 Syndecans, Heparan Sulfate Proteoglycans, Maintain the Proteolytic Balance of Acute Wound Fluids*  

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An imbalance between proteases and antiproteases is thought to play a role in the inflammatory injury that regulates wound healing. The activities of some proteases and antiproteases found in inflammatory fluids can be modified in vitro by heparin, a mast cell-derived glycosaminoglycan. Because syndecans, a family of cell surface heparan sulfate proteoglycans, are the major cellular source of heparin-like glycosaminoglycan, we asked whether syndecans modify protease activities in vivo.

Syndecan-1 and syndecan-4 ectodomains are shed into acute human dermal wound fluids (Subramanian, S. V., Fitzgerald, M. L., and Bernfield, M. (1997) J. Biol. Chem. 272, 14713–14720). Moreover, purified syndecan-1 ectodomain binds cathepsin G (Kd = 56 nM) and elastase (Kd = 35 nM) tightly and reduces the affinity of these proteases for their physiological inhibitors. Purified syndecan-1 ectodomain protects cathepsin G from inhibition by α1-proteinase inhibitor and squamous cell carcinoma antigen 2 and elastase from inhibition by α1-proteinase inhibitor by decreasing second order rate constants for protease-antiprotease associations (kass) by 3700-, 32-, and 60-fold, respectively. Both enzymic degradation of heparan sulfate and immunodepletion of the syndecan-1 and -4 in wound fluid reduce these proteolytic activities in the fluid, indicating that the proteases in the wound environment are regulated by interactions with syndecan ectodomains. Thus, syndecans are shed into acute wound fluids, where they can modify the proteolytic balance of the fluid. This suggests a novel physiological role for these soluble heparan sulfate proteoglycans.

Multiple factors orchestrate the inflammatory response to tissue injury. These include proteases, antiproteases, cytokines, chemokines, and the growth factors derived from the plasma and cells associated with the injury, as well as from cells invading the injury site (1). Emigrating polymorphonuclear leukocytes release proteolytic enzymes into the injury site, including the most potent serine proteases, neutrophil elastase and cathepsin G (CatG).1 These enzymes aid wound repair by digesting extracellular proteins, releasing growth factors from extracellular matrix, and remodeling the tissue (2–4). However, these enzymes can also destroy tissues when proteolysis is prolonged, inappropriate, or excessive (5, 6).

Enormous local concentrations of proteases, estimated to be in the millimolar range for elastase and cathepsin G, are released into extracellular spaces during the leukocyte activation associated with tissue injury (7). Serine protease inhibitors (serpins), provide efficient control mechanisms to prevent undesirable extracellular protein degradation at the injury site (8). These antiproteases, mostly derived from plasma, share three principal properties: (i) they form 1:1 covalent complex with proteases, (ii) complex formation results in both inactivation of the protease and proteolytic cleavage of the serpin, and (iii) inhibition is essentially irreversible (9). The balance of proteases and antiproteases at the site of injury can regulate the extent of the inflammatory response during the repair process (10, 11).

Dermal wound repair requires harmonious protease-antiprotease interactions or proteolytic balance. Excessive elastase action in the wound bed can account for endothelial damage (12), degradation of the epidermal/dermal junction (13), and the development of chronic skin ulcers (14). Physiological neutrophil elastase inhibitors include plasma-derived α2-macroglobulin and, most importantly, α1-proteinase inhibitor (also known as α1-PI, α1-antitrypsin, or α1-AT). The importance of α1-PI in regulating the response to tissue injury is emphasized by the extensive elastin and collagen fiber destruction leading to pulmonary emphysema in the lungs of individuals with congenital α1-PI deficiency (5). The major physiological cathepsin G inhibitor is α1-antichymotrypsin (α1-Ach), another plasma-derived serpin (15). Inherited α1-Ach deficiency is pleiomorphic, but it is often associated with chronic active hepatitis and increased residual lung volumes (16). Another serpin that inhibits cathepsin G is the squamous cell carcinoma antigen 2 (SCCA2), a newly described product of skin and respiratory tract epithelia (17).

Although the activity of one class of serpins is accelerated by binding to heparin or other glycosaminoglycans (GAGs) (9, 18), α1-PI and α1-Ach belong to the class of serpins that function independently of heparin and other GAGs. However, heparin can bind with high affinity to both neutrophil elastase and cathepsin G (19, 20). This binding inhibits the enzymatic activities, but most importantly, it reduces the ability of the enzymes to interact with serpins (19, 20). The heparin used clinically and in these studies is a pharmaceutical product derived from processing of the heparin proteoglycan within mast cells (21). The major physiological source of the heparin-
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like GAG, heparan sulfate, is found in proteoglycans within cells, at the cell surface and in the extracellular matrix (22).

