Inhibition of thrombin by heparin cofactor (HCII) is accelerated ~1000-fold by heparin or dermatan sulfate. We found recently that the mutation Arg103 → His decreases the affinity of HCII for dermatan sulfate but not for heparin (Blinder, M. A., Andersson, T. R., Abildgaard, U., and Tollefsen, D. M. (1989) J. Biol. Chem. 264, 5128–5133). Other investigators have implicated Arg47 and Lys185 of anti-thrombin (homologous to Arg103 and Lys185 of HCII) in heparin binding. To investigate the corresponding residues in HCII, we have constructed amino acid substitutions (Arg103 → Leu, Gln, or Trp; Lys185 → Met, Asn, or Thr) by oligonucleotide-directed mutagenesis of the cDNA and expressed the products in Escherichia coli. The recombinant HCII variants were assayed for binding to heparin-Sepharose and for inhibition of thrombin in the presence of various concentrations of heparin or dermatan sulfate. All of the Arg103 variants bound to heparin with normal affinity. Furthermore, inhibition of thrombin by the Arg103 → Leu variant occurred at a normal rate in the absence of a glycosaminoglycan and was accelerated by normal concentrations of heparin and dermatan sulfate. These results indicate that HCII, unlike anti-thrombin, does not require a positive charge at this position for the interaction with heparin or dermatan sulfate. The Arg103 → Gln and Arg103 → Trp variants inhibited thrombin at about one-third of the normal rate in the absence of a glycosaminoglycan, suggesting that these mutations exert an effect on the reactive site (Leu144→Ser146) of HCII. All of the Lys185 variants bound to heparin with decreased affinity but inhibited thrombin at approximately the normal rate in the absence of a glycosaminoglycan. These variants required >10-fold higher concentrations of heparin to accelerate inhibition of thrombin and were not stimulated significantly by dermatan sulfate, suggesting that heparin and dermatan sulfate interact with Lys185 of HCII. These results provide evidence that the glycosaminoglycan-binding site in HCII includes Lys185 but not Arg103, both of which were predicted to be involved by homology to anti-thrombin.

The serine proteinase inhibitors (serpins) include a variety of homologous plasma proteins that inactivate specific target proteinases (1). Inhibition occurs when the proteinase attacks a peptide bond in the serpin, termed the reactive site, and becomes trapped in a 1:1 complex. Certain glycosaminoglycans increase the rate of proteinase inhibition by heparin cofactor II (HCII) and anti-thrombin (AT) ~1000-fold (2), which distinguishes these inhibitors from other serpins. Heparin, heparan sulfate, and dermatan sulfate accelerate the interaction between HCII and thrombin (3). In contrast, only heparin and heparan sulfate accelerate inactivation of target proteinases (e.g. coagulation factors IXa, Xa, and thrombin) by AT. Glycosaminoglycans increase the rate of proteinase inhibition by providing a template to which both the inhibitor and the proteinase bind or by inducing a conformational change in the reactive site of the inhibitor (4).

Chemical modification experiments and natural mutations suggest that the binding sites for glycosaminoglycans on HCII and AT are composed, in part, of positively charged arginine and/or lysine residues. A natural mutant of HCII, HCIIvar (Arg103→His), does not bind dermatan sulfate; consequently, inhibition of thrombin is not accelerated by dermatan sulfate (5-6). HCIIvar binds heparin, however, and thrombin inhibition is accelerated in a normal manner by this glycosaminoglycan, implying that the binding sites for heparin and dermatan sulfate are not identical. In AT, 2 basic amino acid residues have been strongly implicated as necessary for heparin-stimulated inhibition of thrombin. Naturally occurring mutations at Arg77 have been discovered in several unrelated families, and in each case that has been examined, the AT variant binds heparin or is thrombin inhibition accelerated by heparin (7-9). In addition, selective chemical modification of Lys185 greatly reduces the affinity of the modified AT for heparin (10). This residue occurs in a region containing 4 positively charged amino acids (Lys125, Arg129, Arg132, and Lys135) that have been proposed to lie adjacent to Arg77 in the tertiary structure of the molecule and form the heparin-binding site (11). Based on alignment of the amino acid sequences, HCII contains Arg103 and Lys185 in positions homologous to Arg77 and Lys185 of AT, respectively (12). In the current investigation, we have constructed mutations in the codons for Arg103 and Lys185 of HCII and have expressed the recombinant products to investigate the role of these amino acids in heparin binding and thrombin inhibition.

