Secondary Structure of Human Plasma Fibronectin: Conformational Change Induced by Calf Alveolar Heparan Sulfates†

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ABSTRACT: The quantitative analysis of circular dichroic spectra of native human plasma fibronectin according to the method of Provencher and Glöckner [Provencher, S. W., & Glöckner, J. (1981) *Biochemistry 20*, 33–37] indicated the presence of β-sheet (79%), β-turn (21%), but no α-helix or random coil in the secondary structure. The calf alveolar heparan sulfates induced a change in the conformation of fibronectin: the magnitude of the change depended on the molecular properties of the particular heparan sulfate preparations.

Since the discovery of an intimate relationship between the major transformation-sensitive glycoprotein associated with fibroblast surface and a high molecular weight serum protein (Ruoslahti et al., 1973), considerable effort has been spent to elucidate the structural and functional properties of these proteins, now known as the cellular and soluble forms of fibronectin, respectively. The molecular structure emerging from these studies is a dimeric glycoprotein with subunits of similar size [M_r 220 000; for recent reviews, see Mosher (1980), Ruoslahti et al. (1981), Hynes & Yamada (1982), and Furcht (1983)]. The subunit chains seem to be identical except for their carboxy-terminal regions (Richter et al., 1981; Hayashi & Yamada, 1983; Sekiguchi & Hakomori, 1983). The two subunits are interconnected by disulfide bridges close to the carboxy-terminal ends of their polypeptide chains (Jilek & Hörmann, 1977; Petersen et al., 1983). Cellular and soluble fibronectins have a similar gross structure; however, minor but probably significant differences have been found between cellular and plasma fibronectin (Yamada & Kennedy, 1979; Hayashi & Yamada, 1981).

Fibronectin has distinct binding functions: it has affinities to collagen/gelatin (Engvall & Ruoslahti, 1977), fibrin (Stemberger & Hörmann, 1976), glycosaminoglycans (Hayashi et al., 1980; Sekiguchi & Hakomori, 1980; Yamada et al., 1983), bacteria (*Staphylococcus aureus*) (Kuusela, 1978), actin (Keski-Oja et al., 1980), DNA (Zardi et al., 1979), sphingolipids (Kleinman et al., 1979), acetyl cholinesterase (Emmerling et al., 1981), and complement factor C1q (Menzel et al., 1981). Fibronectin is substrate for transglutaminase (Mosher et al., 1979). Fibronectin has a hemagglutinating activity (Yamada et al., 1975; Vuento, 1979), and it binds to cell surfaces (Grinnell et al., 1982).

The binding sites for macromolecular ligands are located at protease-resistant domains on the subunit polypeptide chains. As a consequence, fibronectin is able to simultaneously bind multiple ligands such as glycosaminoglycans, collagen, and the cell surface. This multifunctional nature of fibronectin probably is significant for its biological activity. Fibronectin mediates a number of adhesive interactions between cells and between cells and various substrata. It promotes attachment and spreading of cells on plastic and on collagen or fibrin substrata. It is thought that fibronectin functions in vivo in anchoring cells to the extracellular matrix (see above reviews). Soluble fibronectin has been suggested to have a function as a nonspecific opsonin (Saba & Jaffe, 1980).

Recently, considerable interest has been focused in the interaction of fibronectin with glycosaminoglycans. Fibronectin binds to heparin (Yamada et al., 1983), heparan sulfate (Laterra et al., 1980; Stamataglou & Keller, 1982), and hyaluronic acid (Yamada et al., 1983; Isemura et al., 1982). Immunofluorescent studies have demonstrated a codistribution of fibronectin and collagen with sulfated glycosaminoglycans in the extracellular matrix (Hedman et al., 1982). Fibronectin has been shown to be cross-linked to sulfated proteoglycans at the cell surface (Perkins et al., 1979). It has been suggested that heparan sulfate proteoglycans might function as receptors for fibronectin in the attachment and spreading of fibroblasts on fibronectin-coated substrata (Rollins et al., 1982).

It is interesting that the presence of heparin seems to enhance the binding of fibronectin to collagen (Jilek & Hörmann, 1979; Johansson & Höök, 1980; Ruoslahti & Engvall, 1980). This suggests that the binding functions of fibronectin could be cooperative, the binding of one ligand being affected, e.g., by conformational changes induced by other ligands. In the present study we demonstrate that fibronectin has an ordered secondary structure and that there is a change in the conformation of fibronectin induced by heparan sulfate.

