Regulated Shedding of Syndecan-1 and -4 Ectodomains by Thrombin and Growth Factor Receptor Activation*

Sukanya V. Subramanian‡, Marilyn L. Fitzgerald‡, and Merton Bernfield§

From the Joint Program in Neonatology, Harvard Medical School, Boston, Massachusetts 02115

The syndecan family of transmembrane heparan sulfate proteoglycans is abundant on the surface of all adherent mammalian cells. Syndecans bind and modify the action of various growth factors/cytokines, proteases/antiproteases, cell adhesion molecules, and extracellular matrix components. Syndecan expression is highly regulated during wound repair, a process orchestrated by many of these effectors. Each syndecan ectodomain is shed constitutively by cultured cells, but the mechanism and significance of this shedding are not understood. Therefore, we examined (i) whether physiological agents active during wound repair influence syndecan shedding, and (ii) whether wound fluids contain shed syndecan ectodomains.

Using SVEC4–10 endothelial cells we find that certain proteases and growth factors accelerate shedding of the syndecan-1 and -4 ectodomains. Protease-accelerated shedding is completely inhibited by serum-containing media. Thrombin activity is duplicated by the 14- amino acid thrombin receptor agonist peptide that directly activates the thrombin receptor and is not inhibited by serum. Epidermal growth factor family members accelerate shedding but FGF-2, platelet-derived growth factor-AB, transforming growth factor-β, tumor necrosis factor-α, and vascular endothelial cell growth factor 165 do not. Shed ectodomains are soluble, stable in the conditioned medium, have the same size core proteins regardless whether shed at a basal rate, or accelerated by thrombin or epidermal growth factor-family members and are found in acute human dermal wound fluids. Thus, shedding is accelerated by activation of at least two distinct receptor classes, G protein-coupled (thrombin) and protein tyrosine kinase (epidermal growth factor). Proteases and growth factors active during wound repair can accelerate syndecan shedding from cell surfaces. Regulated shedding of syndecans suggests physiological roles for the soluble proteoglycan ectodomains.

The response to tissue injury is orchestrated by multiple soluble effectors. These are derived from the blood plasma, immigrant cells from the circulation and resident cells at the wound site, and include proteases, antiproteases, growth factors, cytokines, and chemokines (1). Heparin modifies the action of several of these effector molecules such as thrombin, antithrombin III, heparin-binding epidermal growth factor-like growth factor (HB-EGF), vascular endothelial cell growth factor, basic fibroblast growth factor, and interleukin-8 (2–4). These interactions focus attention on the potential regulatory role of the heparan sulfate that is at the surface of all adherent cells.

Much of the heparan sulfate at the cell surface is derived from the syndecan family of transmembrane proteoglycans. These four gene products consist of single polypeptides which comprise at their COOH termini a short cytoplasmic domain (28–34 amino acids) containing three invariant tyrosines, and at their NH2 termini an extracellular domain (ectodomain) that places heparan sulfate chains distal from the plasma membrane. The syndecans bind a variety of growth factors, cytokines, proteases, antiproteases, and cell adhesion molecules (5, 6), are individually expressed in distinct cell-, tissue-, and development-specific patterns (7), and show cell-specific variations in the structure of their heparan sulfate chains (8, 9).

Syndecan expression is highly regulated during development, neoplasia, and wound repair (2, 4, 6, 10). Following skin wounds in mice, the keratinocytes at the leading edge migrating into the wound show a loss of cell surface syndecan-1. Concomitantly, syndecan-1 expression increases on the endothelial cells and syndecan-4 expression increases on the dermal fibroblasts that comprise the forming granulation tissue (11, 12). Cell surface syndecan-1 and -4 and their transcripts are induced in cultured endothelia and fibroblasts by PR-39, a pig neutrophil-derived antimicrobial peptide, and analogous induc- tive activity is found in human wound fluid (13). While syndecan expression can be regulated at or following transcription, depending on the cell type or pathophysiological situation, the precise mechanism(s) underlying these changes is unknown (11, 14–17).

A diverse group of transmembrane proteins is regulated by proteolytic cleavage of their ectodomains which are then released into the surrounding milieu (18–20). These cell surface proteins often have soluble counterparts in vivo, and can be detected in various body fluids (18, 21). This shedding can result in solubilization of the functional domains of the cell surface proteins. Recently, this shedding has been shown to be a highly regulated process and a common system has been proposed for regulating the shedding of several transmembrane proteins released by the action of calcium ionophores or...
protein kinase C activators such as phorbol 12-myristate 13-acetate (PMA) (22–24).

It has been known for many years that syndecan-1 is shed constitutively by cultured cells (25) and that this shedding involves release of the soluble ectodomain (26). Indeed, each of the syndecan family members is shed (7) and the site of cleavage has been suggested to be a dibasic sequence (syndecan-1, -2, -3) or a basic residue (syndecan-4) adjacent to the plasma membrane (6, 25).

