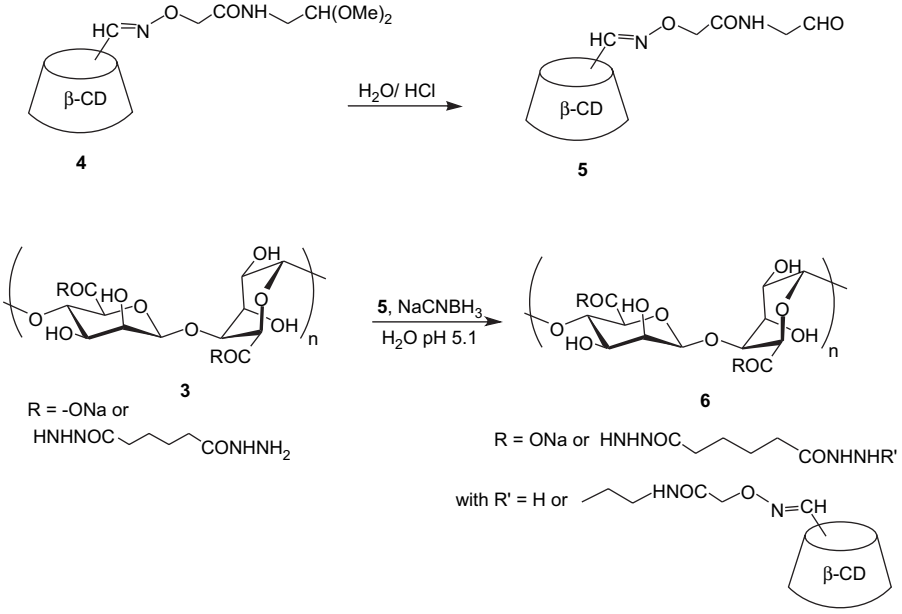
was shown to be more reactive as a DS of 0.12 was obtained. The value of the DS was determined by digital integration of the NMR signals arising from the anomeric protons of alginate and the CH_2 protons of ADH after partial enzymatic hydrolysis of alg-ADH. Indeed, partial enzymatic depolymerization was required because NMR spectra obtained from this product, and initial alginate as well, are characterized by broad proton signals due to the high viscosity of the solutions, which does not allow a correct determination of the DS by NMR integration. Fig. 1 displays the ^{1}H NMR spectra of alg-ADH **3** before and after partial enzymatic hydrolysis. It can be noticed in particular that the intensities of the CH_2CO (k and i) signals of the grafted ADH groups dramatically change after enzymatic hydrolysis. Their similar values after hydrolysis provide evidence of the grafting of the ADH groups through only one side as this theoretically leads to two non-equivalent CH_2CO signals with the same intensity. However, analysis of alg-ADH by size exclusion chromatography equipped with three on-line detectors (see Section 2) indicated the presence of aggregates and only 13% of the product was eluted. This was attributed to the presence of the pendant ADH groups. In order to limit the formation of such physical aggregates, other alg-ADH samples with lower DS were synthesized. For this purpose the quantity of EDC used with respect to alginate was decreased. Moreover, the amount of ADH added was also reduced since alginate appears to be rather reactive. Table 1 summarizes the different reaction conditions used and the structural and macromolecular characteristics of the products. These data seem to demonstrate that a decrease of the DS allows to avoid the formation of aggregates. Indeed, the products with DS of 0.03 and 0.06 are correctly eluted and their weight-average molecular weight is close to that of initial alginate ($M_w = 2.96 \times 10^5$ g/mol). Among these derivatives, we selected those having a DS of 0.06 (Table 1, entry 5) for the coupling reaction with β-CD. The latter was performed according to the procedure recently described for the synthesis of HA-CD [14], which involved the four-step synthesis of a β-cyclodextrin derivative **4** possessing an aldehyde function on the primary face, starting from natural β-CD. Thus, alg-ADH was reacted with excess β-CD derivative **4** (3.8 M equiv with respect to the pendant ADH group) in aqueous solution at an optimal pH value of 5.1 in the presence of sodium cyanoborohydride (Scheme 2). The reductive amination-like reaction was allowed to proceed at room temperature while maintaining the pH at constant value by the dropwise addition of 0.1 M HCl. Indeed, 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 [23]. The expected alg-CD derivative **6** was isolated by a diafiltration process followed by freeze-drying.

