BASEMENT MEMBRANE GLYCOPROTEIN LAMININ BINDS TO HEPARIN

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1. Introduction

Extracellular matrix and basement membranes contain collagens, proteoglycans and non-collagenous glycoproteins [1]. Fibronectin is one of the glycoproteins. It is present in the cell surface matrix of many cell types and mediates cell attachment [2]. Another high molecular weight, non-collagenous glycoprotein consisting of two polypeptides with mol. wt ~200 000 and 400 000, has been found in the extracellular matrix elaborated by endodermal cells derived from mouse embryonal carcinoma [3,4]. A protein with a similar polypeptide composition and immunological crossreactivity with the endodermal cell protein has been isolated from a basement membrane-forming mouse tumor and has been named laminin [5]. The functional role of laminin is unknown, but the presence of this (or of immunologically similar) material in basement membranes [4–6] suggests that it could, as is thought to be the case with fibronectin, be involved in attachment of cells to basement membranes.

The various extracellular matrix components, collagen, fibronectin,and glycosaminoglycans, interact with one another [7–11] and such multiple interactions could be the basis for formation of extracellular matrix. Since laminin is also a matrix component, it was of interest to see whether it would interact with other components of the extracellular matrix. We show here that laminin from cultures of mouse endodermal cells interacts with heparin and heparan sulfate as evidenced by binding to heparin–Sepharose and inhibition of this binding. The interaction of laminin with glycosaminoglycans may be important in the formation of basement membranes. Chromatography on heparin–Sepharose could be a useful step in the isolation of laminin.

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2. Materials and methods

2.1. *Isolation and identification of laminin*

The endodermal cell line PF HR-9 originally derived from the mouse endodermal carcinoma line PCC4-F [12] was used. The cell culture and the preparation of extracellular matrix were done as in [4]. The main glycoprotein of the matrix was extracted by incubating the isolated matrix in 0.5 M NaCl for 48 h at 4°C. This protein is called laminin, based on earlier comparison of laminin and the glycoprotein of endodermal cell matrix [5] and results presented here.

Antibodies to laminin were prepared by immunizing rabbits with laminin released from PF HR-9 matrix by treatment with collagenase and with isolated 400 k component obtained by SDS gel electrophoresis as in [6].

2.2. *Affinity chromatography*

Heparin–Sepharose (Pharmacia Fine Chemicals, Uppsala), heat-denatured calf-skin collagen–Sepharose and fibronectin–Sepharose [13] were used. PF HR-9 cells were seeded on bacteriological Petri dishes and labeled 7 days later with 20 μCi [^{35}S]methionine or 10 μCi [^{3}H]glucosamine per 10 ml culture medium for 6 h. After labeling, the media were dialyzed against phosphate-buffered saline (PBS) [0.137 M NaCl, 0.008 M Na_2HPO_4, 0.015 M KH_2PO_4, pH 7.4] and passed through a column of Sepharose 4B to eliminate possible components binding to Sepharose [13]. The material that did not bind to Sepharose was then applied to a heparin (calf-skin collagen, fibronectin)–

Sepharose column using 20 ml medium/ml gel. The columns were washed with PBS and eluted with increasing concentrations of NaCl in the above phosphate buffer (heparin–Sepharose), or 8 M urea in 0.05 M Tris–HCl buffer (pH 7.5) (collagen–Sepharose and fibronectin–Sepharose).

2.3. *Inhibition of binding of culture medium proteins to heparin–Sepharose*

Dialyzed medium (70 000 cpm, 0.1 ml) from cultures labeled with [^{3}H]glucosamine was incubated with 0.1 ml 50% suspension of heparin–Sepharose with or without added soluble heparin (Sigma Chemical Co., St Louis, MO) or heparan sulfate (a gift from Upjohn Company, Kalamazoo, MI) in duplicate tubes. After incubation for 16 h at room temperature with shaking, the particles were washed 4 times with 2 ml PBS and the bound material was eluted with 0.5 ml 0.5 M NaCl and radioactivity was measured.

