SC1/Hevin

AN EXTRACELLULAR CALCIUM-MODULATED PROTEIN THAT BINDS COLLAGEN I*

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SC1, a member of the BM-40 family of extracellular matrix proteins, was recombinantly expressed in a eukaryotic expression system. The full-length protein as well as truncated versions were purified to homogeneity under non-denaturing conditions. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry of full-length SC1 revealed a mass of 87.8 kDa of which 16.8 kDa is contributed by posttranslational modifications. In electron microscopy, after negative staining, SC1 was revealed as a globule attached to a thread-like structure. A calcium dependence of the SC1 conformation could be demonstrated by fluorescence spectroscopy. In the extracellular matrix of cultured osteosarcoma cells SC1 was found associated with collagen I-containing fibrils, and binding of SC1 to reconstituted collagen I fibrils could be demonstrated by immunogold labeling and electron microscopy. SC1 showed a broad expression in a variety of tissues.

SC1 was originally cloned from a rat brain expression library by screening with a polyclonal antibody raised against synaptic junction glycoproteins (1). Its mRNA is expressed widely in the brain and could be detected in many types of neurons. In immunoblots of brain proteins SC1 was detected as a doublet band of 116 and 120 kDa, whereas the molecular mass predicted from sequence was 70.6 kDa (1). A homologous cDNA called hevin was also cloned from a library derived from human high endothelial venule cells (2). Human high endothelial venule cells form specialized postcapillary vascular sites (high endothelial venules) that mediate extensive extravasation of lymphocytes from the blood into lymphoid organs and sites of chronic inflammation. The high sequence identity suggests that hevin is the human SC1 ortholog. SC1/hevin is expressed in many tissues as a secreted glycoprotein of the extracellular matrix.

Sequence analysis showed that SC1/hevin belongs to the BM-40/SPARC/osteonectin family of proteins and is composed of a highly acidic domain I without homology to any known protein, a follistatin-like domain (FS) and an extracellular calcium-binding domain (EC) (Fig. 1). The presence of the FS and the EC domain is the hallmark of the BM-40/SPARC/osteonectin family, which consists of BM-40/SPARC/osteonectin, SC1/hevin, QR1, tsc36/flk, the testisicans, and the SMOCs (3).

Functional assays suggest that SC1/hevin may serve as an antagonist of cell adhesion, an effect also studied extensively for SPARC (4). Hevin was shown to inhibit the attachment and spreading of endothelial cells on fibronectin substrates, and it was suggested that hevin modulates the high endothelial venule cell adhesion and phenotype, thus facilitating lymphocyte transendothelial migration (5). The effects of SC1/hevin on cell adhesion are of particular interest due to the fact that SC1/hevin is down-regulated in many types of cancer cells and may serve as a negative regulator of cell growth and proliferation (6–8). Furthermore, expression of SC1/hevin is associated with the migration of myotomes during somitogenesis in early mouse embryos and undergoes a rapid down-regulation just before myotome emigration from the somitic environment (9). In another line of work, SC1/hevin was shown to bind to B-lymphocyte precursors (10) and to augment B cell lymphopoiesis (11, 12), effects that are mediated by the N-terminal acidic domain I. Gene targeting of SC1 in mouse did not show any obvious phenotype (13), a finding that is not too surprising considering the number of members of the BM-40 family that may play a compensatory role.

The major objective of this investigation was to characterize SC1/hevin at the protein level, to investigate the functionality of its predicted calcium-binding EC domain, and to identify mechanisms by which it can be integrated into the extracellular matrix. For this purpose we expressed full-length SC1 as well as fragments with domain-specific truncations. These were analyzed for structural features as well as for the ability to bind calcium and collagen fibrils. The purified SC1 FS-EC domain pair was used to raise an antibody with which we could demonstrate a colocalization of SC1/hevin with collagen I in the extracellular matrix of SAOS-2 cells and determine the distribution of SC1 in many tissues as well as the central nervous system.

