Direct Measurement of the Interactions of Glycosaminoglycans and a Heparin Decasaccharide with the Malaria Circumsporozoite Protein†

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ABSTRACT: Circumsporozoite (CS) protein is a predominant surface antigen of malaria sporozoites, the infective form of the parasite, and has been used for making anti-malaria vaccines. For the first time we have examined the interaction of CS protein with various glycosaminoglycans in real time using surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC). Heparin was the best binder among the glycosaminoglycans tested and bound to CS protein with nanomolar affinity. Using purified and structurally defined small heparin oligosaccharides, we identified a decasaccharide to be the minimum sized CS protein-binding sequence. In an indirect competition assay, this decasaccharide blocked the CS protein interaction with HepG2 cells with an ID_{50} of less than 60 nM. The decasaccharide has a structure commonly found in hepatic heparan sulfate, and the same sequence has recently been shown to bind specifically to apolipoprotein E. Examination of porcine liver heparan sulfate in this indirect competition assay showed that it and heparin were the only glycosaminoglycans that could effectively block CS protein interaction with HepG2 cells in culture. These data support the hypothesis that the invasion of liver cells by the parasite shares a common mechanism with the hepatic uptake of lipoprotein remnants from the blood.

Plasmodium falciparum sporozoites are inoculated into the mammalian host by the bite of an infectious mosquito. These sporozoites traverse the cytosol of several cells before invading a hepatocyte (*1*). The invasion of liver cells by these sporozoites has been ascribed to the interaction of circumsporozoite (CS) protein, a 40−45 kDa antigen (*2*) that covers the plasma membrane of sporozoites, with glycosaminoglycan side chains of proteoglycans located on the surface of the hepatocytes (*3, 4*).

The nature of the glycosaminoglycan involved in this interaction has been extensively studied using assays that measure the ability of exogenously added glycosaminoglycans and other polyanions to interfere with CS binding on liver cells (*4*−*7*). In most studies, heparin (Figure 1), a highly sulfated glycosaminoglycan having the major repeating disaccharide sequence →4)-α-L-IdoAp2S(1→4)-α-D-GlcNpS-6S(1→ (where IdoAp is iduronic acid, S is sulfate, and GlcNp is glucosamine), has been shown to bind to CS (*4*−*7*). Heparan sulfate, an undersulfated glycosaminoglycan containing a small number of the trisulfated repeating disaccharide sequences found in heparin, also displaces CS from target cells albeit with lower efficiency (*4*). Highly sulfated polyanions of nonmammalian origin, such as fucoidan and dextran sulfate, can also displace CS from target cells (*4, 5*).

The nature of the binding site within the CS protein has also been extensively studied. Along with CS protein, thrombospondin-related anonymous protein (TRAP), another sporozoite surface protein, has also been implicated in infection (*8*). Both the proteins contain thrombospondin type I repeat (TSR) motifs that are present in numerous parasite and mammalian proteins, and interact with various cell surface moieties (*9, 10*). These TSR motifs, contained in β-sheet domains, mediate heparin binding in heparin-binding growth-associated molecule (HB-GAM) (*10*), midkine (*11*), and laminin (*12*). In CS protein, an 18 amino acid sequence (EWSPCSVTCGNGIQVRIK) named region II plus, located within the TSR motif, has been suggested to be involved in binding (*4*).

Recent studies by our laboratory have demonstrated the importance of the two cysteine residues in region II plus (*13*). Conversion of cysteines to alanine, either individually or in combination, resulted in a complete loss of binding to target cells, but binding could be effectively restored by incubating mutant CS proteins with low quantities of heparin