Materials and Methods

Purification of Fibronectin. Fibronectin (FN)[1] was purified from citrated human plasma by affinity chromatography procedures under nondenaturing conditions (Vuento & Vaheri, 1979). In brief, FN was adsorbed from human plasma to gelatin–agarose and eluted with 1 M arginine at pH 7.5. A second affinity chromatographical step on arginine–agarose completed the procedure. FN was stored at 4 °C at a concentration of 1–3 mg/mL in 50 mM Tris-HCl, pH 7.5, containing 100 mM NaCl and 0.02% (w/v) NaN_3. For circular dichroic (CD) measurements, samples were dialyzed against 50 mM sodium phosphate buffer, pH 7.5, and centrifuged (12000*g*, 20 min, 24 °C) to remove any FN aggregates.

For affinity chromatography, FN was coupled to cyanogen bromide activated Sepharose 4B gel (Pharmacia, Uppsala,

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[1] Abbreviations: FN, fibronectin; HS, heparan sulfate; CD, circular dichroic (dichroism); UV, ultraviolet; CPC, cetylpyridinium chloride; Tris, tris(hydroxymethyl)aminomethane; EDTA, (ethylenedinitrilo)-tetraacetic acid.

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Sweden) according to the instructions of the manufacturer. The gel contained 1 mg of FN/mL. It was extensively washed before use with 0.2 M EDTA, pH 7.4, and with 4 M NaCl and equilibrated with 50 mM Tris-HCl buffer, pH 7.5.

Preparation of Glycosaminoglycan Fractions. Calf lungs obtained from a local slaughter house were lyophilized. Lipids were removed by extraction with acetone and acetone–diethyl ether (1:1). A total of 90 g of dried tissue was cut in pieces and homogenized (Serval high-speed tissue homogenizer) with 1500 mL of acetate buffer (Wasteson et al., 1972), and 750 mg of papain (Merck, Darmstadt, Germany, Art. 7144) was added. After an incubation at 60 °C for 24 h, proteins and nucleic acids were removed by adding solid trichloroacetic acid (10%) and allowing the mixture to stand overnight at 4 °C. The mixture was then centrifuged (25000*g*, 20 min, 4 °C), and the precipitate was washed twice with 10% trichloroacetic acid. Combined supernatant and washings were dialyzed exhaustively against deionized water. A small precipitate formed during dialysis was removed, and HCl was added to the supernatant to a concentration of 0.05 M.

The acidified supernatant was applied on a DE-52 column (2.5 × 15 cm, equilibrated with 0.05 M HCl), and the column was washed with 0.05 M HCl until no more hyaluronic acid was detected in the eluate. Glycosaminoglycans were eluted with 1 and 2 M NaCl in 0.5 M HCl. Combined eluates were neutralized and dialyzed against deionized water. Sulfated glycosaminoglycans except keratan sulfate were precipitated with 1% cetylpyridinium chloride (CPC, twice recrystallized from acetone) and converted to their Na salts and further purified with repeated ethanol precipitation (2.5 volumes) as presented by Rodén et al. (1972). Dermatan sulfate was removed with alkaline copper precipitation (Fransson et al., 1980). Dialyzed supernatant was precipitated with 1% CPC and converted to Na salt with ethanol precipitation. The crude heparan sulfate (HS) fraction was finally separated with ethanol fractionation as Ca salt, saving the material obtained at ethanol concentrations between 18% and 36% (Rodén et al., 1972). HS was converted to its Na salt, dried with diethyl ether, and weighed. The yield was 127 mg.

The purified HS preparate was fractionated with different NaCl concentrations as presented by Fransson et al. (1980). A total of 100 mg of the dried HS preparation was precipitated with 2.5% CPC in 0.05 M NaCl, and the precipitate was added to a Celite pad on a Buchner funnel. Celite was washed with 0.05 M NaCl/0.05% CPC and 0.2 M NaCl/0.05% CPC under suction. Elution was achieved with 0.6, 0.8, 1.0, 1.2, and 1.8 M NaCl in 0.05 M CPC (HS fractions 1–5, respectively), and the preparations were precipitated by diluting eluates slightly below lower NaCl concentrations. CPC was removed from precipitates, and heparan sulfates were converted to their Na salts by precipitation with ethanol (2.5 volumes). Final products were precipitated from water with 2 volumes of ethanol and washed with ethanol and diethyl ether. Dried HS fractions were dissolved in water at concentrations of 1 mg/mL and stored at −20 °C.

Total glycosaminoglycan concentrations in HS samples were measured by the *m*-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973). Ratios of heparan sulfates to other glycosaminoglycans in fractions were measured by determining the ratio of glucosamine to galactosamine after acid hydrolysis of samples (Ludowieg & Benmaman, 1967).

