Human Fibrinogen of Relatively High Solubility. Comparative Biophysical, Biochemical, and Biological Studies with Fibrinogen of Lower Solubility*

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ABSTRACT: Naturally occurring human fibrinogen of relatively high solubility (fraction I-8) was compared with fibrinogen of lower solubility (fraction I-4). The average molecular weight computed from sedimentation velocity–diffusion data and from sedimentation equilibrium data was 269,000 for I-8 and 325,000 for I-4. Electrophoretic studies of these fractions in acrylamide gels of 5–12% concentration demonstrated that the over-all mobility of I-8 was greater than that of I-4, a result consistent with differences in size and/or symmetry between these fractions; in addition, each fibrinogen subfraction could be separated into two more or less distinct bands, a phenomenon probably related to the heterogeneity demonstrable by DEAE-cellulose gradient chromatography.

The sialic acid, hexose, and hexosamine content of I-8 was higher than that of I-4 per gram of protein. Computation of these data on a molar basis indicated that the sialic acid and hexosamine contents of I-8 and I-4 were the same, probably true as well for the hexose content. There were no differences between I-8 and I-4 in the absorbancy coefficients ($A^{1\%}_{1\,cm}$) at 282 mμ or in the ultraviolet spectral curves (254–330 mμ) in alkaline urea; the tyrosine:tryptophan ratios computed from these data did not differ significantly. There were no qualitative nor detectable quantitative differences in the N-terminal amino acids of I-8 and I-4; each contained one to two residues of alanine and tyrosine per mole as well as smaller amounts of aspartic acid. Both I-8 and I-4 were denatured at approximately the same rate at 56° but were stable with regard to loss of clottability at 37°. The plasminogen and fibrin-stabilizing factor contents of I-8 were lower than that of I-4.

The nature of the prolongation of the thrombin time of high solubility fibrinogen relative to that of lower solubility was investigated. No detectable differences in the enzymatic phase of the fibrinogen–fibrin conversion, as judged from the thrombin-catalyzed release of trichloroacetic acid soluble nitrogen from either fibrinogen fraction, were observed but the onset of polymerization of fibrin monomer derived from I-8 fibrinogen was delayed when compared with I-4.

Recently, a method was described for the isolation and purification of naturally occurring human fibrinogen(s) of higher solubility than those derived from plasma fraction I (Mosesson and Sherry, 1966) and representing approximately 25% of the total fibrinogen of plasma. When compared with fibrinogen of relatively lower solubility, the higher solubility fibrinogen(s) clotted more slowly in the presence of thrombin but were indistinguishable from classically prepared fibrinogen (*i.e.*, plasma fraction I-4) by electrophoresis on cellulose acetate strips, immunoelectrophoresis in agar gel, and the gross appearance of formed clots. Detailed solubility studies indicated that although no fraction was homogeneous by phase-rule criteria, the procedures employed did serve to isolate fractions highly enriched in one solubility type or another. A system of nomenclature based upon that introduced by Cohn *et al.* (1946) and further developed by Morrison *et al.* (1948) and Blombäck and Blombäck (1956) was employed. The higher the over-all solubility of a given fraction, the higher was the assigned number (*e.g.*, I-1, I-2, etc.).

In the studies to be reported, further comparative examinations of a fibrinogen subfraction representative of high solubility fibrinogen (*i.e.*, fraction I-8) with that of lower solubility fibrinogen (*i.e.*, fraction I-4) were made.

Materials and Methods

Fibrinogen subfractions I-4 and I-8 employed in these studies were prepared from outdated human ACD plasma as described by Mosesson and Sherry (1966). All I-4 preparations were at least 97% clottable; I-8 preparations were at least 95% clottable. Plasminogen-free fibrinogen was prepared from fraction I-4 by the method of Mosesson (1962). The fractions were exten-

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sively dialyzed against 0.3 M NaCl and stored frozen at −20°.

The concentration of dialyzed fibrinogen solutions was determined with a Brice–Phoenix differential refractometer. A specific refractive increment of 0.188 ml/g at 546 mμ was assumed (Armstrong *et al.*, 1947).

Spectrophotometric measurements were made in a Beckman Model DU spectrophotometer. Tyrosine:tryptophan ratios were computed from ultraviolet spectral data according to Bencze and Schmid (1957). Corrections for light scattering were made (when appropriate) from the ratio of the fourth power of the wavelength at 330 mμ to that at lower wavelengths.

DEAE-cellulose chromatography was performed at 3–5° using a continuous Tris-phosphate salt and pH gradient from 0.005 M phosphate (pH 8.6) to 0.5 M phosphate (pH 4.1–4.3) essentially as described for the chromatography of fibrinogen (Finlayson and Mosesson, 1963). The system was modified for the examination of small samples (10–40 mg) by reduction of the column size (22 × 1 cm), the number of chambers comprising the gradient and the total gradient volume (seven chambers each containing 50 ml of solution, the molarities of which with respect to phosphate were consecutively: 0.005, 0.02, 0.03, 0.05, 0.10, 0.20, and 0.50; these solutions were prepared by mixing appropriate proportions of starting and limit buffer). Fractions (3.3–3.6 ml) were collected in a Beckman-refrigerated fraction collector. The fraction volume was constant (±0.1 ml) for any given column. A flow rate of 100–130 ml/hr was maintained with a Sigma motor pump; the gradient was completed by tube 95–100.

