

Lack of sialic acid in synovial fluid fibronectin

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1. INTRODUCTION

Fibronectins are high M_r glycoproteins present in an insoluble form in interstitial matrices, basement membranes, newly formed connective tissue, differentiating cartilage and bone, and rheumatoid synovial membrane. Fibronectins are also present, in a soluble form, in blood plasma and other body fluids including amniotic, synovial, cerebrospinal and pleural fluids, urine and milk (for reviews on distribution, structure and biological functions of fibronectin see [1–6]).

The functions attributed to fibronectin such as cell adhesion to substrata, cell spreading, opsonization of bacteria and other particulate matter, ability to induce a more normal phenotype in transformed cells, wound healing and chemotaxis are all related to its affinity to cell surface and a large number of different macromolecules (see reviews above).

In previous studies fibronectin has been reported to be present in synovial fluid from patients with various rheumatic diseases and exceed those in plasma samples from the same individual, suggesting local synthesis [7–10].

Recently it was reported that synovial fluid fibronectin from rheumatoid patients has a different molecular charge with respect to plasma fibronectin [10]. This different molecular charge has been attributed to proteolytic cleavage of

fibronectin by proteolytic enzymes present in synovial fluid [10].

Here we demonstrate that charge differences between plasma and synovial fluid fibronectin are not due to proteolytic cleavage but to lack of sialic acid in synovial fluid fibronectin.

2. MATERIALS AND METHODS

Synovial fluid and blood were supplemented with EDTA (final conc. 0.5%, w/v). Synovial fluids were incubated with hyaluronidase (final conc. 5 $\mu\text{g/ml}$) at 37°C for 1 h. Samples were centrifuged at 7000 $\times g$ for 10 min at 20°C. Sodium azide at a final conc. of 0.1% (w/v) and 5 kunits/ml of aprotinin (Sigma, St. Louis, MO) were added. In experiments comparing plasma and synovial fluid fibronectin the plasma was also incubated with hyaluronidase (5 $\mu\text{g/ml}$). Human plasma and synovial fluid fibronectin were purified as in [11,12]. Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was done as in [13]. Separation of plasma and synovial fluid proteins according to their net molecular charge was carried out either on 5% polyacrylamide gels as in [14] or on 2% agarose in high resolution buffer (Gelman Sciences, Ann Arbor, MI).

Plasma and synovial fluid proteins were transferred from polyacrylamide gels to nitrocellulose sheets as in [15]. Fibronectin was located on nitrocellulose sheets using monospecific

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rabbit antibodies to human plasma fibronectin and peroxidase conjugated goat anti-rabbit Ig as the second antibody [15,16].

Neuraminidase (Neuraminidase-Test, Behringwerke, Marburg, FRG) treatment of purified plasma and synovial fluid fibronectin was carried out incubating fibronectin (0.5 mg/ml final conc.) in 0.05 M sodium acetate buffer, 0.154 M NaCl, 9 mM CaCl₂ (pH 5.5) with neuraminidase (0.5 units/ml final conc.) for 1 h at 37°C.

Thermolysin (Protease type X, Sigma, St. Louis, MO) digestion of purified fibronectin was performed as in [17]. Purified fibronectin (1 mg/ml) in 25 mM Tris (pH 7.6), 0.5 mM EDTA, 50 mM NaCl was digested with 5 µg/ml thermolysin for 4 h at 22°C. The digestion was terminated by adding EDTA (5 mM final conc.) to inactivate thermolysin. Purification of the gelatin binding fragments from the fibronectin thermolysin digest was carried out as in [17].

3. RESULTS

Fig.1A shows an SDS-PAGE of purified plasma and synovial fluid fibronectin; no significant differences in apparent M_r are detectable. No fibronectin fragments are visible in either fibronectin preparation. Identical results were obtained with fibronectin from synovial fluid from healthy donors or from patients suffering from inflammatory rheumatic diseases. To exclude the possibility of artifacts or selection of fibronectin molecules during the purification procedures total plasma and synovial fluid proteins were transferred from a 10% SDS-PAGE onto a nitrocellulose sheet followed by immunoenzymatic localization of fibronectin. Results identical to those observed with purified fibronectin were obtained (fig.1B).

