Isolation and characterization of fibronectin from bovine aqueous humor

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Aqueous humor collected from freshly enucleated bovine eyes was passed over a gelatin affinity column, and bound material was eluted with 4M urea. When fractionated by sodium dodecyl sulfate electrophoresis, the eluted material co-migrated with human plasma fibronectin and protein standards of approximately 220,000 daltons. Amino acid analyses demonstrated a composition similar to that of human plasma fibronectin and fibronectin synthesized by cultured cells. The concentration of fibronectin in aqueous humor is approximately 100 times less than in human plasma. (INVEST OPHTHALMOL VIS SCI 22:57-61, 1982.)

Key words: fibronectin, aqueous humor, cornea, bovine, endothelial cells

Fibronectin is a disulfide-bonded glycoprotein with a subunit molecular weight between 200,000 and 250,000. Plasma fibronectin or "cold-insoluble globulin" has been isolated from plasma, serum, and amniotic fluid.1-7 Cell surface fibronectin is synthesized in vitro by numerous diverse cell types, including fibroblasts, myoblasts, amniotic fluid cells, intestinal epithelial cells, vascular endothelial cells, and corneal endothelial cells.5-7-8 The plasma and cellular fibronectins are clearly similar but not identical in structure and types of biological activity.9

In vivo immunofluorescence studies show fibronectin associated with mesenchymal tissues and basement membranes.10-12 In vitro fibronectin is localized in extracellular fibrils and aggregates.13 Therefore it is not surprising to find that fibronectin has specific binding sites for hyaluronic acid, heparin, and collagen (see ref. 14 for review). Since there is also a binding site for cell surfaces, it has been suggested that fibronectin is important in cell-cell aggregation and cell-substratum adhesion. Other possible biological roles for fibronectin have been discussed elsewhere.14

It appears that when fibronectin is found in a fluid (e.g., amniotic fluid or plasma), it can also be synthesized in vitro by cells closely associated with that fluid, i.e., isolated amniotic fluid cells and vascular endothelial cells, respectively.7-8 Since the anterior chamber of the eye is lined with corneal endothelial cells and these cells produce fibronectin in culture,8 it seemed possible that fibronectin might be a component of aqueous humor. We report here the isolation and characterization of fibronectin from bovine aqueous humor.

Materials and methods

All reagents and chemicals were of the highest grades available. Fresh bovine eyes were pur-
Fig. 1. Upper, Affinity chromatography of bovine aqueous humor. A 100 ml volume of aqueous humor was applied to the pre-equilibrated column, followed by a wash with 100 ml of calcium- and magnesium-free phosphate-buffered saline. Fibronectin was eluted with 4M urea in 0.05M Tris buffer (pH 7.5) and was contained in the 280 to 295 ml fractions. Lower, Affinity chromatography of outdated, citrated human plasma. A 12.5 ml volume of plasma was diluted 1:4 with a citric acid:sodium citrate:dextrose solution and applied to the columns. The columns were washed and fibronectin was eluted as described above. Fibronectin was contained in the 280 to 295 ml fractions.

Chased from a local slaughter house and transported on ice to the laboratory. The aqueous humor, which was aspirated with a syringe and 25-gauge needle from the anterior chamber, was processed immediately. Out-dated, citrated human plasma was purchased from a local blood bank and diluted 1:4 with a citric acid:sodium citrate:dextrose solution and applied to the columns. Aqueous humor (100 ml) or human plasma (12.5 ml) was loaded on columns pre-equilibrated in calcium- and magnesium-free phosphate-buffered saline (pH 7.2) containing 0.01M sodium citrate. The fractionation was carried out with two columns in series—the first containing cyanogen bromide (CnBr)-activated Sepharose 4-B only and the second column with gelatin coupled to the CnBr-activated Sepharose 4-B. Bound material was eluted from the second column with 4M urea/0.05M Tris buffer at pH 7.5, protease inhibitors were added, and samples were dialyzed against 0.005M NaOH prior to analyses.

Protein estimations were made with the Hartree method. Five percent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a Tris borate buffer system according to the method of Benya et al. For amino acid analyses, samples were hydrolyzed in 6M HCl at 110°C for 24, 48 and 72 hr. Serine and threonine concentrations were determined by extrapolation to zero time from these time points. No adjustment was made for decreased methionine and tyrosine concentrations due to hydrolytic destruction.