Most cellular heparan sulfate derives from the syndecan family of cell surface proteoglycans. This family (currently known as syndecan 1–4 in mammals) consists of single transmembrane proteins containing conserved cytoplasmic and transmembrane domains and less well conserved extracellular domains (ectodomains), which bear variable numbers of GAG chains. All syndecans bear heparan sulfate, although syndecan-1 and -3 can also bear chondroitin sulfate. Syndecans bind many of the factors that orchestrate the inflammatory response to tissue injury as well as a variety of extracellular matrix components and adhesion molecules via their heparan sulfate chains and are individually expressed in distinct cell-, tissue-, and development-specific patterns (23).

Syndecan expression is highly regulated during wound repair. During cutaneous wound repair, keratinocytes migrating from the wound edge show loss of cell surface syndecan-1 (24). Concomitantly, syndecan-1 expression increases on the endothelial cells, and syndecan-4 expression increases on the dermal fibroblasts that form the granulation tissue (24,25), apparently due to inductive action of neutrophil-derived antimicrobial peptides (26). Syndecans on cell surfaces can be cleaved near the plasma membrane, which releases the now soluble intact proteoglycan ectodomains into the surrounding milieu (27). This shedding is accelerated by activation of protease (e.g. thrombin) and growth factor receptors (epidermal growth factor family members) and by the direct action of proteases (e.g. plasmin) involved in wound repair (27). Moreover, soluble syndecan-1 and -4 ectodomains are detected in acute dermal wound fluids (27). Although syndecan expression and shedding are highly regulated during the response to tissue injury, the role these processes play in this response is not clear.

A key aspect of the response to tissue injury is the establishment and maintenance of proteolytic balance at the wound site. The action of the major proteases, neutrophil elastase and cathepsin G, must be countered by their major inhibitors, α1-PI and α1-Achy, for normal wound repair to ensue. Loss of this balance can prevent normal repair, potentially leading to chronic wounds, which in the skin are difficult to treat satisfactorily (14). We postulated that because activities of the major proteases in acute wound fluids can be modified in vitro by heparin, soluble syndecan ectodomains could be involved in establishing and maintaining the proteolytic balance in wounds in vivo. We found syndecan-1 and -4 ectodomains in acute human dermal wound fluids. We also found that purified syndecan-1 ectodomain binds to both neutrophil elastase and cathepsin G, markedly reducing their affinity for serpins and thus protecting these enzymes from their physiological inhibitors. Moreover, both degradation of endogenous heparan sulfate and removal of syndecan-1 and -4 from wound fluids reduce proteolytic activities in the fluid. Thus, syndecan ectodomains maintain the proteolytic balance in acute wound fluids, a novel physiological role for soluble heparan sulfate proteoglycans.

EXPERIMENTAL PROCEDURES

Materials—Flat bottomed, low binding 96-well microtiter plates were obtained from Costar (Cambridge, MA). Human trypsin and mouse IgG were from Sigma, human neutrophil cathepsin G and human plasma α1-PI were from Athens Research & Technology Inc. (Athens, GA). Human neutrophil elastase was from Calbiochem (La Jolla, CA), and human plasma α1-Achy was from Biodiagnostics International (Kennebunk, ME). Purified glutathione S-transferase-SCCA2 fusion protein was a kind gift from Dr. Gary Silverman, Children’s Hospital, Boston, MA (17). For enzyme substrates, succinyl-Ala-Ala-Pro-Phe-pNA (Suc-AAPF-pNA) for cathepsin G and N benzoyloxy carbonyl (CBZ)-Ala-Ala-Ala-Ala-Ac-110 for elastase was from Molecular Probes Inc. (Eugene, OR). Heparin (porcine intestinal mucosa) was from Hepar Industries Inc. (Franklin, OH), and chondroitin-6-sulfate, chondroitin sulfate ABC lyase (chondroitinase ABC, EC 4.2.2.4), heparan sulfate lyase III (heparitinase, EC 4.2.2.8), and chondroitin-sulfate lyase I (chondroitinase EC 4.2.2.7) were from Seikagaku America Inc. (Rockville, MD). The syndecan-1 ectodomain was purified to homogeneity from the conditioned medium of NMuMG mouse mammary epithelial cells (28). One mg of this syndecan-1 core protein contains 5 mg of HS,2 cDNA for ectodomain of human syndecan-1 and -4 in glutathione S-transferase-expressing pGEX-2T (American International Biotechnologies Inc.) was expressed as fusion proteins in Escherichia coli, induced by 0.2 mM isopropyl-β-D-thiogalactopyranoside for 6 h at 37°C, solubilized with 1% Triton X-100, and centrifuged 12,000 × g for 10 min. Supernatants were purified on glutathione-agarose beads (Sigma); washed with PBS; eluted with 50 mM Tris, pH 8, and 5 mM reduced glutathione (Janssen Chimica, New Brunswick, NJ); subjected to 10% SDS-polyacrylamide gel electrophoresis; and detected with Coomassie Blue. Antibodies used were polyclonal antisera H1-1 against the recombinant human syndecan-1 ectodomain (25); monoclonal antibodies MCA-681 from Serotec (Oxford, United Kingdom) and p1-101 against human syndecan-1, 5G9 and 8C7 (25) against human syndecan-4, and horseradish peroxidase (HRP)-conjugated anti-rabbit IgG and horseradish peroxidase-conjugated anti-mouse IgG were from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA).