EXPERIMENTAL PROCEDURES

Materials—The chromogenic substrate tosyl-Gly-Pro-Arg-p-nitroanilide (Chromozym TH) was obtained from Boehringer Mann-
hein. Thrombin and HCII derived from human plasma were prepared and assayed as described previously (6). Pyridoxylated HCII was prepared as described by Blackburn and Schachman (13); the modified HCII retained <1% of the original heparin cofactor activity but inhibited thrombin at ~13 times the normal ratio in the absence of heparin. Affinity-purified rabbit anti-HCII antibodies were prepared and labeled with Na[125]I as described previously (12). Bovine lung heparin and porcine skin dermatan sulfate were obtained from Sigma, and the dermatan sulfate was treated with nitrous acid to remove contaminating heparin prior to use (14). Heparin-Sepharose was prepared as described previously (15).

The reagents used for oligonucleotide-directed mutagenesis were purchased from Amer sham Corp. Restriction enzymes were obtained from New England Biolabs or Amersham Corp. DNA sequencing was performed using T7 DNA polymerase from United States Biochemical Corp. Deoxyadenosine-[α-32P]thiotriphosphate (α-32P)dATTP was obtained from Du Pont-New England Nuclear. The protein chemistry facility of Washington University provided the synthetic oligonucleotides.

**Mutagenesis of HCII cDNA**—The cDNA for HCII was isolated previously from a human fetal liver library in agt11 (12). Twenty-one base oligonucleotides corresponding to regions of the coding strand of the HCII cDNA were synthesized with the desired nucleotide mismatch at the middle position. Mutagenesis was performed in M13mp18 containing the normal strand by the method of Nakanishi and Eckstein (16). For each of the nucleotide substitutions, four clones containing the mutated HCII cDNA were isolated and grown in 2 ml of YT medium. Single-stranded plague DNA wasprepared from the bacterial supernatant, and the region of the DNA containing the nucleotide substitution was sequenced using the deoxy chain termination method of Sanger et al. (17). The replicative form of M13mp18 containing the correctly mutated sequence was then isolated from one clone for each of the constructs (18). The 1000-base pair PstI-XhoI fragment of the mutated cDNA, which contained the codons for Ala19 to Arg33 of plasma HCII, was isolated from the replicative vector and ligated into the expression vector pMON-HCII (6) digested with the same enzymes. Correct ligation was verified by restriction endonuclease digestion, and the mutation was confirmed in the final plasmid construct by deoxy chain sequencing of both strands. Mutation of the cDNA resulting in the replacement Arg33 → His was reported previously (6).

**Expression and Quantification of Recombinant HCII and HCII Variants**—For each recombinant HCII (rHCII) preparation, transformed Escherichia coli were grown to an optical density (550 nm) of 1.0–1.2 in 500 ml of medium, and expression was induced with 1 ml of isopropyl-β-d-thiogalactopyranoside. The concentrated cell lysates were dialyzed against 0.05 M NaCl, 0.01 M Tris-HCl, pH 7.5, for all other experiments.

The concentration of HCII was determined by a slot blot immunoblot technique using an immuno blot of the gel probed with 125I-labeled anti-HCII antibodies. Relative quantities of HCII in each of the fractions were determined by densitometry of autoradiographs of the immunoblots.

**Inhibition of Thrombin by Recombinant HCII**—The effect of glycosaminoglycans on the rate of inhibition of thrombin by rHCII was determined by incubating 90 μl of concentrated cell lysate containing 56–69 nM rHCII with 5 μl of 280 nm thrombin and 5 μl of various concentrations of heparin or dermatan sulfate in a disposable polystyrene cuvette. After a 90-s incubation at room temperature, 500 μl of 78 μM Chromozym TH in 0.15 M NaCl, 0.01 M Tris-HCl, 1 mg/ml polyethylene glycol, pH 7.5, was added, and the rate of change of absorbance at 405 nm was determined. The rate of change of absorbance was proportional to the concentration of active thrombin remaining in the incubation. When lysates prepared from E. coli transformed with pMON-5840 were assayed under identical conditions, no thrombin inhibition was detected with or without heparin or dermatan sulfate. Thrombin-inhibitory activity in the absence of a glycosaminoglycan was determined using thrombin and rHCII at the concentrations indicated above in a total volume of 1 ml. At specific times between 1.5 and 250 min, a 100-μl aliquot removed from the incubation was added to 600 μl of Chromozym TH, and the rate of change of absorbance at 405 nm was determined as above.

