An Abnormal Fibrinogen Fukuoka II (Gly-Bβ15 → Cys) Characterized by Defective Fibrin Lateral Association and Mixed Disulfide Formation

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goat anti-rabbit immunoglobulin and horseradish peroxidase-conju-
gated rabbit anti-chicken immunoglobulin were from Bio-Rad Labora-
tories (Richmond, CA) and from Zymed Laboratories (South San Fran-
cisco, CA), respectively. Immobilon polyvinylidene difluoride mem-
brane and by single radial immunodiffusion using chicken anti-human fibrin-
in plasmawere determined by the thrombin time method of Clauss (19)
into aliquots and stored at
al.
propositus and his family and of normal healthy controls as described
in Table I. In the propositus and his father, the fibrinogen concentration determined by thrombin time assay was much lower than that determined by the immunological assay.
The reptilase as well as thrombin clotting times for plasma and purified fibrinogen were also prolonged with or without calcium ions. After digesting fibrinogen molecules of the propositus and his father with a low concentration of thrombin (0.1 unit/ml) at 37°C for 1 h, the release of FPB from fibrinogen was about 50% of that from normal fibrinogen, whereas the release of FPA from the propositus molecule was normal (Fig. 1a). The retarded release of FPB, however, was restored to almost 80% of normal when the molecule was extensively digested with a high concentration of thrombin; under these conditions fibrinogen molecules were cleaved at multiple additional sites in the Aα and Bβ chains (Fig. 1b). Essentially the same results were obtained using fibrinogen from the father of the propositus (data not shown). In subsequent experiments, plasma and pur-
ified fibrinogen molecules from the father were used unless otherwise noted. The abnormal fibrinogen in this study is des-
ignated hereafter as fibrinogen Fukuoka II. Release of FPA by reptilase instead of thrombin was not different between normal fibrinogen and fibrinogen Fukuoka II (Fig. 1c).
Fibrin Monomer Repolymerization—Normal fibrinogen and

Mixed Disulfide Formation in Fibrinogen Fukuoka II

29393

RESULTS

Fibrinopeptide Release—Coagulation studies on plasma and purified fibrinogen from the propositus and his family are shown in Table I. In the propositus and his father, the fibrinogen concentration determined by thrombin time assay was much lower than that determined by the immunological assay.

Electron Microscopy—Fibrin fibrils (2 mg/ml) produced by reptilase (2 μg/ml) were observed in acetic acid, diluted with 25-fold TBS, and mounted on a glow discharged carbon/formvar-coated grid. After incubation for 15 min at room temperature, the samples were negatively stained with 2% (w/v) uranyl acetate. Electron microscopy was performed using a JEM 2000EX transmission electron microscope (Japan Electron Optics Laboratory Co., Ltd., Tokyo, Japan). Fibrinogen Fukuoka II and normal fibrinogen were diluted to 20 μg/ml in TBS containing 50% glycerol, sprayed onto mica discs, and rotary shadowed with carbon platinum at an angle of 3 degrees. The rotary shadowed sample was floated on distilled water, loaded on a glow discharged carbon/formvar-coated grid, and examined by transmission electron microscopy.

DNA Amplification and Sequence Analysis—Genomic DNA was iso-
lated from peripheral blood cells according to Haar (30). Exon II of the fibrinogen Bβ chain was amplified by polymerase chain reaction with a sense primer (5′-TGGGGAATCCGTAGTATGATCT-3′) and an antisense primer (5′-CTTTTCTTAGTGGAGACCCCCACT-3′) (31). The polymerase chain reaction products were inserted into pUC19 plasmid and transfected into competent cells derived from E. coli strain DH5α; plasmids were isolated from the transformed E. coli by the method of Sambrook et al. (32). Following alkaline treatment of plasmids, the inserts were sequenced using the dideoxynucleotide chain termination method (33).

Analytical Procedures—Total protein concentrations were deter-
mined by the method of Lowry et al. (34). Immunoblot analysis was performed after blotting SDS-PAGE gels to Immobilon polyvinylidene difluoride membranes using the method of Towbin et al. (35). Free sulphydryl groups of fibrinogen were determined using Ellman’s proce-
dure (36). Amino acid analysis of Bβ 1–42 fragments was performed using a Picotag system (Waters, Millipore Corp.) according to the method of Heinriksson and Meredith (37). After treatment with pyro-
glutamate aminopeptidase, Bβ 1–42 fragments were sequenced using a gas phase sequencer (Applied Biosystems, model 476A, Foster, CA), and the phenylthiohydantoin-derivatives were identified using an on-line Applied Biosystems 20A phenylthiohydantoin-derivative analyzer.