Results

Separation of bovine aqueous humor proteins by methods previously used to isolate fibronectin showed the presence of an absorbance peak that co-chromatographed with human plasma fibronectin (Fig. 1). The protein concentration for outdated human plasma was 72 mg/ml. Fibronectin concentrations in this plasma were 290 μg/ml, or 0.4% of the total plasma proteins. Bovine aqueous humor contained 0.65 mg/ml protein of which fibronectin constituted 0.38% (2.46 μg/ml). SDS-PAGE of the "fibronectin" peaks after reduction with 0.1M β-mercaptoethanol showed the presence of a single protein band, indicating the dimer form of fibronectin.

Fig. 2. SDS polyacrylamide gel showing fibronectin isolated from bovine aqueous humor and human plasma. 1, Rat acid-soluble collagen, showing α1, α2, and β chains; 2, human plasma fibronectin; 3, aqueous humor fibronectin (FN).
Table I. Amino acid composition (residues/1000)

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<th>Bovine aqueous humor fibronectin</th>
<th>Human plasma fibronectin</th>
<th>Human^a plasma fibronectin</th>
<th>Chick^b fibroblast fibronectin</th>
<th>Type IV^c collagen</th>
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Bovine aqueous humor fibronectin

Discussion

We have demonstrated that fibronectin isolated by conventional methods^15 from bovine aqueous humor has an electrophoretic mobility and amino acid composition similar to those of fibronectins from plasma and chick fibroblasts. The concentration of fibronectin in aqueous humor is 2.46 μg/ml, or approximately 1/100 of its concentration in human plasma (290 μg/ml). Expressed as a percentage of total protein, however, bovine aqueous humor and plasma fibronectins are very similar and comprise 0.38% and 0.4%, respectively. In contrast, fibronectin in amniotic fluid constitutes a considerably higher percentage of total protein (1.1%). This is consistent with other studies that demonstrate increased production of extracellular matrices by embryonic cells. Since the amniotic fluid is associated with a developing system, however, we did not compare it directly with the tissue fluid systems of plasma and aqueous humor from adults.

The difference in fibronectin concentrations in plasma and aqueous humor may be related to different shear forces in the two systems. It is postulated that fibronectin functions in the attachment of cells to extracellular matrix components (see ref. 14 for review). Vascular endothelial cells produce fibronectin into their extracellular matrix,^8 which may allow them to remain attached to their basal laminae in the face of high shear forces of circulating blood. In contrast, the relatively low concentration of fibronectin in
aqueous humor might reflect the fact that the intraocular pressure pushes the corneal endothelial cells against their basement (Descemet's) membrane with minimal flow, so that there is little need for large quantities of fibronectin. Until the function of fibronectin is more clearly elucidated, however, this theory remains speculative.

There are a number of possibilities for the source of fibronectin in aqueous humor. It has been demonstrated that in vitro corneal endothelial cells produce fibronectin. It seems reasonable to postulate that these cells synthesize the fibronectin in vivo and release it into the aqueous humor. In support of this idea, it has been shown that in other systems such as amniotic fluid and plasma, the closely associated cell types (amniotic fluid cells and vascular endothelial cells, respectively) produce fibronectin in vitro. Although there is less supportive evidence for it, another possibility is that aqueous humor fibronectin is produced by the ciliary body, since it is the source of aqueous humor. At the present time, however, the ciliary body has not been demonstrated to produce fibronectin in vitro. A third possible source of aqueous humor fibronectin is the lens capsule because it is a basement membrane and fibronectin is considered to be an extracellular matrix glycoprotein. The fourth possibility is that the fibronectin diffuses post-mortem from iridial vessels into the anterior chamber. This seems unlikely because these experiments were repeated numerous times and values for protein and fibronectin contents were always consistent. In addition, although most of our experiments utilized aqueous humor removed immediately from freshly enucleated bovine eyes, when aqueous humor was not collected immediately, the fibronectin content did not change. Whatever the source of aqueous humor fibronectin, we do not believe that it is a postmortem diffusion phenomenon since there is not a direct correlation between fibronectin concentration and postmortem time.

The presence of fibronectin in the aqueous humor offers exciting dimensions to the biology of corneal endothelial cells, a highly structured monolayer. Fibronectin is thought to be involved in (1) the maintenance of normal tissue architecture, (2) cellular motility, and (3) alteration of chondrocyte synthesis from type II to type I collagen. All these are significant in light of known loss of corneal endothelial cell organization during disease processes, and recent studies have demonstrated an altered collagen production by rabbit model retrocorneal fibrous membrane cells in vitro. We are presently investigating the effect of fibronectin on collagen synthesis by corneal endothelial cells in vitro and possible variations of fibronectin concentrations in aqueous humor during formation of retrocorneal fibrous membranes.

REFERENCES