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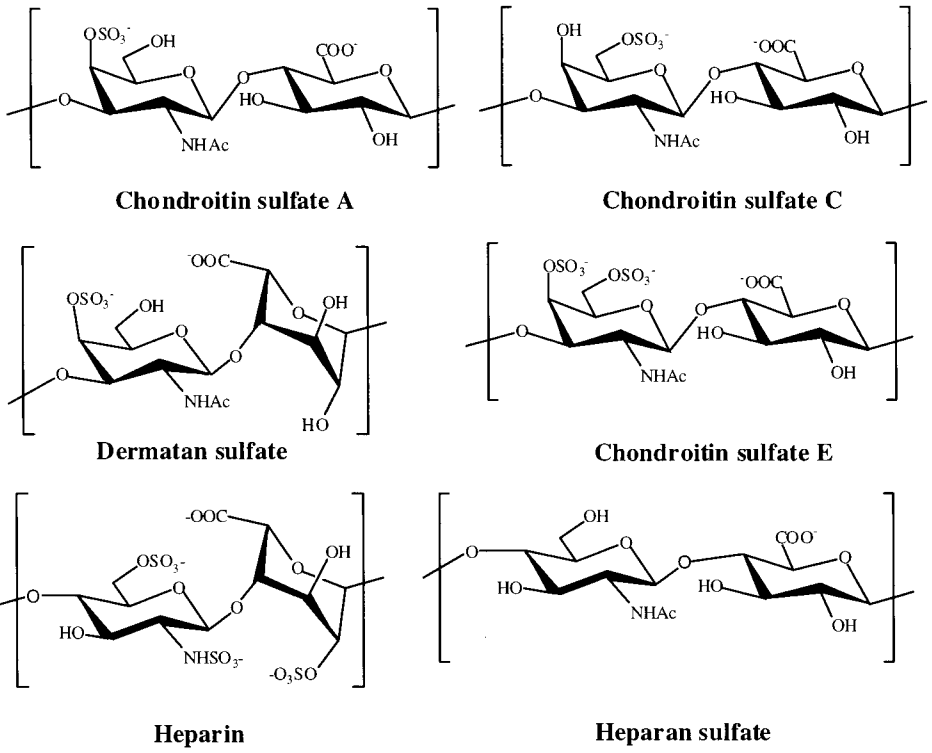

FIGURE 1: Structures of glycosaminoglycans tested for binding to CS protein.

(*13*). We further demonstrated that exogenous heparin could regulate the binding of CS protein to HepG2 cells in a concentration-dependent manner (*7*). At high concentrations, heparin inhibited CS binding as previously reported (*4*, *5*). At low concentrations, heparin unexpectedly enhanced CS binding, suggesting that it may act as a cross-linking agent effectively promoting the formation of multimeric CS complexes, and thus was more effective in the binding of CS to target cells (*7*, *13*). Lyon and co-workers (*14*) have characterized liver heparan sulfate, the natural CS receptor, and shown that it is a highly sulfated glycosaminoglycan rich in →4)-α-L-IdoAp2S(1→4)-α-D-GlcNpS6S(1→ sequences (*14*) that also comprise from 70% to 90% of heparin's structure (*15*).

Here we have examined the binding of CS protein to glycosaminoglycans by surface plasmon resonance spectrometry (SPR) and isothermal titration calorimetry (ITC). This is the first report where the binding of CS protein to glycosaminoglycans has been studied directly. These measurements have provided information on the binding kinetics, thermodynamics, and the minimum heparin sequence capable of tightly binding to CS protein.

MATERIALS AND METHODS

Materials

The sodium salts of porcine intestinal heparin (150 units/mg), porcine intestinal heparan sulfate, and porcine skin dermatan sulfate were obtained from Celsus (Cincinnati, OH). The sodium salts of bovine lung heparin (150 units/mg) and bovine trachea chondroitin sulfate A (70% A, 30% C) were from Sigma (St. Louis, MO). The sodium salt of shark cartilage chondroitin sulfate E was obtained from Seikagaku America (Falmouth, MA). The sodium salt of porcine liver heparan sulfate was purified as previously described (*16*). Heparin lyase I (EC 4.2.2.7) and bovine serum albumin were supplied by Sigma. Hepes-buffered saline (HBS), *N*-ethyl-*N*-(dimethylaminopropyl)carbodiimide (EDC), and *N*-hydroxysuccinimide (NHS) were from BIAcore International AB (Uppsala, Sweden). All other chemicals and reagents were obtained from Sigma and were of the highest purity available. A 50 mL pressure filtration apparatus and MWCO 5000 membranes, 4.3 cm diameter, were from Amicon, W. R. Grace and Co. (Beverly, MA). Size exclusion chromatography was performed using Sephadex G-50 (superfine) resin from Pharmacia Biochemicals (Piscataway, NJ) on a glass column, 4.8 cm × 1.0 m, from Kontes Scientific Glassware (Morton, Grove, IL). Bio Gel P-2 (BioRad, Hercules, CA) resin was used for a desalting column, 5 cm × 0.5 m. Gradient PAGE was performed with the BioRad Protean II system, BioRad. Oligosaccharide standards were from Seikagaku America.