For production of monoclonal antibodies specific to human syndecan-1, recombinant syndecan-1 was used for immunization of mice, and production of monoclonal antibody was by Maine Biotechnology Services, Inc. (Portland, ME). Mice were immunized and boosted with 100 μg of recombinant syndecan-1. Out of 12 hybridoma clones, which produced antibodies reacting with human syndecan-1 fusion protein, only 1 (p1-101) reacted specifically with native human syndecan-1 ectodomain purified from conditioned medium of human A431 cells. This was assessed as reactivity on Western blotting with a 300-kDa proteoglycan smear, which reduced to a 70-kDa core protein after treatment with heparitinase and chondroitinase ABC as described previously (30). Affinity Capture-electrophoresis (ACE) Analysis—NMuMG cells were labeled with radiosulfate, and [35S]Sulfate-labeled syndecan-1 ectodomain from conditioned medium was purified by DEAE and immunoaffinity chromatography (28). Binding of this ectodomain to elastase and cathepsin G was assessed by ACE as described elsewhere (31, 32). Briefly, 1% (w/v) low melt agarose gels were cast containing distinct lanes with various concentrations of protease (indicated in Fig. 1). [35S]Sulfate-labeled syndecan-1 ectodomain (12,500 cpm) was electrophoresed through these lanes. In competition assays, the same amount of syndecan-1 was mixed with 1 mg/ml chondroitin-6-sulfate or heparin prior to electrophoresis. The migration of syndecan-1 on a Phospholmager (Molecular Dynamics, Sunnyvale, CA). The pixel intensities were integrated and used to determine the migration distance of the major peak of 35S-labeled syndecan-1 in each protease-containing lane. These mobilities were plotted as a function of ligand concentration and used to estimate the apparent Kd values as described earlier (32). Assays for Enzyme Inhibition—The amounts of proteases and serpins were calibrated by the method of Chase and Shaw (33). Trypsin was calibrated by using p-nitrophenyl-p’-guanidinobenzoate (Sigma), except that 100 mM Tris-HCl, pH 8.3, was used in place of sodium barbiturate buffer. The concentration of α1-PI was standardized against calibrated trypsin. Elastase and cathepsin G were calibrated against the standardized α1-PI. α1-Achy was calibrated against the standardized cathepsin G. Reaction buffers were 50 mM Hepes, 150 mM NaCl, 5% N,N-dimethylformamide, pH 7.4, for cathepsin G, and 50 mM Tris, 150 mM NaCl, 0.1 mM/ml bovine serum albumin, pH 7.4, for elastase. Enzyme inhibition was determined by mixing enzyme with increasing concentrations of syndecan-1 in the appropriate reaction buffer and incubating for 15 min at 25°C. The inhibitor was added, and residual enzyme activity was determined by adding the appropriate substrate and measuring hydrolysis at 405 nm with a UVMax microplate reader ( Molecular Devices) or at 488 nm with a FluorImager 575 ( Molecular Dynamics). For storage of cathepsin G, 34 mM α1-Achy or SCCA2, and 3 mM Suc-AAPF-pNA. The concentrations for elastase assays were 34 nM elastase, 34 nM α1-PI, and 5 μM (CBZ-Ala-Ala-Ala-Ala)-R110. In some experiments, syndecan-1...
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FIG. 1. Syndecan-1 ectodomain binds cathepsin G and elastase via its heparan sulfate chains. ACE of the soluble syndecan-1 ectodomain with cathepsin G (A and B) and elastase (C and D) is shown. 

RESULTS

Syndecan-1 Ectodomain Binds Elastase and Cathepsin G—Because heparin can protect elastase and cathepsin G against inhibition by certain plasma-derived serpins (19, 20), we speculated that the soluble syndecan ectodomains in wound fluid might act similarly. 

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up to 7.5 μg as (HS) was pretreated with heparinase (150,000 units/ml), heparitinase (150,000 units/ml each) or chondroitinase ABC (1.0 units/ml) in 10 μl of 50 mM Tris, 10 mM NaCl, pH 7.4, for 6 h at 37 °C and boiled for 10 min.

Protease-Serpin Binding Stoichiometry—Constant concentrations of syndecan-1 ectodomain or heparin were preincubuated with protease for 15 min at 25 °C with increasing concentrations of serpin in the appropriate reaction buffer, and the residual enzyme activities were measured with an appropriate substrate. The concentrations for cathepsin G assays were 34 nm cathepsin G and 3 mM Suc-AAPF-pNA in cathepsin G reaction buffer. The concentrations for elastase assays were 34 nm elastase and 5 μM (CBZ-Ala-Ala-Ala-Ala)-R110 in elastase reaction buffer.

