Buffalo plasma fibronectin: A physico-chemical study

Nizamuddin Ahmed*, Ramesh Chandra and H G Raj

*Dr B R Ambedkar Center for Biomedical Research, University of Delhi, Delhi 110 007; 
Department of Biochemistry, V.P. Chest Institute, University of Delhi, Delhi 110 007, India

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Plasma fibronectin (FN) of buffalo (Bubalis bubalis) was purified to apparent homogeneity, using gelatin-Sepharose and heparin-Sepharose affinity columns. It was found to have two subunits of molecular mass 246 kDa and 228 kDa, on SDS-gel. Its immunological cross-reactivity with anti-human plasma FN was confirmed by Western blotting. The amino acid composition was found to be similar to that of human and bovine plasma FNs. Buffalo plasma FN contained 2.23% neutral hexoses and 1.18% sialic acids. No titratable sulphydryl group could be detected in the absence of denaturant. Reaction with DTNB indicated 3.4 sulphydryl groups in the molecule, whereas BDC-OH titration gave a value of 3.8 –SH groups in buffalo plasma FN. Stoke’s radius, intrinsic viscosity, diffusion coefficient and frictional ratio indicated that buffalo plasma FN did not have a compact globular conformation at physiological pH and ionic strength. Molecular dimensions (average length, 120 nm; molar mass to length ratio, 3950 nm^-1 and mean diameter, 2.4 nm) as revealed by rotary shadowing electron microscopy further supported the extended conformation of buffalo plasma FN. These results show that buffalo plasma FN has similar properties as that of human plasma FN.

Fibronectin (FN) is an extracellular glycoprotein, found as a soluble dimer in plasma and other body fluids and as an insoluble multimeric form in extracellular matrix. It is involved in embryogenesis, wound healing, haemostasis and thrombosis. It has also been implicated in many pathological conditions including metastatic diseases. Human plasma FN is a dimer with subunit molecular mass approximately 220-250 kDa and joined by two disulfide bonds near the carboxyl terminus of the protein. Various isoforms of FN are generated by alternative splicing of FN pre-mRNA. Complete primary structures of FNs from human and bovine plasma have been determined. However, no information is available on the structure and function of buffalo plasma FN. The present paper describes purification and physico-chemical properties of buffalo plasma FN with the view to understand the role of this protein in the physiology and pathology of domestic animals.

Materials and Methods

Blue Dextran 2000, BDC-OH, gelatin-Sepharose, heparin-Sepharose, protease inhibitors and TBA were purchased from Sigma Chemical Co. Sephacryl S-300 HR and gel filtration molecular weight kit were from Pharmacia Biotech. The reagents for electrophoresis were procured either from BioRad or from Sisco Research Laboratories, India. Nitrocellulose paper was from Schleicher & Schuell. Rabbit anti-human plasma FN was a kind gift from Prof. D F Mosher, University of Wisconsin, Madison, USA. All other reagents were of analytical grade from standard commercial sources.

Collection of blood and isolation of plasma

Blood from buffalo jugular vein was collected in 15% ACD solution containing 5 mM B-HCl and 0.4 mM PMSF. The blood containing anticoagulant and protease-inhibitors was centrifuged at 11,000 x g for 10 min at 4°C. The supernatant was further spun at 23,000 x g for 15 min at 4°C to get clear plasma. The plasma was treated with STI, 0.1 mg/ml; B-HCl, 10 mM; EDTA, 10 mM; e-ACA, 100 mM; PMSF, 2 mM and sodium azide, 0.025%.
Protein estimation

FN concentration was estimated spectrophotometrically, using extinction coefficient $\varepsilon_{1 	ext{cm}}^\text{nm} = 12.8$ at 280 nm.$^9$

Purification of FN

After removal of vitamin K-dependent clotting factors by BaCl$_2$ and (NH$_4$)$_2$SO$_4$ treatments at 4°C (ref. 10), buffalo plasma was pre-filtered on Sepharose 4B column and applied to gelatin-Sepharose affinity column.$^{11}$ The column was extensively washed with PBS, containing 10 mM EDTA, 5 mM B-HCl and 100 mM e-ACA. It was further washed with the same buffer containing 1 M NaCl and subsequently with the buffer without NaCl. The bound FN was eluted with 4 M urea in 50 mM Tris-HCl buffer, pH 7.5, containing 10 mM EDTA and 2 mM PMSF.

