Heparin Enhances Serpin Inhibition of the Cysteine Protease Cathepsin L

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The glycosaminoglycan heparin is known to possess antimitotic activity in experimental models and preclinical studies, but there is still uncertainty over its mechanism of action in this respect. As an anticoagulant, heparin enhances inhibition of thrombin by the serpin antithrombin III, but a similar cofactor role has not been previously investigated for proteases linked to metastasis. The squamous cell carcinoma antigen (sentrins B3 and B4) are tumor-associated proteins that can inhibit papain-like cysteine proteases, including cathepsins L, K, and S. In this study, we show that SCCA-1 (B3) and SCCA-2 (B4) can bind heparin as demonstrated by affinity chromatography, native PAGE gel shifts, and intrinsic fluorescence quenching. Binding was specific for heparin and heparan sulfate but not other glycosaminoglycans. The presence of heparin accelerated inhibition of cathepsin L by both serpins, and in the case of SCCA-1, heparin increased the second order inhibition rate constant from $5.8 \times 10^6$ to $>10^8$, indicating a rate enhancement of at least 180-fold. A templating mechanism was shown, consistent with ternary complex formation. Furthermore, SCCA-1 inhibition of cathepsin L-like proteolytic activity secreted from breast and melanoma cancer cell lines was significantly enhanced by heparin. This is the first example of glycosaminoglycan enhancement of B-clade serpin activity and the first report of heparin acting as a cofactor in serpin cross-class inhibition of cysteine proteases. Most importantly, this finding raises the possibility that the anticancer properties of heparin may be due, at least partly, to enhanced inhibition of prometastatic proteases.

Serpin B3 was originally isolated as squamous cell carcinoma antigen (SCCA), a tumor marker antigen associated with cervical cancer that has served as a diagnostic serum marker for squamous cell carcinomas of the cervix, head, neck, and lung (1, 2). SCCA is an atypical serpin in that it exhibits class-cross activity, inhibiting papain-like cysteine proteases (cathepsins L, K, and S) rather than serine proteases as targeted by most family members (3, 4). A closely related human gene encoding serpin B4 (SCCA-2) was subsequently isolated (5, 6) and has 92% protein sequence identity to the original antigen (SCCA-1), with the most significant divergence in the reactive center loop region. SCCA-2 shows some overlap in inhibitory profile with SCCA-1, but it can inhibit the serine proteases cathepsin G and mast cell chymase and shows significantly weaker inhibition of cysteine proteases in comparison with SCCA-1 (7). In addition, SCCA-1 can inhibit parasite-derived cysteine proteases (8), and SCCA-2 inhibits the D. p. f. 1 mite allergen cysteine protease activity (9).

Overexpression of SCCA-1 in F12 cell clones and keratinocytes has been shown to block tumor necrosis factor-α and UV light-induced apoptosis, respectively (10, 11), and SCCA-2 overexpression can protect Hela cells from tumor necrosis factor-α-induced apoptosis (12). It has been proposed by Silverman et al. (13) that along with other human B-clade members, the major function of these serpins may be to protect cells against promiscuous proteolysis. However, the implied prosurvival role in cancer has not been clearly established, and in contrast, SCCA-1 overexpression in head and neck squamous carcinoma cells significantly inhibits in vitro migration in Matrigel assays and in vivo growth and tumor invasion in nude mice (14). Similarly, the depletion of SCCA-1 using an antisense approach results in increased invasive activity of SiHa cervical carcinoma cells (15).

SCCA-1 and SCCA-2 lack a recognizable secretory signal sequence, and they appear to be predominantly cytoplasmic in the normal epithelium (16). However, the antigens are routinely found extracellularly in the plasma of patients with various malignant and nonmalignant diseases (17). In addition, SCCA-1 can be secreted upon treatment of keratinocytes and Hek293 cells with interleukin-4 and interleukin-13, and this appears to be independent of the endoplasmic reticulum/Golgi pathway (8). This may implicate the immune system in triggering SCCA-1 secretion in the allergic response and perhaps also during malignancy. Extracellular mammalian targets have not yet been characterized, but it is known that cysteine cathepsins are also up-regulated in many cancers, including malignant melanoma, where secreted cathepsins contribute to the breakdown of the extracellular matrix during metastasis (18, 19).