N-terminal amino acid analysis was performed by the DNFB[1] method of Sanger as described by Fraenkel-Conrat *et al.* (1955). Thin layer chromatography analysis of ether-extractable dinitrophenyl amino acids was carried out on 20 × 20 cm glass plates coated with silica gel G[2] using the toluene–2-chloroethanol–pyridine–0.8 N NH_4OH solvent system (30:18:9:18) for the first dimension and chloroform–methanol–acetic acid (95:5:1) for the second (Brenner *et al.*, 1961).

Ultracentrifugal studies were made in a Spinco Model E analytical ultracentrifuge employing either schlieren or Rayleigh optics. Diffusion constants were determined in the synthetic boundary cell by the method of Schachman (1957). For molecular weight estimations from sedimentation velocity and diffusion data, a partial specific volume of 0.725 cc/g was assumed (Armstrong *et al.*, 1947); frictional ratios (f/f_0) from these data were calculated from the equation $f/f_0 = 10^{-8}(1 - (\bar{v}\rho/D^2\bar{s}\bar{v}))^{1/3}$ (Shulman, 1953). Molecular weight estimations by sedimentation equilibrium were performed in a six-chamber Yphantis cell, and the results were calculated by the method of Yphantis (1964).

Electrophoresis in polyacrylamide gels was performed essentially by the method of Davis (1964).[3] Separating gel concentration was varied between 5 and 12%. The samples in 40% sucrose were layered on a 4% stacking gel. The runs were made in 0.025 M Tris–0.192 M glycine–0.01 M ε-ACA buffer (pH 9.2). Samples were run for 5–6 hr at 2.5 ma/tube for the first hour and then at 5 ma/tube for the remainder of the run. Densitometric scans of the gels, stained with Amido Black, were performed in a Chromoscan.[4]

Hexose analysis was performed by the orcinol method as described by Winzler (1955) using a galactose–mannose (1:1) standard. Estimation of the hexosamine content was made by the Elson–Morgan technique as described by Winzler (1955); a glucosamine standard was employed. Sialic acid content was estimated by the thiobarbituric acid method (Warren, 1959) and calculated as *N*-acetylneuraminic acid.

Studies of the rate of release of peptide nitrogen from fibrinogen by thrombin were undertaken employing a modification of the procedure described by Lorand (1952). To a series of tubes containing 9.6 mg of fibrinogen in 1.0 ml of 0.225 M NaCl and 0.025 M sodium phosphate (pH 7.5) solution was added 0.10 ml of a thrombin solution (approximately 0.2–1.0 NIH unit/ml) in 0.1 M sodium phosphate (pH 7.5) buffer; the thrombin solution was prepared fresh in nonwettable containers immediately before the experiment from a stock solution of approximately 100 NIH units/ml in 50% glycerol which had been stored at −20°. The thrombin was Parke Davis bovine thrombin which had been further purified by the procedure of Rasmussen (1955). At approximately timed intervals, 1.1 ml of 2% (w/v) MCA solution was added to stop the reaction and to dissolve any clot which had formed. To this was added 0.80 ml of 20% (w/v) TCA solution. The precipitated protein was centrifuged at 3000 rpm and the supernatant solution was filtered through well-packed glass wool to remove any particulate matter. The clear filtrate was analyzed for its nitrogen content in the micro-Kjeldahl distillation apparatus described by Jenden and Taylor (1953).

Fibrin monomer solutions were prepared by a modification (Latallo *et al.*, 1962) of the method described by Donnelly *et al.* (1955). Protein concentration of fibrin monomer solutions was determined from absorbancy readings at 282 mμ in alkaline urea solution (5 M urea–0.1 N NaOH). Studies of the fibrin polymerization process were carried out by adding 0.1 ml of fibrin monomer solution in 1 M NaBr (pH 5.3) to a ten- to twentyfold excess of 0.10 M sodium phosphate buffer (pH 6.0) (Latallo *et al.*, 1962), and followed by periodic readings at 350 mμ.

An estimate of the plasminogen content of various

[1] Abbreviations used: DNFB, dinitrofluorobenzene; MCA, monochloroacetic acid; TCA, trichloroacetic acid; UK, urokinase; ε-ACA, ε-aminocaproic acid; SK, streptokinase; CTA, committee on thrombolytic agents.

[2] Available from Brinkman Instruments, Westbury, Long Island, N. Y.

[3] Apparatus and reagents purchased from Canalco Corp., Bethesda, Md.

[4] Available from the National Instrument Laboratories, Rockville, Md.

fibrinogen preparations was made in the fibrinogenolytic assay system described previously by Mosesson and Finlayson (1963a). UK[5] at a final concentration of 50 CTA units/ml was used to activate any plasminogen present. For studies of the sensitivity of fibrinogen to plasmin, SK-activated plasmin prepared as previously described (Fletcher *et al.*, 1966) was substituted for UK. The final concentration of plasmin in the assay system was approximately 0.025 casein[6] unit/ml.

Assay for the presence of fibrin-stabilizing factor activity was performed in the presence of cysteine by the method of Loewy *et al.* (1961). Fibrin-stabilizing factor was prepared from fraction I according to the method of Loewy *et al.* (1961). Fibrin-stabilizing factor free fibrinogen was prepared by the chromatographic procedure described by Mosesson and Finlayson (1963b).