The biosensing system used for SPR measurements consisted of a BIAcore 2000, BIAevaluation software 3.2, and a CM5 chip (BIAcore International AB). Isothermal titration calorimetry (ITC) experiments were performed on a Hart Scientific 4209 microtitration calorimeter (Pleasant Grove, UT). The SAX-HPLC system consisted of dual-face programmable LC-7A titanium-based pumps (Shimadzu, Kyoto, Japan), a Rhoedyne (Cotati, CA) titanium injector, a Waters semipreparative column (Milford, MA), a Pharmacia LKB variable-wavelength UV detector, and a Shimadzu Chromatopac C-R2A integrating recorder. Capillary electrophoresis was performed using a Dionex Capillary Electrophoresis system equipped with a model I advanced computer interface and high-voltage power supply capable of constant or gradient voltage control. The capillary selected for determination of the heparin-derived oligosaccharides was fused silica, 75 μm i.d. × 375 μm o.d., 78 cm long, from Dionex Corp. (Sunnyvale, CA). UV spectroscopy was

performed using a Shimadzu model UV 160 spectrophotometer equipped with a thermostatic cell. A Bruker AMX 600 NMR spectrometer equipped with a X32 computer was used for the identification of oligosaccharide structures.

Methods

Expression and Purification of Plasmodium falciparum CS Protein. Recombinant CS protein was obtained by expressing plasmid pCS1 in *E. coli* strain BL21(λDE3). Plasmid construction, protein expression, and purification have been described elsewhere (*17*). Briefly, pCS1 was expressed in BL21 cells, and the protein was localized in the periplasm. Periplasm was isolated by a mild osmotic shock without lysing the cell membrane. CS protein was purified using a heparin-Sepharose affinity column followed by gel filtration chromatography.

Preparation of Heparin-Derived Oligosaccharides. A mixture of oligosaccharides was prepared from bovine lung heparin by controlled enzymatic depolymerization with heparin lyase I as previously described (*18*). Briefly, the low molecular weight (M_r < 5000) portion of this mixture, obtained by pressure filtration through a 5000 MWCO membrane, was fractionated on a 4.8 × 100 cm column of Sephadex G-50 (superfine) in 200 mM sodium chloride. The fractions containing tetrasaccharide through tetradecasaccharide were recovered, exhaustively dialyzed using 500 MWCO membranes, and freeze-dried. Repetitive strong anion exchange−high-performance liquid chromatography (SAX-HPLC), using a 2.5 × 25 cm semipreparative 5 μm SAX column, allowed separation and recovery of the highly sulfated oligosaccharides. The purity of each oligosaccharide was determined using capillary electrophoresis (CE) and gradient polyacrylamide gel electrophoresis (PAGE). Additionally, capillary electrophoresis enabled the determination of oligosaccharide structures via co-injection with known standards. The structure of each oligosaccharide was verified with ^{1}H NMR, and the spectra were identical to standards of known structure. The structures of the isolated oligosaccharides were ΔUAp2S (1[→4)-α-D-GlcNpS6S(1→4)-α-L-IdoAp2S(1]$_n$→4)-α-D-GlcNpS6S (where n = 1, 2, ..., 6, corresponding to tetrasaccharide, hexasaccharide, octasaccharide, decasaccharide, dodecasaccharide, and tetradecasaccharide, and ΔUA is 4-deoxy-α-L-*threo*-hexenopyranosyluronic acid).

Preparation of CS Biosensor Chip. CS protein was immobilized on the surface of a CM5 sensor chip through amine coupling, where the protein's primary amino groups reacted with carboxymethylated dextran, yielding a covalent bond and a stable surface. Carboxymethylated dextran covers the surface of the gold film in the sensor chip, providing a hydrophilic environment conducive for the interaction of biological molecules. The carboxymethylated dextran surface was activated using an injection pulse (7 min, 35 μL) containing a mixture of 0.05 M NHS and 0.05 M EDC. Protein immobilization was accomplished by manually injecting 80 μL of CS protein, prepared at a concentration of 50 μg/mL in a 3 mM sodium citrate buffer at pH 5.0. The remaining unreacted sites on the sensor surface were blocked with a 35 μL injection of 0.1 M ethanolamine. The surface was then cleaned with the extra clean step available in the instrument. All steps were carried out in a continuous flow of HBS running buffer at 5 μL/min, and all buffers were degassed and deoxygenated prior to use.

SPR Measurements of CS Protein Interaction with Glycosaminoglycan and Oligosaccharides. Each glycosaminoglycan or oligosaccharide sample was prepared in HBS buffer and injected at 5 μL/min for 3 min. The sensor surface was regenerated using 10 μL of 2 M NaCl following a 2 min dissociation phase. Reequilibration between the sensor surface and running buffer was established prior to injection with the next sample. Response was monitored as a function of time (sensogram) at 25 °C. Kinetic parameters were evaluated using the BIAevaluation software.

