Electron Microscopy of Fibrin Paris I

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Fibrinogen Paris I, a congenital fibrinogen abnormality, is characterized by delayed fibrin aggregation and poor clot retraction owing to the replacement of normal γ-chains by mutant γ-chains, which are termed γ-Paris I. Available evidence indicates that the structural abnormality involves the amino acid sequence near the COOH-terminus of the mutant chain and probably includes the region containing the normal γ-chain crosslinking site. Electron microscopy was carried out on Paris I fibrin. In place of the normally interwoven network of branching cross-striated fibers, negatively or positively contrasted Paris I fibrin was characterized by nonfibrous clumps of material connected by distinct fibrous strands tending to be thinner and more irregular in width than normal fibrin. Most Paris I fibrin fibers tended to be aperiodic, although cross-striations were observed occasionally in negatively contrasted specimens and rarely in positively contrasted specimens. In addition, Paris I fibrin frequently showed relatively short, abruptly terminating fibers. The gross ultrastructural differences between normal and Paris I fibrin suggest that for fibrin assembly to take place normally, a region(s) in the fibrin molecule near to or possibly overlapping the COOH-terminal γ-chain crosslinking site must be preserved or at least not sterically hindered.

Fibrinogen Paris I1 is a congenital fibrinogen abnormality due to a γ-chain defect.2,3 The thrombin and reptilase clotting times of fibrinogen Paris I are markedly prolonged owing to delay in the aggregation phase of clot formation,4,5 clot retraction is poor.1 These abnormal molecules also possess the ability to delay the aggregation1,4 and factor XIIIa-catalyzed crosslinking of normal fibrin molecules.3 These properties can be accounted for by the presence of mutant γ-chains, termed γ-Paris I, that replace 50% or more of the normal γ-chain population in circulating plasma molecules.2,3 Unlike normal γ-chains, γ-Paris I chains do not undergo intermolecular crosslinking in the presence of factor XIIIa to form covalently linked γ-dimers,2,3 nor do they incorporate the fluorescent amine donor, dansylcadaverine.3 These observations show that the crosslinking site located in the COOH-terminal region of the normal γ-chain either is not present or is unavailable for crosslinking in γ-Paris I chains. Together with other evidence,3,8,9 they indicate that the structural abnormality involves the amino acid sequence in the COOH-terminal region of the mutant chain, although the extent of the difference is not yet known.

The functional abnormalities of congenital fibrinogen abnormalities frequently are reflected by ultrastructural abnormalities, as assessed by electron microscopy of fibrin clots.8,9,14 This seems to be the case for fibrinogen Paris I as well, judging from the appearance of a low-power micrograph of an ultrathin section of a Paris I fibrin clot;15 that photograph showed nondescript collections of material giving no suggestion of normal fiber formation. In this article, we report recent electron microscopic observations of Paris I fibrin that extend existing data and permit a more detailed ultrastructural comparison with normal fibrin.

MATERIALS AND METHODS

Normal human fibrinogen fraction I-4 was prepared as previously described14 and stored frozen as a stock solution of 11 mg/ml in 0.3 M NaCl. Fibrinogen Paris I was prepared from citrated plasma by precipitation with 2.1 M glycine17 and stored frozen as a stock solution of 2 mg/ml in 0.3 M NaCl.

Fibrin for microscopy was deposited on formvar-coated copper grids or on these same grids that had been coated with carbon film. Clots were formed in the cold (~2°C) in order to optimize conditions for observing periodic banding.18 The fibrinogen preparations had been diluted to a final protein concentration of 500–700 μg/ml and an NaCl concentration of 0.075 M. The solution was buffered at pH 7 with a Na phosphate buffer (0.025 or 0.050 M) and was maintained at ~2°C by immersion in an ice bath. Purified human thrombin (lot H-1, Bureau of Biologies, FDA), 10–20 US U/ml, was added to a purified fibrinogen solution in a volume ratio of 1:10. Immediately after this addition, a drop of the as yet ungelled mixture was placed on a glass slide that had been resting on a bed of crushed ice. Formvar-coated grids were then placed face down on the drop and allowed to remain in that position for 10–20 min in the case of normal fibrin samples, or for 20 min or longer in the case of Paris I samples. Following this incubation period, the grids were removed by pulling them from the clot in a tangential direction to the clot surface. Each grid was washed twice with a solution of 0.15 M NaCl and then with H2O. Negative staining was carried out for 5–10 min with a 1% solution of uranyl acetate, pH 4.3.15 Positive staining was carried out for 5–10 min with a 2% solution of phosphotungstic acid, pH 4, followed by several washes with H2O.18,20

For certain experiments, fibrin was formed directly from citrated plasma by diluting the plasma with two parts of ice cold water.
followed by the addition, in rapid succession, of ice cold CaCl₂ (final concentration, 15 mM) and thrombin (final concentration, 5 US U/ml). The clotting solutions were then immediately deposited on ice-cold glass slides and processed exactly as described above for fibrin derived from purified fibrinogen.