Determination of Rate Constant k_{app} for Enzyme-Inhibitor Association—The association rate constant for the interaction of serpins with free and syndecan-1-bound enzymes was determined under second order rate conditions (15). Equimolar amounts (34 nm) of enzyme with or without the syndecan-1 ectodomain and inhibitor were incubated at 25 °C for varying periods of time. The reaction was quenched by the addition of substrate, for which the enzyme has higher affinity, and the release of pNA or rhodamine was measured. The residual enzyme activities were used to calculate the concentration of free enzyme E. Protease standard curves used for calculation of free enzyme were done in the presence and absence of syndecan-1 ectodomain. The rate of change in the amount of free enzyme over time is described as

\[
\frac{1}{E} = k_{\text{app}} \times t + \frac{1}{E_0}
\]

where the slope of the plot of reciprocal remaining free enzyme (1/E) over time (t) yields a second order rate constant (k_{app}).

Collection of Acute Wound Fluids—Acute human dermal wound fluids were collected from reduction mammoplasty patients (samples were kindly provided by Dr. E. Eriksson, Brigham and Women’s Hospital, Boston, MA). Wound fluids were collected at 1-day intervals from sterile closed-suction drains routinely placed in the subcutaneous space after mammoplasty. After collection, fluids were centrifuged for 15 min at 200 × g and 4 °C to remove cells and further for 15 min at 3300 × g and 4 °C to remove debris. The supernatants were stored at −70 °C until use. Percutaneous wound fluid was produced, collected, and processed as described elsewhere (26, 34).

Informed consent was obtained for all procedures, and the use of anonymous discarded material was approved by the Human Research Committee (protocol 95-7509-01, Brigham and Women’s Hospital). Immuno depletions—Acute wound fluids collected from several patients were sequentially incubated (at 4 °C for 30 min each) with a mixture of 20 μg/ml of each monoclonal antibody to syndecan-1 (DL101 and MCA-681) and syndecan-4 (5G9 and 8C7) or with 80 μg/ml of mouse IgG, 80 μg/ml rabbit anti-mouse IgG (Dako Corp., Carpinteria, CA), and protein A-Sepharose beads (Amersham Pharmacia Biotech). Beads were centrifuged, and supernatants were assayed for elastolytic activity in elastase reaction buffer by adding 5 μM (CBZ-Ala-Ala-Ala-Ala)-R110 elastase substrate and measuring hydrolysis with time at 488 nm with a FluorImager 575 (Molecular Dynamics).

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[S]Sulfate-labeled syndecan-1 ectodomain was electrophoresed through agarose gels containing various concentrations of cathepsin G (A) or elastase (C) as described under “Experimental Procedures.” The effects of chondroitin 6-sulfate (1 mg/ml) and heparin (1 mg/ml) on the [35S]sulfate-labeled syndecan-1 ectodomain binding to cathepsin G (B) and elastase (D) are shown. Equal amounts of [35S]sulfate-labeled syndecan-1 ectodomain were introduced into each slot, the electrophoresis was run, and distribution of radioactivity on the gel was visualized by autoradiography. The concentrations of proteases are indicated below each lane.

RESULTS

Syndecan-1 Ectodomain Binds Elastase and Cathepsin G—Because heparin can protect elastase and cathepsin G against inhibition by certain plasma-derived serpins (19, 20), we speculated that the soluble syndecan ectodomains in wound fluid might act similarly. [35S]Sulfate-labeled syndecan-1 ectodomain purified from the conditioned media of NMuMG cells was incubated with nitrocellulose filters containing dots of purified human neutrophil cathepsin G and elastase, and serum-derived α1-PI and α1-Achy. The syndecan-1 ectodomain bound to cathepsin G and elastase at picomolar levels of protease, whereas no binding to the antiproteases was detected at 10-fold higher concentrations (data not shown). ACE (32) of [35S]sulfate-labeled syndecan-1 ectodomain with cathepsin G and elastase confirmed this binding and yielded apparent K_d values of 56 nM for cathepsin G and 35 nM for elastase (Fig. 1, A and C). Heparin (1 mg/ml) completely abolished binding to the proteases, whereas chondroitin sulfate (1 mg/ml) had little or no effect, indicating that the binding is mainly due to the heparan sulfate chains on syndecan-1 (Fig. 1, B and D). The ACE profiles with both enzymes showed heterogeneity in syndecan-1 ectodomain binding at concentrations near the K_d values (80 nM), suggesting that there are subfractions of the ectodomain that differ in their avidity for the proteases (data not shown).