Interaction between CS and Heparin Measured by ITC. ITC was used to measure the interaction between CS protein and porcine intestinal heparin. CS protein (1.3 mL, 14 μM) was titrated with 25 injections of heparin (10 μL, 75 μM) at 25 °C. Samples were prepared in buffer containing 50 mM sodium phosphate and 150 mM sodium chloride, pH 7.4. Peak area was analyzed using ITC DataWorks and BindWorks software version 1.0, from CSC (Spanish Fork, UT), and used to fit the data. Experimental protocols and data processing have been described elsewhere (*19*).

Inhibition of CS Binding by Glycosaminoglycans and Heparin-Derived Saccharides. HepG2 cells, maintained in RPMI 1640 containing 10% fetal bovine serum, were seeded in a 96-well plate at a density of 5 × 10^4 per well and allowed to adhere overnight at 37 °C. The following day, cells were fixed by the addition of a 4% paraformaldehyde solution and incubated on ice for 15 min. Empty areas of the well were blocked with 1% BSA in Tris-buffered saline, TBS (50 mM Tris, pH 7.4, containing 135 mM NaCl), for 2 h. To evaluate the binding inhibition potential, 50 nM CS protein in TBS was co-incubated with various concentrations of the carbohydrate at 37 °C for 15 min. The protein−carbohydrate mixture was added to the fixed cells and incubated (described above) at 37 °C for 1 h. Unbound material was removed, and the cells were incubated with anti-CS monoclonal antibody in TBS for 30 min. This was followed by the addition of anti-mouse antibody−alkaline phosphatase conjugate diluted in TBS containing 0.1% Tween 20 and further incubation at 37 °C for 30 min. Unbound conjugate was removed, and the cells were incubated with 1 mM 4-methylumbelliferyl phosphate, used as substrate, at 37 °C for 15 min. The amount of protein binding onto the cells was measured in a fluorometer with excitation at 350 nm and emission at 460 nm. Binding was compared to a control where the CS protein was incubated with buffer alone.

RESULTS

Expression, Purification, and Immobilization of CS Protein. Recombinant CS protein was expressed in *E. coli* and was purified to homogeneity by two-column chromatography. The purity of the protein was determined by SDS−PAGE analysis. Purified CS protein was immobilized on the surface of the CM5 sensor chip. The immobilization on the chip was successful as it resulted in an increase of 6000 response units. The CS protein was stable on the sensor surface as more than 90% of the heparin-binding activity remained even after 10 repeated injections of the sample and subsequent regeneration using 2 M sodium chloride.

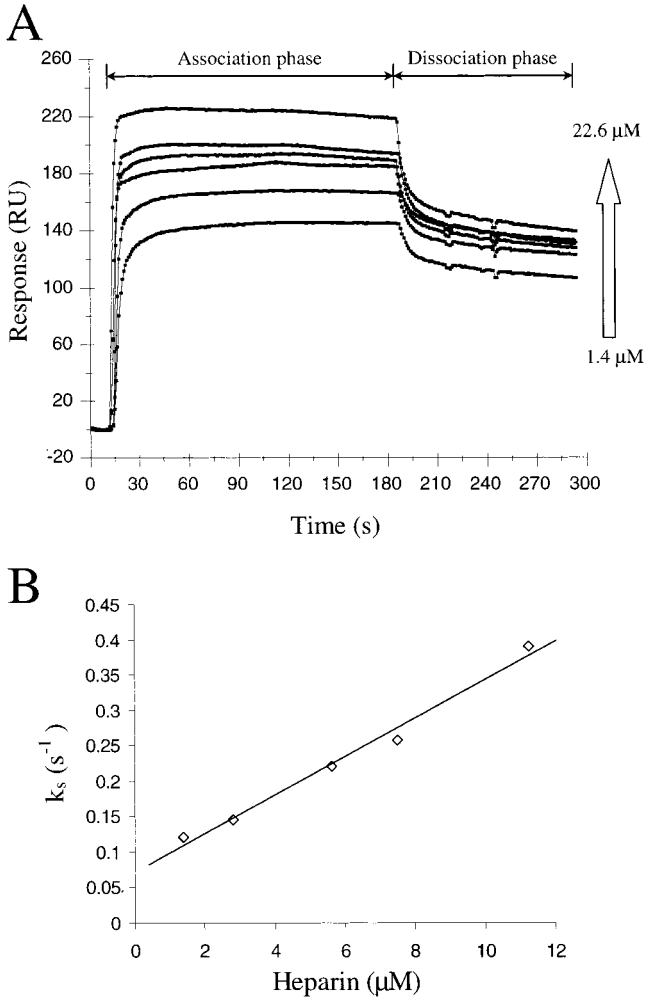

FIGURE 2: SPR analysis of heparin−CS interaction. (A) Sensograms of CS protein−heparin interaction at heparin concentrations of 1.4, 2.8, 5.7, 7.5, 11.3, and 22.6 μM. (B) Plot of the observed association rate, k_s, as a function of the concentration of heparin.

