"Fibrinogen Tokyo II"

AN ABNORMAL FIBRINOGEN WITH AN IMPAIRED POLYMERIZATION SITE ON THE ALIGNED DD DOMAIN OF FIBRIN MOLECULES

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Abstract A hereditary dysfibrinogenemia associated with defective aggregation of fibrin monomers was found in a 39-yr-old female and in the members of her immediate family, who had all been asymptomatic.

The abnormality was probably due to an impaired polymerization site exposed in the DD domain of two adjacent fibrin molecules, because plasmic fragment DD derived from the propositus' cross-linked fibrin bound far less tightly to insolubilized normal fragment E than that from the normal one. Its complementary polymerization site in the E domain of fibrin, which was exposed by thrombin cleavage, and the polymerization site in the D domain of fibrinogen, which was available without activation by thrombin, were both found to be normal.

More anodal migration of the abnormal fragment DD than the normal one, as shown by immunoelectrophoresis, seemed to support our concept that the mutation most likely resides in the D domain of the abnormal fibrinogen molecule at or near a region closely related to the polymerization site that is exposed when two fibrin molecules are linearly aligned.

The work of others on the polymerization of normal fibrin with different techniques yielded results consistent with our conclusions.

We tentatively designate this type of abnormal fibrinogen "fibrinogen Tokyo II," but its possible identity with other abnormalities of fibrinogen reported heretofore is not excluded.

INTRODUCTION

Many of the dysfibrinogenenias reported thus far fall into a category with abnormal polymerization of fibrin monomer with or without altered releases of fibrinopeptides (1–3). The functional abnormalities associated with abnormal polymerization have not been located on a molecular level, except in fibrinogen Detroit (4, 5), to the replacements of amino acid residues or to the functional domains where the polymerization sites have been identified.

In this paper, we report a congenital dysfibrinogenemia, designated as fibrinogen Tokyo II, with a substantially delayed polymerization of fibrin monomer that is probably due to an abnormality in the polymerization site on the DD domain of fibrin molecules, which are unfolded so that they can function when two fibrin molecules are linearly aligned.

METHODS

Blood collections. Venous blood was collected by the two-syringe method. A 2-ml portion was placed in a plain glass tube and allowed to clot at 37°C for 2 h to harvest serum. Another 2-ml portion was transferred to a glass tube containing EDTA and used for blood cell counting and preparation of EDTA plasma for assays of plasma proteins including fibrinogen. The other portion was anticoagulated with 1/9 vol of 3.8% trisodium citrate; platelet-rich and platelet-poor plasmas were prepared for the platelet aggregation and blood coagulation studies, respectively.

Hemostasis and coagulation tests. Hemostatic and coagulation studies were performed by standard procedures (6, 7) or as described elsewhere (8), unless otherwise stated. Factor XIII (XIII)1 was determined immunochemically by

1Abbreviations used in this paper: XIII, factor XIII; XIIIa, activated factor XIII; IBS, imidazole-buffered saline; KIU, kallikrein inhibitor unit; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
Laurell's method (9) using antisera purchased from Behring-Werke AG, Marburg/Lahn, West Germany as well as by measuring the fluorescence of incorporated monodansyl cadiaverine into casein, basically according to Lorand et al. (10). Plasma fibrinogen was determined by four methods: (a) the thrombin time method essentially according to Clauss (11) using a fibrinogen determination kit (Dade, Miami, FL), (b) the method of Ratnoff and Menzie (12), (c) the method of Laurell (9), and (d) the turbidimetric method—0.1 ml of EDTA-plasma diluted with 3.0 ml of 0.5% EDTA-K₃ in physiological saline was heated at 50°C for 10 min and the resulting turbidity was measured at 600 nm. Concentrations of fibrinogen in the sample were obtained on a calibration curve made with dilutions of a pooled plasma containing a known amount of fibrinogen. The thrombin time was performed as follows: 0.1 ml of either imidazole-buffered saline (IBS; one part of 0.257 M imidazole buffer, pH 7.4, and nine parts of physiological saline) or 0.025 M CaCl₂ was added to 0.1 ml of plasma, and the mixture was warmed at 37°C for 2 min. 0.1 ml of 10 NIH-U/ml thrombin (Thrombin Topical from Parke, Davis & Co., Detroit, MI) was dissolved in 0.1 ml of physiological saline containing 0.8% NaCl, and diluted as previously described with IBS just prior to use) was then added, and a clotting time was obtained in duplicate with a fibrinometer (Becton, Dickinson & Co., Cockeysville, MD). The Reptilase time was performed by replacing thrombin with a 50 μg/ml solution of Reptilase, venom of Bothrops atrox (Reptilase-R, Pentapharm, Switzerland).

