

# Structural Studies of the Interaction of *Crataeva tapia* Bark Protein with Heparin and Other Glycosaminoglycans

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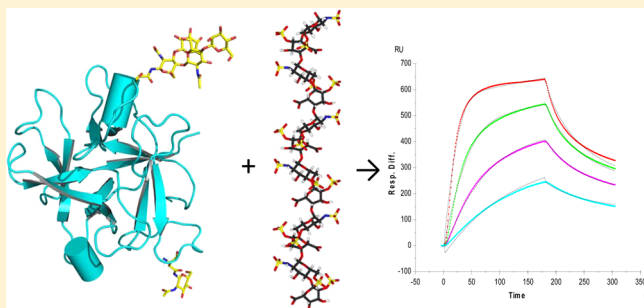
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## Supporting Information

**ABSTRACT:** CrataBL, a protein isolated from *Crataeva tapia* bark, which is both a serine protease inhibitor and a lectin, has been previously shown to exhibit a number of interesting biological properties, including anti-inflammatory, analgesic, antitumor, and insecticidal activities. Using a glycan array, we have now shown that only sulfated carbohydrates are effectively bound by CrataBL. Because this protein was recently shown to delay clot formation by impairing the intrinsic pathway of the coagulation cascade, we considered that its natural ligand might be heparin. Heparin is a glycosaminoglycan (GAG) that interacts with a number of proteins, including thrombin and antithrombin III, which have a critical, essential pharmacological role in regulating blood coagulation. We have thus employed surface plasmon resonance to improve our understanding of the binding interaction between the heparin polysaccharide and CrataBL. Kinetic analysis shows that CrataBL displays strong heparin binding affinity ( $K_D = 49$  nM). Competition studies using different size heparin-derived oligosaccharides showed that the binding of CrataBL to heparin is chain length-dependent. Full chain heparin with 40 saccharides or large oligosaccharides, having 16–18 saccharide residues, show strong binding affinity for CrataBL. Heparin-derived disaccharides through tetradecasaccharides show considerably lower binding affinity. Other highly sulfated GAGs, including chondroitin sulfate E and dermatan 4,6-disulfate, showed CrataBL binding affinity comparable to that of heparin. Less highly sulfated GAGs, heparan sulfate, chondroitin sulfate A and C, and dermatan sulfate displayed modest binding affinity as did chondroitin sulfate D. Studies using chemically modified heparin show that *N*-sulfo and 6-*O*-sulfo groups on heparin are essential for CrataBL–heparin interaction.



It is well-known that glycans can mediate a wide variety of specific physiological or pathophysiological functions by interacting with glycan-binding proteins (GBPs). These interactions are broadly classified into two major groups, lectins and glycosaminoglycan (GAG)-binding proteins.<sup>1</sup> Interaction of lectins with glycans on different cellular surfaces can promote hemagglutination and other responses, such as antimicrobial, mitogenic, antitumor, and insecticidal activities.<sup>2,3</sup> Interactions of heparin, heparan sulfate (HS), and other GAGs with proteins mediate diverse biological processes, such as blood coagulation, cell growth and differentiation, host defense and viral infection, lipid transport and metabolism, cell-to-cell and cell-to-matrix signaling, inflammation, and cancer.<sup>4–8</sup> From the molecular structural view of the interactions,

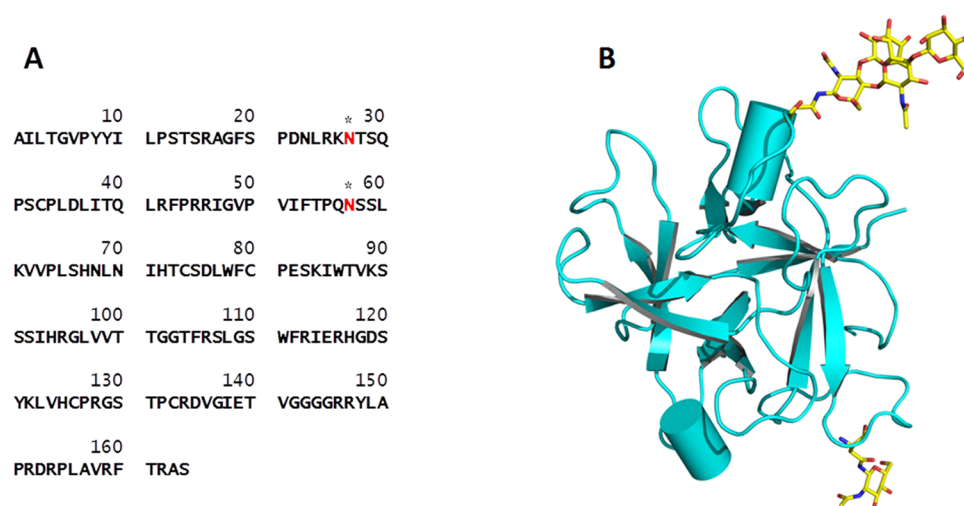
most lectins have defined “carbohydrate-recognition domains” (CRDs) with specific features of amino acid sequence or three-dimensional structure.<sup>1</sup> These CRDs bind to their carbohydrate ligands primarily through hydrogen bonding interactions. In contrast, most interactions of protein with sulfated GAGs seem to involve surface clusters of positively charged amino acids that align to bind the anionic GAG chains primarily through ion pairing interactions.<sup>4,9,10</sup> Thus, an understanding of glycan–protein interactions at the molecular level is of fundamental

