Carbohydrate Polymers 87 (2012) 822–829

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Semi-synthesis of chondroitin sulfate-E from chondroitin sulfate-A

Chao Cai [a], Kemal Solakyildirim [a], Bo Yang [a], Julie M. Beaudet [a,b], Amanda Weyers [a], Robert J. Linhardt [a,b,c,d], Fuming Zhang [c,*]

[a] Department of Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York 12180, USA
[b] Department of Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York 12180, USA
[c] Department of Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York 12180, USA
[d] Department of Biomedical Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York 12180, USA

ARTICLE INFO

Article history:
Received 11 July 2011
Received in revised form 8 August 2011
Accepted 23 August 2011
Available online 30 August 2011

Keywords:
Chondroitin sulfate-E
Chondroitin sulfate-A
Chemical semi-synthesis
NMR
PAGE
LCMS
Surface plasmon resonance

ABSTRACT

Chondroitin sulfate-E (chondroitin-4,6-disulfate) was prepared from chondroitin sulfate-A (chondroitin-4-sulfate) by regioselective sulfonation, performed using trimethylamine sulfur trioxide in formamide under argon. The structure of semi-synthetic chondroitin sulfate-E was analyzed by PAGE, ^{1}H NMR, ^{13}C NMR, 2D NMR and disaccharide analysis and compared with natural chondroitin sulfate-E. Both semi-synthetic and natural chondroitin sulfate-E were each biotinylated and immobilized on BIAcore SA biochips and their interactions with fibroblast growth factors displayed very similar binding kinetics and binding affinities. The current semi-synthesis offers an economical approach for the preparation of the rare chondroitin sulfate-E from the readily available chondroitin sulfate-A.

1. Introduction

Chondroitin sulfate (CS) is a family of sulfated glycosaminoglycans (GAGs) composed of a repeating disaccharide motif of glucuronic acid (GlcA) and *N*-acetylgalactosamine (GalNAc) (Fig. 1), (Sugahara et al., 2003) and has been implicated in various physiological functions including cell division and morphogenesis (Mizuguchi et al., 2003; Nandini & Sugahara, 2006) central nervous system (CNS) development (Sugahara & Mikami, 2007) and signal transduction (Sato et al., 2008). CS is found on the plasma membranes of cell surfaces and in the extracellular matrix (ECM) (Basappa et al., 2009) of various kinds of human cells. It is particularly abundant in bone, tendons, blood vessels, nerve tissue, and cartilage (Deepa et al., 2007; Garnjanagoonchorn, Wongekalak, & Engkagul, 2007). Naturally occurring chondroitin sulfate GAG, which is widely distributed in animal tissues, has been reported to have an average molecular weight of 20 kDa (Sugahara et al., 2008). Like most GAGs, however, a range of average molecular weights has been reported that are dependent on the tissue source, extraction and purification methods and the analytical technique used to determine molecular weight.

Chondroitin sulfate-E (CS-E) is one member of the CS family that was originally isolated from squid cartilage (Kawai, Seno, & Anno, 1966; Suzuki et al., 1968), and the structure of CS-E, is [4)-β-D-GlcA-(1→3)-β-D-4,6-*O*-disulfo-GalNAc-(1→]$_n$ (Fig. 1). CS-E is also found in bone marrow-derived mast cells and mucosal mast cells (Stevens & Adachi, 2007) and plays several important biological roles such as: interaction with heparin-binding factors including midkine, L-selectin and P-selectin, CD44 and chemokines (Deepa, Umehara, Higashiyama, Itoh, & Sugahara, 2002; Kawashima et al., 2002; Ueoka et al., 2000); neurite elongation (Clement, Sugahara, & Faissner, 1999; Nandini & Sugahara, 2006); bone formation and biomineralization (Miyazaki et al., 2008); and blocking HSV invasion of cells at substantially lower concentrations (Bergefall et al., 2005).

Abbreviations: CS-E, chondroitin sulfate-E; CS-A, chondroitin sulfate-A; GAG, glycosaminoglycan; ge, gradient-enhanced; GalNAc, N-acetyl-galactosamine; GlcA, glucuronic acid; IdoA, iduronic acid; HMQC, Heteronuclear Multiple-Quantum Coherence experiment; NMR, nuclear magnetic resonance; TOCSY, total correlation spectroscopy; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; SPR, surface plasmon resonance; SA, streptavidin; FC, flow cell; RU, resonance unit; FGF, fibroblast growth factor; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; EDTA, ethylenediaminetetraacetic acid; HXA, n-hexylamine; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; MWCO, molecular weight cut off; PAGE, polyacrylamide gel electrophoresis; 2D, two-dimensional.

* Corresponding author. Tel.: +1 518 276 6839; fax: +1 518 276 3405.
E-mail address: zhangf2@rpi.edu (F. Zhang).