SPR Analysis of CS−Glycosaminoglycan Interaction. Varying concentrations of heparin were passed over the CM5 chip containing immobilized CS protein, and the change in response was evaluated as a function of time. Figure 2A depicts the sensograms for the binding of different concentrations of heparin to CS protein. The characteristic shape of these curves can be divided into three distinct phases. Initially, HBS (running buffer) was injected over the sensor chip, and the response was taken as the reference value to which the heparin−CS protein interactions were compared. Next, when heparin solution came in contact with the chip, there was an increase in response and the rising part of the curve corresponds to the association of the sample with CS protein present on the surface of the sensor chip. In the final phase, the stream flowing over the sensor chip contained only running buffer, which allowed the carbohydrate to dissociate from the protein due to a concentration gradient, thereby causing a decrease in the measured response.

The affinity of CS protein for heparin was quantified by determining the overall dissociation constant ($K_d = k_d/k_a$), where k_d and k_a represent dissociation and association rate constants calculated from dissociation and association phase of the curve, respectively. The dissociation rate constants (k_d) were obtained from direct analysis of the dissociation phase of the sensograms in Figure 2A. To determine association rate constants, first, k_s was calculated from the association phase of the sensograms. The k_s values were subsequently plotted as a function of the concentration of heparin bound to CS protein (Figure 2B). The resulting linear relationship was used to estimate the association rate constants (k_a) for each concentration of heparin injected over the sensor chip. On calculation, heparin had an overall dissociation constant (K_d) of 41 nM (Table 1).

Table 1: SPR Data for Interaction of Malaria CS Protein with Glycosaminoglycans

glycosaminoglycans	k_a ($M^{-1}\,s^{-1}$)	k_d (s^{-1})	K_d
heparin	2.7×10^4	1.1×10^{-3}	41 nM
dermatan sulfate	1.4×10^4	3.0×10^{-3}	220 nM
chondroitin sulfate E	1.5×10^4	9.6×10^{-3}	640 nM
heparan sulfate	7.8×10^3	1.7×10^{-2}	2.2 μM
chondroitin sulfate A	8.1×10^3	3.6×10^{-2}	4.4 μM

Other glycosaminoglycan samples, which included porcine intestinal heparan sulfate, dermatan sulfate, chondroitin sulfate A, and chondroitin sulfate E (Figure 1), gave similar absorption and desorption binding curves (Figure 3). The binding of these glycosaminoglycans was also characterized by the overall dissociation constantsm K_d, which are summarized in Table 1. Heparin, with a K_d of 41 nM, showed the strongest binding affinity for CS protein, followed by dermatan sulfate and chondroitin sulfate E, with a K_d of 220 and 640 nM, respectively. This suggested that dermatan sulfate and chondroitin sulfate E bind CS protein with 5- and 15-fold less affinity in comparison to heparin. Porcine intestinal heparan sulfate and chondroitin sulfate A, though interacted with CS, also had a much weaker binding. Porcine intestinal heparan sulfate had 50-fold less affinity than porcine intestinal heparin with a K_d value of 2.2 μM, while chondroitin sulfate A had a K_d of 4.4 μM, indicating a 100-fold difference in binding in comparison to heparin.

Highly purified and structurally characterized heparin-derived oligosaccharides in sizes ranging from tetrasaccharide to tetradecasaccharide were investigated in an attempt to identify the smallest size of a heparin-based saccharide that could interact with CS protein. The SPR sensograms of heparin oligosaccharides showed adsorption and desorption curves similar to those observed for the heparin (data not shown). The k_a values ranged from 0.2×10^3 to 1.0×10^3 $M^{-1}\,s^{-1}$, and k_d values ranged from 0.6×10^{-2} to $4.0 \times 10^{-2}\,s^{-1}$, which gave K_d values between 1×10^{-5} and 5.0×10^{-5} M (data not shown).