Serpin inhibition of proteases proceeds via a well-characterized conformational switch mechanism, resulting in a stable complex containing a distorted inactivated protease (20). For a number of serpins, this activity can be enhanced by the presence of cofactors, most notably the effects of glycosaminoglycans on plasma serpin inhibition. Other ligands can also modulate serpin activity, and Ong et al. (21) showed that SCCA-1 activity against cathepsin V can be enhanced in the presence of DNA, but that unlike the nuclear serpin MENT, SCCA-1 does
not bind DNA directly, and enhancement appears to be mediated via the protease.

In this study, we initially investigated the possibility that SCCA-1 and SCCA-2 could bind glycosaminoglycans despite the fact that they possess an overall negative isoelectric point. Using a combination of solid phase and solution phase techniques, we found that these serpins bind heparin and heparan sulfate but not other glycosaminoglycans. Kinetic analysis showed that heparin significantly enhanced inhibition of the cysteine protease cathepsin L but had no effect on SCCA-2 inhibition of the serine protease cathepsin G. We also found that proteolysis of a cathepsin L substrate by extracellular fractions from MDA-MB-231 and WM793 cancer cell lines was more potently inhibited by SCCA-1 when in the presence of heparin.

**EXPERIMENTAL PROCEDURES**

**Recombinant Protein Production**—The full-length open reading frame cDNAs for SCCA-1, SCCA-2, and ovalbumin were previously cloned into the pRSET-C expression vector (22). Escherichia coli BL21(DE3) cells transformed with plasmid were grown in 50 ml of Overnight Express autoinducing medium (Merck) containing 100 µg/ml ampicillin for 16 h at 37 °C, and this was used to inoculate 0.5–1 liters of Overnight Express medium. Following growth at 37 °C for 24 h, cells were harvested by centrifugation at 11,000 rpm for 30 min and lysed using BugBuster lysis reagent (Merck). Soluble material was clarified by centrifugation of the cell lysate at 15,000 rpm for 30 min at 4 °C, followed by 0.22 µm filtration. The recombinant serpin was purified from this extract using a HiTrap purification kit (Merck), routinely yielding >5 mg of protein from 500 ml of culture.

**Binding to Heparin by Affinity Chromatography**—Heparin HiTrap 1-ml columns (GE Healthcare) were equilibrated with buffer A (50 mM Tris and 20 mM NaCl pH 6.9). 0.5 mg of recombinant SCCA (rSCCA)-1, rSCCA-2, recombinant ovalbumin, and antimithrin III in buffer A were applied, followed by 10 column volumes of buffer A. Bound proteins were eluted using a stepwise NaCl gradient (0–1 M) in buffer A at 1.5 column volumes per fraction.

**Glycosaminoglycan Specificity**—20 µg of rSCCA-1 or rSCCA-2 was added to 50 µl of a 50% slurry of heparin-agarose beads in buffer A containing 100 mM NaCl in a final volume of 200 µl, followed by incubation at 4 °C for 1 h. The beads were washed three times with buffer A to remove unbound protein. Heparin, heparan sulfate, acetyl heparin, de-N-sulfated heparin, or chondroitin sulfate A or B (all from Sigma) in buffer A (50 or 500 µg/ml) was added to the beads and incubated for 10 min at 4 °C. Beads were pelleted by centrifugation at 1000 rpm for 5 min, and supernatants were analyzed by SDS-PAGE.

**Intrinsic Tryptophan Fluorescence**—The tryptophan fluorescence of rSCCA-1 and rSCCA-2 was monitored in both the presence and absence of heparin to investigate if heparin binding induces a conformational change. 1 µM rSCCA was incubated with 1, 2, and 5 µM heparin in cathepsin assay buffer (200 mM sodium acetate, 8 mM dihydroethrol, 4 mM EDTA, and 0.1% Brij-35, pH 5.5) in a final volume of 600 µl. Using a Hitachi F4500 fluorometer, each sample was excited at 295 nm, and the emission was scanned over a wavelength range of 320–400 nm at a rate of 60 nm/min using excitation/emission slit widths of 10 nm. The buffer fluorescence spectra (with or without heparin) were subtracted from each sample. Each sample was scanned five times, and the mean of these values is displayed.

For titration analysis, SCCA-1 and SCCA-2 (1 µM) in cathepsin assay buffer were titrated with heparin (0.5–10 µM), and fluorescence measurements (using an excitation wavelength of 295 nm and an emission wavelength of 340 nm) were recorded. The change in fluorescence (∆F) divided by the initial fluorescence value (F0) was plotted against heparin concentration, and binding constants were estimated using nonlinear regression analysis.