MATERIALS AND METHODS

Construction of Expression Vectors for Full-length and Truncated Forms of SC1—The cDNA clone for mouse SC1 was kindly supplied by Dr. Peter J. McKinnon. Four cDNA fragments, representing the full-length SC1, the pair of I and FS domains, the pair of FS and EC domains, and the EC domain, were generated by PCR using the following primers: forward primer-1, 5′-CGGCGCCTAGCCCGACAAGTAGC-CAATGACTGCGGCCGCTCAAAAGA-GCCCCGCTAGCCCCGACAGTAC-AAAAGGTTTTCTAT, forward primer-3, 5′-GCCCCGCTAGCCCGCCAACACT-CTCAAAAAAGAGGAGTTTTCTAT, forward primer-3, 5′-GCCCCGCTAGCCCGCCAACACT-CTCAAAAAAGAGGAGTTTTCTAT, forward primer-4, 5′-GCCCCGCTAGCCCGCCAACACT-CTCAAAAAAGAGGAGTTTTCTAT, and reverse primer-5, 5′-CAATGACTGGGGGCGGTGAAGTTTTAC-CTCAAAAAAGAGGAGTTTTCTAT, forward primer-5, 5′-CAATGACTGGGGGCGGTGAAGTTTTAC-CTCAAAAAAGAGGAGTTTTCTAT, and reverse primer-5, 5′-CAATGACTGGGGGCGGTGAAGTTTTAC-CTCAAAAAAGAGGAGTTTTCTAT. Primers 1 and 2 were used for full-length SC1, primers 1 and 5 were used for the pair of I and FS domains, primers 3 and 2 were used for the pair of FS and EC domains, and primers 4 and 2 were used for the EC domain. The primers introduced
new restriction sites (NdeI, NotI) and a stop codon. The NdeI/NotI-restricted PCR products were purified and cloned into the corresponding restriction sites of pCEP-Pu/EM40 (14) to obtain the final expression vectors pCEP-Pu/SC1, pCEP-Pu/SC1-1-FS, pCEP-Pu/SC1-EC, and pCEP-Pu/SC1-EC. The C-terminal fragment of I-FS was additionally cloned in the modified expression vector pCEP-Pu with an N-terminal tag consisting of six histidines, a Myc epitope, and an enterokinase recognition site for cleavage of the tag (15). The eukaryotic expression vector pRCMV (Invitrogen) was used to produce a modified vector pRCCMV/EM40 (5.5 kilobases). Therefore, a HindIII/NotI fragment containing the signal peptide sequence and some of the 5'-untranslated region of BM-40 (105 bp) together with the full-length SC1 sequence (1.9 kilobases) was cleaved from pCEP-Pu/SC1, purified, and ligated to the same restriction sites in pRCMV. The resulting vector was designated pRCMV/SC1. The correct insertion and sequences of all amplified fragments were verified by cycle sequencing of both strands using an ABI Prism 377 Automated Sequencer (PE Biosystems).

**Cell Culture and Transfection**—The human embryonic kidney cell line 293 (Invitrogen), which constitutively expresses the EBNA-1 protein from Epstein-Barr virus was used for transfection with pCEP-Pu/SC1-1-IFS, pCEP-Pu/SC1-EC, and pCEP-Pu/SC1-EC and selected with puromycin as described (14). The fibrosarcoma cell line HT1080 (kindly provided by Dr. M. Aumailley) was transfected with pRCMV/SC1. Two days after transfection the cells were split 1:10, and selection was started by adding 800 μg/ml G418 (Invitrogen). Resistant clones were isolated after 4–5 weeks at 4 °C over a DEAE-Sepharose (Amersham Biosciences) column equilibrated in the same buffer. Fractions that eluted between 0.26–0.35 M NaCl showed immunoreactivity with the antibody to SC1. These were dialyzed against 50 mM BisTris, pH 7.0, and applied to a Q-Sepharose (Amersham Biosciences) column equilibrated in the same buffer. The recombinant protein was eluted at 0.35–0.77 M NaCl and was further purified by gel filtration on a column of Sephadex G-75 (Amersham Biosciences) in 0.2 M NaCl, 50 mM BisTris, pH 7.4. The pure SC1 was dialyzed against 5 mM Tris-HCl, pH 7.4.