ITC Analysis of CS and Heparin Saccharide Interactions. As SPR was unable to distinguish any remarkable differences in the binding affinity of CS for heparin-derived oligosaccharides, this interaction was further examined using ITC, in which the minimum size sequence within heparin capable of strongly interacting with CS protein was determined. The heats of titration were calculated and were fit to a sigmoidal curve (Figure 4). Curve-fitting was performed allowing ΔH and n (binding stoichiometry) to vary, and the values of ΔH and n that give the best fit were determined. The interaction revealed a ΔH of −184 kcal/mol, a K_d of 160 nM, and a stoichiometry (n) of 3.75 proteins per heparin chain. This represents a binding site sequence within heparin of MW 3200, corresponding in size to a decasaccharide, and suggests that →4)α-L-IdoAp2S(1→[4)-α-D-GlcNpS6S(1→4)-α-L-

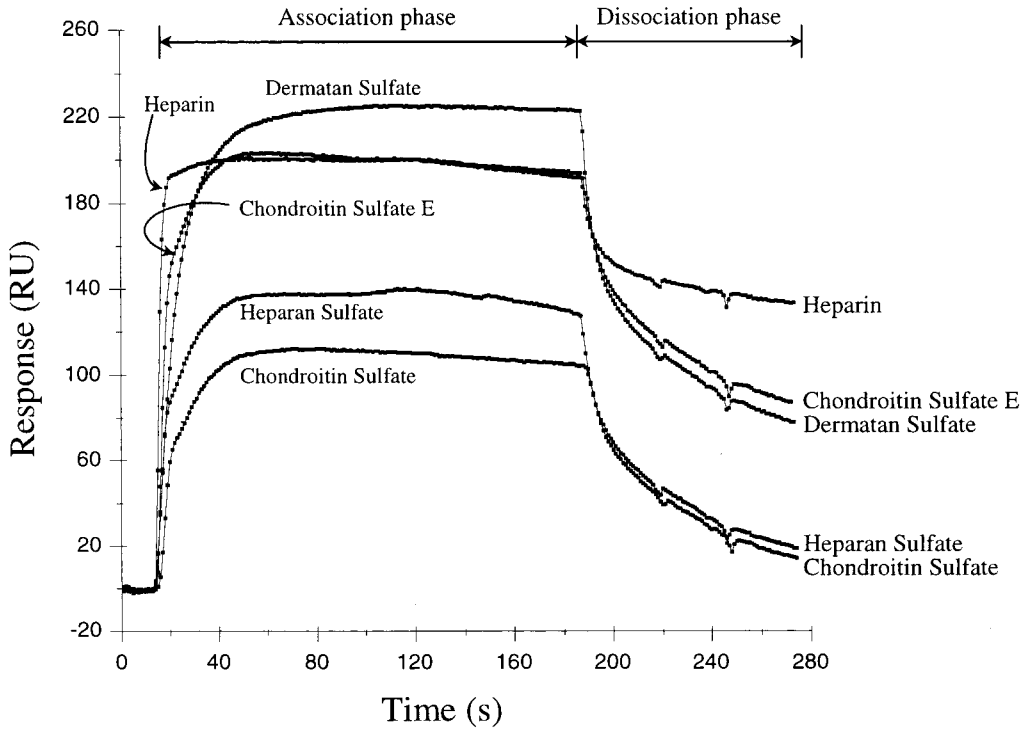

FIGURE 3: SPR sensograms of glycosaminoglycan−CS interactions. A concentration of 10 μM of each glycosaminoglycan was used in this experiment.

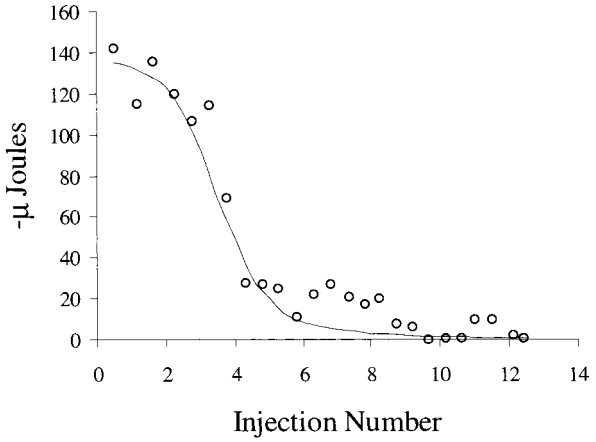

FIGURE 4: ITC analysis of heparin interacting with CS protein. The measured heats (○) after each injection are plotted, and the fitted line was used to calculate ΔH, K_d, and n.

IdoAp2S(1→]$_4$4)-α-D-GlcNS6(1→ is the minimum size CS-binding site in heparin.