The HS samples were analyzed by using cellulose–acetate electrophoresis in cupric acetate (Hronowski & Anastassiades, 1979). Hyaluronic acid (Sigma) and heparin (Medica, Finland) were used as reference materials. The purity of fractions was further checked by cellulose–acetate electrophoresis in 0.1 M barium acetate (Wessler, 1968) before and after treatment with testicular hyaluronidase (Sigma H-3506, type 1-s) (Friman et al., 1974). Glycosaminoglycans were stained with Alcian Blue (M_w 1298.88, purchased from Eastman). Digestion with hyaluronidase was done as follows: 0.5 mg of hyaluronidase in 1 mL of 0.15 M citric acid/phosphate buffer, pH 6.0, was incubated with 1 mL of HS sample (0.05–0.15 mg in H_2O) at 37 °C for 24 h with dialysis against the buffer, then further dialyzed against water for 24 h, lyophilized, and electrophoresed.

Affinity Chromatography with Fibronectin–Sepharose. A part of the crude HS preparation in 50 mM Tris-HCl, pH 7.5, with 0.02% NaN_3 was applied on the FN–Sepharose column (3 mL, equilibrated with 50 mM Tris-HCl, pH 7.5, 0.02% NaN_3), and the column was washed with the buffer. The material adsorbed to the column was eluted with increasing NaCl concentration in equilibration buffer, 0.1, 0.2, 0.5, and 2 M NaCl, and at last with 2 M NaCl/8 M urea. Fractions were dialyzed exhaustively against deionized water, lyophilized, and subjected to cellulose–acetate electrophoresis in cupric and barium acetates.

Circular Dichroic (CD) Measurements. The CD spectra were measured on a Cary Model 61 spectropolarimeter with a thermostated cell holder. Calibration was done with D-(+)-10-camphosulfonic acid. All spectra were measured with a 10-s time constant, a full-scale sensitivity of 20×10^{-3} deg, and a scan rate of 1 nm/min. The spectral slit width was changed automatically by the instrument. Cells of 1- and 10-mm path lengths were used in far- and near-ultraviolet (UV) regions, respectively. Triplicate scans were made, and reproducibility was invariably within 1×10^{-3} deg.

Spectra were obtained in the far-UV region between 250 and 190 nm, at 24 °C. FN concentrations varied from 0.085 to 0.15 mg/mL, and 50 mM sodium phosphate buffer, pH 7.5, was used for all spectral studies. The buffer blanks were subtracted for each sample. Mean residue ellipticities (deg cm^2 dmol^{-1}), $[\theta]$, were calculated according to

$$[\vartheta] = \frac{[\theta]_{obsd} \times 108}{10lc}$$

where $[\theta]_{obsd}$ is the manually digitalized observed ellipticity with 0.5-nm increments, 108 is the mean residue molecular weight obtained from the amino acid composition of FN (Vuento et al., 1977), l is the path length in centimeters, and c is the concentration of FN in grams per milliliter. Final data reduction including secondary structure analysis was performed on the Burroughs 7800 computer of the University of Helsinki with the method of Provencher & Glöckner (1981). The final spectra shown are drawn with 1-nm increments, taking the mean value of three consecutive background subtracted experimental ellipticities.

Near-UV CD spectra were measured between 310 and 270 nm. Appropriate blanks were subtracted, and molecular ellipticities (deg cm^2 dmol^{-1}), $[\theta]$, were calculated from the above formula with an average molecular weight of 450 000. FN samples were incubated with HS solutions for 3–24 h at room temperature (24 °C) before measurements of CD spectra.

Estimation of Fibronectin Concentrations. Concentrations of FN solutions used were estimated spectrophotometrically, assuming a value of $A(1\%,1\text{ cm}) = 12.8$ at 280 nm (Mosesson & Umfleet, 1970).

Results

Fractionation of Heparan Sulfates. Cetylpyridinium chloride complexes of heparan sulfates were dissociated ac-

Table I: Characteristics of Heparan Sulfate Fractions

NaCl concn (M)	heparan sulfate fraction	yield (%)	yield (%)[a]	galactosamine (% of total hexosamines)	uronic acid (mg/mg of HS)
0.2–0.6	1	14	16	3.9	250
0.6–0.8	2	17	25	5.4	238
0.8–1.0	3	21	21	24.9	258
1.0–1.2	4	25	10	38.5	213
1.2–1.8	5	23	28	14.3	234

[a] Fransson et al., 1980.

