ELECTRON MICROSCOPY STUDY OF FIBRONECTIN STRUCTURE

V. E. KOTELIANSKY, M. V. BEJANIAN and V. N. SMIRNOV
USSR Cardiology Research Center, USSR Academy of Medical Sciences, Petroverigsky Pereulok 10, Moscow, USSR

Received 3 September 1980

1. Introduction

Much attention has been paid recently to the study of fibronectins (reviewed [1–4]). Fibronectins are high molecular-weight glycoproteins present in plasma, tissues and in various types of cells [1–4]. They specifically interact with collagen, fibrin, heparin, hyaluronic acid, DNA and display a high affinity to the cell surface [1–4]. It is believed that fibronectins participate in cell adhesion, hemostasis, functions of the reticuloendothelial systems; fibronectin synthesis decreases at malignant transformation [1–4]. Fibronectin is a dimer of M_r 4.4–5.0 × 10^5 consisting of two polypeptide chains interconnected by one or two disulfide bridges [5,6]. In physiological conditions fibronectin has a sedimentation coefficient of 13–14 S and a frictional ratio of 1.7 [5–7]. In evaluating the secondary structure of fibronectin from circular dichroism measurements no organized structures, either α-helices or β-forms, were observed [5,7,9]. However, the study of the fibronectin denaturation process registered by optical and sedimentation methods has shown the presence of highly organized tertiary structure elements in the protein [7–9]. It has been suggested that fibronectin has an elongated form containing domains which are interconnected by randomly organized polypeptide chain segments [7–9].

This work is the first to present an electron microscopy study of the structural organization of fibronectin. Vasiliev's method was used to prepare the sample by freeze-drying in vacuum and shadowing with tantalum–tungsten [10–12]. It was shown that fibronectin has a compact, somewhat elongated structure with an axial ratio of 2:1.1, a length of 155 ± 13 Å and a width of 88 ± 17 Å.

2. Materials and methods

Fibronectin was isolated from human plasma by gelatin–Sepharose affinity chromatography [13]. Plasma (100 ml) was passed through a Sepharose 4B column (200 ml) and then through a gelatin–Sepharose column (400 ml). The gelatin–Sepharose was washed with a 200 ml buffer containing 0.02 M Tris–HCl, 0.05 M α-amino caproic acid ($pH_{20°C}$ 7.5) and then with 200 ml of buffer consisting of 0.2 M Tris–HCl, 1 M NaCl ($pH_{20°C}$ 7.5) until the A_{280} was ≤0.01 units. The protein bound to the gelatin–Sepharose was eluted by a buffer with 0.05 M Tris–HCl, 2 M KBr ($pH_{20°C}$ 5.3). The obtained fibronectin preparation was dialyzed overnight against buffer with 0.05 M Tris–HCl, 0.1 M NaCl ($pH_{20°C}$ 7.5). Immediately before the experiment the studied fibronectin preparation was transferred for electron microscopy into a buffer containing 0.05 M CH_3COONH_4 (pH 7.5) using a column with Sephadex G-25. The protein concentration was 0.1–0.2 A_{280} units/ml.

The sample was prepared for electron microscopy by the method developed in [10–12] consisting of fast freezing to liquid nitrogen temperature, freeze-drying in vacuum and shadowing with tungsten–tantalum. Fibronectin preparations were studied in the JEM-100C electron microscope at 80 kV at a magnification of 50 000.

3. Results

A protein preparation studied by electron microscopy must by highly homogeneous and so immediately before the experiment the fibronectin preparation was analyzed by high speed sedimentation and polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Figures 1 and 2 present the

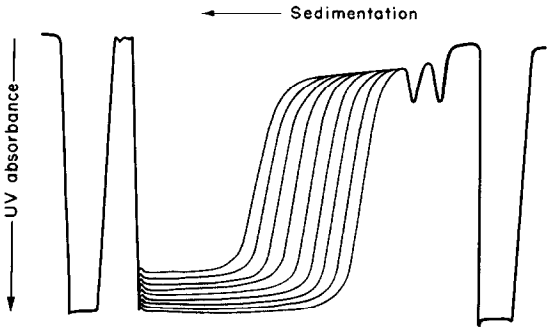

Fig.1. Sedimentation diagram of the fibronectin preparation in buffer containing 0.05 M CH_3COONH_4 (pH 7.5). Centrifugation was done in a UCA-10 analytical centrifuge (USSR) at 500 000 rev./min, 20°C; records were taken at 4 min intervals.