**Biotinylation of Lysine Residues**—Biotin was covalently linked to rSCCA-1 or rSCCA-2 using Sulfo-NHS-Biotin (sulfo-N-hydroxysuccinimide-LC-biotin; Pierce) following the manufacturer's protocol. Briefly, 0.5 mg of recombinant protein in phosphate-buffered saline was added to 250 µl of 1 mg/ml Sulfo-NHS-Biotin and incubated for 1 h at room temperature, and the reaction mixture was dialyzed to remove any free biotinylation reagent. Both biotinylated and unmodified proteins were subjected to heparin affinity chromatography as described above, and eluted fractions were analyzed by SDS-PAGE.

**Determination of the Association Rate Constant (kobs)**—Heparin Accelerates Serpin Inhibition of Cathepsin L

The association constants for inhibition of cathepsin L (EC 3.4.22.15) were determined using the discontinuous method at pH 5.5 in cathepsin assay buffer at room temperature with excitation/emission wavelengths of 370/460 nm (Hitachi F4500 fluorometer). For assays in the absence of heparin, the concentration of cathepsin L (Athena Research) was held at 10 nM, and the concentration of rSCCA-1 was at least 3-fold higher, ranging from 30 to 80 nM. In the presence of heparin, the enzyme concentration was lowered to 2.5 nM with rSCCA-1 concentrations of 7.5 nM. The unfracionated heparin concentration of 0.32 µg/ml used in these assays represents a concentration of ~30 nM (taking an average molecular mass of 11,000) or 4-fold higher than the concentration of serpin. Following the addition of serpin (in the presence or absence of heparin), the residual cathepsin L activity was calculated at various time points in a final volume of 600 µl containing 40 µM N-benzoyloxycarbonyl-Arg-7-amido-4-methylcoumarin (Z-GR-MC) in assay buffer. Substrate cleavage was measured for 2 min, and the natural logarithm of the residual activity was plotted against the time elapsed. Data were analyzed using linear regression analysis in GraphPad Prism software. The slope of this line represents the observed rate of inhibition (−kobs), and the association rate constant was determined from the slope of a plot of kobs versus inhibitor concentration. For cathepsin G assays, the chromogenic substrate succinyl-Alpha-Leu-Pro-Phe-p-nitroanilide (Calbiochem) was used, and residual activity was followed at 405 nm.

**Stoichiometry of Inhibition**—The stoichiometry for cathepsin L inhibition by SCCA-1 and SCCA-2 was determined in the absence and presence of heparin. Briefly, 25 nM cathepsin L was incubated with a range of serpin concentrations (5–25 nM) with or without 50 nM heparin. Reactions were incubated for 1 h at 37 °C in cathepsin assay buffer, and fractional residual activity was assayed using a Hitachi F4500 fluorometer.
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was plotted against the serpin/cathepsin L ratio (1/[L]/1[EL]). The stoichiometry of inhibition value was determined as the x intercept value, calculated by linear regression analysis using Prism.

Inhibition of Extracellular Cysteine Protease Activity of Cancer Cell Lines—The cancer cell lines MDA-MB-231 (breast carcinoma) and WM793 (melanoma-derived) were grown in 100-mm dishes to 80-90% confluence, and conditioned media from these cells were collected. Phenylmethylsulfonyl fluoride (1 mM) and EDTA (1 mM) were added, and the cathepsin L-like activity was determined. Briefly, 50 μl of conditioned medium was incubated with SCCA-1 (200 nM), heparin (1 mg/ml), or both in combination for 30 min, in addition to a control buffer only incubation. Subsequently, 50 μl of cathepsin assay buffer was added, and samples were assayed for cathepsin L-like activity over 10-20 min using the fluorogenic substrate Z-FF-AMC (40 μM). The sensitivity of activity to E-64 (10 μM) and resistance to CA-074 (10 μM) were determined to verify that activity was not due to cathepsin B. The control assay was taken as 100% activity. Each assay was performed in triplicate.

RESULTS

Serpins SCCA-1 and SCCA-2 Bind the Glycosaminoglycan Heparin—Although many serpins are known to bind and be modulated by glycosaminoglycans, most notably heparin, this has not previously been investigated for SCCA-1 and SCCA-2. As members of the B-clade subfamily, they are generally regarded as intracellular proteins, but along with other members such as PAI-2 and ovalbumin, an extracellular presence is evident despite the lack of a classical secretion signal sequence (23).