We have recently found that features of syndecan shedding are similar to those of the common system proposed to be responsible for shedding of several membrane-anchored growth factors, growth factor receptors, cell adhesion, and other membrane proteins. As with the common system, syndecan-1 and -4 are shed at basal levels, but this shedding is accelerated within minutes of treating cells with PMA (27). Drosophila syndecan lacks basic residues adjacent to the plasma membrane, yet is readily shed from cultured cells (28). Thus, as with cleavage by the common system, the amino acid sequence may not be of primary importance to the cleavage process. Finally, the apparent loss of cell surface syndecan-1 during wound repair, a process involving various proteases and growth factors, recalled the findings that receptor stimulation can activate the common shedding system (29–31).

Therefore, we examined whether (i) agents that are active during acute wound repair influence syndecan shedding and (ii) dermal wound fluids contain shed syndecans. We find that plasmin, thrombin, and epidermal growth factor (EGF) family members accelerate the shedding of the syndecan-1 and -4 ectodomains from cultured endothelial cell surfaces, that activation of at least two distinct receptor classes (G protein-coupled and protein tyrosine kinase) accelerates shedding, and that the syndecan-1 and -4 ectodomains are in acute human dermal wound fluids. Regulated shedding of the syndecans suggests a physiological role for the soluble proteoglycan ectodomains.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human (rHu) HB-EGF was obtained from Dr. J. Abraham (Scios-Nova, Mountain View, CA); rHu EGF, TGF-α, and FGF-2 from Intergen (Purchase, NY); rHu platelet derived growth factor AB from Upstate Biotechnology (Lake Placid, NY); rHu vascular endothelial cell growth factor 165 from Dr. G. Neufeld (Israel Institute of Technology, Haifa, Israel); rHu TNF-α and porcine platelet t-PA from R & D Systems (Minneapolis, MN). Plasmin (human plasma), thrombin (human plasma), thrombin receptor agonist peptide (TRAP), soybean trypsin inhibitor, and genistein were from Calbiochem (La Jolla, CA). Tyrophostin 25 and methyl 2,5-dihydroxycinnamate were from Toronto Research Chemicals (Ontario, Canada). Urokinase-type plasminogen activator, PMCA, and TPCK-treated trypsin were from Sigma. Heparan sulfate lyase (heparitinase, EC 4.2.2.8) and chondroitin sulfate ABC lyase (chondroitinase ABC, EC 4.2.2.4) were from Seikagaku America Inc. (Rockville, MD).

Immunochromics—Horseradish peroxidase-conjugated goat anti-rat IgG and horseradish peroxidase goat anti-rabbit IgG were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA) or Amersham Life Sciences. Antibodies specific to syndecan ectodomains included monoclonal antibodies 281-2 against the mouse syndecan-1 ectodomain (32), 529 and 8C7 against the syndecan-4 ectodomain (12), polyclonal antisera MSE-2, MSE-3, and MSE-4, against their respective recombinant mouse syndecan ectodomains (7) and polyclonal antisera HSE-1, against the recombinant human syndecan-1 ectodomain (12).

Serum antibodies prepared against the syndecan-1 and -4 cytoplasmic domains, which are identical in sequence across species, detect both mouse and human syndecan-1 and -4, respectively. Polyclonal antisera STC, against a 7-amino acid synthetic peptide (KQEEFYA) corresponding to the COOH terminus of syndecan-1, has been described (26). Antibodies specific for the syndecan-4 cytoplasmic domain were prepared against a 13-amino acid peptide (LGKGPITYPKAPPTN) unique to the syndecan-4 cytoplasmic domain. The immunogen was synthesized using the multiple antigen peptide system and used for immunization and boosts of rabbits at Quality Controlled Biochemicals Inc. (Hopkinton, MA). The antisera was called SCD-4 for syndecan cytoplasmic domain antibodies prepared by the corresponding number to the specific syndecan family member.

Specificity of SCD-4 was determined by immunoprecipitation of [35S]SO4-labeled SVEC4–10 cell lysates prepared in RIPA buffer (RIPA: 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 1% deoxycholate, 0.1% SDS, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride) with antisera specific for each of the four syndecan ectodomains (281–2, MSE-2, MSE-3, and MSE-4). Each antigen-antibody complex contained radioactivity, indicating that these cells express all four syndecans. The complexes were eluted from protein A beads by boiling in 1.1 (v/v) of 2% SDS in 100 mM Tris-HCl, pH 7.4, the eluate was diluted to 0.1% SDS with RIPA buffer without SDS and equal portions were re-immunoprecipitated with SCD-4 sera and preimmune sera as a control. [35S]SO4 counts in the resulting immune complexes were found only in the complex obtained with MSE-4. Thus, SCD-4 is selective for the cytoplasmic domain of syndecan-4.

