FIBRINOGEN PETOSKEY: IDENTIFICATION OF A NEW DYSFIBRINOGENEMIA
CHARACTERIZED BY ALTERED RELEASE OF FIBRINOPEPTIDE A[1]

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ABSTRACT

A new congenital dysfibrinogenemia, designated fibrinogen Petoskey,
which was traced through four generations of a Michigan family, was
found to exhibit an abnormally slow rate of release of fibrino-
peptide A upon treatment with thrombin and batroxobin. Batroxobin
only partially hydrolyzed and polymerized the fibrinogen from
affected individuals, suggesting that these patients had both normal
and abnormal fibrinogen in their circulation and that batroxobin
was not capable of releasing fibrinopeptide A from the abnormal
fibrinogen. Polymerization of fibrin monomers from fibrinogen
Petoskey and plasmin mediated digestion of fibrinogen Petoskey were
normal. The Factor XIIIa-catalyzed cross-linking of fibrinogen
Petoskey was slightly delayed at low (but not at high) concen-
trations of thrombin. This delayed cross-linking appeared to be a
secondary effect of the lower rate of release of fibrinopeptide A.

INTRODUCTION

The first report of a functional abnormality in fibrinogen appeared in
1958 (1). Since then over 50 congenital dysfibrinogenemias have been report-
ed. (See 2,3,4 for reviews discussing most of the known dysfibrinogenemias).
Although in most affected individuals the concentration of fibrinogen and

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other clotting factors is normal, fibrinogen from affected individuals usual-
ly exhibits a prolonged thrombin clotting time or a prolonged prothrombin
time (or both). The affected step in the clotting process has been determin-
ed in many of these dysfibrinogenemias. In some cases, the rate of proteoly-
sis and release of fibrinopeptide A or B is decreased due to an alteration in
interactions between the thrombin and fibrinogen. In other cases, altered
rates of spontaneous polymerization of fibrin monomers or altered rates of
Factor XIIIa-mediated cross-linking of fibrin are observed. In addition to
showing one or more of these functional defects, fibrinogen from individuals
with certain dysfibrinogenemias exhibits altered degradation rates and
products upon digestion with plasmin.

Only two dysfibrinogenemias (fibrinogens Paris I and Detroit) have been
characterized on a molecular level. In fibrinogen Paris I (5), a fraction of
the γ-chains is elongated, and in fibrinogen Detroit (6), Arg-19 of the
Aα-chains is replaced by Ser. Detailed functional and structural studies of
inherited abnormal fibrinogens provide a powerful method for characterizing
interactions of fibrinogen which are important in the formation of a
functionally competent blood clot. In the case of fibrinogen Detroit such a
study (6) has revealed that interactions involving aminoacyl residues at the
amino termini of the α-chains are necessary for polymerization of fibrin and
thrombin mediated cleavage of fibrinopeptide B from the Bβ-chains.

The present report describes a new congenital abnormality in a Michigan
family (7), characterized by fibrinogen which shows abnormally slow release
of fibrinopeptide A with thrombin and resistance to proteolysis by batroxobin
(reptilase). It is anticipated that further study of this fibrinogen will
increase our understanding of the structural determinants of the interaction
between thrombin and fibrinogen.

METHODS

Normal human plasma was obtained from the American Red Cross. It had
been prepared from 450 ml of whole blood collected into 63 ml of citrate
phosphate dextrose anticoagulant. Plasma from affected individuals was
prepared from whole blood collected in a similar manner. All plasma was
stored frozen.

Fibrinogen was purified from plasma by glycine precipitations to obtain
fraction I-2 as described previously (8), and was further purified to frac-
tion I-4 by the method of Blombäck and Blombäck (9). Contaminating plasmino-
gen was removed by passing fraction I-4 through a lysine-Sepharose column
(10). The resulting fibrinogen preparations were at least 95% clottable.

