Immunoochemical Characterization of Fibrinogen, Fibrin I, and Fibrin II in Human Thrombi and Atherosclerotic Lesions

By Alessandra Bini, John Fenoglio Jr., Joan Sobel, John Owen, Marcela Fejgl, and Karen L. Kaplan

Arterial thrombi and atherosclerotic lesions were analyzed immunochromatically and examined histologically. The extent of in vivo proteolytic cleavage of the amino-terminal end of fibrinogen by thrombin and plasmin was determined and quantitated by specific radioimmunoassays. The samples were treated with cyanogen bromide (CNBr), and the total amount of fibrinogen and fibrin-derived protein was determined as NDSK, the NH$_2$-terminal disulfide knot of fibrinogen. Thrombin-releasable fibrinopeptides A and B were used to quantitate fibrinogen and fibrin I. Previous plasmin cleavage of the B142-43 chain was inferred from the amount of B142-43 and B15-42 in undigested NDSK. The results obtained in both acute and organized thrombi indicate that ~60% of the total protein (as determined by amino acid analysis) was fibrinogen-derived and that 70% to 80% of the fibrinogen-derived material was fibrin II. These findings support the hypothesis that fibrin II as distinct from fibrin I is the predominant component in a thrombus. In samples from normal and atherosclerotic aortas, fibrinogen-derived protein comprised <10% of the total protein. Samples from grossly normal aortas contained only fibrinogen and fibrin I. Fibrinogen concentration decreased and fibrin II concentration increased with increasing severity of the lesions, suggesting that increased fibrin II formation is associated with progression of atherosclerosis.

Several in vitro studies have defined the biochemical structure of fibrin in vitro. Little information is available on the composition of human thrombi and atherosclerotic plaques with respect to the content of fibrinogen and resulting fibrin products.

Previous studies on thrombi recovered from patients have been devoted to the carboxy-terminal end of the fibrinogen molecule and have shown fibrin with a high degree of $\alpha$ and $\gamma$ chain crosslinking. Recently, Francis and colleagues demonstrated that the degree of fibrin degradation in thrombi increases progressively with the age of the thrombus and it is more prominent in the deeper layers of chronic thrombi.

The association between thrombus formation and atherosclerotic lesions has long been recognized. The presence of fibrinogen and fibrin-related antigens in normal aortas and atherosclerotic plaques has been shown by immunofluorescence and electron microscopy by several investigators. Smith and colleagues documented that fibrinogen and fibrin in soft gelatinous human aortic intimal thickenings and cholesterol-rich advanced plaques were, respectively, four and ten times more concentrated than in normal vessels.

Shainoff and Page reported that atherosclerotic plaques contained crosslinked fibrinogen, based on their estimates of fibrinopeptide content and on insolubility in 5 mol/L of urea. In normal aortas and in aortas with atherosclerotic lesions, York and Benjamin found a correlation between the content of fragment D antigen and the severity of the atherosclerotic lesion. No correlation was found with the fibrinogen content of plaques.

Nossel postulated that thrombi should consist mainly of fibrin II, and, in a study of postcraniotomy venous thrombosis, the patients who developed venous thrombosis showed evidence of an imbalance of thrombin and plasmin action that would favor fibrin II formation.

We therefore studied the biochemical composition of the amino-terminal ends of fibrinogen and fibrin in thrombi and atherosclerotic lesions with respect to proteolysis by thrombin and plasmin. Using specific radioimmunoassays, we determined the relative amounts of fibrinogen, fibrin I, fibrin II, and fragment X, and compared the data obtained on thrombi with results in normal and atherosclerotic arterial intima.

**MATERIALS AND METHODS**

**Preparation of fibrin in vitro.** Platelet-poor plasma was prepared from blood collected into 1/10 vol of an anticoagulant solution consisting of 3.8% trisodium citrate, 100 KIU/mL of aprotinin (TrasyloI, Mobay Chemical, FBA, New York), 10 mg/mL soybean trypsin inhibitor ( Worthington Biochemical, Freehold, NJ), and without 80 U/mL of heparin (Heparin sodium, Hyson Westcott and Dunning, Baltimore), and separated by centrifugation for 20 minutes at 1,700 g at 4°C in a Sorvall RC-2 centrifuge (Newton, CN).