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**Figure 1.** Structure of CrataBL. (A) Amino acid sequence of CrataBL. Asterisks denote glycosylation sites. (B) Chain tracing (prepared with PyMol<sup>30</sup>) showing the three-dimensional structure of a CrataBL monomer. Two glycosylated residues and the attached carbohydrates are shown as sticks.

importance to biology and to the development of highly specific therapeutic agents.<sup>4,10</sup>

CrataBL (see structure in Figure 1), a lectin from *Crataeva tapia* tree bark (belonging to the Capparaceae family, found in northeastern Brazil), has been purified using reversed micelles in isooctane<sup>11</sup> and through chromatographic processes.<sup>12</sup> Previous research demonstrated that CrataBL shows some specificity for binding glucose and galactose.<sup>12</sup> A number of biological properties associated with CrataBL have been characterized, including anti-inflammatory, analgesic, antitumor,<sup>13</sup> and insecticidal activities.<sup>12</sup> A recent study showed that CrataBL delayed clot formation by impairing the intrinsic pathway of the coagulation cascade, suggesting this protein might interact with heparin.<sup>14</sup> Heparin and heparin-derived low-molecular weight heparins (LMWHs) are the most widely used clinical anticoagulants. The heparins bind specifically and with high affinity ( $K_D \sim 20$  nM) to the serine protease inhibitor antithrombin III (AT), resulting in its conformational activation and leading to the inhibition of major coagulation cascade proteases, including thrombin [factor IIa (FIIa)] and FXa. In this study, we have shown, using a lectin array, that CrataBL binds effectively only sulfated oligosaccharides, thus confirming the prediction that it might be a heparin-binding protein. We have further analyzed molecular interactions of heparin and other GAGs with CrataBL to improve our understanding of the possible mechanism of the disruption of the intrinsic pathway of the coagulation cascade by CrataBL. This was accomplished with the BIAcore system (BIAcore 3000), which utilizes surface plasmon resonance (SPR) and allows a direct quantitative analysis of the label-free molecular interactions in real time.

