

Fibrinogen-Fibrin Degradation Products

HAU C. KWAAN, M.D.

*Department of Medicine,
Northwestern University Medical School,
and VA Lakeside Hospital,
Chicago, IL 60611*

ABSTRACT

The identification of the molecular structure of fibrinogen has given a better understanding of many clinical conditions associated with abnormalities of fibrinogen. Proteolysis of fibrinogen or fibrin by its natural lytic enzyme, plasmin, leads to the formation of degradation products which are of great importance in the laboratory diagnosis of many pathological conditions. The biochemical and biological properties of these fibrinogen-fibrin degradation products and their value in the diagnosis of disseminated intravascular coagulation are presented.

Introduction

When fibrin is laid down intravascularly to form a hemostatic barrier or when it is formed extravascularly in exudates in inflammatory tissues for the localization of infections, foreign bodies or tumor cells, it is subjected to proteolysis. Most of this is carried out by the fibrinolytic enzyme plasmin. The resulting breakdown products are particles of different molecular sizes collectively known as fibrin degradation products. In addition, plasma fibrinogen itself can also be subjected to the proteolytic action of plasmin whenever there is excessive fibrinolytic activity in blood. The resulting breakdown products are known as fibrinogen degradation products. The biological behavior of these latter products is essentially similar to that of fibrin

degradation products, and they shall all be referred to as fibrinogen-fibrin degradation products (FDP) in this article. Excellent reviews on this topic are available in recent literature.^{10, 12, 13, 17}

Structural Considerations of the Fibrinogen Molecule

Mammalian fibrinogen has a dimeric structure, each consisting of three peptide chains. It can be represented^{2, 4} by the formula $(\alpha (A) \beta (B) \gamma)^2$. It has a molecular size of 340,000 with two symmetrical monomers of 170,000 joined by disulphide bonds between the respective $\alpha (A)$ and γ chains. When fibrinogen is clotted by thrombin, the process involves the cleavage of a part of the $\alpha (A)$ chain at the N-terminal known as fibrinopeptide A

and of a part of the β (B) chain at the N-terminal known as fibrinopeptide B at the arginyl-glycyl bonds. Besides thrombin, other proteases can also convert fibrinogen to fibrin.^{3,4} Ancrod and reptilase, two different glycoproteins extracted from snake venom, remove only fibrinopeptide A from fibrinogen but not fibrinopeptide B.^{11,15} This property has been utilized in the Reptilase time or Ancrod time tests which will be noted. Interestingly, the cleavage of only fibrinopeptide B but not fibrinopeptide A, such as seen by the action of another venom of the viper *Agkistrodon contortrix*, will not result in clotting until sufficient fibrinopeptide A is split off as well.³

Fibrinogen-fibrin Degradation Products

The proteolytic action of plasmin on the fibrinogen molecule, on the other hand, results in the cleavage of peptide bonds on other loci, leading to the formation of the FDP's. Several stages of the fragmentation of fibrinogen are known.¹⁶ The earliest product is termed fragment X, with a molecular size of 240,000. Proteolysis of this fragment X results in the formation of fragment Y, with a molecular size of 155,000, along with a smaller piece, fragment D with a molecular size of 83,000. The final stage of fragmentation involves the breakdown of fragment Y into a second fragment D and another smaller moiety, fragment E, with a molecular size of 50,000.

The proteolysis of fibrin by plasmin involves essentially the same steps as that of fibrinogen. It has been shown that fragment E is located at the N-terminal of the fibrinogen molecule and contains the fibrinopeptide A. Thus, the fragment E derived from fibrinogen would be different from that derived from fibrin. Fragment D is located at the COOH-terminal and, as such, has the same structure whether it is derived from fibrin or fib-

rinogen.¹⁴ Fragment D also contains the site for fibrin cross-linking.⁷

FDP is a potent anticoagulant, being able to inhibit the polymerization of fibrin as well as competitively inhibit the clotting action of thrombin.¹ In addition, the smaller FDP fragments impair platelet adhesiveness, aggregation and release reactions, while the larger FDP and FDP-fibrinogen monomer complexes have the opposite effect.²⁰ In the presence of a mixture of FDP's varying in size, the net effect on platelet function would depend on the composition of the FDP. Thus, in disseminated intravascular coagulation, increased platelet aggregation may be encountered since large FDP fragments predominate.