*Definition of terms: fibrin I, formed from fibrinogen by cleavage of Aa16-17; fibrin II, formed from fibrinogen by cleavage of both Aa16-17 and Bb14-15; NDSK, amino terminal CNBr fragment of fibrinogen; fragment X, formed by plasmin cleavage of the Bb42-43 bond and $\alpha$-chain bonds of fibrinogen, fibrin I, or fibrin II (includes fragments Y and Z as calculated in this study).
Fibrinogen concentration was measured in citrated plasma as described by Ellis and Stransky23 and by the clot weight method.24 Plasma obtained from the heparin-containing anticoagulant mixture was clotted overnight at 37°C with batroxobin 1 mg/mL of plasma (Reptilase-R, Abbott Laboratories, North Chicago). Crosslinked fibrin was prepared by adding 1.0 National Institutes of Health (NIH) U of human α-thrombin (3.7 × 10^7 NIH U/mg) (prepared by Dr J. Fenton and kindly given to us by Dr G. Murano, Bethesda, MD) and 10 μL of a 2.5 mol/L of calcium chloride solution per milliliter of citrated plasma (final concentration 0.025 mol/L of CaCl_2), followed by overnight incubation at 37°C. The clots were removed by winding them on a wooden stick; they were rinsed three times for 15 minutes in distilled water, cut longitudinally with a scalpel, stirred for 1 hour in 70% vol/vol of formic acid, and incubated with 300-fold molar excess cyanogen bromide (CNBr),23 for 20 to 24 hours with continuous stirring. The clots were then divided into grossly homogenous parts, and areas of atherosclerotic aortas were collected with paired normal adjacent areas. Each specimen was individually placed in preweighed siliconized glass vials and frozen and stored at −70°C until processed. Prior to freezing, a portion of each sample was cut and fixed in 10% neutral buffered formaldehyde for histologic examination.

The specimens for histology were paraffin embedded, and 8-μm sections were cut from the blocks and stained with hematoxylin-phloxine-safran and Mason's trichrome. The stain allows distinction between cells, nuclei, "fibrin," and collagen. The samples were classified as acute or organized thrombi or fibrous, fatty, or complicated plaques according to standard histological criteria (Table 1).

### Table 1. Clinical Data of Patients From Whom Samples Were Obtained

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Disease</th>
<th>Procedure</th>
<th>Histology of Specimen</th>
</tr>
</thead>
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<td></td>
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<td>Surgical specimens</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>55</td>
<td>M</td>
<td>Atherosclerosis, thrombosis left popliteal artery</td>
<td>Endarterectomy</td>
<td>Acute thrombus</td>
</tr>
<tr>
<td>2</td>
<td>73</td>
<td>M</td>
<td>R superior</td>
<td>Embolectomy</td>
<td>Acute thromboembolus from atheromatous plaque in abdominal aortic aneurysm</td>
</tr>
<tr>
<td>3</td>
<td>84</td>
<td>M</td>
<td>R common iliac artery aneurysm</td>
<td>Repair atheroembolus</td>
<td>A: Complicated atheroembolic plaque</td>
</tr>
<tr>
<td>8</td>
<td>74</td>
<td>M</td>
<td>Abdominal aortic aneurysm</td>
<td>Aneurysmectomy</td>
<td>B: Complicated atheroembolic plaque</td>
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<tr>
<td>9</td>
<td>88</td>
<td>M</td>
<td>Abdominal aortic aneurysm</td>
<td>Aneurysmectomy</td>
<td>A: Organized thrombus</td>
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<tr>
<td>11</td>
<td>93</td>
<td>F</td>
<td>Peripheral vascular disease</td>
<td>Popliteal endarterectomy</td>
<td>B: Acute thrombus</td>
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<tr>
<td>12</td>
<td>52</td>
<td>M</td>
<td>Atherosclerosis, abdominal aneurysm</td>
<td>Repair atheroembolus</td>
<td>C: Organized thrombus</td>
</tr>
<tr>
<td>13</td>
<td>62</td>
<td>M</td>
<td>R carotid stenosis</td>
<td>Endarterectomy</td>
<td>B: Organized thrombus</td>
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<tr>
<td>14</td>
<td>62</td>
<td>M</td>
<td>Thrombosed A-V fistula</td>
<td>Thrombectomy</td>
<td>Complicated atheroembolic plaque</td>
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<tr>
<td>15</td>
<td>75</td>
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<td>Abdominal aortic aneurysm</td>
<td>Aneurysmectomy</td>
<td>Acute thrombus</td>
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<td>16</td>
<td>49</td>
<td>M</td>
<td>Mitral stenosis</td>
<td>Mitral valve commissurotomy</td>
<td>B: Organized thrombus</td>
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<td>18</td>
<td>53</td>
<td>M</td>
<td>Left ventricular aneurism with mural thrombus formation</td>
<td>Aneurysmectomy</td>
<td>A: Acute thrombus</td>
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<td>Autopsy specimens</td>
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<td>4</td>
<td>60</td>
<td>F</td>
<td>Myocardial infarction</td>
<td>A: Fibrous atheroembolic plaque</td>
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<tr>
<td>5</td>
<td>64</td>
<td>F</td>
<td>Myocardial infarction</td>
<td>A: Fatty atheroembolic plaque</td>
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<tr>
<td>6</td>
<td>82</td>
<td>M</td>
<td>Myocardial infarction</td>
<td>A: Normal aorta</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>65</td>
<td>F</td>
<td>Cirrhosis, sepsis</td>
<td>A: Normal aorta</td>
<td></td>
</tr>
</tbody>
</table>