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doi:10.1016/j.carbpol.2011.08.075

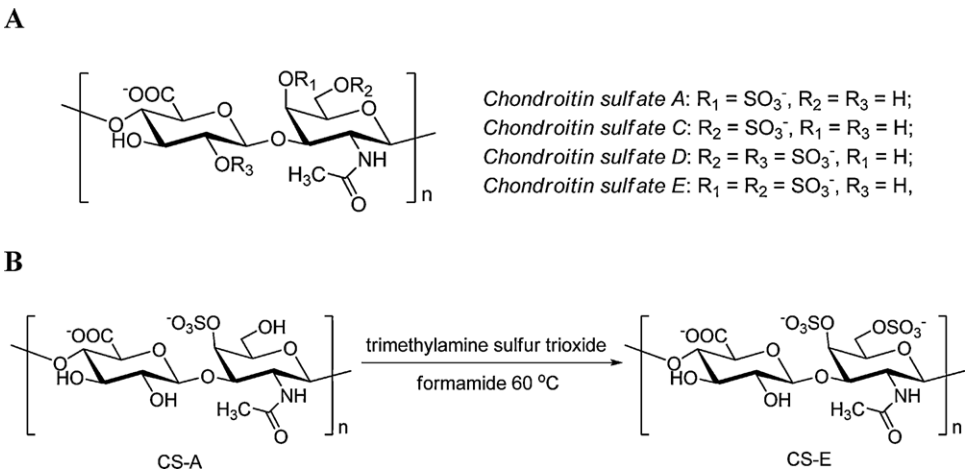

Fig. 1. (A) The structures of chondroitin sulfates, (B) synthesis of CS-E from CS-A.

Commercial CS GAG is generally derived from bovine, porcine cartilage (CS-A) (Liu et al., 2010) and shark cartilage (CS-C) (Ogamo, Yamada, & Nagasawa, 1987). Commercially available CS-A and CS-C typically do not exist as structurally homogenous substances. A CS-A chain can contain some CS-C sequences and a CS-A purified from tissue can contain CS-B or CS-C chains. Moreover, rare forms of CS are obtained from other animals such as crab, squid or hagfish. CS-E, obtained from squid cartilage, contains substantial quantities of other forms of CS making its extraction and purification difficult, resulting in an exorbitant price (Kinoshita et al., 1997). In this paper, we describe a facile and efficient semi-synthetic chemical route, using trimethylamine sulfur trioxide, to prepare the expensive CS-E from commercially available and inexpensive CS-A {[4)-β-D-GlcA-(1→3)-β-D-4-O-sulfo-GalNAc-(1→]$_n$ (Fig. 1)}. Chemical sulfonation is widely used for the chemical synthesis of sulfated oligosaccharides (Blanchard, Turecek, & Gelb, 2009; Marra, Dong, Petitou, & Sinay, 1989; Zsiska & Meyer, 1991). This semi-synthesis is scalable potentially offering a new and relatively inexpensive method for the preparation of large amount of CS-E for use in biological and medical applications. The potential side effects of chemically sulfonated chondroitin sulfate should be seriously considered and further investigation is clearly required before semi-synthetic CS-E can be used in vivo for clinical applications. Disaccharide analysis, using liquid chromatography and mass spectrometry (LC–MS), structural analysis relying on ^{1}H, ^{13}C and 2D (two-dimensional) NMR spectroscopy and surface plasmon resonance (SPR) for protein binding studies, were used to characterize this semi-synthetic CS-E.

2. Materials and methods

2.1. Materials

CS-A, from bovine trachea, was purchased from Celsus Laboratories (Cincinnati, OH), and CS-E from squid cartilage, was purchased from Seikagaku Biobusiness Co. (Tokyo, Japan), respectively. Unsaturated disaccharides standards of CS/DS (ΔDi-0S: ΔUA-GalNAc, ΔDi-4S: ΔUA-GalNAc4S, ΔDi-6S: ΔUAGalNAc6S, ΔDi-2S: ΔUA2S-GalNAc, ΔDi-diSB: ΔUA2S-GalNAc4S, ΔDi-diSD: ΔUA2S-GalNAc6S, ΔDi-diSE: ΔUA-GalNAc4S6S, ΔDi-triS: ΔUA2SGalNAc4S6S, where ΔUA corresponds to 4-deoxy-α-L-*threo*-hex-enopyranosyluronic acid, S corresponds to sulfo, and Ac corresponds to acetyl), chondroitin lyases ABC and ACII were purchased from Associates of Cape Cod, Inc. (East Falmouth, MA). Trimethylamine sulfur trioxide, formamide, deuterium oxide, n-hexylamine (HXA), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), were purchased from Sigma–Aldrich (St. Louis, MO). 4–20% precast Mini-PROTEAN TGX gels were purchased from Biorad (Hercules, California). Recombinant FGF-1 an FGF-2 were prepared from *Escherichia coli* (Mach et al., 1993) and had a purity of >95% by sodium dedecylsulfate polyacrylamide gel electrophoresis (PAGE).

2.2. Semi-synthesis of CS-E

CS-A (6.7 g, ~0.335 mmol) was dissolved in formamide (90 mL, previously dried over 4 Å molecular sieves), and trimethylamine sulfur trioxide (10 g, ~5 equiv. free hydroxyl group, based on disaccharide units) was added into the solution under Ar. The mixture was stirred vigorously at 60 °C for 24 h to achieve clear solution, which was then transferred into 95% aqueous ethanol (100 mL) at room temperature and held for 30 min before the addition of 1% aqueous NaCl (500 mL). After the pH was adjusted to neutral with 2 M NaOH, the solution was dialyzed against distilled water for 2 days and the dialysate was lyophilized to give the crude sulfated product as off-white powder. The powder was redissolved in 16% aqueous NaCl (100 mL), which was followed by the addition of ethanol (350 mL), and the precipitate was centrifuged at 4000 rpm for 10 min to afford purified semi-synthetic CS-E (3.6 g) as white powder.