Table 1
Reaction conditions used for the synthesis of alg-ADH samples and characterization by ^{1}H NMR and SEC

Reaction	EDC[a]	ADH[a]	DS[b]	M_w[c] (g/mol)	% Eluted product[d]
1	0.15	10	0.12	1.87×10^5	13
2	0.03	10	0.03	2.70×10^5	73
3	0.03	5	0.03	2.96×10^5	69
4	0.06	5	0.06	2.72×10^5	69
5	0.06	1	0.06	2.69×10^5	70

[a] Number of molar equivalents/mole of carboxylate.
[b] Obtained by ^{1}H NMR.
[c] Determined by SEC.
[d] Calc. mass/injected mass ratio.

The grafting of CD molecules onto alginate was demonstrated by ^{1}H NMR spectroscopy. However, owing to the complexity of the one-dimensional ^{1}H NMR spectrum (spectral overcrowding of the protons of the β-CD and alginate, and in particular of the anomeric protons of the β-CD and guluronic



Scheme 2. Synthesis of alg-CD.

residue as shown in Fig. 2), two-dimensional COSY experiments, allowing to make the distinction between the H-2 proton signals of the sugar units of alginate and glucose units of β-CD, were carried out. Fig. 3 compares the partial 2D COSY spectra obtained with initial alginate and alg-CD. From the present data, the presence of two additional cross-peaks can be observed on the spectrum of alg-CD, corresponding to the scalar coupling between the H-1 and H-2 protons of the glucose units of the grafted β-CD. The smaller cross-peak attributed to the scalar coupling between the H-1′ and H-2′ protons of the monosubstituted glucose unit allows in addition to confirm the specific grafting of the CD molecule onto the polysaccharide through only one spacer arm.

The degree of substitution of alg-CD **6** was derived from gas chromatography after complete hydrolysis of the polysaccharide by heating under acidic conditions. Indeed, under such conditions the content of glucose in alg-CD could be quantitatively achieved. This leads to a DS of 0.05, indicating that almost all the ADH groups are substituted by a CD cavity. Analysis of alg-CD by size exclusion chromatography suggested the homogeneous substitution of the polysaccharide with no formation of physical aggregates as shown by the molecular weight distribution profile (data not shown) and also by the fact that 81% of the modified polysaccharide was eluted. The weight-average molecular weight of alg-CD was found to be 3.2×10^5 g/mol. The higher value of M_w for alg-CD compared to that for initial alginate and alg-ADH can be easily explained by the presence of pendant cyclodextrins.

3.2. Complexation properties of alg-CD

The inclusion ability of the alg-CD **6** was investigated by isothermal titration calorimetry using sodium adamantane acetate (ADAc) **7** (Fig. 4) 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 high association constants ($K_a \sim 80\,000\ M^{-1}$). In this study, experiments were performed in 0.1 M NaCl in order to screen electrostatic interactions between negatively charged ADAc and alg-CD **6**. 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 [24]. The dependence of heat evolved upon titration of alg-CD **6** by a solution of adamantane acetate (Fig. 5) yielded an overall stoichiometry of 0.84 consistent with a 1:1 stoichiometry of binding (see Table 2). Fig. 5 shows the titration data for the grafted β-CD/ADAc system fitted with the simplest model in which a single set of identical binding

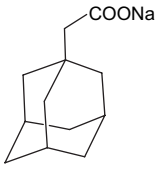


Fig. 4. Chemical structure of sodium adamantane acetate.

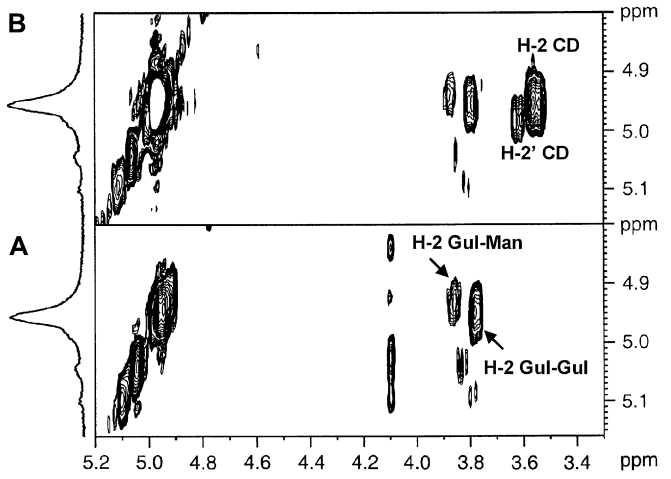


Fig. 3. Partial contour plots of COSY experiments (400 MHz, 80 °C, D_2O) performed on: (A) initial alginate **1** and (B) alg-CD **6** in D_2O (6 mg/mL) after partial enzymatic hydrolysis.