Studies of the rate and degree of denaturation of fibrinogen solutions (1 mg/ml in 0.3 M NaCl–0.01 M sodium phosphate (pH 6.4) solution) at 56° were performed by incubating a series of tubes containing equal volumes of fibrinogen solution in a 56° water bath; at timed intervals an aliquot solution was removed and rapidly cooled in an ice bath. The insoluble material which had formed was centrifuged at 3000 rpm and the clear supernatant solution was read at 280 mμ in the spectrophotometer. Results were expressed as per cent total absorbancy remaining in solution.

Results

Ultracentrifugal and Related Studies of Fractions I-8 and I-4. Upon analytical ultracentrifugation both fibrinogen fractions I-8 and I-4 sedimented as single peaks throughout the run. The $s^0_{20,w}$ of I-8 was slightly less (7.52 S) than that of I-4 (7.85 S) although the concentration dependence of both was the same ($-1.3c$) (Figure 1). The diffusion coefficient ($D_{20,w}$) determined in the ultracentrifuge was 2.54×10^{-7} cm²/sec for I-8 and 2.18×10^{-7} cm²/sec for I-4.[7] The frictional ratio (f/f_0) computed from these data was 1.99 for I-8 and 2.17 for I-4 and indicated that I-8 was less asymmetrical than I-4; the molecular weights calculated from these data by the Svedberg equation were 261,000 for I-8 and 318,000 for I-4. The molecular weights determined by equilibrium ultracentrifugation were in close agreement and were 276,000 for I-8 and 333,000 for I-4. For later computations, the molecular weights of I-8 and I-4, respectively, were taken as the average of these values, namely, 269,000 and 325,000.

Acrylamide Gel Electrophoresis. Electrophoresis of fractions I-8 and I-4 in acrylamide gels of 5–12% concentration demonstrated distinct differences in the behavior of these fractions (Figure 2). At any given gel concentration the over-all mobility of I-8 was greater than that of I-4. Since previous electrophoretic studies (Mosesson and Sherry, 1966) on cellulose acetate strips or in agar gel had demonstrated no differences in mobility of these fractions, the differences demonstrable by electrophoresis of I-8 and I-4 in this medium appeared to be related to differences in size and/or shape rather than charge.

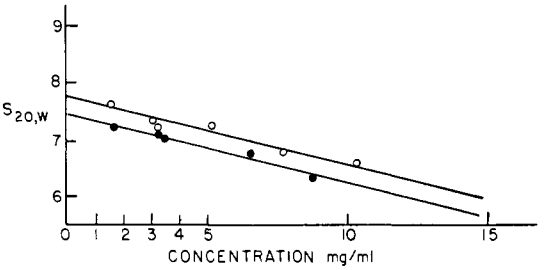

FIGURE 1: $s_{20,w}$ *vs.* concentration of fractions I-8 and I-4. Ultracentrifugation was carried out in 0.29 M NaCl–0.005 M (pH 6.4) sodium phosphate buffer. Fraction I-4 = 0; I-8 = ●.

Of additional interest was the observation that both fractions I-8 and I-4 separated into two major more or less distinct bands most apparent in the densitometric scan pattern (Figure 2). As estimated by densitometry, the faster band (*i.e.*, more anodol) represented 15–25% of the total. Although no firm conclusions regarding the nature of this phenomenon can be drawn, the distribution of these bands suggested that the phenomenon was related to the known heterogeneity of human fibrinogen when chromatographed on DEAE-cellulose (Finlayson and Mosesson, 1963)[8] (Figure 3).

DEAE Chromatography. Gradient chromatographic patterns of three preparations each of I-8 and I-4 prepared from different plasma pools were similar and revealed the two major chromatographic types of human fibrinogen discernible by this technique (Figure 3). The first chromatographic type in the I-8 preparations examined appeared to peak slightly earlier (two to five tubes) than the corresponding peak in the I-4 preparation. The appearance of the second chromatographic type was in approximately the same position for both types of fibrinogen fractions. There were

[5] Abbott Laboratories, North Chicago, Ill.

[6] This casein unit is approximately equivalent to 0.66 original Remmert and Cohen unit.

[7] $D_{20,w}$ determined by the immunochemical method of Allison and Humphrey (1960) was in good agreement, being 2.40 and 1.94×10^{-7} cm²/sec for a single preparation each of I-8 and I-4, respectively.

[8] Preliminary studies to explore this possibility were undertaken by comparing the pattern of human fraction I-4 in 7–10% gels with that of the major chromatographic fraction (*i.e.*, peak 1) obtained from DEAE chromatography (Figure 3). Electrophoresis of chromatographic peak 1 fibrinogen resulted in a single peak corresponding in position to the major band (*i.e.*, least anodal mobility) of unchromatographed I-4. The peak 1 band was somewhat more diffuse than the corresponding band of I-4. It is of interest that purified bovine fibrinogen yielded two bands upon acrylamide electrophoresis (B. Sweet, unpublished results) but only a single peak upon DEAE chromatography (Finlayson and Mosesson, 1964); thus, further studies regarding the exact nature of these electrophoretic bands are indicated.