The FN eluted from gelatin-Sepharose column was further purified by heparin-Sepharose affinity column.$^{12}$ The affinity column was washed with 50 mM Tris-HCl buffer, pH 7.5, containing 10 mM EDTA, 2 mM PMSF and 4 M urea and subsequently washed with the same buffer without urea. The bound FN was eluted with 0.5 M NaCl in 50 mM Tris-HCl buffer, pH 7.5, containing 10 mM EDTA and 2 mM PMSF. Purification of FN was carried out at room temperature.

Slab gel electrophoresis and Western blotting

Reductive SDS-PAGE was performed by the methods of Laemmli.$^{13}$ Protein bands on the gel were stained by Coomassie Brilliant Blue.

Western blotting was performed as described by Towbin et al.$^{14}$ After reductive SDS-PAGE, FN was transferred to nitrocellulose membrane by electrotransfer in Tris-glycine buffer, pH 8.3, with 10 % methanol. After transfer was completed, membranes were blocked overnight with 4% BSA in PBS containing 0.1% Tween-20 and 0.025% sodium azide. After incubation with rabbit anti-human FN (1:250) and further treatment with goat anti-rabbit HRP-conjugate (1:500), the blot was developed using DAB reagent (40 mg DAB, 15 μl H$_2$O$_2$ and 300 μl of 8 % (w/v) NiCl$_2$ in 50 ml of 100 mM Tris-HCl buffer, pH 7.35).

Gel filtration on Sephacryl S-300 HR column

Pre-swollen Sephacryl S-300 HR was packed in Pharmacia column (2.5 x 100 cm; bed volume 400 ml) as recommended by Pharmacia Biotech. The column was equilibrated with PBS containing 5 mM EDTA, 1 mM PMSF and 0.025% sodium azide. Protein (10-20 mg) in 1-2% of the packed volume of the column was applied to the bed surface of the gel. Proteins were eluted at a flow rate of 50 ml/hr by the column equilibrating buffer.

Carbohydrate analysis

The hexose content was determined as described by Johansen et al.$^{15}$ by orcinol-H$_2$SO$_4$ reagent, using galactose-mannose (1:1) mixed solution as standard. TBA reaction$^{16}$ was employed to determine sialic acids content of FN after hydrolysis with 0.1 M H$_2$SO$_4$ at 80°C for 60 min. The amount of N-acetyl neuraminic acid present in the sample was calculated by using molar extinction coefficient $\varepsilon_{492} = 5700$ for N-acetyl neuraminic acid.

Analysis of sulphydryl groups

The thiol content was determined by modification of Ellman assay as described by Raddles et al.$^{17}$ Guanidinium chloride was used to unfold the protein and make all thiols of protein reactive. The thiol content was determined by measuring the thionitrobenzene anion (TNB$^-$) released upon the reaction of protein with DTNB using $\varepsilon_{412}$ for TNB$^-$ as 13,700 M$^{-1}$ cm$^{-1}$ in the presence of guanidinium chloride and 14,150 M$^{-1}$ cm$^{-1}$ in the absence of guanidinium chloride.

The -SH content of FN was determined by BDC-OH titration as described by Rohrach et al.$^{18}$. FN was dialysed against 40 mM sodium acetate buffer pH 5.1 containing 4.0 M guanidinium chloride and centrifuged at 4000×g at 4°C. Aliquots from 40 to 200 μl of the protein solution were added to a series of 10 ml volumetric flasks. To each flask was added exactly 100 μl of stock BDC-OH solution (2.2-2.6 mM) and sufficient sodium acetate buffer containing guanidinium chloride to dilute the volume. All solutions were thoroughly mixed and allowed to stand for approximately 25 min and the absorbance was recorded against the reagent blank containing acetate buffer with guanidinium chloride. The -SH content was determined from a plot of $A_{412} \ versus$ protein concentration and by dividing the slope of the line (determined by least squares method) by molar absorption coefficient of BDC$^+$ as given by the following equation:

\[
\text{Number of } -\text{SH groups} = \frac{-\text{Slope of the line}}{\varepsilon_{412}} \quad \cdots (1)
\]
using $\hat{\varepsilon}_{\text{Mapp}}$, the apparent molar coefficient of $\text{BDC}^+$

\[
\text{BOC}^+ = 70,800 \text{ M}^{-1} \text{ cm}^{-1}.
\]

**Amino acid analysis**

FN was hydrolyzed with 6 N HCl for 20 to 24 hr at 110°C under vacuum and amino acid analysis was carried out by Moore and Stein's method as described by Ozols.9

**Rotary shadowing electron microscopy**

A sample of buffalo plasma FN was dialysed against 0.1 M ammonium bicarbonate solution at 4°C and diluted to 20 µg/ml with the same solution and rotary shadowed according to Engel and Furthmeyer20.

