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B R I E F C O M M U N I C A T I O N

FIBRINOGEN BERGAMO III AND FIBRINOGEN TORINO: TWO FURTHER VARIANTS WITH HEREDITARY MOLECULAR DEFECTS IN FIBRINOPEPTIDE A

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INTRODUCTION

Dysfibrinogenemia has been recognized as a frequent cause of prolonged clotting time of plasma. About half of the reported cases were asymptomatic and most fibrinogen variants have been discovered incidentally. The majority of molecular defects reported thus far are clustered around the primary functional site of fibrinogen, i.e. in the vicinity of the thrombin-sensitive peptide bond in the Aα-chain. In most functionally abnormal fibrinogen variants, the arginine residue in position 16 of the Aα-chain has been found to be substituted by either histidine or cysteine (1). Pending more specific terminology, each abnormal fibrinogen has been designated by the city where it was first detected.

In this communication, we describe two additional fibrinogen variants, originating from Bergamo and Torino, in which Aα16Arg is replaced by His and Cys, respectively.

Key words: Dysfibrinogenemia, fibrinogen Bergamo III, fibrinogen Torino

MATERIALS AND METHODS

Thrombin time and reptilase time assays were performed in a Greiner Microcoagulometer (Greiner Electronics, Langenthal) by incubating 40 µl citrated plasma with 20 µl thrombin (7.5 NIH-units/ml bovine thrombin, Hofmann-La Roche, Basel) or with 20 µl reptilase reagent (Boehringer, Mannheim), respectively. Concentration of plasma fibrinogen was determined by functional (2), immunologic (3) and heat precipitation (4) methods.

Fibrinogen was isolated from plasma by affinity chromatography on fibrin monomer Sepharose CL-2B (5). Cysteine residues in mercaptolyzed fibrinogen were chemically modified by aminoethylation, methylation or carboxamidomethylation as recently reported (6). Kinetics of fibrin aggregation was assayed by recording the turbidity curve at 350 nm following addition of thrombin. Fibrinopeptide release was estimated by high performance liquid chromatography (HPLC) according to Kehl et al. (7) with some minor modifications (6).

RESULTS

Case reports:

Fibrinogen Bergamo III: The propositus was a 6-year-old boy. His plasma showed a prolonged thrombin time and reptilase time (Table 1), as well as decreased fibrinogen concentration when assayed by the functional test. Further studies revealed delayed clotting of his mother's plasma, while no coagulation defect was noted in his father. Both affected members of the family were asymptomatic. Biochemical studies were performed on fibrinogen of the propositus' mother.

TABLE 1

Results of laboratory assays on citrated plasma.

	Bergamo III		Torino		Normal plasma pool
	son	mother	daughter	mother	
Clotting time (sec)					
- thrombin	37	37	40	42	18
- reptilase	74	70	53	76	20
Fibrinogen (g/L)					
- functional	0.3	0.7	0.2	0.2	2.0
- immunologic	2.5	3.7	2.3	2.4	2.0
- heat precipitated	2.5	3.7	2.7	3.0	2.0

Fibrinogen Torino: The proposita, a 21-year-old female patient, was admitted to the hospital for recurrent edema of the legs. The results of phlebography were negative. Her plasma and the plasma of her mother suggested abnormal fibrinogen function resulting in prolonged clotting and reptilase times (Table 1). Normal coagulation values were found in the proposita's father and sister. There was no known bleeding or thrombotic tendency in other members of the family.

Biochemical characterization:

Upon addition of thrombin to purified preparations of fibrinogen Bergamo III or Torino, we observed a delayed onset of turbidity increase when compared to turbidity curve of the normal fibrinogen. The delay was potentiated following removal of calcium ions with 1 mM EDTA.

Fibrinopeptides A and B were completely cleaved from normal fibrinogen by thrombin (final concentration 0.04 NIH units/ml) within 24-h-incubation at 37°C (Fig. 1, panel 1). Under the same experimental conditions, the release of fibrinopeptide B from fibrinogen Bergamo III was also complete whereas a double peak of fibrinopeptide A was observed on HPLC (Fig. 1, panel 2); both the abnormal peak, denoted with an asterisk, and the normal peak were of approximately equal size. Normal amount of fibrinopeptide B was cleaved during 24 h from fibrinogen Torino while only about one half of the normal fibrinopeptide A was detected in elution diagram (Fig. 1, panel 3).