The clottability of fibrinogen was determined by a modification of the
method of Mosesson and Sherry (8) and Laki (11), in which the absorbance at
280 nm was measured before and after clotting. In this procedure, 0.94 ml of
fibrinogen in 0.3 M NaCl was mixed with 50 µl of a 0.64 M potassium phosphate
buffer which brought the pH of the resulting solution to 6.4. The solution
was clotted by the addition of 1 U of human thrombin (labeled greater than
3000 U/mg as obtained from Sigma Chemical Company, St. Louis, Missouri) in
10 µl. After 3 hours at room temperature the clot was removed and the
absorbance (280 nm) of the clot supernatant was determined and compared to
the absorbance of a diluted sample of the original fibrinogen solution. The
clottability of fibrinogen from affected individuals was determined in a

similar manner, except that 5 U of human thrombin in 10 μl was used to clot the fibrinogen.

Clinical coagulation assays, including thrombin clotting time (12), prothrombin time (13) and partial thromboplastin time (14), were performed by standard procedures, and the results obtained with patients' plasma were compared to those obtained with pooled normal plasma. Factor V and Factor VIII levels were measured by modifications of the methods used to determine prothrombin time (13) and partial thromboplastin time (14), respectively. Factor XIII levels were measured as described by Duckert et al. (15). Fibrinogen concentrations were determined by the thrombin clotting time assay of Clauss (16) or by assay of the fibrin tyrosine content (17).

Thrombin- and batroxobin-mediated polymerizations were studied by the following modification of the method of Gralnick et al. (18). A fibrinogen solution (0.15 ml) in 0.3 M NaCl was equilibrated at 25°C in a cuvette with 1.35 ml of 0.06 M sodium phosphate buffer, pH 6.8. Either 10 μl thrombin or 50 μl batroxobin (reconstituted Reptilase-R obtained from Abbott Laboratories, North Chicago, Illinois) was added and the absorbance at 350 nm was continuously monitored in a thermostated cell compartment of a GCA/McPherson spectrophotometer.

Radioimmunoassays of FPA were done with a kit from IMCO Corp., Ltd. AB Stockholm, Sweden. Samples were prepared using hirudin to inhibit the thrombin (19).

The total amount of fibrinopeptides released upon treating fibrinogen with thrombin was measured as described previously (18) using the method of Lowry (20) to determine trichloroacetic acid (TCA)[4] soluble peptides in the clot supernatant.

Fibrin monomers were prepared and subsequently aggregated by a modification of the method of Gralnick et al. (18 and references cited therein). Fibrinogen in 0.15 M Tris-HCl buffer, 0.15 M NaCl, pH 7.4 was clotted with human thrombin. After approximately 90% of the fibrinogen had clotted, the clot was wound around a glass rod and then dissolved in 0.02 M acetic acid. Polymerization of the fibrin monomers was initiated by adding a portion of the monomer solution to a 31-fold excess of 0.06 M potasium phosphate buffer at pH 6.8 which either contained no NaCl (for runs at an ionic strength of 0.12) or 0.3 M NaCl (for runs at an ionic strength of 0.42) at 25°C.

The cross-linking of fibrin was monitored as described previously (21).

Digestion of fibrinogen with plasmin was carried out by a method similar to that described by Sherman et al. (22). Plasminogen (profibrinolysin, labeled 2.5 U/mg as obtained from Sigma Chemical Co., St. Louis, Missouri) at 5 mg/ml in 0.046 M Tris-HCl, 0.115 M CaCl$_2$ pH 8.1 was activated with 100 Plough U/ml of urokinase (obtained from Calbiochem-Behring Corp., La Jolla, California) for one hour, prior to the start of digestion.

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[4]Abbreviations used in this paper: TCA, Trichloroacetic acid; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) was done on slab gels as described by Laemmli (23).

Circular dicroism spectra were obtained with a Jasco, J-40C automatic recording spectropolarimeter.

RESULTS

Case History. The propositus, an 80 year old Caucasian male, was first studied in 1977 prior to total hip replacement surgery. He was in excellent health, except for severe degenerative arthritis in the left hip. When studies of his clotting system indicated an abnormality, he was referred to The University of Michigan Hospital. He had no history of unusual bleeding, except in 1973 when he required two units of blood after a cholecystectomy.

Coagulation studies (Table I) demonstrated that the patient's prothrombin time and thrombin clotting times were abnormally long. The partial thromboplastin time, and Factor V and XIII levels of the propositus were normal, whereas his Factor VIII level was elevated. The fibrinogen concentration in the plasma of the propositus was abnormally low when measured using the indirect thrombin clotting time assay, and normal when measured from the tyrosine content of the fibrin. These studies suggested a diagnosis of dysfibrinogenemia, wherein the actual level of fibrinogen in the plasma was normal, but some of the fibrinogen was functionally deficient so as to give an abnormally long thrombin clotting time.