2.3. Polyacrylamide gel electrophoresis (PAGE) analysis

PAGE was used to examine the molecular weight properties of the CS-A sample, the semi-synthetic CS-E sample and the natural CS-E sample. A 4–20% precast gel was loaded with aliquots of 3–5 μg of a sample into each lane of the gel and then subjected to electrophoresis. The samples included a standard ladder comprised of oligosaccharides collected from the digestion of CS-A. The gel was washed for 1 h, stained with alcian blue, destained with 10% acetic acid/25% ethanol (v/v) solution, and calculations were made using UN-scan–it software (Silk Scientific, Utah) and the molecular weight characteristics of each sample was calculated.

2.4. Nuclear magnetic resonance (NMR) analysis

The purified semi-synthetic CS-E (~2.5 mg) and natural CS-E (~2 mg) polysaccharides were prepared for NMR analysis by dissolving each in 0.4 mL of 99.996 atom% deuterium oxide (^2H_2O) and freeze dried to remove exchangeable protons. All NMR data were acquired on a Bruker Avance II Ultrashield 600 MHz (14.1-T) NMR instruments equipped with an ultrasensitive HCN cryoprobe with a *z*-axis gradient. The ^{13}C NMR spectra were recorded at 150 MHz. The spectra were acquired at a probe temperature of 298 K. For one-dimensional ^{1}H NMR spectra, sweep width of 20.5 ppm and

acquisition time of 2.66 s were employed. For two-dimensional NMR experiments, 128 experiments resulting in 4096 data points for a spectral width of 10 ppm were measured. Proton-detected HMQC experiments used 12 ppm and 78 ppm spectral widths in the ^{1}H dimension and ^{13}C dimension, respectively. The 2D NMR data sets were processed by Topspin version 2.1.4 and cross-peak assignments were carried out using an NMR assignment software Sparky (Goddard & Kneller, 2001).

2.5. Disaccharide analysis using LC/MS

LC–MS analyses were performed on an Agilent 1100 LC/MSD instrument (Agilent Technologies, Inc., Wilmington, DE) equipped with an ion trap and a UV detector. The column used was a 1.7 μm Acquity UPLC BEH C18 column (2.1 × 150 mm, Waters, Milford, MA, USA). Solutions A and B for UPLC were 0 and 75% acetonitrile, respectively, containing the same concentration of 15 mM HXA as an ion-pairing reagent and 100 mM HFIP as an organic modifier. The column temperature was maintained at 45 °C. Solution A for 10 min, followed by a linear gradient from 10 to 40 min of 0 to 50% solution B at the flow rate of 100 μL/min was used for disaccharides analysis. The electrospray interface was set in positive ionization mode with the skimmer potential 40.0 V, capillary exit 40.0 V, and a source of temperature of 350 °C to obtain maximum abundance of the ions in a full-scan spectra (350–2000 Da, 10 full scans/s). Nitrogen was used as a drying gas (8 L/min) and a nebulizing gas (40 psi) (Solakyildirim, Zhang, & Linhardt, 2010).

2.6. Surface plasmon resonance (SPR) analysis

CS-E (2 mg) and amine-PEG3-biotin (2 mg, Pierce, Rockford, IL) were dissolved in 200 μL H_2O and 10 mg $NaCNBH_3$ was added to prepare biotinylated CS-E. The reaction mixture was heated at 70 °C for 24 h, after that a further 10 mg $NaCNBH_3$ was added and the reaction was heated at 70 °C for another 24 h. After cooling to room temperature, the mixture was desalted with the spin column (3000 molecular weight cut off (MWCO)). Biotinylated CS-E was collected, freeze-dried and used for streptavidin (SA) chip preparation. Since the SA chip captures only biotinylated GAGs, no purification of biotinylated CS-E from CS-E starting material was required.

SPR experiments were performed on a BIAcore 3000 operated using the version software (GE Healthcare, Uppsala, Sweden). Biotinylated CS-E was immobilized to SA chip (GE Healthcare, Uppsala, Sweden) based on the manufacturer's protocol. In brief, 20 μL solution of the CS-E–biotin conjugate (1 mg/mL) in HBS-EP buffer (10 mM 4-(2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES), 150 mM sodium chloride, 3 mM ethylenediaminetetraacetic acid (EDTA), 0.005% polysorbate surfactant P20 pH 7.4 buffer) (GE Healthcare, Uppsala, Sweden) was injected over flow cells 2 and 3 (FC2 and FC3 for semi-synthetic CS-E and natural CS-E, respectively) of the SA chip at a flow rate of 10 μL/min. The successful immobilization of CS-E was confirmed by the observation of a 100–250-resonance unit (RU) increase in the sensor chip. The control flow cell (FC1) was prepared by 1 min injection with saturated biotin.