**RESULTS**

**Expression of Recombinant HCII Variants**—Single nucleotide substitutions were made in the HCII cDNA to alter the codons for Arg103 and Lys185 (Fig. 1). Each of the mutations eliminated the positive charge on the side chain of the amino acid residue. The 1060-base pair PstI-XhoI fragment of the mutated cDNA in M13mp18 was subcloned into pMON-HCII for expression in E. coli. This vector, which has been characterized previously, contains the entire coding sequence for HCII except that the codons for the signal peptide (19 amino acid residues) and the N-terminal 18 amino acid residues of plasma HCII are replaced by the sequence encoding Met-Ala-Val-Leu (6). The term "native rHCII" is used to designate the product of pMON-HCII. Each of the rHCII variants appeared to be identical in size to native HCII and had a molecular weight of 55,000 determined by immunoblot analysis of concentrated lysate subjected to SDS-polyacrylamide gel electrophoresis (not shown). This molecular weight is close to that predicted for the recombinant protein without post-translation modifications (53,416).

**Binding of rHCII to Heparin-Sepharose**—Heparin-Sepharose chromatography was performed to determine the relative affinities of the rHCII variants for heparin (Fig. 2). Native rHCII was eluted from the heparin-Sepharose column with a peak at 0.56 M NaCl. Substitution of either tryptophan or leucine for Arg103 did not affect the ionic strength at which the rHCII variant eluted (panel A). The Arg103 → Glu mutation resulted in a molecule that eluted one fraction later and thus may have a slightly higher affinity for heparin in comparison with native rHCII. These experiments indicate that

**ATIII** + Arg + Ala-Lys-Leu-Aan-Cys-Arg-Leu-Tyr-Arg-Lys-Thr-His-Leu-Thr-AArg

**HCII** + Arg + Arg-Lys-Leu-Thr-His-Arg-Leu-Phe-Arg-AArg

**rHCII Variants** + Arg + Arg-Lys-Leu-Thr-His-Arg-Leu-Phe-Arg-AArg

**Fig. 1.** Alignment of the putative glycosaminoglycan-binding domains of AT, HCII, and rHCII variants. The amino acid residues of AT and HCII are numbered as reported previously (12).
These results suggest that a small fraction (20-30%) of the column had been exceeded, the flow-through material was column as determined by quantitative immunoblot analysis of SDS-polyacrylamide gel electrophoresis, is unable to bind heparin-Sepharose chromatography, purified plasma HCII was treated with pyridoxal phosphate under conditions in which a limited number of lysine residues are modified. Church and Griffith (21) have reported that this modification greatly decreases the affinity of HCII for heparin but does not diminish the rate of thrombin inhibition by HCII in the absence of heparin. As expected, pyridoxylated plasma HCII mixed with concentrated control E. coli lysate did not bind to the heparin-Sepharose column (not shown).

Glycosaminoglycan-independent Inhibition of Thrombin by rHCII Variants—The time course of inhibition of thrombin was determined in the absence of a glycosaminoglycan for native rHCII and for each of the rHCII variants (Fig. 3). Second-order rate constants for the reactions were calculated by dividing the slope of each progress curve (i.e. the pseudo first-order rate constant) by the concentration of HCII in the incubation (Table I). The average of the two values

![Graph](http://www.jbc.org/)

**FIG. 2.** Heparin-Sepharose chromatography of rHCII and rHCII variants. Lysates of E. coli containing ammonium sulfate (1.5 ml) containing native rHCII or HCII variants (56-70 nm) were applied to a 2-ml heparin-Sepharose column and eluted with a linear gradient of NaCl. An aliquot of each fraction was subjected to SDS-polyacrylamide gel electrophoresis, and the rHCII was detected on an immunoblot probed with [125I]-labeled anti-HCII IgG. The amount of HCII in each fraction was determined by densitometry of the immunoblot. The relative amounts normalized against the peak fraction (=100) are shown. The concentration of NaCl in each fraction was determined from the conductivity. Panel A: ○, native rHCII; □, rHCII (Arg103 → Trp); ■, rHCII (Arg103 → Gin); △, rHCII (Arg103 → Leu). Panel B: ○, native rHCII; ○, rHCII (Lys185 → Met); ■, rHCII (Lys185 → Asn); △, rHCII (Lys185 → Thr). Panel C: ○, native rHCII; □, rHCII (Arg103 → His).