Electron microscopy was carried out using a Siemens Elmiskop model IA or JOEL model 100 C microscope at 80 kV.

RESULTS AND DISCUSSION

Electron microscopy of negatively stained normal fibrin at relatively low magnification reveals an interwoven network of branching fibers that occasionally become gradually tapered and terminating (Fig. 1). Most fibers, particularly when clotting takes place in the cold, usually possess prominent cross-striations, having an interval of about 20–24 nm. Observation at higher power reveals finer intraperiodic structural details (Fig. 2). Many fibers, in addition to the major stain-excluding band, show three other minor stain-excluding band-like structures—this characteristic staining pattern has been well described. Other fibers, often in the same field, do not display the minor banding and the cross-striations, but appear instead as a series of somewhat irregular, relatively broad, stain-excluding bands. Aperiodic fibers can usually also be found. These well described variations in the negative staining pattern could be due to differences in the degree of stain penetration, to minor differences in packing and organization of individual fibrin molecules, to differences in preparative procedures, and/or to other less well definable factors.

Positively stained normal fibrin fibers appear as alternating thin and wide dark bands. Each of these bands has the same periodic interval (i.e., 20–24 nm), as in negatively stained preparations (Fig. 3).

Fibrin fibers prepared from purified Paris I fibrinogen or from Paris I plasma differed from normal fibers in several respects. Images taken at relatively low magnification (Fig. 1) showed that the Paris I clot was organized differently than normal fibrin. A prominent feature of negatively or positively contrasted specimens of fibrin Paris I was nonfibrous clumps of material that were invariably connected by distinct fibrous structures tending to be thinner and less regular in width than normal fibrin fibers. These fibers often were extensively branched or overlapped to form a network. In addition, Paris I fibrin frequently showed relatively short, abruptly terminating fibers. When such tapering fibers are observed in normal fibrin, they usually do not terminate as abruptly.

At higher magnification, most negatively stained Paris I fibers were aperiodic although occasional fields did reveal cross-striations displaying the same type of banding as for normal fibrin fibers (Fig. 2). Finer intraperiodic structural details, such as those described above for normal fibrin fibers, could not be
appreciated. Fibrin fibers from positively stained specimens were almost always aperiodic, although in rare fields faint periodic banding was suggested (Fig. 3). We assume that these departures from normal are due to the presence of γ-Paris I chains, but it is not clear whether the occasional cross-striated fibers that are seen are a reflection of the population of normal γ-chains known to be present in the preparations we studied.

The tendency for Paris I fibrin to appear as nonfibrous clumps of protein or as aperiodic fibers is not a feature of other abnormal fibrins. However, the finding of frequent abruptly terminating fibers was a feature of at least two other fibrins and the tendency to form relatively thin fibers has also been noted.

Mutant γ-Paris I chains are defective with respect to their crosslinking function. It is useful to consider this aspect of normal γ-chains in terms of the electron microscopic characteristics of fibrin. In contrast to γ-Paris I chains, normal fibrin γ-chains in the presence of factor XIIIa, readily undergo reciprocal intermolecular covalent bridging near their COOH-termini to form γ-dimers. Under most circumstances, this process takes place much more rapidly in fibrin than it does in fibrinogen. Evidently, crosslinking is facilitated after the fibrin self-assembly process has resulted in proper alignment of the sites to be crosslinked. In other words, none of the complementary polymerization sites in the fibrin molecule, utilization of which result in the assembly of fibrin fibers, is in the same location as the γ-chain crosslinking site (although one or more may conceivably be situated quite close by). This conclusion is consistent with reports that there are no distinguishable differences between crosslinked and noncrosslinked fibrin with respect to fine details of ultrastructure. Our own unpublished comparative electron microscopic studies on crosslinked and noncrosslinked normal fibrin support such observations. Furthermore, we have also observed (unpublished experiments) that when fibrin crosslinking is carried out in the presence of dansylcadaverine, there are no apparent changes in the cross-striated staining pattern of the resulting fibrin fibers. Considering these results, it seems unlikely that changes involving only the potential crosslinking site of γ-Paris I chains could account for the grossly disordered clot structure displayed by its fibrin, and we postulate that a more extensive mutation has taken place that affects one or more of the primary polymerization sites in the molecule. The results therefore suggest that in order for normal fibrin assembly to take place, a region(s) in the fibrin molecule (probably representing a polymerization site) near to or possibly even overlapping the γ-chain crosslinking site, must be preserved or at least not sterically hindered.

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REFERENCES


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