The protein sample fibroblast growth factor (FGF)-1 or -2, was diluted in HBS-EP buffer to do the kinetic measurements of protein–CS-E interactions. Different dilutions of protein samples in buffer were injected at a flow rate of 30 μL/min. At the end of the sample injection (120 s), the same running buffer was passed over the sensor surface to facilitate dissociation for 120 s. After dissociation, the sensor surface was regenerated by injecting 2 M NaCl. The response was monitored as a function of time (sensorgram) at 25 °C. SPR experiments were run in duplicate at each concentration to confirm the bindings were repeatable.

Table 1
Proton (^{1}H) and carbon (^{13}C) chemical shift values of semi-synthetic and natural CS-E polysaccharides.

Residue/position[a]	Natural CS-E		Semi-synthetic CS-E	
	^{13}C	^{1}H	^{13}C	^{1}H
GlcA1	103.87	4.40	104.00	4.43
GlcA 2	72.14	3.29	72.07	3.29
GlcA 3	73.75	3.50	73.72	3.53
GlcA 4	82.00	3.68	81.38	3.71
GlcA 5	76.48	3.57	75.40	3.71
GalNAc1	102.80	4.48	101.35	4.47
GalNAc 2	51.40	3.97	51.14	3.94
GalNAc 3-4S6x[b]	75.85	3.96	75.58	3.96
GalNAc 3-6S	nd[c]	nd	79.97	3.76
GalNAc 4	76.29	4.67	76.14	4.69
GalNAc 5-6S4x	72.23	4.03	72.30	4.03
GalNAc 6-4S	60.74	3.81	60.98	3.70
GalNAc 6′-4S	60.95	3.67	nd	nd
GalNAc 6-6S4x	67.67	4.13	67.37	4.13
GalNAc (CH_3)	22.48	1.95	22.43	1.93

[a] Assignments made based on the literature data (Bociek et al. 1980; Kinoshita et al., 1997, 2001; Kinoshita-Toyoda et al., 2001).
[b] x represents either an OH or sulfate.
[c] nd, not determined.

3. Results and discussion

3.1. Chemical semi-synthesis of CS-E from CS-A

Semi-synthetic CS-E was prepared from CS-A using trimethylamine sulfur trioxide (Blanchard et al., 2009; Marra et al., 1989; Zsiska & Meyer, 1991) in formamide under argon. The commercial CS-A as starting material had a molecular weight of 16 kDa (estimated by PAGE analysis) and was monosulfated with 79% of its sulfo groups at carbon 4 of GalNAc (CS-A) and 21% of its sulfo groups at carbon 6 of GlcNAc (CS-C) (Table 2). The most sterically accessible hydroxyl group in CS-A is the primary hydroxyl group at carbon-6 of the GalNAc residue. Regioselective 6-*O*-sulfonation of dermatan sulfate, [4)-α-L-IdoA-(1→3)-β-D-4-*O*-sulfo GalNAc (1→]$_n$ had previously been reported affording dermatan-4,6-disulfate (Brister, Buchanan, Griffin, Van Gorp, & Linhardt, 1999). As anticipated sulfonation was regioselective at C-6 of GalNAc, with a modest level of sulfonation also occurring at the secondary hydroxyl group of the C-2 on GlcA, due to the use of excess trimethylamine sulfur trioxide. Optimal conditions affording a maximum level of CS-E product based on the consumed CS-A with minimum 2-*O*-sulfonation was found to be 5 equiv. of trimethylamine sulfur trioxide at 60 °C for 24 h. Semi-synthetic CS-E was initially purified by dialysis, resulting in some residual trimethylamine in crude product. Precipitation of the crude product from 16% NaCl aqueous solution by the addition of ethanol afforded purified semi-synthetic CS-E in 46.2% overall yield.

3.2. Polyacrylamide gel electrophoresis (PAGE) analysis

Initial characterization of the molecular weight properties of semi-synthetic CS-E by PAGE with alcian blue staining (see Supplemental figure) suggested it to have an average molecular weight (~16 kDa) and polydispersity comparable to that of the CS-A starting material that had been used. It is noteworthy that this is considerably smaller than that of natural CS-E obtained from squid, having an average molecular weight of ~56 kDa based on PAGE analysis. The results of these analyses suggest that the polysaccharide backbone remains intact during chemical sulfonation and that higher molecular weight CS-A starting material will be required if a semi-synthetic CS-E having identical molecular weight properties to squid CS-E is desired. In this study, however, our goal was only to prepare a semi-synthetic CS-E with comparable