Case Report—A 1-year-old Japanese boy was referred to us for an-
investigation of hypofibrinogenemia. The propositus and his family had no other clinical symptoms, and consanguinity was not found in his pedigree.

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Fibrin Monomer Repolymerization—Normal fibrinogen and
Fibrinogen Fukuoka II were digested with thrombin or reptilase to form fibrin clots, and fibrin monomers were obtained by dissolving the fibrin clots in acetic acid as described under "Experimental Procedures." In contrast to normal fibrin monomers, Fukuoka II fibrin monomers started to repolymerize after a longer lag time with a decreased maximum rate and a lower ultimate turbidity at physiological ionic strength as well as at low ionic strength (\(\mu = 0.04–0.14\)) (Fig. 2, a and b). When fibrin monomers obtained with reptilase instead of thrombin were compared, Fukuoka II fibrin monomers also showed a slow maximum rate of repolymerization and a decreased ultimate turbidity at all ionic strengths examined (\(\mu = 0.04–0.14\)) (Fig. 2, c and d). At physiological ionic strength (\(\mu = 0.14\)), Fukuoka II fibrin monomers obtained with either thrombin or reptilase were most extensively impaired (Fig. 2, b and d). These observations were consistent with the prolonged thrombin and reptilase times of propositus plasma and purified fibrinogen Fukuoka II.

**C. atrox** proteinase III cleaves the B\(_b\) chain of fibrinogen at the carboxyl side of Arg-42 (9). Des-(B\(_b\)1–42)-fibrinogens prepared from either normal fibrinogen or fibrinogen Fukuoka II

| TABLE I | Coagulation studies on plasma and purified fibrinogen from the propositus and his family |
|---------|--------------------------------------------------------------------------------------------------|
| **Plasma** |                                                                                               |
| Prothrombin time (s) | 12.2 | 12.8 | 12.1 | 10.8–12.2 |
| Activated partial thromboplastin time (s) | 34.9 | 30.7 | 31.9 | 25.0–32.1 |
| **Fibrinogen (mg/dl)** |                                                                                               |
| Thrombin time method | 73 | 133 | 178 | 154–358 |
| Immunologic method | 223 | 277 | 165 | 154–358 |
| Thrombin time (s) |                                                                                               |
| Without calcium ions | 24.1 | 19.2 | 8.8 | 7.7–9.1 |
| With calcium ions | 10.3 | 9.4 | 5.6 | 5.3–6.5 |
| Reptilase time (s) |                                                                                               |
| Without calcium ions | 46.1 | 44.7 | 18.6 | 18.1–21.6 |
| With calcium ions | 25.6 | 23.8 | 15.0 | 13.5–15.5 |
| **Purified fibrinogen** |                                                                                               |
| Thrombin time (s) |                                                                                               |
| Without calcium ions | 8.8 | 8.6 | 5.7 | 5.5–6.3 |
| With calcium ions | 7.3 | 7.2 | 4.7 | 4.6–5.2 |
| Reptilase time (s) |                                                                                               |
| Without calcium ions | 18.5 | 18.1 | 13.2 | 12.9–14.6 |
| With calcium ions | 15.3 | 15.1 | 11.5 | 11.0–12.8 |

**Fig. 1.** HPLC analysis of FPA and FPB. Fibrinogen was incubated with a low concentration of thrombin (0.1 units/ml) at 37°C for 1 h (a), with a high concentration of thrombin (50 units/ml) at 37°C for 6 h followed by incubating at 4°C for another 12 h (b), and with reptilase (2 \(\mu\)g/ml) at 37°C for 1 h (c). The released FPA and FPB were analyzed by HPLC on a reversed-phase column of Cosmosil 5C18-P (4.6 × 250 mm) with a linear gradient of 5–20% CH\(_3\)CN (dotted line) containing 0.025 M ammonium acetate (pH 6.0), at a flow rate of 0.5 ml/min (25). A, FPA that consists of A\(_a\)1–16; AP, phosphorylated FPA (A\(_a\)1–16) at the 3rd Ser of A\(_a\); AY, FPA that consists of A\(_a\)2–16; B, FPB that consists of B\(_b\)1–14; BR, FPB that consists of B\(_b\)1–13 (des-Arg-B); N, normal fibrinogen; F, fibrinogen Fukuoka II.