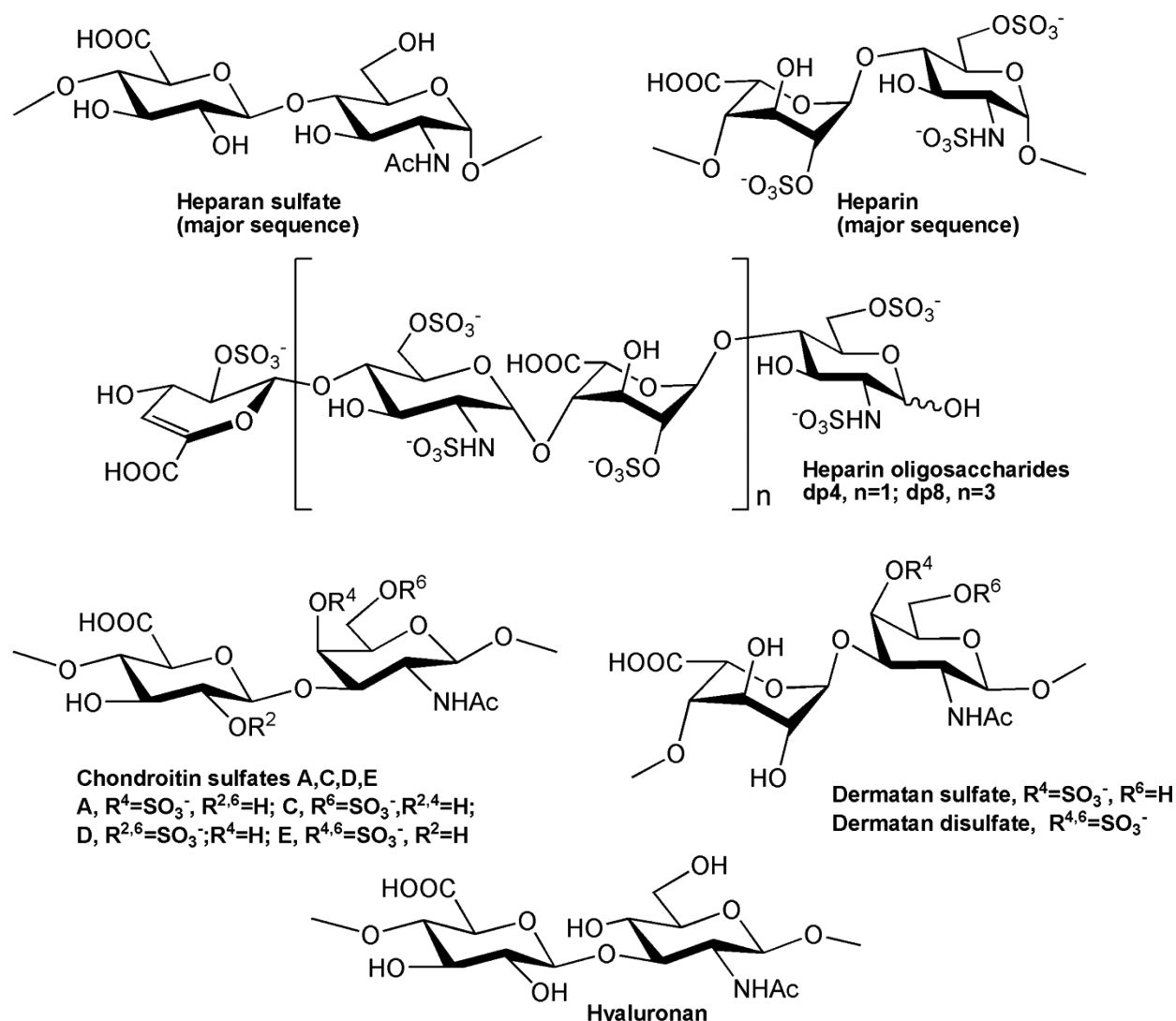
## EXPERIMENTAL PROCEDURES

**Isolation of CrataBL.** The protein was isolated according to the procedure of Araújo et al.<sup>12</sup> Briefly, extracts from *C. tapia* bark 10% (w/v) were fractionated with 30–60% ammonium sulfate. The fraction was dialyzed against 10 mM sodium citrate/sodium phosphate buffer (pH 5.5) and applied to a column of CM-cellulose equilibrated with the same buffer, also containing 0.5 M NaCl. Adsorbed protein was eluted with equilibration buffer containing 0.5 M NaCl. The single peak from ion exchange chromatography was submitted to size

exclusion chromatography on a Superdex 75 column, equilibrated in 0.15 M NaCl using an ÄKTA Purifier (GE Healthcare, Uppsala, Sweden), followed by high-performance liquid chromatography (HPLC) on a C18 column, to confirm the homogeneity of the sample. Elutions were monitored at 280 nm. This glycosylated protein has a molecular mass of 20.2 kDa, including ~1170 Da N-linked carbohydrates bound to two asparagine residues (R. S. Ferreira et al., manuscript submitted for publication).

**Carbohydrate Array Studies.** Purified CrataBL was labeled with Alexa Fluor 488. The protein (781  $\mu$ g/mL) was dissolved in 0.1 M NaHCO<sub>3</sub> containing 50 mM GlcNAc, 50 mM glucose, and Alexa Fluor 488 carboxylic acid, 2,3,5,6-tetrafluorophenyl ester (Life Technologies) at a final concentration of 0.3 mM. After 1 h at room temperature, the reaction mixture was applied to a Bio-Gel P2 column (5 mL) and eluted with phosphate-buffered saline (PBS) in 0.5 mL fractions to separate the labeled protein from unreacted dye and the monosaccharides, which were included to protect the glycan binding site during the labeling process. The fraction containing the highest protein concentration (estimated using a calculated extinction coefficient) with a molar ratio of dye to protein of 0.6 was used to interrogate version 5.0 of the glycan microarray produced by the Consortium for Functional Glycomics (<http://www.functionalglycomics.org>) at concentrations of 200 and 20  $\mu$ g/mL, according to the standard protocol.<sup>15,16</sup> Key sulfated glycans in the composition of the array were synthesized as described previously.<sup>17,18</sup> After being processed, the microarray slide was scanned using a PerkinElmer ProScanArray for detecting Alexa Fluor 488, and the data were displayed as histograms of relative fluorescence units (RFU) corresponding to individual glycan structures (see the Supporting Information).

**GAGs and Heparin Oligosaccharides.** The GAGs used in this study were porcine intestinal heparin (16 kDa) (Celsus Laboratories, Cincinnati, OH), low-molecular weight (LMW) heparin (4.8 kDa) (Celsus), porcine intestinal heparan sulfate (HS) (Celsus), chondroitin sulfate A (CS-A) (20 kDa) from porcine rib cartilage (Sigma, St. Louis, MO), dermatan sulfate (DS) (also known as chondroitin sulfate B, 30 kDa, from porcine intestine, Sigma), dermatan disulfate (Dis-DS) (4,6-



**Figure 2.** Chemical structures of GAGs and heparin-derived oligosaccharides.

disulfo DS, 33 kDa, Celsus) prepared through the chemical 6-O-sulfonation of dermatan sulfate,<sup>19</sup> chondroitin sulfate C (CS-C) (20 kDa, from shark cartilage, Sigma), chondroitin sulfate D (CS-D) (20 kDa, from whale cartilage, Seikagaku, Tokyo, Japan), chondroitin sulfate E (CS-E) (20 kDa, from squid cartilage, Seikagaku), and hyaluronic acid sodium salt (100 kDa, from *Streptococcus zooepidemicus*, Sigma). Fully desulfated heparin (14 kDa), N-desulfated heparin (14 kDa), and 2-O-desulfated heparin (13 kDa) were all prepared using the method of Yates et al.<sup>20</sup> The 6-O-desulfated heparin (13 kDa) was a generous gift from L. Wang of the Complex Carbohydrate Research Center of the University of Georgia (Athens, GA). Heparin oligosaccharides included disaccharide (dp2), tetrasaccharide (dp4), hexasaccharide (dp6), octasaccharide (dp8), deca-saccharide (dp10), dodecasaccharide (dp12), tetradecasaccharide (dp14), hexadecasaccharide (dp16), and octadecasaccharide (dp18) and were prepared from controlled partial heparin lyase 1 treatment of bovine lung heparin (Sigma) followed by size fractionation.<sup>21</sup> Chemical structures of these GAGs and heparin oligosaccharides are shown in Figure 2.

