Adsorption of Vitronectin in Human Serum onto Plastics is Augmented by Sodium Dodecyl Sulfate

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ABSTRACT. We have investigated the adsorption of cell-spreading activity in human serum onto polystyrene plates after treatment of the serum with sodium dodecyl sulfate (SDS). Vitronectin in human serum was remarkably adsorbed onto the plate after boiling the serum with 0.1% SDS for 5 min. SDS was effective over the concentration range from 0.05 to 0.25%. Increase of the vitronectin adsorption was accompanied by an increase of cell spreading on the plates. The cell-spreading activity in SDS-treated serum was impeded by anti-vitronectin antibody but not by anti-fibronectin antibody. After treatment with SDS, fibronectin-depleted serum could induce cell spreading but vitronectin-depleted serum could not. These results indicate that vitronectin alone was the cell-spreading factor in SDS-treated human serum. However, SDS-treated pure vitronectin itself did not retain the cell-spreading activity. The activity was recovered when bovine serum albumin was added to pure vitronectin before or after boiling with 0.1% SDS. Therefore, vitronectin adsorbed from SDS-treated serum might retain the cell-spreading activity with the aid of serum protein. Treatment of serum with SDS provides an easy, specific, and efficient method of coating polystyrene plates with vitronectin.

It is well known that a variety of animal cells can be grown in vitro with synthetic media supplemented with animal sera, but most cultured cells need to attach and spread on a substrate to proliferate in vitro (9, 10). Adhesive factors are provided by serum in the medium and/or secreted by cultured cells themselves (5, 12, 15, 24).

The adhesive factors in serum are glycoproteins, mostly fibronectin (2, 19) and vitronectin (4, 16). The latter has been called “serum spreading factor” (3), “S-protein” (29), and “epibolin” (33). The two adhesive glycoproteins differ in molecular size, amino acid sequence, and cDNA sequence (3, 22, 35, 36). Moreover, their antibodies do not cross-react with each other (4, 16). The peripheral cytoplasm of cells expands more widely with fibronectin than with vitronectin (39). Both glycoproteins have, however, the same cell-attachment sequence of Arg-Gly-Asp (22, 28, 35), bind to heparin (13, 38) and to collagen (8, 20), and exist in human serum at an
almost equal concentration, approximately 0.2 mg/ml (4, 16, 26, 31).

In fetal bovine serum, commonly used for cell culture, vitronectin rather than fibronectin appears to be the main adhesive glycoprotein, as is reported below. Human plasma vitronectin is known to adsorb avidly to polymers (6). The cell-attachment activity attributable to vitronectin is 8-16 fold greater than that of fibronectin in the cell-blotting assay of fetal bovine serum (17). The fibronectin concentration in fetal bovine serum is only about 0.03 mg/ml (18), probably about 1/7 of the vitronectin concentration (17). The specific activity of vitronectin for cell spreading is approximately 10 fold greater than that of fibronectin (3, 39). Fibronectin in human serum adsorbs onto tissue culture plates and induces cell spreading at serum concentrations lower than 1% (11). It adsorbs very little at 10% serum (11), however, which is the concentration mostly used for cell culture. At concentrations of 3% serum and above, cell spreading is mediated by vitronectin (23).

However, little is known about vitronectin adsorption onto polystyrene tissue culture plates. We have previously developed a modified sandwich ELISA, termed "ELISA-SDS" (1), to quantitate vitronectin in human serum reliably. During treatment of human serum with sodium dodecyl sulfate (SDS) for the quantitation, we noticed that vitronectin became highly adhesive to the polystyrene plates. In this paper we describe that SDS activated the adsorption of vitronectin in human serum to polystyrene plates and that the adsorbed vitronectin could spread cultured cells.

**MATERIALS AND METHODS**

*Whole Serum, fibronectin-depleted serum, and vitronectin-depleted serum.* Human plasma was clotted by adding CaCl₂ to the final concentration of 20 mM, left to stand at room temperature for 1 h, then at 4°C overnight, and finally centrifuged at 10,000 rpm for 10 min at 4°C. The resulting serum was stored frozen until use. The protein concentration of the whole serum was 64 mg/ml. Fibronectin-depleted serum was obtained as the flow-through fraction of gelatin-Sepharose affinity chromatography of the whole serum (8). The fibronectin concentration in the serum decreased from 6.3 µg/mg of total protein to 0.024 µg/mg, while the vitronectin concentration in the serum remained at 3.8 µg/mg of total protein, similar to the value in the whole serum (4.1 µg/mg). Vitronectin-depleted serum was obtained as the flow-through fraction after two cycles of anti-vitronectin-Sepharose 4B column chromatography. The vitronectin concentration in the serum decreased from 4.1 µg/mg of total protein to 0.29 µg/mg. However, the fibronectin concentration in the serum also decreased from 6.3 µg/mg to 1.3 µg/mg. The serum was amended with pure fibronectin up to 6.3 µg/mg for the vitronectin-depleted serum. The concentrations of vitronectin and fibronectin in the above sera were determined with ELISA-SDS (1) and sandwich ELISA (30), respectively.

