SIMILARITY OF FIBRONECTINS ISOLATED FROM HUMAN PLASMA AND SPENT FIBROBLAST CULTURE MEDIUM

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1. Introduction

Fibroblasts contain a major surface glycoprotein, fibroblast surface antigen [1] or fibronectin, which is secreted by cultured fibroblasts into the medium [2]. Immunological evidence indicates, that a protein similar to fibronectin is present in human plasma [3]. We describe here the isolation of the fibronectin antigen from human plasma and spent fibroblast culture medium by use of chromatography on immobilized antibody to fibronectin followed by isoelectric focusing. The two forms of fibronectin were found to have the same amino acid, carbohydrate, and polypeptide compositions. This and their previously known immunological identity strongly suggest that the circulating fibronectin is identical to the one secreted by cultured fibroblasts.

2. Materials and methods

The antisera to fibronectin used in these experiments have been described [4]. After absorption with fetal calf serum, the antiserum gave a single identical precipitation line against whole plasma and fibroblast extracts and did not react with calf serum. Radioimmunoassays of fibronectin were carried out by a double antibody method using ^{125}I-labeled [5] plasma fibronectin as tracer. The IgG fraction of antiserum was coupled to Sepharose 4B gel beads (Pharmacia Fine Chemicals, Piscataway, NJ) by the cyanogen bromide method [6]. Samples of heparinized human plasma or spent fibroblast culture medium obtained from two different lines of human skin fibroblasts grown in 10% fetal calf serum were incubated with the antibody containing gel for 4 h at 22°C. The beads were washed with 100 bed vol. PBS and eluted with 8 M urea in 0.05 M Tris–HCl buffer, pH 7.5. The eluates were dialyzed against 9 M urea. To protect the amino groups, Ampholine (pH 3.5–10.0, LKB, Sweden) was added to the solution to a concentration of 0.1% prior to dialysis. The dialyzed material was subjected to isoelectric focusing in a 200 ml LKB column for 24 h with 800 V. A pH gradient of 3.5–10.0 in 9 M urea was used.

Fractions with high specific activity from isoelectric focusing were pooled and dialyzed against 1 M NaCl, then 0.1 M acetic acid, and lyophilized. For amino acid and sugar analyses, purified material was taken up in a small volume of deionized 4 M urea and precipitated in cold with 50% ethanol. Samples were hydrolyzed with *p*-toluensulfonic acid [7] and analyzed with a Beckman Model 121 amino acid analyzer. Tyrosine/tryptophan ratio was determined spectroscopically [8]. Monosaccharide composition was determined by gas chromatography after methanolysis and trifluoroacetylation of the protein samples [9]. SDS–polyacrylamide gel electrophoresis was carried out by the method of Fairbanks et al. [10] using 4% acrylamide. A commercial preparation (BDH, Poole, England) of crosslinked oligomers of a protein with a 53 000 mol. wt monomer was used as

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a standard. Peptide mapping by limited proteolysis in sodium dodecyl sulphate followed by gel electrophoresis was carried out by the method of Cleveland et al. [11]., except that trypsin at pH 8.0 was used. Cyanogen bromide cleavage was performed in 70% formic acid at a protein and cyanogen bromide concentration of 2 mg/ml (100-fold molar excess to methionine). After 16 h incubation at 25°C, the samples were lyophilized and analyzed in gel electrophoresis [11].

3. Results

The purification procedure resulted in a 170- and 380-fold increase in specific activities of plasma and culture medium fibronectins, respectively. The yield in both cases was about 30%.

Fibronectin, isolated by immunoadsorbent, focused as detected by radioimmunoassay in a pH-range of 5.5–6.2, the main peak being at pH 6.0 (fig.1). Refocusing in a shallower pH gradient (pH 3.5–7.0), revealed further heterogeneity. In shallow gradients, losses due to precipitation and absorption to glass were appreciable. Similar patterns were obtained with plasma and culture medium fibronectins.

In SDS–polyacrylamide electrophoresis reduced plasma and culture medium fibronectins showed two closely migrating bands with approx. mol. wt 250 000. In addition, a weak doublet band with approx. mol. wt 220 000 was consistently observed (fig.2). The amino acid compositions of plasma and culture medium fibronectins were very similar (table 1). The tyrosin/tryphophan ratio was 1.6 in both cases. The only appreciable dissimilarity was in the value assigned to threonin. The fibronectin preparations contained about 4.5% carbohydrate (table 2). The carbohydrate portion of the molecule is made up of galactose, mannose, glucosamine, and sialic acid. The carbohydrate composition of plasma and culture medium fibronectins differs significantly in the content of sialic acid only. The specific activities of the two fibronectins in radioimmunoassay were identical.