**Fig. 2.** Fibrin monomer repolymerization. Three kinds of fibrin monomers (a and b; c and d; e and f) were repolymerized in Tris buffer of various ionic strength: 1, 0.05 M Tris-HCl, pH 7.4 (ionic strength, \(\mu = 0.04\)); 2, 0.05 M Tris-HCl in 0.03 M NaCl, pH 7.4 (\(\mu = 0.07\)); 3, 0.05 M Tris-HCl in 0.06 M NaCl, pH 7.4 (\(\mu = 0.1\)); 4, 0.05 M Tris-HCl in 0.1 M NaCl, pH 7.4 (\(\mu = 0.14\)). a and b, repolymerization of fibrin monomers pretreated with thrombin. c and d, repolymerization of fibrin monomers pretreated with reptilase. e and f, repolymerization of des-(B\(_b\)1–42)-fibrin monomers pretreated with reptilase. a, c, and e, fibrin monomers or des-(B\(_b\)1–42)-fibrin monomers obtained from normal fibrinogen. b, d, and f, fibrin monomers or des-(B\(_b\)1–42)-fibrin monomers obtained from fibrinogen Fukuoka II.
by pretreating with C. atrox proteinase III were digested with
reptilase to form fibrin clots. Repolymerization of normal des-(B\(\beta\)1–42)-fibrin monomers did not occur at physiological ionic
strength but did occur at lower ionic strengths, although the
ultimate turbidity was lower than when uncleaved fibrin mono-
mers were used (Fig. 2, c and d). Repolymerization of Fukuoka II des-(B\(\beta\)1–42)-fibrin monomers was identical to normal des-
(B\(\beta\)1–42)-fibrin monomers (Fig. 2, e and f), suggesting that the
susceptible fibrinogen Fukuoka II structural abnormalities are
located within the first 42 residues of the B\(\beta\) chain.

Fibrin Structure Determined by Electron Microscopy—The
ultrastructure of repolymerized fibrin monomers at physiological
ionic strength was examined. In repolymerized normal fibrin monomers produced by reptilase, the dot matrix was
predominantly composed of thick branching striated fibers
with few thin fibrils (Fig. 3a). In contrast to normal fibrin monomers, the dot matrix of fibrin monomers Fukuoka II
contained mainly thin fibrils with only a few thick fibers
(Fig. 3b).

DNA Sequence Analysis—Using genomic DNA from the pro-
positus as well as the father, we amplified exon II of B\(\beta\) chain, which codes for amino acids from B\(\beta\)9 to 72, inserted it into a
pUC19 plasmid, and sequenced it. As shown in Fig. 4, a single
base substitution was found. This mutation changed the codon
GGT (Gly) to GTT (Cys) at the 15th residue of B\(\beta\) chain. The
normal sequence was also observed, indicating that fibrinogen
Fukuoka II is heterozygous.

SDS-PAGE and Immunoblot Analysis of Purified Fibrino-
gen—Purified fibrinogens from a normal control and the af-
fected individuals were analyzed by SDS-PAGE using nonre-
ducing conditions. Gels were stained with Coomassie Blue (Fig.
5a). Normal fibrinogen showed two bands with molecular masses of
340,000 and 300,000 daltons, corresponding to high molecular
mass fibrinogen and low molecular mass fibrinogen (38) (lane 1 in Fig. 5a). Fibrinogen Fukuoka II contained two
additional minor bands with molecular masses of 400,000 and
700,000 daltons as well as several faint bands (lanes 2 and 3 in
Fig. 5a). After digesting with C. atrox proteinase III, the des-
(B\(\beta\)1–42)-fibrinogen Fukuoka II showed only two bands,
which migrated slightly faster than the high or low molecular
mass fibrinogen (lane 4 in Fig. 5a). Immunoblot analysis using an anti-fibrinogen antibody showed that the additional slow
moving bands as well as the major bands were fibrinogen (lanes
2 and 3 in Fig. 5b) and that these slow moving bands disap-
ppeared when des-(B\(\beta\)1–42)-fibrinogen Fukuoka II was formed
(lane 4 in Fig. 5b). Immunoblot analysis using anti-albumin
antibody showed that the band of high molecular mass at
400,000 daltons contained albumin (lanes 2 and 3 in Fig. 5c).
The other band of molecular mass at 700,000 daltons was
determined to be a dimer of fibrinogen molecules by eluting the
band followed by reanalysis using SDS-PAGE after reducing the
sample (data not shown). Thus, the replacement of Gly with
Cys at residue 15 of the B\(\beta\) chain in fibrinogen Fukuoka II
results in the formation of albumin-fibrinogen complexes as
well as dimeric fibrinogen complexes through a disulfide bond
involving the mutated Cys residue.

