PERSISTENCE OF A 140 000 M_r SURFACE GLYCOPROTEIN IN CELL-FREE MATRICES OF CULTURED HUMAN FIBROBLASTS

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1. Introduction

Detergent treatment of cultured cells has been used to study the composition of the pericellular matrix formed by fibroblasts [1–3] and other types of cells in culture [4,5]. Fibronectin seems to be one of the major components of the pericellular matrix [1,2,6,7] and it plays an important role in cell attachment [8–10]. Here, we show that the cell-free matrix material from human fibroblast cultures contains also a 140 000 M_r cell-surface glycoprotein (cf. [11]) which seems to be closely associated with the pericellular fibronectin.

2. Materials and methods

2.1. *Cell culture and treatments with sodium deoxycholate and trypsin*

Human embryonic fibroblasts were obtained from a local source and cultured in RPMI 1630 medium supplemented with 10% fetal calf serum and antibiotics. Sodium deoxycholate (DOC, 0.5%) extraction, to produce cell-free matrix of cultured fibroblasts, was performed on confluent, substratum-attached cells according to [1].

For trypsin treatment, the cells were incubated in 75 μg/ml of trypsin–TPCK (Worthington, Freehold, NJ) in NaCl–P buffer (140 mM NaCl, 10 mM sodium phosphate, pH 7.2) at 37°C for 30 min. Soybean trypsin inhibitor (100 μg/ml; Sigma, St Louis, MO) was used to terminate the enzymatic digestion.

2.2. *Radioactive labelling of the cells*

The externally disposed glycoproteins of cultured fibroblasts were labelled radioactively using the galactose oxidase/NaB^3H_4 technique [12] or the periodate/NaB^3H_4 technique [13]. Details are given in the figure legends. For metabolic labelling experiments, the subconfluent cells were grown in the presence of 2-[^{3}H]glycine (10 μCi/ml; spec. act. 14 Ci/mmol) for 48 h. The radiochemicals were purchased from the Radiochemical Centre (Amersham).

2.3. *Immunofluorescence microscopy*

Purified antibodies against human plasma fibronectin and fluorescein isothiocyanate (FITC)-coupled goat anti-rabbit IgG were obtained from Cappel Labs. (Cochraneville, CA) and tetramethyl rhodamine isothiocyanate-coupled wheat germ agglutinin (TRITC–WGA) from Vector Labs. (Burlingame, CA). For fluorescence staining, the cell or cell-free matrices were washed first in NaCl buffer and then fixed in 3.5% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 10 min. Thereafter the cells were incubated with anti-fibronectin antibodies followed by FITC–goat anti-rabbit IgG and then with TRITC–WGA. No binding of fluorochrome-coupled lectin by immunoglobulins was found [14,15].

2.4. *Polyacrylamide gel electrophoresis*

SDS–polyacrylamide gel electrophoresis was done as in [16]. For fluorography, the gels were immersed in EN^{3}HANCE (Radiochemical Centre, Amersham), dried and exposed to Kodak X-Omat film.

3. Results

Treatment of the cultured fibroblasts with 0.5% DOC led to a rapid disappearance of substratum-attached cellular material as judged by phase-contrast microscopy (fig.1). After such a treatment 10% of the cellular proteins remained on culture dishes.