*Preparation of fibronectin, vitronectin, and their antibodies.* Vitronectin was purified from outdated human plasma according to our recent procedure (39). Fibronectin was purified from outdated human plasma using a gelatin affinity column (8, 14). The concentrations of pure vitronectin and pure fibronectin were determined from absorbance measurements at 280 nm with a 1-cm path-length cell using \( E_{1\text{mg/ml}} \) values of 1.38 (7) and 1.28 (26), respectively. Preparation of rabbit anti-human vitronectin antibody and rabbit anti-human fibronectin antibody, and their conjugation with horseradish peroxidase were described previously (1, 30).

*Adsorption and quantitation of vitronectin.* In the standard procedure, human serum was diluted to 0-4% in phosphate-buffered saline (PBS) containing 0.1% SDS, and boiled
for 5 min. Polystyrene 96-well plates for ELISA (No. MS-3496F, Sumitomo Bakelite, Tokyo) were incubated with 50 µl of SDS-treated serum and untreated serum at room temperature for 1 h. After rinsing with PBS 3 times, the plates were incubated with 1% (w/v) bovine serum albumin in PBS at room temperature for 1 h. After rinsing with PBS 3 times again, the plates were incubated with horseradish peroxidase-conjugated anti-vitronectin antibody diluted at 1/2,000 in PBS containing 1% bovine serum albumin at room temperature for 1 h. After rinsing with PBS 5 times, an orange color was generated by incubation of 100 µl of 0.4 mg/ml o-phenylenediamine, 2.5 mM H2O2, 0.1 M citric acid, and 0.2 M Na2HPO4 for 10 min in the wells. The adsorbed vitronectin on the plates was quantitated by the absorbance at 492 nm for the orange color.

**Cell-spreading activity.** BHK cells were grown in Dulbecco’s modified Eagle medium supplemented with 10% calf serum, 50 U/ml penicillin, and 50 µg/ml streptomycin sulfate at 37°C in a 5% CO2-humidified air mixture. Polystyrene 96-well plates for tissue culture (No. 167008, Nuclon Delta SI, Nunc, Denmark) were incubated at room temperature for 1 h with 50 µl of human serum, fibronectin-depleted serum, vitronectin-depleted serum, pure vitronectin, and pure fibronectin at indicated concentrations before and after boiling with 0.1% SDS for 5 min. After rinsing with PBS 3 times, the plates were incubated at 37°C for 1.5 h with 100 µl of BHK cell suspension (2 × 10⁵ cells/ml) in a Grinnell’s adhesion medium (12) of 150 mM NaCl, 1 mM CaCl2, 3 mM KCl, 0.5 mM MgCl2, 6 mM Na2HPO4, and 1 mM KH2PO4, pH 7.3. After rinsing gently with PBS to remove unattached cells, attached cells were fixed with 100 µl of 2% glutaraldehyde, 5% formaldehyde, and 5% sucrose in PBS at room temperature for 30 min. The percent of spread cells (number of spread cells per 100 attached cells) in 4 areas of 0.7 × 0.7 mm² were counted microscopically.

To test the effects of antibody on cell spreading, 50 µl of 0-1.5 mg/ml rabbit anti-human vitronectin antibody, rabbit anti-human fibronectin antibody, or rabbit normal IgG in PBS containing 1% bovine serum albumin was incubated with the serum-coated wells at 37°C for 3 h before receiving the BHK cells.

**RESULTS**

*Increased adsorption of vitronectin.* We previously developed a modified sandwich ELISA, ELISA-SDS (1), in which vitronectin was boiled with 2% SDS for 5 min, diluted 1 to 20 to lower the concentration of SDS to 0.1%, and added to polystyrene plates pre-coated with anti-vitronectin antibody. After the establishment of ELISA-SDS, we noticed that boiling with 0.1% SDS remarkably enhanced the adsorption of vitronectin to the plates. The amount of adsorption increased with increasing concentrations of SDS-treated serum (Fig. 1). A limited range of SDS concentrations (0.05-0.25%) was effective (Fig. 2a). Although boiling was not necessary for the activation, we always boiled human serum with 0.1% SDS in the following experiments to ensure the SDS treatment.

