Functional Mapping of SPARC: Peptides from Two Distinct Ca^{++}-binding Sites Modulate Cell Shape

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Abstract. Using synthetic peptides, we have identified two distinct regions of the glycoprotein SPARC (Secreted Protein Acidic and Rich in Cysteine) (osteonectin/BM-40) that inhibit cell spreading. One of these sites also contributes to the affinity of SPARC for extracellular matrix components. Peptides representing subregions of SPARC were synthesized and antipeptide antibodies were produced. Immunoglobulin fractions of sera recognizing an NH_2-terminal peptide (designated 1.1) blocked SPARC-mediated anti-spreading activity. Furthermore, when peptides were added to newly plated endothelial cells or fibroblasts, peptide 1.1 and a peptide corresponding to the COOH terminal EF-hand domain (designated 4.2) inhibited cell spreading in a dose-dependent manner. These peptides exhibited anti-spreading activity at concentrations from 0.1 to 1 mM. The ability of peptides 1.1 and 4.2 to modulate cell shape was augmented by an inhibitor of protein synthesis and was blocked by specific anti-peptide immunoglobulins. In addition to blocking cell spreading, peptide 4.2 competed for binding of [^{125}I]SPARC and exhibited differential affinity for extracellular matrix molecules in solid-phase binding assays. The binding of peptide 4.2 to matrix components was Ca^{++}-dependent and displayed specificities similar to those of native SPARC. These studies demonstrate that both anti-spreading activity and affinity for collagens are functions of unique regions within the SPARC amino acid sequence. The finding that two separate regions of the SPARC protein contribute to its anti-spreading activity lead us to propose that multiple regions of the protein act in concert to regulate the interactions of cells with their extracellular matrix.

Although our understanding of the molecular bases of cell attachment and spreading has increased appreciably, the processes that regulate these phenomena are complex and less well defined. It is clear that cellular adhesion is a dynamic phenomenon. Migratory or transformed cells have fewer focal contacts and exhibit reduced spreading as compared to stationary or nontransformed cells. In nontransformed cells, focal contacts disassemble during mitosis and cell migration, with a resultant temporary change in cell shape (Burridge et al., 1988; Couchman and Rees, 1979; Woods and Couchman, 1988). Molecules that have been implicated as regulators of cell shape because of their effects on focal contacts include proteases, growth factors, phorbol esters, and agents that increase intracellular cAMP (Burridge et al., 1988; Herman and Pledger, 1985; Herman et al., 1987; Schliwa et al., 1984). In addition, several extracellular glycoproteins have recently been described as anti-spreading factors; i.e., proteins that regulate the adhesive characteristics of cells by altering contacts between cells and their extracellular matrix.

SPARC[1] (osteonectin/BM-40), a developmentally regulated, secreted glycoprotein, is associated with cellular events requiring tissue remodeling, cell movement, and/or proliferation (Termine et al., 1981; Holland et al., 1987; Howe et al., 1988; Wewer et al., 1988; Mason et al., 1986*b*; Mann et al., 1987; Sage et al., 1984; Sage et al., 1989*a*). Levels of SPARC protein are elevated in response to cellular injury after endotoxin treatment, or as a result of culture shock (Sage et al., 1986). Recently, SPARC has been shown to induce cell rounding in spread monolayers of cultured endothelial cells and fibroblasts (Sage et al., 1989*b*) and may thus be classified, along with thrombospondin (Tsp) (Lahav, 1988; Murphy-Ullrich and Höök, 1989) and tenascin (Chiquet-Ehrismann et al., 1988), as a negative mediator of cellular spreading. The actions of these glycoproteins are distinct from those of adhesive proteins such as fibronectin and laminin.

While our information is limited, the mechanisms by which Tsp, tenascin, and SPARC influence spreading appear to be different. Tsp and tenascin have both adhesive and anti-adhe-

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1. *Abbreviations used in this paper*: BAEC, bovine aortic endothelial cell; CHX, cycloheximide; FCLF, fetal calf ligament fibroblast; SPARC, secreted protein acidic and rich in cysteine; Tsp, thrombospondin.

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sive properties that may be cell-specific (Chiquet-Ehrismann et al., 1988; Kruse et al., 1985; Lahav, 1988; Lahav et al., 1987; Tuszynski et al., 1987). Recently, unique adhesive and anti-adhesive regions have been identified in tenascin with fragments of the recombinant protein (Spring et al., 1989). The effects of Tsp, which contains an RGD sequence (Lawler and Hynes, 1986), are apparently mediated via specific cell surface integrins (Lawler et al., 1988). Although tenascin has no RGDS sequences, both Tsp and tenascin interfere with the binding of fibronectin to cells (Chiquet-Ehrismann et al., 1988; Lahav et al., 1987). In contrast, SPARC has no demonstrated adhesive characteristics for cells and disrupts spreading in a variety of cultured cell strains. This anti-spreading activity of SPARC appears to act by an integrin independent pathway (Sage et al., 1989*b*). That Tsp binds to SPARC with a K_d of 0.7 nM (Clezardin et al., 1988) suggests the possibility of an integrated regulatory pathway.

Synthetic peptides have now been used by a number of investigators to identify functional regions of complex proteins (Stewart and Young, 1984). The identification of adhesive regions of fibronectin (Ruoslahti and Pierschbacher, 1986; Ruoslahti and Pierschbacher, 1987; Dufour et al., 1988) and laminin (Graf et al., 1987*a*,*b*; Tashiro et al., 1989; Kleinman et al., 1989) demonstrated the feasibility of peptide reagents for studying cell–substrate adhesion. Important contributions have also been made in the identification of receptor binding sites (Morris et al., 1990) and in the analysis of specific amino acids involved in ligand binding sites (Parraga et al., 1990; Reid, 1990). In addition, peptide immunogens have proven valuable for the production of antisera against molecules for which sequence information was available (Tamura et al., 1983; Kamboj et al., 1989).

Here we report that antibodies raised against a peptide from the NH_2-terminal region of SPARC block SPARC-mediated anti-spreading activity. In addition, both the same NH_2-terminal peptide and a peptide derived from the COOH-terminal Ca^{++}-binding site inhibit cell spreading specifically. These results support the idea that SPARC acts via a Ca^{++}-dependent mechanism to modulate cell attachment (Sage et al., 1989*b*). We propose that this anti-spreading activity results from disruption of cell–substrate contacts and the subsequent reorganization of cytoskeletal elements. The synthesis and release of SPARC may be one of the options available to cells in the process of diminishing adhesive interactions with their substrates. The functional consequences of such actions include the regulation of cell shape, migratory potential, and the ability to divide.

Materials and Methods

Peptide Synthesis and Labeling

A series of 20-mers representing regions of the mouse SPARC amino acid sequence were synthesized by Dr. Patrick Chou (Howard Hughes Medical

Table I. Diagrammatical Representation of the SPARC Protein and the Location of Synthetic Peptides with Their Relative Anti-Cell Spreading Activities

A

Domains	I	II	III	IV
	Ca^{++}-Binding	Cysteine Rich	Helical Domain	Ca^{++}-Binding

NH_2 — CHO — COOH

Peptide	1.1	1.3	2.1	2.3	3.2	3.4	4.2
Anti-spreading	+	–	–	ND	–	–	+

B

Peptide	Sequence		Sequence location	$E^{1\ mM/280}_{1\ cm}$
1.1	QTEVAEEIVE	EETVVEETGV	5–23	1.28*
1.3	QVEMGEFEDG	AEETVVEEVVA	31–50	1.28*
2.1	CQNHHCKHG	KVCELDESNTP	54–73	0.36
2.3	TLEGTKKGHK	LHLDYIGP	113–130	1.28
3.2	KNVLVTLYER	DEGNNLLTEK	154–173	1.28
3.4	NEKRLEAGDI	PVELLARDFE	184–203	1.28*
4.2	TCDLDNDKYI	ALEEWAGCFG	254–273	7.21

(*A*) Peptides are named with reference to the predicted domain structure of murine SPARC (Engel et al., 1987). (+) Inhibition of spreading; (–) no inhibition observed; (ND), not determined. Bars indicate 20 amino acids. (*B*) Sequences of synthetic peptides used in these experiments. Sequences were derived from the predicted amino acid sequence of murine SPARC (Mason et al., 1986*a*). To determine peptide concentration, extinction coefficients ($E^{mM/280}_{cm}$) were calculated based on amino acid composition (Gill and von Hippel, 1989). Cysteine residues are underlined. (*) Extinction coefficients derived for peptides synthesized with an additional NH_2-terminal tyrosine residue. The single letter amino acid code has been used.