Binding of Syndecan-1 Ectodomain to the Protease Reduces the Effect of Antiprotease—To determine whether the binding of the syndecan-1 ectodomain to the proteases affects their rate of interaction with a serpin, rate constants (k_{app}) for these interactions were measured in the presence and absence of soluble syndecan-1 ectodomain (Table I). The protease and serpin form a 1:1 complex. Because neither heparin or the syndecan-1 ectodomain alters this stoichiometry (Fig. 2), the k_{app} were determined under second order conditions (15). Equimolar amounts (34 nm) of protease and serpin were incubated in the presence or absence of the syndecan-1 ectodomain at concentrations indicated in Table I. After various times,
complex formation was quenched by adding substrate, and the remaining free enzyme activity was measured as described under “Experimental Procedures.” The $k_{\text{obs}}$ for the interaction was calculated from linear regressions (Equation 1). The $k_{\text{obs}}$ for cathepsin G with $\alpha_1$-antichymotrypsin decreased over 3700-fold and with SCCA2 over 32-fold in the presence of the syndecan-1 ectodomain (Table I). The $k_{\text{obs}}$ for elastase with $\alpha_1$-proteinase inhibitor was decreased 60-fold by the syndecan-1 ectodomain (Table I). For comparison, second order rate constants were also measured for protease-serpin complex formation involving concentrations of serpins (Fig. 3). Syndecan-1 ectodomain alone reduced cathepsin G activity in a concentration-dependent manner, reaching maximal inhibition (35%) at 2 $\mu$M and with SCCA2 over 32-fold in the presence of the syndecan-1 ectodomain (Table I). The $k_{\text{obs}}$ for elastase with $\alpha_1$-proteinase inhibitor was decreased 60-fold by the syndecan-1 ectodomain (Table I). For comparison, second order rate constants were also measured for protease-serpin complex formation.

Soluble Syndecan-1 Ectodomain Modifies Protease Activities via Interactions of its Heparan Sulfate Chains—The effect of the purified syndecan-1 ectodomain on protease activity was assessed in the presence and absence of serpin. The ectodomain was preincubated with cathepsin G or elastase for 15 min and assayed for protease activity with or without equimolar concentrations of serpins (Fig. 3). Syndecan-1 ectodomain alone reduced cathepsin G activity in a concentration-dependent manner, reaching maximal inhibition (35%) at 2 $\mu$M as core protein (Fig. 3A). However, the syndecan-1 ectodomain markedly decreased the ability of both $\alpha_1$-Achy and SCCA2 to inhibit cathepsin G activity (Fig. 3A). In the absence of ectodomain, these serpins completely inhibit the protease, but with increasing concentrations of ectodomain, their inhibitory activity is reduced and ultimately abolished (Fig. 3A). The syndecan-1 ectodomain was more effective in reducing cathepsin G inhibi-

TABLE I

| Protease/serpin                  | $k_{\text{obs}}$ No addition | $k_{\text{obs}}$ Syndecan-1 ectodomain | Decrease |
|---------------------------------|-------------------------------|---------------------------------------|----------|
|                                 | $\mu^{-1} s^{-1} \times 10^4$| $\mu^{-1} s^{-1} \times 10^4$          | $\mu g/ml$ | $\mu^{-1} s^{-1} \times 10^4$ | $\mu g/ml$ | $-\text{fold}$ |
| Cathepsin G/$\alpha_1$-Achy     | 820                           | 0.22                                  | 16.5     | 9                         | 16.5   | 3727          | 91         |
| Cathepsin G/SCCA2               | 35                            | 1.1                                   | 10.0     | 1.9                       | 10.0   | 32            | 18         |
| Elastase/$\alpha_1$-PI          | 720                           | 12                                    | 7.5      | 8                         | 7.5    | 60            | 90         |

FIG. 2. Syndecan-1 ectodomain does not alter the stoichiometry of protease-serpin complex formation. Increasing concentrations of serpin were mixed with a constant concentration of protease without addition (□) and in the presence of syndecan-1 ectodomain (●) or heparin (●). Final concentrations: A, $[\text{CatG}] = 34$ nm, $[\text{Suc-AAPF-pNA}] = 3$ nm, 16.5 $\mu$M syndecan-1 ectodomain HS, 16.5 $\mu$M heparin; B, $[\text{CatG}] = 34$ nm, $[\text{Suc-AAPF-pNA}] = 3$ nm, 10 $\mu$M syndecan-1 ectodomain HS, 10 $\mu$M heparin; C, $[\text{neutrophil elastase}] (\text{NE}) = 34$ nm, $[\text{CBZ-Ala-Ala-Ala-Ala}_{2}-\text{R110}] = 5$ nm, 7.5 $\mu$M syndecan-1 ectodomain HS, 7.5 $\mu$M heparin. Relative activity is the rate relative to the rate observed in the absence of serpin. The lines are drawn by the least squares method from data obtained without GAG. Addition of GAG did not statistically affect the 1:1 stoichiometry.