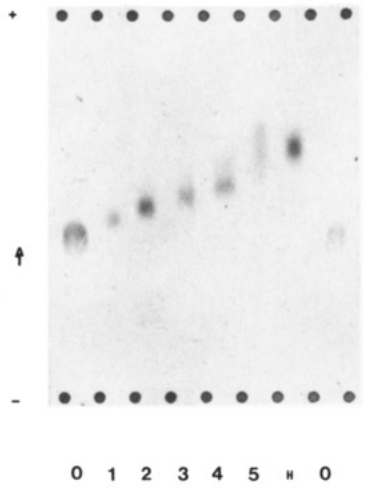

FIGURE 1: Analysis of purified heparan sulfates by cellulose–acetate electrophoresis in the presence of cupric acetate. (1–5) Heparan sulfate fractions eluted from CPC precipitate on Celite bed with 0.2–0.6, 0.6–0.8, 0.8–1.0, 1.0–1.2, and 1.2–1.8 M NaCl, respectively; H is commercial heparin, and O is hyaluronic acid. The direction of migration is shown by an arrow.

cording to their acidity by increasing sodium chloride concentration (Table I). Percentages of HS fractions of total heparan sulfates were the same magnitude as obtained by Fransson et al. (1980), whose results are shown for comparison in Table I. A cellulose–acetate electrophoresis of the fractions is shown in Figure 1. Heparan sulfates obtained below 0.8 M NaCl were named low-sulfated ones (fractions 1 and 2), and the material eluted with 1.2–1.8 M NaCl (fraction 5) was named high-sulfated HS. HS fraction 5 consisted three components, one of which had mobility similar to that of commercial heparin, and the two others had mobilities near it (Figure 1). The material obtained with 0.8–1.2 M NaCl (fractions 3 and 4) was contaminated with chondroitin sulfates (25%–40%) (Table I); the other glycosaminoglycans detected in barium acetate electrophoresis were susceptible to digestion with testicular hyaluronidase (not shown).

Affinity Chromatography on Fibronectin–Sepharose. The electrophoretic analysis of HS fractions bound to FN–Sepharose from a preparate of crude HS mixture is shown in Figure 2. The sample applied on the column contained all the molecular components shown in Figure 1. HS material was eluted from the FN column with 0.1 and 0.2 M NaCl (A and B in Figure 2). Traces of high-sulfated HS material were also eluted with 0.5 M NaCl (C in Figure 2). HS eluted with 0.1 M NaCl had a migration rate between those of commercial chondroitin sulfate and heparin. Materials eluted with 0.2 and 0.5 M NaCl had migration rates close to that of heparin (Figure 2), and low-sulfated HSs failed to bind to the FN–Sepharose column (not shown).

Far-Ultraviolet Circular Dichroism. The CD spectrum of native FN presented (Figure 3) is an average of nine different scans and four different FN preparations. The CD spectrum

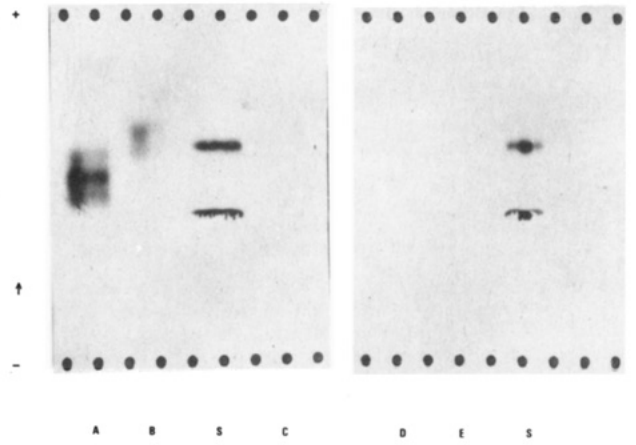

FIGURE 2: Cellulose–acetate electrophoresis of heparan sulfates fractionated by affinity chromatography on FN–Sepharose. (A–E) Fractions eluted from fibronectin–Sepharose with 0.1, 0.2, 0.5, and 2 M NaCl and 2 M NaCl/8 M urea, respectively; S is commercial chondroitin sulfate and hyaluronic acid. The direction of migration is shown by an arrow.