Institute, University of Washington, Seattle, WA) and Kathy Walker (ZymoGenetics Corp., Seattle, WA). Peptides were synthesized using either t-boc or f-moc chemistry (Applied BioSystems Inc., Foster City, CA). An NH_2-terminal Tyr residue was added in some cases to allow for iodination of the peptide. The location and amino acid sequences of the peptides used in this study are shown in Table I. Sequences were derived and numbered from the predicted amino acid sequence of mature mouse SPARC after removal of the signal sequence (Mason et al., 1986*a*). Thus, Ala_{18} of pre-SPARC is considered to be Ala_1 of the mature protein. Peptide nomenclature is based on the four structural regions deduced from the amino acid sequence (Engel et al., 1986). Thus, peptides 1.1 and 1.3 represent 20-mer peptides derived from the NH_2-terminal region 1. Peptides 2.1 and 2.2 are derived from region 2, etc.

Purity and homogeneity were achieved by analytical HPLC on a PRP-3 column (Hamilton Co., Reno, NV) with a linear gradient of 0–30% acetonitrile in 50 mM NaOH and 0.1% trifluoroacetic acid. For some experiments, peptides were purified on a semipreparative PRP-3 column with similar gradients in a 20 mM NH_4HCO_3 (pH 8.5) buffer. HPLC-purified peptides were lyophilized repeatedly to remove salts before use in cell culture experiments. Peptides were solubilized in dilute NaOH and neutralized with PBS (10 mM phosphate, pH 7.5, 150 mM NaCl). For some experiments, peptides 1.1, 3.2, and 4.2 were labeled with Na[^{125}I] using Iodogen according to protocols described by the supplier (Pierce Chemical Co., Rockford, IL). Specific activity was ~300 cpm/fmole peptide, but slight variations for each peptide were noted. Amino acid composition and concentration of peptide stock solutions were performed by amino acid analysis (ZymoGenetics Corp., Seattle, WA). Alternatively, peptide concentrations were determined by optical density using extinction coefficients calculated for each peptide (Gill and von Hippel, 1989) (Table I).

Cells and Cell Culture

All cells were maintained in DME medium (Gibco Laboratories, Grand Island, NY) containing 10% by volume FCS and antibiotics (Sage et al., 1989*b*). Primary cultures of bovine aortic endothelial cells (BAEC) were isolated as described by Schwartz (1978). Primary cultures of fetal calf ligamentum nuchae fibroblasts (FCLFs) (Sage and Mecham, 1982) were a gift of Dr. L. Fouser (University of Washington, Seattle, WA). Cells, used between passages 3 and 8, were thawed from stocks maintained in liquid N_2 and were passaged with trypsin/EDTA (Sage et al., 1989*b*). The mouse parietal yolk sac endodermal line PYS-2 was a gift from Dr. J. Lehman (Albany Medical College, Albany, NY).

Antibody Production and Characterization

Peptides were selected for antibody production based on our interest in their Ca^{++}-binding properties (1.1, 1.3, and 4.2) or based on predicted antigenicity. Antigenicity was assessed by the hydrophilicity algorithm of Hopp and Woods (1981). The sequence YERDEG (aa 161–166) contained within peptide 3.2 proved to have the highest hydrophilic character by these calculations. Peptides were conjugated to keyhole limpet hemocyanin (Sigma Chemical Co., St. Louis, MO) with carbodiimide (Story Chemical Corp., Muskegon, MI), according to protocols by which the amino (N-KLH)- or carboxy (C-KLH)-terminal residue is preferentially cross-linked (Tamura et al., 1983). Female New Zealand White rabbits were injected every 2 wk with 3 mg of a mixture of N-KLH, C-KLH, and free peptide (1:1:1) in Freund's adjuvant (Sigma Chemical Co.). Addition of tracer amounts of iodinated peptides suggested that 5–20 mol of peptide were bound per mole KLH (data not shown). Immunoglobulin fractions were prepared by precipitation of whole sera with 20% ammonium sulfate (wt/vol) ratio, and precipitates were dialyzed against PBS.

Titer and specificity of antisera were monitored by an ELISA in conjunction with an alkaline-phosphatase goat anti-rabbit IgG detection system as previously described (Sage et al., 1984). Peptides were conjugated to rabbit serum albumin (Sigma Chemical Co.) with glutaraldehyde (Harlow and Lane, 1988). Anti-peptide antisera were assayed for binding to homologous and heterologous peptide–rabbit serum albumin conjugates and to a variety of purified proteins.

Immune Precipitation of SPARC

Immunoprecipitations from cell culture supernatants were performed as previously described (Sage et al., 1984) with a few modifications. Subconfluent monolayers of PYS-2 cells were incubated with 20 μCi [^{35}S]Met/ml for 18 h in media lacking Met. Media were centrifuged at 12,000 *g* to remove cell debris, and a mixture of protease inhibitors was added (final concentrations: 0.25 mM EDTA, 5 mM *N*-ethylmaleimide, 0.1 μg/ml pepstatin A, 0.4 mM PMSF). Proteins in the media were preabsorbed with protein A-Sepharose (Pharmacia-LKB, Piscataway, NJ) to reduce nonspecific binding. Antibodies were added and immune complexes were precipitated with protein A-Sepharose. Precipitates were washed three times, and immune complexes were released with SDS-PAGE buffer at 95°C (0.1 M Tris-HCl pH 6.8, 2% SDS, 1 M urea, 10% glycerol) (Laemmli, 1970). Proteins released from protein A were reduced with 50 mM DTT and heated 1–3 min at 95°C. SDS-PAGE was performed on discontinuous acrylamide gels (4% stacking and 12.5% separating gels) at 25 mA. Molecular weight standards (Bethesda Research Laboratories, Gaithersburg, MD) included myosin H-chain (200 kD), phosphorylase b (97.4 kD), BSA (68 kD), chicken egg albumin (43 kD), α-chymotrypsinogen (27.5 kD), and lysozyme (14.6 kD). Gels were fixed and stained with Coomassie brilliant blue R-250 (0.25% in 45% methanol, 9% acetic acid). Destained gels were treated with 2,5-diphenyloxazole in DMSO and dried before exposure to x-ray film (X-Omat-RP, Kodak) at −70°C.

Antibody Specificity for Proteolytic Fragments of SPARC

SPARC was purified to >95% homogeneity from PYS cell culture medium as described by Sage et al. (1989*b*). 5 μg of SPARC was suspended in 20 μl TBS (50 mM Tris-HCl, pH 7.5, 150 mM NaCl) and treated for 30 min at 37°C with buffer alone, 0.1 μg TPCK-trypsin (Worthington Diagnostics, Freehold, NJ), or 2 μg TPCK-trypsin. The reaction was stopped with a 100-fold molar excess of soybean trypsin inhibitor (Sigma Chemical Co.) and SDS-PAGE buffer. Fragments were reduced with DTT, resolved by SDS-PAGE (4% stacking gel, 12.5% and 20% separating gels), and transferred to nitrocellulose (BA-85; Schleicher & Schuell Inc., Keene, NH). Transferred proteins were stained with amido black (Bio-Rad Laboratories, Richmond, CA), blocked in MT-buffer (PBS containing 1% nonfat dried milk, 0.05% Tween-20; Bio-Rad Laboratories), and incubated with antibodies as described in the figure legends. In some cases, antibodies were preincubated with MT-buffer containing 0.2 mM free peptide (Sage et al., 1989*b*).

Neutralization of SPARC Activity with Anti-peptide Antibodies

SPARC protein (10–20 μg/ml) was preincubated with 500 μg/ml of a purified immunoglobulin fraction from each antiserum (see above). BAEC were plated for 24 h as described above, except that the serum used was depleted of complement by heating at 55°C for 1 h. SPARC alone, or as immune complexes, was then added to BAEC and cell morphology was monitored for 24 h.

Effects of SPARC Peptides on Cellular Morphology

BAEC and FCLF were cultured as described above. Morphological effects of SPARC and peptides were determined by two procedures. (*a*) Cells were trypsinized, washed in 10% FCS/DME, washed in 1% FCS/DME, and subsequently plated on 24- or 48-well plates of tissue culture plastic (Costar, Cambridge, MA) in the presence of SPARC (1–40 μg/ml) or peptides (0.1–2 mM). For some experiments, peptides were preincubated with anti-peptide antibodies. Control experiments showed that spreading was not inhibited by antibodies alone. (*b*) Trypsinized cells were plated for 24 h on glass chamber slides (Lab-Tek, Nunc, Inc., Naperville, IL) in 10% FCS/DME. Cells were washed in serum free-DME and peptides added as above. Media were replaced with fresh 10% FCS/DME containing 5 μg/ml cycloheximide (CHX/DME; Sigma Chemical Co.) for 1 h after which peptides diluted in CHX/DME were added in fresh media to the cells. Cell morphology was assessed by visual inspection using an inverted phase photomicroscope (Carl Zeiss Inc.) and the images recorded on Ektachrome film (ET 135, 160 ASA; Kodak). Cells surrounded by a halo of refractility, as compared to untreated cells plated under similar conditions, were classified as "rounded."