In the peptide mapping experiments, fifteen

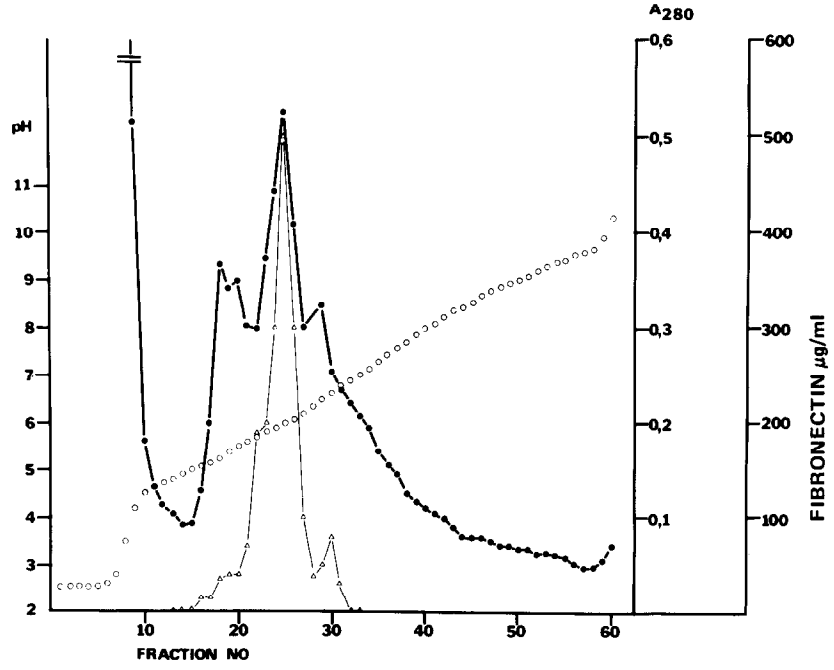

Fig.1. Isoelectric focusing of plasma fibronectin purified by immunoadsorption chromatography. Open circles: pH-gradient. Closed circles: absorbance at 280 nm. Triangles: fibronectin activity in radioimmunoassay.

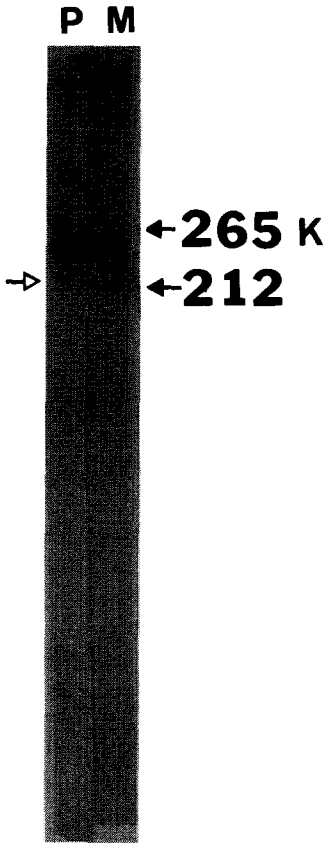

Fig.2. SDS–polyacrylamide electrophoresis in 4% gel of plasma (P) and culture medium (M) fibronectins. Migration positions of mol. wt markers (265 000, 212 000) are shown by arrows on the right side of picture. Migration position of weak doublet bands is indicated by the arrow on left side of the picture.

major bands were observed in the electrophoretical analysis of tryptic peptides from medium fibronectin (fig.3). Analysis of tryptic peptides from plasma fibronectin revealed the same bands with slight differences in the intensity of some of the bands. These differences were not reproducible when several plasma and medium fibronectins were analyzed. An identical electrophoretical pattern was obtained with cyanogen bromide peptides of medium and plasma fibronectins (fig.3).