**Preparation of the Heparin Biochip.** The biotinylated heparin was prepared by reaction of sulfo-N-hydroxysuccini-

imide long chain biotin (Pierce, Rockford, IL) with free amino groups of unsubstituted glucosamine residues in the polysaccharide chain following a published procedure.<sup>22</sup> The biotinylated heparin was immobilized to a streptavidin (SA) chip (GE Healthcare) based on the manufacturer's protocol. In brief, a 20  $\mu$ L solution of the heparin-biotin conjugate (0.1 mg/mL) in HBS-EP running buffer was injected over flow cell 2 (FC2) of the SA chip at a flow rate of 10  $\mu$ L/min. The successful immobilization of heparin was confirmed by the observation of an  $\sim$ 250 resonance unit (RU) increase in the sensor chip. The control flow cell (FC1) was prepared by a 1 min injection with saturated biotin. SPR measurements were performed on a BIAcore 3000 (GE Healthcare) operated using BIAcore 3000 control software and BIAevaluation version 4.0.1.

**Measurement of the Interaction between Heparin and CrataBL Using BIAcore.** The protein samples were diluted in HBS-EP buffer [0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, and 0.005% surfactant P20 (pH 7.4)]. Different dilutions of protein samples were injected at a flow rate of 30  $\mu$ L/min. At the end of the sample injection, the same buffer was passed over the sensor surface to facilitate dissociation. After a 3 min dissociation time, the sensor was regenerated via injection

with 30  $\mu$ L of 2 M NaCl. The response was monitored as a function of time (sensorgram) at 25  $^{\circ}$ C.

**Competition between Heparin on the Chip Surface and Heparin-Derived Oligosaccharides.** CrataBL protein (125 nM) mixed with 1000 nM heparin oligosaccharides, including disaccharide (dp2), tetrasaccharide (dp4), hexasaccharide (dp6), octasaccharide (dp8), decasaccharide (dp10), dodecasaccharide (dp12), tetradecasaccharide (dp14), hexadecasaccharide (dp16), and octadecasaccharide (dp18), in HBS-EP buffer were injected over heparin chip at a flow rate of 30  $\mu$ L/min. After each run, dissociation and regeneration were performed as described above. For each set of competition experiments on SPR, a control experiment (only CrataBL protein without any heparin or oligosaccharides) was performed to make sure the surface was completely regenerated and that the results obtained between runs were comparable.

**Competition between Heparin on the Chip Surface and GAGs.** For testing the ability of other GAGs and chemically modified heparins to inhibit the CrataBL–heparin interaction, CrataBL at 125 nM was premixed with 1000 nM GAG or chemically modified heparin and injected over the heparin chip at a flow rate of 30  $\mu$ L/min. After each run, a dissociation period and regeneration protocol was performed as described above.

## RESULTS AND DISCUSSION

**Glycan Array Studies.** The results of binding Alexa Fluor 488-labeled CrataBL to the glycan targets on the CFG glycan microarray indicated that this lectin preferred sulfated oligosaccharides (see the Supporting Information). Of the 611 glycans on this array, 67 of the glycans are sulfated and are primarily di-, tri-, and tetrasaccharides. The results of binding of CrataBL to selected sulfated and related nonsulfated disaccharides are shown in Table 1. Among this group, the strongest binding glycans were di- and trisulfated derivatives of Gal $\beta$ 1–3/4GlcNAc (glycans 1–7 and 18) and LacdiNAc (glycan 2a). All binding was lost when the sulfates were not present on these structures (glycans 13 and 20), and addition or substitution of a different negatively charged group, such as sialic acid (glycans 14–17 and 19) or phosphate (glycans 11 and 12), did not enhance binding. With the limited number of sulfated structures on the CFG array, it is difficult to identify an obvious binding motif, but the preference for binding to sulfated derivatives of lactosamine strongly suggests that CrataBL may be a GAG-binding protein as has been observed previously in many studies using the CFG glycan array.

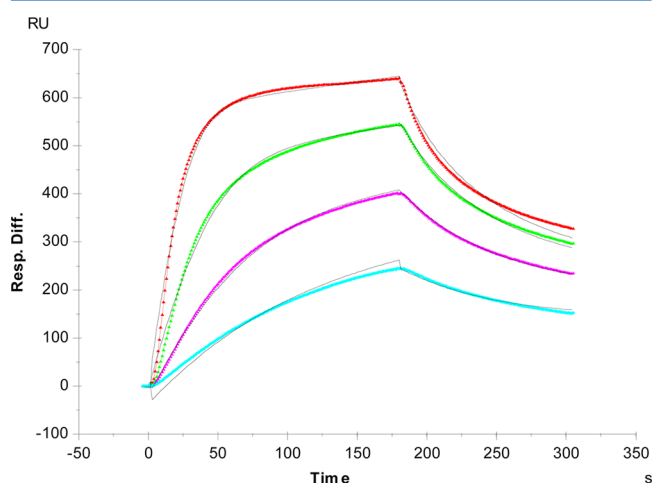
The disaccharide (6S)(3S)Gal $\beta$ 1–4(6S)GlcNAc, which exhibited the best binding to CrataBL, in the form of -OCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>- $\beta$ -glycoside, was utilized in experiments aimed at creating ligand–carbohydrate complexes suitable for crystallographic investigations. Although crystals could be grown from a mixture of CrataBL and the disaccharide, analysis of the electron density has not shown any appreciable binding. This result, most likely caused by the still relatively low binding constant for a short oligosaccharide, prompted us to search for other, longer sulfated carbohydrates that that might exhibit higher-affinity binding.