**Purification of the FS-EC Domain Pair**—Conditioned serum-free medium was dialyzed against 50 mM BisTris, pH 7.0, containing 0.5 mM phenylmethylsulfonyl fluoride and passed over a column of DEAE-Sepharose (Amersham Biosciences) column equilibrated in the same buffer. Fractions that eluted between 0.26–0.33 M NaCl showed immunoreactivity with the antibody to SC1. These were dialyzed against 50 mM BisTris, pH 7.0, and applied to a Q-Sepharose (Amersham Biosciences) column equilibrated in the same buffer. The recombinant protein was eluted at 0.35–0.77 M NaCl and was further purified by gel filtration on a HiTrap heparin column (Amersham Biosciences) equilibrated in 10 mM sodium phosphate, pH 7.0. The protein was eluted at 0.21 M NaCl. After concentration on a Resource Q (Amersham Biosciences) column equilibrated in 20 mM BisTris, pH 6.0, the protein was further purified by gel filtration on a Sephadex G-75 (Amersham Biosciences) in 0.2 M NaCl, 50 mM BisTris, pH 7.4. The purified FS-EC domain pair was used to immunize a rabbit. The antiserum was purified by affinity chromatography on a column with the antigen coupled to CNBr-activated Sepharose (Amersham Biosciences).

**Binding of SC1 to Reconstituted Collagen I Fibrils**—Solutions of highly purified chicken collagen I were prepared in 0.4 M NaCl, 0.1 M Tris-HCl, pH 7.4. Samples were degassed under vacuum and then transferred to microcuvettes (light path, 1 cm) and mixed with an equal volume of distilled water at 4 °C. The cuvettes were sealed and placed into a spectrophotometer (Beckman UV 640, equipped with a multiecell holder Micro Auto 12) connected to a water bath at 37 °C. Aggregation was followed by monitoring turbidity at 313 nm. When a plateau was reached, aliquots were spotted onto Paraffin sheets. Nickel grids coated with 1% uranylacetate in 2% glutaraldehyde were used for 5 min on dropcoated samples for electron microscopy. The grids were washed with PBS and treated for 30 min with 5% bovine serum albumin in PBS. The adsorbed fibrils were allowed to react with full-length SC1 or the FS-EC domain pair in 0.15 M NaCl, 50 mM Tris-HCl, pH 7.4, containing 10 mM CaCl2 for 90 min. After washing 5 times with PBS, the grids were incubated with the affinity-purified SC1 antibody in PBS containing 0.2% (w/v) dried skim milk and, after renewed washing with a suspension of colloidal gold particles (18 nm), coated with goat antibodies to rabbit IgG (Jackson Immunoresearch) in PBS containing 0.2% (w/v) dried skim milk. Finally, the grids were washed with distilled water and negatively stained with 2% uranyl acetate for 10 min. In control experiments SC1 or the first antibody was omitted. Electron micrographs were taken at 80 kV with a Philips CM 10 electron microscope.

**Immunofluorescence Microscopy of SaOS-2 Cell Layers**—The human osteosarcoma cell line SaOS-2 (DSMZ, Braunschweig) was cultivated in McCoy’s 5A medium supplemented with 10% of fetal calf serum, 2 mM
FIG. 1. Domain composition of recombinant full-length and truncated SC1 proteins. FS denotes the follistatin-like domain, and EC denotes the globular extracellular calcium binding domain. The black bars represent the acidic N-terminal domain I. Signal peptides as well as the tag on the I-FS protein have been omitted. /f, full-length.