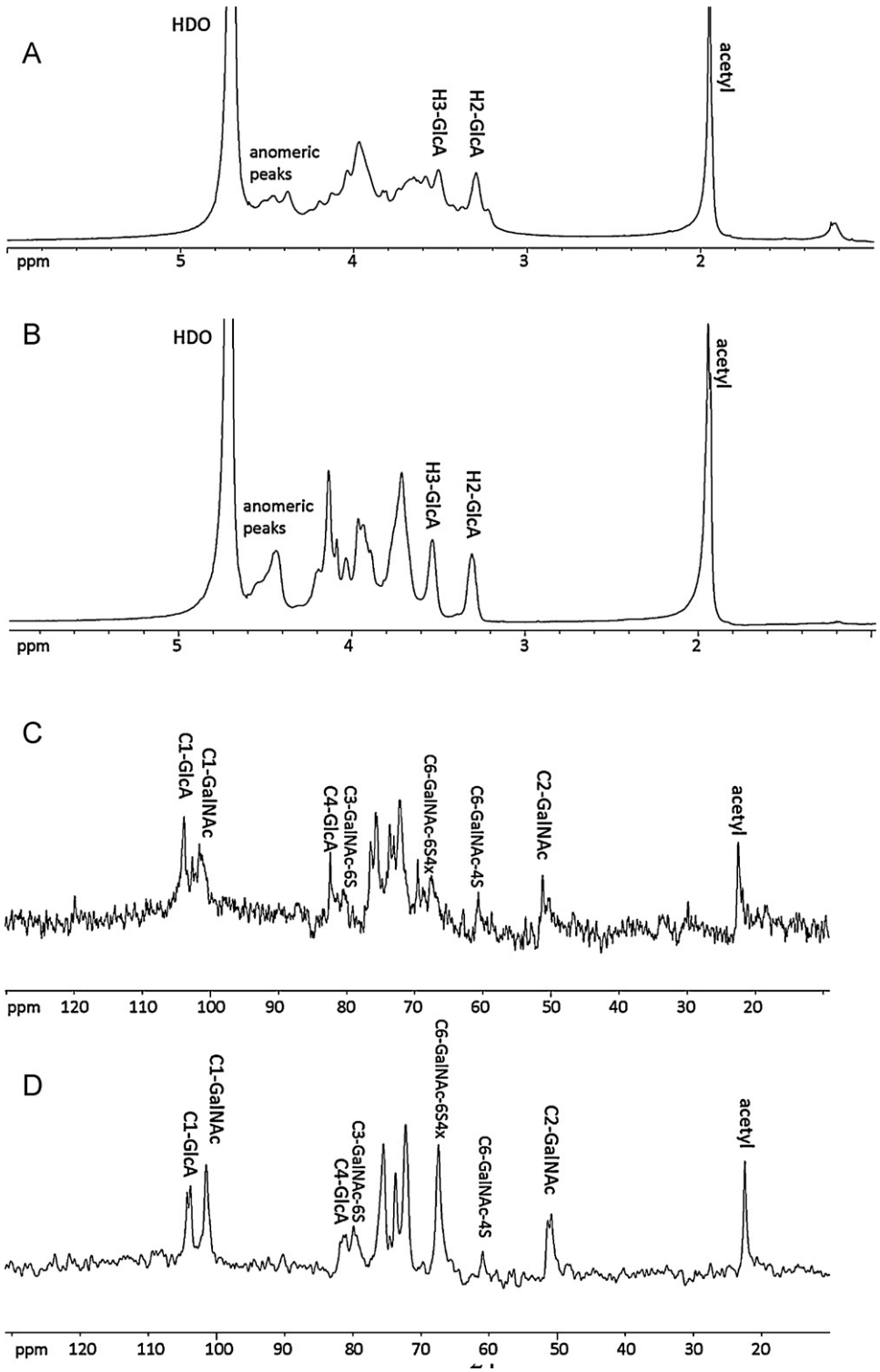

Fig. 2. ^{1}H NMR spectra of the natural CS-E (panel A) and semi-synthetic CS-E (panel B) polysaccharides recorded in $^{2}H_2O$ at 298 K. ^{13}C NMR spectra of the natural CS-E (panel C) and semi-synthetic CS-E (panel D) polysaccharides recorded in $^{2}H_2O$ at 298 K.

disaccharide sequence/and a similar protein binding characteristics to that of the natural product.

3.3. NMR results

The purified semi-synthetic CS-E and natural CS-E polysaccharides were analyzed by NMR spectroscopy (Figs. 2 and 3, and Table 1). Chemical shift assignments relied on one-dimensional ^{1}H and ^{13}C, and two-dimensional (2D) HMQC (^{13}C–^{1}H), ge–HMQC–TOCSY (data not shown), and literature data (Bociek, Darke, Welti, & Rees, 1980; Kinoshita et al., 1997, 2001; Kinoshita-Toyoda et al., 2001). The ^{1}H and ^{13}C NMR data of the natural CS-E and the semi-synthetic CS-E (Fig. 2A–D) shows the Ac-CH_3 signal at ~2 ppm and resonances at ~3.29 ppm and ~3.50 ppm, characteristic of the H-2 and H-3 protons of the GlcA residue.

