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Deglycosylation of the chymotryptic collagen-binding fragment of human plasma fibronectin does not modify its affinity to denatured collagen

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N-Glycanase deglycosylation of purified 44 kDa chymotryptic collagen-binding domain from human plasma fibronectin does not significantly modify its behavior on gelatin affinity chromatography. This indicates that carbohydrates do not play any role in the binding affinity of fibronectin to collagen. The influence of changes in glycosylation on the biological functions of fibronectin is discussed.

Fibronectin; Collagen; Peptide-N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase; Glycosylation

1. INTRODUCTION

Plasma fibronectin (pFn) is a high-M_r adhesive glycoprotein constituted of two similar subunits joined at the carboxy-terminal ends by disulfide bonds [1]. pFn has been implicated in numerous biological phenomena including cellular adhesion and migration, embryonic differentiation, chemotaxis, wound healing and hemostasis. These functions are associated with the presence in the pFn molecule of different domains which bind a variety of macromolecules including cell membrane receptors [2], collagen [3], heparin and other glycosaminoglycans [4], fibrin [5] and actin [6].

The role of carbohydrate residues in these binding functions is not yet well understood. The results of Olden et al. [7], suggesting that the carbohydrate moiety of Fn of chicken embryo fibroblasts is not required for the mediation of a number of biological activities played by this glycoprotein, have been recently re-examined by Jones et al. [8]. These authors provide evidence that carbohydrates weaken the binding affinity of human cellular Fn to gelatin and cellular receptors. Here, we show that enzymatic deglycosylation of the pFn collagen-binding domain does not affect its affinity for denatured collagen.

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Abbreviations: BSA, bovine serum albumin; Fn, fibronectin; pFn, human plasma fibronectin; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; TBS, 10 mM Tris-HCl/0.154 M NaCl (pH 7.2) buffer

2. MATERIALS AND METHODS

2.1. *Reagents*

N-Glycanase [peptide:*N*-glycosidase F, peptide-N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase, EC 3.5.1.52] (260 U/ml) was purchased from Genzyme (Boston, MA). α-Chymotrypsin type II (EC 3.4.21.1) (48 U/mg) was from Sigma (St Louis, MO), [^{125}I]IMS.30 from Amersham (Amersham, England). Iodo-Gen (1,3,4,6-tetrachloro-3α,6α-diphenylglycoluryl) was obtained from Pierce (Rockford, IL), gelatin-Ultrogel and Ultrogel AcA 44 from IBF (Villeneuve la G., France) and heparin-Sepharose 6B from Pharmacia (Uppsala).

2.2. *Purification of pFn and preparation of the chymotryptic collagen-binding domain*

pFn was prepared from citrated human plasma by gelatin-Ultrogel affinity chromatography as described [3] and eluted by 4 M urea. Prior to digestion, purified pFn was dialysed vs TBS.

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Hydrolysis was performed with α-chymotrypsin (pFn concentration, 0.8 mg/ml; enzyme/substrate ratio, 1:100, w/w) for 3 h at 20°C [9]. The chymotryptic hydrolysate was applied to heparin-Sepharose 6B and gelatin-Ultrogel columns serially (both 3 ml bed volume) equilibrated in TBS. After washing, the gelatin-Ultrogel column was eluted with 6 M urea.

2.3. *Radioiodination*

The chymotryptic collagen-binding domain was radioiodinated using [^{125}I]IMS.30 by the Iodo-Gen method according to Fraker and Speck [10]. ^{125}I-labelled collagen-binding domain was further purified by gel permeation on an Ultrogel AcA 44 column (1.6 × 100 cm) equilibrated in TBS supplemented with BSA (1 mg/ml).

2.4. *Deglycosylation*

The ^{125}I-labelled collagen-binding domain was deglycosylated with *N*-glycanase without previous denaturation. First, labelled glycopeptides were equilibrated in 0.22 M Tris-HCl (pH 8.6) buffer by gel permeation on a Sephadex G-25 column (10 ml bed volume). The reaction mixture containing, in a final volume of 90 μl, 80 μl glycopeptide solution (18000 cpm/10 μl), 9 μl of 100 mM 1,10-phenanthroline hydrate (in methanol solution) and 1 μl enzyme solution, was incubated for 48 h at 37°C. The action of *N*-glycanase was analyzed by SDS-PAGE (5–25% gradient gel run under reducing conditions) and autoradiography [11].