FIG. 3. Syndecan-1 ectodomain reduces cathepsin G and elastase activities and their inhibition by serpins. A, increasing concentrations of syndecan-1 ectodomain were incubated for 15 min with cathepsin G (●), cathepsin G and $\alpha_1$-Achy (□), or cathepsin G and SCCA2 (●), and the enzymatic activity was measured with the synthetic substrate Suc-AAPF-pNA. B, increasing concentrations of syndecan-1 ectodomain were incubated for 15 min with elastase (●) or elastase and $\alpha_1$-PI (○), and the enzymatic activity was measured with synthetic substrate CBZ-Ala-Ala-Ala-Ala$_2$-R110. Final concentrations: A, $[\text{CatG}] = 34$ nm, $[\alpha_1$-Achy$] = 34$ nm, $[\text{SCCA2}] = 34$ nm, $[\text{Suc-AAPF-pNA}] = 3$ nm; B, $[\text{neutrophil elastase}] = 34$ nm, $[\alpha_1$-PI$] = 34$ nm, $[\text{CBZ-Ala-Ala-Ala-Ala}_{2}-\text{R110}] = 5$ $\mu$b. Relative activity is the rate relative to the rate observed in the absence of the ectodomain.
Soluble Syndecan-1 and -4 Ectodomains Modify Proteolytic Balance of Wound Fluids—Wound fluids accumulating during dermal wound repair contain syndecan-1 and -4. Aliquots of cell-free human acute dermal wound fluids, collected days 1–3 after mammaplasty, were applied to cationic membranes. Antiserum to human syndecan-1 (HSE-1) and monoclonal antibody to human syndecan-4 (5G9) detected syndecan-1 and -4, respectively (Fig. 5). However, antibodies against the cytoplasmic domains of syndecan-1 and -4 failed to detect these proteoglycans (data not shown) (27), and neither proteoglycan was detected in human plasma (data not shown) (27).

We then asked whether these syndecan ectodomains could modify the balance between the endogenous proteases and their inhibitors in the wound fluid. To demonstrate this balance, we added heparin to wound fluids before and after degrading the endogenous heparan sulfate in the fluid. Direct addition of as little as 2 μg/ml heparin increased elastolytic activity, suggesting that heparin shifts the balance in favor of the protease (Fig. 6A). Degrading the endogenous heparan sulfate with the Hase/HSase mixture, as in Fig. 4, markedly reduced elastolytic activity (Fig. 6B). This enzymatic degradation similarly affected chymotryptic activity in pig wound fluids (data not shown). Adding heparin to the enzyme-treated samples shifted the balance in favor of the protease and nearly reversed the loss of activity (Fig. 6B). Thus, the activity of proteases and their inhibitors is balanced in wound fluid, and this balance can be shifted by the endogenous heparan sulfate in the fluid. To evaluate whether the syndecan ectodomains comprised the active heparan sulfate in the fluid, wound fluids were treated with monoclonal antibodies against the syndecan-1 and -4 ectodomains or, as a control, with equal concentrations of mouse IgG, and the elastolytic activity was measured. Removal of syndecan-1 and -4 ectodomains from each of 5 wound fluids reduced elastolytic activity 33–60% (Fig. 7). Adding heparin to immunodepleted samples shifted the balance in favor of the proteases and nearly reversed the loss of activity, identical to that observed in Fig. 6B (data not shown). These data indicate that soluble syndecan ectodomains maintain the proteolytic balance of wound fluids but do not complex with all the protease in the fluid.

DISCUSSION

In this study, we provide new insights into the regulation of protease-antiprotease balance during tissue injury. We show that syndecan-1 and -4, cell surface heparan sulfate proteogly-
Soluble Syndecan Ectodomains as Heparin-like Mediators at the Wound Site—

Wound repair requires precise temporal and spatial regulation of a panoply of effectors, including chemo-
kines, growth factors, extracellular components, cell adhesion proteins, proteases, and antiproteases. Many of these proteins bind heparin and heparan sulfate under physiological condi-
tions and with high affinities (35). During repair of skin injury, cellular expression of syndecan-1 and -4 is altered (24, 25), and cell surface syndecan-1 and -4 are converted to soluble mole-
cules by juxtamembrane cleavage of their extracellular do-
main (ectodomains), a process known as shedding (27). Recent
studies have shown that syndecan shedding is a highly regu-
lated process that is stimulated by certain agents released at the
site of tissue injury (27). Shedding instantly converts a cell
surface proteoglycan into a soluble effector.

The functions of these soluble ectodomains are not clear. 
Syndecans on cell surfaces can act as co-receptors for heparin-
binding growth factors; notably, the action of FGF-2 requires a
growth factor-heparan sulfate proteoglycan-FGFR1 complex
(36). However, because the soluble ectodomains retain all their
HS, they can bind the same ligands as the cell surface synde-
cans, enabling them to be potential inhibitors of these ligand
interactions. On the other hand, the soluble ectodomains place
HS chains containing heparin-like domains into the wound
environment. These chains can interact with heparin-binding
proteins and peptides involved in the repair.

The inflammatory phase of tissue repair is characterized by
plasma exudation and the involvement of neutrophils that
produce and secrete the matrix remodeling enzymes elastase
cathepsin G. Although heparin binds and accelerates ac-
tivity of some serpins (9), heparin does not interact with the
serpins that regulate these enzymes. Rather, the enzymes
themselves bind heparin, which reduces their affinity for the
serpin and protects them from inhibition (19, 20).