**Purification of fibrinogen.** About 130 ml of acid-citrate-dextrose-plasma derived from the propositus or healthy adults was passed through lysine-agarose (13) and gelatinagarose (14) columns connected in tandem at 22°C. The agarose was Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden). Fibrinogen was precipitated from this plasma by either 25% ammonium sulfate saturation (repeated three times) or the method of Blomback and Blomback (15). The fibrinogen fractions were dissolved in appropriate volumes of 0.3 M NaCl which was extensively dialyzed against the same solution and stored frozen in small aliquots at −80°C until used. In general, the purity of fibrinogen in various samples prepared by these two methods fell between 90 and 95% as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (16).

**Studies on purified fibrinogen and its derivatives.** The thrombin and Reptilase times were measured similarly as described above on plasma samples using 0.05 M Tris-HCl, pH 7.6, containing 0.1 M NaCl, instead of IBS. Thrombin clotting time was estimated by the ratio of thrombin clottable protein to total protein, as determined by the method of Ratnoff and Menzie (12). Immuno electrophoresis was performed at a constant current of 3 mA/cm width for 90 min using 0.8% agarose (Nakarai Chemicals, Ltd., Kyoto, Japan) in barbital buffer, ionic strength 0.05, pH 8.6. Gels were stained with Coomasie Brilliant Blue R-250 (Nakarai Chemicals). DEAE-cellulose chromatography was carried out as described by Finlayson and Mosesson (17). Cross-linking of fibrin mediated by activated factor XIII (XIIIa) were examined by SDS-PAGE using 5.0 or 7.5% gels as well as by the clot solubility test in 1.0% monochloracetic acid (18). Total release of fibrinopeptides by either thrombin or Reptilase and aggregation of fibrin monomer were studied essentially according to Gralnick et al. (19).

**Preparation of plasma digests of cross-linked fibrin.** The patient's (30 mg) and normal (200 mg) fibrinogen, 2% in 0.3 M NaCl, were enriched with 50 U of purified human XIII (25 U/ml) prepared by the method of Curtis and Lorand (20) per gram of fibrinogen and diuted 100-fold with 0.05 M Tris-HCl, pH 7.6, containing 0.1 M NaCl, 0.01 M CaCl₂, and 25 kallikrein inhibitor unit (KIU)/ml aprotinin (Ono Pharmaceutical Co., Ltd., Osaka, Japan). Cross-linked fibrin clots were formed by adding 2,000 NIH-U of purified bovine thrombin (21) per gram of fibrinogen under gentle stirring for more than 30 s. The clots were then incubated for the initial 2 h at 37°C and for another 12 h at 4°C to induce a maximal conversion of fibrinogen to fibrin. They were transferred onto filter paper placed in funnels and the clot liquid was squeezed out. The fibrin clots were then repeatedly washed with the buffer without aprotinin and cut into small pieces. To harvest fragments DD and E₁ + E₂, the subfractions of fragment E that retain polymerization capacity (22–24), the minced fibrin clots derived from 200 mg of normal or 30 mg of abnormal fibrinogen were suspended in 4.0 and 0.6 ml, respectively, of 0.05 M Tris-HCl, pH 7.6, containing 0.1 M NaCl, 0.01 M CaCl₂, 0.04% Na₃Citrate, and 12.5 U/ml streptokinase-activated human plasmin (22.5 Committee on Thrombolytic Agents U/ml). After 8 h of gentle stirring at 25°C, the remaining clots were separated by centrifugation and again digested similarly with one-half of the original volume of the plasmin-containing buffer for another 8 h. The digestion was repeated once again so that the fibrin clots were almost completely digested. The lysates, which had been separated by centrifugation and treated each time with 500 KIU of aprotinin per unit of plasmin, were combined and subjected to two-step chromatography on Sepharose 6B (Pharmacia Fine Chemicals), essentially as described by Olea and Budzynski (22). The fractions that retained the molecular weight for either fragment DD or fragments E₁ and E₂, as verified by SDS-PAGE, were combined, concentrated, and used for the binding study or stored frozen until used.