2.5. *Gelatin affinity chromatography*

Native or deglycosylated collagen-binding fragments were applied to a 10 ml gelatin-Ultrogel column and eluted with a 400 ml linear gradient from 0 to 6 M urea in TBS, supplemented with 0.1 mM PMSF.

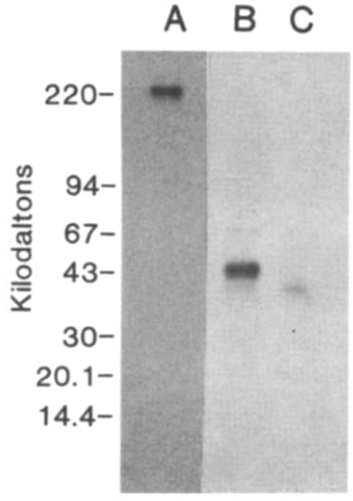

Fig.1. SDS-PAGE of purified pFn stained with Coomassie blue (lane A) and of ^{125}I-labelled native (B) and deglycosylated (C) collagen-binding fragments characterized by autoradiography. The left lane indicates the position of marker proteins for molecular mass estimation (in kDa): ferritin (220), phosphorylase *b* (94), albumin (67), ovalbumin (43), carbonic anhydrase (30), trypsin inhibitor (20.1) and α-lactalbumin (14.4).

3. RESULTS

3.1. N-*Glycanase treatment*

Affinity chromatography on heparin-Sepharose and gelatin-Ultrogel columns, followed by gel permeation on an Ultrogel AcA 44 column, led to isolation of the 44 kDa collagen-binding domain from the chymotryptic hydrolysate of purified pFn. Fig.1 shows SDS-PAGE of affinity-purified pFn and of its native and deglycosylated chymotryptic collagen-binding domains. Electrophoresis was run under reducing conditions and the absence of lower molecular mass bands indicates that there is no internal cleavage of the 44 kDa glycopeptides. After *N*-glycanase treatment, we observed a shift of the apparent molecular mass by about 6 kDa. Given that the collagen-binding domain of pFn is substituted by three biantennary *N*-glycans [12] with an expected mass of 5800 Da, the shift can therefore be attributed to total deglycosylation of the fragment.

3.2. *Gelatin affinity chromatography*

Collagen-binding domains treated or not with *N*-glycanase quantitatively bound to the gelatin-Ultrogel column and the elution profiles obtained

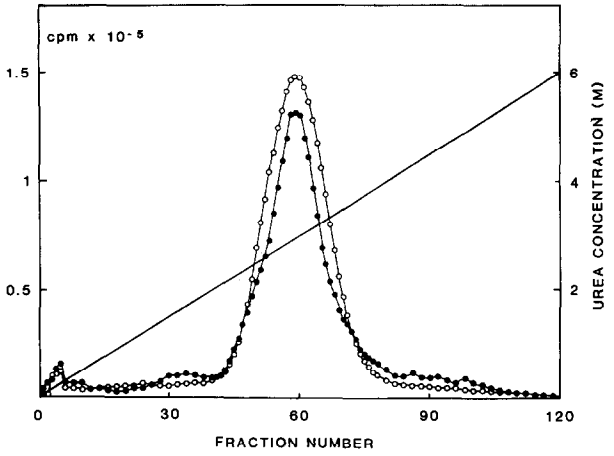

Fig.2. Elution profiles of chymotryptic collagen-binding fragment from gelatin-Ultrogel column. Both fragments, native (○—○) or deglycosylated (●——●), were eluted with 400 ml of a 0–6 M urea gradient. 3.35-ml fractions were collected. Elution peak of both fragments appeared at 2.95 M. (——) Urea concentration.

with a linear gradient of 0–6 M urea are shown in fig.2. The peak for elution of the native 44 kDa collagen-binding domain appeared at 2.95 M urea. We could observe that the apparent affinity of the isolated domain is enhanced vs native pFn (not shown). There is no significant difference in the profile obtained with the deglycosylated fragment, eluted at the same concentration in urea. Experiments performed in duplicate after 2 or 6 days incubation with the enzyme gave identical results.