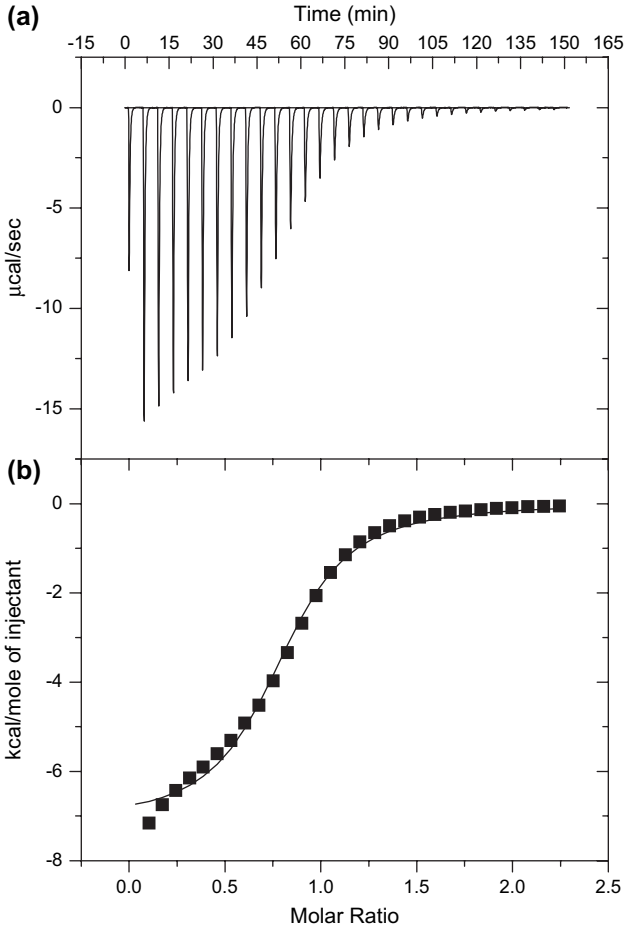


Fig. 5. Calorimetric titration of alg-CD with sodium adamantane acetate in 0.1 M NaCl at 25 °C. (a) Raw data for 30 sequential injections (10 μL per injection) of ADAc (7 mM) injected into alg-CD solution ([CD] = 0.545 mM). (b) The integrated curve showing experimental points and the best fit for titration of alg-CD with ADAc.

Table 2
Thermodynamic parameters for inclusion complex formation of sodium adamantane acetate with natural β-CD [14] and with the CD of alg-CD in 0.1 M NaCl, derived from calorimetric titration experiments

CD derivative	[CD cavity] (mM)	[AD] (mM)	K_a (M^{-1})	ΔH^0 (kJ/mol)	$T\Delta S^0$ (kJ/mol)	n (nAD:1CD derivative)
Natural β-CD	0.798	8.78	$(8.5 \pm 1.1) \times 10^4$	-25.9 ± 0.9	2.21 ± 0.98	0.97 ± 0.08
Grafted β-CD	0.545	7	$(5.0 \pm 0.5) \times 10^4$	-27.8 ± 0.4	0.99 ± 0.26	0.84 ± 0.09

sites is present. Comparison of the thermodynamic parameters derived from the theoretical titration curve for the binding of ADAc to the grafted β-CD with those for the binding to natural β-CD shows similar ΔH^0 values, suggesting similar mechanisms of binding. However, the association constant for the grafted β-CD is slightly lower as a result of a less favorable entropy.

Thus, the calorimetric studies performed with ADAc as a model guest clearly demonstrated that almost all the grafted CD cavities are available for inclusion and their binding properties towards ADAc are similar to those of natural β-CD.