For quantitation of anti-spreading activity of peptides on freshly plated cells (procedure 1), a scoring system was devised to characterize the degree of spreading within a group of cells. Unattached cells were removed by washing and the culture was photographed as above. Cell counts were determined and cells were scored as (*a*) spread, flattened cells with diminished cellular refractility; (*b*) unspread, rounded cells projecting short processes in the initial stages of spreading; and (*c*) round, highly refractile cells with no apparent processes. Cells representative of these three groups are indicated in Fig. 4. The number of cells in each group was then converted into a "rounding index" by the formula: rounding index = $[(1 \times a) + (2 \times b)$

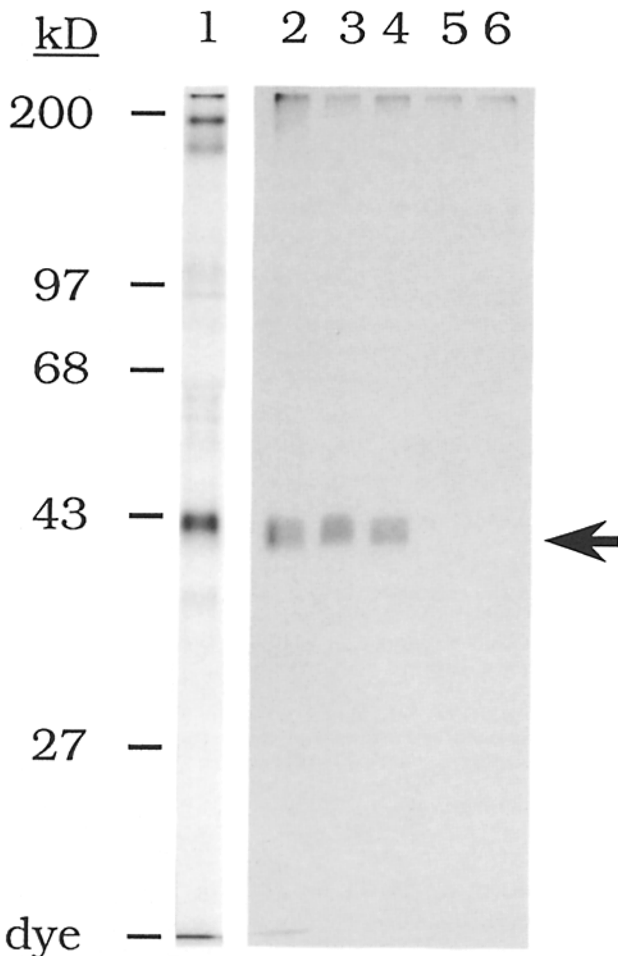


Figure 1. Anti-peptide 1.1 IgG precipitates SPARC from tissue culture media. PYS cells were labeled for 12 h with [^{35}S]Met, and the media were centrifuged and treated with protease inhibitors. Proteins were resolved by SDS-PAGE of (lane *1*) unfractionated media; (lane *2*) anti-SPARC immunoprecipitate; (lanes *3* and *4*) antipeptide 1.1 immunoprecipitate, serum from two different rabbits; (lanes *5* and *6*) anti-peptide 4.2 immunoprecipitate, serum from two different rabbits. Proteins were resolved in the presence of DTT and were visualized by fluorescent autoradiography. Arrow indicates the location of the SPARC doublet.

$+ (3 \times c)]/(a + b + c)$, where a, b, and c are the number of cells in each group. An index of 1 thus represents a culture with only spread cells. A culture with increasing numbers of unspread and round cells would approach the maximum rounding index of 3. Values were calculated for two independent cultures and graphed as the average ± SE.

Binding to Purified Extracellular Proteins

Solid-phase binding assays were conducted in polystyrene microtiter wells as previously described (Sage et al., 1989*b*). BSA (Pentex grade; Miles Laboratories, Inc., Naperville, IL) and ovalbumin (Sigma Chemical Co.) were solubilized in TBS. Native collagen types I (lathyritic rat skin), II (bovine cartilage), III (bovine skin), IV (bovine placenta), V (human amnion), and VIII (bovine Descemet's membrane) were isolated as described (Sage et al., 1989*b*). SPARC and Tsp were prepared as described (Sage et al., 1989*b*). Collagens were solubilized in 0.1 N acetic acid, and dialyzed against TBS. Solubilized proteins were coated onto microtiter wells for 4–12 h (Removawell strips, Immulon; Dynatech Laboratories Inc., Alexandria, VA).

To assay for peptides that competed for the binding of SPARC to collagens, collagen types I and III were coated on microtiter wells and additional binding sites were blocked with Hepes binding buffer (20 mM Hepes, 130 mM NaCl, 1 mM $CaCl_2$, 3 mM KCl, 1 mM $MgCl_2$, 0.1 mM $CuSO_4$) containing 0.1% ovalbumin (Badet et al., 1989). ^{125}I-Labeled SPARC was added to wells containing substrate-adsorbed proteins for 4–12 h, at 4°C. Wells were washed three times with Hepes binding buffer and bound radioactivity was measured by a gamma counter (Beckman Instruments Inc., Palo Alto, CA). Assays were done in triplicate and values graphed as the mean ± SD. To determine specific binding, excess unlabeled ligand was mixed with labeled ligand before addition to substrate-coated wells. In competition assays, label was mixed with individual peptides before addition to wells.

To determine whether peptides would bind extracellular matrix components directly, purified proteins were plated in microtiter wells and excess binding sites blocked with TBS containing 1% ovalbumin. ^{125}I-Labeled peptides were then added for 4–12 h, at 4°C with or without 5 mM $CaCl_2$; unbound radioactivity was removed with TBS containing 5 mM $CaCl_2$ and 1% ovalbumin. Quantitation of substrate protein bound to plates was performed by amino acid analysis of material solubilized by constant boiling HCl at 110°C. Bound radioactivity was corrected for pmoles of substrate on each well. Assays were done in triplicate, and values were graphed as the mean ± SD.

Results

Anti-peptide Antibodies Recognize SPARC in a Conformation-specific Manner

In preliminary studies, affinity for immunizing (homologous) peptide and for native SPARC were assessed by ELISA (data not shown). All sera reacted with homologous peptides (titers of 10^{-3}–10^{-4}) but not with heterologous peptides. Furthermore, sera did not react with BSA, ovalbumin, or 70 K protein (a serum protein often bound to native SPARC). Only anti-peptide 1.1 antibodies reacted with native SPARC in these assays. Antibodies displayed negligible cross-reactivity with heterologous peptide–rabbit serum albumin conjugates. Reactivity to SPARC depended on the peptide immunogen and did not depend on the immunized animal ($n = 2$).

To confirm these results, radioimmune precipitations were performed on media from PYS cells. Anti-SPARC (Fig. 1, lane *2*) and anti-peptide 1.1 antibodies from two different rabbits (Fig. 1, lanes *3* and *4*) precipitated SPARC as a doublet (due to differential glycosylation) from these cultures. Antibodies generated to other peptides did not precipitate the 43-kD species (Fig. 1, lanes *5* and *6*). Radioimmune precipitations of ^{125}I-labeled SPARC were also performed. Precipitations with anti-SPARC and anti-peptide 1.1 antibodies precipitated 43-kD SPARC. Preincubation of anti-peptide 1.1 antibodies with 0.2 mM peptide 1.1 competed for this binding by >95%. Antibodies against peptide 3.2 had no reactivity and antibodies against peptide 4.2 precipitated only trace amounts of the ^{125}I-labeled SPARC (data not shown).

Upon reduction or certain types of denaturation, additional epitopes were exposed. Western blots of partially purified SPARC (Fig. 2 *b*) confirmed that unreduced SPARC was specifically recognized by anti-peptide 1.1 antibodies and to a lesser extent by anti-peptide 1.3 antibodies, but not by antibodies raised against other peptides. After reduction, SPARC was recognized by all antibodies (Fig. 2 *b*). These results, which are summarized in Table II, suggested that the anti-peptide antibodies showed conformational specificity and that denatured SPARC does not display the same epitopes present on the native molecule. Only the NH_2 terminus of native SPARC seemed to provide an antigenic surface similar to that of its homologous synthetic peptide (1.1).