*Cell-spreading on the adsorbed plates.* The polystyrene plates incubated with human serum were examined for cell-spreading activity using BHK cells. Interestingly, BHK cells spread on the plates coated with SDS-treated serum. The effective range of SDS concentrations for cell spreading (Fig. 2b) was in good agreement with that for vitronectin adsorption (Fig. 2a). Human serum treated with more than 0.5% SDS induced neither vitronectin adsorption nor cell spreading. When 4% serum treated with 0.5% SDS was serially diluted, both cell spreading and vitronectin adsorption occurred at 0.016-0.008% SDS (data not shown). Therefore, the lack of cell-spreading activity in more than 0.5% SDS was mainly due to the lack of
vitronectin adsorption, not to the denaturation of its binding activity to cells. The percent of cell spreading increased with increasing concentrations of SDS-treated serum and became saturated at around 3% serum (Fig. 3). These results suggest that the cell-spreading activity adsorbed onto the plates from SDS-treated serum can be attributed to vitronectin.

Without treatment with SDS, serum also increased cell spreading on the polystyrene plates. The maximal activity of cell spreading was observed at lower concentrations of native serum, ranging from 0.1 to 0.5% (Fig. 3), which was in good agreement with the profile of fibronectin adsorption reported by Grinnell and Feld (11). Therefore, this activity can probably be attributed to at least fibronectin in the serum.

The morphology of BHK cells spread on the coated plates is illustrated in Fig. 4. Cell spreading was promoted by native 0.5% serum (Fig. 4a) but not by native 4% serum (Fig. 4b), and, inversely, promoted by SDS-treated 4% serum (Fig. 4d) but not by SDS-treated 0.5% serum (Fig. 4c). Cell attachment was sparse only in a well coated with native 4% serum (Fig. 4b). Fibronectin (Fig. 4e) promoted wider lateral expansion of the peripheral cytoplasm of spread cells than vitronectin did (Fig. 4f), as was discovered very recently (39). Native 0.5% serum (Fig. 4a) promoted slightly
wider lateral expansion of the peripheral cytoplasm of spread cells than SDS-treated 4% serum did (Fig. 4d). The latter shapes resembled those with pure vitronectin (Fig. 4f), and native 0.5% serum (Fig. 4a) induced shapes intermediate between those with pure fibronectin (Fig. 4e) and those with pure vitronectin (Fig. 4f). The morphology of spread cells suggested that vitronectin dominated the cell spreading in SDS-treated 4% serum but that the cell-spreading activity induced by native 0.5% serum was not due to only vitronectin.

**Fibronectin and vitronectin in the adsorbed cell-spreading activity.** Cell-spreading activity was examined more specifically with respect to fibronectin and vitronectin. Both anti-fibronectin antibody and anti-vitronectin antibody interfered with cell spreading induced by native 0.5% serum (Fig. 5a). Cell spreading induced by SDS-treated 4% serum was also reduced by anti-vitronectin antibody, but not by anti-fibronectin antibody (Fig. 5b).

The action of fibronectin and vitronectin in native and SDS-treated sera was also examined using whole serum, fibronectin-depleted serum, and vitronectin-depleted serum. When treated with SDS, all types of 0.5% sera and vitronectin-depleted 4% serum failed to promote cell spreading, while fibronectin-depleted 4% serum pro-
moted cell spreading to a similar degree to whole 4% serum (Table 1). These results indicate that the cell-spreading activity in SDS-treated sera can be attributed to only vitronectin. This result was consistent with the result that cell spreading induced by SDS-treated 4% serum was impeded by anti-vitronectin antibody but not by anti-fibronectin antibody (Fig. 5b).

Without SDS treatment, on the other hand, none of the 4% sera substantially promoted cell spreading (Table 1), which suggests that neither fibronectin nor vitronectin could sufficiently adsorb to the plates, probably due to the abundant presence of serum albumin. Lower concentrations of sera did promote cell spreading. The percent of cell spreading induced by fibronectin-depleted 0.5% serum and vitronectin-depleted 0.5% serum was approximately 1/3 and 1/5 of that induced by whole 0.5% serum (Table 1). This result suggests that the effects of fibronectin and vitronectin in native serum are additive, consistent with the results on antibody interference (Fig. 5a).