3.3. Gelation ability of alg-CD in the presence of calcium

The ability of alg-CD to give rise to a physical gel in the presence of calcium ions was finally examined. Dialysis of a solution of alg-CD in water ($C_p = 27$ g/L) against a 1 M $CaCl_2$ aqueous solution provided a gel. The latter was cut into disks which were then subjected to dynamic rheology and swelling experiments. Table 3 gives the swelling ratios, together with the values of the dynamic rheological moduli at 1 Hz of the gel of alg-CD and of the gels of initial alginate and alg-ADH prepared under identical conditions. Although the values of the swelling ratios are similar for the three hydrogels, large differences in the values of the dynamic moduli can be noticed, on the other hand. This is illustrated in Fig. 6, which shows the frequency dependence of the dynamic rheological moduli. The storage and loss moduli for all the gels are weakly dependent on frequency, reflecting the network-type structure. The values of the storage and loss moduli of the gel of initial alginate appear larger than those obtained for the gel of alg-ADH, the latter being larger than those of the gel of alg-CD. Since the value of the G′ modulus is related to the number of interchain junction points, these results clearly indicate that chemical modification of alginate at the carboxylate groups hampers ionic cross-linking. Moreover, as the G-blocks are exclusively involved in ionic cross-linking [25], this suggests that some G moieties in G-blocks have been modified. Additionally, although β-CD has been linked to alginate through the ADH group, the steric effect of this cyclic oligosaccharide plays a slight negative role in ionic cross-linking. Indeed, the values of the storage and loss moduli of the alg-CD based hydrogel are lower than those of the alg-ADH hydrogel, which can be attributed to the rigidity and big volume of the CD molecule that reduces the flexibility of the polymer chain and thus, its ability to form interchain junctions. However, it can be noted that the presence of adamantane acetate included in the CD cavities had no effect on the values of the storage and loss moduli, which provides evidence of the absence of specific interactions between the calcium ion and carboxylate of adamantane acetate.

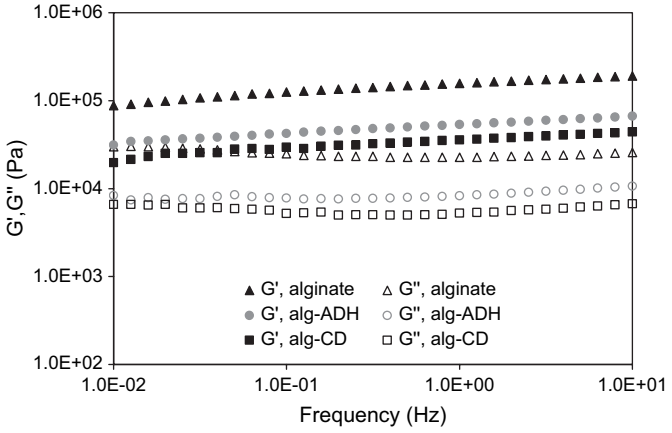


Fig. 6. Comparison of the storage and loss moduli as a function of frequency for calcium cross-linked hydrogels in 1 M $CaCl_2$ of initial alginate **1** (20 g/L or 0.1 monomol/L), alg-ADH **3** (20 g/L or 0.1 monomol/L) and alg-CD **6** (27 g/L or 0.1 monomol/L) at 25 °C.

Table 3
Properties of initial and modified alginate hydrogels at 25 °C

Sample	G′ (Pa, at 1 Hz)	G″ (Pa, at 1 Hz)	SR[a] (g/g)
Alginate **1**	157 000	22 760	29
alg-ADH **3**	53 440	8285	27
alg-CD **6**	35 700	5262	27

[a] Swelling ratio.

4. Conclusion

A monoaldehyde derivative of β-CD was prepared and then grafted by a reductive amination-type reaction onto an alginate derivative selectively modified with adipic dihydrazide (alg-ADH). The new polymeric materials obtained, alg-ADH intermediate and β-CD-grafted alginate were fully characterized in terms of chemical integrity and purity by high-resolution NMR spectroscopy. Inclusion of sodium adamantane acetate as a model guest, into the grafted β-CD, was investigated by isothermal titration calorimetry. Similar enthalpy values were obtained, suggesting similar mechanisms of binding. However, the association constant for the grafted CD was found to be slightly lower as a result of a less favorable entropy due to the fixation on polymer. All alginate derivatives were shown to give rise to hydrogels in the presence of calcium ions. However, rheological measurements performed on the hydrogels demonstrated that backbone chemical modification leads to

a lower cooperative ability for calcium interaction as a consequence of a G-blocks decrease. In addition, the steric effect of the cyclic oligosaccharide (CD) grafted onto alginate plays a negative role on ionic cross-linking. Therefore, the G′ and G″ values decrease in the following order: alg > alg-ADH > alg-CD, which shows that softer gels are generated.

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