2 We initially examined if the recombinant proteins could bind heparin using heparin-agarose affinity chromatography. Although both proteins are acidic overall, with a predicted and determined pl of ≤6.5 (20), we found that they bound relatively tightly to heparin-agarose at pH 6.9 (Fig. 1). Elution required 0.3 M NaCl for SCCA-2 and 0.4-0.5 M NaCl for SCCA-1. In comparison, a similarly tagged and purified recombinant ovalbumin did not bind heparin-agarose, and antithrombin III was eluted at 1 M NaCl.

Binding was also investigated using mobility shift analysis on native PAGE gels. As shown in Fig. 1b, both proteins showed a mobility shift toward the positive electrode following incubation with heparin. We also examined the ability to bind DNA using agarose gel mobility shift analysis but found no binding for either serpin (data not shown), in agreement with the findings of Ong et al. (21) for SCCA-1.

Glycosaminoglycan Specificity—To determine the specificity of SCCA-1 and SCCA-2 for various glycosaminoglycans, we used recombinant serpin immobilized by pulldown on heparinagarose beads. The ability of heparin, heparan sulfate, chondroitin sulfates A and B, acetyl heparin, and de-N-sulfated heparin to elute the protein was examined (Fig. 2a). Heparin and heparan sulfate eluted the protein, but as expected, the modified heparin molecules acetyl heparin and de-N-sulfated heparin were unable to elute rSCCA-1 or rSCCA-2, as they lack the negatively charged sulfide groups that mediate the ionic interaction. However, we also noted that chondroitin sulfates A and B were similarly unable to elute rSCCA-1 or rSCCA-2 even at a 10-fold higher concentration than that required for heparin and heparan sulfate, indicating a high degree of specificity for heparin.

Further evidence for an ionic interaction was obtained from modification of lysine residues by biotinylation (Fig. 2b). This significantly reduced binding of SCCA-1 and SCCA-2 to heparin-agarose, indicating that surface lysines are important for the interaction and consistent with the lack of binding of desulfated heparin seen in Fig. 2a.

Binding of Heparin Quenches the Intrinsic Tryptophan Fluorescence of SCCA-1 and SCCA-2—Cofactor-induced conformational change is a common occurrence in serpin activity, and we examined this by monitoring the change in intrinsic fluorescence. SCCA-1 and SCCA-2 contain 4 and 5 tryptophan residues, respectively, (Trp103, Trp186, Trp201, and Trp300, with Trp119 in SCCA-2 alone). The tryptophan fluorescence spectra showed a substantial shift in the presence of 1-5 μM heparin for both proteins. A titration curve of change in fluorescence against heparin concentration yielded dissociation constants of 4.20 ± 0.46 μM for SCCA-1 and 2.03 ± 0.15 μM for SCCA-2 (Fig. 3).

Heparin Accelerates the Inhibition of Cathepsin L by SCCA-1 and SCCA-2—Heparin is known to enhance the inhibitory activity of plasma serpins toward their target proteases, with antithrombin III activity toward thrombin increased by >1000-fold in the presence of heparin. However, heparin enhancement of a serpin in the context of cysteine protease inhibition has not previously been reported. We initially investigated if heparin had functional relevance on the ability of SCCA-1 to inhibit lysosomal cathepsin L. We found that, in the presence of heparin, this inhibition was remarkably rapid and complete (Fig. 4a) to the extent that second order inhibition rate constants
Heparin Accelerates Serpin Inhibition of Cathepsin L

could not be determined and were estimated at $>10^8 \text{M}^{-1} \text{s}^{-1}$. In the absence of heparin, the $k_e$ was determined as $5.4 \times 10^9 \text{M}^{-1} \text{s}^{-1}$, which is in close agreement with a previous estimate of $3.0 \times 10^9 \text{M}^{-1} \text{s}^{-1}$ (4). Thus, in the presence of heparin, the rate of inhibition appears to be increased by at least 180-fold.

SCCA-2 shows greater specificity toward serine proteases cathepsin G and mast cell chymase, and we examined effects on cathepsin G activity using the chromogenic substrate succinyl-Ala-Ala-Pro-Phe-p-nitroanilide. Interestingly, no increase in inhibitory activity was observed in the presence of heparin in this case (Fig. 4B). SCCA-2 could inhibit cathepsin L but less efficiently than SCCA-1. We found that heparin could also enhance this inhibition but with only a 4.1-fold increase in rate of inhibition (Fig. 4E). For both serpins, the stoichiometry of inhibition was closer to 1:1 in the presence of heparin, indicating that less of the protein is partitioned to the substrate pathway (Table 1).