**Kinetic Measurement of CrataBL–Heparin Interactions.** Previous research on CrataBL bioactivities showed that it delayed formation by impairing the intrinsic pathway of the coagulation cascade, indicating this lectin might be a heparin-binding protein.<sup>14</sup> SPR was used to characterize the binding of CrataBL to heparin. Heparin was immobilized on SA SPR

**Table 1. Best Binding Glycans Found Using the Glycan Array [20  $\mu$ g/mL CrataBL (see the Supporting Information for details)]**

glycan	structure on the master list	average RFU	standard deviation	% CV	chart number
1	(6S)(3S)Gal $\beta$ 1–4(6S)GlcNAc	9604	427	4	22
2	(3S)Gal $\beta$ 1–4(6S)GlcNAc	4868	1065	22	35
2a	(6S)(4S)GalNAc $\beta$ 1–4(6S)GlcNAc	4834	377	8	512
3	(6S)(3S)Gal $\beta$ 1–4(6S)GlcNAc	3119	1673	54	298
4	(3S)Gal $\beta$ 1–4(6S)GlcNAc	2917	178	6	34
5	(6S)(3S)Gal $\beta$ 1–4GlcNAc	2776	189	7	23
6	(6S)(4S)Gal $\beta$ 1–4GlcNAc	2191	362	17	39
7	(4S)(3S)Gal $\beta$ 1–4GlcNAc	1405	87	6	297
8	(3S)Gal $\beta$ 1–4GlcNAc	242	52	21	36
9	(6S)Gal $\beta$ 1–4GlcNAc	143	34	24	44
10	(4S)Gal $\beta$ 1–4GlcNAc	109	13	12	40
11	Gal $\beta$ 1–4(6P)GlcNAc	287	24	8	518
12	(6P)Gal $\beta$ 1–4GlcNAc	34	16	48	519
13	Gal $\beta$ 1–4GlcNAc	202	46	23	169
14	Neu5Ac $\alpha$ 2–3Gal $\beta$ 1–4(6S)GlcNAc	1294	83	6	252
15	Neu5Ac $\alpha$ 2–6Gal $\beta$ 1–4(6S)GlcNAc	466	36	8	268
16	Neu5Ac $\alpha$ 2–3Gal $\beta$ 1–4GlcNAc	39	14	35	260
17	Neu5Ac $\alpha$ 2–6Gal $\beta$ 1–4GlcNAc	54	14	27	269
18	(6S)Gal $\beta$ 1–3(6S)GlcNAc	7347	2689	37	445
19	Neu5Ac $\alpha$ 2–3Gal $\beta$ 1–3(6S)GlcNAc	454	38	8	239
20	Gal $\beta$ 1–3GlcNAc	17	9	54	150

sensor chips, and sensorgrams of CrataBL–heparin interaction were obtained (Figure 3). These sensorgrams fit well to a 1:1 Langmuir binding model, and the binding kinetics are



**Figure 3.** SPR sensorgrams of CrataBL–heparin interaction. Concentrations of CrataBL of 250, 125, 63, and 32 nM (from top to bottom, respectively). The black curves are the fitting curves using models from BIAevaluation 4.0.1.



presented in Table 2. The SPR data show that CrataBL binds to heparin with dissociation constants ( $K_D$ ) of  $\sim 49$  nM. Although

**Table 2. Summary of Kinetic Data of CrataBL–Heparin Interactions<sup>a</sup>**

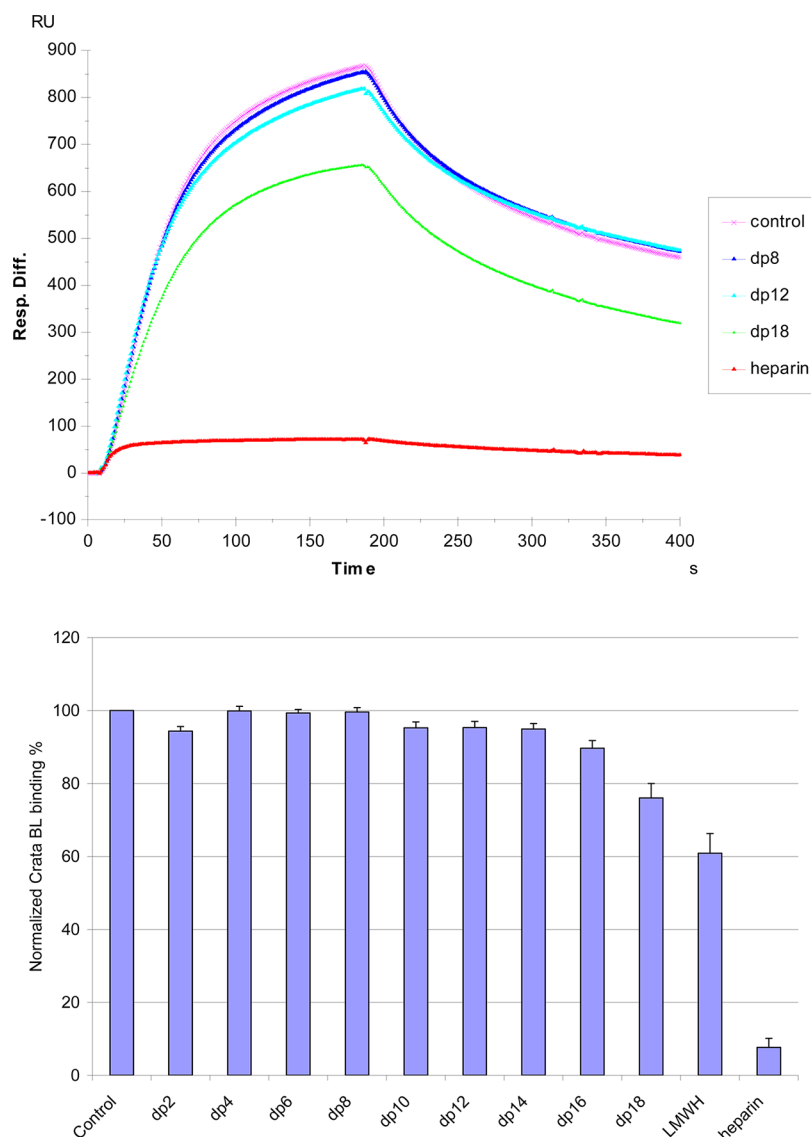
interaction	$k_a$ ( $M^{-1} s^{-1}$ )	$k_d$ ( $s^{-1}$ )	$K_D$ (M)
CrataBL–heparin	$(6.3 \pm 3.5) \times 10^5$	$0.03 \pm 0.02$	$(4.9 \pm 0.8) \times 10^{-8}$