The albumin-fibrinogen complex was selectively removed
from an aliquot of purified fibrinogen Fukuoka II using an
immobilized anti-human albumin antibody column. At physio-
logical ionic strength, repolymerization of the fibrinogen
Fukuoka II was not improved after removing the albumin-
fibrinogen complex (data not shown).

Titration of Free Sulfhydryl Groups in Normal Fibrinogen
and Fibrinogen Fukuoka II Molecules—The number of free
sulfhydryl groups in fibrinogen molecules were examined using
Ellman’s procedure (36). Less than 0.05 mol of -SH group/mol
of fibrinogen (0.05 ± 0.01, mean ± S.D., n = 3) was observed in
normal fibrinogen as well as fibrinogen Fukuoka II, indicating that
the mutated Cys at residue 15 of the B\(\beta\) chain in fibrinogen
Fukuoka II is disulfide cross-linked to a sulfhydryl group in other
molecules including albumin, cysteine, and fibrinogen
Fukuoka II itself (see below).

Analysis of B\(\beta\)1–42—Normal fibrinogen or fibrinogen
Fukuoka II molecules were digested with C. atrox proteinase
III, and the B\(\beta\)1–42 fragments were separated from the pre-
cipitable des-(B\(\beta\)1–42)-fibrinogen molecules by heating at
56°C for 10 min. The supernatant was analyzed using SDS-
Mixed Disulfide Formation in Fibrinogen Fukuoka II

SDS-PAGE and immunoblot analysis of Bβ 1–42 fragments. Normal fibrinogen or fibrinogen Fukuoka II (60 μg of protein each) was incubated with or without C. atrox proteinase III (1 μg/ml) at 37 °C for 2 h. The reaction was stopped by heating at 56 °C for 10 min and centrifuged at 10,000 × g for 5 min. Bβ 1–42 fragments in the supernatant were analyzed by SDS-PAGE (7-20% acrylamide) under nonreducing (a and c) and reducing conditions (b and d). a and b, Coomassie Blue staining. c and d, Immunoblot analysis with anti-human albumin IgG. Lanes 1, normal fibrinogen without C. atrox proteinase III treatment; lanes 2, Bβ 1–42 from normal fibrinogen; lanes 3, Bβ 1–42 from fibrinogen Fukuoka II; lanes 4, fibrinogen Fukuoka II without C. atrox proteinase III treatment.

Fig. 6. SDS-PAGE and immunoblot analysis of Bβ 1–42 fragments. Normal fibrinogen or fibrinogen Fukuoka II (60 μg of protein each) was incubated with or without C. atrox proteinase III (1 μg/ml) at 37 °C for 2 h. The reaction was stopped by heating at 56 °C for 10 min and centrifuged at 10,000 × g for 5 min. Bβ 1–42 fragments in the supernatant were analyzed by SDS-PAGE (7-20% acrylamide) under nonreducing (a and c) and reducing conditions (b and d). a and b, Coomassie Blue staining. c and d, Immunoblot analysis with anti-human albumin IgG. Lanes 1, normal fibrinogen without C. atrox proteinase III treatment; lanes 2, Bβ 1–42 from normal fibrinogen; lanes 3, Bβ 1–42 from fibrinogen Fukuoka II; lanes 4, fibrinogen Fukuoka II without C. atrox proteinase III treatment.