Definitions: Acute thrombus, fibrin clot with discernible lines of Zahn; organizing thrombus, partial replacement of fibrin clot by granulation tissue; organized thrombus, total replacement of fibrin clot by maturing granulation tissue; fibrous plaque, focal intimal accumulation of collagen and fibroblasts; fatty plaque, focal intimal accumulation of "foam cells," collagen, and cholesterol crystals; complicated plaque, focal accumulation of foam cells, collagen, and cholesterol crystals extending into the media and associated with calcification.
Sample processing. The tissue samples were freeze-dried in siliconized glass vials and dry-weighed. They were then homogenized in distilled water (10 to 100 mg sample per milliliter of distilled water). If the dry weight was >100 mg, samples were initially roughly homogenized on a melting ice bath with a Polytron (Brinkmann Instruments, Westbury, NY). All samples were finely homogenized at 4°C in a glass homogenizer (Arthur H. Thomas Scientific Apparatus, Philadelphia), kindly made available by Dr S. DiMauro, Department of Neurology, Columbia University. The homogenate was then diluted 1:5 with 90% formic acid and treated with cyanogen bromide as described above. The amount of CNBr added to the tissue samples was five times the dry weight of the sample. Amino acid analysis performed in a preliminary experiment demonstrated that ~50% of the dry weight was protein. This amount of CNBr corresponds to the 300-fold molar excess used for CNBr digestion of fibrin prepared in vitro.

The precipitates present in two samples were extracted a second time with formic acid and CNBr to determine whether residual NDSK antigen was present. The results are shown in Table 2. NDSK antigen was assayed on the initial extract and on the extract from the repeated CNBr treatment of the precipitate that remained after the first extraction. The recovery of fibrinogen-related material in the first extract was 95% from the intima sample and >99% from the organized thrombus. Therefore, all samples in which a precipitate was present after the first extraction were centrifuged at 12,300 g for 30 minutes, and assays for fibrinogen-related antigens were performed on the supernate.

Radioimmunoassays. NDSK, native fibrinopeptide A (FPA) and fibrinopeptide B (FPB), and Bf I-42 were prepared as previously described. Tyrosinated FPA was from Schwarz-Mann, Becton Dickinson, Orangeburg, NY. Tyrosinated FPB was prepared by G. Wilner. Antiserum to NDSK was lot Q78; to FPB, it was lot R2, R3, R5 to FPB it was lot R28, and to Bf I-42 it was lot R2-3. The Bf I-42 assay kit was purchased from IMCO (MICO USA, New York).