2.4. *Electrophoresis and fluorography*

SDS–Polyacrylamide slab gel electrophoresis was done as in [14] using 5% acrylamide with 3.75% stacking gels. Fluorography was performed according to [15]. Myosin (mol. wt 200 000, Sigma) and fibronectin (monomer mol. wt 220 000; dimer mol. wt 440 000) were used as molecular weight markers.

2.5. *Immunoprecipitation*

Precipitation of proteins with antibodies and subsequent gel analysis was carried out using staphylococci (Pansorbin, Calbiochem, La Jolla, CA) to isolate the immune complexes as in [16].

3. Results

When culture medium from PF HR-9 cells, labeled metabolically with [^{3}H]glucosamine or [^{35}S]methionine, was fractionated on heparin–Sepharose, ~10% of the non-dialyzable radioactivity was bound to the column. The bound material eluted at high concentrations of NaCl as a peak (fig.1). No significant radioactivity from media of labeled PF HR-9 cells bound to insolubilized calf-skin collagen or fibronectin. The bound and eluted fractions were found to contain the immunoreactive laminin present in the original culture medium. This was shown by immunodiffusion against our anti-laminin which showed an

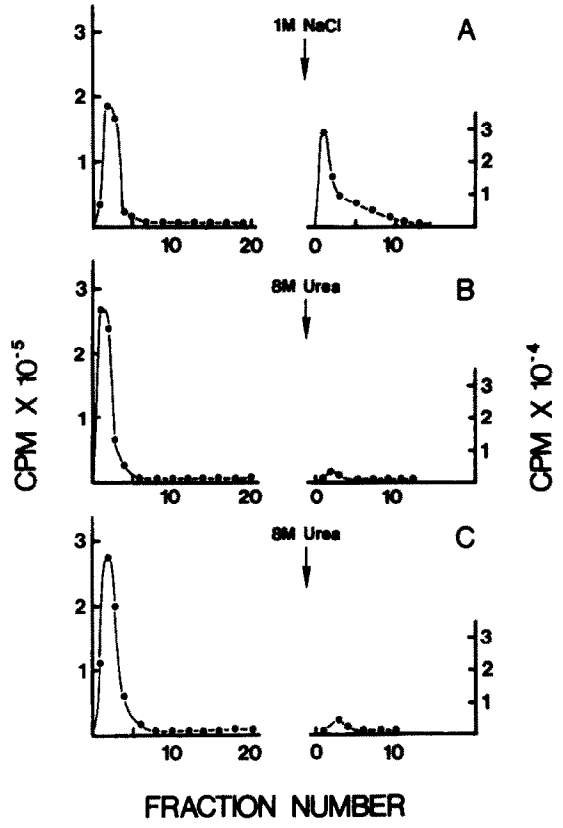

Fig.1. Affinity chromatography of culture medium from PF HR-9 cells labeled with [^{35}S]methionine on: (A) heparin–Sepharose; (B) collagen–Sepharose; (C) fibronectin–Sepharose.

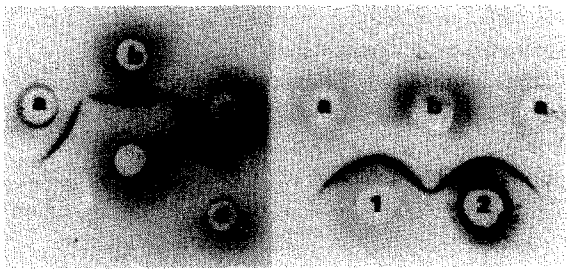

Fig.2. Immunodiffusion analaysis. Left: (a) laminin isolated from the matrix of PF HR-9 cells; (b) original culture medium sample from PF HR-9 cells fractionated on heparin–Sepharose; (c) bound and eluted fraction from heparin–Sepharose; (d) non-bound fraction from heparin–Sepharose. The samples in (b,d) were concentrated 10-fold prior to analysis. Center well: antiserum to laminin from PF HR-9 cells. Right: (a) Laminin from PF HR-9 cells; (b) and reference laminin tested against (1) antiserum to laminin from PF HR-9 cells and (2) reference anti-laminin.

identical reactivity to reference anti-laminin provided by Dr George Martin, National Institute of Dental Research (Bethesda, MD) (fig.2). SDS–polyacrylamide gel electrophoresis showed that the heparin-bound material contained non-radioactive proteins originating from the fetal bovine serum and, in addition, two polypeptides (fig.3A,1) which had incorporated radioactivity (fig.3B). These polypeptides migrated with the same mobility as reference laminin. The binding to heparin–Sepharose of the radioactivity associated with these polypeptides was inhibited by soluble heparin and heparan sulfate (table 1).