Family studies. The dysfibrinogenemia was traced through four generations (Fig. 1). Twenty-seven of the descendants of the propositus were tested and eleven had prolonged thrombin clotting times. The apparent fibrinogen concentration based on the thrombin clotting times of affected individuals ranged from 59 mg/dl to 101 mg/dl. The Reptilase time determined for plasma from the propositus' daughter (III-4 in Fig. 1) was also found to be prolonged (31.5 s vs. 14.8 s for normal plasma). All affected individuals were asymptomatic; several had undergone surgery, including hysterectomy, appendectomy, cholecystectomy, and tonsillectomy with no unusual bleeding or requirements for blood products. There was no known consanguinity in the

TABLE I

Coagulation Assays on Plasma from the Propositus

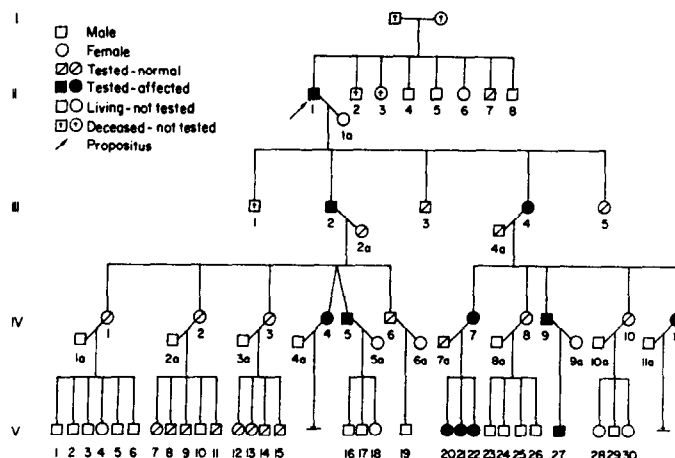| Assay	Patient	Normal
Thrombin Clotting Time	13.6 s	8-9 s
Prothrombin Time	13.0 s	11 s
Partial Thromboplastin Time	30.1 s	35 s
Factor V	111%	80-120%
Factor VIII	208%	80-120%
Factor XIII	Insoluble Clots	
Fibrinogen Concentration		
Based on the Thrombin Clotting Time	96 mg/dl	200-300 mg/dl
Based on the Fibrin Tyrosine Content	298 mg/dl	200-300 mg/dl

FIG. 1.

Pedigree of fibrinogen Petoskey

family and the family pedigree (Fig. 1) indicated that the defect was inherited as an autosomal dominant trait.

Localization of the Defect to Fibrinogen. Fibrinogen purified from patients' plasma was only 46% clottable after three hours with one unit of thrombin per ml of fibrinogen solution, whereas normal fibrinogen was greater than 95% clottable. When the thrombin concentration was increased to five U/ml, however, both the patient's fibrinogen and normal fibrinogen were greater than 95% clottable. Polyacrylamide gel electrophoresis in SDS of fibrinogen, as well as reduced Aα-, Bβ-, and γ-chains obtained from normal and affected individuals indicated that the molecular weights of the polypeptide chains of fibrinogen from normal and affected individuals were similar. Circular dichroism spectra of both fibrinogens were also identical, suggesting that there was no major conformational alteration in the abnormal fibrinogen. Immunodiffusion of fibrinogen from normal individuals and affected family members against goat antibodies to human fibrinogen revealed complete lines of identity. These results suggested a close structural resemblance between the normal and abnormal fibrinogens.

Mixing a patient's plasma with normal plasma in various ratios revealed no inhibitory effect in thrombin clotting time when compared with normal plasma mixed with barbital buffered saline. A similar result was obtained when thrombin-mediated clotting of purified fibrinogen was studied at several ratios of normal fibrinogen, abnormal fibrinogen and buffer. These results suggest that the slower clotting of the fibrinogen from affected individuals was not due to an inhibitor to clotting in their plasma and that the fibrinogen from affected individuals had no inhibitory effect on the clotting of normal fibrinogen.