All fragments were labeled by the method of Hunter and Greenwood. Sodium iodide (^131I) (1 to 2 mCi) was obtained from New England Nuclear (Boston). After iodination, NDSK was passed over Sephadex G-10, and the other peptides and fragments were passed over Sephadex G-10 (Pharmacia Fine Chemicals, Piscataway, NJ). NDSK was assayed with a slightly different technique from that previously described. The test was performed in 12 x 75-mm polypropylene tubes (Sarstedt, Princeton, NJ). Both tracer and anti-NDSK antibody were diluted in 0.15 mol/L of Tris-NaCl pH 8.5 containing 1% bovine serum albumin (BSA) and 0.01% NaN3 (wt/vol). After overnight incubation at 4°C, goat anti-rabbit IgG antiserum (lot 0050, Miles Scientific, Naperville, IL) was added at 1/3 dilution in Tris-NaCl containing 1% bovine serum albumin (BSA) and 2% polyethylene glycol (wt/vol) (PEG 8000, J.T. Baker Chemical, Phillipsburg, NJ). The samples were incubated for 2 hours at 4°C, and the bound antigen was separated by centrifugation, as previously described. Radioimmunoassays for FPA, FPB, Bf I-42, and Bf I-42 were performed by our published double-antibody technique, substituting first antibody, tracer, standard, and second antibody as appropriate.

The assay buffer for all of these assays is 0.15 mol/L of Tris-NaCl pH 8.0 containing 1% PEG 8000, heparin (0.7 U/ml), 0.01% Tween 20, and 0.01% Na3. The bound antigen was separated by centrifugation at 1,700 g for 20 minutes, the supernate was aspirated, and the precipitate was counted for 1 minute with an LKB 1270 Rackgamma II, (LKB Instruments, Rockville, MD).

Calculation of fibrinogen-derived material. The radioimmunoassays applied to identify and quantitate the fibrinogen NH2-terminal antigens present in the CNBr-treated samples are shown in Table 3.

The radioimmunoassay (RIA) for NDSK detects fibrinogen and fibrin quantitatively. Experiments were made to characterize the anti-NDSK antiserum used. Comparable immunoreactivity was observed in the following preparations: NDSK, thrombin-treated NDSK, plasmin-treated NDSK, and CNBr-degraded fibrinogen derived from early (5 to 20 minutes), and late (60 minutes and overnight) plasmin digest. Non-CNBr-treated fragment E is 30 times less reactive than the other preparations. The NDSK assay accurately measured all fibrinogen and fibrin-related material in the samples, however, since all samples were treated with CNBr before assay.

The RIA for fibrinopeptide A measures only the free peptide. Thus, the fibrinopeptide B released by thrombin treatment of the samples provides a measure of residual fibrinogen. The FPB RIA similarly measures only the free peptide. Fibrinopeptide B released by thrombin treatment thus gives the sum of the fibrinogen and fibrin I in the samples examined.

In the Bf I-42 RIA, the antibody used requires an intact Bf I-45 peptide bond, and it recognizes the Bf I-42 fragment either as a free peptide or within fibrinogen or NDSK. It is therefore a measure of both fibrinogen and fibrin I, but not of fibrin II or fragment X, which lack FPB or Bf I-42, respectively.

The antibody used in the Bf I-42 RIA crossreacts completely with all the Bf I-42-containing fragments derived from CNBr treatment. Therefore, Bf I-42 provides a measure of fibrinogen, fibrin I, and fibrin II, but not of fragment X.

The total fibrinogen-derived material and the proportions of fibrinogen, fibrin I, fibrin II, and fragment X in samples can be calculated from these data using the formulae shown in Table 4.

The molar amount of NDSK represents fibrinogen plus fibrin I, fibrin II, and fragment X. The percentage of intact fibrinogen is calculated as the difference in FPA immunoreactivity before and after thrombin treatment. Because measurement of either releasable FPB or of Bf I-42 provides an index of fibrin I, the mean of these two

| Table 2. Recovery of Fibrinogen-Related Material by CNBr Extraction |
|------------------|-----------------|-----------------|
| Sample           | NDSK (pmol/mL) |
|                  | First Extraction | Repeat Extraction | Recovery in First Extraction (%) |
| Fibrinous plaque | 50              | 2.5             | 95                               |
| Organized thrombus | 28,180         | 7.0             | >99                              |

NDSK, NH2-terminal disulfide knot of fibrinogen; CNBr, cyanogen bromide.
contains the B(15-42 fragment. It is therefore calculated by subtracting the percentage of B13-5-42 from the B$15-42 immunoreactivity. Total fibrinogen-related material in each sample. Because 1 mol of NDSK contains 2 mol of each of the fibrinopeptides, the amount of NDSK is multiplied by a factor of 2 in the above formulae.

measurements minus the previously calculated value for intact fibrinogen is used to calculate the percentage of fibrin I more accurately than does a measurement based only on fibrinopeptide B release from NDSK. Fibrin II lacks both fibrinopeptides but contains the B15-42 fragment. It is therefore calculated by subtracting the molar amount of B15-14-containing molecules from the total B15-42 immunoreactivity.