Inhibition of Cell Binding by Glycosaminoglycans. The glycosaminoglycans were next examined for their ability to inhibit CS binding to HepG2 cells. Out of the six glycosaminoglycans examined, only porcine intestinal heparin and highly sulfated porcine liver heparan sulfate inhibited the binding of CS to HepG2 cells (Figure 5A). Porcine intestinal heparan sulfate, chondroitin sulfate A, dermatan sulfate, and chondroitin sulfate E failed to inhibit the binding. The inhibition by heparin and liver heparan sulfate was dose-dependent with almost complete inhibition at the highest concentrations of the two GAGs evaluated. At low concentrations, heparin, liver heparan sulfate, and chondroitin sulfate E promoted the binding of CS to HepG2 cells by 300, 180, and 250%, respectively. A similar heparin-mediated enhancement of CS binding to HepG2 cells has been demonstrated previously (*7*, *13*) which suggests that heparin, liver heparan sulfate, and chondroitin sulfate E could be acting as a template to form CS multimers.

Inhibition of Cell Binding by Heparin-Derived Oligosaccharides. A heparin tetrasaccharide and decasaccharide were tested for their ability to inhibit the binding of CS to HepG2 cells. Both the molecules inhibited CS binding at all the concentrations tested and in a dose-dependent manner (Figure 5B). The heparin decasaccharide potently inhibited CS protein binding to HepG2 cells, showing complete inhibition at a concentration of 20 μg/mL and an ID_{50} concentration estimated at <0.1 μg/mL (<60 nM). The heparin tetrasaccharide was more than 250-fold less effective and had an ID_{50} of 20 μg/mL (15 μM).

DISCUSSION

Numerous bacteria, parasites, and viruses bind to cellular and extracellular glycosaminoglycans and initiate an infection (*20*). In the case of malaria parasite *P. falciparum*, heparan sulfate side chains of hepatocyte cell surface proteoglycans interact with CS protein and help in the binding of the parasite to liver cells (*3*, *4*, *21*). Most studies to date have examined the ability of glycosaminoglycans and the other polyanions to act as competitors to block the binding of CS protein to target cells (*4*−*7*, *22*). In the current study, we took direct measurements of the binding kinetics between CS protein and various glycosaminoglycans by SPR. The results indicated that heparin has fast on-rate (k_a) and slow off-rate (k_d) kinetics for CS protein with a K_d of 41 nM. While it is unclear whether the CS molecules bound to the chip are sufficiently close to one another to oligomerize, it is interesting to note that an IC_{50} of ∼50 nM was reported for heparin interaction with a CS tetramer as determined by gel permeation chromatography (*23*). Multivalency is a well-