<sup>a</sup>The standard deviations are from triplicate binding experiments.

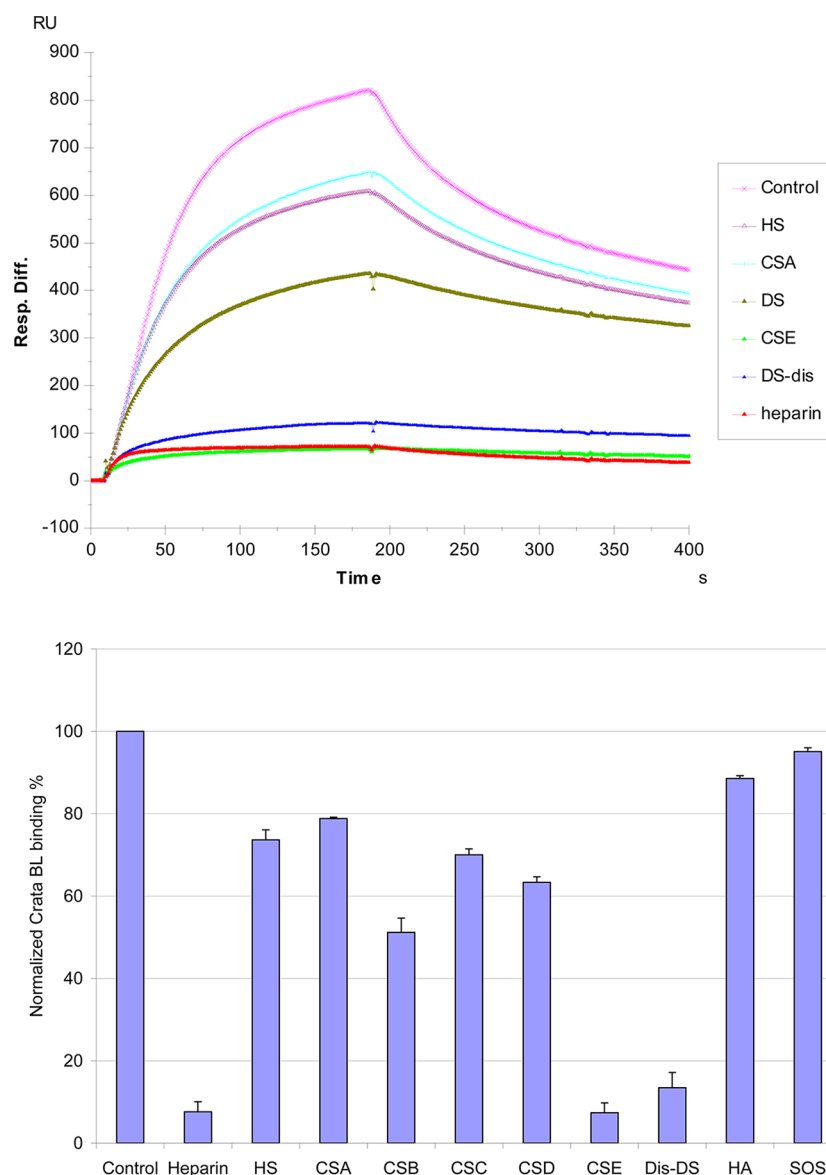
lectins were first discovered in plants, they are now known to be present throughout the kingdoms of life, i.e., plant, animal, bacteria, and virus. Most lectins have defined CRDs with specific features for binding a specific sequence of glycans. In general, single-site binding affinities in many lectins appear to be low (with  $K_D$  values in the micromolar range), although some lectins recognize glycans with a much higher affinity (with  $K_D$  values in the nanomolar range).<sup>1</sup> Despite the numerous

lectins that exist throughout nature, only a few heparin-binding proteins, from human placenta, fish, clams, and cloned thymic myoid cells, have been reported in the literature.<sup>23–26</sup> The affinity of interactions between these lectins and heparin has not been rigorously determined. The kinetic data of CrataBL–heparin interaction could be used for explaining some of the CrataBL bioactivities.

**Solution Competition Study by SPR of the Interaction of Surface-Immobilized Heparin with Protein Using Heparin-Derived Oligosaccharides.** Solution–surface competition experiments were performed by SPR to examine the effect of the saccharide chain size of heparin on the heparin–protein interaction. Different size heparin-derived oligosaccharides (from dp2 to dp18) were used in the competition study. The same concentration (1000 nM) of heparin-derived oligosaccharides was present in the CrataBL (125 nM)/heparin interaction solution. Only negligible competition was observed when 1000 nM heparin-derived oligosaccharides (from dp2 to dp14) were present in the CrataBL protein solution. In



**Figure 4.** Sensorgrams of solution heparin oligosaccharide–surface heparin competition (top). The CrataBL concentration was 125 nM, and concentrations of heparin oligosaccharides in solution were 1000 nM. Bar graphs (based on triplicate experiments with the standard deviation) of the normalized CrataBL binding preference for surface heparin via competition with differently sized heparin oligosaccharides in solution (bottom).