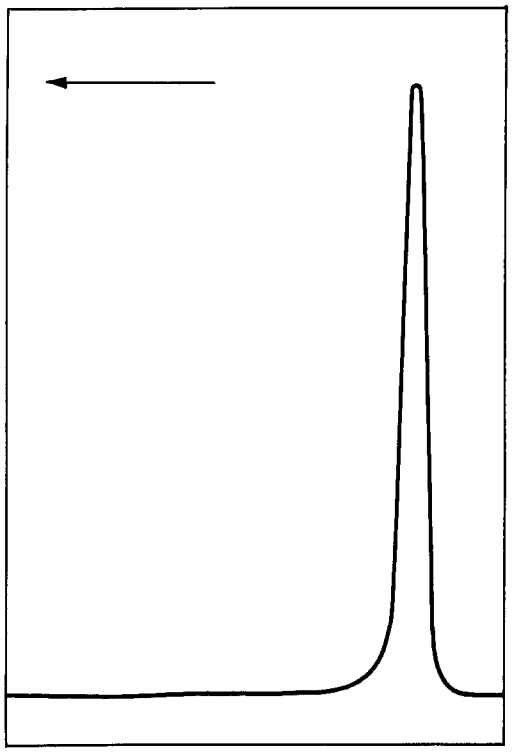

Fig.2. Densitogram of the gel after discontinuous polyacrylamide gel electrophoresis of the fibronectin preparation in the presence of sodium dodecyl sulfate and dithiothreitol according to [14]. The acrylamide concentration was 5%. Scanning was done on a Joyce-Loeble densitometer.

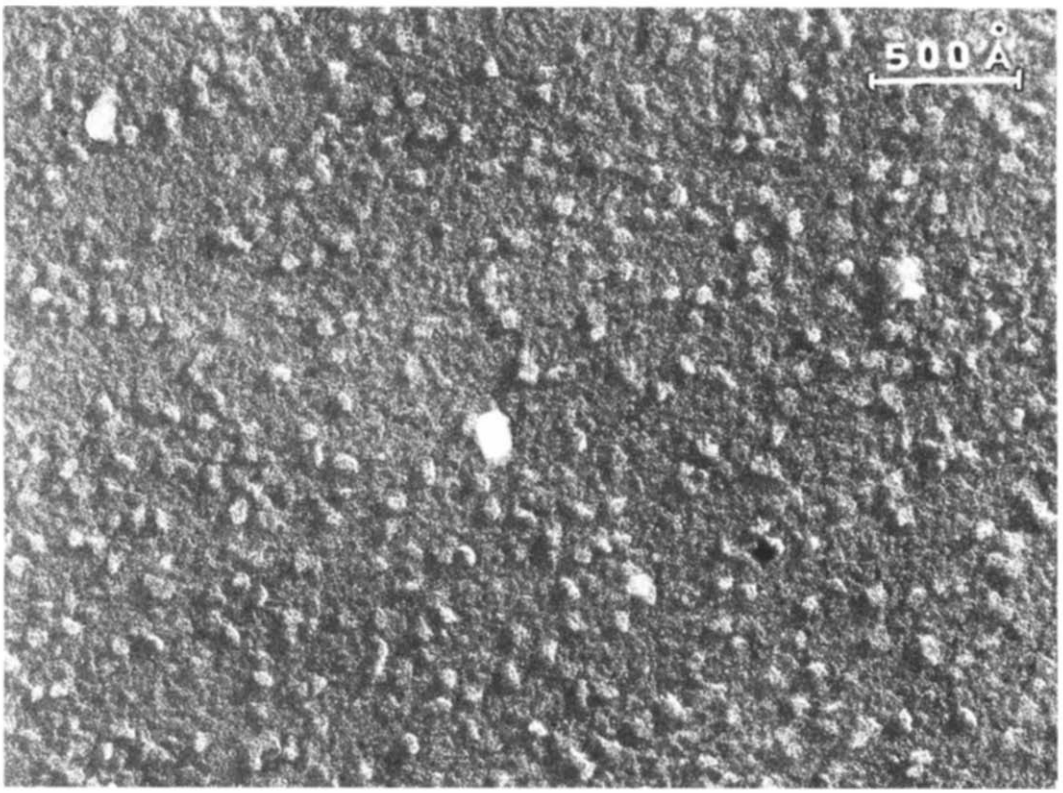

Fig.3. Electron micrograph of the fibronectin preparation freeze-dried in vacuum. Shadowing with tungsten–tantalum. Metal layer thickness was 15 Å. Magnification 200 000 ×.