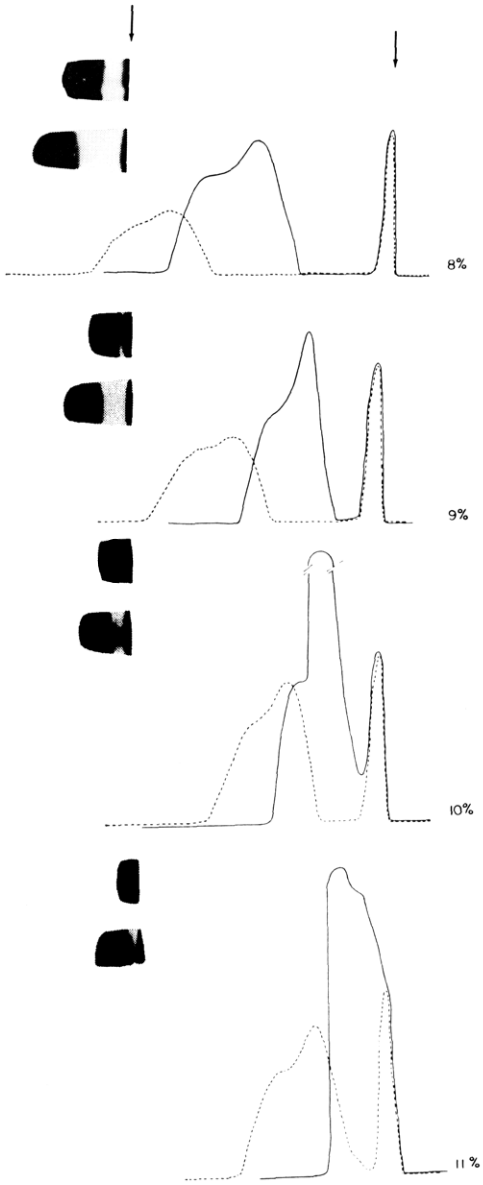

FIGURE 2: Acrylamide gel electrophoresis of fibrinogen subfractions at 8–11% gel concentration. Separator gel concentration is indicated in each instance. The densitometric tracing of the I-4 gel is indicated by the solid line whereas I-8 is indicated by the dashed line. Photographs of the gels from which the tracings were made are in the left portion of the figure (upper gel, I-4; lower gel, I-8). The vertical arrow marks the junction of the stacking (4%) and separating gel.

marginal differences in the distribution of the peaks; the average distribution of absorbancy between peaks 1 and 2 for the three I-4 fractions examined was 75% peak 1 (range 72–78) and 25% peak 2 (range 22–28) in close agreement with previously published data (Finlayson and Mosesson, 1963). For the three I-8 preparations examined, peak 1 amounted to 82% (range 81–84) and peak 2, 18% (range 16–19). No corrections for peak overlapping were applied to these calculations. The average chromatographic recovery was only slightly higher for I-8 than it was for I-4 (80 and 75%, respectively).

Ultraviolet Absorbance Studies. The mean absorbancy coefficients ($A^{1\%}_{1\,cm}$) in alkaline urea (5 M urea–0.1 N NaOH) solution at 282 mμ for five preparations of I-8 and four preparations of I-4 were 16.8 ± 0.5 and 16.7 ± 0.2, respectively. These differences were not significant.

The ultraviolet spectra of two preparations each of I-8 and I-4 in alkaline urea were compared between 254 and 330 mμ (Figure 4) and revealed the typical tryptophan (absorption maximum 282–283 mμ) and tyrosine:tryptophan peaks (absorption maximum 290 mμ) for each. No apparent differences were observed in the spectral curves; the tyrosine:tryptophan ratios calculated by the method of Bencze and Schmid (1957) were reasonably similar for both preparations and the average ratio for two I-8 fractions was 1.37:1 whereas that for two preparations of I-4 was 1.32:1.

N-Terminal Amino Acid Analysis. Five preparations of I-4 and I-8 were analyzed for their content of aminos terminal residues (Table I). Hydrolysis of DNP protein-

TABLE I: N-Terminal Amino Acid Content of Fractions I-4 and I-8.[a]

	Residues/Mole[b] Mean (range)	
Amino Acid	I-4	I-8
Alanine	1.3 (0.8–2.0)	1.1 (0.5–1.6)
Tyrosine	1.3 (0.9–2.1)	1.0 (0.5–1.5)
Aspartic	0.4 (0.1–0.8)	0.4 (0.1–0.9)

[a] Five preparations each of I-4 and I-8 were analyzed. [b] Based on a molecular weight of 269,000 for I-8 and 325,000 for I-4.

was carried out for 14–16 hr at 105°. The major N-terminal amino acids found for I-4, consistent with the findings of others (Blombäck and Yamashina, 1958; Von Korff *et al.*, 1963; Blombäck *et al.*, 1966), were alanine and tyrosine, present to the extent of one to two residues per mole. The same was true of I-8. Smaller amounts of aspartic acid were consistently found and amounted to 0.4 residue/mole of fibrinogen for both subfractions. Trace amounts of other amino acids were found.