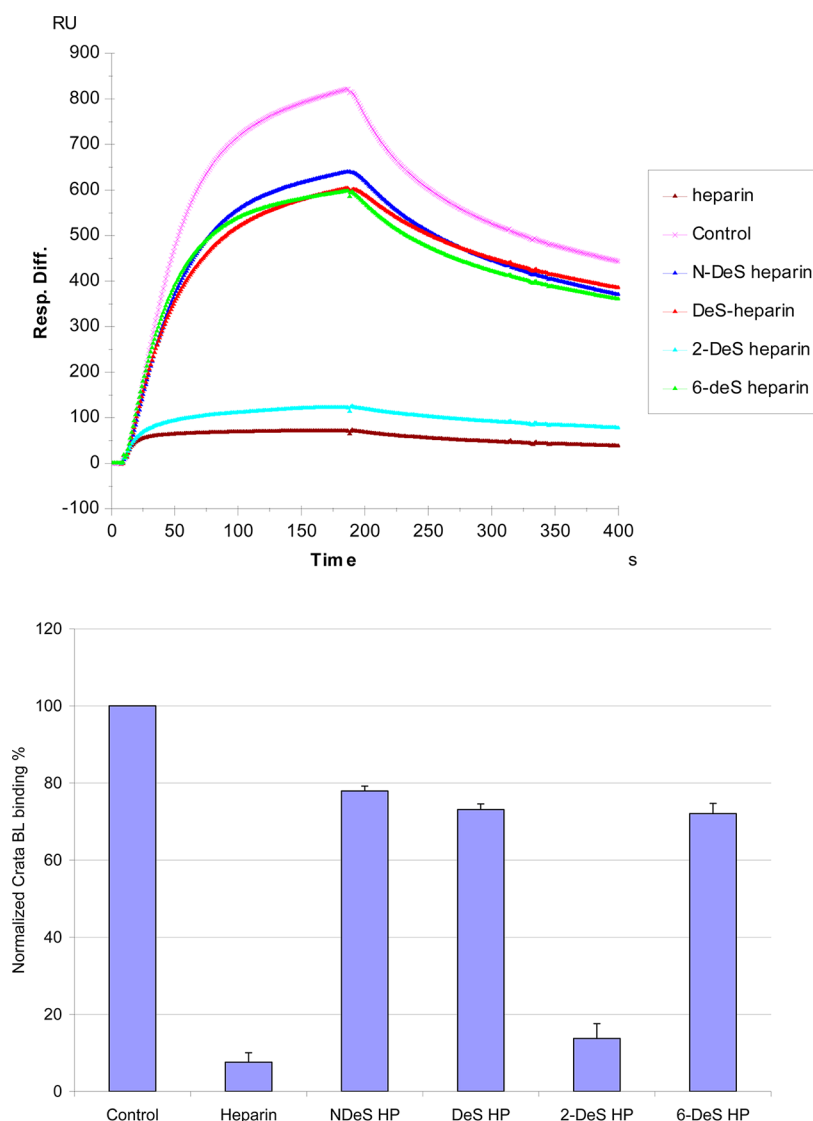
**Figure 5.** Sensorgrams of solution GAG–surface heparin competition (top). The CrataBL concentration was 125 nM, and concentrations of GAGs in solution were 1000 nM. Bar graphs (based on triplicate experiments with the standard deviation) of CrataBL binding RU to surface heparin by competing with different GAGs (bottom).

contrast, heparin-derived oligosaccharides dp16 and dp18, LMWH (average dp of ~20), and full chain heparin (average dp of ~40) showed a level of competition with immobilized heparin that increased with increasing oligosaccharide size (Figure 4). These results suggest that the interaction between CrataBL and heparin is chain length-dependent and CrataBL requires a relatively long heparin chain (>dp16) to interact. While chain length requirements for heparin–protein interactions were reported for binding to other proteins, including Shh–heparin, FGF–heparin, and IL-7–heparin interactions,<sup>27–29</sup> this chain size requirement is larger than those observed previously. The large minimal heparin chain length requirement for CrataBL binding appears to be critical for accommodation of the protein as shorter saccharide chains may not suffice in neutralizing the positively charged heparin binding regions of the protein.

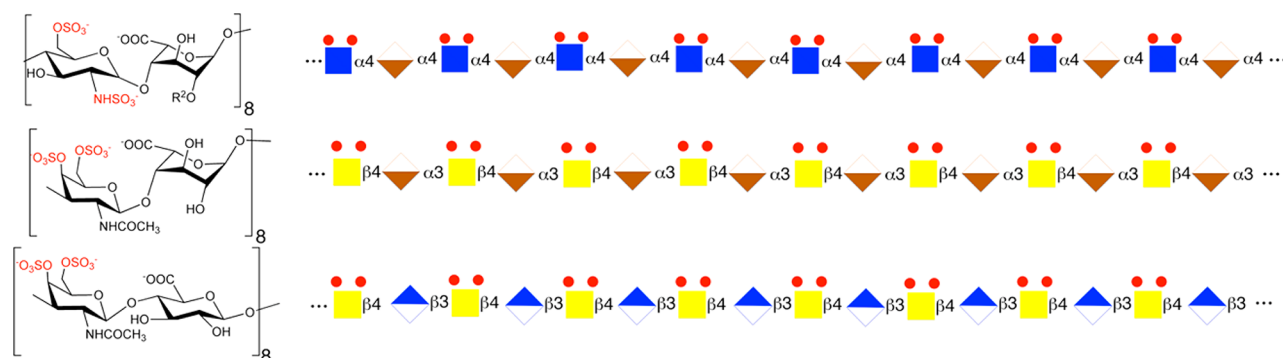
**SPR Solution Competition Study of Different GAGs.** The SPR competition assay was also utilized to determine the binding preference of CrataBL for various GAGs (Figure 2).