4. DISCUSSION

Most of the fibronectin carbohydrates are located on the gelatin-binding domain of the molecule and Bernard et al. [13] have clearly shown that they play an important role in the stabilization of this domain against proteolytic degradation. On the other hand, the role of carbohydrates in the binding function is not yet well established and contradictory results have been reported [7,8]. Zhu and Laine [9] have recently shown that polylactosaminic glycosylation of human placental Fn weakens its affinity to gelatin. Here, we show that removal of biantennary glycans by *N*-glycanase does not affect the binding capacity of the collagen-binding domain of pFn. From these results, it appears that the binding capacity of Fns to collagenous structures can be modulated by the nature of the glycan. Several studies report evidence that Fns glycans can be modified under physiological [14,15] or pathological conditions such as neoplastic transformation [16–19]. With this in mind, further work is needed to determine the effect of the enrichment in tri- and tetra-antennary glycans and of high sialylation on the binding capacity of Fns secreted by transformed cells.

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REFERENCES

[1] Hynes, R.O. and Yamada, K.M. (1982) J. Cell Biol. 95, 369–377.
[2] Pierschbacher, M.D., Hayman, E.G. and Ruoslahti, E. (1985) J. Cell. Biochem. 28, 115–126.
[3] Engvall, E. and Ruoslahti, E. (1977) Int. J. Cancer 20, 1–5.
[4] Lark, M.W. and Culp, L.A. (1984) J. Biol. Chem. 259, 6773–6782.
[5] Steinberger, A. and Hörmann, H. (1976) Hoppe-Seyler's Z. Physiol. Chem. 367, 1003–1005.
[6] Keski-Oja, J., Sen, A. and Todaro, G.J. (1980) J. Cell Biol. 85, 527–533.
[7] Olden, K., Pratt, R.M. and Yamada, K.M. (1979) Proc. Natl. Acad. Sci. USA 76, 3343–3347.
[8] Jones, G.E., Arumugham, R.G. and Tanzer, M.L. (1986) J. Biol. Chem. 103, 1663–1670.
[9] Zhu, B.C.R. and Laine, R.A. (1985) J. Cell Biol. 260, 4041–4045.
[10] Fraker, P.J. and Speck, J.C. (1978) Biochem. Biophys. Res. Commun. 80, 849–857.
[11] Laemmli, U.K. (1970) Nature 227, 680–685.
[12] Hayashi, M. and Yamada, K.M. (1983) J. Biol. Chem. 258, 3332–3340.
[13] Bernard, B.A., Yamada, K.M. and Olden, K. (1982) J. Biol. Chem. 257, 8549–8554.
[14] Zhu, B.C.R., Fisher, S.F., Pande, H., Calaycay, J., Shively, J.E. and Laine, R.A. (1984) J. Biol. Chem. 259, 3962–3970.
[15] Krusius, T., Fukuda, M., Dell, A. and Ruoslahti, E. (1985) J. Biol. Chem. 260, 4110–4116.
[16] Wagner, D.D., Ivatt, R., Destree, A.T. and Hynes, R.O. (1981) J. Biol. Chem. 256, 11708–11715.
[17] Delannoy, P., Debeire, P., Montreuil, J. and Debray, H. (1983) in: Proc. 7th Int. Symp. Glycoconjugates, Lund-Roneby, July 17–23 (Chester, M.A. et al. eds) pp.173–174, Rahms, Sweden.
[18] Murayama, K., Nichols, E.J., Levery, S.B., Carter, W.G. and Hakomori, S.I. (1984) Glycoconjugate J. 1, 155–169.
[19] Delannoy, P., Debray, H. and Montreuil, J. (1985) CR Acad. Sci. 301, 767–772.