Published in final edited form as:

Carbohydr Polym. 2012 January 4; 87(1): 822–829. doi:10.1016/j.carbpol.2011.08.075.

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

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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, ¹H NMR, ¹³C 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.

Keywords

Chondroitin sulfate-E; chondroitin sulfate-A; chemical semi-synthesis; NMR; PAGE; LCMS; surface plasmon resonance

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 and Sugahara, 2006) central nervous system (CNS) development (Sugahara and Mikami, 2007) and signal transduction (Nandini and Mikami, 2007; 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, et al., 2007).

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Naturally occurring chondroitin sulfate GAG, which is widely distributed in animal tissues, has an average molecular weight of 20 kDa (Sugahara, et al., 2008).

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

Commercial CS GAG is generally derived from bovine, porcine cartilage (CS-A) (Liu, et al., 2010) and shark cartilage (CS-C) (Ogamo, et al., 1987). Commercially available CS-A typically contains small amounts of CS-C and CS-C typically contains small amounts of CS-A. 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 to prepare the expensive CS-E from commercially available and inexpensive CS-A $\{[4\text{-}\beta\text{-D-GlcA-(1}\rightarrow\text{3)-}\beta\text{-D-4-O-sulfo-GalNAc-(1}\rightarrow\text{)}_n$, (Fig. 1)}. 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. Disaccharide analysis, using liquid chromatography and mass spectrometry (LC-MS), structural analysis relying on ^1H , ^{13}C and 2D (two-dimensional) NMR spectroscopy and surface plasmon resonance (SPR) for protein binding studies, was 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 ($\Delta\text{Di-0S}$: $\Delta\text{UA-GalNAc}$, $\Delta\text{Di-4S}$: $\Delta\text{UA-GalNAc4S}$, $\Delta\text{Di-6S}$: $\Delta\text{UAGalNAc6S}$, $\Delta\text{Di-2S}$: $\Delta\text{UA2S-GalNAc}$, $\Delta\text{Di-diSB}$: $\Delta\text{UA2S-GalNAc4S}$, $\Delta\text{Di-diSD}$: $\Delta\text{UA2S-GalNAc6S}$, $\Delta\text{Di-diSE}$: $\Delta\text{UA-GalNAc4S6S}$, $\Delta\text{Di-triS}$: $\Delta\text{UA2SGalNAc4S6S}$, where ΔUA corresponds to 4-deoxy- $\alpha\text{-L-threo-hex-enopyranosyluronic acid}$, 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).

2.2 Semi-synthesis of CS-E

CS-A (6.7 g, ~ 0.16 mmol) was dissolved in formamide (90 mL, previously dried over 4 Å molecular sieves), and trimethylamine sulfur trioxide (10 g, ~ 20 equiv. free hydroxyl group) 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 4,000 rpm for 10 min to afford pure 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 μ g 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 CSA. 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 ($^2\text{H}_2\text{O}$), and freeze dried to remove exchangeable protons. All NMR data were acquired on a Bruker Avance II Ultrashield 600 MHz (14.1-Tesla) 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 298K. For one-dimensional ^1H -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 ^1H 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, T.D. and Kneller, D.G. 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 μ m Acquity UPLC BEH C18 column (2.1 $\times$ 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 $^\circ\text{C}$. 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 $\mu\text{L}/\text{min}$ 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 $^\circ\text{C}$ 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, et al., 2010).

2.6 Surface Plasma 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 $^\circ\text{C}$ for 24 h, after that a further 10 mg NaCNBH_3 was added and the reaction was heated at 70 $^\circ\text{C}$ for another 24 h. After cooling to room temperature, the mixture was desalted with the spin column (3,000 molecular weight cut off (MWCO)). Biotinylated CS-E was collected, freeze-dried and used for streptavidin (SA) chip preparation.

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 μ L 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 μ L/min. The successful immobilization of CS-E was confirmed by the observation of a 100 to 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 μ L/min. At the end of the sample injection (120 sec), the same running buffer was passed over the sensor surface to facilitate dissociation for 120 sec. 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.

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 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 $\rightarrow$ 3)- β -D-4-*O*-sulfo GalNAc (1 $\rightarrow$)_n had previously been reported affording dermatan-4,6-disulfate (Brister et al., 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 20 equiv of trimethylamine sulfur trioxide at 60 °C for 24 h. Semi-synthetic CS-E was initially purified by dialysis, resulting in some residual triethylamine 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 (not shown) 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. 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 disaccharide sequence to that of the natural product.

3.3 NMR Results

The purified semi-synthetic CS-E and natural CS-E polysaccharides were next analyzed by NMR spectroscopy (Figures 2 and 3, and Table 1). Their chemical shift assignments were determined using a combination of data obtained from one-dimensional ^1H and ^{13}C , and two-dimensional HMQC (^{13}C - ^1H), *ge*-HMQC-TOCSY (data not shown), and literature data (Bociek, et al. 1980; Kinoshita, et al., 1997; Kinoshita, et al., 2001; Kinoshita-Toyodo, et al., 2001). These polysaccharides had nearly identical spectral properties. The anomeric signals of each type of monosaccharide residue were assigned based on their characteristic downfield positions. The ^1H and ^{13}C NMR data of the natural CS-E and the semi-synthetic CS-E are illustrated in Figs. 2 (A, B, C, and D). Besides the Ac-CH₃ signal ~ 2 ppm, all proton resonances are found between 3 and 5 ppm for both CS-E samples in ^1H -NMR. The resonances at ~ 3.29 ppm and ~ 3.50 ppm are characteristic of the H-2 and H-3 protons of the GlcUA residue, respectively, for both polysaccharides. Anomeric peaks are not clearly resolved in ^1H -NMR spectra requiring use of ^1H - ^{13}C HMQC spectroscopy.