Because SPARC isolated from tissues is often proteolyti-

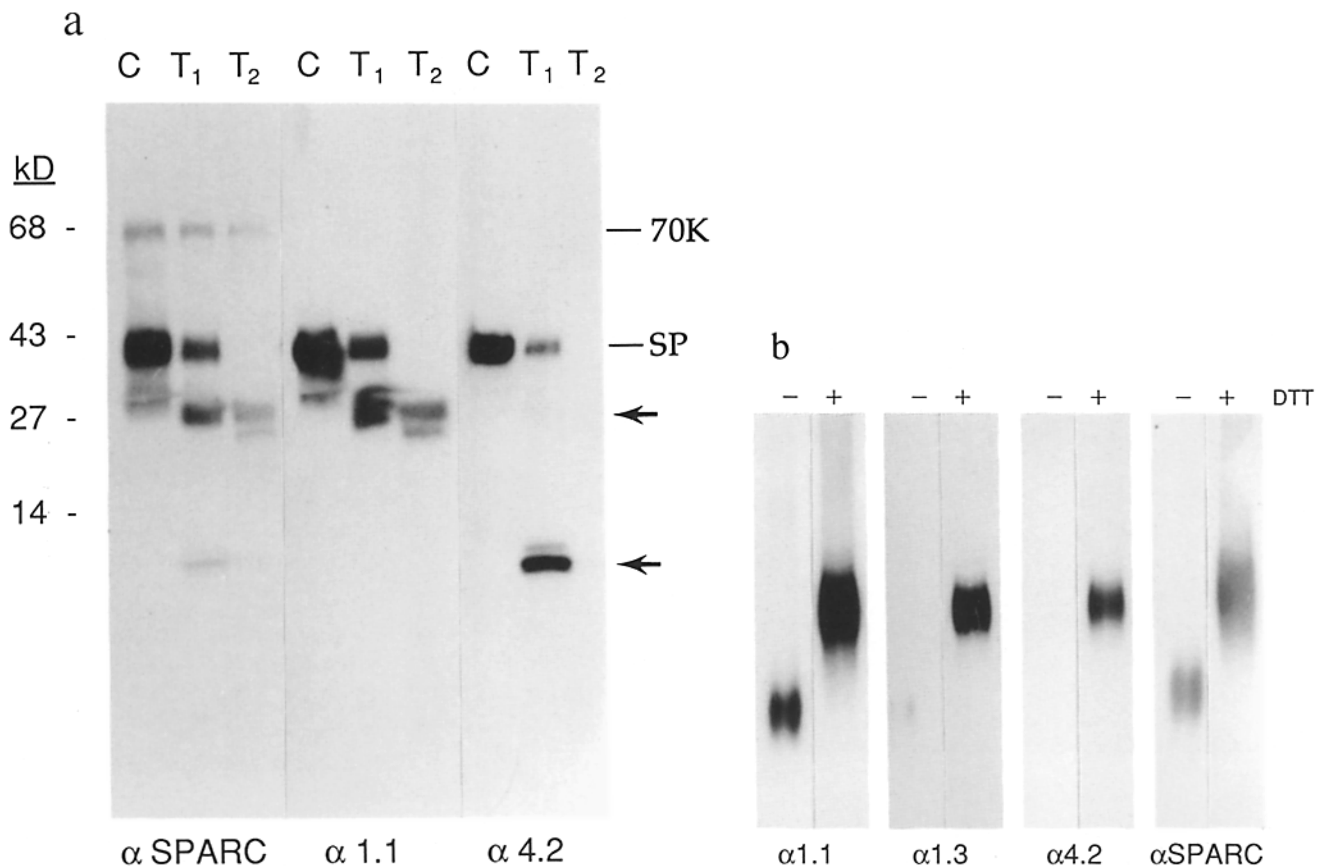


Figure 2. Anti-peptide antibodies recognize SPARC in a regional and conformation-specific manner. (*a*) Partially purified SPARC was subjected to limited proteolysis. Fragments were resolved by SDS-PAGE, transferred to nitrocellulose, and probed with antisera. (*C*) Reaction without enzyme; (T_1) 0.1 μg trypsin; (T_2) 2 μg trypsin. 43-kD SPARC (*SP*) is cleaved to 28-kD NH_2-terminal fragment and a 10-kD COOH-terminal fragment (*arrows*). The 70-kD protein (*70K*) recognized by anti-SPARC (*αSPARC*) polyclonal sera is a serum protein structurally unrelated to SPARC (Sage et al., 1986). Replicate blots were probed with anti-SPARC (*αSPARC*) polyclonal sera; antipeptide 1.1 (*α1.1*); and anti-peptide 4.2 (*α4.2*). (*b*) Samples of SPARC were resolved by SDS-PAGE in the absence (−) or presence (+) of a reducing agent (*DTT*) and transferred to nitrocellulose. Blots were probed serially with sera, stripped with 0.2 M glycine (pH 2.2), and reprobed. Blots were exposed to film for 20 h, and stripped blots were exposed for 48 h before incubation with second antibody to ensure no carryover between probings.

cally processed to a 28-kD polypeptide, we wanted to determine whether anti-peptide antibodies could identify SPARC fragments. Partially purified SPARC was digested with trypsin and the proteolytic fragments were separated by SDS-PAGE under denaturing conditions (Fig. 2 *a*). Western blots of the resolved fragments demonstrated the specificity of the various antibodies. Anti-SPARC antibodies (αSPARC) reacted with intact SPARC (43 kD), the NH_2-terminal fragment (a doublet at 28 kD), a COOH-terminal fragment (10 kD), and a 70-kD serum component (70 K) that copurifies with SPARC. Anti-peptide 1.1 (α1.1) recognized intact SPARC and the 28-kD NH_2-terminal doublet but failed to detect the COOH-terminal fragment. Anti-peptide 4.2 (α4.2) recognized intact SPARC and the COOH-terminal 10-kD fragment. Consistent with the COOH-terminal location of region IV, anti-peptide 4.2 antibodies failed to recognize the NH_2-terminal fragment. Neither of the anti-peptide antibodies detected the 70 K protein.

Anti-peptide Antibodies Block SPARC

Peptide 1.1 antibodies blocked the anti–cell spreading activ-

Table II. Recognition of SPARC by Anti-peptide Antibodies is Dependent on Conformation

	Immunogen	Antibody reactivities: Self*	Native‡ SPARC	Reduced§ SPARC	Block‖ SPARC rounding
1.	1.1	++	++	++	++
2.	1.3	++	+/−	+	−
3.	2.1	++	−	+	−
4.	3.2	++	−	+/−	−
5.	3.4	++	−	+	−
6.	4.2	++	+/−	++	−
7.	SPARC	++	++	++	++

Anti-peptide antisera specific for different regions of the SPARC molecule were scored in three separate assays for their ability to bind the intact SPARC protein.

* Specific binding to each immunogen was measured by ELISA (Self).

‡ Binding to native SPARC was measured by an immunoprecipitation assay.

§ Binding to denatured SPARC was measured after reduction and Western blotting.

‖ Ability to inhibit bioactivity of SPARC was measured by preincubating SPARC with antisera before adding to cells.

(+) Positive; (−) negative.