Adsorption and cell-spreading activity of SDS-treated pure vitronectin. Pure vitronectin after boiling with 0.1% SDS was adsorbed onto polystyrene plates as well as native pure vitronectin was (Fig. 6). However, adsorption of pure vitronectin onto the polystyrene plates was impeded by the presence of bovine serum albumin. The interference was much greater for native pure vitronectin than for SDS-treated pure vitronectin (Fig. 6).

We examined whether SDS-treated pure vitronectin adsorbed onto plates retained cell-spreading activity. Unexpectedly, the cell-spreading activity of SDS-treated pure vitronectin decreased terribly (Table 2). We supposed that serum protein influenced the cell-spreading activity of vitronectin. Bovine serum albumin was
Fig. 4. Morphology of spread BHK cells induced by SDS-treated serum, native serum, fibronectin, or vitronectin. Photographs (a-d) correspond to the results in Fig. 3. a, Native 0.5% serum; b, native 4% serum; c, SDS-treated 0.5% serum; d, SDS-treated 4% serum; e, 8 µg/ml fibronectin; and f, 8 µg/ml vitronectin.

Fig. 5. Inhibition of cell-spreading by antibodies. Cell-spreading activity was examined as described in the legend to Fig. 2, except for treatment with antibodies as follows. A serum-coated 96-well tissue culture plate was incubated with 50 µl of 0-1.5 mg/ml anti-vitronectin antibody, anti-fibronectin antibody, or normal IgG in 1% bovine serum albumin at 37°C for 3 h before receiving BHK cells. (a) The plate was coated with native 0.5% serum, (b) The plate was coated with SDS-treated 4% serum. Anti-vitronectin antibody, ○; anti-fibronectin antibody, △; and normal IgG, □.
therefore added to pure vitronectin before or after boiling with SDS. Almost all cell-spreading activity was recovered, although the relative amounts of adsorbed vitronectin decreased by 29-32% (Table 2). SDS-treated pure vitronectin once adsorbed alone, however, could not be activated by the following addition of bovine serum albumin. These results suggest that bovine serum albumin was a co-factor for the cell-spreading activity of vitronectin and a weak inhibitor for its adsorption onto the plates.

**TABLE 1. CELL-SPREADING ACTIVITY OF FIBRONECTIN AND VITRONECTIN-DEPLETED SERA**

<table>
<thead>
<tr>
<th>Concentrations of serum</th>
<th>Treatment with SDS</th>
<th>Cell spreading (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whole serum</td>
</tr>
<tr>
<td>0.5%</td>
<td>No</td>
<td>58.3</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>0.0</td>
</tr>
<tr>
<td>4%</td>
<td>No</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>44.7</td>
</tr>
</tbody>
</table>

Fibronectin concentrations in whole, fibronectin-depleted, and vitronectin-depleted sera were 6.3 μg/mg, 0.024 μg/mg, and 6.3 μg/mg of total protein, respectively. Vitronectin concentrations in whole, fibronectin-depleted, and vitronectin-depleted sera were 4.1 μg/mg, 3.8 μg/mg, and 0.29 μg/mg of total protein, respectively. The concentrations of sera were normalized by taking the protein concentration of 64 mg/ml as 100%. Treatment with SDS meant boiling the sera with 0.1% SDS for 5 min. Cell-spreading activity was examined as described in the legend to Fig. 2.

Therefore added to pure vitronectin before or after boiling with SDS. Almost all cell-spreading activity was recovered, although the relative amounts of adsorbed vitronectin decreased by 29-32% (Table 2). SDS-treated pure vitronectin once adsorbed alone, however, could not be activated by the following addition of bovine serum albumin. These results suggest that bovine serum albumin was a co-factor for the cell-spreading activity of vitronectin and a weak inhibitor for its adsorption onto the plates.

**Fig. 6. Adsorption of pure vitronectin in the presence of bovine serum albumin.** Adsorption of 8 μg/ml native pure vitronectin and 8 μg/ml SDS-treated pure vitronectin in the presence of the indicated concentrations of bovine serum albumin (BSA) was examined as described in the legend to Fig. 1. SDS-treated pure vitronectin, ♂; native pure vitronectin, △.
DISCUSSION

We have found that 0.1% SDS remarkably activated the adsorption of vitronectin in human serum to polystyrene plates intended for tissue culture and ELISA. Our novel method provides an efficient way to coat vitronectin onto the plates without purifying it.