**Results**

**Purification of buffalo plasma FN, size and charge homogeneity of the preparation**

FN from buffalo plasma (70 ml) was isolated by gelatin-Sepharose affinity chromatography and further purified on heparin-Sepharose column. The electrophoretic separation of plasma proteins on reductive SDS-gel at various stages of FN purification is shown in Fig. 1. The yield of purified FN was 219±6 µg ml⁻¹ plasma (mean ± SD of 6 experiments).

The FN preparation was homogeneous with respect to size and charge as analysed by analytical gel filtration and SDS-PAGE.

**Subunits, immunological cross-reactivity and molecular mass of FN**

On reductive SDS-gel, the FN preparation showed two closely spaced protein bands, FN1 and FN2, which have very little difference in their electrophoretic mobility on the gel (Fig. 1, lanes 11-13 and Fig. 2, lanes 2-4). The migration of FN and marker proteins on reducing SDS-gel is shown in Fig. 2. Molecular mass of FN subunits, FN1 and FN2, were determined by the linear relation between Log M and relative mobility of SDS-protein complexes ($R_m$) on the gel. The relation after least squares analysis yields a straight line represented by the following equation:

\[
\text{Log} \ M = (-1.5931) \ R_m + 5.6630 \quad \ldots (2)
\]

The substitution of $R_m$ values (0.1707 for FN1 and 0.1910 for FN2) in the above equation yields molecular mass of 246 kDa for FN1 and 228 kDa for FN2. The apparent molecular mass of buffalo plasma FN was computed to be 474 kDa.

The immunoblot of buffalo plasma FN (with anti-human plasma FN) gave two closely spaced bands (doublet) as shown in Fig. 3. It is evident that some degraded products of buffalo plasma FN also cross-react with rabbit anti-human plasma FN.

**Biochemical properties**

Amino acid composition of buffalo plasma FN is shown in Table 1. Neutral hexoses and sialic acid

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**Fig. 1**—Separation of plasma proteins on SDS-gel [At different stages of FN isolation proteins were analysed by SDS-PAGE (5-15% gradient gel) as described under Methods. Plasma samples were diluted 1:60. Gel was stained with Coomassie Blue. Lanes 1 & 2, protease inhibitors-treated plasma (50 µl & 100 µl); lanes 3 & 4, BaCl₂-treated plasma (50 µl & 100 µl); lanes 5 & 6, FN-depleted plasma (50 µl & 100 µl); lanes 7 & 8, FN eluted from gelatin column (30 µl, 60 µl); lanes 9 & 10, Flow through of heparin column (100 µl); lanes 11, 12 & 13, purified FN from heparin column; 5 µg, 10 µg & 15 µg, respectively]
contents are shown in Table 2. No titratable -SH group of FN could be deducted in the absence of guanidinium chloride by DTNB assay, whereas in the presence of the denaturant, the number of -SH groups were determined to be 3.4. The titration of -SH groups of FN with BDC-OH (in presence of denaturant) is shown in Fig. 4. The slope of straight line was determined to be \(-0.26904 \times 10^6\). Thus, the number of -SH groups as calculated by the equation (1) was found to be 3.8. Table 3 shows -SH groups of FN determined with DTNB and BDC-OH under different experimental conditions.