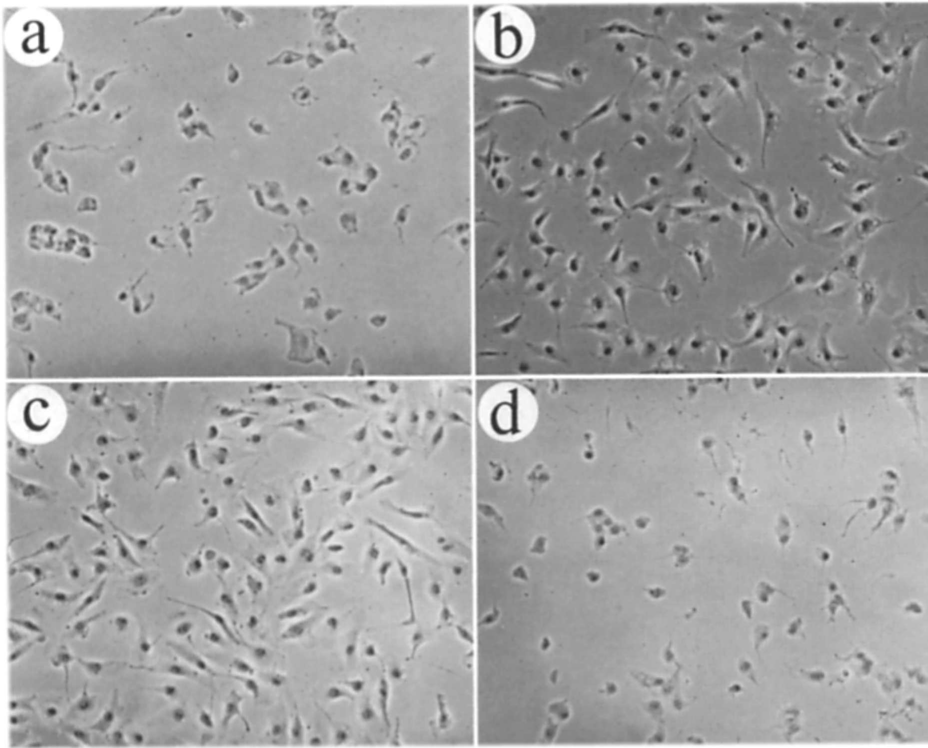


Figure 3. Anti-peptide 1.1 IgG blocks SPARC-mediated anti-spreading activity. BAECs were plated for 24 h on glass coverslips in 10% heat-inactivated FCS/DME. Cells were then washed in 1% heat-inactivated FCS/DME, and treated with 10 μg/ml SPARC for 4 h. (*a*) SPARC alone; (*b*) preincubated with anti-SPARC immunoglobulins; (*c*) preincubated with anti-peptide 1.1 immunoglobulins; (*d*) preincubated with anti-peptide 4.2 immunoglobulins. Cells incubated with PBS alone, or with immunoglobulins alone, were indistinguishable from untreated cells (data not shown).

ity of SPARC (Fig. 3). BAEC were plated for 24 h in media containing 10% heat-inactivated FCS. After treatment for 4 h with 10 μg/ml SPARC, most cells had assumed a rounded morphology (Fig. 3 *a*). Pretreatment of the SPARC with anti-SPARC antibodies (Fig. 3 *b*) or anti-peptide 1.1 antibodies (Fig. 3 *c*) blocked this activity. However, anti-peptide 4.2 antibodies were ineffective at blocking SPARC (Fig. 3 *d*). The fact that the anti-peptide 1.1 antibodies could neutralize SPARC activity suggested that the NH_2 terminus of SPARC could participate in the anti-spreading activity of SPARC. To test whether peptide 1.1 or other peptides had a direct effect, peptides were added directly to cells in culture.

Peptides Corresponding to Ca++-binding Regions Inhibit Cell Spreading

BAEC and FCLF were trypsinized and plated on tissue culture plastic in the presence of 1% FCS and increasing amounts of peptide. Under these conditions, control BAEC assumed a spread morphology within 1 h (Fig. 4 *a*), and FCLF within 20 min (Fig. 4 *e*). In the presence of 1% FCS, peptide 1.1 at a concentration of 0.8 mM permitted attachment, but not spreading, of 70% of the BAEC after 3 h (Fig. 4 *b*). Peptide 4.2 had a similar effect on 85% of the cells (Fig. 4 *d*); i.e., 85% of the cells displayed a rounded phenotype. The effect was no longer apparent after 24 h (data not shown). Peptide 3.2 (Fig. 4 *c*), as well as peptides 1.3, 2.1, and 3.4 (data not shown), had no effect up to concentrations of 1.5 mM peptide.

FCLF were two to five-fold more sensitive with respect to the anti-spreading effect of peptides 1.1 and 4.2. When FCLF were plated on dishes coated with type I collagen, 0.3 mM peptide 1.1 or 4.2 was capable of inhibiting spreading in 80–90% of the cells (Fig. 4, *f* and *h*) 2.5 h after plating; in contrast, peptide 3.2 exhibited no effect on spreading (Fig. 4 *g*). Preincubation of 1 mM peptide with homologous anti-peptide immunoglobulins eliminated the anti-spreading effect of peptides 1.1 and 4.2 on both cell types. The use of heterologous antibodies did not block the anti-spreading effect. These observations argue strongly that the anti-spreading effects are due to specific actions of the peptides themselves and not to trace contaminants in the peptide preparations or to cytotoxicity.

The dose response of BAEC to peptides was assessed by scoring the degree of cell reattachment and spreading in newly plated cultures. BAEC displayed a dose-dependent response to peptides 1.1 and 4.2, but not to inactive peptides or to an equivalent amount of PBS carrier (Fig. 5). 3 h after plating, control cells had a rounding index of 1.12 (SE ± 0.033) consistent with cells that were completely spread. In contrast, cultures exposed to 0.6 mM peptide 1.1 had an index of 1.39 (±0.062) that was indicative of a significant decrease in cell spreading ($p \leqslant 0.05$). 1.0 mM peptide 1.1 still permitted cell spreading, but the process was delayed. By 3 h, 21.1% of cells exposed to 1 mM peptide 1.1 were scored as spread. By 24 h, the effect of the peptide was overcome. For comparison, only 7.1% of cells exposed to 1.0 mM peptide 4.2 were spread at 3 h. There was no statistical difference between cells treated with peptide 3.2 and control (PBS) at 3 or 24 h. The morphological effects of all peptides used in this study are summarized in Table I.

Serum Concentration and Preplating Diminished the Anti-spreading Activity

Initial studies had shown that BAEC, preplated 20 h in 10% FCS, did not change shape in response to peptides at a concentration of 0.8 mM in the presence of 2.5% FCS (see for

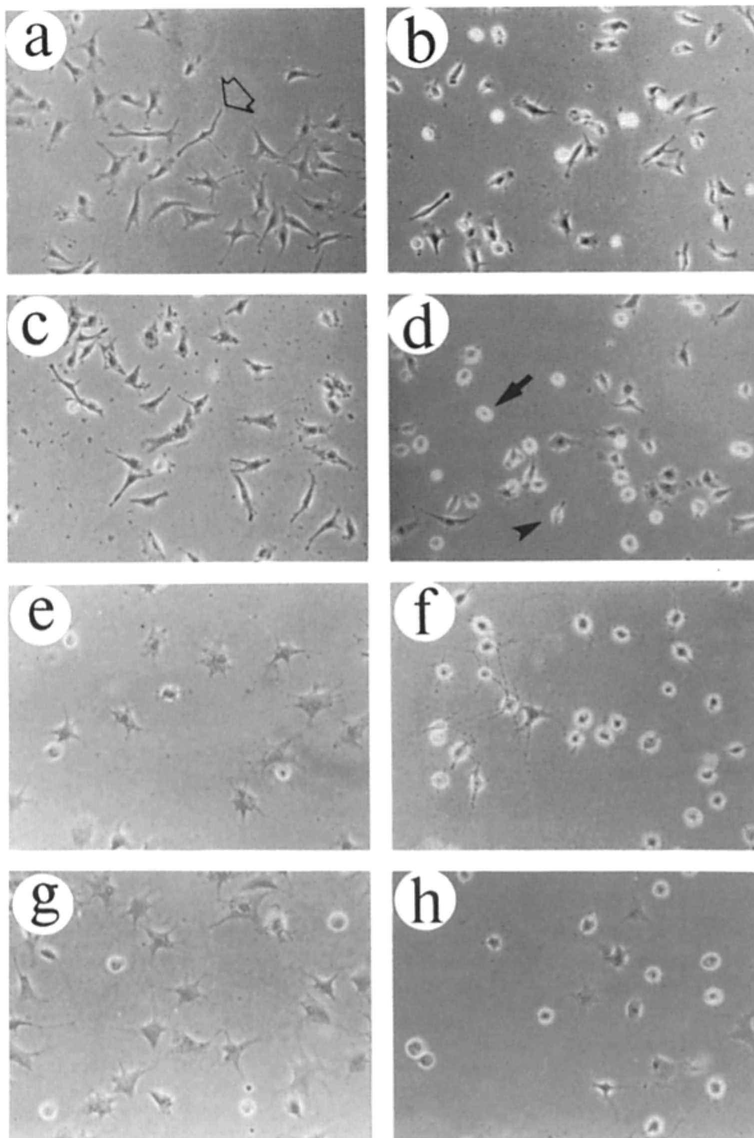


Figure 4. SPARC peptides 1.1 and 4.2 delay spreading of endothelial cells and fibroblasts. BAECs were plated on plastic dishes in 1% FCS/DME ± peptides (*a*, *b*, *c*, and *d*). Cells were photographed after 3 h. (*a*) PBS control; (*b*) 0.8 mM peptide 1.1; (*c*) 1.0 mM peptide 3.2; (*d*) 0.8 mM peptide 4.2. FCLFs were plated on collagen coated dishes in 1% FCS/DME ± peptides (*e*, *f*, *g*, and *h*). Cells were photographed after 2.5 h. (*e*) PBS control; (*f*) 0.3 mM peptide 1.1; (*g*) 0.3 mM peptide 3.2; (*h*) 0.3 mM peptide 4.2. Peptides derived from other regions of SPARC were inactive in this assay when added at concentrations up to 1.5 mM (data not shown). Open arrow (⇨) indicates a spread cell (group a); solid arrowhead (➤) indicates a cell that has begun to spread (group b); and the solid arrow (➔) indicates an attached round cell that has not initiated spreading (group c).