Purification of SVEC4–10 Cell Lysates and Conditioned Media—The shed form of syndecan-1 and -4 was partially purified from the conditioned media (CM) of confluent cells cultured for 3 days by QAE-Sephadex A-25 (Pharmacia) and cesium chloride density gradient separation similar to the method of Rapraeger and Bernfield (33). One μl of this partially purified CM represents the amount of heparin sulfate proteoglycan purified from 1 ml of CM. The native transmembrane form of syndecan-1 and -4 was prepared from a RIPA lysate of confluent cells in a 75-cm2 flask (Falcon). The lysate was partially purified on a DEAE-Sephacel column, precipitated with 95% ethanol containing 1.3% potassium acetate, and dissolved in deionized water. Five hundred μl of this partially purified lysate represents the amount of heparin sulfate proteoglycan in the lysate collected from 2.5 × 107 cells.

Wound Fluid and Plasma Samples—Acute human dermal wound fluids were obtained and treated essentially as described by Grinnell et al. (34) and Wysocki et al. (35). Briefly, wound fluid was collected at 1-day intervals for three consecutive days from sterile closed-suction drains routinely placed in the subcutaneous space following mammoplasty. Fluids were centrifuged at 200 × g for 15 min to pellet cells and debris, and further clarified at 3300 × g and stored frozen (−70 °C). Wound fluids were kindly provided by Dr. E. Eriksson, Brigham and Women’s Hospital, Boston, MA. The use of this anonymous discarded material was approved by the Human Research Committee of the Brigham and Women’s Hospital, protocol number 92–5416-01. Blood plasma was collected from human blood diluted 1:1 (v/v) with phosphate-buffered saline, pH 7.4, at 37 °C following separation of cellular components on a Ficoll (Histopaque 1083, Sigma) gradient by centrifugation at 1000 × g for 30 min at room temperature and stored frozen (−70 °C).

Cell Culture and Shedding Assay—SVEC4–10 cells were cultured in 96-well and 6-well tissue culture plates (Costar or Falcon) for 16 and 8 h, respectively. Cells were grown to confluence in Dulbecco’s modified Eagle’s medium containing glucose at 4.5 g/liter (Life Technologies, Grand Island, NY), supplemented with 10% fetal calf serum (FCS, Intergen, Purchase, NY) and L-glutamine. At the time of treatment, cell culture media was replaced with fresh media containing the indicated amounts of FCS and test agents. Following incubation for the indicated times, cells were examined by phase microscopy for survival and morphology and the conditioned media harvested for dot blot analysis. Cells in 96-well plates were fixed with 2% paraformaldehyde (in Hapes-buffered saline, pH 7.4, with 1 mM CaCl2, 0.5 mM MgCl2), washed with Tris-buffered saline (TBS) and extracted with 1% sodium dodecyl sulfate (SDS) / 100 mM Tris-HCl, pH 7.4, 1 mM EDTA, for 30 min at 100 °C. Protein was measured using the BCA protein assay (Pierce) with bovine serum albumin as the standard. The 96-well plate (16 h) assays were performed in triplicate with 100 μl of media per well. The 6-well plate (8 h) assays yielded 600 μl of media per well, which was divided into three equal portions for dot blot analysis. All assays were performed at least three times.

Cell Surface Tryptsinization—Tryptsinization of cell surface syndecans from SVEC4–10 cell monolayers in 6-well plates was performed essentially as described (25). Briefly, following harvesting of the conditioned media (600 μl), cell layers were washed twice with ice-cold 0.5 mM EDTA-TBS and incubated with 600 μl of 10 μg/ml TPCK-treated trypsin in the same buffer for 10 min on ice. After incubation, soybean trypsin inhibitor was added to 50 μg/ml, cells were counted with a hemocytometer and centrifuged (200 × g) for 5 min at 4 °C. Supernatants containing the ectodomains were used immediately or stored at 4 °C for protein determination and dot immunosassay. Total RNA was extracted from pellets as described below.
Regulated Shedding of Syndecan-1 and -4

**RESULTS**

**Agents Involved in Response to Tissue Injury Accelerate Syndecan-1 and -4 Shedding from Cultured Cells**—We assessed whether agents known to be involved in wound repair regulate the shedding of syndecan ectodomains from the cell surface. SVEC4–10 cells, a mouse SV40 transformed endothelial cell line, were used as a test cell line (36). These cells were chosen because they remain viable in the presence or absence of serum, express both syndecan-1 and -4, and shed the ectodomains of these syndecans at a low basal rate.

Shedding was assessed by measuring the appearance in the culture media of immunoreactive syndecan ectodomains. Shed proteoglycans are not endocytosed by these cells during a 16-h incubation and thus are stable within the conditioned medium (data not shown). NMuMG mouse mammary epithelial cells were previously shown to shed the syndecan-1 ectodomain by demonstrating that the material in conditioned medium (i) migrated as a GAG-free core protein at the same Mr, as the tryptic-released ectodomain (25) but smaller than the transmembrane proteoglycan (37), and (ii) failed to react with an antibody directed against the cytoplasmic domain (26). We confirmed these results for syndecan-1 and -4 shed into conditioned media by SVEC4–10 cells in the presence or absence of serum. Proteoglycans in conditioned media and RIPA cell lysates were partially purified as described under “Experimental Procedures,” treated with tryptase to remove GAG chains, and subjected to SDS-PAGE and transfected to cationic polyvinylidene difluoride membranes. As expected, the glycosaminoglycan-free core proteins derived from the conditioned media were smaller than those from lysates (data not shown). The proteoglycans were immobilized on cationic membranes and probed with core protein-specific antibodies directed against the ectodomains and the cytoplasmic domains (Fig. 1). Antibodies against the syndecan-1 and -4 ectodomains detected these proteoglycans in cell lysates and conditioned media, as expected. Antibodies against the cytoplasmic domains detected proteoglycans in the cell lysates, but failed to detect proteoglycans in conditioned media, indicating that these are the ectodomains.