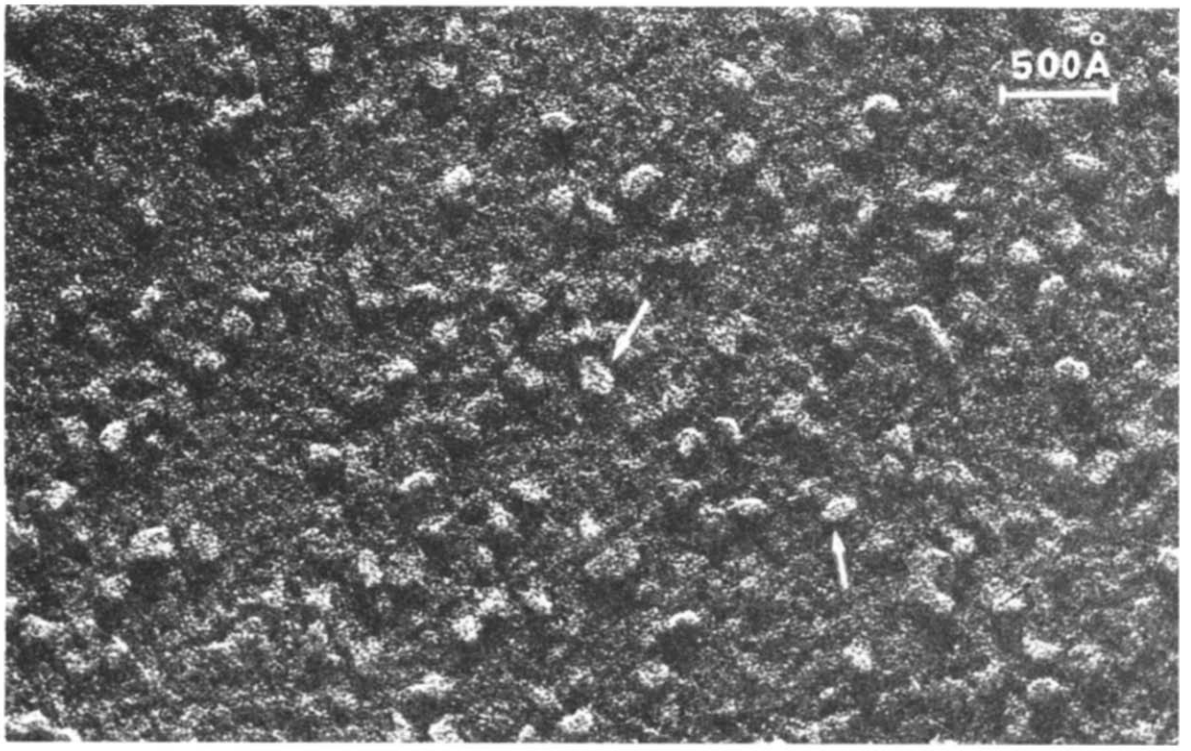

Fig.4. Electron micrograph of the fibronectin preparation. Conditions as in fig.3. Magnification 300 000 ×.

results of homogeneity analysis of the fibronectin preparation. Sedimentation analysis demonstrates the high homogeneity of the studied preparation (fig.1). The content of high molecular-weight aggregates was ⩽10%. In the buffer for electron microscopy fibronectin had a sedimentation coefficient of $s^{o}_{20,w} = 13.5 \pm 0.5$. On electrophoresis in polyacrylamide gel in the presence of sodium dodecyl sulfate and dithiothreitol (fig.2), fibronectin migrates as one band corresponding to a fibronectin monomer with an app. M_r of $2.2–2.5 \times 10^5$. The contaminants in the fibronectin preparations, as seen in fig.2, were ⩽5–10%. Thus, the studied fibronectin preparations satisfied the requirements for studies by physical methods. It must be noted that the fibronectin preparations also possessed a high functional activity. ⩾90% of the protein was capable of forming complexes with gelatin–Sepharose. Fig.3 shows a general view of a field of fibronectin molecules at a small magnification. It is readily seen that the studied protein preparation consists predominantly of homogeneous material with the majority of particles being close in dimensions and shape, and does not contain any noticeable amounts of denatured or degraded fibronectin molecules.