example Fig. 6, *c* and *e*). However, when the peptides were presented in serum-free medium, cells rounded up and remained attached but unspread (not shown). This result suggested that serum factors, or a metabolic activity of the cells supported by serum, affected the anti-spreading activity of the peptides. To analyze the role of cellular metabolism on SPARC function, we presented the SPARC and peptides to cells pretreated with an inhibitor of protein synthesis, cycloheximide (CHX). CHX (5 μg/ml) pretreatment inhibited protein synthesis by >95%. SPARC or peptides were added to control BAEC or to CHX-pretreated BAEC in 2.5% FCS, plus or minus CHX. After 2 h, cells were fixed and photographed (Fig. 6). CHX markedly augmented the response to added SPARC (compare Fig. 6 *a*; 10 μg/ml SPARC) with Fig. 6 *b* (0.02 μg/ml SPARC). The activities of peptides 1.1 (Fig. 6 *d*) and 4.2 (Fig. 6 *f*) were also enhanced, since these peptides had little effect on preplated cells in the presence of serum (Fig. 6, *c* and *e*). CHX pretreatment had no effect on the morphology of control cells or on cells incubated with peptide 3.2 (not shown). These observations suggested that, while SPARC can affect metabolically active cells, it is significantly more active when protein synthesis is reduced. The failure of peptides 1.1 and 4.2 to round previously spread, metabolically active cells may be due to the diminished activities of these peptides when compared to native SPARC.

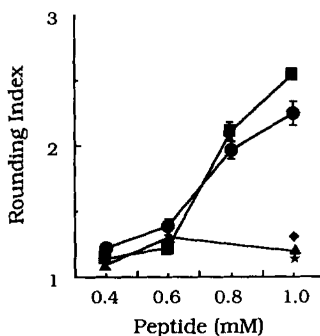


Figure 5. The antispreading activity of peptides 1.1 and 4.2 is dose dependent. Peptides were added to BAECs released from their substrate by brief exposure to trypsin. After 3 h, cultures were photographed and scored for degree of cell spreading. A score of 3 indicates rounded cells that have not initiated spreading, while a score of 1 indicates fully spread cells. Further details of the scoring procedure are given in Materials and Methods. (●) Peptide 1.1; (◆) peptide 1.3; (▲) peptide 3.2; (■) peptide 4.2; (★) PBS control.

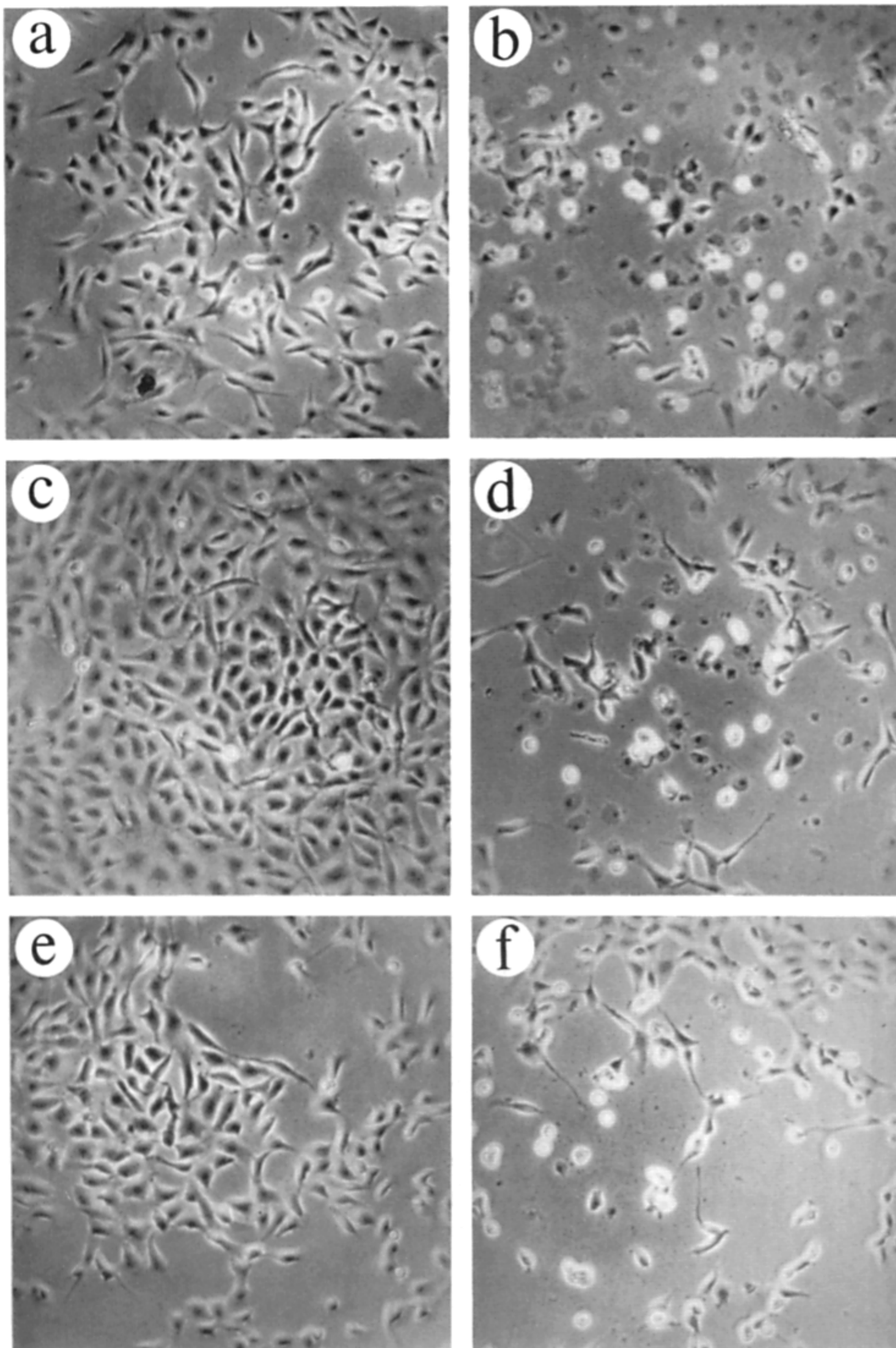
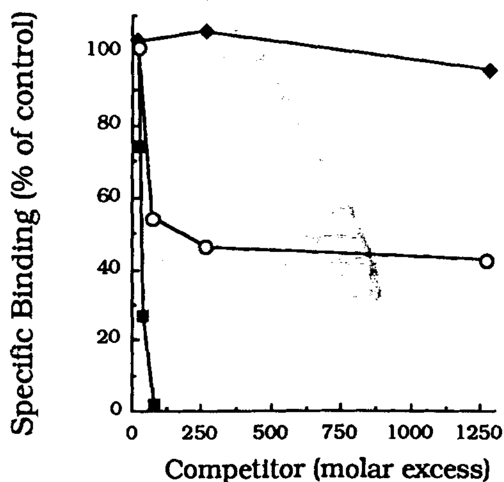


Figure 6. Antispreading activity of SPARC and peptides is augmented by inhibition of protein synthesis. BAECs were plated for 24 h on glass coverslips in 10% FCS/DME. Cells were then preincubated for 1 h with 2.5% FCS/DME alone (*a*, *c*, and *e*), or in the same media containing 5 μg/ml CHX for 1 h to block protein synthesis (*b*, *d*, and *f*). Media were then replaced with fresh media containing SPARC or peptides. After 2 h, cells were fixed and photographed. (*a*) 10 μg/ml SPARC; (*b*) 0.02 μg/ml SPARC; (*c* and *d*) 0.8 mM peptide 1.1; (*e* and *f*) 0.8 mM peptide 4.2.