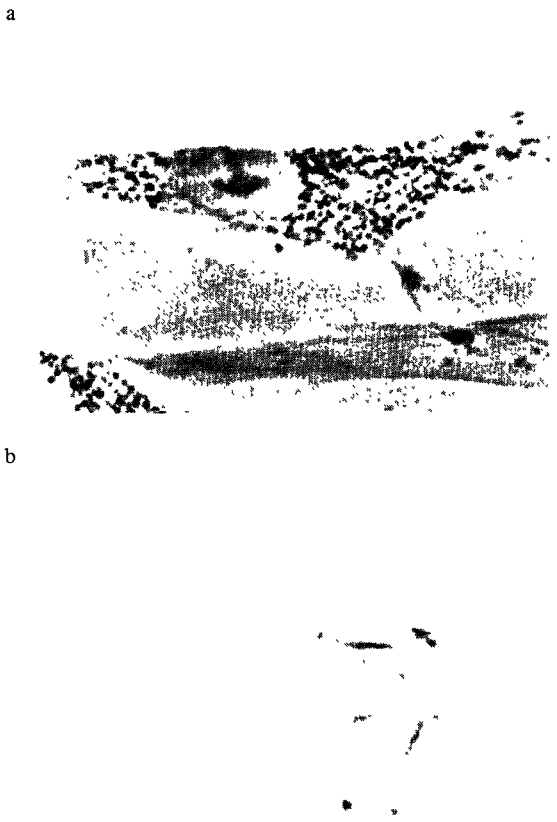
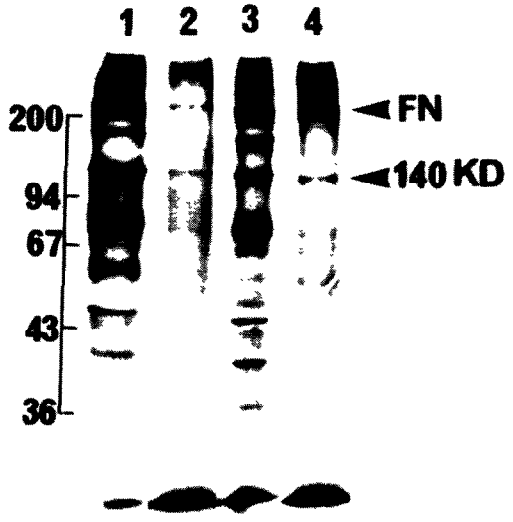


Fig.1. Phase contrast microscopy of untreated cultured fibroblasts shows a distinct nucleus and a dense cytoplasm (a) whereas after DOC-treatment only faint fibrils are left on the culture dishes (b). ×700.



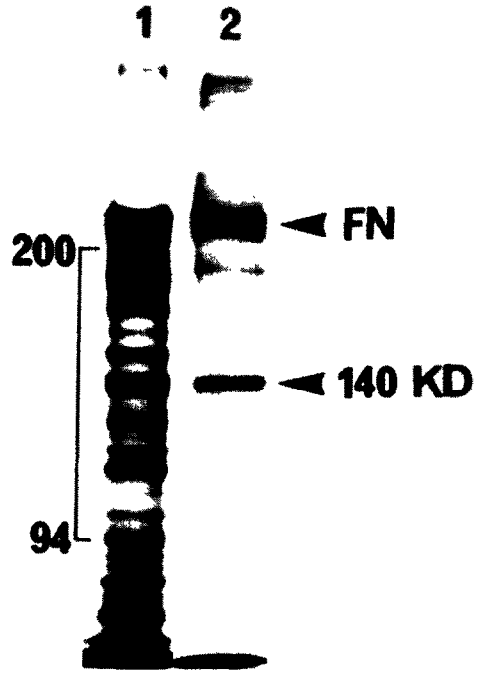


Fig.2. SDS–polyacrylamide gel electrophoresis of [^{3}H]glycine-labelled whole cells (1) and cell-free matrices produced by 0.5% DOC-treatment (2).

Fig.3. SDS–polyacrylamide gel electrophoresis after surface labelling by neuraminidase/galactose oxidase/NaB^3H_4 method of untreated fibroblasts (1) and of cell free matrices (2) and by metaperiodate/NaB^3H_4 method of whole cells (3) and of cell-free matrices (4). For surface labelling with neuraminidase/galactose oxidase/NaB^3H_4 method the cells on culture dishes were treated with neuraminidase (10 U/ml; *Vibrio cholera* neuraminidase, Behringwerke, Marburg-Lahn) and galactose oxidase (5 U/ml; Kabi, Stockholm) in NaCl–P buffer (140 mM NaCl, 10 mM sodium phosphate, pH 7.2) supplemented with Ca^{2+} at 37°C for 30 min. After washing, the cells were scraped into suspension and incubated with NaB^3H_4 (0.5 mCi/ml; 9.8 Ci/mmol; Radiochemical Centre, Amersham) at 22°C for 30 min. The cells were washed and dissolved in electrophoresis sample buffer. For metaperiodate/NaB^3H_4 labelling, the cells were treated first with ice-cold 2 mM sodium metaperiodate in NaCl–P buffer for 10 min, washed, scraped into suspension and reduced by NaB^3H_4 as above. To label the cell-free matrices, the cells were treated first with neuraminidase/galactose oxidase or metaperiodate as above and then extracted with 0.5% DOC. The material remaining on the culture dish was collected and treated with NaB^3H_4 as above.