Carbohydrate Analysis. An analysis of the carbohydrate content of several preparations of I-8 and I-4 indicated that I-8 contained a higher amount of sialic acid, hexose, and hexosamine per gram of protein (Table II). The differences in the sialic acid and hexose content were highly significant ($p < 0.001$), whereas that in the hexosamine content was significant only

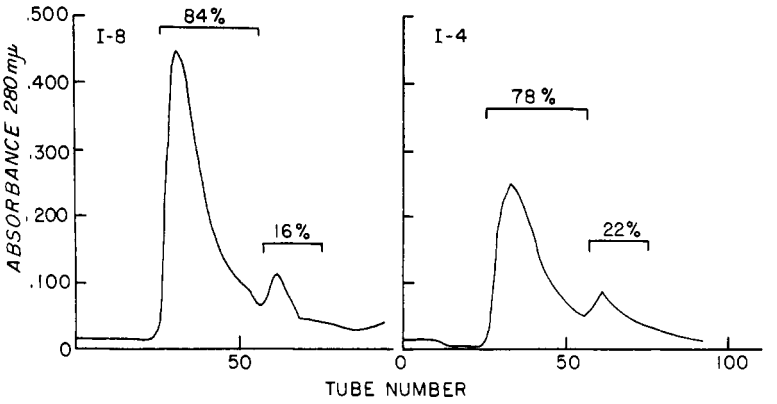

FIGURE 3: DEAE-cellulose gradient elution chromatography showing the elution profile of 22 mg of fraction I-8 (left) and 14 mg of I-4 (right) prepared from the same plasma. The distribution (per cent total) of the absorbancy of peaks 1 and 2 for these columns is indicated.

TABLE II: Carbohydrate Content of Fractions I-4 and I-8.

Carbohydrate Determination	I-4			I-8		
	n^a	%	Residues/Mole[b]	n^a	%	Residues/Mole[b]
Sialic acid	11	0.61 ± 0.03	6.4 ± 0.3	9	0.70 ± 0.05	6.1 ± 0.4
Hexose	6	1.30 ± 0.2	23.0 ± 3.0	5	1.80 ± 0.3	27.0 ± 4.0
Hexosamine	7	1.10 ± 0.2	20.0 ± 3.0	6	1.30 ± 0.1	20.0 ± 2.0
Total carbohydrate		3.0			3.8	

[a] Number of preparations analyzed. [b] Based on a molecular weight of 269,000 for I-8 and 325,000 for I-4.

at the 10% level. When these values were computed on a molar basis, there were no differences in the molar content of sialic acid or hexosamine; the hexose content of I-8 was computed to be 27 residues/mole and that of I-4 to be 23 residues/mole. The variance of the results suggested these differences might not be significant.

Studies of the Fibrinogen–Fibrin Conversion. The thrombin time of fraction I-8 is longer than that of I-4 or other fibrinogen fractions of lower solubility (Mosesson and Sherry, 1966). In order to determine the nature of this prolongation, studies of the release of peptide nitrogen and the polymerization of isolated preparations of fibrin monomer were undertaken. Preliminary studies of two preparations of I-4 and one of I-8 indicated that the rate of release of TCA-soluble nitrogen from I-8 after the addition of thrombin was at least as fast as that from I-4, and that the ultimate amount of TCA-soluble nitrogen (per cent total nitrogen) released from I-8 was higher than from I-4. Under the conditions of these experiments, in which visible clot formation occurred within 1–2 min in the case of fraction I-4, the release of nitrogen was too rapid for precise study of the early reaction rate. Reduction of the thrombin concentration, to approximately one-fifth, permitted an investigation of the early release of TCA-soluble nitrogen (Figure 5). These latter studies demonstrated that TCA-soluble nitrogen was released even faster from I-8 than from I-4, in the period preceding the formation of visible clot. When the respective molecular weights of each fraction were taken into consideration and the data were plotted as micrograms of nitrogen per micromole of fibrinogen, a single line could be drawn through all the points indicating that within the time period studied, the same amount of peptide material was released by thrombin from either fibrinogen species.

On the other hand, studies of the polymerization of fibrin monomer solutions (Figure 6) clearly indicated that there was a delay in the onset of polymerization of I-8 monomer as compared with I-4, a phenomenon readily demonstrable at several concentrations of monomer. Mixtures of I-8 and I-4 monomer yielded curves intermediate between those for I-8 and I-4. From these studies it could be concluded that the delayed clot formation of I-8 fibrinogen was related primarily, if not solely, to the polymerization phase of the thrombin-catalyzed fibrinogen–fibrin conversion.

Heat Stability, Plasminogen, Fibrin-Stabilizing Factor Content, and Related Studies. The rate and degree of

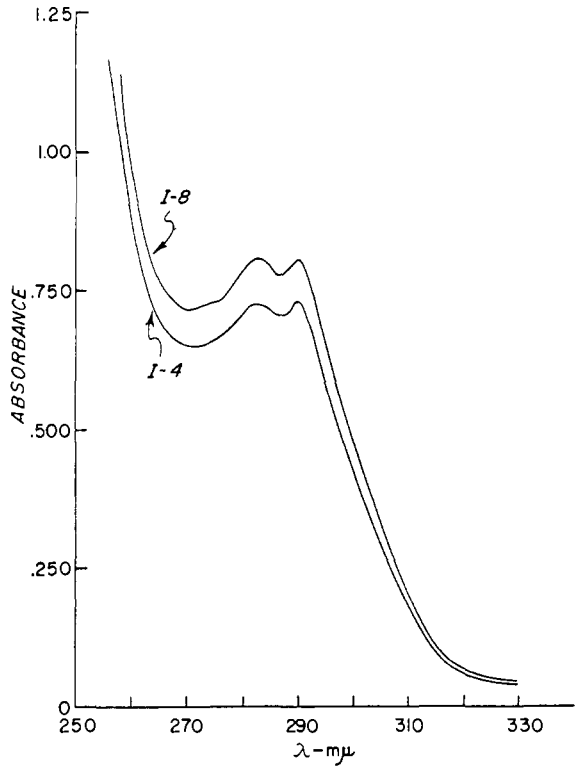

FIGURE 4: Ultraviolet absorption spectra of a single preparation each of I-4 and I-8 in 5 M urea–0.1 N NaOH. The concentration of I-4 was approximately 0.44 mg/ml whereas I-8 was approximately 0.48 mg/ml.