Biophysical properties

Sephacryl S-300 column was calibrated by passing marker proteins, of known Stoke's radii, on the column. Elution profile of marker proteins and buffalo plasma FN is shown in Fig. 5. Gel filtration parame-

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Mole %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>9.628</td>
</tr>
<tr>
<td>Threonine</td>
<td>9.865</td>
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<tr>
<td>Serine</td>
<td>6.348</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>13.534</td>
</tr>
<tr>
<td>Proline</td>
<td>7.412</td>
</tr>
<tr>
<td>Glycine</td>
<td>5.070</td>
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<tr>
<td>Alanine</td>
<td>3.258</td>
</tr>
<tr>
<td>Cystine</td>
<td>1.899</td>
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<tr>
<td>Valine</td>
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<tr>
<td>Methionine</td>
<td>1.395</td>
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<td>Isoleucine</td>
<td>4.993</td>
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<tr>
<td>Tyrosine</td>
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<td>Phenylalanine</td>
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<td>Histidine</td>
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</tr>
<tr>
<td>Lysine</td>
<td>4.402</td>
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<tr>
<td>Arginine</td>
<td>7.715</td>
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*Tryptophan analysis was not performed

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>mg/100 mg of group</th>
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<tr>
<td>Neutral hexoses</td>
<td>2.23±0.13</td>
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<tr>
<td>Sialic acids</td>
<td>1.81±0.10</td>
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</table>

*The values are mean of 4 estimations±SD
ters for marker proteins and buffalo plasma FN are shown in Table 4.

Stoke's radius of buffalo plasma FN was computed\(^{22}\) to be 80.75 Å from gel filtration data. Similarly, by using standard equations\(^{23}\), other biophysical properties were also determined as shown in Table 5.

Electron microscopic analysis after rotary shadowing revealed a good population of intact buffalo plasma FN molecules (Fig. 6). As shown in the figure, the intact FN molecules can be visualized as 120 nm long (average length) strands with V-shaped kink in the middle dividing the molecules in two arms of

**Table 3**—Titration of -SH group of buffalo plasma FN with DTNB and BDC-OH

<table>
<thead>
<tr>
<th>Denaturant</th>
<th>Titrant</th>
<th>-SH groups (*moles/**moles of buffalo plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>DTNB</td>
<td>Not Detectable</td>
</tr>
<tr>
<td>Guanidinium chloride (6 M)</td>
<td>DTNB</td>
<td>3.2±0.2</td>
</tr>
<tr>
<td>Guanidinium chloride (6 M)</td>
<td>BDC-OH</td>
<td>3.8±0.2</td>
</tr>
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*Values are the means of 4 estimations±SD
**Using the molecular weight of 474,000 for buffalo plasma FN
Table 4—Gel filtration parameters of marker proteins and buffalo plasma FN*

<table>
<thead>
<tr>
<th>Protein</th>
<th>$V_c$ (ml)</th>
<th>$V_c/V_o$</th>
<th>$K_d = V_c/V_o/V_i$</th>
<th>$K_i = V_c V_o/V_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldolase</td>
<td>245</td>
<td>1.4411</td>
<td>0.3219</td>
<td>0.3275</td>
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<tr>
<td>Catalase</td>
<td>239</td>
<td>1.4059</td>
<td>0.2961</td>
<td>0.3013</td>
</tr>
<tr>
<td>Ferritin</td>
<td>215</td>
<td>1.2647</td>
<td>0.1931</td>
<td>0.1965</td>
</tr>
<tr>
<td>Thyroglobulin</td>
<td>191</td>
<td>1.1235</td>
<td>0.0901</td>
<td>0.0917</td>
</tr>
<tr>
<td>Buffalo plasma FN</td>
<td>194</td>
<td>1.1411</td>
<td>0.1030</td>
<td>0.1048</td>
</tr>
</tbody>
</table>

*Each protein was passed on Sephacryl S-300 HR column (1.25 x 81.5 cm) separately and eluted with PBS containing 5 mM EDTA and 1 mM PMSF. $V_e$, elution volume of proteins; $V_o$, $V_i$ and $V_i$ represent void volume, bed volume and internal volume of the column, respectively.

Table 5—Biophysical properties of buffalo plasma FN

<p>| | | | | |</p>
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<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stoke’s radius</td>
<td>80.75 Å</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrinsic viscosity</td>
<td>7.04 ml/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$D_{20, w}$</td>
<td>2.71 x 10^{-7} cm² sec⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frictional ratio, $b/f$</td>
<td>1.59 min</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Determined by analytical gel filtration in PBS containing 5 mM EDTA, 0.025% sodium azide and 1 mM PMSF; pH 7.2

equal length. The FN preparation also contained some fragments, the latter mostly a half of the full length, about 55-60 nm. The molar mass to length ratio, M/L, for buffalo plasma FN was found to be 3950 nm⁻¹. The mean diameter, $d$, of buffalo plasma FN was computed to be 2.45 nm.