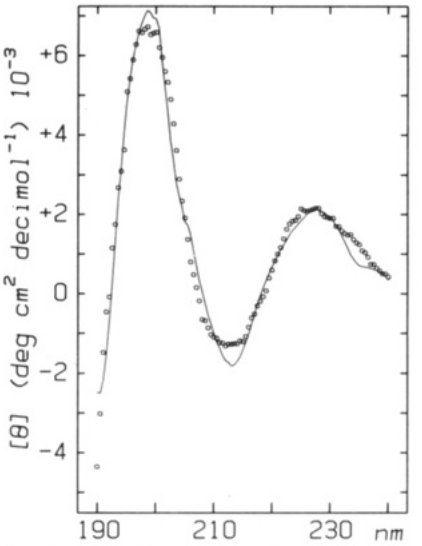

FIGURE 3: Circular dichroic spectrum of native human plasma fibronectin in 50 mM sodium phosphate, pH 7.5 ([FN] = 0.1 mg/mL), in the far-ultraviolet region between 240 and 190 nm (□) and the theoretical spectrum as calculated by the method of Provencher & Glöckner (1981) (—).

of FN in the far-UV region between 240 and 190 nm contained a maximum at 198.5 nm, a minimum at 212.5 nm, and a broad maximum at 227 nm. An analysis of the spectral data by the method of Provencher & Glöckner (1981) showed that FN contains 79% β-sheet structure and 21% of β-turn structure. A theoretical spectrum predicted for such a structural composition is shown in Figure 3 for comparison.

The amounts of heparan sulfates added to FN solutions were so low that their contribution to measured ellipticities was negligible and could be subtracted with a buffer background from the measured sum spectra of FN and HS (Figure 4). These calculated CD spectra of FN at different HS fraction 5 concentrations are shown in Figure 5.

In Table II it is shown how the CD spectrum of FN was changed by different HS types and by different HS amounts: 79% β-sheet and 21% β-turn of native FN varied to 74% and 26% of high-sulfated HS interrupted FN, respectively. The effect was saturable with HS fraction 5 at the concentration of 0.01 mg/mL, and the far-UV spectra of FN were not further changed when the concentration of HS fraction 5 was raised to 0.05 mg/mL (Table II).

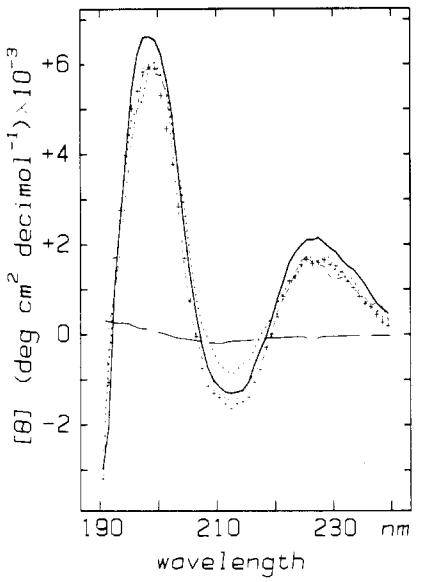

FIGURE 4: Far-ultraviolet circular dichroic spectrum of native plasma fibronectin (the same one as in Figure 1) (—) and the circular dichroic spectra of fibronectin with different heparan sulfate additions: FN/HS1 = 7.5 (···), FN/HS2 = 7.5 (– –), and FN/HS3 = 10 (+). The circular dichroic spectrum of heparan sulfate fraction 1 (–·–) is also shown; this has been subtracted from the respective FN/HS1 circular dichroic spectrum.

Table II: Effects of Heparan Sulfates on Secondary Structure of Fibronectin Calculated from Far-Ultraviolet Circular Dichroic Data[a]

heparan sulfate fraction	FN concn/ HS concn (w/w)	% β-sheet	% β-turn	% random coil
		79	21	0
1	1.3	76	24	0
1	7.5	78	22	0
2	7.5	74	26	0
3	9.7	77	23	0
4	7.3	76	24	0
5	2930	76	24	0
5	293	75	25	0
5	11.3	74	26	0
5	2.9	74	26	0

[a] Fibronectin concentration is 0.1 mg/mL.

Near-Ultraviolet Circular Dichroism. A near-UV CD spectrum of native FN (1 mg/mL) and spectra of FN at different HS concentrations (fraction 5) are given in Figure 6A. A change of CD spectrum of FN is seen already, when the heparan sulfate concentration is 0.002 mg/mL.

Changes in CD ellipticities of FN as a function of additions of HS fraction 5 at 275, 281.5, 285, 290, and 296 nm are drawn in Figure 6. These were the five most prominent bands in the spectrum of FN at this region. When 1.5 M NaCl was added to the solution of FN and HS fraction 5 (FN:HS5 = 43) in the 50 mM sodium phosphate buffer, pH 7.5, transitions regained their original strengths at 290 and 296 nm, did not change at 285 and 281.5 nm, and reverted partly at 275 nm.