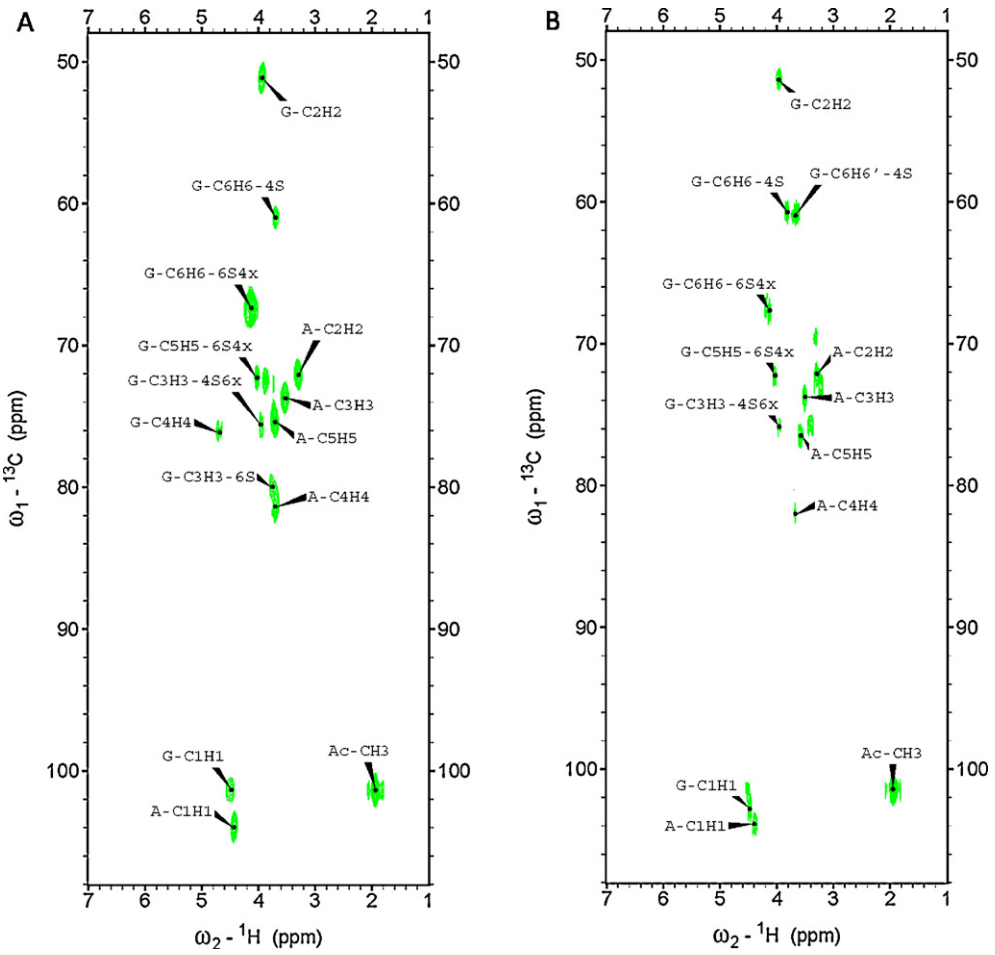

Fig. 3. 2D HMQC spectra of the semi-synthetic CS-E (panel A) and natural CS-E (panel B) polysaccharides recorded in ^2H_2O at 298 K. The letter x represents either an OH or a sulfate.

Table 2
CS/DS disaccharide composition analysis by LC–MS.

Sample	CS/DS disaccharides composition							
	ΔDi-0S	ΔDi-2S	ΔDi-6S	ΔDi-4S	ΔDi-diS_D	ΔDi-diS_B	ΔDi-diS_E	ΔDi-TriS
Natural CS-E	–	–	0.9	21.3	–	–	77.3	–
CS-A	–	–	21.2	78.8	–	–	–	–
Semi-synthetic CS-E	–	–	15.4	24.2	5.5	2.7	52.2	–

2D-NMR experiments, particularly HMQC (^1H–^{13}C), permitted the resolution and identification of almost all the proton resonances (Fig. 3 and Table 1). The resonances for C-4/H-4 of GlcA (81.38/3.71 ppm) and C-3/H-3 of GalNAc-6S (79.97/3.76 ppm) were made using a ge–HMQC–TOCSY experiment (data not shown). The synthetic CS-E sample showed nearly identical resonances to the commercial CS-E polysaccharide. However, the chemical shift values for the ^1H and ^{13}C signals for the GalNAc residue show some minor differences in Figs. 2A–D. For instance, the H-4 proton of GalNAc of the natural CS-E was not observed in the HMQC spectrum, which could be due to the low intensity of the H-4 proton resonance, possibly associated with its higher molecular weight. The presence of H-4 proton of GalNAc residue could be observed in a ^1H NMR spectrum at 328 K (data not shown) resulting in a downfield shift in the H-4 proton of GalNAc residue and permitting its easy identification at 4.95–5.00 ppm.