Our results indicate that the HS chains on the soluble syn-
decan ectodomains mimic this action of heparin and thus regu-
late the activity of the neutrophil-derived proteases in the
wound environment (Fig. 4). Indeed, the syndecan-1 ectodo-
main HS chains are at least as effective in decreasing the
protease-antiprotease interaction as an equal concentration of
heparin (Table I). The binding affinities of the syndecan-1
ectodomain for the protease approximate that for heparin (Fig.
3) but does not alter the stoichio-
metry of protease binding to the serpin (Fig. 2).

Proteolytic Balance in Wound Repair—Proteolysis is im-
portant for fibrinolysis, growth factor mobilization and activation,
cell migration into the wound site, reepithelialization, angio-
genesis, and extracellular matrix degradation (37). An imbal-
ance of proteolytic activity disrupts normal wound repair and
capillary morphogenesis (38, 39). If the soluble ectodomains
also act like heparin to accelerate the activity of heparin-acti-
vatable serpins (viz. antithrombin III, protease nexin I, plas-
minogen activator inhibitor-1, and others), the ectodomain
could regulate several aspects of proteolysis during wound
repair.

Proteases in wounds co-exist with their physiological inhib-
itors, and thus their activity is finely regulated to provide
optimal activity for repair. This activity results from a balance,
involving enzyme production and activation counterpoised by
enzyme degradation and inhibition. The involvement of syn-
decan ectodomains in regulating proteolytic balance could explain
several observations, including the variability of elastase ac-
tivity and the inconsistency of fibronectin degradation
in wound fluids (11). Our finding that both HS degradation and
immunodepletion of syndecan ectodomains reduce the proteo-
lytic activity of acute wound fluids (Figs. 6 and 7) indicates that
Syndecan Ectodomains Modify Proteolytic Balance

these soluble proteoglycans contribute to balanced proteolytic activity in the wound environment. Alterations in proteolytic balance are thought to be one reason why acute wounds do not heal properly and become chronic (11, 40). The high levels of proteolytic activity in chronic wound fluid have led to the proposal that misregulated proteases contribute to the inability of chronic wounds to heal even when treated with exogenous matrix and growth factors (41, 42). Whether alterations in the levels of syndecan ectodomains could lead to loss of proteolytic balance and thus to development of chronic wounds needs investigation.

Abnormalities in Proteolytic Balance—Optimal proteolytic activity is needed for normal wound repair. Formation of the fibrin-rich provisional matrix produced after tissue injury is an initial step in the repair process. Once the fibrin clot has formed, migrating keratinocytes at the wound edge and emigrating neutrophils produce a variety of serine proteases and matrix metalloproteases to degrade this matrix and close the wound. An imbalance of proteases and serpins contributes to chronic inflammatory conditions, such as rheumatoid arthritis, pulmonary fibrosis, emphysema, and the development of vascular plaques of atherosclerosis and amyloid plaques in the central nervous system in Alzheimer’s disease (9). Whether or not syndecans have a role in regulating proteolytic activities in these events is not known, but in light of our data, this possibility seems worth investigating.

We have found that syndecan-1 and -4 ectodomains act within human acute wound fluids to maintain proteolytic balance. Although no evidence so far exists, the ectodomains of other heparan sulfate proteoglycans that can be shed, such as glypican-1 and CD-44 (Refs. 43 and 29, respectively), might act similarly. Altered proteolytic balance in the wound environment has the potential to interfere with therapeutic procedures ranging from growth factor application to skin grafting. Thus, syndecan expression and shedding should be considered in evaluating and attempting to modify the response to tissue injury.