Discussion

The spectral parameters of fibronectin obtained by us with circular dichroic measurements in the far-ultraviolet region (Figure 3) are in line with those obtained by other laboratories (Alexander et al., 1979; Koteliansky et al., 1981; Marković et al., 1983). The far-UV CD spectra of FN have been restricted to a spectral region between 190 and 240 nm, the lower limit being imposed by the buffer system used. The accuracy of the analysis could be improved by extending the spectra down to 165 nm (Brahms & Brahms, 1980), but with the

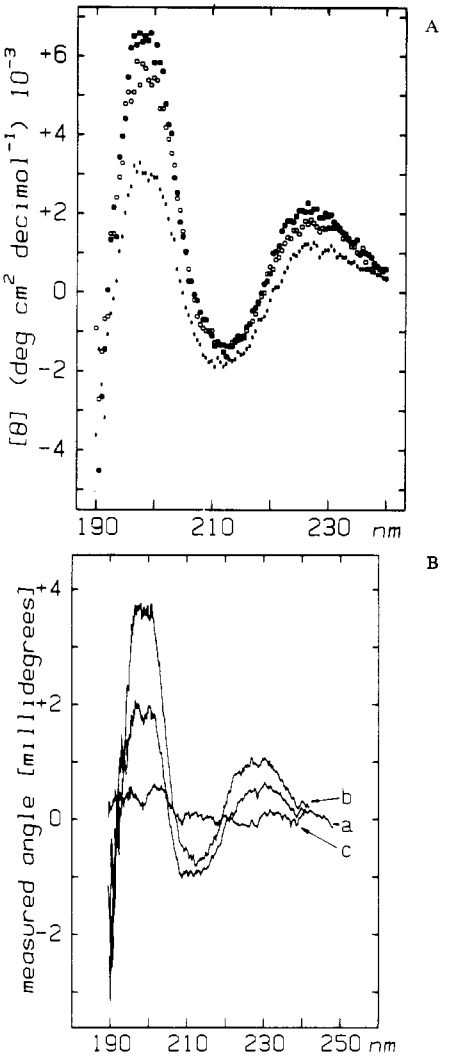

FIGURE 5: (A) Individual far-ultraviolet circular dichroic spectra of fibronectin with different high-sulfated heparan sulfate (fraction 5) additions: FN/HS4 = 67 (□), FN/HS5 = 24 (*), and FN (■). Fibronectin concentrations were 0.1 mg/mL. (B) Actual tracings of the far-ultraviolet circular dichroic spectra of fibronectin (a) with (FN/HS5 = 24) and (b) without heparan sulfate added; (c) background (50 mM sodium phosphate, pH 7.5).

commercial apparatus available to us such measurements were not possible.

In our interpretation of the far-UV CD spectra of FN in terms of secondary structure, we have used the method of Provencher & Glöckner (1981). As in any spectroscopical method of analyzing the secondary structure of proteins and peptides, interference from chromophores other than the amide group will complicate the analysis. This is particularly evident in the region between 220 and 240 nm, where contributions from aromatic residues and disulfide bridges are well documented (Sears & Beychok, 1973). Accumulating evidence from pH titrations and temperature studies, from CD and NMR spectroscopy, on a series of homologous or nearly related compounds indicates that changes in measured ellipticity in the region between 220 and 240 nm, albeit of nonpeptide origin, nonetheless are correlated to changes in the peptide backbone (Dufton & Hider, 1983). Such spectral features, if present in our collection of standard protein CD spectra, will be recognized and, accordingly, the corresponding proteins given more weight in the regularization process, while other proteins in the linear basis set will be suppressed. We therefore decided to cover the whole wavelength interval between 190