3.4. Disaccharide analysis using LC/MS

Compositional analysis of disaccharides gives important structural information and is a sensitive and highly reliable method to detect variation of GAG structures. CS/DS GAGs contain different disaccharide sequences including those corresponding to the eight CS/DS disaccharide standards. An LC/MS analysis method that relies on ion-pairing reversed-phase capillary HPLC was used to determine the GAG disaccharide composition (Solakyildirim et al., 2010). This method affords good resolution in the separation of eight CS/DS disaccharide standards (Fig. 4a). The disaccharide analysis of CS-A natural CS-E and semi-synthetic CS-E (Fig. 4b–d and Table 2) show that in CS-A is the 6S and no 4S6S (SE) disaccharide is detected. In contrast, the major disaccharide in natural and semi-synthetic CS-E was ΔDi-diS_E, over 77% and 52% in natural and semi-synthetic CS-E, respectively. Semi-synthetic CS-E also shows a small amount of some 2S4S (SB) and 2S6S (SD) disaccharide, 2.7%

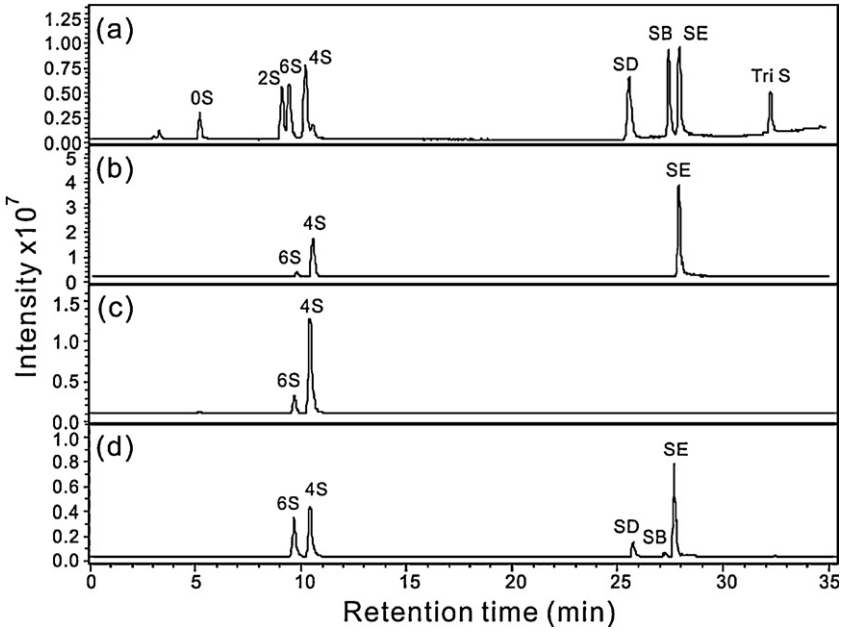

Fig. 4. CS/DS disaccharides analysis by LC–MS (a) extracted ion chromatography (EIC) of CS/DS disaccharide standards; (b), (c) and (d) EIC of CS/DS disaccharides from natural CS-E CS-A starting material and semi-synthetic CS-E.