Table 1
The amino acid compositions (mol %) of fibronectins isolated from spent fibroblast culture medium (I) and from plasma (II), and of the cold insoluble globulin of plasma (III, IV)

Residue	I[a]	II[b]	III[d]	IV[e]
Tyr	4.21	4.34	4.54	4.3
Phe	2.54	2.56	2.71	2.4
Trp	2.63[c]	2.71[c]	2.77	
Lys	5.13	4.35	3.65	3.6
His	1.96	1.80	2.07	2.3
Arg	4.51	4.48	5.16	5.4
Asp	9.52	10.08	9.25	9.5
Thr	8.42	10.42	9.67	10.8
Ser	8.33	8.55	6.79	8.0
Glu	11.63	11.78	11.61	11.5
Pro	8.46	7.01	7.61	8.2
Gly	8.85	8.27	7.97	8.6
Ala	4.93	5.10	4.29	4.2
1/2 Cys	2.77	2.14	2.63	2.5
Val	6.16	6.01	8.06	8.2
Met	0.86	1.09	1.12	0.8
Ile	3.56	3.12	4.40	4.5
Leu	5.52	6.17	5.71	5.4

[a]Mean of 2 determinations from 3 independent samples
[b]Mean of 2 determinations from 2 independent samples
[c]Calculated from determined tyrosin/tryptophan ratio
[d]Calculated from the data of Mosesson et al. [12]
[e]Data from Mosher [13]

Table 2
Carbohydrate composition of fibronectins isolated from spent fibroblast culture medium (I) and from plasma (II)

Residue	Spent medium (%) I[a]	Plasma (%) II[b]
Galactose	1.2	0.9
Mannose	1.4	1.2
Glucosamine	1.4	1.5
Sialic acid	0.5	0.8
Total	4.5	4.4

[a]Mean of determinations from 3 independent preparations
[b]Mean of determinations from 2 independent preparations

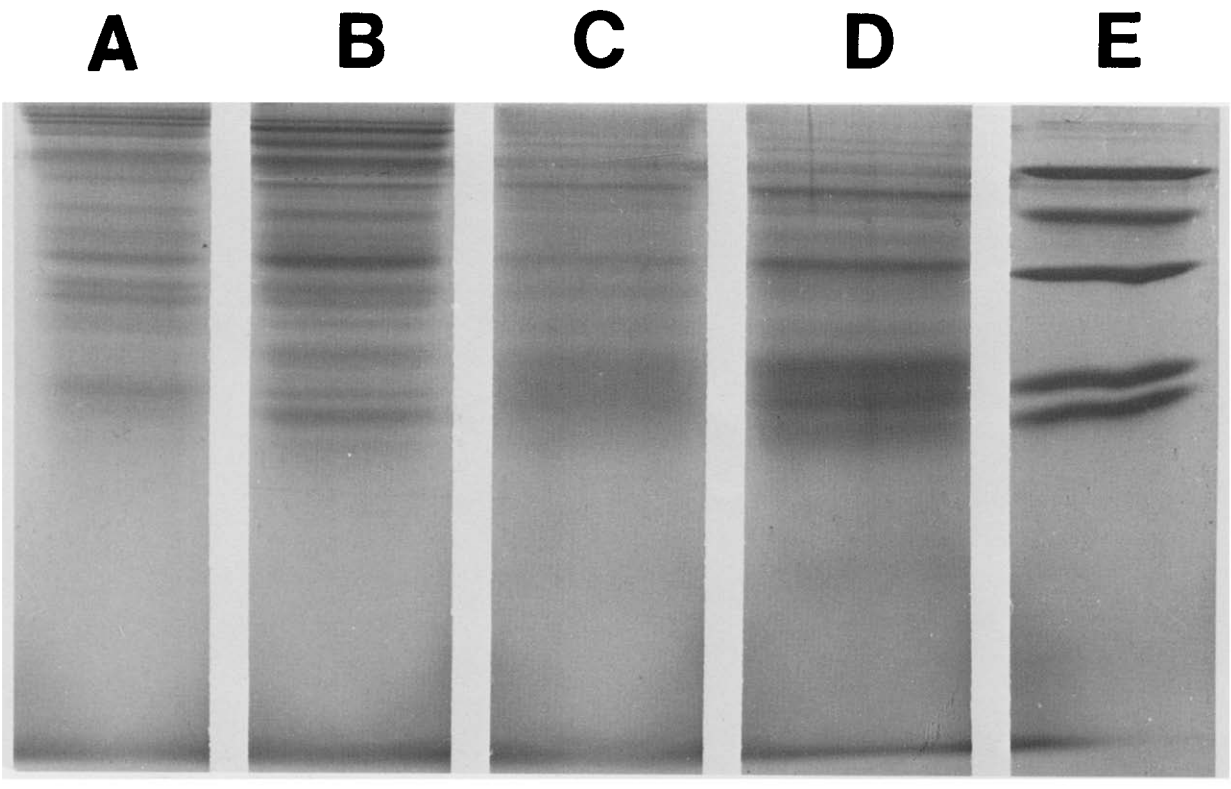

Fig.3. Discontinuous SDS–polyacrylamide electrophoresis (11% gel) of peptides obtained by limited tryptic proteolysis of plasma (A) and medium (B) fibronectins and by cyanogen bromide cleavage of plasma (C) and medium (D) fibronectins. Standards shown on gel E are: bovine serum albumin (mol. wt 68 000), ovalbumin (mol. wt 43 500), carbonic anhydrase (mol. wt 28 000), β-lactoglobulin (mol. wt 18 000) and lysozyme (mol. wt 14 500).