RESULTS

Recombinant Production of Full-length and Truncated SC1 Proteins—Eukaryotic cells were used for protein expression to ensure proper folding and posttranslational modifications. The use of the BM-40 signal peptide gave targeting through the endoplasmic reticulum and Golgi apparatus and secretion into the medium from which recombinant proteins could be purified. The human embryonic kidney cell line ERNA-293 was initially used for expression of all four constructs, corresponding to full-length SC1, the I-FS and FS-EC domain pairs, and the EC domain (Fig. 1). Although the shorter constructs were produced at acceptable levels, expression of full-length SC1 corresponded. The human embryonic kidney cell line EBNA-293 was initially used for expression of all four constructs, corresponding to full-length SC1, the I-FS and FS-EC domain pairs, and the EC domain (Fig. 1). Although the shorter constructs were produced at acceptable levels, expression of full-length SC1 corresponded.

Electron Microscopic Appearance of Full-length SC1—The purified full-length SC1 was visualized by negative staining electron microscopy (Fig. 3). SC1 was seen as a monomeric particle with a length of about 25 nm composed of a globule with a diameter of 9.5 nm attached to a thread-like structure with a length of about 15 nm. Because the x-ray structure of the EC-FC domain pair from the homologous protein BM-40 shows a rather compact fold (20), the globule is likely to correspond to the FS and EC domains that make up the C-terminal part of SC1, whereas the thread-like structure is contributed by the highly acidic N-terminal domain I.

Conformation and Calcium Binding of Full-length and Truncated SC1—The conformations of the recombinant proteins were analyzed by circular dichroism in the far-ultraviolet region. Spectra of full-length SC1 and the I-FS domain pair exhibited a marked negative band at 200 nm, indicating a large content of unordered structures (Fig. 4). In contrast the FS-EC domain pair and the isolated EC domain displayed shoulders at 222 nm typical for a-helices. Analysis of the CD spectra (Table II) suggested that the EC domain has the highest content of a-helices followed by the FS-EC domain pair, which also shows a

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SC1 fl  
I  
FS  
EC  

I-FSag  
I  
FS  

FS-EC  
I  
FS  
EC  

EC  

FIG. 2. SDS-PAGE of purified recombinant SC1 proteins. The purified full-length (f) SC1 (lanes 1 and 5), I-FSag (lanes 2 and 6), FS-EC (lanes 3 and 7), and EC (lanes 4 and 8) were submitted to SDS-PAGE under non-reducing (lanes 1–4) and reducing (lanes 5–7) conditions. The 12% polyacrylamide gel was stained with Coomassie Brilliant Blue. The molecular mass of marker proteins are given at the left margin.

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high content of $\beta$-sheet. Domain I consists largely of unordered structures, which it contributes to full-length SC1 and the I-FS domain pair. All four proteins gave similar CD spectra when equilibrated in 2 mM CaCl$_2$ or 4 mM EDTA (Fig. 4).

The potential calcium dependence of SC1 conformation was also studied by intrinsic fluorescence. Upon excitation at 280 nm all proteins showed emission maxima between 339 and 351 nm that were not changed by the addition of 2 mM CaCl$_2$ (Fig. 5). However, calcium addition led to a decrease in fluorescence intensity for all proteins containing the EC domain, i.e., full-length SC1 (24%), the pair of FS and EC domains (64%), and the single EC domain (97%) itself. The conformational change was reversible when EDTA was added in excess. In contrast, the fluorescence signal of the I-FS domain pair was not influenced by calcium (results not shown).