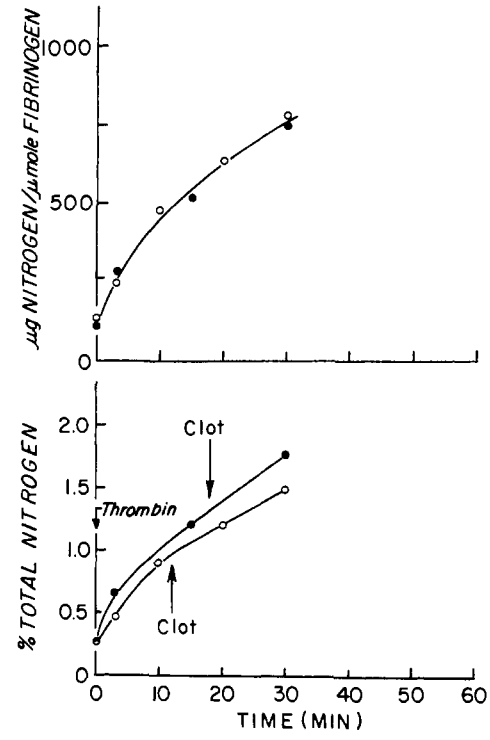

FIGURE 5: The release of TCA-soluble nitrogen from fibrinogen after the addition of thrombin. Fraction I-4 = 0; I-8 = ●. In the lower portion of the figure the per cent total TCA-soluble nitrogen is plotted against time. The first appearance of visible clot for each particular preparation is indicated by vertical arrows. In the upper portion of the figure, the same data are plotted as micrograms of nitrogen per micromole of fibrinogen. Molecular weight of I-8 and I-4 are assumed to be 269,000 and 325,000, respectively.

denaturation of a single preparation each of I-8 and I-4 at 56° were studied (Figure 7). Both fibrinogen subfractions were heat precipitable, and the rate at which this reaction took place was approximately the same for each; the data suggested, however, a slightly delayed onset of denaturation for I-8 compared with I-4. Both I-8 and I-4 fibrinogen were stable with respect to clottability when incubated for 20 hr at 37°.

Observations on the loss of clottability of two preparations of I-8 after the addition of UK suggested that the plasminogen content was lower than that of I-4. In one of the I-8 preparations which was 96.5% clottable before the addition of UK, 94% of the fibrinogen was still clottable after 20 hr at 37°. In the other I-8 preparation studied, 76% of the fibrinogen was clottable (original clottability 96%) after 20 hr at 37° in the presence of UK. In contrast, fraction I-4 was rendered nonclottable in less than 5 hr under the same conditions. The possibility that the relatively slow lysis rate of the I-8 fractions was due to the presence of a plasmin inhibitory activity, such as has been described in certain fibrinogen fractions (Mosesson and Finlayson, 1963a), was excluded by demonstrating that the rate of loss of clottability was approximately the same for I-4 and I-8 after the addition of plasmin. Furthermore, the possibility that inhibition of UK activation of plasminogen in I-8 could account for the observed lysis rates was reasonably excluded by the following experiment. Fraction I-4 was mixed with plasminogen-free fibrinogen (1:1), and I-8 was mixed with I-4 (1:1). UK was added and the rate of loss of clottability was determined. Since there were no significant differences in the lysis rates, it was concluded that the plasminogen content of I-8 was indeed lower than that of I-4.

"Stabilization" of I-4 preparations in the presence of calcium ions, cysteine, and thrombin occurred within 1-hr incubation at 37°, whereas I-8 did not form acetic acid insoluble clots for at least 2 hr under the same conditions. This was not due to an inability of I-8 to be "stabilized;" fibrin-stabilizing factor added to I-8 resulted (when activated) in acetic acid stable clots within the same period of time as fibrin-stabilizing factor added, in similar amounts, to fibrin-stabilizing factor free fibrinogen prepared from fraction I-4.

Discussion

Estimates of the molecular weight of human fibrino-

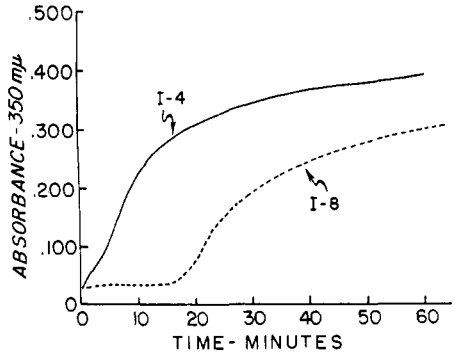

FIGURE 6: Polymerization of fibrin monomer solutions (1 M NaBr, pH 5.3) of I-8 and I-4 diluted with 0.1 M sodium phosphate (pH 6.0). One part of monomer (each 5.3 mg/ml) was mixed with ten parts of phosphate buffer (final concentration = 0.48 mg/ml). Absorbance at 350 mμ (ordinate) is plotted against time (abscissa).