Polyacrylamide gel electrophoresis of [^{3}H]glycine-labelled cells revealed only a few polypeptides in the DOC-resistant material remaining on the culture dishes (fig.2, lanes 1,2). The 220 000 M_r polypeptide represents pericellular fibronectin (cf. [1–3]) and the 180 000 M_r polypeptide procollagen type I chain [1,2]. In addition, a prominent polypeptide of 140 000 M_r appeared in the electrophoresis of the DOC-extracted cell cultures (fig.2, lanes 1,2). The 140 000 M_r polypeptide could be seen distinctly after DOC-treatment of cultured fibroblasts labelled by galactose oxidase/NaB^{3}H$_4$ or metaperiodate/NaB^{3}H$_4$ technique (fig.3, lanes 1–4). Trypsin-treatment led to a rapid detachment of the cultured fibroblasts from the growth substratum.

After surface labelling, the 140 000 M_r glycoprotein was found to remain in the detached cells which now lacked pericellular fibronectin [9,17] (fig.4, lanes 1–3). No cellular material could be revealed on the culture dishes after trypsin-induced cell-detachment in accordance with [3].

Immunofluorescence microscopy of the cell cultures treated with DOC revealed fibronectin either as distinct fibrillar structures or as spots (fig.5). The distribution of the 140 000 M_r glycoprotein was studied using TRITC–WGA, a sialic acid-binding lectin [18] which reacts with the 140 000 M_r sialoglycoprotein of cultured fibroblasts (V.-P. L., T. V., I. V., submitted). In double-staining microscopy, TRITC–WGA showed a membrane residue-like staining in the DOC-treated cultures. Large lace-like plasma residues, probably representing cell-to-substratum attachment sites (cf. [11,19]), could be seen attached to the growth substratum in the immediate vicinity of extracellular fibronectin (fig.5).

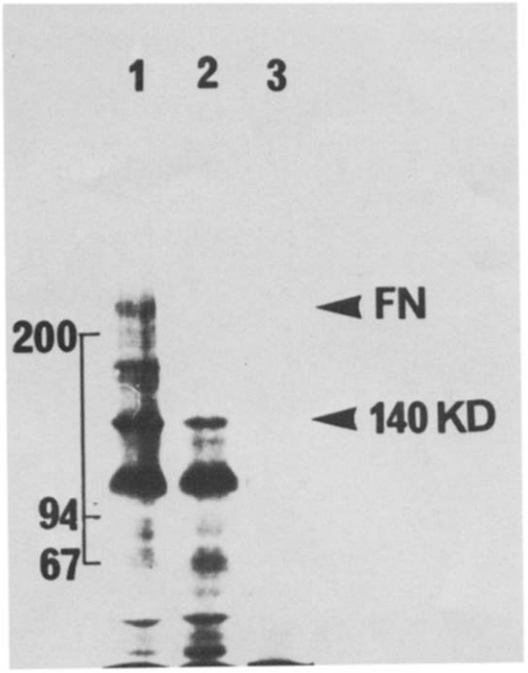
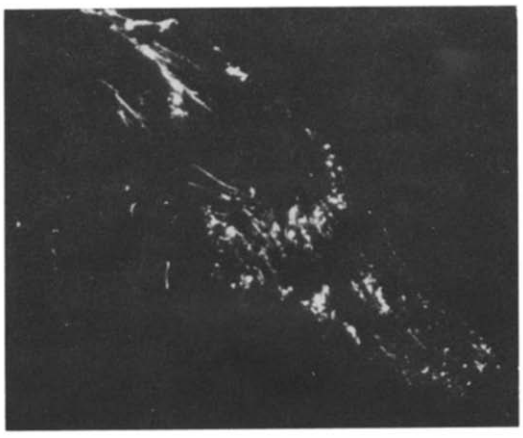


Fig.4. SDS–polyacrylamide gel electrophoresis after metaperiodate/NaB^{3}H$_4$-labelling of untreated (1), and of trypsin-treated cells (2) and of the material remaining on the culture dish after the trypsin-treatment (3). Note the disappearance of the 220 000 M_r (fibronectin) and the 180 000 M_r (procollagen) polypeptides [12] and the preservation of the 140 000 M_r polypeptide in the trypsin-treated cells.

a



b

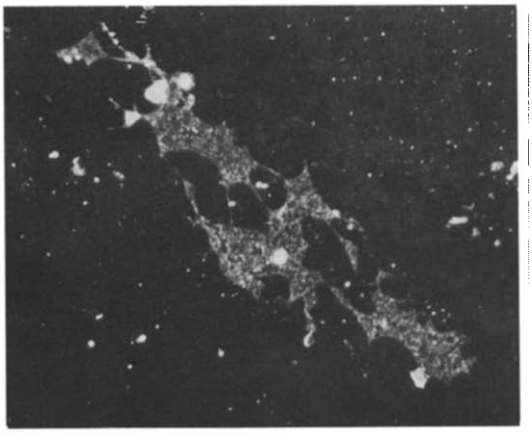


Fig.5. Double immunofluoresence of cell free matrices with anti-fibronectin antibodies (a) and TRITC–WGA (b). Note the close coalignment of the fibrillar fibronectin-specific fluorescence and the lace-like WGA binding. ×700.