Other biological actions of FDP are weak and, therefore, of less clinical significance. The smaller FDP fragments may cause the induction of smooth muscle contraction, enhancement of the contractive action of bradykinin, increase in capillary permeability and chemotaxis of granulocytes.

There are several ways with which the FDP may be quantitatively measured in blood, urine or other body fluids. The choice of these methods depends on the degree of sensitivity of the test results desired and the need for a rapid answer, such as required in a clinical case with massive bleeding. During the collection of the test samples for assay, precautions must always be taken against artifactual production of FDP *in vitro* by the spontaneous proteolysis that may occur. It is common practice to use soybean trypsin inhibitor, aprotinin or epsilon aminocaproic acid as effective protease inhibitors in the collection tubes.

The most popular clinical method is based on immunological identification of the FDP. One common antigenic determinant is shared by fibrinogen, fibrin and fragments X, Y, D and E. To increase the sensitivity of the test to the smaller fragments, most commercially available test

TABLE I

Clinical Disorders In Which FDP's* May Be Present
In Abnormally High Quantities In the Blood

Intravascular origin
Derived from fibrinogen
Pathologic fibrinolysis
Thrombolytic therapy
Derived from fibrin
Disseminated intravascular coagulation
Pulmonary embolism
Extensive deep vein thrombosis
Therapeutic defibrination with ancrod or reptilase
Extravascular origin
Large haematoma
Tumor
Sepsis
After extensive surgery
Pregnancy
Normal labor
Abruptio placentae
Toxemia of pregnancy
Renal diseases
Acute glomerulonephritis
Proliferative glomerulonephritis
Renal homograft rejection
Nephrotic syndrome
Lupus nephritis
Schonlein-henoch nephritis

*Fibrin degradation products.

systems such as the Wellcotest* utilizes the antiserum against fragments D and E. Immunologic agglutinations using tanned red cells or latex beads are the two most commonly used tests. Precipitin, immunodiffusion, or even flocculation methods are also available. The most sensitive test seems to be the tanned red cell hemagglutination inhibition immunoassay (TRCHII),¹⁸ which can detect as little as 0.6 μg per ml of fragment X or Y or 1.2 μg per ml of fragment D, but is relatively less sensitive in the detection of fragment E.

Since the FDP has strong anticoagulant properties, all tests based on the clotting of fibrinogen as the end-point are prolonged by the presence of FDP. Consequently, the activated clotting time, prothrombin time, partial thromboplastin time and thrombin time are all affected by FDP. As mentioned previously, two

extracts from viper venom, Ancrod and Reptilase, can convert fibrinogen to fibrin. This process is inhibited by the anticoagulant action of FDP, but not by the anticoagulant action of heparin. Therefore, the performance of an Ancrod time test or a Reptilase time test is often useful to differentiate in a sample, the anticoagulant activity of FDP from that of heparin.⁶

An interesting property of fibrinogen and FDP in producing the clumping of a suspension of staphylococci has been utilized in a test for FDP.⁸ This test has the drawback of lack of sensitivity to fragments D and E and is less commonly used. One other group of tests for FDP deals with the phenomenon of paracoagulation. When fibrin monomers or fragment X are formed, they may form soluble complexes with the other FDP fragments. On the addition of ethanol or protamine sulphate, these soluble complexes will dissociate leaving the fibrin monomer or the fragment X to undergo gel or fibrin strand formation. This paracoagulation forms the basis of the ethanol gelation test⁵ and the protamine sulphate test,¹⁹ and is often used to differentiate plasma in acute disseminated intravascular coagulation (when they are often positive) from that in pathological fibrinolysis.

Clinical Significance of FDP

Since fibrinogen or fibrin may be found both intravascularly and extravascularly, FDP resulting from proteolysis may be detected in blood as well as in many other body fluids in a wide variety of pathological conditions. Even under physiological conditions, fibrinogen catabolism would release small amounts of FDP, with as much as 8 μg per ml detectable in blood.

In table I, an attempt is made to list a few common pathological disorders in which abnormally high FDP levels may be found in the blood. It can be seen that a careful study of the clinical and other lab-

* Prepared by the Burroughs Wellcome Company.

oratory findings is needed in each case before a firm diagnosis can be made. A common error is to assume that a high blood FDP level is diagnostic of disseminated intravascular coagulation. This is frequently not the case even though the highest FDP levels are found in this condition.