Thrombin-Mediated Polymerization of the Abnormal Fibrinogen. The
thrombin-catalyzed cleavage of normal and abnormal fibrinogen near the amino
termini of the Aα- and Bβ-chains, to release fibrinopeptides A and B, and the
subsequent spontaneous polymerization of the fibrin monomers (24) was
monitored by following the increase in turbidity of the solution caused by
the polymerization reaction. Although the increase in turbidity of the solu-
tion reflects polymerization of fibrin monomers, subsequent to the thrombin-
mediated proteolysis of the fibrinogen, the rate at which the turbidity of a
solution of fibrinogen increases upon addition of thrombin may be determined
either by the rate of proteolysis or polymerization, whichever is slower. As
shown in Fig. 2A, a fifteen-fold increase in the concentration of thrombin
(from 2.9 U/ml to 43.7 U/ml) had no effect on the rate of thrombin-mediated
polymerization of normal fibrinogen as measured from the time dependent in-
crease in turbidity. Since the increased thrombin concentration should have
increased the rate of proteolysis, this result suggests that polymerization
and not proteolysis was rate limiting under the conditions used with normal
fibrinogen. The fibrinogen from an affected family member displayed markedly
different behavior (Fig. 2B). At a low thrombin concentration (2.9 U/ml),
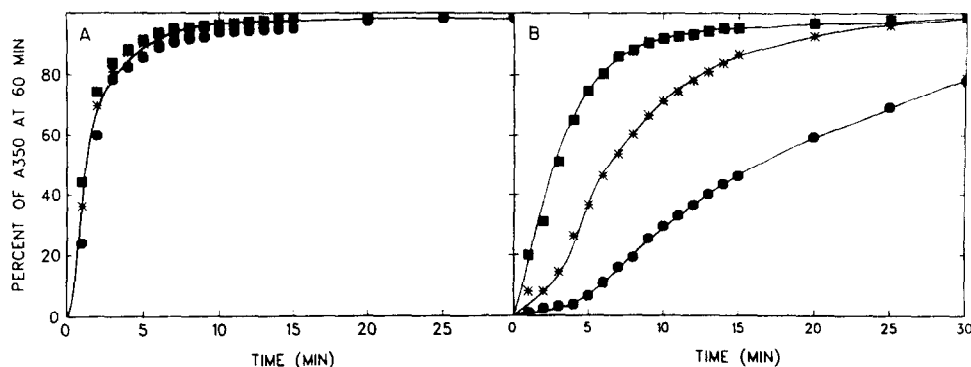thrombin-mediated polymerization of the patient's fibrinogen was much

FIG. 2.
Thrombin-mediated polymerization of normal fibrinogen (A), and fibrin-
ogen from an affected individual (B). The fibrinogen (0.052 mg/ml) in
0.054 M potassium phosphate buffer, 0.027 M NaCl, pH 6.8 was treated
with ■, 43.7 U/ml; *, 11.3 U/ml; and ●, 2.9 U/ml thrombin.

slower than thrombin-mediated polymerization of normal fibrinogen.
Moreover, increasing the concentration of thrombin, which presumably in-
creased the rate of release of fibrinopeptide A and B, markedly increased the
rate of polymerization (Fig. 2B). These observations suggested that pro-
teolysis of abnormal fibrinogen was rate limiting under the same conditions
where polymerization of fibrin monomers was rate limiting with normal
fibrinogen. Differences in the rate of thrombin-mediated polymerization of
abnormal and normal fibrinogen similar to those depicted in Fig. 2 were
observed at all fibrinogen concentrations studied (0.05 mg/ml to 5.7 mg/ml).

Although thrombin-mediated polymerization of the patient's fibrinogen
was slower (at low thrombin concentrations) than that of normal fibrinogen,

the amounts of fibrinopeptides ultimately released were within experimental error (±5%) of each other as judged by Lowry (20) determinations of TCA soluble peptide material in the clot supernatants (18). The low sensitivity of this procedure, however, made it impractical to use it to compare rates of release of fibrinopeptides.[5]

Radioimmunoassay of Fibrinopeptide A. The release of fibrinopeptides from fibrinogen was monitored using a radioimmunoassay to obtain additional evidence in support of the conclusion that the abnormal fibrinogen exhibits a decreased rate of proteolysis. Normal fibrinogen and fibrinogen from an affected family member at 2.82 mg/ml (8.3 μM) were each incubated with equal aliquots of a dilute solution of thrombin, so as to achieve a slow rate of release of fibrinopeptide A which could be measured conveniently. Radio-immunoassay of the fibrinopeptide A released after 6 hours indicated that the normal fibrinogen had released 50% of fibrinopeptide A, whereas the abnormal fibrinogen had released only 33% of fibrinopeptide A. This result provided direct evidence that the rate of release of fibrinopeptide A from the abnormal fibrinogen is decreased.