PAGE followed by staining with Coomassie Blue and immunoblotting using anti-human albumin antibody (Fig. 6). Both undigested and digested samples contained several bands above 67,000 daltons, which must be due to contamination in the purified fibrinogen fractions (lanes 1–4 in Fig. 6, a and b). Residual undigested fibrinogen (lanes 1 and 4 in Fig. 6a) or des-(Bβ 1–42)-fibrinogen molecules (lanes 2 and 3 in Fig. 6a) remained at the top of the gel under nonreducing conditions. When normal fibrinogen was digested with C. atrox proteinase III, the Bβ 1–42 fragment was detected by SDS-PAGE as a single band at 4,500 daltons under either reducing or nonreducing conditions (lane 2 in Fig. 6, a and b). Two new bands of 9,000- and 65,000-dalton molecular mass were observed when fibrinogen Fukuoka II was digested with C. atrox proteinase III under nonreducing conditions (lane 3 in Fig. 6a). The 9,000-dalton band disappeared under reducing conditions (lane 3 in Fig. 6b). Immunoblot analysis using anti-human albumin antibody confirmed that the protein at 65,000-dalton molecular mass was albumin (lane 3 in Fig. 6, c and d).

Further analysis of the Bβ 1–42 fragments using either reducing or nonreducing conditions was performed by reversed-phase HPLC (TSK gel ODS-120T, 4.6 × 250 mm) using a linear gradient of 20–30% CH₃CN containing 0.1% trifluoroacetic acid. Bβ 1–42 fragments in the purified fibrinogen fractions (lanes 1–4 from normal fibrinogen (i), Bβ 1–42 from fibrinogen Fukuoka II (ii), and reduced S-pyridylethylated Bβ 1–42 from fibrinogen Fukuoka II (iii)) were analyzed on a TSK gel ODS-120T reversed-phase column (4.6 × 250 mm) with a linear gradient of 20–30% CH₃CN (dotted line) containing 0.01% trifluoroacetic acid. B1–B9 were analyzed by SDS-PAGE (7-20% acrylamide) under nonreducing (b) and reducing conditions (c).

Cys mutation at residue 15 as deduced from the genomic DNA analysis. These results are consistent with the genomic DNA analysis, which shows that the propositus and his father are heterozygous (Fig. 4).

The Bβ 1–42 fragments from peaks B10 and B12 confirmed that they were the mutated fragments, and those from peaks B11 and B13 had the normal sequence. Judging from the peak heights of B10–B13 in Fig. 7a (iii), we concluded that fibrinogen Fukuoka II was expressed at almost the same level as normal fibrinogen in these heterozygosis patients.

Cysteine was shown to disulfide cross-link to fibrinogen Fukuoka II in an equimolar ratio. The results of amino acid composition and sequence analysis of all Bβ 1–42 fragments were consistent with the exception of peaks B3 and B5. Amino acid composition of those two fragments indicated that an additional Cys residue was associated with Bβ 1–42 in peaks B3 and B5 (Table III). The additional Cys was lost after reducing those samples with dithiothreitol (see B3' and B5' in Table III). Thus, a cysteine-fibrinogen Fukuoka II complex was formed through a disulfide bond at the mutated Cys of residue 15.

Rotary Shadowing of Fibrinogen Molecules—Fibrinogen molecules were directly observed by electron microscopy. Examination of normal fibrinogen molecules revealed the usual tridomainal structures (Fig. 8a), whereas in addition to the tridomainal structures, extra globular domains situated near the fibrinogen E domain and dimers of the tridomainal structures were observed in fibrinogen Fukuoka II (Fig. 8b). It is reasonable to assume that the extra globular domain structure and the dimer of tridomainal structure correspond to the albumin-fibrinogen complex as reported in fibrinogen Dusart (40) and the dimers of fibrinogen Fukuoka II. Statistic analysis on electron micrographs showed that about 5% (10 out of 200) and
2% (4 out of 200) of fibrinogen Fukuoka II molecules formed albumin-fibrinogen complexes and intermolecular dimeric fibrinogen complexes, respectively. This proportion was consistent with the density of bands on SDS-PAGE (Fig. 5).

**DISCUSSION**

A congenital heterozygous abnormal fibrinogen designated as fibrinogen Fukuoka II is characterized by prolonged thrombin and reptilase times (Table I), retarded release of FPB, normal release of FPB, normal release of FPA (Fig. 1), and disrupted repolymerization behavior of intact fibrinogen Fukuoka II is characterized by prolonged thrombin and reptilase times (Table I), retarded release of FPB, normal release of FPA (Fig. 1), and disrupted repolymerization of intact fibrinogen Fukuoka II (Fig. 2). This proportion was consistent with the density of bands on SDS-PAGE (Fig. 5).