**Binding study using Sepharose conjugated with fibrin monomer or with plasmin digests of cross-linked fibrin.** Fibrinmonomer-Sepharose was prepared from normal fibrinogen essentially according to Heene and Matthias (25), packed into a small column (gel vol = 4 ml), and equilibrated with 0.05 M Tris-H₂PO₄, pH 7.6, containing 0.1 M NaCl, 0.005 M EDTA, and 5 KIU/ml aprotinin (buffer A). A gradient employing pH (7.6 – 4.1) and NaCl (0.1 – 2.0 M) was applied for elution using 0.05 M Tris-H₂PO₄, pH 4.1, containing 2.0 M NaCl, 0.005 M EDTA, and 5 KIU/ml aprotinin (buffer B). 1-ml fractions were collected.

Sepharose conjugated with the plasmin digests of normal cross-linked fibrin was prepared as follows: 57.5 mg of fragment DD and 19.8 mg of fragments E₁ + E₂ (fragment E₁+₂), as estimated on the basis of an extinction coefficient of E₂₈₀ = 20.8 for fragment DD and 10.2 for fragment E (26), were mixed with 5 ml each of CNBr-Sepharose 4B gel (27). Fragment DD was confirmed to be fairly homogeneous, but fragment E₁+₂ was an approximately 1:1 mixture of E₁ and E₂ which contained a trace amount of E₃ if any, as depicted in Fig. 1. Approximately 86.5% of fragment DD and 93.6% of fragment E₁+₂ were conjugated to the Sepharose gels. After treatment with 1 M monoethanolamine, pH 8.0, the gels were extensively washed with chilled 0.1 M NaHCO₃, pH 8.9, containing 0.5 M NaCl and packed into small columns. The packed DD- and E₁+₂-conjugated Sepharose gels (3.5 ml) were successively washed with 100 vol each of 6 M urea in 0.05 M Tris-H₂PO₄, pH 4.1, containing 1 M NaCl, 0.005 M EDTA, and 5 KIU/ml aprotinin (buffer B); 0.1 M CH₃COOH; and buffer A. After the columns were equilibrated with buffer A, samples of ~0.5 mg were applied to the columns. The adsorbed materials were eluted after the A₂₈₀ became <0.005 by employing pH (7.6 – 4.1), NaCl

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(0.1 – 1.0 M), and urea (0 – 6 M) gradients, using buffer B2. 1-ml fractions were collected.

The affinity chromatography was performed by using the same column for normal and abnormal samples in order to compare chromatograms as precisely as possible. For each run, these columns were regenerated by washing with sufficient amounts of buffer B1 for fibrinmonomer-Sepharose and buffer B2 for DD- or E1+E2-Sepharose as well as with 0.1 M CH3COOH. The gels were then equilibrated with buffer A for repeated use. The binding capacity of the gels was confirmed to be unaltered since it was observed that the chromatograms were almost similar as long as identical samples were applied.