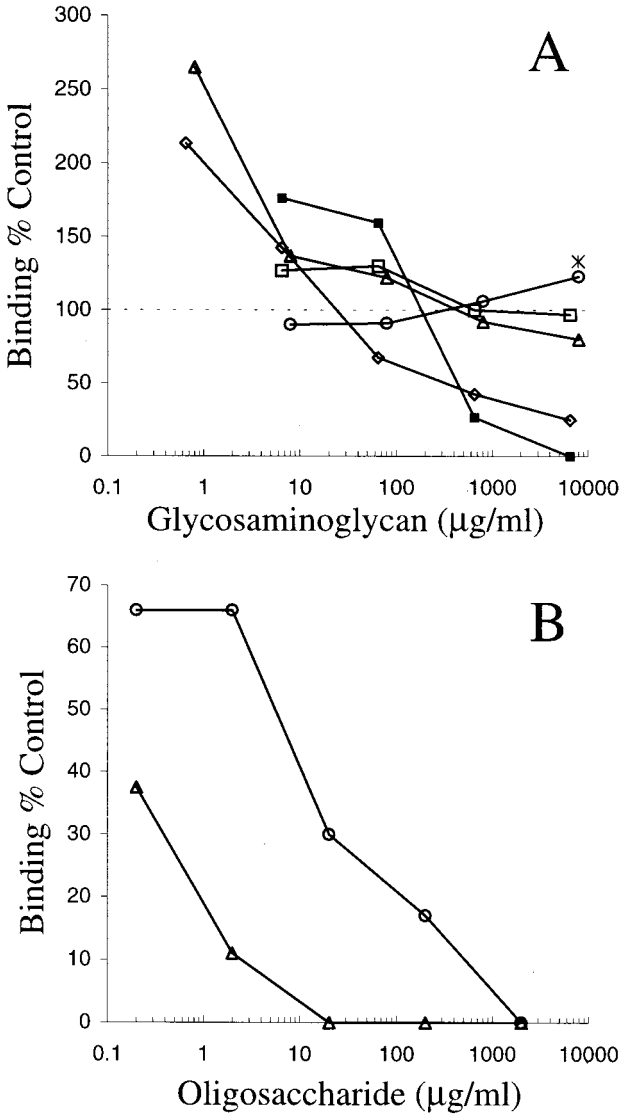

FIGURE 5: Inhibition of CS binding to HepG2 cells. (A) Glycosaminoglycans were added at varying concentrations to block CS binding to HepG2 cells. Heparin (◇), chondroitin sulfate E (△), heparan sulfate from porcine liver (■), heparan sulfate from porcine intestinal mucosa (□), dermatan sulfate (○), chondroitin sulfate A (★). (B) Heparin-derived tetrasaccharide (○) and decasaccharide (△) were added at varying concentrations to stop CS from binding to HepG2 cells.

known property that can greatly enhance the affinity of protein−carbohydrate interactions (*24*) and particularly those interactions involving GAGs (*25*). Heparin's nanomolar binding affinity might result from its multivalent interaction with CS oligomers on the biochip. The reduced binding affinity observed for the heparin oligosaccharides using SPR also suggests that they are simply too small to participate in multivalent interactions with CS immobilized on the biochip. Heparan sulfate from porcine intestine, while structurally related to heparin, has a low level of sulfation and contains a D-glucuronic acid residue in place of the flexible L-iduronic acid residue found in heparin (Figure 1), which showed 50-fold weaker ($K_d = 2.2\ \mu M$) binding to CS. The intermediate levels of CS binding shown by dermatan sulfate containing the flexible L-iduronic acid residue and the highly sulfated chondroitin sulfate E, suggest that both polymer flexibility and a high level of negative change are crucial factors governing this interaction. It is interesting to note that chondroitin sulfate E interacts with midkine, a mammalian neuroregulatory protein containing TSR motifs, with the same affinity as does heparin (*11, 26*).

By ITC we found that the minimum sequence within the heparin polymer capable of a strong interaction with the CS protein is a decasaccharide and that heparin had a K_d of 160 nM. Next we examined the ability of a heparin decasaccharide and a considerably smaller heparin tetrasaccharide to inhibit the binding of CS protein to HepG2 cells. In comparison to the tetrasaccharide, the decasaccharide was found to be 250-fold more active in inhibiting the binding of CS protein, suggesting an increase in inhibition with the increase in chain length of the oligosaccharides. Carbohydrate−protein associations are weak in nature and are enhanced by the cooperative effect of multivalent binding sites, which could be the reason for heparin decasaccharide to be a more potent inhibitor of binding. A similar competition experiment performed with intact glycosaminoglycans showed that only heparin and the highly sulfated porcine liver heparan sulfate inhibit CS binding to HepG2 cells and only at relatively high concentrations. At low concentrations of heparin, porcine liver heparan sulfate and chondroitin sulfate E promoted CS interaction with HepG2 cells by presumably acting as cross-linking reagents (*7*).

It is of interest to compare the results of this study with a similar study performed in our laboratory on apolipoprotein E (apoE)−heparin interaction (*27*). ApoE is involved in the rapid removal of lipoprotein remnants from the blood by the liver. Its activity is mediated through its binding to a highly sulfated hepatic heparan sulfate (*28*). The study by Dong and co-workers (*27*) demonstrated that apoE interacted most strongly with a fully sulfated heparin-derived octasaccharide having the same sequence as the decasaccharide used in the current study. The theory that the heparan sulfate mediated clearance of lipoprotein remnants from the blood by the liver involves a mechanism comparable to the uptake of malaria sporozoites by hepatocytes was first put forward by Sinnis and co-workers (*29*). ApoE contains a sequence with homology to region II plus of CS, and both of these domains have been suggested to be involved in heparin binding (*27, 28*). Moreover, both apoE and CS contain linearly noncontiguous domains that also have been implicated in heparin binding (*27, 30*). The current study supports this hypothesis and further suggests that an octasaccharide and decasaccharide domain of sequence →4)-α-D-GlcNpS6S(1→4)-α-L-IdoAp2S(1→, in the unusually highly charged hepatocyte heparan sulfate (*14*), is required for the binding of both CS and apoE.

In conclusion, we provide evidence supporting a decasaccharide of heparin to be the minimum binding sequence. A decasaccharide incapable of promoting the formation of CS multimers might represent a specific inhibitor of sporozoite attachment. This size dependence might also suggest a therapeutic potential for low molecular weight heparins in preventing malarial infection and in treating atherogenesis.

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