**TABLE I**

| Cycle Position | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 |
|----------------|----|----|----|----|----|----|----|----|----|
| Gly           | 206.4 | 713.5 | Gly | 385.7 | Gly | 220.0 | Gly | 322.2 | Gly | 593.2 | Gly | 115.1 | Gly | 77.8 | Gly | 87.0 |
| Val           | 236.6 | 702.1 | Val | 381.8 | Val | 202.5 | Val | 335.9 | Val | 580.9 | Val | 79.5 | Val | 51.5 | Val | 61.7 |
| Asn           | 172.5 | Asn | 376.4 | Asn | 299.9 | Asn | 115.4 | Asn | 270.0 | Asn | 356.7 | Asn | 64.5 | Asn | 40.9 | Asn | 43.7 |
| Asp           | 197.1 | Asp | 387.0 | Asp | 321.1 | Asp | 123.2 | Asp | 253.3 | Asp | 326.1 | Asp | 57.1 | Asp | 41.7 | Asp | 46.7 |
| Asn           | 140.6 | Asn | 421.0 | Asn | 269.1 | Asn | 130.2 | Asn | 233.8 | Asn | 353.5 | Asn | 55.6 | Asn | 36.8 | Asn | 34.5 |
| Glu           | 148.3 | Glu | 374.6 | Glu | 274.6 | Glu | 106.2 | Glu | 201.5 | Glu | 299.9 | Glu | 52.6 | Glu | 33.5 | Glu | 38.6 |
| Glu           | 150.0 | Glu | 594.8 | Glu | 257.0 | Glu | 131.2 | Glu | 191.4 | Glu | 409.8 | Glu | 44.6 | Glu | 30.6 | Glu | 33.1 |
| Glu           | 123.0 | Glu | 321.7 | Glu | 206.5 | Glu | 82.7 | Glu | 176.6 | Glu | 260.1 | Glu | 49.3 | Glu | 34.5 | Glu | 39.7 |
| Phe           | 152.9 | Phe | 338.8 | Phe | 271.2 | Phe | 93.5 | Phe | 189.2 | Phe | 265.1 | Phe | 47.5 | Phe | 31.4 | Phe | 37.9 |
| Phe           | 161.8 | Phe | 575.3 | Phe | 268.9 | Phe | 120.0 | Phe | 193.0 | Phe | 373.0 | Phe | 44.4 | Phe | 30.9 | Phe | 35.4 |
| Ser           | 53.5 | Ser | 46.0 | Ser | 88.4 | Ser | 23.9 | Ser | 75.6 | Ser | 59.5 | Ser | 18.6 | Ser | 13.9 | Ser | 14.4 |
| Ala           | 73.3 | Ala | 259.5 | Ala | 160.0 | Ala | 56.0 | Ala | 145.1 | Ala | 196.5 | Ala | 34.2 | Ala | 22.2 | Ala | 24.2 |
| Arg           | 98.8 | Arg | 172.5 | Arg | 194.1 | Arg | 43.8 | Arg | 160.0 | Arg | 151.5 | Arg | 37.0 | Arg | 25.4 | Arg | 22.7 |

 Titration of sulfhydryl groups showed that fibrinogen Fukuoka II contained no detectable free sulfhydryl groups, indicating that the mutated Cys residue forms a disulfide bond with a sulfhydryl group from other compounds. SDS-PAGE and immunoblot analysis showed that this mutated Cys residue could form a disulfide bond with a sulfhydryl group in albumin as shown in other abnormal fibrinogens (14, 40). In addition, dimeric fibrinogen Fukuoka II was also formed via an intermolecular disulfide bond (Fig. 5). Rotary shadowing of the affected fibrinogen molecules indicated that 5 and 2% of fibrinogen Fukuoka II fraction formed albumin-fibrinogen complexes and intermolecular dimeric fibrinogen complexes, respectively. This means that approximately 10 and 4% of the mutated fibrinogen forms albumin-fibrinogen and dimeric fibrinogen complexes, because the examined patients were heterozygous for this mutation.