**Table I**

|                      | SC1 fl | I-FS$_{tag}$ | FS-EC | EC | FS | I |
|----------------------|--------|--------------|-------|----|----|---|
| $M_r$ calculated [Da]| 70,866 | 57,632       | 27,473| 18,585 | 8,888 | 43,513 |
| $M_r$ [Da]           | 87,779 | 74,367       | 29,583| 18,589 | 10,994 | 58,192 |
| $M_r$ after N-glycosidase F [Da]| 83,017 | 69,577 | 27,474 | 18,806 | 8,868 | 55,543 |
| $M_r$ of N-glycans [Da]| 4,755  | 4,792 | 2,109 | 0 | 2,126 | 2,649 |
| $M_r$ of other posttranslational modifications [Da]| 12,031 | 11,943 | 1 | 21 | 0 | 12,030 |
| $M_r$ after endoglycosidase H [Da]| 85,335 | 71,984 | 27,769 | 18,606 | 9,163 | 57,566 |
| $M_r$ of high mannose and/or hybrid glycans [Da]| 2,440 | 2,383 | 1,814 | 0 | 1,831 | 626 |

* Experimentally determined by MALDI-TOF mass spectrometry and used to calculate the other values.

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a true SC1 fragment or variant, recombinant full-length SC1 protein and kidney tissue extract were tested in an inhibition experiment. A dilution of the affinity-purified antibodies was preincubated with the recombinant FS-EC domain pair against which the antibodies were originally raised. This completely abolished antibody binding to both the full-length recombinant SC1 and to the 55-kDa band from kidney extracts, whereas a control sample of the same antibody dilution without the addition of inhibitor gave strong signals (Fig. 8B).

**DISCUSSION**

SC1 has been proposed to perform a number of important functions in development and regeneration of the central nervous system (1, 16, 24–26) in the adhesion of high endothelial venule cells (2, 5), in muscle differentiation (9), and in the maturation of B-lymphocytes (10–12). However, the structural features of SC1 have not been characterized beyond sequence interpretation (1, 2), and even though it is described as an extracellular matrix protein, its mode of integration into extracellular matrices has not been investigated. We therefore undertook the systematic expression of full-length and truncated SC1 proteins to be able to determine some of their important structural features and to provide a basis for future mechanistic studies of SC1 functions. Two important properties of BM-40 are the functionality of the EC domain in calcium binding and the ability to bind fibrillar collagens. Therefore, we investigated if both are conserved in SC1. To determine whether a collagen binding activity can be of physiological relevance, we studied SC1 to determine if it occurs in close association with fibrillar collagens and is expressed in tissues rich in a collagenous matrix.

Full-length SC1 was recombinantly expressed in the human fibrosarcoma cell line HT1080 and the truncated SC1 proteins (Fig. 1) in the human embryonal kidney cell line EBNA-293. Both of these cell types have been used frequently for the expression of other mammalian proteins and have proven to reliably provide a correctly folded product with the right pattern of disulfide bonds. We recently compared the glycosylation pattern of human bone sialoprotein expressed in EBNA-293 cells with that of the same protein purified from human bone and found that, although differences were detected in both N- and O-linked glycans, functional properties such as enhancement of cell attachment and hydroxyapatite binding were retained in both forms (15). Because there is no abundant tissue source for purification, it has not been possible to perform an equally detailed analysis of SC1. Nevertheless, the consistency of the mobilities of the tissue-derived and the recombinant full-length protein in SDS-PAGE (Fig. 8) indicate considerable similarities in processing.

Both full-length SC1 and the I-FS domain pair showed a lower mobility in SDS-PAGE than expected from the molecular mass calculated from the amino acid sequence. A part of the discrepancy could be explained by the presence of N-glycans on...
both the I and the FS domain (Table I). Digestion with endoglycosidase H indicated that the N-glycans on the FS domain are mainly of high mannose or hybrid type, whereas those on domain I are predominantly of complex type. However, analysis of SC1 proteins that had been digested with N-glycanase F with MALDI-TOF mass spectrometry indicated the presence of a further $\mu$H11011 kDa of posttranslational modifications. Likely candidates are O-glycosidically linked oligosaccharides and, with an algorithm for prediction of O-glycosylation sites (NetOGlyc) (27), eight potential O-glycosylation sites encompassing six threonines and two serines are predicted within domain I. As expected from the MALDI-TOF analysis, no O-glycosylation sites are predicted for the FS and the EC domains. The most likely explanation for the discrepancies in measured molecular mass determined by SDS-PAGE (116 kDa) and MALDI-TOF mass spectrometry (88 kDa) is that the highly negatively charged domain I (106 acidic residues of 400 residues) causes an atypical binding of SDS to SC1, leading to an anomalous electrophoretic behavior.