We first examined the effect of various agents on syndecan shedding by SVEC4–10 cells during a 16-h incubation in the presence and absence of 5% fetal calf serum. Basal shedding of syndecan-1 and -4 was barely detectable, but shedding was markedly increased by PMA treatment (Fig. 2), as described previously (27). Both EGF and HB-EGF (10 ng/ml) accelerated shedding, however, vascular endothelial cell growth factor 165, FGF-2, platelet-derived growth factor AB, TGFβ, and TNFα (10

---

**FIG. 1.** Syndecans shed from cultured endothelial cells are ectodomains. Proteoglycans partially purified from lysates and conditioned media (CM) of SVEC4–10 mouse endothelial cells cultured for 3 days in the presence of serum were applied to Immobilon-N membranes and analyzed by dot blot using ECL detection. Blots carrying increasing amounts of lysate (2, 5, 10, and 20 μl) and CM (0.1, 0.2, 0.5, and 1.0 ml), prepared as described under “Experimental Procedures,” were probed with antibodies specific for the ectodomains of mouse syndecan-1 (mAb 281-2) and -4 (antisera MSE-4) (Antibody = Ectodomain). Syndecan cytoplasmic domains were assessed using serum antibodies that recognize the syndecan-1 (STC) and -4 (SCD-4) cytoplasmic domains (Antibody = Cytoplasmic Domain).
Regulated Shedding of Syndecan-1 and -4

**Fig. 2. Growth factors accelerate syndecan shedding.** SVEC4–10 cells were incubated for 16 h with PMA (0.5 μM) or growth factors (10 ng/ml) in the presence of 5% fetal calf serum. Conditioned media were harvested and proteoglycans partially purified by application to cationic membranes were analyzed by dot blot. Syndecan-1 and -4 were detected by ECL using mAb 281-2 and MSE-4 antiserum, respectively. The PMA-treated samples are from a different experiment than the growth factor-treated samples. VEGF, vascular endothelial growth factor.

ng/ml did not (Fig. 2, and data not shown). This growth factor-accelerated shedding was unaffected by incubation of the cells in serum-containing medium. Both thrombin and plasmin accelerated shedding, but urokinase-type plasminogen activator did not (Fig. 3). This protease-accelerated shedding was completely inhibited by incubation of the cells in serum-containing media.

All members of the EGF family tested accelerated the shedding of syndecan-1 and -4 from SVEC4–10 cells (Figs. 4, A and B). EGF was the most potent while amphiregulin was the least potent. Amphiregulin, FGF-2, and HB-EGF bind heparin; this binding does not appear to influence whether syndecans are shed in response to growth factors.

Thus, shedding of syndecan-1 and -4 appears to be a regulated process. PMA and only certain proteases and growth factors accelerate shedding from SVEC4–10 cells and shedding of both proteoglycans is affected similarly. Shedding accelerated by proteases is inhibited by serum, suggesting that serum contains inhibitors which prevent proteolytic scission of the core protein. On the other hand, PMA and growth factor accelerated shedding is not altered by incubation with serum, suggesting a different mechanism.

**Accelerated Shedding Yields the Same Size Ectodomain Core Proteins**—To evaluate whether the shed ectodomains differ in size when released from SVEC4–10 cells by distinct agents, cells were incubated for 16 h with various accelerating agents in the absence of serum. The media were harvested, proteoglycans were precipitated with ethanol, and incubated with heparitinase and chondroitinase ABC, applied to SDS-PAGE, and the ectodomain core proteins analyzed by Western blots. The GAG-free syndecan-1 and -4 ectodomain core proteins (Figs. 5, A and B) were the same size regardless whether shed at a basal rate, or accelerated by PMA, thrombin, EGF or HB-EGF, or TGF-α. These results suggest that the shedding mechanism yields the same size product whether shed constitutively or by a phorbol ester, protease, or growth factor.

**Accelerated Shedding of Syndecans by Thrombin Is Receptor-mediated**—Because the thrombin-released ectodomain was the same M̄, as ectodomains released by other agents, we assessed whether syndecan shedding accelerated by thrombin may result from thrombin receptor activation rather than direct proteolytic cleavage. Increasing thrombin concentrations significantly enhanced (p < 0.01) syndecan-1 ectodomain but reduced syndecan-4 ectodomain levels in the culture media (Figs. 6, A and B), suggesting proteolytic degradation of the syndecan-4 ectodomain. Thrombin receptor activation requires the serine protease activity of its ligand, thrombin (38). Therefore, cells were treated with TRAP, a 14-amino acid peptide which directly activates the thrombin receptor and has no inherent proteolytic activity (40). TRAP treatment caused a significant increase (p < 0.01) in the levels of both the shed syndecan-1 and syndecan-4 ectodomains in the culture media (Figs. 6, A and B). Thus, direct activation of the thrombin receptor can accelerate syndecan-1 and -4 shedding.

