Preferential in vitro interaction of fibronectin with histone H2A + H3

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The interactions of fibronectin with unfractionated histone and histone subfractions (H1, H2A + H3, H2B and H4) were studied under physiological conditions by laser nephelometry. It was found that fibronectin interacted preferentially with histone H2A + H3. Due to self-aggregation, fibronectin showed a very low relative light scattering.

Fibronectin *Histone* *Laser nephelometry* *DNA synthesis* *Chromatin* *Heparan sulfate*

1. INTRODUCTION

Fibronectin is present on the surface of various cells where it forms the pericellular matrix by interacting with collagens, proteoglycans (glycosaminoglycans), laminin and gangliosides [1–8]. Fibronectin is also present in the plasma [9], where it is known to interact with fibrin, fibrinogen and factor XIIIa [6]. Pericellular fibronectins are reported to be associated with cytoplasmic actin via transmembrane components [10].

The intracellular localization of fibronectins has been reported by authors in [11]. They demonstrated the presence of fibronectin in cultured human fibroblast chromatin by immunological and physicochemical techniques. They considered fibronectins, which consisted of 0.7% of the total chromatin proteins, as DNA binding proteins. Recently, the ability of fibronectin fragments to stimulate DNA synthesis in normal hamster fibroblasts was reported in [12].

In our previous study, exogenously added heparin was shown to stimulate DNA synthesis in nuclei isolated from normal rat livers probably by complexing with histones and leading to the release of template restrictions [13,14]. During these studies, we observed that fibronectin also stimulated DNA synthesis [13]. This study was conducted to elucidate the possible interaction of fibronectins with histones. A preliminary report has been presented [15].

2. MATERIALS AND METHODS

2.1. *Isolation of fibronectin from human plasma*

Fibronectin was isolated from human plasma by affinity chromatography on a gelatin–Sepharose column [16]. Human plasma (40 ml) was mixed with an equal volume of phosphate-buffered saline (pH 7.2) consisting of 10 mM sodium citrate and chromatographed on a column of gelatin–Sepharose (~2 mg gelatin/ml gel). The column was washed at room temperature with 300 ml phosphate-buffered saline (pH 7.2) containing 10 mM sodium citrate and the proteins bound to the gelatin–Sepharose eluted with 8 M urea in 50 mM Tris–HCl (pH 7.5). The eluate was dialyzed against 50 mM Tris–HCl (pH 7.5) to remove urea and applied on a column (1.5 × 28 cm) of DEAE–Sepharose. Fibronectin was eluted with

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0.7 M NaCl in 50 mM Tris–HCl (pH 7.5) and recovered by dialysis against 50 mM Tris–HCl (pH 7.0), and ultrafiltration.

The purity of fibronectin was checked by polyacrylamide slab gel electrophoresis in the presence of sodium dodecyl sulfate and β-mercaptoethanol as in [17].

2.2. *Subfractionation of histones*

Calf thymus histones (Calbiochem, La Jolla, CA) were solubilized with dilute perchloric acid and repurified as in [18]. Purified histones were fractionated into histone H1, H2A + H3, H2B and H4 by chromatography on a column (2.5 × 150 cm) of Bio-Gel P-60 as in [19]. Rechromatography of the histone subfractions on Bio-Gel P-60 yielded single peaks characteristic of these components.

3. RESULTS AND DISCUSSION

Fibronectin used in this study was found to be pure as judged by SDS–polyacrylamide slab gel electrophoresis. A single band of M_r 220000 was observed. On acid–urea slab gel electrophoresis (15% acrylamide gel in 2.5 M urea–0.9 M acetic acid, pH 2.7) histone subfractions H1, H2B and H4 gave single bands and subfraction H2A + H3 gave the expected two bands.

Interaction of fibronectin with histones was determined by relative light scattering measurements using a laser nephelometer. This method has been used for the detection of circulating immunocomplexes and for studying antigen–antibody interaction and lectin–glycoprotein association [20–22]. Relative light scattering is apparently based on the number and size of complexes formed.