References

1. BANG, N. U., FLETCHER, A. P., ALKJAERSIG, N., and SHERRY, S.: Pathogenesis of the coagulation defect developing during pathological plasma proteolytic (fibrinolytic) states. III. Demonstration of abnormal clot structures by electron microscopy. *J. Clin. Invest.* 41:935, 1962.
2. BLOMBACK, B.: Fibrinogen to fibrin transformation. *Blood Clotting Enzymology*. Seegers, W. H., ed. New York, Academic Press, 1967, p. 143.
3. BLOMBACK, B.: Studies on the action of thrombin enzymes on bovine fi-frinogen as measured by N-terminal analysis. *Arkiv Kemi.* 12:321, 1958.
4. BLOMBACK, M., BLOMBACK, B., HESSEL, B., IWANAGA, S., and WOODS, K. R.: N-terminal disulphide knot of human fibrinogen. *Nature* 218:130, 1968.
5. BREEN, F. A., JR. and TULLIS, J. L.: Ethanol gelation. A rapid screening test for intravascular coagulation. *Ann. Int. Med.* 69:7, 1968.
6. FUNK, C., GMUR, J., HEROLD, R., and STRAUB, P. W.: Reptilase. A new reagent in blood coagulation. *Brit. J. Hematol.* 21:41, 1971.
7. GAFFNEY, P. H.: Subunit relationships between fibrinogen and fibrin degradation products. *Thromb. Res.* 2:201, 1973.
8. HAWIGER, J., NIEWIAROWSKI, S., GUREWICH, V., and THOMAS, D. P.: Measurement of fibrinogen and fibrin degradation products in serum by staphylococcal clumping test. *J. Lab. Clin. Med.* 75:93, 1970.
9. HERZIG, R. N., RATNOFF, O. B., and SHINOFF, J. R.: Studies on the procoagulant factor of southern copperhead snake venom: The preferential release of fibrinopeptide. *Brit. J. Lab. Clin. Med.* 76:451, 1970.
10. KWAAN, H. C. and BARLOW, G. H.: Nature and biological activities of degradation products of fibrinogen and fibrin. *Ann. Rev. Med.* 24:335, 1973.
11. KWAAN, H. C. and BARLOW, G. H.: The mechanism of action of Arvin and Reptilase. *Thromb. Diath. Hemorrh. Suppl* 47:361, 1971.
12. KWAAN, H. C. and HATEM, A. A.: Clinical significance of fibrinogen/fibrin degradation products. *Thromboembolism: Advances in Aetiology, Prevention and Management*. Nicolaides, A. N., ed. Lancaster, England, MTP Medical Publishing Co., 1975, p. 29.
13. KOWALSKI, E.: Fibrinogen derivatives and their biological activities. *Seminar Hematol.* 5:45, 1968.
14. LATALLO, Z. S., TEISSEYRE, T., WEGRZYNOWICZ, Z., and KIPEC, M.: Degradation of fibrinogen by proteolytic enzymes. *Scand. J. Hematol. Suppl.* 13:15, 1970.
15. LAURENT, T. C. and BLOMBACK, B.: On the significance of the release of two different peptides from fibrinogen during clotting. *Acta Chem. Scand.* 12:1875, 1958.
16. MARDER, V. J., BUDZYNSKI, A. Z., and JAMES, J. L.: High molecular weight derivatives of human fibrinogen produced by plasmin III. Their NH-terminal amino acids and comparison with the NH₂-terminal Di-sulfide knot. *J. Biol. Chem.* 24:4755, 1972.
17. MARDER, V. J., MATCHETT, M. O., and SHERRY, S.: Detection of serum fibrinogen and fibrin degradation products. Comparison of six techniques using purified products and application in clinical studies. *Amer. J. Med.* 51:71, 1971.
18. MERSKEY, C., LALEZARE, P., and JOHNSON, A. J.: A rapid, simple sensitive method for measuring fibrinolytic split products in human serum. *Proc. Soc. Exp. Biol. Med.* 131:871, 1960.
19. NIEWIAROWSKI, S. and GUREWICH, V.: Laboratory identification intravascular coagulation. The serial dilution protamine sulfate test for the detection of fibrin monomer and degradation products. *J. Lab. Clin. Med.* 77:665, 1971.
20. NIEWIAROWSKI, S., REAN, V. J., and THOMAS, D. P.: Effect of fibrinogen derivatives on platelet aggregation. *Thromb. Diath. Hemorrh. Suppl.* 42:49, 1970.