Discussion

FN, a large protein, is highly susceptible to proteolytic cleavage, mainly, by serine and metallo-proteases of plasma. Several protease inhibitors were used during isolation of buffalo plasma FN. B-HCl, a peptidase inhibitor, competitively inhibits trypsin, thrombin, and plasmin. e-ACA is a plasmin inhibitor, PMSF irreversibly inhibits serine proteases and EDTA is a potent inhibitor of metallo-proteases. Other reports also suggest that use of protease inhibitors and removal of vit.K-dependent clotting factors are essential to protect FN from proteolytic degradation during its isolation.

Buffalo plasma FN was purified using two affinity columns: gelatin-Sepharose and heparin-Sepharose. As FN-loaded gelatin column retains FN-bound heparin and serum albumin, these contaminants were removed by washing the column with buffer containing 1 M NaCl. We have used 4 M urea in Tris buffer to elute FN from gelatin column. Tris buffer is preferred for elution as it scavenges any cyanate ions produced from urea, which, otherwise modify free amino groups in FN. Initially, 8 M urea was used to elute FN from gelatin column. But fluorescence studies suggest that elution of FN with high concentration of urea (> 4 M) unfolds the molecule. The use of gradient elution has revealed that elution of FN begins at 2 M urea and completes at 4 M urea. Alternatively, FN can also be eluted with 2 M urea and 1 M NaCl from the gelatin column. Although, use of 4 M urea for FN elution causes subtle irreversible changes in conformation, the urea-eluted FN retains all biological properties.
We have used heparin-Sepharose affinity column for further purification of FN preparation eluted from gelatin column. Several studies suggest that FN preparation isolated from gelatin column usually requires further purification by affinity, ion-exchange or gel filtration columns. Plasma gelatinase is the usual contaminant of FN eluted from gelatin column. Our studies also suggest that FN preparation eluted from gelatin column is contaminated with at least two proteins (Fig. 1, lanes 7 and 8). Plasma gelatinase does not bind to heparin-Sepharose in presence of 4 M urea. During purification of buffalo plasma FN, gelatinase probably appears in the flow through of heparin-Sepharose column. The protein band above the albumin band in Fig. 1 (lanes 9 and 10) indicates presence of gelatinase in the FN preparation. Smilenov et al. have used metal affinity chromatography to remove gelatinase from FN preparation of gelatin column. Buffalo plasma FN eluted from heparin column is highly purified and free from any contamination (Fig. 1, lanes 11-13). The two bands in the figure represent two subunits, namely, FN1 and FN2. Recently, Poulouin et al. have used a three step combination of gelatin- and heparin-cellulose affinity columns to isolate FN from human plasma. This preparation was highly purified and did not contain any proteolytic activities.

The appearance of two closely-spaced bands, FN1 and FN2 (sharp doublet) (Fig. 1, lanes 11-13; Fig. 2, lanes 2-4) implies that buffalo plasma FN contains at least two different subunits. Several groups have reported that human plasma FN analysed on high resolution reducing SDS-gels migrates as a doublet. The mobility of FNs from different sources varies on SDS-gels and the characteristic migration pattern of plasma FN distinguishes it from the cellular FNs and FNs of other body fluids. The difference observed in SDS-PAGE patterns among FNs from different sources may be due to variation in glycosylation of FN molecule. FN of human amniotic fluid, which differs more markedly from plasma FN in its electrophoretic mobility, has a carbohydrate content of 9.5%, whereas plasma FN has only 5% carbohydrate.

Buffalo plasma FN1 and FN2 have molecular mass of 246 kDa and 228 kDa, respectively as revealed by SDS-PAGE. It had been reported that the molecular mass of human plasma FN was 450±25 kDa with two closely similar subunits of 220 kDa. Bovine plasma FN is also a dimer with molecular mass of about 225-250 kDa. Our results show that molecular mass and subunit mass of buffalo plasma FN are similar to those of human and bovine plasma FNs.