The predicted domain structure of SC1 could be partially confirmed by electron microscopy of recombinant SC1. The full-length protein is seen as a flexible tail of about 15-nm length connected to a globular structure of 9.5-nm diameter (Fig. 3). The x-ray structure of the FS-EC domain pair from BM-40 shows a comparatively compact fold and a close interaction between these domains, yielding a roughly globular structure of about the same dimensions as the SC1 globule seen by electron microscopy (20). Accordingly, the flexible tail must be the domain I, a conclusion that is also supported by the high content of unordered secondary structure in this domain (see below). This domain has been shown to increase the survival and proliferation of B lineage lymphocytes and their precursors (10–12). Its extended flexible structure will make it well available to cells also if SC1 is anchored in the extracellular matrix. It may be that its high negative charge density and potential O-linked glycosylation plays a role in its receptor interactions.

Circular dichroism spectroscopy showed that the EC domain is rich in $\alpha$-helix and that the FS domain has a comparatively high content of $\beta$-structure (Fig. 4, Table II). This is in good agreement with the detailed knowledge of the three-dimensional structure of the homologous domains in BM-40 (28, 20) and structure-based sequence alignments. On the other hand, the domain I contains largely unordered secondary structure,
in agreement with its thread-like appearance in electron microscopy. The circular dichroism spectra obtained in the presence of saturating amounts of calcium or with excess EDTA were closely similar, pointing to a rearrangement of secondary structure in SC1 upon calcium binding that is too small to be detected by circular dichroism. In contrast, large and reversible changes in intrinsic fluorescence were detected for all SC1 proteins containing the EC domain when calcium was added and removed (Fig. 5). This demonstrates that the SC1 EC domain is active in calcium binding, which is an agreement with the fact that both EF-hands are conserved when compared with BM-40. EF-hand 2 is a classic EF-hand as seen in multiple cytosolic EF-hand proteins in which the acidic side chains of residues at position 1, 3, 5, and 12 within the EF-hand loop mainly contribute to calcium binding. The EF-hand 1 of BM-40 is of a non-classical type as it contains a one-amino acid insertion and a proline in cis conformation in the EF-hand loop. This leads to a structural rearrangement such that the carbonyl oxygen of the proline coordinates the calcium ion instead of an oxygen from the side chain of residue 3 (28). In SC1 the residues of EF-hand 1 including the insertion and the proline are strictly conserved compared with BM-40; therefore, calcium coordination is most probably identical to the one seen in BM-40. The fluorescence change upon calcium addition further shows that the calcium binding induces a conformational change even though this could not be detected by circular dichroism. Calcium-induced conformational changes as well as a specific proteolytic cleavage that may have related structural consequences have been shown to enhance the binding of BM-40 to collagen (21, 22, 29). Only the I-FS domain pair was unaffected by calcium binding, as determined by intrinsic fluorescence, making it likely that these domains do not bind.

**Fig. 7.** Immunofluorescence laser scanning microscopy of SC1 and collagen I in monolayer cultures of SaOS-2 osteosarcoma cells. Cells were cultivated in the presence (A–C) or absence of ascorbate (D–F). In A and D SC1 was detected with affinity-purified rabbit antibodies raised against the purified FS-EC domain pair and a DTAF-conjugated affinity-pure goat anti-rabbit IgG (green). In C and F collagen I (Col. I) was detected with a mouse monoclonal antibody followed by a Cy3™-conjugated goat anti-mouse IgG (red). B and E are merged images. The bar corresponds to 40 μm.