the positive charge on the amino acid at position 103 of HCII is not required for heparin binding when assessed by affinity chromatography. In contrast, substitutions of asparagine, methionine, or threonine for Lys103 resulted in elution of rHCII from the heparin-Sepharose column with a peak at 0.22 M NaCl (panel B), suggesting that these rHCII variants have a moderately decreased affinity for heparin. The Arg103 → His variant bound to heparin-Sepharose with the same affinity as native rHCII (panel C); this variant contains the amino acid substitution found in the natural mutant HCIIO,~,, which appears to have a normal affinity for heparin (5, 6).

Between 17 and 33% of each of the rHCII preparations, including native rHCII, did not bind to the heparin-Sepharose column as determined by quantitative immunoblot analysis (not shown). To determine whether the binding capacity of the column had been exceeded, the flow-through material was reapplied to the column. Less than 5% of the rechromatographed rHCII antigen bound to the heparin-Sepharose. These results suggest that a small fraction (20-30%) of native rHCII, despite being normal in size by immunoblot analysis of SDS-polyacrylamide gel electrophoresis, is unable to bind heparin and is probably denatured. As a control for the heparin-Sepharose chromatography, purified plasma HCII was treated with pyridoxal phosphate under conditions in which a limited number of lysine residues are modified. Church and Griffith (21) have reported that this modification greatly decreases the affinity of HCII for heparin but does not diminish the rate of thrombin inhibition by HCII in the absence of heparin. As expected, pyridoxylated plasma HCII mixed with concentrated control E. coli lysate did not bind to the heparin-Sepharose column (not shown).

**TABLE I**

<table>
<thead>
<tr>
<th>Variant</th>
<th>Final rHCII concentration</th>
<th>Second-order rate constant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nM</td>
<td>M⁻¹ min⁻¹</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rHCII (native)</td>
<td>59</td>
<td>6.3 x 10⁴</td>
</tr>
<tr>
<td>rHCII (Arg103 → Leu)</td>
<td>60</td>
<td>6.3 x 10⁴</td>
</tr>
<tr>
<td>rHCII (Arg103 → Gin)</td>
<td>68</td>
<td>2.6 x 10⁵</td>
</tr>
<tr>
<td>rHCII (Arg103 → Trp)</td>
<td>50</td>
<td>1.3 x 10⁴</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rHCII (native)</td>
<td>56</td>
<td>5.0 x 10⁴</td>
</tr>
<tr>
<td>rHCII (Lys185 → Met)</td>
<td>57</td>
<td>3.7 x 10⁴</td>
</tr>
<tr>
<td>rHCII (Lys185 → Asn)</td>
<td>58</td>
<td>4.4 x 10⁴</td>
</tr>
<tr>
<td>rHCII (Lys185 → Thr)</td>
<td>60</td>
<td>3.7 x 10⁴</td>
</tr>
</tbody>
</table>
obtained for native rHCII (5.7 × 10^8 M^-1 min^-1) was similar to the value reported by Griffith et al. (22) and 9 fold lower than the value reported by Tollefsen et al. (15) for purified plasma HCII. The mutations Arg(lo) → Leu and Lys(150) → Met, Asn, or Thr produced only minor changes in the second-order rate constant for thrombin inhibition in the absence of a glycosaminoglycan, providing evidence that the recombinant proteins were folded properly and that the reactive sites, in particular, were intact. The differences in the rate constants among these variants were similar in magnitude to the difference in rate constants obtained with two preparations of native rHCII (cf. Table I, A and B) and may be related to imprecision in the determination of rHCII concentration by the immunoblot assay described in “Experimental Procedures.” Substitution of tryptophan or glutamine for Arg(lo) appeared to decrease the second-order rate constant for thrombin inhibition to a greater extent than the other mutations (Table I). These observations suggest that certain mutations of Arg(lo) may affect the conformation of the reactive site of HCII or its accessibility to proteinases.

