ULTRASTRUCTURE OF UNSTABILIZED FIBRIN

A. M. Ul'yanov and E. S. Zhitnikova

UDC 612.115.13-086.3

The results of electron-microscopic investigations showed that unstabilized fibrin consists of a network of fibers (fibrils) from 2 to 10 μ long and up to 2000 Å wide. Negative staining with uranyl acetate showed that unstabilized fibrils are composed of 5 to 10 longitudinally organized protofibrils and possess cross-striation with a long period (about 200 Å). Comparison of the ultrastructure of unstabilized fibrin fibers with fibers stabilized by factor $XIII_a$ revealed their morphological similarity.

KEY WORDS: stabilized fibrin; unstabilized fibrin.

In the modern view the process of fibrin formation incorporates the following basic stages; the enzymic action of thrombin on fibrinogen with the formation of fibrin monomer; aggregation of molecules of fibrin monomer into a fibrin aggregate (unstabilized fibrin); conversion of the latter under the influence of factor XIII, activated by thrombin (factor $XIII_a$) into stabilized fibrin [10-12]. From the biochemical point of view, stabilized fibrin is characterized by the presence of γ-glutamyl-ε-lysine cross linkages between the γ and α chains of residues of fibrinogen molecules [11-12].

There are certain differences between the physiological properties of stabilized and unstabilized fibrin. For instance, stabilized fibrin dissolves under the influence of the enzyme plasmin or its activated form – the plasmin–heparin complex [3]. A characteristic property of unstabilized fibrin is its ability to go into solution both under the influence of the agents already mentioned and also through the action of complexes of heparin with certain plasma proteins and some biogenic amines formed in the blood stream during excitation of the anticlotting system [3, 7-9]. Unstabilized fibrin is also rendered soluble by urea, monochloroacetic acid, and other agents [1-3]. It is not clear whether, besides differences in the biochemical and physiological properties of stabilized and unstabilized fibrin, any other differences exist in their ultrastructure.

The object of this investigation was to make an electron-microscopic study of the structure of unstabilized fibrin and to compare it with that of fibrin stabilized by factor $XIII_a$.

EXPERIMENTAL METHOD

Fibrinogen and thrombin from the Kaunas Bacteriological Preparations Factory and factor XIII obtained from bovine plasma by the method of Loewy et al. were used.

Unstabilized fibrin was prepared by the method of Kudryashov et al. [4] directly on 200-mesh grids from Tesla (Czechoslovakia), coated with Formvar film. To one drop of a solution of fibrinogen with inhibitors of stabilization and enzymic fibrinolysis, applied to the grid, an equal volume of thrombin in a concentration of 0.1 mg/ml (1 unit/ml) was added. After the formation of visible fibrin threads (after 15-60 min) the excess of fluid was soaked up with filter paper, after which the grids with the preparations were washed in 0.85% NaCl solution (pH 7.34). The preparations were negatively stained in 2% phosphotungstic acid (PTA) or 2% uranyl acetate. Before staining with uranyl acetate they were fixed with 0.5% glutaraldehyde solution, pH 7.34. The grids with the preparations were then dried. The specimens were examined in the IEOL electron microscope.

Stabilized fibrin was obtained by treating the fibrin formed without addition of inhibitor of stabilization to the fibrinogen solution with a solution of factor XIII, activated with thrombin in the presence of Ca^{2+}. Preparations of factor XIII with an activity of 500-800 units/ml were used. After the addition of factor $XIII_a$ the grids with the preparation of fibrin were incubated for 15-20 min at 37°C, after which they were treated as described above.

Laboratory of Physiology and Biochemistry of Blood Clotting, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 2, pp. 237-239, February, 1978. Original article submitted July 1, 1977.

