Conformational state of circulating human plasma fibronectin

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Fibronectin *Conformational state*

1. INTRODUCTION

Fibronectin (FN) is a multifunctional glycoprotein present in the soluble form in plasma and other body fluids and in the insoluble form in the extracellular matrices and basement membranes. The functions attributed to fibronectin such as cell-cell and cell-substrate adhesion, cell spreading, enhancement of phagocytosis, cell migration, wound healing and ability to induce a more normal phenotype in transformed cells seem to be all based on its affinity to cell surfaces and to many different macromolecules including collagens, fibrin, fibrinogen, heparin, DNA, actin, complement component C1q and gangliosides (for reviews on distribution, structure and biological functions of fibronectin see [1–7]).

The human plasma fibronectin molecule is composed of two polypeptides (α and β chains) with a relative molecular mass of about 220 kDa. These subunits may have slight differences in sequence and are linked as a dimer via interchain disulfide bonds near the C-terminus. These subunits are chains of independent globular domains connected by short flexible segments of polypeptide chain. The length of a fibronectin subunit is about 70 nm and its width 2 nm. Physicochemical and electron microscopy studies [8–12] on purified fibronectin indicate that the shape of the purified fibronectin molecule varies from an almost extended conformation to a compact form depending on solvent conditions. Recent studies on purified fibronectin suggested a rather compact conformation under near physiological conditions and a more elongated structure at high ionic strength, pH or glycerol content [13–17]. Since all the studies on fibronectin conformation have been done on purified fibronectin we have investigated with the aid of immunological procedures the hydrodynamic properties of fibronectin in whole plasma. These data indicate that fibronectin is present in plasma in a compact conformation.

2. MATERIALS AND METHODS

Human plasma fibronectin and monospecific antibodies to fibronectin were prepared as in [18]. Diffusion coefficient ($D_{20,w}$) and molecular exclusion chromatography measurements were both carried out at two different NaCl concentrations, 137 and 600 mM, in 8 mM Na-phosphate buffer (pH 7.2), 2.7 mM KCl, 1 mM Na_2EDTA, 10 units/ml kallidogenase inhibitor (KIR, Richter, Milan), 0.02% (w/v) NaN_3. All samples were extensively dialyzed against the buffer in which the measurements were to be done. Diffusion coefficient measurements in 0.8% agarose plates were carried out as in [19]. Stokes' radii (R_s) were calculated from the $D_{20,w}$ as in [20].

Molecular exclusion chromatography was car-

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ried out on a column of 2.6 cm diameter filled with Ultrogel AcA-22 (LKB, Bromma, Sweden) to a bed height of 91.6 cm, for a total volume (V_t) of 486 ml. Flow rate was 10 ml/h with upward elution. Elution volumes (V_e) of purified proteins were determined by continuous monitoring of the eluate's absorbance at 280 nm. When whole plasma was analyzed, fractions were collected and analyzed using the fused rocket immunoelectrophoresis technique [21] with antibodies specific to IgM, α_2-macroglobulin (Behring, Marburg) and fibronectin. The column was calibrated by determining the elution volumes of 5 proteins of known R_s: human IgM, R_s = 12.5 nm, human α_2-macroglobulin (α_2-M), R_s = 9.3 nm, bovine thyroglobulin, R_s = 8.5 nm, horse ferritin, R_s = 6.1 nm, and bovine catalase, R_s = 5.22 nm. The elution volumes of the first two proteins were measured directly from experiments with whole human plasma. The last 3 proteins were part of a commercial high-M_r calibration kit (HMV Calibration Kit, Pharmacia, Uppsala) as well as the Blue Dextran 2000 (M_r 2 × 10^6) that was used for the column void volume (V_0) determination. A plot of $\sqrt{-\log K_{av}}$ ($K_{av} = (V_e - V_o)/V_t - V_o$) [22]) *vs* R_s was linear from the bovine catalase value to the human α_2-M value while the human IgM value fell in the non-linear portion of the curve. Since molecular asymmetry can strongly affect the elution behaviour of a macromolecule [23], the R_s values calculated this way have been taken as 'apparent R_s' (R_s(app)). All measurements have been carried out at room temperature.