The adsorbed vitronectin in human serum was active for cell spreading and the trace amount of SDS possibly adsorbed was not toxic. However, SDS-treated pure vitronectin did not retain the cell-spreading activity, but recovered the activity when bovine serum albumin was added to pure vitronectin before or after boiling with 0.1% SDS (Table 2). These results suggest that the resistance of the cell-spreading activity of vitronectin to boiling with 0.1% SDS was not only due to an intrinsic property of vitronectin, but also depended on a serum protein.

Cell-spreading activity of fibronectin can be duplicated by fibronectin synthetic peptides containing Arg-Gly-Asp (RGD) (28). Intact fibronectin induces wider lateral expansion of peripheral cytoplasm of spread cells than vitronectin (39). Some researchers, but not all, have reported that RGD peptides-coated substrate induces poorer cell spreading in several respects, including the area of spread cells, number and size of focal contacts, and development of actin cables (21, 32, 34, 37). They suggest that a second site, the heparin-binding domain of fibronectin, is required for full spreading of cells. Although the heparin-binding site of vitronectin is exposed, the area of cells spread by vitronectin is similar to that by the RGD peptides rather than by fibronectin (39). Thus, heparin-binding activity alone does not support full spreading of cells. The third site found recently in fibronectin may be required (27).

There is an unsolved problem. Although vitronectin was adsorbed more in native 4% serum than 0.5% serum (Fig. 1), the 4% serum did not promote cell spreading, whereas the 0.5% serum did (Fig. 3). There are two possibilities. First, the cell-spreading activity, as well as cell-attachment activity, might be inhibited by the abundant presence of serum proteins in the 4% serum but not in the 0.5% serum. Second, in native 0.5% serum, both fibronectin and vitronectin have cell-spreading activity (Fig. 5a and Table 1). Fibronectin may synergistically promote the cell-spreading activity of vitronectin in the native serum. Grinnell and Feld (11) have reported that the amount of fibronectin adsorption onto plates was maximal at

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**TABLE 2. EFFECT OF BOVINE SERUM ALBUMIN ON CELL-SPREAD ACTIVITY OF VITRONECTIN**

<table>
<thead>
<tr>
<th>Coating solution</th>
<th>Cell spreading (%)</th>
<th>Adsorbed vitronectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine serum albumin (BSA)</td>
<td>1.5</td>
<td>—</td>
</tr>
<tr>
<td>Vitronectin</td>
<td>49.4</td>
<td>1.99</td>
</tr>
<tr>
<td>Vitronectin → boiling with SDS</td>
<td>3.3</td>
<td>2.11</td>
</tr>
<tr>
<td>Vitronectin + BSA → boiling with SDS</td>
<td>47.0</td>
<td>1.50</td>
</tr>
<tr>
<td>Vitronectin → boiling with SDS → + BSA</td>
<td>47.2</td>
<td>1.44</td>
</tr>
</tbody>
</table>

To examine the effect of bovine serum albumin on the cell-spreading activity of pure vitronectin, the albumin was added to SDS-treated pure vitronectin (8 μg/ml) before or after boiling with 0.1% SDS. The concentration of the albumin was 0.25%. Polystyrene plates were incubated with the samples treated as indicated in "coating solution". The amount of adsorbed vitronectin and its cell-spreading activity were examined as described in the legends to Figs. 1 and 2, respectively.
0.1% serum and then decreased with increasing serum concentration. The decrease of fibronectin adsorption might critically contribute to the decrease of cell-spreading activity of vitronectin in the native 4% serum.

Our previous report (1) indicated that our anti-vitronectin antibody reacts with vitronectin dependent on its conformation. Therefore, the amounts of adsorbed vitronectin assayed in this report should be interpreted as relative values. To clarify more quantitatively, we plan to use purified and radiolabelled preparations of vitronectin and fibronectin in future studies. In the purified preparations, conformational change of vitronectin will also be measured after coupling it to fluorescent dyes, as we recently did with fibronectin (25).

Barnes et al. (5) have reported that vitronectin supports cell growth in a variety of cell lines in serum-free medium. Applied to serum-free cell culture, treatment of serum with SDS will provide an instant and specific coating of tissue culture polystyrene plates with vitronectin. Although bovine and porcine sera instead of human serum have not been examined yet, they appear to contain similar concentrations of vitronectin (17, 40). Therefore, coating the plates with bovine or porcine serum in the presence of 0.1% SDS is economically suitable for serum-free cell culture. We also have not used either 10% serum nor fetal bovine serum yet. The same conditions as for routine cell culture should be studied in the near future with respect to the adsorption of fibronectin and vitronectin as well as the morphology of spread cells.

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