RESULTS

Case history and family study

A 39-yr-old female inpatient at the gynecological ward had been examined for hemostasis and blood coagulation before she underwent hysterectomy for a uterine myoma. She had experienced neither excessive bleeding nor thrombotic tendency. Since the preoperative coagulation study revealed a markedly prolonged thrombin time and variable levels of plasma fibrinogen, we were prompted to further investigate her plasma. Some of her immediate family members investigated were also found to have a similar abnormality of fibrinogen, although they are all asymptomatic. Thus, the abnormality appears to be compatible with an autosomal dominant trait; no consanguinity was known to the family (Fig. 2).

Hemostasis and coagulation studies

The bleeding time, whole blood clotting time, platelet counts, and platelet aggregation were normal but the one-stage prothrombin time, 15.8 s (control, 11.3 s), and activated partial thromboplastin time, 37.9 s (control, 21.3 s), were moderately prolonged. In thrombelastography, there was a moderately prolonged clot formation time, 13.0 min (normal, 5.4±1.0 min), and reduced maximum amplitude, 35.0 (normal, 53.4±4.5). The means±SD (n = 50) are given. Blood coagulation factors including XIII were found to be within normal ranges. Levels of plasminogen and antithrombin III were normal both in activity and antigen. Concentrations of α2-plasmin inhibitor, 6.9 mg/dl, and plasma fibronectin, 31.3 mg/dl, were also normal; the incorporation of both, which were recently demonstrated to be cross-linked to the α-chain of fibrin by XIIIa (28, 29), into fibrin was normal as evidenced by ~33 and 23% decreases in the amounts found in serum, respectively. As has been shown in many of the disfibrinogenemia reported thus far, the thrombin and Reptilase times were markedly prolonged, but could be partially corrected by the addition of calcium ions. There was also a great discrepancy between the markedly reduced level of plasma fibrinogen determined by the thrombin clotting time method and normal levels obtained by other techniques, as shown in Table I.

| Table I: Thrombin and Reptilase Times and Fibrinogen Concentrations Determined on Propositus' Plasma
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<td>Thrombin time (s)</td>
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Studies on purified fibrinogen and its plasmic fragments

Cross molecular structure. Molecular weights of fibrinogen Tokyo II and its three subunit polypeptides and the cross-linking pattern made by XIIIa were identical with those of normal fibrinogen when examined by SDS-PAGE (pattern not shown). Fibrinogen Tokyo II was also indistinguishable from normal fibrinogen by DEAE-cellulose chromatography (profile not shown) and immunoelectrophoresis (Fig. 7, wells 1 and 2).

Thrombin and Reptilase times. As shown in Table II, the thrombin and Reptilase times were markedly prolonged; virtually no solid fibrin clots were formed in more than 40 min. The addition of CaCl₂ corrected the clotting times remarkably, but still only partially.

Release of total fibrinopeptides. When the release of total fibrinopeptides was studied at timed intervals after the addition of thrombin or Reptilase, no substantial differences were noted between the patient’s and normal fibrinogens (pictures not shown).

Aggregation of fibrin monomer. Markedly altered aggregation was observed in the patient’s fibrin monomer when compared with normal ones. The alteration depends on the ionic strength and thus virtually no aggregation was observed when the concentration of NaCl was increased to 0.05 M or more in 0.06 M potassium phosphate, pH 6.8. When normal fibrin monomer was mixed with 1/3 vol of either 0.02 M acetic acid or the patient’s fibrin monomer, the aggregation profiles were almost similar; this shows that the patient’s fibrin monomer per se would not inhibit the polymerization of normal fibrin monomer (pictures not shown).

Binding studies by means of affinity chromatography

Fibrinogen on the fibrinmonomer-Sepharose. To see if the proposed binding site on the D domain of fibrinogen (5, 30, 31) is malfunction in fibrinogen Tokyo II, 5.5 mg each of highly purified normal and abnormal fibrinogen fractions (fractions recovered in the study by DEAE-cellulose chromatography were combined and repeatedly used here) were subjected to affinity chromatography on fibrinmonomer-Sepharose. Gradient elutions with 50 ml each of buffers A and B₁ were performed. As shown in Fig. 3, adsorbed fractions of normal and abnormal fibrinogens were eluted nearly at the same positions, which suggests that the binding site on the D domain in fibrinogen Tokyo II was indistinguishable from that in normal fibrinogen.