3. RESULTS

We have studied the elution behaviour of fibronectin either purified or in whole plasma from a molecular exclusion chromatography column (Ultrogel AcA-22) at two different NaCl concentrations (137 and 600 mM). The elution profile of fibronectin in whole plasma was obtained using fused rocket immunoelectrophoresis. To obtain the relative elution position of fibronectin with respect to other proteins we used an intermediate gel containing antibodies to IgM and α_2-M. Fig.1A and B shows the elution profiles of total human plasma protein from the Ultrogel AcA-22 molecular exclusion chromatography column. The insets show the immunochemical elution profile of IgM, α_2-M and fibronectin. In table 1 are summarized the elution volumes and the calculated

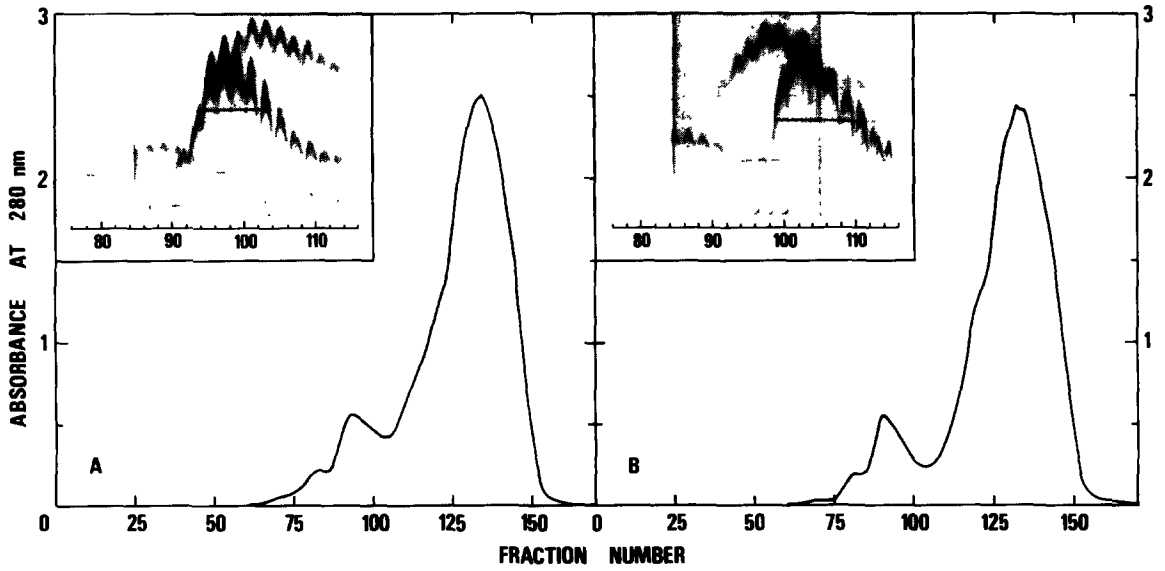

Fig.1. Elution profiles of total plasma proteins from the Ultrogel AcA-22 column. Conditions as in section 2. (A) In 137 mM NaCl, 2.9 ml/fraction; the peaks in the immunochemical elution profile in the inset are, from left to right, IgM, α_2-M and FN. (B) In 600 mM NaCl, 2.86 ml/fraction; the peaks in the immunochemical elution profile in the inset are, from left to right, IgM, FN and α_2-M.

Table 1

Elution volumes and apparent Stokes' radii from molecular exclusion chromatography

Sample	[NaCl]			
	137 mM		600 mM	
	V_e (ml)	R_s(app) (nm)	V_e (ml)	R_s(app) (nm)
FN, purified	275.5 ± 2[a]	8.4	246 ± 2[a]	10.1
FN, whole plasma	276 ± 0.5[b]	8.4	245 ± 2[b]	10.2
α_2-M, whole plasma	262 ± 0.5[b]	9.1	259 ± 2[b]	9.3
IgM, whole plasma	236 ± 3[b]	10.9	229 ± 3[b]	11.3
Blue Dextran 2000	183	–	181	–

[a] Mean of 3 expts ± SD
[b] Mean of 2 expts ± mean deviation

R_s(app), at the two different NaCl concentrations, of fibronectin (purified and in whole plasma), IgM, and α_2-M.