Heparin Enrichment Is a Template Effect—The mechanism for heparin enhancement could depend on binding to serpin alone or on a templating effect whereby both protease and inhibitor are bound, effectively increasing the reactant concentration and rate of inhibition. This template mechanism is found for most plasma serpin enhancement by heparin, but for SCCA-1 rate enhancement in the presence of DNA, a non-templating saturation effect was seen as a result of protease binding only (21). Using a range of heparin concentrations (Fig. 5), we found that a saturating effect did not occur and that the curve obtained was consistent with the formation of a ternary complex, i.e. at high heparin concentrations, the protease and serpin are more likely to bind different heparin molecules, thus decreasing the template effect and rate of inhibition. Furthermore, we found that cathepsin L was able to bind heparin-agarose (eluting at $\sim 0.4 \text{M NaCl}$ under the same conditions used for serpin analysis) but

![Figure 2. Glycosaminoglycan specificity of SCCA-1 and SCCA-2 and importance of lysine residues for heparin binding.](image)

![Figure 3. Effect of heparin on rSCCA-1 Intrinsin fluorescence.](image)
that the intrinsic fluorescence of cathepsin L was not altered, suggesting that conformational change in the protease is not induced following heparin binding (data not shown).

**Heparin and SCCA-1 Combine to Inhibit Cancer Cell Line-derived Proteolytic Activity**—Overexpression of cathepsin L has been identified in many human malignancies, including melanomas and colorectal cancers (19, 24, 25), and there is also previous evidence that SCCA-1 and heparin can independently inhibit metastasis (14). We investigated if SCCA-1 can inhibit secreted cathepsin L-like activity from two invasive human cancer cell lines and if heparin can enhance this inhibition. We found that conditioned media from the breast cancer cell line...
MDA-MB-231 and the melanoma-derived cell line WM793 possessed substantial ability to cleave the cathepsin L substrate over a 20-min incubation. This cleavage could be largely abolished by the addition of the broad-specificity cysteine protease inhibitor E-64. However, the addition of the cathepsin B inhibitor CA-079 reduced activity by just 15%, and this may account for most of the remaining activity following heparin and SCCA-1 treatment. (Fig. 6). The addition of SCCA-1 alone could partially inhibit the activity, but in the presence of heparin, activity was further reduced to ~22% of the original.

### Table 1
Kinetic parameters of cathepsin L inhibition by SCCA-1 and SCCA-2

<table>
<thead>
<tr>
<th></th>
<th>Increase</th>
<th>S.I.</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCCA-1</td>
<td>(5.4 ± 0.3) × 10^5</td>
<td>1</td>
<td>1.38 ± 0.06</td>
</tr>
<tr>
<td>SCCA-1 + heparin</td>
<td>&gt;5 × 10^4</td>
<td>1</td>
<td>1.55 ± 0.10</td>
</tr>
<tr>
<td>SCCA-2</td>
<td>(1.0 ± 0.06) × 10^5</td>
<td>1</td>
<td>1.40 ± 0.07</td>
</tr>
<tr>
<td>SCCA-2 + heparin</td>
<td>(4.1 ± 0.28) × 10^4</td>
<td>1</td>
<td>1.17 ± 0.06</td>
</tr>
</tbody>
</table>

a S.I., stoichiometry of inhibition.

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**Heparin Accelerates Serpin Inhibition of Cathepsin L**

Cardin and Weintraub (29) have identified the motifs B0B3BxxBx and B0Bx0Bx as heparin-binding sequences, where B = basic and x = non-basic residues. We examined the SCCAs for such motifs and noted an B0Bx0Bx motif at residues 19–24 (FRKSK) in helix A. We carried out site-directed mutagenesis of the motif. Basic residues (25FRKSK to AASA) in SCCA-1 using the QuikChange mutagenesis method (Stratagene). However, the resulting mutant recombinant protein displayed similar affinity for heparin binding and a similar degree of heparin enhancement toward cathepsin L inhibition compared with the wild-type protein (data not shown).