Binding of fragment E₁⁺₂ to fragment DD-Sepharose. The binding of fragment E₁⁺₂ to fragment DD-conjugated Sepharose was tested. A sample of 0.46 mg (0.5 ml) of fragment E₁⁺₂ that was derived from fibrinogen Tokyo II or one from normal fibrinogen was applied to a fragment DD-conjugated Sepharose column, and 1-ml fractions were collected. Approximately 30% of each fragment E₁⁺₂ was not adsorbed; this was probably because an excess amount of protein had been applied as judged from the SDS-PAGE of the eluted fractions. The adsorbed materials were eluted in a similar fashion at 3.4 M or higher urea concentrations (Fig. 4). The eluted fractions were combined as indicated, concentrated, and subjected to SDS-PAGE to confirm the molecular size and structure. As shown in Fig. 5, left panel, they were found to be composed solely of fragments E₁ and E₂ that had been applied to the column. Thus, it appears that the

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<td>&gt;2,400</td>
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<td>With calcium ions</td>
<td>18.8</td>
<td>11.6</td>
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<tr>
<td>Reptilase time (s)</td>
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<tr>
<td>Without calcium ions</td>
<td>&gt;2,400</td>
<td>25.3</td>
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<tr>
<td>With calcium ions</td>
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<td>16.8</td>
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<tr>
<td>Thrombin clottability (%)</td>
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<tr>
<td>Without calcium ions</td>
<td>72</td>
<td>92</td>
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<tr>
<td>With calcium ions</td>
<td>90</td>
<td>96</td>
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**Figure 3** Affinity chromatography of fibrinogen Tokyo II (○) and normal fibrinogen (●) on fibrinmonomer-Sepharose. A 5.5-mg sample of highly purified fibrinogen (fractions recovered in the study of DEAE-cellulose chromatography were combined and used in this study) was applied to a fibrinmonomer-Sepharose 4-ml column at 25°C, and adsorbed protein was eluted with 50 ml each of buffers A and B₁ by employing gradients of NaCl (0.1 → 2.0 M) and pH (7.6 → 4.1). 1-ml fractions were collected.

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polymerization site postulated in the NH2-terminal region of fragment E (which is present in E1 and E2, but not in E3) (31) may function normally and bind to a unique complementary site on the DD domain formed by two adjacent fibrin molecules.

Binding of fragment DD to fragment E1+2-Sepharose. The reverse of the situation discussed above was investigated. 0.5 mg (1.0 ml) each of fragment DD derived from fibrinogen Tokyo II and from normal fibrinogen were applied to fragment E1+2-Sepharose, and the chromatograms were compared. As shown in Fig. 6, fragment DD of fibrinogen Tokyo II was eluted much earlier between fractions 6 and 13, which corresponded to 1.0-2.2 M urea. On the other hand, fragment DD derived from the normal cross-linked fibrinogen was eluted between fractions 23 and 32, which corresponded to much higher concentrations of 3.4-4.8 M urea. The eluted proteins of fragment DD derived from abnormal as well as normal fibrinogens both exhibited the DD-structure as shown by SDS-PAGE (Fig. 5, right panel). The discrete elution profiles clearly show that fragment DD derived from fibrinogen Tokyo II has a significantly lower affinity than that from normal fibrinogen to fragment E1+2.

Immunelectrophoresis of fragment DD. When fragment DD derived from fibrinogen Tokyo II was compared with that from normal fibrinogen, more anodal migration was observed (Fig. 7, wells 3 and 4).

FIGURE 4 Affinity chromatography of plasmic fragment E1+2 (E1 + E2) derived from fibrinogen Tokyo II (○) and normal fibrinogen (●) on fragment DD-conjugated Sepharose. Samples of 0.46 mg of fragment E1+2 were individually applied to a 3.5-ml column at 25°C, and adsorbed proteins were eluted by employing gradients of NaCl (0.1 → 1.0), pH (7.6 → 4.1), and urea (0 → 6 M), using 20 ml each of buffers A and B. 1.1 ml fractions were collected. Fractions containing proteins were combined as indicated by horizontal bars, concentrated, and subjected to SDS-PAGE to ascertain the molecular weight of eluted protein fractions (see Fig. 6).