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REFERENCES

1. Clark, R. A. F. (1996) The Molecular and Cellular Biology of Wound Repair 2nd Ed., Plenum Press, New York
2. Taylor, J. C., and Mittman, C. (1987) Pulmonary Emphysema and Proteolysis, Academic Press Inc., New York, NY
3. Döring, G. (1994) Am. J. Respir. Crit. Care Med. 150, S114–S117
4. Taipale, J., Lohi, J., Saarinen, J., Kotvanen, P. T., and Keski-Oja, J. (1995) J. Biol. Chem. 270, 4689–4696
5. Bieth, J. G. (1986) in Biology of Extracellular Matrix: Regulation of Matrix Accumulation (Mecham, R. P., ed) pp. 217–320, Academic Press Inc., Orlando, FL
6. Campbell, E. J., Senior, R. M., and Welgus, H. G. (1987) Chest 92, 161–167
7. Campbell, E. J. (1986) Am. Rev. Respir. Dis. 134, 435–437
8. Travis, J., and Salvesen, G. S. (1995) Annu. Rev. Biochem. 52, 655–709
9. Gettins, P. G. W., Patatoy, P. A., and Olson, S. T. (1996) Serpins: Structure, Function and Biology, R. G. Landes Co., Austin, TX
10. Potempa, J., Korusza, E., and Travis, J. (1994) J. Biol. Chem. 269, 15957–15960
11. Grinnell, P., and Zhu, M. (1996) J. Invest. Dermatol. 106, 335–341
12. Coleridge-Smith, P. D., Thomas, P., Scyr, J. H., and Dormandy, J. A. (1988) Br. Med. J. 296, 1726–1727
13. Braggman, R. A., Schechter, N. M., Fraki, J., and Lazarus, G. S. (1984) J. Exp. Med. 160, 1027–1042
14. Falanga, V. (1993) J. Invest. Dermatol. 100, 721–725
15. Beatty, K., Bieth, J., and Travis, J. (1980) J. Biol. Chem. 255, 3931–3934
16. Eriksson, E., Lindmark, B., and Lilja, H. (1986) Acta Med. Scand. 220, 447–453
17. Schick, C., Kamachi, Y., Bartusse, A. J., Schechter, N. M., Pemperton, P. A., and Silverman, G. A. (1997) J. Biol. Chem. 272, 1849–1855
18. Pratt, C. W., Whinnin, H. C., and Church, F. C. (1992) J. Biol. Chem. 267, 8795–8801
19. Frommherz, K. J., Faller, B., and Bieth, J. G. (1991) J. Biol. Chem. 266, 15356–15362
20. Ermolieff, J., Boudier, C., Laine, A., Meyer, B., and Bieth, J. G. (1994) J. Biol. Chem. 269, 29602–29608
21. Svec, R. L. (1989) Prog. Clin. Biol. Res. 297, 131–143
22. Yanagishita, M., and Hascall, V. C. (1992) J. Biol. Chem. 267, 9451–9454
23. Bernfield, M., Kokenyesi, R., Kato, M., Hinke, M. T., Spring, J., Gallo, R. L., and Lose, E. J. (1992) Annu. Rev. Cell Biol. 8, 365–398
24. Ehrlich, R., Vainio, S., Lehto, M., Savimivita, M., Thesleff, I., and Jalkanen, M. (1991) J. Cell Biol. 114, 585–595
25. Gallo, R. L., Kin, C., Kokenyesi, R., Adzick, N. S., and Bernfield, M. (1996) J. Invest. Dermatol. 107, 676–683
26. Gallo, R. L., Ono, M., Povsic, T., Page, C., Eriksson, E., Klagsbrun, M., and Bernfield, M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11035–11039
27. Subramanian, S. V., Fitzgerald, M. L., and Bernfield, M. (1997) J. Biol. Chem. 272, 14713–14720
28. Jalkanen, M., Raapaeag, S., Saunders, S., and Bernfield, M. (1987) J. Cell Biol. 105, 3087–3096
29. Bennett, K. L., Jackson, D. G., Simon, J. C., Tanezos, E., Pears, R., Modrell, B., Stamenkovic, I., Plowman, G., and Aruffo, A. (1995) J. Cell Biol. 129, 687–698
30. Sanderson, R. D., and Bernfield, M. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 9562–9566
31. Kato, M., Wang, H., Bernfield, M., Gallagher, J. T., and Turnbull, J. G. (1994) J. Biol. Chem. 269, 1881–1980
32. Lee, M. K., and Lander, A. D. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 2768–2772
33. Chase, T. J., and Shaw, E. (1967) Biochem. Biophys. Res. Commun. 29, 508–514
34. Breuing, K., Eriksson, E., Liu, P., and Miller, D. R. (1992) J. Surg. Res. 52, 50–58
35. Lindahl, U., Lidholt, K., Spillman, D., and Kjellén, L. (1994) Thromb. Res. 57, 1–32
36. Stenfors, R., Van Den Berghe, H., and David, G. (1996) J. Cell Biol. 133, 405–416
37. Mignatti, P., Rikfin, D. B., Welgus, H. G., and Parks, W. C. (1996) in The Molecular and Cellular Biology of Wound Repair (Clark, R. A. F., ed) 2nd Ed., pp. 427–461, Plenum Press, New York
38. Montesano, R., Pepper, M. S., Möhle-Steinlein, U., Risau, W., Wagner, E. F., and Orci, L. (1990) Cell 62, 435–445
39. Pepper, M. S., Montesano, R., Mandriota, S. J., Orci, L., and Vassalli, J.-D. (1990) Enzyme Protein 49, 153–162
40. Rao, C. N., Ladin, D. A., Liu, Y. Y., Chilikuri, K., Hou, Z. Z., and Woodley, D. T. (1995) J. Invest. Dermatol. 105, 572–578
41. Grinnell, P., Ho, C., and Wysocki, A. (1992) J. Invest. Dermatol. 98, 445–416
42. Martin, P. (1997) Science 276, 75–81
43. Bashkin, P., Neufeld, G., Gitay-Goren, H., and Vlodavsky, I. (1992) J. Cell Physiol. 151, 126–137
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