Analysis by HPLC of the B3-42 peptides from fibrinogen Fukuoka II produced seven peaks (B3-B9), whereas normal fibrinogen produced only two peaks (B1 and B2). Peak B1 contained hydroxyproline instead of proline at normal residue 31 of the Bβ chain, and peak B2 contained proline at this position (Table II). When multiple individuals were examined, the ratio of peak B1 to peak B2 ranged from 0.2 to 0.7. Bβ 1–42 peaks in peaks B7 and B9 were homodimers of the mutated Bβ 1–42 fragment that contained hydroxyproline and proline at position 31, respectively (Table II). Peak B8 was a heterodimer of Bβ 1–42 that contained hydroxyproline or proline. These data show that normal fibrinogen molecules have a random distri-
Mixed Disulfide Formation in Fibrinogen Fukuoka II

TABLE III

Amino acid compositions of the aberrant Bβ 1–42 fragments

| Peptides                  | B3          | B3'         | B5           | B5'          | Normal Bβ 1–42 | Fukuoka II Bβ 1–42 |
|---------------------------|-------------|-------------|--------------|--------------|---------------|-------------------|
| **Amino acid composition**|             |             |              |              |               |                   |

| Residue | B3 (%) | B3' (%) | B5 (%) | B5' (%) | Normal (%) | Fukuoka II (%) |
|---------|--------|---------|--------|---------|------------|----------------|
| Asx     | 4.04 ± 0.07 | 3.83 ± 0.14 | 3.78 ± 0.22 | 3.83 ± 0.18 | 4          | 4               |
| Glx     | 5.42 ± 0.08 | 5.16 ± 0.13 | 5.05 ± 0.15 | 5.20 ± 0.31 | 5          | 5               |
| Ser     | 2.40 ± 0.08 | 2.61 ± 0.31 | 2.37 ± 0.01 | 2.52 ± 0.11 | 3          | 3               |
| Gly     | 5.14 ± 0.08 | 4.95 ± 0.11 | 5.36 ± 0.02 | 5.24 ± 0.03 | 6          | 5*              |
| His     | 0.97 ± 0.02 | 1.02 ± 0.17 | 0.95 ± 0.03 | 0.94 ± 0.06 | 1          | 1               |
| Arg     | 4.90 ± 0.07 | 4.71 ± 0.08 | 4.90 ± 0.10 | 4.89 ± 0.21 | 5          | 5               |
| Ala     | 2.79 ± 0.09 | 2.81 ± 0.18 | 2.58 ± 0.05 | 2.95 ± 0.12 | 3          | 3               |
| Pro     | 5.16 ± 0.13 | 5.24 ± 0.35 | 5.99 ± 0.19 | 6.35 ± 0.73 | 5/6         | 5/6             |
| Tyr     | 0.94 ± 0.02 | 0.88 ± 0.11 | 0.94 ± 0.02 | 0.92 ± 0.05 | 1          | 1               |
| Val     | 0.77 ± 0.05 | 0.78 ± 0.24 | 0.77 ± 0.04 | 0.80 ± 0.17 | 1          | 1               |
| Ile     | 0.73 ± 0.07 | 0.83 ± 0.23 | 0.59 ± 0.07 | 0.78 ± 0.21 | 1          | 1               |
| Leu     | 1.87 ± 0.08 | 1.82 ± 0.23 | 1.72 ± 0.07 | 1.80 ± 0.12 | 2          | 2               |
| Phe     | 2.01 ± 0.07 | 1.84 ± 0.05 | 1.94 ± 0.05 | 1.89 ± 0.08 | 2          | 2               |
| Lys     | 1.95 ± 0.03 | 1.78 ± 0.21 | 1.93 ± 0.05 | 1.92 ± 0.14 | 2          | 2               |
| Hyp     | 0.73       | 0.83       | 0.73       | 0.83       | 10          | 10              |
| CySO₃H  | 2.21 ± 0.16 | 2.31 ± 0.13 | 0          | 1          | 1            |                  |
| Pe-Cys  | 0.55 ± 0.05 | 0.56 ± 0.05 | 0          | 1          | 1            |                  |

| **Note**: Amino acid compositions of normal or Fukuoka II Bβ 1–42 were calculated on the basis of the data from amino acid sequences.
| **Note**: Average values of three experiments (means ± S.D.) are given.
| **Note**: This aberrant Bβ 1–42 had a Gly to Cys substitution at residue 15 of the Bβ chain.
| **Note**: Cystic acid.
| **Note**: Pyridylethylcysteine.

The observed two residues of Cys indicate that the mutated Cys is disulfide cross-linked with free cysteine.

**FIG. 8.** Electron micrographs of rotary shadowed molecules of normal fibrinogen (a) and fibrinogen Fukuoka II (b). Extra globular domains and dimers of the tridomainal structures are indicated by the arrows and the arrowhead, respectively. The bars indicate 200 nm.
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