Inhibition of Thrombin by rHCII Variants in the Presence of Heparin or Dermatan Sulfate—The effects of heparin and dermatan sulfate on the extent of inhibition of thrombin by rHCII during a 90-s incubation are shown in Figs. 4–6. In these experiments, native rHCII inhibited 50% of the thrombin activity in the presence of either heparin or dermatan sulfate (3–4 μg/ml). Most importantly, the mutation Arg(lo) → Leu did not affect the ability of either glycosaminoglycan to accelerate the reaction (Fig. 4). This result is consistent with the data from affinity chromatography which indicate that this variant binds normally to heparin. The mutations Arg(lo) → Gln or Trp decreased the amount of thrombin inhibited at a given concentration of heparin or dermatan sulfate roughly in proportion to the decrease in the rate of glycosaminoglycan-independent thrombin inhibition (Table I).

All of the Lys(150) variants required at least 10-fold higher concentrations of heparin to accelerate inhibition of thrombin in comparison with native rHCII (Fig. 5), which is consistent with the observation that these variants have a lower affinity than native rHCII for heparin-Sepharose. Essentially no acceleration of thrombin inhibition by these variants was observed with dermatan sulfate at concentrations up to 1.25 mg/ml. The rHCII variant Arg(lo) → His was stimulated normally by heparin but required about a 300-fold higher concentration of dermatan sulfate than native rHCII to accelerate inhibition of thrombin (Fig. 6). The results obtained with the Arg(lo) → His variant of rHCII are very similar to those reported previously for the natural mutant HCIIoslo (6).

**Fig. 5.** Effect of glycosaminoglycan concentration on inhibition of thrombin by rHCII variants of Lys(150). Incubations were performed with rHCII (56–60 nM) as indicated in the legend to Fig. 4. ○, native rHCII; □, rHCII (Lys(150) → Met); ■, rHCII (Lys(150) → Asn); □, rHCII (Lys(150) → Thr).

**Fig. 6.** Effect of glycosaminoglycan concentration on thrombin inhibition by rHCII (Arg(lo) → His). Incubations were performed with rHCII (59–63 nM) as indicated in the legend to Fig. 4. ○, native rHCII; ○, rHCII (Arg(lo) → His).
DISCUSSION

HCII and AT are homologous members of the serpin family (1). In contrast to other serpins, the rate of proteinase inhibition by HCII and AT is increased -1000-fold by certain glycosaminoglycans (2). The glycosaminoglycan is thought to serve as a catalytic template to which both the inhibitor and the proteinase bind or to induce a conformational change in the inhibitor, which causes the reactive site to become more accessible to the proteinase (4). In either case, the glycosaminoglycan must bind to the inhibitor to produce the stimulatory effect. Binding is presumed to be mediated by ionic interactions between sulfate and/or carboxylate groups on the glycosaminoglycan and a cluster of basic amino acid residues on the inhibitor, since binding of HCII or AT to heparin-Sepharose is disrupted by solvents of high ionic strength (15, 23). In addition, chemical modification of either lysine or arginine residues in HCII or AT results in a protein that fails to bind heparin (21, 23, 24), which suggests that the binding site for heparin in each protein contains at least 1 lysine and 1 arginine residue.

The binding site for AT in heparin consists of a specific pentasaccharide that contains a unique 3-O-sulfated glucosamine residue in the middle position (25, 26). Only about one-third of heparin molecules contain this structure and bind to AT with high affinity. The remainder have <5% of the activity of the high-affinity molecules in catalyzing proteinase inhibition by AT. Likewise, dermatan sulfate and other glycosaminoglycans that do not contain the pentasaccharide structure are virtually inactive with AT (3). In comparison with AT, HCII elutes from heparin-Sepharose at a lower ionic strength and requires about a 10-fold higher concentration of heparin to accelerate inhibition of thrombin (3, 15). Moreover, stimulation of HCII does not depend on the presence of the pentasaccharide containing 3-O-sulfated glucosamine (27, 28). Dermatan sulfate and a variety of other natural and synthetic polyanions (e.g. chondroitin sulfate E, pentosan polysulfate, and polyaspartic acid) all increase the rate of thrombin inhibition by HCII (3, 29-32). These findings indicate that HCII is able to interact with a broader range of ligands than AT.

It is reasonable to assume that the binding sites for glycosaminoglycans in HCII and AT occur in regions of the two proteins which have similar structures and which are poorly conserved in other serpins. Four of the 94 basic amino acid residues in HCII occur in a peptide (residues 1-47) that can be removed from the N terminus without the loss of glycosaminoglycan-dependent thrombin inhibition (22). Of the remaining 50 basic amino acid residues, 36 can be aligned with a basic amino acid in at least one serpin other than AT, and 11 are not present in AT (for alignments, see Refs. 33 and 34). Only 9 basic amino acid residues in HCII (Lys180, Arg186, and Arg199) are present in the homologous positions in AT but not in other serpins (Fig. 1). The two other basic amino acids in this domain (Arg184 and Arg192) are also present in other serpins. We consider this unusual cluster of positively charged residues in HCII and AT to be a likely candidate for the glycosaminoglycan-binding site.