Complete characterization of the CS-E structure in the one-dimensional ^1H and ^{13}C spectra is prevented by overlapping signals. Two-dimensional NMR experiments, particularly HMQC (^1H - ^{13}C), permits the resolution and identification of all proton resonances (Fig. 3). The resolution of almost all resonances was achieved in the HMQC spectra allowing accurate chemical shift assignments (Table 1). The resonances for C-4/H-4 of GlcUA (81.38/3.71 ppm) and C-3/H-3 of GalNAc-6S (79.97/3.76 ppm) were difficult to assign solely using an HMQC experiment. Instead, this assignment was 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. For example, the anomeric H-1 signals at 4.40/103.87 ppm and at 4.43/104.00 ppm indicate GlcUA residues from the natural CS-E and semi-synthetic CS-E, respectively, and the anomeric H-1 signals at 102.80/4.48 ppm and at 101.35/4.47 ppm show the GalNAc residues from the natural CS-E and semi-synthetic CS-E, respectively. Signals associated with the Glc-A residue (labeled as A in Fig. 3) indicate that the ^1H and ^{13}C resonances are virtually identical (Table 1). However, the chemical shift values for the ^1H and ^{13}C signals for the GalNAc residue show some minor differences in Figs. 2 (A, B, C, and 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 328K (data not shown). At 328 K, a downfield shift in the H-4 proton of GalNAc residue permits 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. 5b,c,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 the major disaccharide was $\Delta\text{Di-diSE}$, 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% 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 trimethylamine

sulfur trioxide might reduce these side products but also would result in reduced levels of 2S4S (SE) disaccharides.

3.5 CS-E-protein interaction analysis by SPR

The numerous biological important activities of glycosaminoglycans are associated with the interactions with diverse proteins (Capila and 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 Figure 5) showed both CS-Es having very similar binding kinetics and affinity to FGF1 and FGF2. 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.

Abbreviations

CS-E	chondroitin sulfate-E
CS-A	chondroitin sulfate-A
GAG	glycosaminoglycan
ge	gradient-enhanced
GalNAc	N-acetyl-galactosamine
GlcA	glucuronic 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
2D	two-dimensional

Acknowledgments

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

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A

Chondroitin sulfate-A: $R_1 = \text{SO}_3^-$, $R_2 = R_3 = \text{H}$

Chondroitin sulfate-C: $R_2 = \text{SO}_3^-$, $R_1 = R_3 = \text{H}$

Chondroitin sulfate-D: $R_2 = R_3 = \text{SO}_3^-$, $R_1 = \text{H}$

Chondroitin sulfate-E: $R_1 = R_2 = \text{SO}_3^-$, $R_3 = \text{H}$

B

Figure 1.

A. The structures of chondroitin sulfates, **B.** Synthesis of CS-E from CS-A.

Figure 2. ^1H -NMR spectra of the natural CS-E (panel **A**) and semi-synthetic CS-E (panel **B**) polysaccharides recorded in $^2\text{H}_2\text{O}$ at 298K. ^{13}C -NMR spectra of the natural CS-E (panel **C**) and semi-synthetic CS-E (panel **D**) polysaccharides recorded in $^2\text{H}_2\text{O}$ at 298K.

Figure 3. 2D HMQC spectra of the semi-synthetic CS-E (panel A) and natural CS-E (panel B) polysaccharides recorded in $^2\text{H}_2\text{O}$ at 298K.

Figure 4. CS/DS disaccharides analysis by LCMS (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.

Figure 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 1

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

Residue/position	Natural CS-E		Semi-synthetic CS-E	
	^{13}C	^1H	^{13}C	^1H
GlcUA1	103.87	4.40	104.00	4.43
GlcUA 2	72.14	3.29	72.07	3.29
GlcUA 3	73.75	3.50	73.72	3.53
GlcUA 4	82.00	3.68	81.38	3.71
GlcUA 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 3-4S _{6x} ^a	75.85	3.96	75.58	3.96
GalNAc 3-6S	nd ^b	nd	79.97	3.76
GalNAc 4	76.29	4.67	76.14	4.69
GalNAc 5-6S _{4x}	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 6-6S _{4x}	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; Kinoshita, et al., 2001; Kinoshita-Toyodo, et al., 2001].

^b nd, not determined.

Table 2

CS/DS disaccharide composition analysis by LC-MS

Sample	CS/DS disaccharides composition							
	Δ Di-0S	Δ Di-2S	Δ Di-6S	Δ Di-4S	Δ Di-diS _D	Δ Di-diS _B	Δ Di-diS _E	Δ Di-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	-

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}