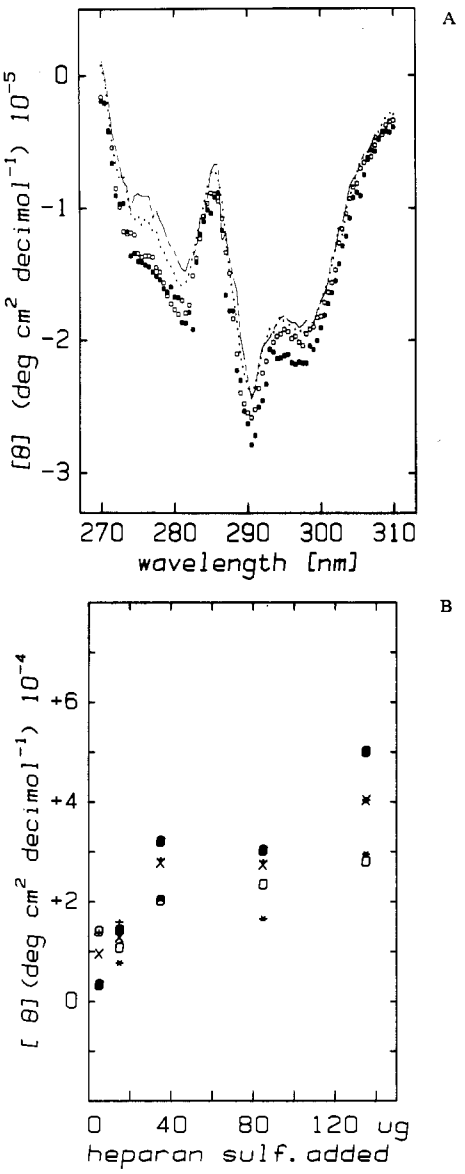

FIGURE 6: (A) Circular dichroic spectrum of human plasma fibronectin in 50 mM sodium phosphate, pH 7.5 ([FN] is 1 mg/mL), in the near-ultraviolet region between 310 and 270 nm (■) and the circular dichroic spectra of fibronectin at different concentrations of heparan sulfate fraction 5: HS5 = 0.002 mg/mL (□), HS5 = 0.012 mg/mL (···), and HS5 = 0.043 mg/mL (– –). (B) Effect of heparan sulfate fraction 5 on the 275-nm (■), 281.5-nm (×), 285-nm (+), 290-nm (*), and 296-nm (□) molar differential ellipticity of fibronectin as compared to the ellipticity of native fibronectin.

and 240 nm in our theoretical evaluation of the secondary structure of FN.

With the above limitations in mind, we have interpreted our far-UV CD spectra of the FN molecule in a native state as well as complexed to heparan sulfates in terms of secondary structural elements, and changes therein, respectively. For the native FN molecule, a best fit analysis gave a 100% β structure (average values: 79% β-sheet, 21% β-turn) in native FN. The amount of α-helix could not be accurately estimated, but it was certainly very low. Koteliansky et al. (1981), using infrared spectroscopy, also found that the ordered secondary structure of FN was exclusively β structure, with no α-helix present. Similar results have been obtained with FN fragments (Venyaminov et al., 1983). It appears that FN belongs to the family of proteins with all-β structure, representative examples of which are concanavalin A and immunoglobulins (Levitt & Chothia, 1976). Although this could be surprising in view of the fact that many all-β proteins are globular and FN certainly does not look that way in electron microscopy (Engel et al., 1981; Price et al., 1982; Erickson & Carrell, 1983), it should be noted that the polypeptide chains of FN are organized into several folded domains (Skorstengaard et al., 1984).

The near-UV CD spectrum obtained here for native human plasma FN (Figure 6) is very similar to that reported by Tooney et al. (1982). However, the ratio of measured value of the rotational strengths of the negative bands at 281.5 and 290.5 nm of our FN sample is 0.41, which is somewhat higher than the corresponding ratio as estimated from Tooney's spectrum. Because buffer identity and ionic strength do have influence on the spatial arrangement of FN domains (Marković et al., 1983), it is reasonable to expect some differences in the spectra of FN in 50 mM sodium phosphate buffer, pH 7.5 (the buffer system used in this work), and in 0.15 M NaCl, 0.02 M *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid, pH 7.0 (the buffer system used in the cited reference).

Formation of FN–HS complexes gives rise to spectral changes, which are evident in far-UV as well as in near-UV spectra. Theoretical analysis on basis of the far-UV data implies that the β-sheet structure increases somewhat in the binding process (Figures 4 and 5; Table II). These results agree with those of Welsh et al. (1983), who reported a conformational change induced in bovine FN by heparin.

The optical activity of FN samples was decreased by all heparan sulfates used: already by a trace amount of HS in the cases of HS fractions 4 and 5 (at the level of nanograms per milliliter). The results (Figures 4 and 5; Table II) show that the ability of various HSs to decrease optical activity of FN correlates roughly with the degree of sulfatation in HS. This may be due to higher binding affinity of high-sulfated HS to FN as compared to the affinity of low-sulfated HS. Affinity chromatography experiments (Figure 2) showed that FN preferentially binds high-sulfated HS, which supports the above concept. It is interesting in this context that only highly sulfated heparin but no low-sulfate HSs was able to enhance the binding of FN to collagen (Johansson & Höök, 1980).