Because fragment X arises by cleavage of the B42-43 bond, with loss of B15-42, the percentage of fragment X in the samples can be calculated by subtracting the percentage of B15-42 from the percentage of total NDSK.

**Amino acid analysis.** The analyses were performed with a Beckman automatic amino acid analyzer model 121 MB (Beckman Instruments, Fullerton, CA), following acid hydrolysis under a vacuum (6 N HCl for 24 hours at 110°C). All chemicals were of reagent grade and were purchased from Fisher Scientific, Springfield, NJ.

**Statistical analysis.** The statistical significance of variation of the content of fibrinopeptides in the thrombi and atheromatous plaques was tested by one-way analysis of variance. In the analysis of variance, a variance is computed from the within-group variation, and the statistical significance of the between-group variation is tested by comparing the ratio of the between-group to within-group variations to the appropriate F value in a table. The Bonferroni procedure was used to control the significance level for multiple comparisons, ie, the significance levels .05 and .01 were divided by the number of comparisons, which was three.

**RESULTS**

**Validation of methods.** The amounts of each peptide expected to be present in the batroxobin and thrombin clots formed in vitro are shown in Table 5(A). In the case of the clot formed with thrombin, all FPA and FPB would be expected to be cleaved. For the batroxobin clot, only FPA would be removed and the amino terminus of the Bβ chain should be intact. Because each molecule of fibrinogen contains two Aα and Bβ chains, the expected molar amounts of the amino terminal peptides are double that of NDSK. The recovery of these peptides was >85% in each case, and was >90% for NDSK. When these data were treated as though these samples were unknowns, the calculated compositions given in Table 5(B) were obtained. These results show the expected predominance of fibrin I in the batroxobin clot and fibrin II in the thrombin clot.

**Tissue samples.** The samples examined and the surgical procedures from which they were obtained are shown in Table 1. The samples were classified histologically as acute or organized thrombi; fibrous, fatty, or complicated atheromatous plaques; or "normal" aortas.

Table 6 shows the fibrinopeptide content of the thrombi examined. The fibrinogen-related material determined immunologically as NDSK comprised 50% to 70% of the total protein as measured by amino acid analysis. In both acute and organized thrombi, the FPA content indicates that <2% of the NDSK was fibrinogen. The amount of releasable FPA in both groups was <5% of the NDSK, indicating that >95% of the Bβ1-14 peptide had been cleaved. Because 87% and 79% of the Bβ15-42 was present, the Bβ1-14 was cleaved by thrombin, leading to fibrin II formation, rather than by plasmin at Bβ1-42. No significant differences in the content of Bβ1-42 and Bβ15-42 or of releasable FPA and FPB were found when acute thrombi were compared with organized thrombi, indicating that they were biochemically similar at the NH2-terminus.

Analysis of the aortic samples (Table 7) showed that <10% of the total protein was fibrinogen derived, ie, a 10- to 25-fold lower fibrinogen-related protein content than thrombi. The samples of normal aorta contained amounts of fibrinogen-related protein similar to those in the various types of plaques. In the samples of normal aorta, 79% of the fibrinogen Aα chains were intact. The amount of intact Aα-chain decreased from normal to fatty, from fatty to fibrous, and from fibrous to complicated plaques, suggesting an increase in Aα-chain proteolysis. The FPA content of complicated plaques was significantly less than that of normal aorta (P < .01), fatty plaques (P < .01), and fibrous plaques (P < .01). No significant differences in FPA content existed between normal aortas and either fatty or fibrous plaques or between fatty and fibrous plaques. The amount of

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**Table 4. Summary of Calculations of Percentage of Fibrinogen, Fibrin I, and Fibrin II in NDSK**

<table>
<thead>
<tr>
<th></th>
<th>Fibrinogen = FPA*(2 NDSK) \times 100</th>
<th>Fibrin I = [(FPB + Bβ1-42)/2 - FPA]/2 NDSK \times 100</th>
<th>Fibrin II = (Bβ15-42 - [FPB + Bβ1-42]/2)/2 NDSK \times 100</th>
<th>Fragment X = (2 NDSK/2 NDSK \times 100) - (Bβ15-42/2 NDSK \times 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbreviations</td>
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</table>