The x-ray crystal structure of SCCA-1 has recently been determined (Protein Data Bank code 2ZVD, 30). One exposed residue is the helix D Lys87, which is equivalent to Lys87 in antithrombin III, previously shown by Schedin-Wies et al. (31) to be important for heparin binding and activation.

### DISCUSSION

The anticancer properties of heparin have been known for many years, but this has not translated to the clinic, and the underlying mechanism is still a subject of some debate (32). Experiments with modified heparins lacking anticoagulant activity suggest that other factors are important, and among those proposed are prevention of platelet interactions with cancer cells by P-selectin binding (33) and competition with cell-surface heparan sulfate proteoglycans for binding proangiogenic growth factors such as fibroblast growth factor-β (34). A comprehensive review of the preclinical data has led Niers et al. (35) to conclude that inhibition of metastasis rather than primary tumor growth is the predominant means by which heparin exerts its anticancer effects. Evidence for prometastatic activity of heparanase also supports this hypothesis (36).

In acting as an anticoagulant, heparin binds and induces a conformational change in the serpin antithrombin III, greatly accelerating its ability to inhibit thrombin and other coagulation factors via a ternary complex, for which the structure has now been solved (37). Heparin also enhances heparin cofactor II...
Heparin Accelerates Serpin Inhibition of Cathepsin L.

and protease nexin I inhibition of thrombin and protein C inhibitor inhibition of activated protein C (28, 39). A number of serpins can also inhibit angiogenesis, and in the case of latent anti-thrombin and kallistatin, heparin is found to be important for antiangiogenic activity (40, 41), which could represent an indirect mechanism for inhibiting metastasis. However, a more rapid and direct role for heparin in the inhibition of metastatic proteases has not previously been suggested, and our data now underpin this novel mechanism in relation to cathepsin L inhibition. Cathepsin L is a widely expressed cysteine protease with a major role in lysosomal proteolysis, protein processing, matrix degradation, and tissue remodeling, and it has been linked to the invasive phenotype in many cancers (24, 25). Inhibition of cathepsin L activity by synthetic inhibitors and by the cathepsin S propeptide can reduce invasiveness of a number of human cancer cell lines (42), and prevention of cathepsin L secretion by introducing an overexpressed intracellular anti-cathepsin single chain variable antibody fragment dramatically reduced melanoma cell metastasis (43). We propose that SCCA-1 and heparin may combine to facilitate an endogenous mechanism for regulation of extracellular cathepsin activity and cathepsin-mediated metastasis. SCCA-1 is expressed in many epithelial tissues prone to carcinoma development, including skin, cervix, lung, and esophagus. It appears to be predominantly intracellular in normal epithelial cells but is secreted in certain malignancies, in benign disorders such as psoriasis, and following stimulation with specific cytokines. Interestingly, intracellular cathepsin L appears to lose a role in processing of propeparane to the active heparanase (44), facilitating a possible synergistic regulatory role for intracellular SCCA-1.

Elucidation of physiologically relevant target proteases for individual serpins can be difficult, and in general, a k, of >10^3 is considered potentially relevant in vivo. The increase to >10^4 in the presence of heparin generates an extremely potent and rapid mechanism for cathepsin L inhibition by SCCA-1. To our knowledge, this degree of hebparin enhancement (>180-fold) for cathepsin L activity is the second most significant after thrombin with regard to the protease targeted. -Fold increases with heparin range from 45 to >2000 for thrombin inhibition by various serpins, but for other proteases, reports range from 4-fold for inhibition of factor XIa to 52-fold for inhibition of activated protein C (28). SCCA-2 is a substantially poorer inhibitor of cathepsin L both alone and in the presence of heparin; we found just a 4-fold heparin-induced increase. Another B-clade serpin, headpin or urpin (serpin B13), can also inhibit cathepsin L (45), and it remains to be seen if heparin enhances this activity. The inhibition of parasitic and allergen proteases may be a primary function of SCCA-1 and SCCA-2 (8, 9), and interestingly, heparin has also been found to have regulatory potential in allergic inflammation (46).

Heparin treatment has not shown antime metastatic effects in all preclinical and clinical studies, but successful outcomes do include malignancies where cathepsin L is overexpressed (e.g., B16 melanomas) and tissues in which SCCA-1 has been detected extracellularly (31). A re-examination of heparin preclinical data in terms of serpin and cathepsin expression, secretion, and activity may prove valuable in predicting which cancers will respond positively to heparin treatment.

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