Table 1
Inhibition of binding of laminin to heparin–Sepharose by heparin and heparan sulfate

Concentration of inhibitor	Radioactivity bound (cpm/tube)	Inhibition (%)
Control without inhibitor	62 800	
Heparin		
1.0 mg/ml	29 400	53
2.0 mg/ml	20 600	67
4.0 mg/ml	9000	86
Heparan sulfate		
2.0 mg/ml	62 500	1
4.0 mg/ml	44 800	29
8.0 mg/ml	42 600	32

4. Discussion

Our results demonstrate that the major non-collagenous polypeptide components of extracellular matrix from endodermal cells bind to heparin. That these are the polypeptides of laminin is shown by the similarity of their molecular weights with authentic laminin, and the identical reactivities of our antisera and antisera to laminin.

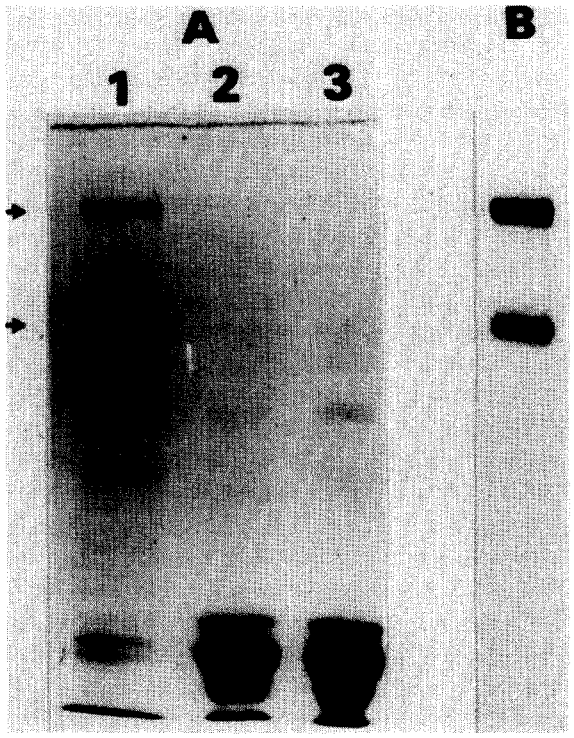

Fig.3. Heparin–Sepharose fractions of culture media from [^{35}S]methionine-labeled PF HR-9 cells analyzed in polyacrylamide gel electrophoresis in the presence of SDS and 2-mercaptoethanol. (A) Protein staining: (1) bound and eluted fraction; (2) non-bound fraction; (3) original culture media. The positions to which the polypeptides of reference laminin migrated are indicated by the arrows. (B) Fluorography of a gel similar to that in lane 1.

The interaction of laminin with heparin shows a significant degree of specificity. Laminin was greatly enriched in the bound and eluted fraction relative to the fetal bovine serum proteins present in the culture medium and it was the only labeled protein released by the endodermal cells to the medium that showed such binding. Another indication of the specificity of the interaction with heparin–Sepharose is that laminin did not bind to plain Sepharose, fibronectin–Sepharose, or calf-skin collagen–Sepharose. The binding to heparin could be reversed by increasing the salt concentration. It is interesting to note that extraction with salt also releases laminin from the insoluble matrix of the mouse tumor used in [5], as well as from the matrix of the endodermal cells used here. Since laminin interacts with heparan sulfate and heparan sulfate proteoglycan has been found to be a constituent of basement membranes [17,18] it seems that the interaction of laminin with glycosaminoglycans we have demonstrated here could contribute to the structural integrity of basement membranes in vivo.

Acknowledgements

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