Fig.2 shows an agarose gel electrophoresis on which purified plasma and synovial fluid fibronectin, before and after neuraminidase treatment, migrated according to their net molecular charge. Before neuraminidase treatment synovial fluid fibronectin shows a slower mobility with respect to plasma fibronectin. Neuraminidase treatment does not change the mobility of synovial fluid fibronectin. In contrast, after neuraminidase treatment, plasma fibronectin assumes a mobility identical to that of synovial fluid fibronectin. These data in-

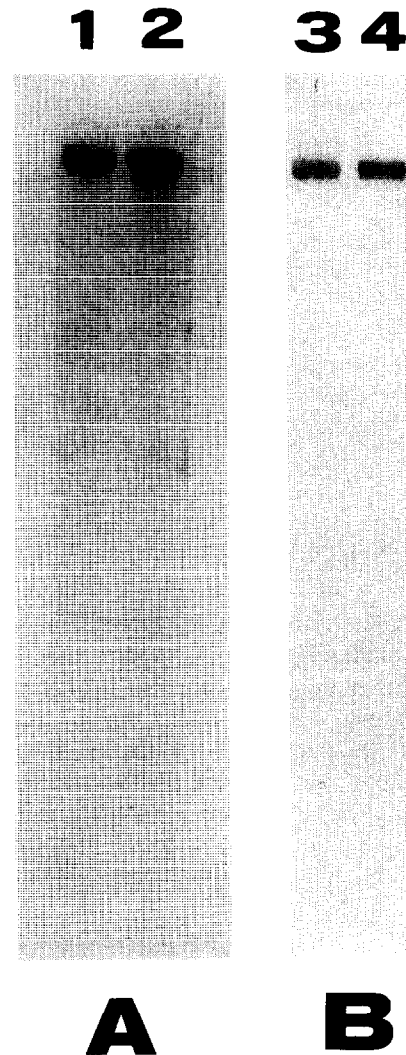


Fig.1. (A) 10% SDS-PAGE of plasma (1) and synovial fluid (2) β -mercaptoethanol reduced purified fibronectin. (B) Nitrocellulose strip on which plasma (3) and synovial fluid (4) proteins were transferred after separation on 10% SDS-PAGE followed by immunoenzymic localization of fibronectin.

dicate that synovial fluid fibronectin lacks sialic acid.

To determine which domain(s) of synovial fluid fibronectin lacks sialic acid, neuraminidase-treated and untreated plasma and synovial fluid fibronectin were digested by thermolysin and the polypeptides obtained analysed on polyacrylamide gel electrophoresis.

Fig.3 shows thermolysin digests of neuramin-

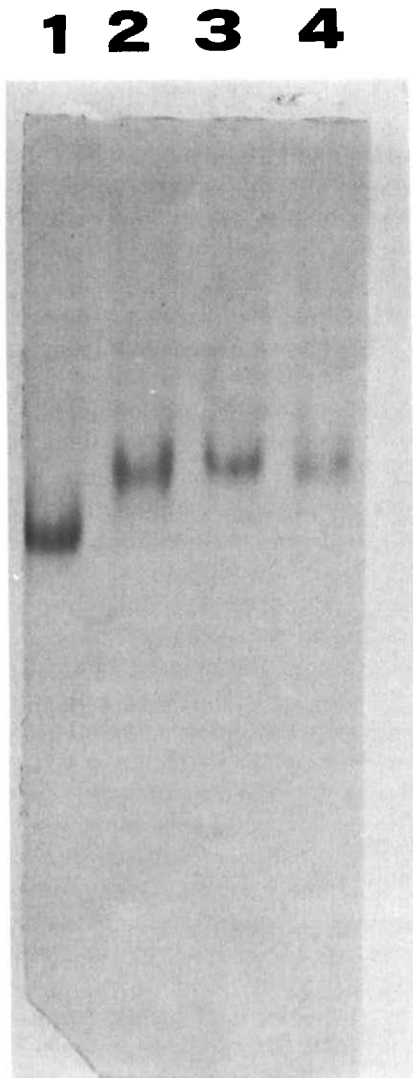


Fig.2. 2% agarose gel of purified fibronectin from: plasma (1); synovial fluid (2); plasma after neuraminidase treatment (3); synovial fluid after neuraminidase treatment (4).