Batroxobin-Mediated Hydrolysis and Polymerization of Fibrinogen. The protease batroxobin, like thrombin, catalyzes hydrolytic cleavage of fibrin-opeptide A from fibrinogen (25). Batroxobin, however, does not cleave the Bβ- chains to release fibrinopeptide B. Thus, it was used to compare the re-lease of fibrinopeptide A from the fibrinogen of an affected individual with that of normal fibrinogen. When batroxobin was added to a solution of normal fibrinogen, the fibrinogen clotted completely, as measured by an increase in turbidity (Fig. 3). Subsequent addition of thrombin had no further effect. This result indicated that removal of fibrinopeptide A from normal fibrinogen resulted in a degree of polymerization which was sufficient to produce the maximal change in turbidity. The addition of batroxobin to the fibrinogen from an affected family member, clotted only about one quarter of the fibrinogen as estimated from the turbidity of the solution (Fig. 3). After 2.5 hours when the maximum batroxobin induced polymerization was complete (as reflected by a constant absorbance at 350 nm), the fibrin product was wound around a glass rod and removed. Subsequent addition of thrombin caused an additional rapid increase in the turbidity of the solution. This result suggested that a portion of the affected family's fibrinogen was totally resistant to proteolysis by batroxobin, but was susceptible to thrombin mediated proteolysis. This view is supported by the data in Table II which show a decreased velocity of batroxobin mediated release of fibrinopeptide A from a patient's fibrinogen. As might be expected if the abnormal fibrinogen molecules in the sample were totally resistant to batroxobin, the yield of fibrinopeptide A from the fibrinogen of a patient was about one half of that obtained from normal fibrinogen even when the patient's fibrinogen was incubated with a large excess of batroxobin for prolonged times. These results, together with the slower thrombin-mediated release of fibrinopeptide A from a patient's fibrinogen seen with the radioimmunoassay, indicated that removal of fibrinopeptide A was the functionally defective step in the clotting of the abnormal fibrinogen.

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[5]A comparative study (using HPLC) of the kinetics of the thrombin catalyzed release of fibrinopeptides A and B from normal fibrinogen and the fibrinogen of an affected family member will be the subject of another study.
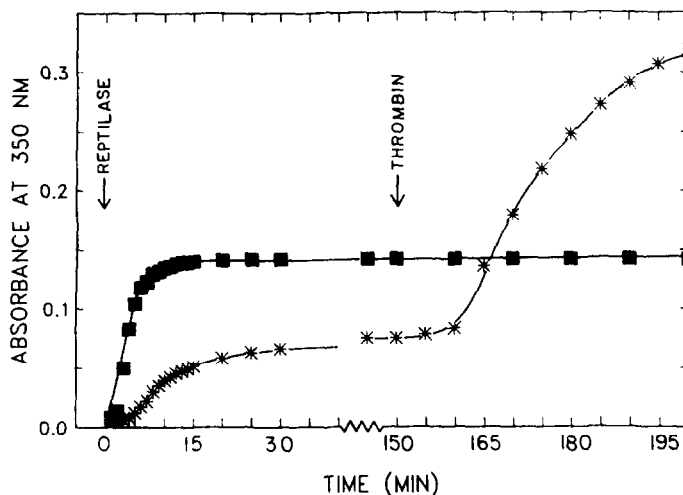
FIG. 3.

Batroxobin- and thrombin-mediated polymerization of normal
fibrinogen and fibrinogen from an affected individual.
Samples were in 0.05 M potassium phosphate buffer, 0.05 M
NaCl, pH 6.8. ■, 0.135 mg/ml normal fibrinogen; *, 0.27
mg/ml fibrinogen from an affected individual. The concen-
tration of the patient's fibrinogen was twice that of the
normal fibrinogen in order to obtain a fair comparison of
the polymerization of the two proteins, since batroxobin
mediated the release of fibrinopeptide A from only one
half of the patient's fibrinogen. At zero time, 50 μl of
reconstituted Reptilase-R was added to each 1.35-ml sample of
fibrinogen. After the maximum amount of polymerization had
occurred (150 min) the clots were removed from the solution,
causing the optical density at 350 nm to return to zero.
Thrombin (5 U) was then added to each sample. Any increase
in the absorbance of the sample is shown here as an addition-
al rise in the absorbance at 350 nm.