**Accelerated Shedding of Syndecans by TRAP and EGF Involves Receptor Signaling**—The kinetics of accelerated shedding were assessed during the initial 8 h of TRAP- and EGF-accelerated shedding. Accelerated shedding of syndecan-1 and -4 was detected as early as 1 h following treatment with either agent and the amount of shed syndecan-1 (Fig. 7A) and syndecan-4 (Fig. 7B) increased with time at a nearly linear rate. Similar responses were seen when NMuMG mouse mammary epithelial cells were used in these assays (data not shown).

To determine whether these agents accelerate syndecan shedding by activating receptor signaling, we tested whether tyrosine kinase inhibitors affected shedding. Genistein, a specific tyrosine kinase inhibitor (39) reduced both TRAP- and EGF-accelerated shedding of syndecan-1 (Fig. 7A) and syndecan-4 (Fig. 7B). Similar results were obtained with the tyrosine kinase inhibitors tyrphostin 25 and methyl 2,5-dihydroxycinnamate (data not shown). Thus, tyrosine phosphorylation is involved in accelerating the shedding of syndecan-1 and -4 from the cell surface.

Receptor-mediated acceleration of syndecan shedding could be due to several possible mechanisms. Thus, we assessed levels of cell surface syndecan-1 and syndecan-1 mRNA during the initial 8 h of TRAP- and EGF-accelerated shedding. The conditioned media of EGF-treated and TRAP-treated cells accumulated 2- and 3-fold more syndecan-1 ectodomain than untreated cells over an 8-h period, respectively (Fig. 8A). During this time, there was no detectable change in the levels of cell surface proteoglycan (Fig. 8B). The levels of syndecan-1 mRNA did not increase (Fig. 8C) during accelerated shedding. These results suggest that accelerated shedding is accompanied by increased syndecan turnover at the cell surface. Furthermore, no change in SVEC4–10 or NMuMG cell morphology occurred during accelerated shedding, an observation which implies that the cells have retained at least 50% of their cell surface syndecan-1 during accelerated shedding (46).

**Syndecan-1 and -4 Ectodomains Are Detected in Wound Fluid**—During cutaneous wound repair in the mouse, keratinocytes migrating into the wound transiently lose syndecan-1. Concurrently, syndecan-1 is transiently induced on the endothelial cells and syndecan-4 on the dermal fibroblasts that comprise the forming granulation tissue (11, 12). Furthermore, plasmin, thrombin, and EGF family members, accelerators of syndecan-1 and -4 shedding from cultured SVEC4–10 cells, are
known to operate during acute wound repair (1). Thus, we examined whether the fluids accumulating in acute wounds contained syndecan-1 and -4. Human dermal wound fluids were immunoprecipitated using antiserum HSE-1 or mAbs specific for the human syndecan-4 ectodomain (5G9 and 8C7) and the precipitates run on PAGE and analyzed by Western blots. Both precipitates contained materials that migrated as proteoglycans (Fig. 9A). Interestingly, the syndecan-1 immunoprecipitate contained both a proteoglycan smear and a lower Mr smear. The latter may represent partially degraded syndecan-1.

To determine whether the wound fluid syndecans were the transmembrane proteoglycans or the ectodomains, the wound fluid proteoglycans were partially purified on cationic polyvinylidene difluoride membranes and probed with antibodies against the syndecan-1 and -4 ectodomains, but not in plasma (Fig. 9B). In contrast, antibodies against the cytoplasmic domains failed to detect these proteoglycans (Fig. 9B). Thus, the synde-
cans in wound fluid are the ectodomains, likely shed from cell surfaces.

**DISCUSSION**

In this study we show that thrombin, plasmin, and members of the EGF family accelerate shedding of the syndecan-1 and -4 ectodomains from cultured endothelial cell surfaces, and that activation of at least two distinct receptor classes (thrombin (G-protein linked) and EGF (tyrosine kinase)) regulate syndecan shedding. Regulation of syndecan shedding appears to be physiologically relevant because EGF and thrombin receptors are both activated during wound repair, and we find the shed syndecan-1 and -4 ectodomains in the fluid that accumulates within acute dermal wounds. Because syndecan shedding is accelerated by multiple effectors that act directly or via receptors, these cell surface proteoglycans likely have additional physiological roles as soluble paracrine effectors.