Peptide 4.2 Competes for the Binding of SPARC to Purified Collagens and Binds to Collagens in a Ca^{++}-dependent Manner

To look more closely at the interactions of SPARC with proteins in the extracellular matrix, we iodinated SPARC and peptides and assayed their binding to various collagen substrates in solid-phase binding assays. In competition assays, peptide 4.2, but not peptide 1.1, competed with [^{125}I]SPARC for binding to type III collagen (Fig. 7 *a*). 46% of the specific binding was competed by addition of a 50-fold molar excess of peptide 4.2 over SPARC, and addition of a 250-fold molar excess of peptide 4.2 competed for 59% of the specific binding. There was no additional competition, even when a 1,250-fold molar excess of peptide 4.2 over SPARC was added (Fig. 7 *a*). Similar effects were seen on type I collagen even though the total binding was less than that observed for type III collagen (Table III). Addition of 1 mM EGTA (fourfold molar excess over available Ca^{++}) eliminated 100% of the specific binding of SPARC to these substrates. Nonspecific binding was 39% in this assay, determined with a 60-fold molar excess of unlabeled SPARC.

When peptides were iodinated directly, peptides 1.1 (with an NH_2-terminal Tyr residue added) and 3.2 failed to bind



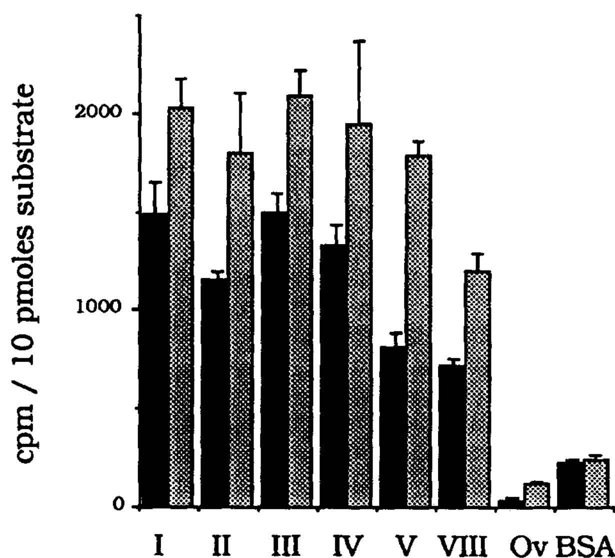


Figure 7. Peptide 4.2 competes for binding of SPARC and binds to collagens in solid phase binding assays. [125I]Peptides and [125I]-SPARC were tested for binding to various purified collagens in solid phase binding assays. Test proteins were bound to plastic microtiter wells and the remaining sites were blocked with ovalbumin. (*A*) Wells were coated with type III collagen. Blocked wells were then exposed to [125I]SPARC, in the presence or absence of cold competitor SPARC (■), peptide 1.1 (♦), or peptide 4.2 (○). Unbound counts were washed off and bound 125I was determined in a γ-counter. Values were expressed as percent of control after correction for nonspecific binding. Values are the average of triplicate determinations. (*B*) Wells were coated with various collagens (Types I, II, III, IV, V, and VIII), ovalbumin (*Ov*), or BSA. Blocked wells were then exposed to [125I]peptide 4.2 in the absence of (*black bars*) or presence of (*hatched bars*) 5 mM $CaCl_2$. Unbound counts were subsequently washed away with buffer containing 5 mM $CaCl_2$. Bound 125I was determined in a γ-counter. Binding is expressed as counts per minute per 10 pmoles substrate. Numbers represent the average of triplicate determinations, ± SD.

to any of the substrates tested. In contrast, [125I]-peptide 4.2 bound several collagens, but did not bind other proteins such as BSA or ovalbumin (Fig. 7 *b*). In similar assays, peptide 4.2 did not bind to SPARC or fibronectin (not shown). Nonspecific binding of peptide 4.2 varied from 20 to 26% in these assays (measured by adding a 100-fold molar excess of unlabeled peptide). Addition of peptide 1.1 had no effect on the binding of peptide 4.2 to collagens (not shown). The binding of peptide 4.2 to collagens was dose dependent (not shown) and could be augmented by addition of excess Ca^{++} (Fig. 7 *b* and data not shown).

In separate experiments, we have observed that peptide 4.2 bound [45Ca] directly (data not shown). The Ca^{++}-dependent binding to collagens and the direct binding of [45Ca] suggested that the EF-hand domain of SPARC was the functional Ca^{++}-binding site reported to be present in the COOH-terminal tryptic fragment of SPARC (Domenicucci et al., 1988; Sage et al., 1989*b*). Moreover, these data support the prediction that the EF-hand domain is a functional Ca^{++}-binding domain (Engel et al., 1986). Finally, our results also suggest that this domain could play a direct role in the binding of SPARC to collagens in the extracellular matrix.

Discussion

Sequences from mouse, bovine, and human cDNAs show that SPARC is highly conserved with >95% amino acid identity between the mouse and human proteins (Mason et al., 1986*a*; Swaroop et al., 1988). There are no regions within SPARC that appear homologous to known attachment-mediating regions; e.g., RGDS (Ruoslahti and Pierschbacher, 1987) or YIGSR sequences (Graf et al., 1987*b*). With the exception of the Ca^{++}-binding regions, it has been difficult to predict the location of functional regions based on conservation of sequence within phylogenetically diverse SPARC genes, or based on comparison with unrelated sequences. Since SPARC is a labile protein and is difficult to purify in large quantity, we chose to look at synthetic peptide analogues in an effort to identify regions involved in the binding of SPARC to cells and to extracellular matrix proteins.

In a previous study (Sage et al., 1989*b*), we demonstrated that SPARC had a specific anti-spreading effect on cultured BAEC. In the present study, we have extended this observation to show that peptides corresponding to sequences in the two Ca^{++}-binding domains of SPARC mimic the anti-spreading activity of the intact protein. Peptide 1.1, derived from the NH_2-terminal Glu-rich Ca^{++}-binding domain, and peptide 4.2, derived from the COOH-terminal EF-hand Ca^{++}-

Table III. Specific binding of [125I]SPARC to Collagen Substrates is Inhibited by Peptide 4.2

Substrate	Competitor	Specific binding (SD) %
Type I Collagen	None	100 (0.9)
	SPARC	0.0 (1.3)
	Peptide 4.2	57.8 (35.2)
	1 mM EGTA	−3.5 (5.0)
Type III Collagen	None	100 (9.4)
	SPARC	0.0 (8.5)
	Peptide 4.2	40.9 (6.4)
	1 mM EGTA	−0.6 (5.0)

Substrate proteins were plated on microtiter wells and nonspecific binding sites were blocked with 0.1% ovalbumin. [125I]SPARC was added in the presence or absence of cold competitor. Unbound radioactivity was washed away and bound [125I] counted in a γ counter. Measurements are averaged from three wells. In three independent experiments, the average nonspecific binding (NSB) was 35.6% on type I collagen and 39.1% on type III collagen.

binding domain, both inhibited cell spreading when added to primary cultures of endothelial cells or fibroblasts. The effect was dose dependent, augmented by the presence of CHX, and inhibited by addition of homologous antipeptide antibodies. Peptides from other regions of the molecule had no effect. Addition of FCS prevented rounding of previously spread cells by peptides, but this effect of serum could be overcome by pretreating the cells with CHX. We have performed other experiments that show that the peptides themselves do not markedly alter the levels of total protein synthesis, although there are alterations in the levels of mRNA and protein corresponding to specific macromolecules (T. F. Lane, M. L. Iruela-Arispe and H. Sage, manuscript in preparation); the activity is therefore apparently not the result of cytotoxicity.

Antibodies raised against peptide 1.1 blocked SPARC-mediated anti-spreading activity. This result suggested that the NH_2 terminus of the protein was required for biological activity. We were also interested in the structure of SPARC as revealed by antibodies directed against other regions of the molecule. However, the pattern of anti-peptide immunoreactivity was found to be highly dependent on the conformation of SPARC. In fact, out of six anti-peptide antibodies raised in this study, only those against peptide 1.1 recognized native SPARC in solution. Antibodies raised against other regions required reduction of SPARC to expose relevant epitopes. Interestingly, Mason et al. (1986*a*,*b*) described antibodies to a peptide corresponding to the COOH-terminal 11 amino acids of murine SPARC. The sequence of this peptide does not overlap with peptide 4.2. Unlike antibodies to peptide 4.2, the antibodies to the COOH-terminal peptide immunoprecipitated SPARC from cell culture media. The immunoreactivity data suggest that the NH_2-terminal Glu-rich region of SPARC is exposed to the solvent and maintains a conformation that is little affected by the remainder of the protein. Since peptide 4.2 competes for the binding of SPARC to collagens, it appears that this domain is exposed to solvent as well. Conservation of sequence and lack of immunogenicity in the EF-hand domain suggest, however, that the exposed portion is not the sequence recognized by the antibodies we have characterized to date.