Near-UV CD spectra of FN recorded after additions of heparan sulfate revealed structural or environmental changes taking place in the vicinity of aromatic residues (Figure 6). It seems, that the changes progress concertedly, involving simultaneous changes in tryptophan (296- and 290-nm transitions) as well as in tyrosine and phenylalanine residues (285-, 281.5-, and 275-nm transitions). It is noteworthy that already at very small HS additions (about 1:100 on molar basis) the effect on the optical activity is pronounced. This could mean that one HS molecule can bind several FN molecules, multimers so formed dissociating finally to HS–FN dimers upon further HS addition. Alternatively, our results imply that one FN molecule has several (at least two) HS binding domains with different affinities. This supports the results obtained by other investigators (Hayashi & Yamada, 1982).

In some final experiments, following formation of the FN–HS complexes, sodium chloride was added to the solution to a final concentration of 1.5 M. Near-UV CD spectra revealed that in this case tyrosines were completely and phenylalanines almost completely unaffected, whereas tryptophan signals were restored to their original strength. These results can be interpreted in terms of strength of interaction for different classes of aromatic residues. The detailed mechanism of binding between FN and HSs remains, however, to be demonstrated.

The conformational change induced in the FN molecule by the binding of HSs, together with possible allosteric effects

on the binding of FN to cell surface and to collagen fibers, could be an important factor controlling the organization of the extracellular matrix and the anchorage of cells to the matrix. Furthermore, such conformational changes could be important in other aspects of FN function.

Heparin is known to enhance the opsonic activity of FN (Molnar et al., 1979; Hörmann & Jelinic, 1980), an effect that is probably due to the association of heparin with FN (Hörmann & Jelinic, 1980). It is also significant that some biological activities like chemotactic activity (Postlethwaite et al., 1981) and opsonin activity (Czop et al., 1981) are more marked in FN fragments than in the intact FN molecule, suggesting that structures essential for these activities are cryptic in the intact molecule. Opening of the molecular structure by proteolysis, or by conformational changes induced by the binding of sulfated glycosaminoglycans, could control the expression of such biological activities. We are sure that the results reported here will evoke further experimentation along the lines of this hypothesis.

Registry No. HS, 9050-30-0.

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Nanosecond Optical Spectra of Iron–Cobalt Hybrid Hemoglobins: Geminate Recombination, Conformational Changes, and Intersubunit Communication†

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ABSTRACT: Hybrid hemoglobins were prepared in which cobalt was substituted for the heme iron in either the α or β subunits. Transient optical absorption spectra were measured at room temperature for these hybrids at time intervals between 0 and 50 ms following photodissociation of the carbon monoxide complex with 10-ns laser pulses. The cobalt porphyrins do not bind carbon monoxide, making it possible to investigate the time-resolved response of the cobalt-containing subunits to photodissociation of carbon monoxide in the iron-containing subunits. At the same time the response of the iron-containing subunits to the photolysis event can be studied, permitting an independent determination of the kinetics of ligand rebinding and conformational changes in the α and β subunits of an intact tetramer. The data were analyzed by using singular-value decomposition to obtain the kinetic progress curve for ligand rebinding, the deoxyheme and cobalt porphyrin spectral changes, and the time course of these spectral changes. The geminate rebinding kinetics following photodissociation of $\alpha(Co)_2\beta(Fe\text{-}CO)_2$ were very similar to those found for unsubstituted hemoglobin, $\alpha(Fe\text{-}CO)_2\beta(Fe\text{-}CO)_2$, indicating equivalence of the geminate kinetics for α and β subunits within the R-state tetramer. The results for $\alpha(Fe\text{-}CO)_2\beta(Co)_2$ were consistent with this conclusion, even though the analysis was complicated by the presence of comparable populations of R- and T-state species. Comparison of the deoxyheme spectral changes and relaxation times among the three molecules indicated that both α and β subunits contribute to the deoxyheme spectral changes that signal tertiary and quaternary conformational changes in the unsubstituted tetramer. The response of the cobalt porphyrins to photodissociation was similar in the two hybrids. No structural changes were detected in the cobalt-containing subunits until the second tertiary conformational change in the iron-containing subunits observed at 1–2 μs. Much larger structural changes, as judged by the amplitude of the spectral changes, occurred in the cobalt-containing subunits concomitant with the R $\rightarrow$ T quaternary change at about 20 μs.

Since the introduction of time-resolved absorption and Raman spectroscopy using picosecond and nanosecond lasers, there have been a number of studies on the photodissociation of oxygen and carbon monoxide from hemoglobin (Hb) [see papers in Ho et al. (1982) and reviews by Friedman et al. (1982) and Noe (1982)]. A major objective of these investigations has been to develop a detailed description of the processes occurring subsequent to photodissociation and to relate these processes to the mechanism for the overall thermal dissociation and binding reactions. In particular, one would like to know the rates of the various conformational changes that are known to take place from X-ray diffraction studies

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