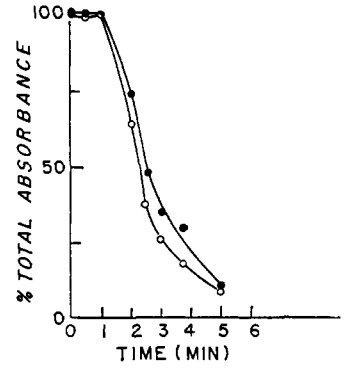

FIGURE 7: Denaturation of fibrinogen fractions I-8 and I-4 at 56°. Per cent of total absorbance remaining in the supernatant (ordinate) is plotted against time after being placed in the water bath. The experiment was terminated after 5 min. Fraction I-4 = 0; I-8 = ●. Initial protein concentration was 1 mg/ml.

gen have ranged from 269,000 (Sowinski *et al.*, 1960) to 700,000 (Holmberg, 1944). In a review of the subject, Edsall (1954) concluded that the most probable molecular weight value of human fibrinogen lay between 325,000 and 340,000. Though differences in molecular weight estimates may be ascribed in part to methodology, the characteristics of the particular preparation of fibrinogen examined must also be considered. For example, the molecular weight estimated in this study for fraction I-4 (325,000) was in agreement with the value proposed by Edsall and with the widely accepted value (341,000) reported by Caspary and Kekwick (1957) who studied fibrinogen prepared in a manner similar to that of fraction I-4. On the other hand, the molecular weight value obtained for fraction I-8 (269,000) agrees very well with the values published by Sowinski *et al.* (1960). Interestingly, the purification method used by these authors included extensive dialysis of fibrinogen against water, a procedure by which only 7–11% of the starting material was recovered in the soluble fraction; such conditions would certainly favor the solution of higher solubility fibrinogen over that of lower solubility. Although the purity of their material was low by standards of clottability, and different methodology was used to arrive at the molecular weight estimates, it seems reasonable to conclude that they characterized a fibrinogen very similar in solubility to I-8.

The stable heterogeneity of human fibrinogen on DEAE-cellulose is well established and appears to be related to differences in net charge of the various chromatographic types, reflected as well by slight differences in electrophoretic mobility of isolated chromatographic fractions on paper (Finlayson and Mosesson, 1963). The present studies have extended these observations to include human fibrinogen(s) of higher solubility (fraction I-8) which demonstrated only minor differences in chromatographic behavior as compared to that previously described for fraction I-4 (Figure 3). These data are consistent with the view that the net charge on corresponding chromatographic types from I-4 or I-8 are very nearly the same, a conclusion supported by previous electrophoretic studies of unchromatographed fibrinogen fractions on cellulose acetate strips or in agar gel (Mosesson and Sherry, 1966).

On the other hand, electrophoretic studies of fractions I-8 and I-4 in acrylamide gel demonstrated that the over-all mobility of I-8 was greater than that of I-4 at gel concentrations of 5–12% (Figure 2). The observed differences between fractions I-8 and I-4 appeared to be related to the molecular sieving properties of the acrylamide gels and reflected the differences in molecular weight and/or symmetry between I-8 and I-4.

In addition, the fibrinogen band of I-8 or I-4 could be separated into two more or less distinct bands (Figure 2). The distribution of these bands estimated by densitometry, and preliminary studies of the electrophoretic behavior of chromatographic peak 1 fibrinogen from fraction I-4, suggested that this phenomenon was related to the previously demonstrated heterogeneity on DEAE-cellulose chromatography (*vide supra*).

The nature of the relative prolongation of the thrombin time of fraction I-8 was investigated by studying the release of peptide nitrogen after the addition of thrombin (Figure 5). This release occurred at the same rate and to the same extent on a molar basis during the period preceding the formation of visible clot for both fractions, indicating that the enzymatic phase of the fibrinogen–fibrin conversion was the same for both I-8 and I-4 over the period of observation. Since the A peptide is released first from human fibrinogen (Abildgaard, 1965), it may be assumed that fractions I-8 and I-4 contain the same amounts of this peptide, an assumption further supported by the N-terminal amino acid analysis of these fractions

(Table I). On the other hand, the study of the polymerization phase of the fibrinogen–fibrin conversion (Figure 6) provided evidence that the polymerization of fibrin derived from I-8 was slower than that of I-4 and enabled us to conclude that it was this phase of clot formation which accounted for the previously observed prolongation of the thrombin time (Mosesson and Sherry, 1966).

The plasminogen content of fraction I-8 was lower than that of I-4; this result was not surprising in view of the observations of others (Kekwick *et al.*, 1955) that the plasminogen present in human fibrinogen tended to precipitate with the less soluble fractions during the course of purification unless specific solubilizing agents such as lysine or ε-ACA were present (Mosesson, 1962). For similar reasons, one could have anticipated that the fibrin-stabilizing factor level of I-8 would tend to be lower than that of I-4. In fact, Loewy *et al.* (1961) used this phenomenon to prepare fibrinogen with a very low fibrin-stabilizing factor content.