**Table 5. Content and Composition of Fibrinogen-Related Material in In Vitro Clots**

<table>
<thead>
<tr>
<th></th>
<th>Content by Radioimmunoassay</th>
<th>Calculated Composition</th>
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<tr>
<td></td>
<td>NDSK</td>
<td>FPA</td>
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<tr>
<td>Batroxobin</td>
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<td></td>
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<tr>
<td>Observed</td>
<td>7.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Expected</td>
<td>7.35</td>
<td>0</td>
</tr>
<tr>
<td>Thrombin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed</td>
<td>6.5</td>
<td>0.13</td>
</tr>
<tr>
<td>Expected</td>
<td>7.35</td>
<td>0</td>
</tr>
</tbody>
</table>

Fibrinogen (Fg), Fibrin I (FbI), Fibrin II (FbII), Fragment X (Frag X), other abbreviations as in Table 2.
intact Bδ-chain in the normal aortas was 91% (mean ± SEM). No significant difference between acute and organized thrombi was found for any of the peptides.

<table>
<thead>
<tr>
<th>Sample</th>
<th>NDSK (%)</th>
<th>Total Protein</th>
<th>FPA</th>
<th>FPB</th>
<th>B(\delta) 1-42</th>
<th>B(\delta) 15-42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute (6)</td>
<td>50 ± 13</td>
<td>1.3 ± 0.3</td>
<td>2.5 ± 0.7</td>
<td>4.4 ± 1.4</td>
<td>87 ± 8</td>
<td></td>
</tr>
<tr>
<td>Organized (6)</td>
<td>68 ± 5</td>
<td>0.6 ± 0.2</td>
<td>1.6 ± 0.4</td>
<td>2.9 ± 0.9</td>
<td>79 ± 11</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Fibrinopeptide Content of Thrombi

Abbreviations as in Table 2.

*Expressed as percentage of total NDSK (mean ± SEM).

DISCUSSION

The purpose of this study was to characterize the insoluble fibrinogen-derived material in thrombi and in atherosclerotic plaques. In vitro studies have shown that when fibrinogen is clotted with thrombin, fibrinopeptides A and B are released, leading to fibrin II formation. A thrombus is believed to contain mainly fibrin II, but this has never been directly demonstrated. To this end, we developed a method to analyze the fibrinogen-derived material in tissues. The peptides and fragments released on thrombin and plasmin proteolysis were quantitated after CNBr degradation. Application of these techniques to clots formed in vitro demonstrated both satisfactory recovery of the fibrinogen-derived material and the retention of immunoreactivity of fibrinopeptides A, B, B\(\delta\)1-42 and B\(\delta\)15-42. Thrombin clots formed in vitro with plasmin inhibitors added consisted mainly of fibrin II, as expected.

The ability to distinguish between fibrin I and fibrin II was confirmed by the data from clots formed in vitro with thrombin or batroxobin (Table 5). In those experiments, the clots contained 6% to 8% fragment X. Because the clots were formed in the presence of aprotinin and soybean trypsin inhibitor, it is unlikely that plasmin was active during that stage. One possible explanation is that this may represent the minor fraction of circulating fibrinogen which has undergone partial degradation.

When these techniques were applied to the examination of pathological human thrombi (Table 6), it was found that the thrombi formed in vivo had undergone proteolysis by thrombin in a manner very similar to that of thrombin clots formed in vitro. Acute and organized thrombi did not differ significantly in fibrinopeptide content. Both contained low amounts of fibrinogen and fibrin I, whereas fibrin II comprised >70% of the fibrinogen-related protein in both groups. Moreover, 50% to 70% of the protein content of the thrombi, measured by amino acid analysis, was fibrinogen-derived in both acute and organized thrombi, confirming that the conversion of fibrinogen to fibrin II is a major factor in thrombus formation.