**Fig. 8.** Immunoblot analysis of SC1 in mouse tissue extracts. A, mouse tissues were extracted with 5 volumes (ml/g) of 0.15 M NaCl, 50 mM Tris-HCl containing 10 mM EDTA, and protease inhibitors. Aliquots were applied to 12% SDS-polyacrylamide gels under reducing conditions, and SC1 was detected by immunoblotting using an affinity-purified antiserum to SC1. B, recombinant full-length SC1 and kidney extracts were submitted to SDS-PAGE followed by immunoblotting as in A. Inhibition of specific antibody binding was performed by incubation of a dilution of the affinity-purified antiserum to SC1 with recombinant FS-EC protein, the antigen against which the serum was originally raised. The control lanes were developed with an identical antiserum dilution that was not treated with inhibitor.
calcium with high affinity or that calcium binding does not strongly affect their fold.

BM-40 has been shown to bind strongly to several collagens (21, 22), and Mov13 mice, which are deficient in collagen I, do not retain BM-40 in their extracellular matrix (23). We therefore determined if the collagen affinity of BM-40 is conserved in SC1 and if this may indeed be a mechanism by which SC1 is anchored in extracellular matrices. Immunogold electron microscopy showed that both full-length SC1 and the FS-EC domain pair bind to the surface of fibrils reconstituted from purified collagen I (Fig. 6). Furthermore, when monolayers of SaOS-2 osteosarcoma cells, which express both SC1 and collagen I, were studied by double immunofluorescence microscopy, we observed a close colocalization between these proteins in filaments of the extracellular matrix (Fig. 7, A–C). When the same cell line was grown in the absence of ascorbate, resulting in intracellular retention of collagen due to decreased activity of the prolyl hydroxylase, such extracellular deposition of SC1 was lost in parallel with that of collagen I (Fig. 7, D–F). Indeed, the intracellular staining for both proteins was increased, creating the impression that SC1 was retained in compartments of the secretory pathway when collagen I could not be processed and externalized. It may be that complexes between collagen I and SC1 may be formed already in the endoplasmic reticulum or in the Golgi apparatus.

A screen performed by immunoblot of tissue extracts showed a broad distribution of SC1, although the largest amounts were found in brain (Fig. 8). The only two organs in which no SC1 could be detected by extraction and immunoblot was liver and adrenal gland, tissues characterized by a high cellularity and a low collagen content. In tissue extracts, SC1 was detected mainly as an SDS-PAGE band at 116 kDa, corresponding to the mature protein. However, some lower bands were also specifically stained, and one of these, at 55 kDa, was the major SC1 reactivity in muscle and the only band in kidney. There is no evidence in the literature for alternative splicing of SC1 mRNA, and it is, therefore, most likely that the 55-kDa band results from a proteolytic cleavage that is particularly prominent in certain tissues. Because the antibodies were raised against the purified FS-EC domain pair, the fragment must contain epitopes from these domains. Considering the flexible and unordered nature of domain I, it is tempting to propose a cleavage in this domain, releasing a large part of it from the rest of the molecule. Because domain I has been implicated in interactions with B-lymphocyte precursor cells (11), it may be that cellular interactions of SC1 may be modulated by such cleavage.