4. Discussion

The anionic detergent, DOC, has been used to study the composition of pericellular matrices because treatment with this detergent appears to leave a cell-free material on the culture substratum [1,2]. This study shows that among the pericellular matrix material, consisting mainly of fibronectin, collagen and glycosaminoglycans [1,2], there is also a cell-surface glycoprotein, easily detectable using surface-specific labelling methods. The 140 000 M_r appears to represent only a small fraction of the total cellular material (cf. [11]), but together with fibronectin, seems to be a major glycoprotein in cell-free matrices. Results on the trypsinized, surface-labelled cells showed that the 140 000 M_r glycoprotein is a membrane glycoprotein (cf. [9,11]), and not an actual component of the pericellular matrix.

Anionic detergents, such as DOC, are known to preserve protein–protein interactions [20] and have been used, for instance, to isolate functionally intact enzyme complexes. The preservation of the 140 000 M_r membrane glycoprotein in the DOC-resistant pericellular matrix material of cultured fibroblasts suggests a specific interaction of the 140 000 M_r glycoprotein with the pericellular fibronectin. This is also supported by our morphological findings, which indicate a close coalignment of the DOC-resistant surface membrane residues with the pericellular material.

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References

[1] Hedman, K., Kurkinen, M., Alitalo, K., Vaheri, A., Johansson, S. and Höök, M. (1979) J. Cell Biol. 81, 83–91.
[2] Vartio, T., Seppä, H. and Vaheri, A. (1981) J. Biol. Chem. in press.
[3] Chen, L. B., Murray, A., Segal, R. A., Bushnell, A. and Walsh, M. L. (1978) Cell 14, 377–391.
[4] Birdwell, C. R., Gospodarowicz, D. and Nicolson, G. L. (1978) Proc. Natl. Acad. Sci. USA 75, 3273–3277.
[5] Gospodarowicz, D. and Ill, C. (1980) J. Clin. Invest. 65, 1351–1364.
[6] Hedman, K., Vaheri, A. and Wartiovaara, J. (1978) J. Cell Biol. 76, 748–760.
[7] Graham, J. M., Hynes, R. O., Davidson, E. A. and Bainton, D. F. (1975) Cell 4, 353–365.
[8] Hughes, R. C., Pena, S. D. J., Clark, J. and Dourmashkin, R. R. (1979) Exp. Cell Res. 121, 307–314.
[9] Grinnell, E. and Feld, M. K. (1979) Cell 17, 117–129.
[10] Yamada, K. M. and Olden, K. (1978) Nature 275, 179–184.
[11] Lehto, V.-P., Vartio, T. and Virtanen, I. (1980) Biochem. Biophys. Res. Commun. 95, 909–916.
[12] Gahmberg, C. G. and Hakomori, S. (1973) J. Biol. Chem. 248, 4311–4317.
[13] Gahmberg, C. G. and Andersson, L. C. (1977) J. Biol. Chem. 252, 5888–5894.
[14] Virtanen, I., Ekblom, P. and Laurila, P. (1980) J. Cell Biol. 85, 429–434.
[15] Kääriäinen, L., Hashimoto, K., Saraste, J., Virtanen, I. and Penttinen, K. (1981) J. Cell Biol. in press.
[16] Laemmli, U. K. (1970) Nature 277, 680–685.
[17] Bornstein, P. and Ash, I. F. (1977) Proc. Natl. Acad. Sci. USA 74, 2480–2484.
[18] Peters, B. P., Ebisu, S., Goldstein, I. J. and Flashner, M. (1979) Biochemistry 18, 5505–5511.
[19] Oppenheimer-Marks, N. and Grinnell, F. (1980) J. Cell Biol. 87, 84a.
[20] Helenius, A., McCastin, D. R., Fries, E. and Tanford, C. (1979) Methods Enzymol. 56, 734–749.