idase-treated and untreated plasma and synovial fluid fibronectin separated on a 5% polyacrylamide gel electrophoresis according to net molecular charge. Thermolysin digest of neuraminidase-untreated plasma fibronectin shows many polypeptides with a higher electrophoretic mobility with respect to those of synovial fluid fibronectin thermolysin digest (fig.3, lanes 1,2). Thermolysin digest of neuraminidase-treated plasma fibronectin shows a pattern identical to

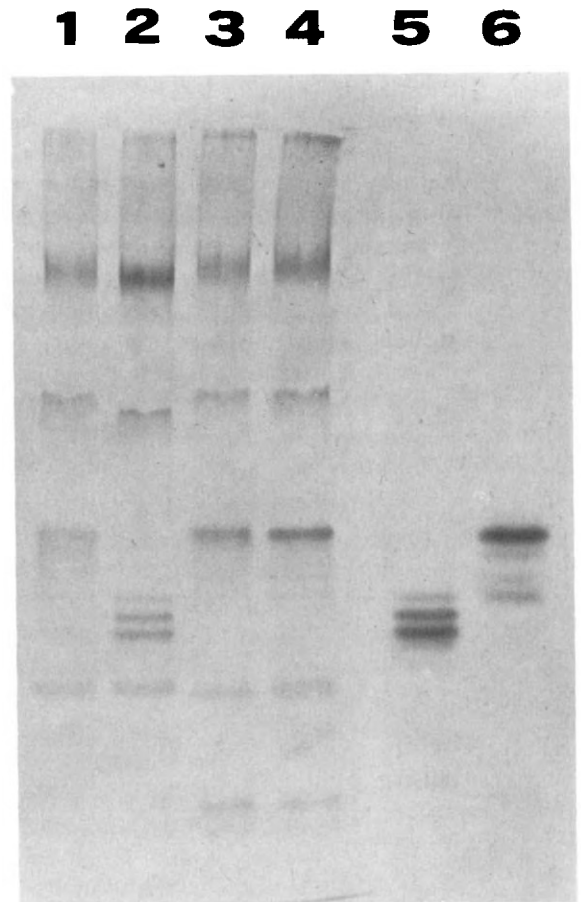


Fig.3. 5% PAGE of thermolysin digested purified plasma and synovial fluid fibronectin. Lane 1, thermolysin digestion of synovial fluid fibronectin, lane 2, thermolysin digestion of plasma fibronectin, lane 3, thermolysin digestion of synovial fluid fibronectin previously treated with neuraminidase, lane 4, thermolysin digestion of plasma fibronectin previously treated with neuraminidase, lane 5, thermolysin digest purified plasma gelatin binding fibronectin fragment, lane 6, thermolysin digest purified plasma gelatin binding fragment after neuraminidase treatment.

that of synovial fluid fibronectin (fig.3, lanes 3,4). In contrast, the patterns of the thermolysin digests of neuraminidase-treated and untreated synovial fluid fibronectin are identical (fig.3, lanes 1,3). Fig.3 also shows the purified plasma fibronectin gelatin binding fragment before and after neuraminidase treatment (lanes 5,6).

4. DISCUSSION

Synovial fluid fibronectin does not show detectable differences in its apparent M_r with respect to plasma fibronectin as determined by SDS-PAGE. This is observed with fibronectin from the synovial fluid of healthy donors and patients suffering from rheumatic inflammatory diseases [18]. In contrast, all synovial fluid fibronectins show a different net molecular charge compared with plasma fibronectins. This difference in molecular charge cannot be explained by proteolytic cleavage of synovial fluid fibronectin, as suggested in [10], since synovial fluid fibronectin shows an M_r similar to that of plasma fibronectin.

Here we demonstrate that the difference between the electrophoretic mobility of synovial fluid and plasma fibronectin is due to a lack of sialic acid in synovial fluid fibronectin. Furthermore, neuraminidase treatment of thermolysin digest fragments indicates that the synovial fluid gelatin binding fragment is completely lacking in sialic acid. The different sialic acid content of synovial fluid fibronectin may be due to synthesis by a cell type which differs from that responsible for the production of the plasmatic pool. Hepatocytes have been proposed as the major producers of plasma fibronectin [19]. Likely candidates for the production of synovial fluid fibronectin, which is at least in part locally synthesized [7-10], would include endothelial cells and synoviocytes. Studies on the electrophoretic mobility of fibronectins synthesized by different kinds of cultured cells should clarify the origin of synovial fluid fibronectin.

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