IDENTITY OF HUMAN PLATELET FIBRINOGEN

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SUMMARY

The controversy on the identity of fibrinogen of human blood platelets is largely due to a lack of proper definition. The fibrinogen fraction isolated directly from platelet granules shows properties which are different from those of plasma fibrinogen.

There is considerable controversy on the identity of fibrinogen isolated from blood platelets (1–8). Much of this controversy, in our opinion, has resulted from the lack of a clear definition of platelet fibrinogen. To define platelet fibrinogen as that fibrinogen which has been isolated from platelets is misleading. In this communication, we present results on fibrinogen isolated directly from human platelet granules. Data are reported which clearly establish platelet fibrinogen as a protein different from plasma fibrinogen.

MATERIALS AND METHODS

Platelets were isolated from fresh human blood, washed with 1% ammonium oxalate – 0.1% EDTA, pH. 7.5, and homogenized in a glass-teflon apparatus in the cold. Intact platelets were removed by low-speed centrifugation. The homogenate was fractionated in a 30% to 60% continuous sucrose density gradient using a Beckman SW 25.1 rotor at 5° C. In this system, it was shown that the platelet homogenate is separated into three fractions – soluble proteins, membranes and granules (9). The granule band was carefully recovered, sucrose was removed by dialysis against 0.1M Tris – 0.1M NaCl, pH. 7.5, and the granules were collected by centrifugation at 20,000g for 1 hour at 5°C. The granule pellet was resuspended in a small volume of Tris-NaCl buffer containing octanol (5 μl/ml) and homogenized at 4°C in a Waring blender. After centrifugation, the clear supernatant was brought to 50% saturation with solid ammonium sulfate. The precipitate was collected by centrifugation and dissolved in Tris-NaCl buffer. This granule protein isolate was extensively dialysed against Tris-NaCl buffer to remove any excess ammonium sulfate. In all steps of the above isolation procedure Trasylol and glutamyl-L-tyrosine were added as proteolytic enzyme inhibitors. The granule protein isolate was clottable with thrombin and the clottability varied between 30% and 60% from preparation to preparation. Fibrinogen from platelet extract was obtained after DEAE-cellulose chromatography as described previously (6). Gel electrophoresis in the presence of sodium dodecyl sulfate and immunoelectrophoresis in agar (Noble agar, Difco) were carried out by established methods (10,11).

RESULTS AND DISCUSSION

Fig. 1 shows the sodium dodecyl sulphate gel electrophoretic patterns of reduced samples of plasma fibrinogen, platelet fibrinogen purified from cell sap, and the granule protein isolate. Plasma fibrinogen, as expected, showed mainly three bands, corresponding to α, β and γ

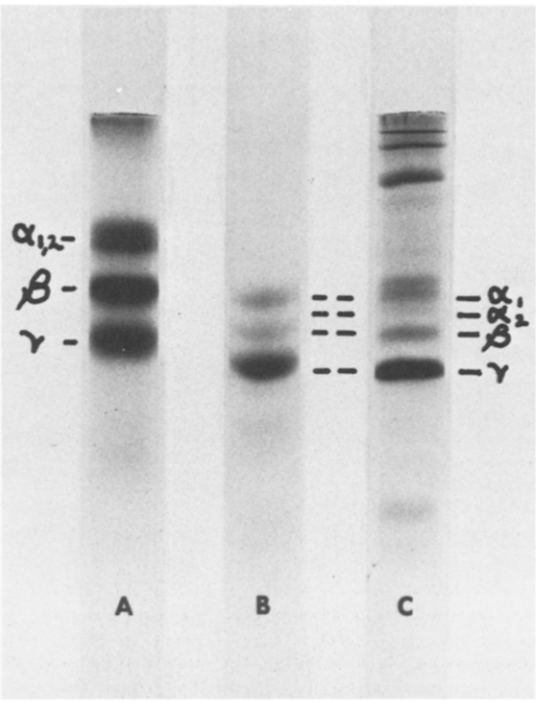

FIG.1 Sodium dodecyl sulphate (0.1%) – acrylamide (10%) gel electrophoretic patterns of mercaptoethanol – reduced samples (approx. 30 μg, 50 μg of protein, respectively) of plasma fibrinogen (gel A), purified platelet fibrinogen (gel B), and the platelet granule protein isolate (gel C). The Greek symbols, α, β and γ, refer to subunit polypeptide chains. Electrophoresis was carried out for 18 hours and the gels were stained with 1% Coomassie Brilliant Blue. Anode at bottom.

subunits. Platelet fibrinogen also showed primarily three bands. However, as reported previously (6,7), platelet fibrinogen subunits have a slightly higher mobility, signifying lower molecular weight, and a different type of distribution compared to plasma fibrinogen. It is evident that the fibrinogen in the granule isolate contains a pattern identical to that of purified platelet fibrinogen. When the granule isolate was treated with thrombin in the presence of coagulation factor XIII, the chain designated as γ is crosslinked, showing that this band is a true component of platelet fibrinogen and is not an impurity.