FIGURE 5 SDS-PAGE of the eluted fractions E1+2 from DD-Sepharose and DD from E1+2-Sepharose. Representative electrophoretograms are those on the combined fractions 26 to 30 for both patient's (P) and normal (N) E1+2 fractions, and the combined peak fractions 8 and 9 for the patient's (P) and 26 and 27 for normal (N) fragment DD. 7.5% gels were stained with Coomassie Brilliant Blue R-250.

FIGURE 6 Affinity chromatography of plasmic fragment DD derived from fibrinogen Tokyo II and normal fibrinogen on fragment E1+2-conjugated Sepharose. 0.5 mg each of fragment DD derived from fibrinogen Tokyo II (○) and normal fibrinogen (●) were applied to a 3.5-ml column, and adsorbed proteins were eluted as mentioned previously (see legend for Fig. 4).

FIGURE 7 Immunelectrophoretic comparison of fibrinogen and fragment DD derived from fibrinogen Tokyo II with those from a normal individual. Electrophoresis was performed at a constant current of 3 mA/cm width for 90 min using 0.8% agarose in barbital buffer, ionic strength 0.05, pH 8.6. Gels were stained with Coomassie Brilliant Blue R-250. Well 1, normal plasma; well 2, propositus' plasma; well 3, fragment DD derived from fibrinogen Tokyo II; well 4, fragment DD derived from normal fibrinogen; troughs A, B, and D, anti-human fibrinogen; trough C, anti-fragment D.
Such an abnormal electrophoretic mobility was not as noticeable in fragment \( E_{1+2} \) (picture not shown) and fibrinogen (Fig. 7, wells 1 and 2).