SPR competition sensorgrams and bar graphs of the GAG competition levels are displayed in Figure 5. Heparin, CS-E, and DiS-DS produced the strongest inhibition in CrataBL binding of immobilized heparin by competing >80% of the CrataBL binding to immobilized heparin on the chip surface. Modest inhibitory activities were observed for HS, CS-A, DS, CS-C, and CS-D. Weak inhibitory activities were observed for hyaluronan (HA) and sucrose octasulfate (SOS, a persulfated disaccharide). The data suggest that the binding interactions of CrataBL with GAGs appear to be structure-dependent and highly influenced by the level of GAG sulfation.

**SPR Solution Competition Study of Different Chemically Modified Heparins.** SPR competition experiments using chemically modified heparins are displayed in Figure 6. The results show that all four chemically modified heparins (fully desulfated heparin, N-desulfated heparin, 2-O-desulfated heparin, and 6-O-desulfated heparin) showed reduced inhibitory activities. Removal of N-sulfo groups and 6-O-sulfo groups from heparin markedly weakened the ability of these chemically



**Figure 6.** Sensorgrams of solution chemical modified heparin–surface heparin competition (top). The CrataBL concentration was 125 nM, and concentrations of chemically modified heparin in solution were 1000 nM. Bar graphs (based on triplicate experiments with the standard deviation) of CrataBL binding RU to surface heparin by competing with different chemical modified heparin in solution (bottom).



**Figure 7.** Proposed CrataBL binding GAG motif, with eight repeating disaccharide units having two sulfo groups on a hexosamine residue linked to a uronic acid having a carboxyl group: heparin dp16 (top), Dis-DS dp16 (middle), and CS-E dp16 (bottom). Sugar symbols: blue squares for GlcNAc, half-tan diamonds for IdoA, yellow squares for GalNAc, and half-blue diamonds for GlcA.

modified heparins to compete with surface-immobilized heparin for CrataBL binding. In contrast, removal of 2-O-sulfo groups afforded a chemically modified heparin that

competed well with surface-immobilized heparin for CrataBL binding.

SPR competition experiments with different GAGs and chemically modified heparin clearly showed an inhibitory

activity that was dependent on the level of GAG sulfation and fine structure. The inhibitory effects of soluble GAGs on the CrataBL–immobilized heparin interaction were greatest for heparin with 2.8 mol of sulfate per disaccharide repeating unit, followed by CS-E and Dis-DS with 1.5–2 mol of sulfate per disaccharide, then HS with 1.2 mol of sulfate per disaccharide, and finally DS, CS-A, and CS-C with <1 mol of sulfate per disaccharide. Surprisingly, CS-D with 1.5–2 mol of sulfate per disaccharide showed a comparable level of competition with GAGs having <1 mol of sulfate per disaccharide. Combining the structural requirements in the heparin and chondroitin/dermatan series of the GAGs suggests a CrataBL binding motif that has eight repeating disaccharide units with two sulfo groups on a hexosamine residue linked to a uronic acid having a carboxyl group (Figure 7). These results suggest that GAG fine structure plays a prominent role in the CrataBL interaction. Two charge groups per disaccharide repeating unit (*N*-sulfo and 6-*O*-sulfo groups) on heparin or the 4-*O*-sulfo and 6-*O*-sulfo groups on CS-E or on Dis-DS appear to be critical for the CrataBL–GAG interaction. These results are similar to the GAG sulfation preference of other proteins, such as Shh, Ihog, FGF1, and FGF2, via interaction with more highly sulfated heparin than the less sulfated HS.<sup>27,28</sup>

In conclusion, SPR analysis shows that CrataBL is a heparin-binding lectin with high affinity ( $K_D \sim 49$  nM). The SPR solution competition study shows that binding of CrataBL to heparin is chain length-dependent. CrataBL prefers to bind full chain heparin or large oligosaccharides, having 16–18 sugars. Higher sulfation levels of GAGs enhance their binding affinities; i.e., CS-E and Dis-DS showed binding affinity for CrataBL comparable to that of heparin, and weakly sulfated GAGs (HS, CS-A, DS, CS-C, and CS-D) having modest binding affinity. *N*-Sulfo and 6-*O*-sulfo groups on heparin are required for the CrataBL–heparin interaction.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

Additional experimental data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

SPR, surface plasmon resonance; GAG, glycosaminoglycan; HS, heparan sulfate; HP, heparin; LMWH, low-molecular weight heparin; CS-A, chondroitin sulfate A; DS, dermatan sulfate; Dis-DS, dermatan disulfate; CS-C, chondroitin sulfate C; CS-D, chondroitin sulfate D; CS-E, chondroitin sulfate E; HA, hyaluronic acid; SA, streptavidin; FC, flow cell; RU, resonance unit.

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