Fig. 2 shows the agar gel immunoelectrophoretic pattern of plasma fibrinogen, platelet fibrinogen isolated from the soluble proteins, and the granule protein isolate. In this system, platelet fibrinogen has a cathodal mobility compared to anodal migration for plasma fibrinogen (12). It is clearly observed that the granule fibrinogen has a distinct cathodal migration. If purified platelet membranes were added to the granule fraction and the total protein isolated, both platelet and plasma fibrinogen were detected. This observation would suggest that the plasma fibrinogen associated with the platelet membrane remains as plasma fibrinogen, with its anodal mobility, during the isolation procedure.

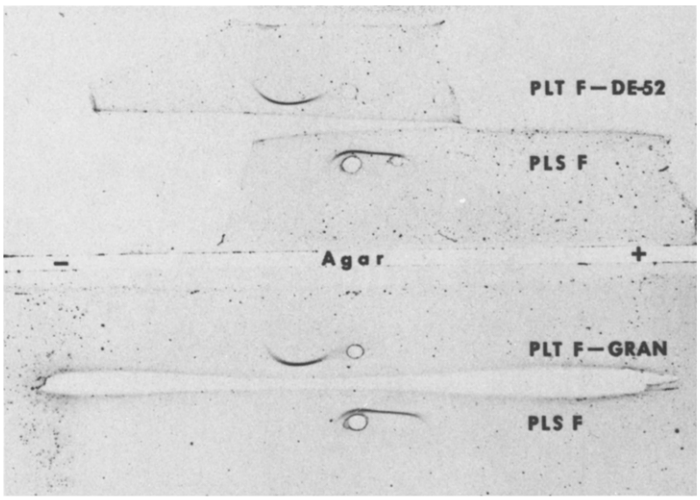

FIG.2 Agar gel immunoelectrophoretic patterns of purified platelet fibrinogen (PLT F-DE-52), the platelet granule protein isolate (PLT F-GRAN), and plasma fibrinogen (PLS F). The buffer employed was sodium barbital-sodium acetate, pH 8.6, at an ionic strength of 0.05. Electrophoresis was carried out at 5°C for 90 minutes at 6 V/cm. Antiserum (75 μl, diluted 1:2 with buffer) to human plasma fibrinogen produced in rabbits was added to both troughs. After washing with isotonic saline, the gels were stained with 1% amido black and destained with 7% acetic acid.

Fibrinogen is associated with blood platelet in two fractions – membranes and granules (13,14). Platelets are known to have an absorbed layer of plasma proteins and the membrane-associated fibrinogen is considered to be plasma fibrinogen. Thus, the true platelet fibrinogen is that which is associated with the granules and which forms only a small part of the total fibrinogen material (13). Previous studies on platelet fibrinogen utilized the cell sap as the starting material The cell sap of washed platelets invariably contains both platelet fibrinogen and fibrinogen material identical to that which occurs in plasma (15). The usual methods employed for following the fibrinogen fraction during its subsequent isolation do not distinguish between these two proteins. Further, we, as well as others (16), have found that platelet fibrinogen is less stable to handling and processing than plasma fibrinogen. Unless proper precautions are taken, it is easy to obtain a pure preparation of plasma fibrinogen using washed platelets as the starting material. Such a preparation, of course, would not show any difference when compared to plasma fibrinogen. A direct approach to this controversy is to isolate the true platelet fibrinogen from the granules and compare it with plasma fibrinogen. In our earlier studies, we demonstrated that the platelet extract contains a fibrinogen fraction which may be distinguished from plasma fibrinogen by its characteristic pattern in gel electrophoresis and agar gel immunoelectrophoresis. We have now shown that fibrinogen isolated directly from the platelet granules satisfy these criteria, establishing this

protein as a distinct entity different from plasma fibrinogen. Whether this fibrinogen is synthesized by the platelet or its precursor, the megakaryocyte, or whether it is adsorbed plasma fibrinogen which becomes modified in a specific way and is then stored in the granules remain to be investigated.

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