The back-calculated R_s(app) value for α_2-M is in agreement with the literature, while the IgM value is considerably lower. Here we have assumed, in the absence of a suitable standard, that the linear portion of the calibration curve extends a little over the α_2-M value to include the FN value at high ionic strength. While α_2-M and IgM at the two different NaCl concentrations do not show significant variations in their elution volumes, the V_e of FN, both purified and in whole plasma, shows a difference of about 30 ml, corresponding to a significant change in the R_s(app) value.

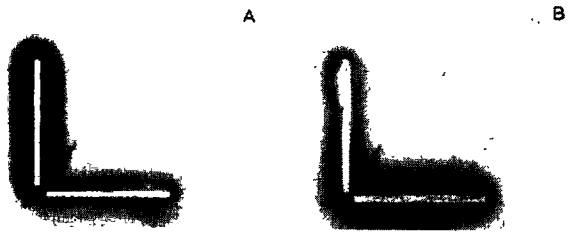

Fig.2. Diffusion coefficient measurements in 0.8% agarose plates. *x*-axis troughs, anti-human plasma FN monospecific antibodies; *y*-axis troughs, whole human plasma. Conditions as in section 2, with 0.1% (w/v) NaN_3. The angle between the antigen (FN)–antibody (IgG) precipitation line and the antigen trough was measured with a protractor. (A) 137 mM NaCl. (B) 600 mM NaCl.

Table 2

Fibronectin diffusion coefficients and Stokes' radii from immunodiffusion experiments with whole human plasma

[NaCl] (mM)	Angle	$D_{20,w} \times 10^7$ (cm^2/s)	R_s (nm)
137	36 50′ ± 50′[a]	2.20 ± 0.10[a]	9.7 ± 0.4[a]
600	33 50′ ± 14′[b]	1.78 ± 0.05[b]	12.0 ± 0.3[b]

[a] Mean of 3 expts ± SD
[b] Mean of 4 expts ± SD

We have also measured the diffusion coefficients of FN in whole plasma at the two different NaCl concentrations using the agarose plates as cited in section 2. Typical experiments are shown in fig.2, and the results are summarized in table 2, which also contains the calculated R_s. Again, we have observed a significant increase in the FN R_s value from near-physiological conditions to high ionic strength.

4. DISCUSSION

Reversible variation of purified fibronectin from a compact to an extended form induced by changing solvent composition has been observed in electron microscopy and physicochemical studies [12–17]. An extended conformation has been observed at high pH, at high ionic strength, in 30%

glycerol and in 1 M urea. However, the conformational state of native fibronectin circulating in plasma, free to interact with other plasma proteins, is unknown.

Here we have studied the diffusion coefficient, using agarose plates, and the elution from a molecular exclusion chromatography column of human plasma fibronectin in whole plasma at two different NaCl concentrations. The R_s calculated from the diffusion coefficients are in agreement with those reported in the literature for purified fibronectin under similar solvent conditions. The R_s(app) values obtained by molecular exclusion chromatography differ from that obtained by coefficients by about 15%. This may be due to the different sensitivity of the two methods to molecular asymmetry. These data indicate that at physiological NaCl concentration, FN is present in whole plasma with a compact form susceptible to change into a more open one at higher [NaCl] as previously demonstrated using purified FN. Although the conformational change of fibronectin from the compact to the open form has been suggested as a means of unmasking previously hidden active sites, it is not known if in vivo there are physiological conformational transitions. Further studies are needed to establish the biological significance of the different fibronectin conformational states and the possible mechanism triggering these conformational changes in vivo.

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