The effects of increasing amounts (2.5–50 μg/ml) of fibronectin on the light scattering by a fixed amount (40 μg/ml) of histones are shown in fig.1. It was observed that the relative light scattering by fibronectin when mixed with the histones was highest for H2A + H3, intermediate for H2B and H4 and lowest for H1 and unfractionated histones. Fibronectin alone showed low relative light scattering at the concentration used, probably due to self-aggregation [16], however, neither unfractionated histone nor the histone subfractions showed relative light scattering up to the highest

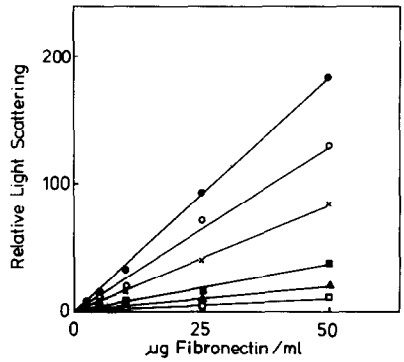

Fig.1. Complex formation between fibronectin and histones as measured by relative light scattering. Histones and fibronectin were dissolved in 0.1 M Tris–HCl (pH 7.0) containing 0.15 M NaCl, 5% (w/v) polyethylene glycol 6000 and 15 mM NaN_3 and the solutions filtered through 0.2–0.6 μm Millipore filters. Varying volumes (2–100 μl) of the fibronectin solution (500 μg/ml) were placed in test tubes, diluted with buffer to 0.98 ml and 0.02 ml of the appropriate histone solutions (200 μg/ml) added. The solution in the test tube was mixed and the relative light scattering measured with a Hyland Laser Nephelometer PDQ at a sensitivity setting of 4.4 as in [14]. Increasing concentrations of fibronectin alone (□—□) and with unfractionated histones (■—■), histone H2A + H3 (●—●), histone 2B (○—○), histone H4 (×—×), and histone H1 (▲—▲).

concentration (50 μg/ml) used here. Bovine serum albumin (fraction V, Sigma), when mixed with various concentrations of fibronectin under conditions identical to those above, did not exhibit significant relative light scattering (table 1). This suggests that the high relative light scattering shown by fibronectin-histone mixtures was not simply due to the result of increased protein concentrations.

Since it has been reported that the glycosaminoglycans, heparan sulfate and heparin [23] are capable of interacting specifically with fibronectin, we measured the relative light scattering of fibronectin and heparan sulfate or heparin mixtures. We found that the light scattering by the fibronectin-histone H2A + H3 mixture was much higher than that by fibronectin-glycosaminoglycan mixtures (table 1). We failed to observe light scattering by varying concentrations of heparan sulfate even though evidence for self-aggregation of this glycosaminoglycan has been obtained by other techniques [24].

Table 1

Complex formation between fibronectin and histone H2A + H3, heparan sulfate, heparin and bovine serum albumin as determined by relative light scattering

Fibronectin (μg/ml)	Relative light scattering				
	None	Histone H2A + H3	Heparan sulfate	Heparin	Bovine serum albumin
10	3.5	34.1	13.2	5.6	2.5
20	9.4	70.0	29.7	17.0	8.1
40	19.6	141.1	55.4	40.0	17.2
60	31.3	>200	82.6	57.6	33.2

Fibronectin at (10–60 μg/ml) was mixed with histone H2A + H3, heparan sulfate, heparin and bovine serum albumin (50 μg/ml) and the relative light scattering measured by a laser nephelometer at a sensitivity setting of 6.6 as in [12]. The mean of 2–3 determinations is presented. Heparan sulfate (Dr M.B. Mathews, University of Chicago, Chicago, IL), heparin (Sigma) and bovine serum albumin (fraction V, Sigma) did not show relative light scattering by themselves

It is most likely that histones interact with the collagen (gelatin, polyamine) binding domain of the fibronectin molecule. This region also contains most of the carbohydrate residues [25,26]. It is, therefore, plausible that fibronectin may interact with histones through the sialic acid residues. If this is the case, then one should expect a higher relative light scattering for the interaction of fibronectin with the more basic histone subfractions H1 and H4 rather than the subfractions H2A + H3 and H2B [27]. However, fibronectins preferentially interacted with H2A + H3. The results suggest that the preferential interaction of fibronectin with histone H2A + H3 is not solely dependent on their charged groups. Since histone H2A + H3 forms a part of the core histones which together with helical double-stranded DNA make up the nucleosomes in cell nuclei, it will be interesting to explore the functional significance of our observation.

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