REFERENCES
1. Johnston, I. G., Paladino, T., Gurd, J. W., and Brown, I. R. (1990) Neuron 4, 165–176
2. Girard, J. P. and Springer, T. A. (1995) Immunity 2, 119–123
3. Vannahme, C., Smyth, N., Misgeo, N., Giesel, S., Frie, C., Paulson, M., Maurer, P., and Hartmann, U. (2002) J. Biol. Chem. 277, 37977–37986
4. Bradshaw, A. D., and Sage, E. H. (2001) J. Clin. Invest. 107, 1049–1054
5. Girard, J. P. and Springer, T. A. (1996) J. Biol. Chem. 271, 4511–4517
6. Nelson, P. S., Plymate, S. R., Wang, R., True, L. D., Ware, J. L., Gan, L., Liu, A. Y., and Hood, L. (1998) Cancer Res. 58, 232–236
7. Bendik, I., Schraml, P., and Ludwig, C. U. (1999) Cancer Res. 58, 626–629
8. Claeskens, A., Ongenae, N., Neefs, J. M., Cheyns, P., Kuijen, P., Cools, M., and Kutz, E. (2000) Br. J. Cancer 82, 1123–1130
9. Ringneste, M., Rogers, I., Varmuza, S., Rush, S., and Brown, I. R. (1998) Dev. Genes Evol. 208, 493–496
10. Oritani, K., and Kincaid, P. W. (1996) J. Cell Biol. 134, 771–782
11. Oritani, K., Kanakura, Y., Aoyama, K., Yokota, T., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Tomiyama, Y., Matsuzawa, Y., and Kincaid, P. W. (1997) Blood 90, 3404–3413
12. Oritani, K., and Kincaid, P. W. (1998) Leuk. Lymphoma 32, 1–7
13. McKinnon, P. J., McLaughlin, S. K., Kasperski, M., and Margulies, R. F. (2000) Mol. Cell. Biol. 20, 656–660
14. Kohfeldt, E., Maurer, P., Vannahme, C., and Timpl, R. (1997) FEBS Lett. 414, 557–561
15. Wuttke, M., Muller, S., Nitsche, D. P., Paulsson, M., Hanisch, F.-G., and Maurer, P. (2001) J. Biol. Chem. 276, 36839–36848
16. McKinnon P. J. and Margulies, R. F. (1996) Brain Res. 709, 27–36
17. Vorm, O., and Mann, M. (1994) J. Am. Soc. Mass Spectrom. 5, 955–958
18. Engel, J., and Furcht, J. (1987) Methods Enzymol. 145, 3–78
19. Gill, S. C., and von Hippel, P. H. (1998) Anal. Biochem. 182, 319–326
20. Hohenester, E., Maurer, P., and Timpl, R. (1997) EMBO J. 16, 3778–3786
21. Maurer, P., Hohenadl, C., Hohenester, E., Gohring, W., Timpl, R., and Engel, J. (1995) J. Mol. Biol. 233, 347–357
22. Sasaki, T., Hohenester, E., Gohring, W., and Timpl, R. (1998) EMBO J. 17, 365–1634
23. Iruela-Arispe, M. L., Vernos, R. O., Wu, H., Jaenisch, R., and Sage, E. (1996) Dev. Dyn. 207, 171–183
24. Mendis, D. B., Shahin, S., Gurd, J. W., and Brown, I. R. (1994) Brain Res. 331, 197–205
25. Mendis, D. B., Shahin, S., Gurd, J. W., and Brown, I. R. (1996) Brain Res. 713, 53–63
26. Mendis, D. B., Ivy, G. O., and Brown, I. R. (1996) Brain Res. 730, 95–106
27. Hansen, J. E., Lund, O., Rapacki, K., and Brunak, S. (1997) Nucleic Acids Res. 25, 278–285
28. Hohenester, E., Maurer, P., Hohenadl, C., Timpl, R., Jansonius, J. N., and Engel, J. (1996) Nat. Struct. Biol. 3, 67–73
29. Sasaki, T., Gohring, W., Mann, K., Maurer, P., Hohenester, E., Knauper, V., Murphy, G., and Timpl, R. (1997) J. Biol. Chem. 272, 9237–9243
30. Hennessey, J. P., Jr. and Johnson, W. C., Jr. (1981) Biochemistry 20, 1085–1094