**Syndecan Ectodomains Are Shed**—Syndecan-1 and -4 are transmembrane proteoglycans but are released both into conditioned media and wound fluid as soluble proteoglycans. Their electrophoretic migration, presence of extracellular epitopes and absence of cytoplasmic domains demonstrate that the syndecans recovered from the conditioned media and wound fluid correspond to the intact ectodomains. Thus, shedding appears to result from a proteolytic process but the precise location of the cleavage site will require analysis of the amino- or carboxy-terminal sequences of the cleavage products. Several different cleavage sites have been shown for shedding of other transmembrane proteins (18). Interestingly, the structure of syndecan-1 and -4 differ in several aspects that are potentially important for shedding, including the number and types of basic residues adjacent to the extracellular leaflet of the plasma membrane. Yet these proteoglycans responded similarly in this study to agents that accelerate shedding.

**Shedding of Syndecan Ectodomains Is Regulated by Physiological Effectors**—Syndecan-1 and -4 belong to the class of transmembrane proteins which undergo proteolytic cleavage and release of their ectodomains into the extracellular milieu when cells are treated with protein kinase C activators. The syndecan-1 ectodomain is shed from cultured adherent cells at a basal rate, but this shedding is markedly enhanced by suspension of the cells (25) or phorbol ester activators of protein

![Figure 7](http://www.jbc.org/)

**FIG. 7.** EGF- and TRAP-accelerated shedding is receptor mediated. SVEC4–10 cells were treated with or without EGF (10 ng/ml) or TRAP (100 μM) in the presence or absence of genistein (5 μg/ml) in media containing 1% FCS over an 8-h period. Conditioned media were harvested at the indicated times and assayed for syndecan-1 (A) and syndecan-4 (B) ectodomains by dot blot analysis as in Fig. 2. Quantitation was as in Fig. 4. Each point represents the mean ± S.D. (n = 3).

![Figure 8](http://www.jbc.org/)

**FIG. 8.** EGF- and TRAP-accelerated shedding is accompanied by increased turnover at the cell surface. SVEC4–10 cells were treated with EGF (10 ng/ml) or TRAP (100 μM) in media containing 1% FCS over an 8-h period. Conditioned media were harvested, cells were trypsinized to release the ectodomain from cell surface syndecan-1, and RNA was extracted from cells as detailed under “Experimental Procedures.” A, conditioned media; and B, trypsinates were assayed for syndecan-1 ectodomains by dot blot analysis as in Fig. 2. Quantitation was as in Fig. 4 and each point represents the mean ± S.D. (n = 3). C, syndecan-1 mRNA was assayed by Northern blot analysis and autoradiography using a mouse syndecan-1 probe (28). Syndecan-1 mRNA levels were quantified by densitometric scanning and analyzed with NIH Image software. Results are expressed as syndecan-1 mRNA level relative to the level of β-actin mRNA.
Regulated Shedding of Syndecan-1 and -4

Regulated Shedding of Syndecan-1 and -4 ectodomains is accelerated by multiple effectors that act through receptor-mediated events at the cell surface. The EGF family of growth factors, including EGF, TGF-β, and TNF-α, stimulate PKC, which is known to accelerate syndecan shedding (27). The common theme of these signaling cascades is change in the levels of serine and/or tyrosine phosphorylation. However, the site(s) of phosphorylation that may augment this shedding is unknown. Thus, syndecan shedding involves several effectors and at least two distinct classes of cell surface receptors. The susceptibility of syndecan shedding to multiple effectors that act directly or via receptors implies that the ectodomains of these cell surface molecules have physiological roles as soluble proteoglycans.

EGF or TRAP enhances the shedding of syndecan-1 and -4 ectodomains within 1–2 h of treatment. However, at least for TRAP-accelerated shedding, the amount of shed proteoglycan cannot be accounted for by a change in the level of cell surface proteoglycan. Thus, as shedding proceeds, the turnover of syndecan-1 at the cell surface may also increase. The syndecan-1 mRNA level is also unchanged, suggesting that the translation rate increases and/or an intracellular pool is depleted. Our results suggest that these cells maintain a steady state level of cell surface syndecan-1 during accelerated shedding. This could account for the lack of apparent change in cell morphology during accelerated shedding, since a marked reduction in cell surface syndecan-1 is accompanied by change in cell shape, rearrangement of actin cytoskeleton (44–46). Such regulation of cell surface syndecan may enable cells to retain their adhesive phenotype while shedding is accelerated.

Our finding that syndecan shedding is accelerated by multiple effectors that act through receptor-mediated events at the cell surface is consistent with the involvement of the common system proposed to regulate shedding of a heterogeneous group of transmembrane proteins (24). This system responds to multiple activators, induces cleavage of ectodomains at diverse sequences, and is inhibited by zinc ion chelators, suggesting the action of membrane-associated metalloproteinases. Whether syndecan shedding follows this same proposed mechanisms remains to be determined.

Receptor-mediated Syndecan Shedding Is Relevant to Wound Repair—The syndecan-1 and -4 ectodomains were readily detected in human dermal wound fluid on day 1 and 2 after wounding. These syndecan ectodomains were not detected in human plasma, indicating that they likely arose in the wound environment as a result of the injury. Syndecans in wound fluids had a relatively high average Mr, similar to that from mesenchymal cells (8, 14), possibly reflecting the cell type(s) from which they are derived. Syndecan-1 from keratinocytes has a lower Mr than from mesenchymal cells or other epithelial cells (14), implying that the endothelial and/or fibroblastic cells at the wound site may be the source of the shed syndecan ectodomains. The shed ectodomains may also undergo partial degradation by proteases and/or heparanases in wound fluid (1).