Direct evidence for the involvement of this domain in heparin binding to AT has been reported by other investigators. Selective chemical modification of Lys185 greatly reduces the affinity of AT for heparin (10), and heparin protects this residue from chemical modification (10, 35). In addition, a proteolytic fragment of AT containing residues 114-156 has been reported to bind heparin (36). No mutations of AT in this area have been reported. In the present study, we have demonstrated that several rHCII variants in which Lys185 (corresponding to Lys125 in AT) has been replaced by a neutral amino acid elute from heparin-Sepharose at a lower ionic strength than native rHCII and, by this criterion, appear to have a decreased affinity for heparin. Furthermore, >10-fold higher concentrations of heparin are required to accelerate thrombin inhibition by these variants in comparison with native rHCII, even though they inhibit thrombin at close to the normal rate in the absence of heparin and thus appear to have assumed a normal conformation, at least in the vicinity of the reactive site (Lys144-Ser145). From the data shown in Fig. 5, mutations of Lys185 appear to affect the interaction with dermatan sulfate to a greater degree than the interaction with heparin. These observations suggest that the binding sites for heparin and dermatan sulfate both contain Lys185. We believe that the effects of these mutations are due to elimination of an ionic bond between the glycosaminoglycan and the side chain of Lys185, although we cannot rule out the possibility that differences in the size or hydrophilicity of the side chain or in the tertiary structure of the glycosaminoglycan-binding domain also have some effect.

We reported previously that the natural mutation Arg187 → His in HCII (HCII1200) results in a protein that fails to bind dermatan sulfate but binds to heparin in a qualitatively normal manner (6). In the present study, we have demonstrated that HCII containing the Arg187 → His mutation elutes from heparin-Sepharose at the same ionic strength as native rHCII and that thrombin inhibition is accelerated by similar concentrations of heparin. Since AT has an arginine residue (Arg129) at the position homologous to Arg187 of HCII but does not interact with dermatan sulfate, this amino acid does not appear to be sufficient for dermatan sulfate binding. Furthermore, since loss of the positive charge on Arg187 does not affect the interaction of HCII with heparin, the binding sites in HCII for the two glycosaminoglycans apparently are not identical.

It has been proposed that Arg47 lies adjacent to the domain containing Lys185 in the tertiary structure of AT, based on a projection of the sequence of AT upon the x-ray crystallographic structure of α₁-anti-trypsin cleaved at the reactive site (11). The natural mutations Arg47 → Cys, His, and Ser have been discovered in AT molecules that fail to bind heparin (7-9), providing support for the hypothesis that this residue is part of the heparin-binding site. As exemplified by AT10man (Arg47 → Cys), mutations of this residue do not generally alter the rate of thrombin inhibition in the absence of heparin (8). Mutations in the homologous position in HCII (Arg194), however, produce different results. In particular, the rate of inhibition of thrombin by the Arg194 → Leu variant is indistinguishable from that of native HCII in the presence or absence of heparin or dermatan sulfate, whereas the Arg194 → Glu and Trp variants appear to inhibit thrombin at a moderately decreased rate in the absence of a glycosaminoglycan. Furthermore, all of these variants, in which the positive charge on the amino acid at position 103 has been eliminated, elute from heparin-Sepharose at the same ionic strength as native rHCII. These findings indicate that Arg187 does not play a major role in the binding of glycosaminoglycans to HCII.

A more detailed understanding of the interactions between glycosaminoglycans and AT or HCII is desirable because of the widespread use of heparin in clinical medicine. Heparin is commonly employed in the prophylaxis and treatment of venous thrombosis and pulmonary embolism, but its use is sometimes complicated by severe bleeding and thrombocytopenia. Animal studies have suggested that dermatan sulfate may produce an antithrombotic effect equivalent to that of heparin and cause less bleeding (37), but this advantage has not yet been confirmed in human studies. Knowledge of the
precise structural requirements for the binding of glycosaminoglycane to AT and HCII may ultimately lead to the design of a better anticoagulant agent.

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