The polyclonal antiserum to NDSK used in the present study recognizes NDSK derived by CNBr treatment of
Fibrinogen, fibrin, and fragment X, and CNBr-treated fragment E. Therefore, incomplete recovery of Bj15-42 in acute and organized thrombi can be assumed to reflect in vivo plasmin cleavage with fragment X formation in both groups. Fragment E formation in vivo would also be detected if the fragment E were noncovalently associated with the fibrin. Whether fragment X was formed by plasmin proteolysis of fibrin I or fibrin II cannot be distinguished in these studies. The comparable recovery of NDSK in the acute and organized thrombi indicates that plasmin cleavage of the NH2-terminal ends of the fibrin in these thrombi was similar. Francis and colleagues7 reported a higher degree of lysis in older thrombi than in more recent ones. They7 and others5,6 examined lysis of the carboxy-terminal region of fibrin. Although in vitro studies in this laboratory have shown parallel cleavage of the NH2-terminal B$ chains and the COOH-terminal Aα chains of fibrinogen,22 the relationship might be different for fibrin, with or without crosslinking.41 In addition, the assays used in the present study do not allow distinction between fragment X and fragment E noncovalently associated with the thrombus.

Samples of aortic intima (Table 7) were very different from thrombi in their content of fibrinogen and fibrin-derived fragments. In all groups, the amount of fibrinogen-related protein was <10% of the total protein and was far lower than the 50% to 70% of total protein in thrombi.

The relative amounts of fibrinogen, fibrin I, fibrin II, and fragment X in the complicated plaques were not significantly different from those in the acute and organized thrombi, although the NDSK content was substantially lower. Both FPA and FPB were significantly lower in complicated plaques than in normal aortas or fatty or fibrous plaques, indicating that a significantly higher proportion of the NDSK in the complicated plaques as compared with the other aortic samples was derived from fibrin II. In contrast, the NDSK in normal aortas was derived largely from intact fibrinogen, and that in fatty and fibrous plaques appeared to be intermediate in its derivation from fibrinogen and fibrin I v fibrin II. The methods used to obtain the samples and the relatively small numbers of samples with specific diagnoses preclude the drawing of conclusions based on disease states.

The amount of fragment X in the complicated and fibrous plaques was slightly higher than that in the other aortic samples, but the amount of plasmin degradation was not large in any of the histologic types.

Previous studies have reported fibrinogen/fibrin in the aortic intima.13-15,42 Quantitative comparisons between those studies and the present one are difficult because of methodologic differences in extraction and assay and in calculation and expression of results. In the present study, it has been possible to determine the molecular nature of the fibrinogen present. The total fibrinogen-related antigen is similar in normal aortas and complicated plaques, whereas the proportions of fibrinogen and fibrin II vary inversely with progression of the lesions. These results support the assertion that fibrinogen-related protein was present in the acute samples in vivo, since in vitro or postmortem acquisition would be expected to show similar proportions of the different antigens. These results also raise the possibility that thrombin proteolysis of fibrinogen occurs within the intima, not only on the vascular surface with subsequent incorporation of fibrin into the intima. Recent studies have shown that fibrinogen can be crosslinked in vitro both by native and activated factor XIII in the presence of calcium ions.43 and ex vivo studies suggested that the insoluble fibrinogen detected in aortic intima might be crosslinked.14 Such crosslinked fibrinogen might be a good substrate for thrombin. If proteolysis of fibrinogen does occur within the vessel wall, the question of possible effects of the released fibrinopeptides on vascular endothelial and smooth muscle cells arises. In addition, the effects of cells present in normal aortas and in atherosclerotic aortas on the proteolysis of fibrinogen with the vessel wall must be considered.

Relatively little plasmin proteolysis of the NH2-terminal end of the intimal fibrin had occurred. A higher degree of plasmin proteolysis of the COOH-terminal Aα-chains may have occurred; this possibility is being investigated in a companion study. Alternatively, little fibrinolytic activity may occur in the arterial wall, due to low levels of plasminogen activator in both normal and atherosclerotic arteries14-48 or to high levels of plasminogen activator inhibitor in the arterial wall.49

In this study, we demonstrated that thrombi formed in vivo consist of fibrin II as Nossel postulated. The molecular
changes in fibrinogen detected in the vessel wall with progression of lesions may result from the triggering of the clotting mechanism in association with the development and/or progression of atherosclerotic lesions. The low degree of plasmin proteolysis of the amino-terminal end of fibrinogen and the fibrin that was detected in both thrombi and plaques suggest either that proteolysis of the carboxy-terminal region occurs preferentially in vivo or that fibrinolysis is limited in the atherosclerotic vessel wall.

REFERENCES


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Immunochemical characterization of fibrinogen, fibrin I, and fibrin II in human thrombi and atherosclerotic lesions

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