Although the sequence represented by peptide 3.2 is predicted to be antigenic by hydrophilicity analysis (Hopp and Woods, 1981), antibodies raised to peptide 3.2 failed to recognize native SPARC and reacted poorly with the denatured protein. Tainer et al. (1984) have suggested that the cross-reactivity of anti-peptide antibodies with native protein is a function of the relative mobility of the corresponding site in the protein. Their study suggests that highly ordered regions would be poorly recognized by anti-peptide antibodies. If this analysis can be extended to SPARC, then one might predict that region 3 (peptide 3.2) and the EF-handlike domain (peptide 4.2) are well-ordered regions of SPARC, whereas the NH_2 and COOH termini are not. The conformational dependence shown by the antipeptide antisera used in this study would support a model of SPARC as a highly ordered protein. Most peptide epitopes were masked in the native structure but became exposed after reduction. A high degree of internal structure could help to explain the high percentage of conserved residues in SPARC. More precise structural studies will be required to understand the native conformation of the SPARC protein. Such studies will improve our understanding of how the Ca^{++}-binding sites function in the disruption of cell-substrate contacts.

The observation that anti-spreading activity is augmented by CHX is the first observation of its kind for SPARC. Interestingly, blockage of protein synthesis increases the activity of other proteins that have anti-spreading activity in endothelial cells, such as Tsp (Murphy-Ullrich and Höök, 1989). Pretreatment with CHX, followed by exposure to native SPARC, resulted in a 50–100-fold increase in anti-spreading activity when compared to cells in which protein synthesis was not affected. Similar effects were seen in serum-free medium in the absence of CHX, but addition of serum to CHX-treated cells did not alter the effect seen with CHX alone. These data suggest the involvement of newly synthesized proteins required for the processing of SPARC or for signal transduction. Specific models of how this effect is mediated could include the inactivation of SPARC by a newly activated protease, or in the turnover of cell surface and extracellular matrix components bound by SPARC. The synthesis of inhibitors of SPARC, or of a protease activity that inactivates SPARC, could be important factors regulating the concentration of SPARC needed to affect cells in vivo.

SPARC has been recognized for its ability to bind extracellular matrix components (Termine et al., 1981; Romberg et al., 1985; Clezardin et al., 1988; Sage et al., 1989*b*). On a molar basis, native SPARC binds preferentially to type III collagen, but also binds to other collagen types including I, II, IV, and V (Sage et al., 1989*b*). In the present study, we used the binding of SPARC to type III collagen as an assay to identify peptides that could affect this process. Peptide 4.2 competed for >50% of the binding of SPARC to type III and type I collagen. In addition, peptide 4.2 showed specific binding to collagens in a Ca^{++}-dependent fashion, but did not bind albumin, ovalbumin, SPARC, or fibronectin. While the intact SPARC protein discriminates among collagens, showing a preference for type III collagen (Sage et al., 1989*b*), peptide 4.2 did not appear to make this distinction. The ability of intact SPARC to discriminate among collagens may thus be dependent on its three-dimensional structure. Other regions of the protein that contribute to the specificity of the collagen binding site may be required. Ongoing studies are being carried out with larger peptides to test this hypothesis. Our present data suggest that a COOH-terminal region of SPARC, which includes the Ca^{++}-binding site represented by peptide 4.2, plays a role in the binding of SPARC to collagens. Continued work with the peptides may identify other regions of SPARC that interact with components of the extracellular matrix, and may lead to additional insights into the structure of active sites within the SPARC protein itself.

While peptide 4.2 competed for the binding of SPARC to collagens at concentrations within one order of magnitude of those required by the intact protein, clearly much larger doses of peptides were required to mimic the effects of native SPARC on cell spreading. The apparent requirement for high concentrations of peptides, relative to those needed for activity of the native protein, is not unique to SPARC. Peptides derived from the cell binding region of fibronectin (RGDS) and laminin (YIGSR), at concentrations between 0.1 and 4 mM, inhibited attachment, while nanomolar concentrations of the native protein displayed similar efficiencies in the assay (Dufour et al., 1988; Chen et al., 1987; Kleinman et al., 1989). Studies that used peptides to map receptor bind-

ing sites claimed that concentrations 10^5-fold greater than that of the native competitor were necessary to inhibit binding (Morris et al., 1990; Longo et al., 1990). The requirement for such high concentrations of a fragmented protein is often attributed to the existence of multiple sites within the native protein that act synergistically to bind receptors (Dufour et al., 1988; Kleinman et al., 1989; Morris et al., 1990). In addition, peptides are likely to occur in several conformations in solution. We interpret the need for high amounts of peptide as a direct consequence of the loss of cooperative sites residing in other regions of the protein and the diminution of conformational constraints provided by the native protein environment.

Even with the limitations that protein fragmentation affords, the ability of specific peptides to modulate cell attachment is now widely appreciated. Anti-spreading activity has been reported in peptides derived from fibronectin (Ruoslahti and Pierschbacher, 1986; Dufour et al., 1988), laminin (Graf et al., 1987*a*,*b*; Kleinman et al., 1989) and in recombinant fragments of tenascin (Spring et al., 1989). In the case of fibronectin, peptides containing the sequence RGDS form the recognition site for cell surface receptors of the integrin class (for review, see Hynes, 1987; Ruoslahti and Pierschbacher, 1987). Proteins such as von Willebrand factor (Dejana et al., 1989; Titani et al., 1986), fibrinogen (Cheresh et al., 1989), and Tsp (Lawler et al., 1988) also have functional RGD-containing sequences. Interestingly, while these RGD domains occur in various proteins, the proteins themselves mediate diverse spreading effects in various cells. These effects may be mediated by different cell surface receptors but are almost surely due to different properties of the intact proteins as well. For example, Tsp allows cell attachment by an RGD-dependent mechanism (Tuszynski et al., 1987) but prevents spreading (Lahav, 1988; Lawler et al., 1988; Murphy-Ullrich and Höök, 1989). Tenascin contains no RGD sequences but has been shown to prevent the interaction of fibronectin with cell surface integrins. It has been proposed that tenascin, by virtue of its size and complex hexabrachion structure, acts by imposing a physical barrier between cells and their substrates (Chiquet-Ehrismann et al., 1988).

It is possible that various extracellular proteins function to diminish the affinity or accessibility of cell-extracellular matrix interactions. Given the need for high concentrations of many extracellular proteins (required for tissue structure and hydrodynamics), as well as a requirement for cells to carry specific receptors for these proteins, it seems reasonable to propose that a class of proteins exists that modulates the ability of cells to interact with the extracellular matrix. Negative modulators of cell-matrix interactions could provide a mechanism for regulation of the overall level of cell-extracellular matrix binding without (or in concert with) the release of degradative enzymes or the reduction of cell surface receptors. We propose that SPARC is a new member of this class of modulators, which also includes Tsp (Murphy-Ullrich and Höök, 1989) and tenascin (Chiquet-Ehrismann et al., 1988). SPARC contains no RGD sequences and there are no data to indicate that it acts via an integrin-mediated mechanism. The nature of the cell surface binding site for SPARC remains to be determined.

The appearance of SPARC mRNA by day 9 of development in the mouse (Howe et al., 1988; Mason et al., 1986*b*), as well as the tissue distribution of SPARC mRNA and protein (Holland et al., 1987; Wewer et al., 1988; Sage et al., 1989*a*), suggested to us that SPARC is associated with cellular events requiring tissue remodeling, cell movement, and/or proliferation. The presence of large amounts of SPARC in the periosteal layer of bone after day 13 of mouse development (Nomura et al., 1988) and in certain invasive tumors (Mann et al., 1987) is consistent with this interpretation. The identification of anti-spreading activity in SPARC was the first function proposed that suggested a unified role for the protein in the various tissues and cultured cells in which it is seen (Sage et al., 1989*b*). Thus, cells in early somites, gut epithelium, or remodeling bone could all require SPARC to modulate their interactions with extracellular matrix. We have now proposed that the synthesis and release of SPARC is one of the options available to cells in the process of diminishing adhesive interactions with their substrate. The regulation of cell shape, migratory potential, and the ability to divide are only a few of the biological consequences. In the present study, we have confirmed the observation of anti-spreading activity of SPARC for BAEC and FCLF cells. We have also shown that unique regions of the protein may contribute to this activity by binding extracellular matrix components and preventing cellular spreading. We are currently investigating the interaction of SPARC with membrane-bound molecules, to identify a pathway by which the presence of SPARC might be linked to the regulation of specific genes.

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