The effectors that accelerate syndecan shedding are known to act during acute wound repair. Tissue injury is accompanied by cell disruption, activation of proteases, and release of growth factors, each of which accelerates syndecan shedding. The accelerated shedding that accompanies cell suspension (25) could involve member(s) of the ADAMS family, widely expressed transmembrane proteins that contain both metalloproteinase and potential integrin binding domains (47). Both thrombin

**Fig. 9.** Syndecan-1 and -4 ectodomains are in human wound fluid. A, acute dermal wound fluid was immunoprecipitated using antiserum against the human syndecan-1 ectodomain (HSE-1, lane 1), or a mixture of mAbs against the human syndecan-4 ectodomain (5G9 and 8C7, lane 3) and analyzed by Western blots. Partially purified A431 human squamous cell carcinoma cell conditioned media was run as a control (lanes 2 and 4). Syndecan-1 and -4 ectodomains were detected by ECL using HSE-1 or mAbs 5G9 and 8C7, respectively. B, proteoglycans in human plasma and dernal wound fluids (day 1 and 2) were applied to Immobilon-N membranes and analyzed for the presence of syndecan-1 and -4 ectodomains by dot blot using HSE-1 antiserum and mAbs 5G9 and 8C7 (Antibody: Ectodomain). Syndecan cytoplasmic domains were assessed using serum antibodies against the syndecan-1 (STC) and syndecan-4 (SCD-4) cytoplasmic domains (Antibody: Cytoplasmic).
and plasmid, formed during the blood coagulation process, the initial event in tissue injury, can accelerate shedding. However, the formation and activity of these proteases is tightly regulated, and their ability to accelerate shedding may be limited in the presence of fluid phase protease inhibitors such as α2-macroglobulin, antithrombin III, and α2-antiplasmin. Indeed, in the presence of serum, shedding by plasmid and thrombin from SVEC4–10 cells was inhibited. However, the platelet activation and aggregation which occurs with hemostasis rapidly releases many mediators into the wound, including EGF, HB-EGF, and TGF-α (1). The shedding activity of these growth factors is not inhibited by serum components. Interestingly, several other transmembrane proteins released by the common shedding system are also involved in the response to tissue injury. These proteins include the receptors for TNF-α (48, 49), CSF-1 (50), interleukin-6 (24, 51, 52), and the growth factors/cytokines TNF-α (53–56), CSF-1 (57, 58), TGF-α (23, 24, 59, 60), and HB-EGF (61), and the cell adhesion molecules V-CAM (62), E- and L-selectin (24, 63–65). Hence, activation of this common shedding system may be a component of wound repair.

The conversion of cell surface syndecans into soluble proteoglycans during wound repair introduces anionic, multivalent shedding system may be a component of wound repair. The significance of the glycans during wound repair introduces anionic, multivalent cytoskeletons TNF-α (53–56), CSF-1 (57, 58), TGF-α (23, 24, 59, 60), and HB-EGF (61), and the cell adhesion molecules V-CAM (62), E- and L-selectin (24, 63–65). Hence, activation of this common shedding system may be a component of wound repair.

The conversion of cell surface syndecans into soluble proteoglycans during wound repair introduces anionic, multivalent shedding system may be a component of wound repair. The significance of the glycans during wound repair introduces anionic, multivalent cytoskeletons TNF-α (53–56), CSF-1 (57, 58), TGF-α (23, 24, 59, 60), and HB-EGF (61), and the cell adhesion molecules V-CAM (62), E- and L-selectin (24, 63–65). Hence, activation of this common shedding system may be a component of wound repair.

Acknowledgments—We thank Shuyuan Zhao, Dmitry Leyfer, and Elena Shneider for technical assistance and Drs. Huiming Wang and Drs. Huiming Wang and Bernfield, M. (1994) Mol. Biol. Cell, suppl. 312a

REFERENCES

1. Clark, R. A. F., and Henson, P. M. (eds) (1996) The Molecular and Cellular Biology of Wound Repair, Plenum Press, New York

2. Bernfield, M., Hinkes, M. T., and Gallo, R. L. (1993) Dev. Suppl. 205–212

3. Bourin, M. C., and Lindahl, U. (1993) Biochem. J. 290, 313–330

4. Gallo, R. L., and Bernfield, M. (1985) in Molecular and Cellular Biology of Wound Repair (Wight, R. N., and Mecham, R. P., eds) Vol. II, pp. 129–154, Marcel Dekker, Inc., New York

5. Jalkanen, M., Jalkanen, S., and Bernfield, M. (1991) in Molecular and Cellular Biology of Wound Repair (Wight, R. N., and Mecham, R. P., eds) Vol. II, pp. 129–154, Marcel Dekker, Inc., New York