4. Discussion

Our results support the idea [4] that the circulating protein known to be immunologically identical with fibronectin in immunodiffusion represents fibronectin shed or secreted by fibroblasts (and possibly other cells synthesizing fibronectin) in vivo. The identical specific activities of fibronectins purified from plasma and culture medium in radioimmunoassay is further proof of their immunological identity. We found the chemical properties of the two fibronectins also very similar. This includes identical mobilities in SDS–polyacrylamide gel electrophoresis, similar peptide maps, similar isoelectric points, and closely similar amino acid and carbohydrate compositions. The identity of plasma and culture medium forms of fibronectin indicates that easily obtainable plasma can be used as a source of fibronectin for studies on the structure and function of this molecule.

Our results also support the suggested identity [3] between fibronectin and cold insoluble globulin (CIg). CIg is known to have two major polypeptide chains with mol. wt 220 000 and 215 000. In SDS–polyacrylamide gel electrophoresis minor bands representing somewhat smaller molecular weights were also detected [12]. This agrees with the present findings. The difference in the absolute values of molecular weights obtained by us and by Mosesson et al. [12] probably is not significant because different molecular weight standards were used. The amino acid and carbohydrate compositions also showed close agreement between our results and those of Mosesson et al.

[12] and the amino acid composition reported by Mosher [13].

Recent results from our laboratory show that both plasma and fibroblast derived fibronectins bind collagen [14] and purified plasma fibronectin agglutinates rabbit red blood cells (Vuento, M., Engvall, E. and Ruoslahti, E. submitted) as has been reported for the cell surface form [15]. These similarities in biological properties and the results presented in this paper form a strong body of evidence for the identity of the plasma and fibroblast derived soluble fibronectins.

The present results bear only on the relationship of plasma fibronectin to that present as a soluble product in a culture medium. It is possible that the fibronectin attached to the cell surface differs from the shed form. It could, for instance, contain a hydrophobic sequence, which is cleaved off when fibronectin leaves the membrane.

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References

[1] Ruoslahti, E., Vaheri, A., Kuusela, P. and Linder, E. (1973) Biochim. Biophys. Acta 322, 352–358.
[2] Vaheri, A. and Ruoslahti, E. (1975) J. Exp. Med. 142, 530–535.
[3] Ruoslahti, E. and Vaheri, A. (1975) J. Exp. Med. 141, 497–501.
[4] Ruoslahti, E. and Vaheri, A. (1974) Nature 248, 790–791.
[5] Greenwood, F. C., Hunter, W. M. and Glover, J. C. (1963) Biochem. J. 89, 114–123.
[6] Axen, R., Porath, J. and Ernback, S. (1967) Nature 214, 1302–1304.
[7] Liu, T.-Y. and Chang, Y. (1971) J. Biol. Chem. 246, 2842–2848.
[8] Bencze, W. L. and Schmid, K. (1957) Anal. Chem. 29, 1193–1196.
[9] Wrann, M. and Todd, C. W. (1977) J. Chromatogr. in press.
[10] Fairbanks, G., Steck, T. L. and Wallach, D. F. H. (1971) Biochemistry 10, 2606–2617.
[11] Cleveland, D. W., Fischer, S. G., Kirschner, M. W. and Laemmli, U. K. (1977) J. Biol. Chem. 252, 1102–1106.
[12] Mosesson, M. W., Chen, A. B. and Huseby, R. M. (1975) Biochim. Biophys. Acta 386, 509–524.
[13] Mosher, D. F. (1975) J. Biol. Chem. 250, 6614–6621.
[14] Engvall, E. and Ruoslahti, E. (1977) Int. J. Cancer 20, 1–5.
[15] Yamada, K. M., Yamada, S. S. and Pastan, I. (1975) Proc. Natl. Acad. Sci. USA 72, 3158–3162.