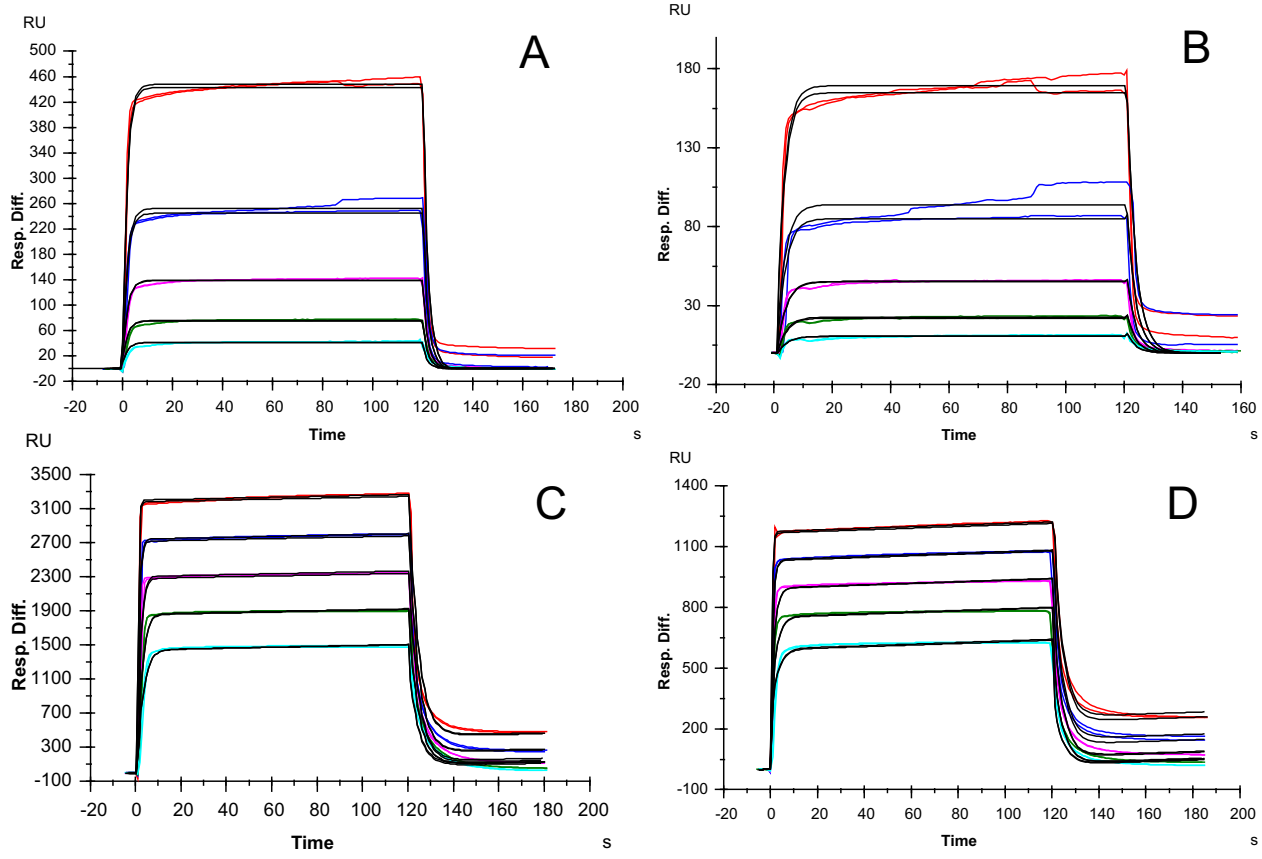

Fig. 5. SPR sensorgrams of the interactions between CS-E and FGF proteins. Concentrations of proteins (from top to bottom): 1000, 500, 250, 125, and 63 nM, respectively. The black curves in all sensorgrams are the fitting curves using 1:1 Langmuir binding model from BIAevaluate 4.0.1. (A) SPR sensorgrams of semi-synthetic CS-E-FGF1 interaction. (B) SPR sensorgrams of natural CS-E-FGF1 interaction. (C) SPR sensorgrams of semi-synthetic CS-E-FGF2 interaction. (D) SPR sensorgrams of natural CS-E-FGF2 interaction.

Table 3
Summary of kinetic data of CS-E–protein interactions.

	ka (1/MS)	kd (1/S)	K_D (M)
Semi-synthetic CS-E-FGF1	8.29×10^4	0.491	5.90×10^{-6}
Natural CS-E-FGF1	7.75×10^4	0.319	4.12×10^{-6}
Semi-synthetic CS-E-FGF2	2.3×10^6	0.244	1.06×10^{-6}
Natural CS-E-FGF2	1.63×10^6	0.265	1.63×10^{-6}

and 5.5%, respectively. This result confirms that some undesired 2-*O*-sulfonation of CS-A has also taken place. Further optimization of sulfonation chemistry, such as a reduction in the number of equivalents of trimethyamine sulfur trioxide might reduce these side products but also would result in reduced levels of 4S6S (SE) disaccharides.

3.5. CS-E–protein interaction analysis by SPR

The numerous biological important activities of GAGs are associated with the interactions with diverse proteins (Capila & Linhardt, 2002). These interactions mediate various physiologic and pathophysiologic processes such as: blood coagulation, cell growth and differentiation, host defense and viral infection, lipid transport and clearance/metabolism, cell–cell and cell–matrix signaling, inflammatory and cancer. It was reported (Deepa et al., 2002) that squid cartilage CS-E binds various heparin-binding growth factors including FGF-2, FGF-10, FGF-16, FGF-18, midkine (MK) and pleiotrophin (PTN) (most of which are expressed in the brain), suggesting that these interactions have physiological significance in brain development. The interaction between the CS-E and proteins from fibroblast growth factor signaling proteins (FGF1 and FGF2) was investigated using SPR to determine if the bioactivities of semi-synthetic and natural CS-E were comparable. The results (Table 3 and Fig. 5) showed both CS-Es having very similar binding kinetics and affinity to FGF1 and FGF2. The binding response for the natural CS-E surface is lower than for the semi-synthetic CS-E in both FGF1 and FGF2 interactions (Fig. 5). This is primarily due to a lower immobilization density for natural CS-E than for semi-synthetic CS-E (~100 RU vs. ~250 RU). The interaction between GAGs (in particular heparin an heparin sulfate are known to only required 4–6 saccharide residues corresponding to a molecular weight of <1800 (Mach et al., 1993). Therefore, we expect that FGF interaction with CS-E would require a polysaccharide chain of similar length and one much shorter than that of either the semi-synthetic CS-E or natural CS-E. We conclude, therefore, that the interaction of the natural CS-E and semi-synthetic CS-E are comparable. Moreover, the binding kinetics and affinity parameters of CS-E FGF1 and FGF2 interactions match with our previous report (Liu et al., 2010). While it is not possible to extrapolate these to other biological activities associated with CS-E, the FGF-binding activity is unique to the CS-E members of the chondroitin family with CS-A failing to show measurable interaction.

In conclusion, the current semi-synthesis offers an economical approach for the preparation of the rare chondroitin sulfate-E (with similar chemical composition and biological activity as the natural CS-E) from the readily available chondroitin sulfate-A. This semi-synthesis is scalable potentially offering an inexpensive method for the preparation of large amount of CS-E for use in biological and medical applications. We have avoided the formation of oversulfated chondroitin sulfate as a byproduct in the semi-synthetic CS-E preparation by carefully controlling the reaction conditions. Despite these precautions, extreme care should be taken before using any chemically sulfonated chondroitin product in vivo as oversulfated chondroitin sulfate has been associated with anaphylactoid-type reactions (Guerrini et al., 2008).

Acknowledgements

This work was supported by grants from the funded by the National Institutes of Health HL101721, HL096972, GM38060 and 2T32GM067545.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.08.075.

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