Polymerization of Preformed Fibrin Monomers. Fibrin monomers were
prepared from normal fibrinogen and the patient's fibrinogen under conditions
where about 90% of the fibrinogen had formed fibrin. When these monomers
were dissociated at low pH, and then returned to a neutral pH, the monomers
polymerized. No significant differences could be detected between the
polymerization (as monitored at 350 nm) of monomers from normal and the
patient's fibrinogen at an ionic strength of 0.12 or 0.42. This result
provided additional support for the view that the proteolysis step and not
the polymerization of fibrin monomers was the altered step in the clotting of
abnormal fibrinogen.

Covalent Cross-linking of Fibrin with Factor XIII. Fibrinogen
preparations which had been subjected to treatment with urea to inactivate
endogeneous Factor XIII (21) contaminants were clotted in the presence of
$CaCl_2$, 4.4 U/ml thrombin and 1 μl/ml normal plasma so as to provide the same
amount of Factor XIII to both samples. The progression of cross-linking was

TABLE II

Batroxobin Mediated Release of Fibrinopeptide A
From Fibrinogen of Normal Individuals and a Patient[a]

| Time (hr) | Fibrinopeptide A Released[b] (nmol/ml) | |
	Normal	Patient
0.25	1.4 (18)	1.0 (13)
0.50	2.7 (34)	1.7 (22)
1	4.3 (55)	2.9 (37)
2	6.3 (80)	3.8 (48)
6	7.3 (93)	4.3 (55)
6[c]	7.8 (99)	4.4 (56)

[a]Fibrinogen (1.34 mg/ml, 3.94 nmol/ml) in 0.25 M NaCl, 30 mM sodium phosphate pH 6.6 was incubated with 1 μl reconstituted Reptilase-R per ml fibrinogen solution at 37°C. At the indicated times 1 volume of 3 N perchloric acid was added to 10 volumes of the reaction mixture to stop the reaction. Following centrifugation, the amount of fibrinopeptide A in each sample was determined using high performance liquid chromatography as described in reference 26.

[b]The numbers in parentheses indicate the % yield of fibrinopeptide A based on two Aα-chains per molecule and a molecular weight of 340,000 for fibrinogen.

[c]This sample contained 100 μl reconstituted Reptilase-R per ml fibrinogen.

evaluated by reducing the fibrin and using PAGE in SDS to determine the extent of cross-linking of α- and β-chains. Both normal fibrinogen and the fibrinogen from an affected family member formed α-polymers and γ-dimers with the concomitant disappearance of the α-monomers and γ-monomers; however, the rate of cross-linking differed in the two samples. After 15 min, the γ-monomers could not be detected in the normal fibrinogen, but were still detectable in the sample of abnormal fibrinogen at one hour. After two hours, γ-monomers also were no longer seen in the sample of abnormal fibrinogen. The disappearance of α-monomers also was delayed in the sample of abnormal fibrinogen during the first hour of incubation.

The difference in the rates of cross-linking of abnormal and normal fibrinogen was abolished, however, when higher concentrations of thrombin (44 U/ml) were present. This result suggested that the delayed cross-linking is a secondary effect due to the slower proteolysis of the fibrinogen from an affected family member. The rate of formation of cross-linked fibrin from fibrinogen will reflect primarily the rate of the slowest step in this multistep process. At high thrombin concentrations where release of fibrinopeptide A is probably not determining the rate of conversion of soluble fibrinogen to cross-linked fibrin, no difference in the rates of formation of normal and abnormal cross-linked fibrins should be observed.