Fig.4 shows the fibronectin molecule images at a greater magnification. Measurements of the image

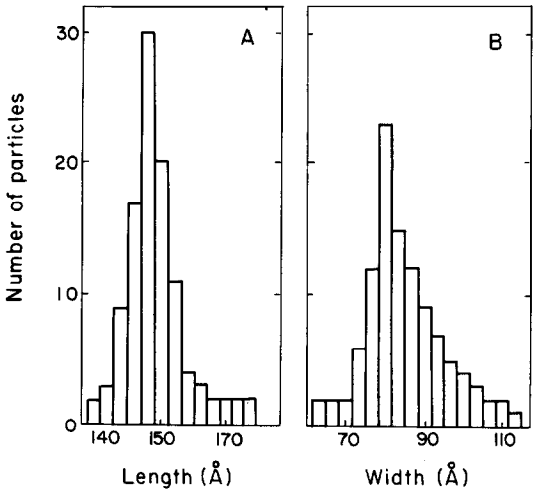

Fig.5. Distribution of fibronectin molecules by dimensions: A, by length; B, by width.

dimensions gave the following: the majority of the 107 analyzed particles had a length of 155 ± 13 Å and a width of 88 ± 17 Å. The histograms of fibronectin molecule distribution by length and width are given in fig.5. The axial ratio of most images is ~2:1.1. It is clearly seen in fig.4 that the fibronectin molecules have a compact, somewhat elongated structure. Some non-uniformity in the distribution of material inside the fibronectin molecule can be discerned in some of the images (these particles are indicated by arrows in fig.4). The observed non-uniformity is not surprising as fibronectin is a dimer and, apparently, contains domains. However, the obtained resolution is insufficient for a more detailed description of the fibronectin intramolecular structure. It should be emphasized that despite some asymmetry (axial ratio 2:1.1) the fibronectin molecule has a compact structure. Thus, the sufficiently high frictional ratio of 1.7 obtained previously can hardly reflect the elongatedness of the fibronectin molecule. Most probably the fibronectin molecule has a high intramolecular mobility.

4. Conclusion

Electron microscopy studies of the fibronectin molecule structure by freeze-drying in vacuum followed by shadowing with tungsten–tantalum has shown that fibronectin has a compact, somewhat elongated structure with an axial ratio of 2:1.1, a length of 155 ± 13 Å and a width of 88 ± 17 Å. The fibronectin monomers are probably not arranged end-to-end to each other, but have a lateral interarrangement.

Acknowledgements

The authors express their gratitude to Dr V. D. Vasiliev for constant attention and valuable discussion, to Dr M. J. Factor for aid, to O. M. Zalite for assistance in carrying out the experiments and to Dr M. A. Glukhova for critical comments.

References

[1] Hynes, R. O. (1976) Biochim. Biophys. Acta 458, 73–107.
[2] Yamada, K. M. and Olden, K. (1978) Nature 275, 179–184.
[3] Vaheri, A. and Mosher, D. F. (1978) Biochim. Biophys. Acta 516, 1–25.
[4] Pearlstein, E., Gold, L. I. and Garcia-Pardo, A. (1980) Mol. Cell. Biochem. 29, 103–128.
[5] Mosesson, M. W., Chen, A. B. and Husbey, R. M. (1975) Biochim. Biophys. Acta 386, 509–524.
[6] Mosher, D. F. (1975) J. Biol. Chem. 250, 6614–6621.
[7] Alexander, S. S. jr, Colonna, G. and Edelhoch, H. (1979) J. Biol. Chem. 254, 1501–1505.
[8] Alexander, S. S. jr, Colonna, G., Yamada, K. M., Pastan, I. and Edelhoch, H. (1978) J. Biol. Chem. 253, 5820–5824.
[9] Colonna, G., Alexander, S. S. jr, Yamada, K. M., Pastan, I. and Edelhoch, H. (1978) J. Biol. Chem. 253, 7787–7790.
[10] Vasiliev, V. D. (1974) Dokl. Akad. Nauk SSSR 219, 994–995.
[11] Vasiliev, V. D. (1974) Acta Biol. Med. Germ. 33, 779–793.
[12] Vasiliev, V. D. and Koteliansky, V. E. (1978) Methods Enzymol. 59, 612–619.
[13] Ruoslahti, E. and Engvall, E. (1977) Int. J. Cancer 20, 1–5.
[14] Studier, F. W. (1973) J. Mol. Biol. 79, 237–248.