6. Vu, T. H., Hung, D. T., Wheaton, V. I., and Coughlin, S. R. (1991) J. Biol. Chem. 266, 1057–1068

7. Akiyama, T., Ishida, J., Nakagawa, S., Ogawa, H., Watanabe, S., Itoh, N., Shibuya, M., and Fukumi, Y. (1987) J. Biol. Chem. 262, 5592–5595

8. Troyer, D., Padilla, R., Smith, T., Kreisberg, J., and Glass, W., II (1992) J. Biol. Chem. 267, 20126–20131

9. Schlessinger, J., and Ullrich, A. (1992) Neuron 9, 383–391

10. Eap, H. S., Dawson, T. L., Li, X., and Yu, H. (1995) Breast Cancer Res. Treat. 31, 115–132

11. Pennington, D. P., and Berndt, M. C. (1994) Clin. Exp. Pharmacol. Physiol. 21, 349–358

12. Leppe, S., Barkin, D., and Jalkanen, M. (1991) Cell Regul. 2, 1–11

13. Leppe, S., Mali, M., Miettinen, H., and Jalkanen, M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 932–936

14. Kato, M., Saunders, S., Nguyen, H., and Bernfield, M. (1995) Mol. Biol. Cell 6, 559–576

15. Wolfensberger, T. G., and White, J. M. (1996) Dev. Biol. 180, 389–401

16. Brakenhuber, C., Farolfemonee, E., Batkin, M., and Wallach, D. (1994) J. Biol. Chem. 269, 32488–32496

17. Crowe, P. D., Walter, B. N., Mohler, K. M., Otten-Evans, C., Black, R. A., and Ware, C. F. (1995) J. Exp. Med. 181, 1205–1210

18. Downing, J. R., Royseel, M. F., and Sherr, C. J. (1989) Mol. Cell. Biol. 9, 2890–2896

19. Mullberg, J., Othoberth, W., Lottspeich, F., Mohr, E., Dittrich, E., Graefe, L., Heinrich, P. C., and Rose-John, S. (1994) J. Immunol. 152, 4958–4968

20. Mullberg, J., Durie, P. H., Otten-Evans, C., Alderson, M. E. R., Rose-John, S., Cosman, D., Black, R. A., and Mohler, K. M. (1995) J. Immunol. 155, 5198–5205

21. Perez, C., Albert, I., DeFay, K., Zacheriades, N., Goodling, L., and Kriegler, M. (1993) J. Biol. Chem. 268, 251–255

22. McGeohan, G. M., Becherer, J. D., Bart, R. C., Jr., Boyer, C. M., Champaing, B., Connolly, K. M., Conway, J. G., Furdon, P., Karp, S., Kidoa, S., McEhenry, A. B., Nichols, J., Pryzwansky, K. M., Schonen, F., Sekut, L., Topoles, A., Verghese, M., Warner, J., and Ways, J. P. (1994) Nature 370, 558–561

23. Mohler, K. M., Sleath, R. H., Fitzner, J. R., Ceretti, D. P., Alderson, M., Kerwar, S. S., Torrance, D. S., Otten-Evans, C., Greenstreet, T., Weerawarna, K., Kronheim, S. R., Petersen, M., Gerhart, M., Kozlosky, J. C., March, C. J., and Black, R. A. (1994) Nature 370, 218–220

24. Stein, J., and Rettenmier, K. (1994) Oncogene 9, 1805–1811

25. Deng, P., Rettenmier, K. W., and Pattegalle, P. K. (1996) J. Biol. Chem. 271, 16338–16343

26. Pandiella, A., Rosenberg, M. W., Huang, E. J., Besmer, P., and Massague, J. (1992) J. Biol. Chem. 267, 24028–24033

27. Rosenberg, M. W., Pandiella, A., and Massague, J. (1993) J. Cell Biol. 122, 95–101

28. Gotoh, K., Higashiyama, S., Klagsbrun, M., Nakano, N., Umeda, T., Ishikawa, M., Mekada, E., and Taniguchi, N. (1995) Mol. Biol. Cell 6, 987–990

29. Lec, O., Manzur, S. E., and Benassou, A. (1995) J. Immunol. 154, 1069–1077

30. Costantini, R., Dillen, L. P., Hemmaty, I. H., and Gearing, A. J. (1992) Biochem. Biophys. Res. Commun. 187, 584–589

31. Kahn, J., Inghram, R. H., Shirley, F., Migaki, G. I., and Kishimoto, T. K. (1989) J. Cell Biol. 105, 410–416

32. Frechau, C., Darlik, K., Kahn, J., Walcheck, B., Spatola, A. F., and Kishimoto, T. K. (1996) J. Biol. Chem. 271, 8019–8024

33. Fernandez-Borlan, R., and Massague, J. (1993) Curr. Opin. Cell Biol. 5, 832–838

34. Steinfield, R., Van Den Bergh, H., and David, G. (1996) J. Cell Biol 133, 405–416

35. Laemmli, U. K. (1970) Nature 227, 680–685