Plasmin-Mediated Degradation of Fibrinogen. Normal fibrinogen and fibrinogen from an affected family member were incubated with plasmin to

determine whether the abnormal fibrinogen exhibited altered degradation
products. At appropriate times 2 mM α-toluenesulfonyl fluoride was added to
samples of the incubation mixture to inhibit the plasmin. PAGE in SDS of the
samples before and after reduction with β-mercaptoethanol indicated that the
two fibrinogens were equally susceptible to proteolysis by plasmin. The
degradation products formed at identical rates in the two fibrinogen samples
and had similar molecular weights. Thus, the structural alteration in the
abnormal fibrinogen did not lead to a detectable alteration in the normal
pattern (27) of degradation of fibrinogen by plasmin.

DISCUSSION

     The propositus described here and several of his descendants have
dysfibrinogenemia, a qualitative alteration in their fibrinogen. The
congenital disorder was traced through four generations. It appeared to be
inherited in an autosomal dominant manner, as described for other dys-
fibrinogenemias (2-4). The observation that prolonged batroxobin treatment
of fibrinogen Petoskey mediated only partial release of fibrinopeptide A
(56%) and the observation that   batroxobin mediated only
partial polymerization of fibrinogen indicated that there are two species of
fibrinogen in the plasma of affected individuals. These observations provide
biochemical proof that affected family members are heterozygotes as suggested
by the mode of inheritance of the dysfibrinogenemia. The normal component of
the fibrinogen Petoskey was presumed to aggregate when it was treated with
batroxobin, because the removal of fibrinopeptide A alone is sufficient to
support end to end polymerization of normal fibrinogen (25).

     The absorbance measurements depicted in Fig. 3 suggest that only about
25% of the patient's fibrinogen polymerized normally upon treatment with
batroxobin. This result taken together with the data in Table II suggests
that batroxobin-mediated hydrolysis of half of an affected individual's
fibrinogen causes polymerization of only one quarter of the fibrinogen.
This apparent discrepancy might be due to the absorbance not being a linear
function of the degree of polymerization. An alternative explanation also
should be considered, however. Assuming that a single structural alteration
in one polypeptide chain is responsible for this abnormality, equal rates of
synthesis of the normal and abnormal polypeptide chains would result in 50%
of either the Aα-, Bβ-, or γ-chains containing the structural abnormality.
For purposes of illustration let us assume the abnormality is in the Aα-
chain.[6] Given that each fibrinogen molecule contains two of each of the
Aα-, Bβ-, and γ-chains, random assembly would result in a mixture in which
25% of the molecules contained two normal Aα-chains, 25% of the molecules
contained two abnormal Aα-chains, and 50% of the molecules contained one
normal and one abnormal Aα-chain. If polymerization (under the experimental
conditions used) required that both Aα-chains in a molecule be converted to
α-chains, batroxobin treatment would be expected to polymerize only 25% of
the fibrinogen upon release of 50% of the fibrinopeptide A. Further work is
underway to verify this hypothesis about the assembly of fibrinogen and the
structural requirements for polymerization.

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[6]The amino acid replacement in fibrinogen Petoskey is established in another
communication (26).

The dysfibrinogenemia reported in this work is one of a class of dysfibrinogenemias which exhibit an abnormality in the release of fibrinopeptide A. Of the 14 dysfibrinogenemias (18, 28-40) in this class only fibrinogen Bethesda I and fibrinogen New Orleans exhibit normal polymerization of fibrin monomers as does the abnormal fibrinogen reported here. Unlike this fibrinogen, however, fibrinogen Bethesda I (18) and fibrinogen New Orleans (37) inhibit the thrombin clotting time of normal plasma. It is interesting to note that fibrinogen Metz (36), like the abnormal fibrinogen reported here, is resistant to proteolysis by batroxobin. Fibrin monomers from fibrinogen Metz, however, exhibit altered rates of polymerization. Therefore, the fibrinogen reported in this work appears to be unique. Following accepted nomenclature, we designate this new dysfibrinogenemia, fibrinogen Petoskey.

Ultimately, the dysfibrinogenemias must be distinguished on the basis of the amino acid replacement responsible for the functional alteration. As exemplified by the characterization of fibrinogen Detroit (6), knowledge of dysfibrinogenemias should greatly enhance our understanding of the complex set of interactions necessary for the conversion of circulating fibrinogen to a covalently cross-linked fibrin clot. Fibrinogen Petoskey offers a unique opportunity to determine an important structural feature of fibrinogen which is essential for its susceptibility to batroxobin and which is important for its interaction with thrombin.

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