The two closely-spaced bands (doublet) on the immunoblot of buffalo plasma FN (Fig. 3) show that both FN1 and FN2 cross-react with rabbit anti-human FN. FNs from different sources are immunologically indistinguishable when polyclonal antibodies are used. Earlier reports also suggest that antisera to FN show broad inter-species cross-reactivity. Rabbit anti-human FN has been widely used to stain cell surface FN in cell lines from a variety of species. Such strong inter-species cross-reactivity clearly indicates a high degree of conservation of at least some structural regions of FN. These observations suggest that the difference among FNs from different sources may result from post-translational modifications (mainly glycosylation) of FN molecules and the polypeptide part is likely to be similar, if not identical. Buffalo plasma FN promotes attachment and spreading of Vero cells and its cell adhesion activity is inhibited by RGD peptide (Results not shown). These observations further confirm the identity of the FN preparation.

Amino acid composition of buffalo plasma FN (Table 1) is also similar to that of FNs from human and bovine plasma. Around 2% variation can be observed in glycine and tyrosine content of buffalo plasma FN. The amino acid composition of FN from many different sources have been found to be similar. Generally, acid residues are more in FN, which account for somewhat acidic pH of 5.5-6.3 for human plasma FN. Some regions of FN are highly enriched in cysteine and some regions of the molecule are basic, while others are acidic.

Buffalo plasma FN contains 2.23% neutral hexoses and 1.81% sialic acids (Table 2). Human plasma FN has around 5% carbohydrates. FNs from human amniotic and synovial fluids have a higher carbohydrate content than plasma FN. The carbohydrate composition of FNs varies depending on their origin. Human plasma FN contains mainly galactose, mannose, N-acetylgalactosamine and sialic acids. Amniotic fluids contain fucose and N-acetylgalactosamine and there is high ratio of galactose and N-acetylgalactosamine to mannose. Vuento et al. reported carbohydrate composition for human cellular FN which showed a lower ratio of sialic acids: galactose and N-acetylgalactosamine: galactose as compared to human plasma FN. The data on carbohydrate analysis of buffalo plasma FN are in good agreement with FNs of other species reported earlier. The difference in glycosylation of FNs from various sources may contribute to differences in their electrophoretic mobility.
The results of titration with DTNB and BDC-OH (Table 3) suggest that, in the absence of denaturant, the free –SH groups of buffalo plasma FN are not accessible to DTNB for titration. The DTNB reaction in the presence of 6 M guanidinium chloride predicts 3.4 free –SH groups in buffalo plasma. The number of –SH groups was determined to be 3.8, when buffalo plasma FN was titrated with BDC-OH (in presence of 4 M guanidinium chloride). BDC-OH titration seems to be more sensitive for analysis of thiol groups of buffalo plasma FN. Smith et al.44 had reported that –SH groups of FN were not accessible for titration in the absence of denaturant. However, titration of FN in presence of 3 M guanidine HCl or 6.3 M urea with DTNB or 2,2’-dipyridyl disulfide indicated 1.3 to 1.6 free –SH groups/200 kDa subunit. Later studies45 also showed that –SH groups in FN could not be exposed by collagen, fibrinogen, heparin, calcium ion, EDTA or deoxycholate. Exposure of –SH groups in physiological salt solution requires at least 1 M guanidine HCl, whereas 3 M guanidine HCl is required for optimal exposure. Our results on free –SH groups of buffalo plasma FN are well in agreement with the data for human plasma FN. Free –SH groups are necessary for binding of FN to cell surface6.

Globular proteins under native conditions have intrinsic viscosity in the range of 3-4 ml/g23. But, buffalo plasma FN has higher intrinsic viscosity (Table 5) which possibly suggests absence of a compact globular conformation at physiological pH and ionic strength. The extended form of human plasma FN and its unfolding at high pH and ionic strength were confirmed by Williams et al.23 and Rococo et al.46 by measurement of intrinsic viscosity [η], determination of the diffusion coefficient, Dη, and radius of gyration. Other studies also suggest that conformation of human plasma FN is ionic strength- and pH-dependent6,47-49.

Electron microscopic analysis after rotary shadowing shows a good population of intact buffalo plasma FN as well as some aggregates and fragments. The changes in FN molecule would have occurred during storage at 4°C for nearly four weeks before analysis. The molecular dimensions of buffalo plasma FN like contour length (120 nm), molar mass to length ratio (3950 nm⁻¹) and the mean diameter (2.45 nm) are comparable to the dimensions of human plasma FN, reported earlier20,24. Other molecular features of buffalo plasma FN such as angle of kink, end-to-end distance and stiffness parameter could not be determined as the quality of the electron micrograph was insufficient for such determinations (Personal communication with Prof J Engel), probably, due to its lower magnification.

Acknowledgement
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