It is well established that fibrinogen is a glycoprotein containing hexose, hexosamine, and sialic acid. Previous estimates of the hexose content of human fibrinogen have ranged from 1.0 to 4.6% (Cohn *et al.*, 1946; Seibert *et al.*, 1948; Consden, 1953; Szára and Bagdy, 1953; Brown, 1963); estimates of the hexosamine content lie between 0.5 and 1.1% (Szára and Bagdy, 1953; Consden, 1953; Brown, 1963) and between 0.6 and 1.0% for the sialic acid content (Böhm and Baumeister, 1955; Mosesson and Finlayson, 1963b; Brown, 1963). In the present studies estimates of the content of these particular carbohydrates in fraction I-4 fell within the reported range, for any given carbohydrate. Fraction I-8 had a higher content of hexose and sialic acid and probably hexosamine than fraction I-4; however, these differences disappeared (with the possible exception of the hexose content) when the molecular weight of the particular fraction was taken into consideration (Table II). Similarly, within the limitations of the DNP method for accurate quantitation of N-terminal amino acids, it was estimated that the same amounts of alanine, tyrosine, and aspartic acid were present in both I-4 and I-8 (Table I).

It is attractive to consider that fibrinogen of lower molecular weight represents a species derived from higher molecular weight fibrinogen by selective cleavage of peptide material from the parent material, resulting in a fibrinogen with somewhat altered biophysical and biological properties but still capable of polymerizing to form a fibrin clot. There is some circumstantial evidence to support this possibility, particularly with regard to degradation of fibrinogen by the enzyme plasmin. Recent detailed studies of plasmin degradation of fibrinogen (Fletcher *et al.*, 1966) demonstrated that early in the plasmin degradation, before clottability was lost, there was a reduction in the molecular weight of fibrinogen from 320,000 to 265,000 concomitant with the release of low molecular weight material. Furthermore, the reduction in the molecular weight was associated with a significant increase in $D_{20,w}$ and no detectable change in the $s_{20,w}$ of the digestion mixture. From these standpoints, *i.e.*, the molecular weight, $s^0_{20,w}$, and $D_{20,w}$, fraction I-8 was very similar to the early plasmin breakdown product(s) described by these investigators. It has also been shown that fibrinogen having the same solubility characteristics as I-8, and still clottable, was produced early in the degradation of fraction I-4 by plasmin (Mosesson and Sherry, 1966) and that the addition of UK or SK to citrated bank plasma resulted in an absolute increase in the clottable fibrinogen fraction which was soluble in 8% ethanol (*i.e.*, super fraction I) (M. W. Mosesson, unpublished data). Other observations relating to the alteration of biologic reactivity to thrombin early in the degradation of fibrinogen by plasmin (Latallo *et al.*, 1964) are consistent with the possibility that an early clottable fibrinogen derivative may contribute significantly to this phenomenon. Nevertheless, our present data do not provide sufficient information to permit any firm conclusions with regard to the relationship between the early plasmin derivatives of fibrinogen and naturally occurring fraction I-8 fibrinogen, particularly since there are other possibilities which could account for the characteristics of this fibrinogen. However, as a working hypothesis, the above concept should prove useful for the interpretation of available data and for the design of future experiments.

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The Nomenclature of Lipids*

Preface

The nomenclature of lipids is the concern both of organic chemists and of biochemists. The systematic names of individual lipids can always be derived by the general rules of organic nomenclature; however, such names are often complex and need to be supplemented by alternative "semisystematic" names (as has been done, *e.g.*, for steroids and corrinoids). Another problem is that of names for groups of related and homologous compounds (including mixtures); such names are hardly ever needed by the pure organic chemist, but are very necessary in biochemical work.

Several attempts have been made in the past to standardize nomenclature in the lipid field, notably by the United States NAS–NRC Subcommittee on the Nomenclature of Biochemistry under the Chairmanship of W. E. M. Lands (Ann Arbor, Mich.) in 1962. At about the same time, proposals were made for names for groups of lipids by a German group (see *Biochem. Z. 335*, 423 (1962)).

The Biological Nomenclature Commission of IUPAC and the Commission of Editors of Biochemical Journals of IUB decided, in 1963, to set up an international Subcommittee on Lipid Nomenclature under the Chairmanship of H. Hirschmann (Cleveland, Ohio); this group discussed and, with the advice of interested colleagues, modified some of the material embodied in the two earlier proposals. The IUPAC–IUB Subcommittee, which later became responsible to the Combined Commission on Biochemical Nomenclature of IUPAC and IUB (CBN), when this was formed in Jan 1964, has consisted of the following: H. Hirschmann (Chairman, U. S. A.), A. Gottschalk (Australia), F. D. Gunstone (U. K.), M. L. Karnovsky (U. S. A.), E. Klenk (Germany), W. E. M. Lands (U. S. A.), J. Polonovski (France), and L. L. M. Van Deenen (The Netherlands). Their discussions were carried out largely by correspondence and resulted in draft proposals that were considered by CBN at its meetings in Paris (1965) and in Gothenburg (1966) and by correspondence between the meetings. The present proposals are the

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Comments on these proposals may be sent to any member of CBN: O. Hoffmann-Ostenhof (Chairman), W. E. Cohn (Secretary), A. E. Braunstein, J. S. Fruton, B. Keil, W. Klyne, C. Liébecq, B. Malmström, R. Schwyzer, E. C. Slater, or corresponding member, N. Tamiya.

Reprints of these proposals may be obtained from W. E. Cohn, Director, NAS–NRC Office of Biochemical Nomenclature, Oak Ridge National Laboratory, Oak Ridge, Tenn. 37830.