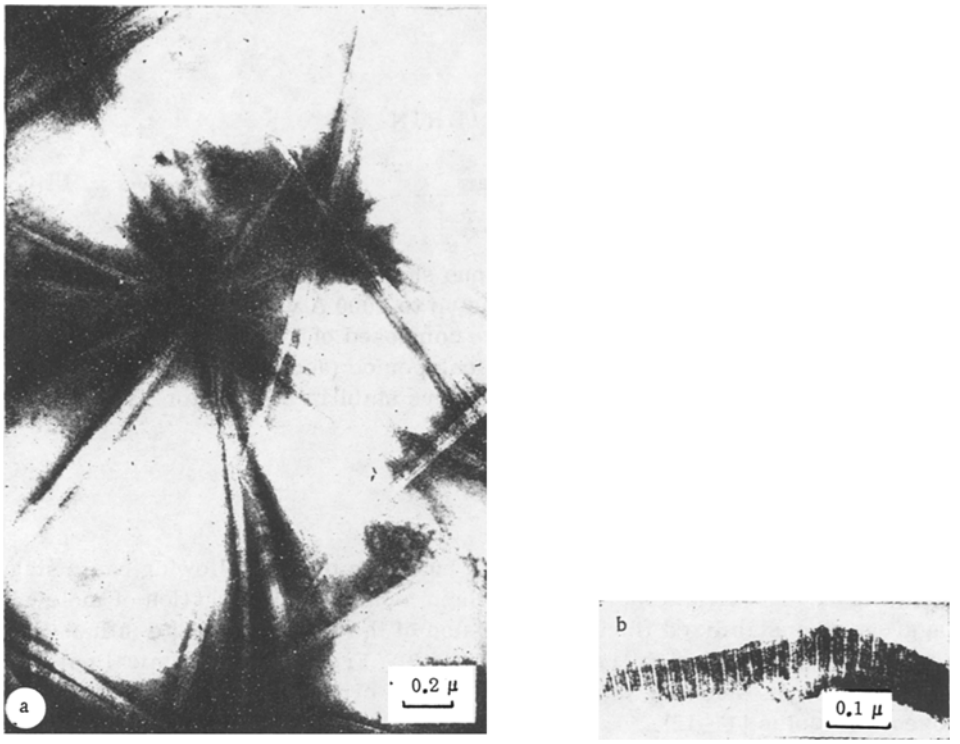

Fig. 1. Unstabilized fibrin: a) Negatively stained with PTA, 40,000×; b) negatively stained with uranyl acetate, 80,000×.

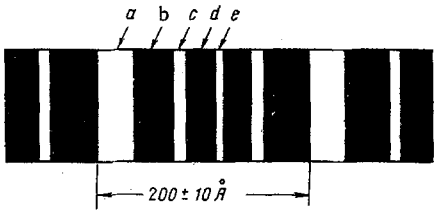

Fig. 2. Scheme of cross-striation of fibers of unstabilized fibrin. Explanation in text.

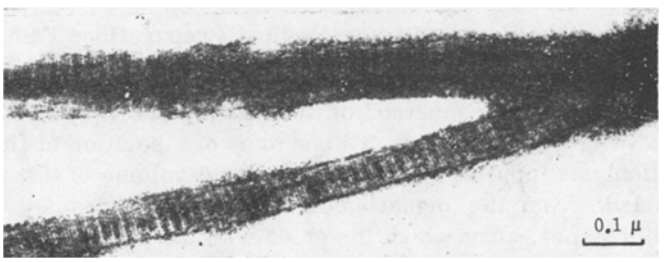

Fig. 3. Stabilized fibrin. Negatively stained with uranyl acetate, 90,000×.

EXPERIMENTAL RESULTS

The electron-microscopic investigation of unstabilized fibrin showed it to be a network of fibrillary structures without any regular arrangement and with high angular spread relative to one another (Fig. 1a). In their external appearance the fibrin fibers (fibrils) consisted of formations of two types: spindle-shaped (thickened in the central part and pointed at the end) and split at the ends, resembling the bristles of a brush. Internal granularity, with a clearly defined longitudinal orientation, was observed in preparations of unstabilized fibrin stained with PTA (Fig. 1a). These were presumably extended molecules of fibrin monomer,

aggregated end-to-end and forming a structure of higher fibrillary organization. The length of the fibers was 10 μ or more and their width 700-2000 Å.

The longitudinal structure of the fibrils was characterized by the presence of protofibrils, forming the basis of the fiber. The investigation showed that the fibrils consisted of 5 to 10 protofibrils about 100 Å in diameter. The distance between the protofibrils was 40-70 Å. The protofibrils were constructed from chains of globular formations, consisting of residues of fibrinogen molecules. These globules as a rule were separated from each other by spaces of 20-50 Å. However, often they were almost touching each other. Between the globules there were intermediate formations consisting of protein material of lower electron density. Large quantities of free globular protein material were observed around the fibrils. Both separate protofibrils and pairs of protofibrils, diverging at an acute angle, were present in the preparation of unstabilized fibrin (Fig. 1). Union of fibrils to form a wider fibrin band could be seen. The structures described above are evidently different degrees of fibrin formation.