**DISCUSSION**

Although impaired polymerization of fibrin monomer is the most common among the dysfibrinogenemias reported thus far (1–3), the affected polymerization sites are not necessarily characterized in relation to the functional domains of fibrinogen molecules. Kudryk et al. demonstrated two functional sites of polymerization—one on the amino-terminal disulfide knot domain that is unfolded by thrombin and the other on the D domain which is already available in the molecule of fibrinogen—by using functional domains of the well-characterized abnormal fibrinogen, fibrinogen Detroit (5, 30). Recently, however, Olexa and Budzynski postulated that four functional sites are involved in the polymerization step and depicted them by their flexible banana model of a bivalent fibrinogen molecule (31). According to their model, there are two sets of binding sites on the NH₂-terminal domain of the fibrinogen molecule that are designated as “A” and “B”, their respective complementary sites are designated as “a” and “b” on the D domain. The “A” and “B” sites become available upon cleavage of fibrinopeptides A and B, respectively. While the “a” site does not need activation and is thus functional in the fibrinogen molecule, the “b” site becomes functional only when two D domains of different molecules have been linearly aligned to form a bivalent “bb.” By using Sepharose conjugated with either normal noncross-linked fibrin, plasmic fragment \( E_{1+2} \) (a mixture of fragments \( E_1 \) and \( E_2 \)), or fragment DD, we showed that fragment DD derived from the patient’s cross-linked fibrin was eluted at distinctly lower concentrations of urea (1.0–2.2 M) and around neutral pH (7.1–6.4) while that derived from cross-linked normal fibrin was recovered at much higher concentrations of 3.4–4.8 M urea and lower pH (5.5–4.8). In the reverse situation, i.e., the elution of the patient’s and the normal fragments \( E_{1+2} \) from the DD-conjugated Sepharose, the elution profiles were nearly identical. Thus, the set “B”–“bb” would not function properly in fibrinogen Tokyo II owing to the impaired “bb” site on the aligned DD domain. It has been suggested that the set of polymerization sites “B”–“bb” induces side-to-side polymerization to form a fibrin sheet (31). The defective binding between the E and DD domains found in fibrinogen Tokyo II could be also explained by the recently proposed model of Fowler et al. (32). This model is based on an electron micrographic study in which they clearly showed the presence of trimer and pentamer complexes. In these complexes, the E domain of a fibrin monomer is attached to the linearly aligned DD domain of fibrinogen molecules, which is covalently cross-linked by XIIIa. In fibrinogen Tokyo II, the formation of a fibrin sheet by side-to-side aggregation or even protofibril formation by the “DE-stag contact” designated by Fowler et al. (32) may thus be impaired. The polymerization site “b” on the D domain may be arranged at or near the XIIIa-mediated cross-link bonds, or these covalent bonds may align and stabilize the sites on the two fragment D moieties, as suggested by Olexa and Budzynski (31). The XIIIa-mediated stabilization or facilitation of an aligned “bb” site may, at least in part, account for an accelerated polymerization and gelation of fibrin in the presence of Ca+++, which is observed in most abnormal fibrinogens (1–3) as well as the normal one (33, 34). The XIIIa-mediated stabilization of the “bb” site may thus contribute to the formation of hemostatic thrombi in vivo where XIII and Ca++ are sufficiently available. This may also explain why little or no hemorrhagic tendency has been observed in many of the dysfibrinogenemias in spite of a greatly prolonged thrombin clotting time in vitro. In our case, the propositus underwent surgery without any pathologic bleeding, though the “B”–“bb” set of Olexa and Budzynski (31) and the “DE-stag contact” of Fowler et al. (32) have been shown to function abnormally. The reason for this is not completely clear, but we presumed that the “bb” site without stabilization by cross-link bonds may not properly react with its complementary “B” site. However, the “bb” site when stabilized, may bind to the “B” site tightly enough to form the (DD)E moiety and promote polymerization of fibrin, although the binding capacity is significantly reduced compared with that of normal molecules. We did not conclusively study another set of polymerization sites, “A”–“a”.

However, we presumed that at least the “a” site on the D domain of fibrinogen Tokyo II functioned normally because the binding of the highly purified fibrinogen fraction to fibrinmonomer-Sepharose was virtually indistinguishable from that of the normal.

Based on these results and presumptions, we constructed a model which may illustrate the abnormal polymerization site on the DD domain in fibrinogen Tokyo II (Fig. 8).

There is a strong possibility that the abnormality resided in the DD domain of linearly aligned fibrin molecules; this was also supported by the abnormal electrophoretic mobility of fragment DD derived from the patient’s fibrin. Since electrophoretic mobilities of fibrinogen in the patient’s and normal plasma are indistinguishable from each other, possible explanations include the presence of conformational changes in the DD domain or a different mode of cleavage by plasmin. Because of a probable conformational change(s) due to mutation at or near the plasmic cleavage site(s),

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the patient's fragment DD may contain a negatively charged polypeptide segment(s) which should be cleaved off by plasmin, or it may have lost a positively charged one(s) which should be retained in normal molecules. Further studies are currently in progress, including an amino acid sequence analysis which is intended to clarify the abnormal structure of fibrinogen Tokyo II in relation to its functional abnormalities.

The other two functions ascribed to fibrinogen were apparently normal in fibrinogen Tokyo II, i.e., the release of total fibrinopeptides and the cross-linking by XIIIa to itself and other plasma proteins (28, 29, 35). Other features of fibrinogen molecules, including the gross molecular weight and subunit compositions as examined by SDS-PAGE, the chromatographic pattern on DEAE-cellulose, the circular dichroism spectra, and the immunologic reactivity, were all found to be normal.

We demonstrated that the impaired polymerization of fibrin monomer found in the dysfibrinogenemia, designated as fibrinogen Tokyo II, is partly, if not completely, attributable to a dysfunction of the polymerization site on the DD domain. Such an abnormality has not been observed in congenital dysfibrinogenemias reported thus far.
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