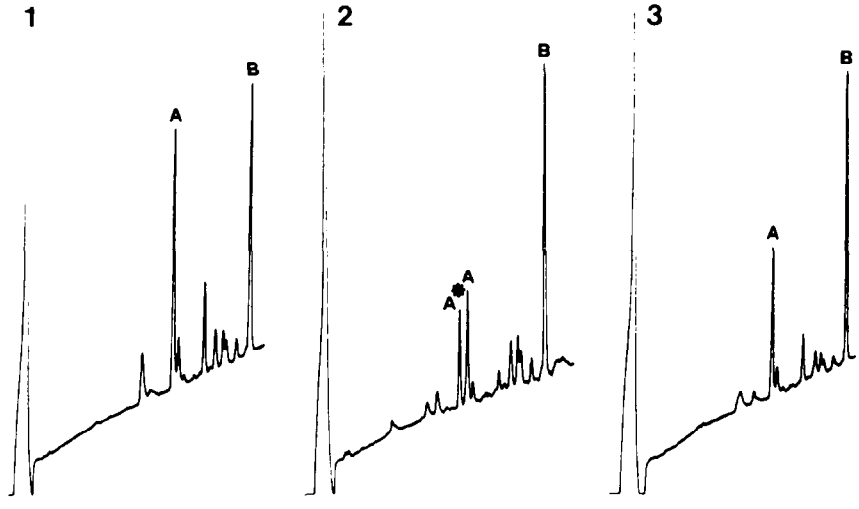

FIG. 1

HPLC diagrams of fibrinopeptides A and B, cleaved from normal fibrinogen (1), fibrinogen Bergamo III (2) and fibrinogen Torino (3) during 24-h-incubation with thrombin. A*, abnormal fibrinopeptide A.

The abnormal peak A* was collected and subjected to amino acid analysis. Its amino acid composition was identical with that of the normal fibrinopeptide A except in that the abnormal peak contained 0.85 mol His and 0.15 mol Arg per mol peptide, while the normal fibrinopeptide A lacks His and contains one mol Arg per mol peptide.

Fibrinogen Torino was mercaptolyzed and the resulting sulfhydryl groups were reacted with either ethylene imine, methyl iodide or iodoacetamide. The chemically modified samples were subjected to treatment with thrombin as described elsewhere (6). The released peptides were analyzed by HPLC. The aminoethylated fibrinopeptide A was eluted closely before its normal counterpart. The methylated extended fibrinopeptide A (Aα 1-19) had a slightly longer retention time than normal fibrinopeptide B, and the carboxymethylated peak Aα 1-19 appeared in the elution diagram midway between normal fibrinopeptides A and B. The observed retention times of the chemically modified peptides perfectly agree with those reported for fibrinopeptide A* derivatives of fibrinogen Bergamo I (6).

DISCUSSION

Two functionally abnormal fibrinogen variants are described, in which the release of fibrinopeptide A is incomplete. Both patients are heterozygotes as judged from the medical history of the affected families. Moreover, upon treatment with thrombin, both fibrinogen preparations produced about 50 % of normal fibrinopeptide A.

From fibrinogen Bergamo III, an abnormal peptide was slowly released by thrombin, which was separated from the normal fibrinopeptide A by HPLC. Results of amino acid analysis indicate that the arginine residue in position 16 of the Aα-chain is replaced by histidine. The same amino acid substitution has up to now been reported in at least ten abnormal fibrinogens (1); in fact, the Aα16Arg $\longrightarrow$ His exchange appears to be the most frequent hereditary functional defect in human fibrinogen.

Only about half the normal amount of fibrinopeptide A was cleaved from fibrinogen Torino, suggesting that this fibrinogen preparation contains, in addition to normal fibrinogen, an abnormal population of fibrinogen molecules with a thrombin-resistant peptide bond Aα16-17. A replacement of Aα16Arg $\longrightarrow$ Cys was confirmed by chemical modifications of the sulfhydryl groups via aminoethylation which rendered the Aα 16-17 bond cleavable by thrombin. Thus, fibrinogen Torino shares its molecular defect with at least eight other hereditary abnormal fibrinogens composing the second largest group of fibrinogen variants (1).

The strikingly frequent substitutions AαArg16 $\longrightarrow$ His as well as Aα16Arg $\longrightarrow$ Cys in apparently unrelated families, compared with only isolated cases of other defects in the amino-terminus of the Aα-chain, e.g. 7Asp $\longrightarrow$ Asn, 12Gly $\longrightarrow$ Val, 19Arg $\longrightarrow$ Ser and

19Arg → Asn (1), suggest that the former two mutations may have repeatedly occurred in several persons rather than having been inherited from a single ancestor.

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REFERENCES

1. Henschen,A., Kehl,M. and Southan,C. Genetically abnormal fibrinogens - strategies for structure elucidation, including fibrinopeptide analysis. In: Variants of human fibrinogen. E.A. Beck and M. Furlan (Eds.) Bern-Stuttgart-Toronto: Hans Huber Publishers, 1984, pp. 273-320.

2. Clauss,A. Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. Acta Haematol. 17, 237-246, 1957.

3. Laurell,C.B. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal. Biochem. 15, 45-52, 1966.

4. Schulz,F.H. Eine einfache Bewertungsmethode von Leberparenchymschäden (volumetrische Fibrinbestimmung). Acta hepatolog. 3, 306-310, 1955.

5. Furlan,M., Rupp,C., Beck,E.A. and Svendsen,L. Effect of calcium and synthetic peptides on fibrin polymerization. Thromb. Haemostas. 47, 118-121, 1982.

6. Reber,P., Furlan,M., Beck,E.A., Finazzi,G., Buelli,M. and Barbui,T. Fibrinogen Bergamo I (Aα16Arg→Cys): Susceptibility towards thrombin following aminoethylation, methylation or carboxamidomethylation of cysteine residues. Thromb. Haemostas. 54, 390-393, 1985.

7. Kehl,M., Lottspeich,F. and Henschen,A. Analysis of human fibrinopeptides by high-performance liquid chromatography. Hoppe-Seyler's Z. Physiol. Chem. 362, 1661-1664, 1981.