The character of the fine morphology of fibers of unstabilized fibrin, shown in Fig. 1a, was obtained by the use of PTA for negative staining, which does not permit the cross-striation of the fibers to be demonstrated. The fibrin fibers shown in Fig. 1a also were relatively young (they were formed 10-15 min after the addition of thrombin to the fibrinogen solution). As Szalontai [15] points out, growing fibers which have not yet reached their largest size are characteristically spindle-shaped.

When uranyl acetate was used as the staining material, a more complex internal structure was revealed in the fibers of unstabilized fibrin. Both transverse and longitudinal striation of the fibrin fibers was observed on the electron micrograph (Fig. 1b). The long period of cross-striation, due to the boundaries between the widest light *a* bands (width 35 Å), was 200 ± 10 Å (Fig. 2). Between the principal bands, two wide dark b bands (each measuring 40 Å), two medium dark d bands (26 Å) and one light central e band (7 Å) were observed to repeat regularly.

The morphological characteristics of the unstabilized fibrin fibers as established above agreed with observations by other workers on stabilized fibrin [2, 5, 6, 14].

However, the results of other investigations [1] showed the absence of cross-striation in fibrin fibers when the activity of factor XIII was reduced. According to these workers, the presence of a transverse structure in fibrin depends directly on an adequate level of factor XIII activity in the medium (in the blood).

The preparations of stabilized fibrin stained with uranyl acetate obtained during the present investigation are illustrated in Fig. 3. Analysis of the ultrastructure of the stabilized fibrin shows that all its basic morphological characteristics (length and width of the fibers, character of the cross-striation) are similar to those established for mature fibers of unstabilized fibrin. However, the number of protofibrils in the fibers of stabilized fibrin is increased up to 20-30 because of their more compact arrangement.

The enzymic action of factor $XIII_a$ on the fibrin monomer aggregate (unstabilized fibrin), giving rise to definite biochemical structural changes and, in particular, causing the appearance of γ-glutamyl-ε-lysine covalent cross-linkages [11], is evidently not reflected in the ultrastructure of the fibrin fiber.

The authors are grateful to Professor Yu. S. Chentsov for continuous attention and help with the work and also for valuable comments during the discussion of the results.

LITERATURE CITED

1. V. P. Baluda, A. G. Aladatov, and Yu. T. Ponomarev, Byull. Éksp. Biol. Med., No. 4, 109 (1971).
2. V. A. Belitser and T. V. Varetskaya, Usp. Sovrem. Biol., 80, No. 4, 5 (1975).
3. B. A. Kudryashov, Biological Problems of Regulation of the Liquid State of the Blood and Its Coagulation [in Russian], Moscow (1975).
4. B. A. Kudryashov, L. A. Lyapina, and I. P. Baskova, Vestn. Mosk. Gos. Univ. Ser., Biol., No. 5, 41 (1974).
5. N. N. Bang, Thromb. Diath. Haemorrh. (Stuttgart), 10, 13 (1966).
6. D. Kay and B. J. Cuddigan, Br. J. Haematol., 13, 341 (1967).
7. B. A. Kudrjashov (B. A. Kudryashov), in: International Society of Haematology. Third Meeting of the European and African Division, Abstracts, London (1975), p. 25: 04.
8. B. A. Kudrjashov (B. A. Kudryashov), Folia Haematol. (Leipzig), 102, 115 (1975).
9. B. A. Kudrjashov (B. A. Kudryashov), et al., Folia Haematol. (Leipzig), 98, 312 (1972).
10. K. Laki and L. Lorand, Science, 108, 280 (1948).
11. A. G. Loewy, Ann. N. Y. Acad. Sci., 202, 41 (1972).

12. L. Lorand, Ann. N. Y. Acad. Sci., 202, 6 (1972).
13. L. Pouit, G. Marcille, M. Suscillon, et al., Thromb. Diath. Haemorrh. (Stuttgart), 27, 559 (1972).