Metabolic Studies of Human Fibrinogen γ/γ' Chain Heterogeneity

By Jose Martinez

Human fibrinogen chromatographed on DEAE-cellulose resolves into two components containing 85% (peak 1) and 15% (peak 2) of the total fibrinogen. Peak 1 and peak 2 differ only in the composition of their γ chains, with peak 1 containing only one type of γ chain and peak 2 containing equal amounts of γ chain and a second chain, called γ'. In an effort to explain the difference in the amount of peak 1 compared to peak 2, metabolic studies were performed with radiolabeled peak 1 and peak 2 in two normal subjects. Autologous peak 1 and peak 2 fibrinogen were pooled separately, rechromatographed, labeled with 131I and 125I, respectively, and injected simultaneously. The half-lives and fractional catabolic rates of peak 1 and peak 2 in the two normal subjects were almost identical. To determine whether one peak might be a metabolic product of the other, plasma or fibrinogen samples purified from plasma obtained 2 and 6 days after infusion of the radiolabeled proteins were chromatographed on DEAE-cellulose. 131I radioactivity eluted only in peak 1 and 125I radioactivity eluted exclusively in peak 2. These studies demonstrate that the difference in concentration between peak 1 and peak 2 is not due to a difference in their catabolic rates and that there is no metabolic interconversion between these two peaks. Our data, together with recent evidence, suggest that the γ/γ' heterogeneity originates from a postsynthetic modification of the fibrinogen molecule prior to its secretion into the peripheral circulation.

NORMAL HUMAN FIBRINOGEN elutes in two major peaks when studied by gradient elution chromatography on DEAE-cellulose. These peaks, designated peak 1 and peak 2, contain approximately 85% and 15%, respectively, of the applied fibrinogen. Analysis of the constituent chains of each peak on acid polyacrylamide gel electrophoresis revealed a difference in the composition of the γ chains of peak 1 and peak 2 fibrinogen. In this system, the γ chain of peak 1 ran as a single band, while that of peak 2 resolved into two bands of similar concentrations. One band had the same electrophoretic mobility as the γ chain of peak 1, and the second band, designated γ', migrated more anodally. Based on this type of heterogeneity, the chain composition of peak 1 may be written as (Aa)2(Bβ)2γ and that of peak 2 as (Aa)2(Bβ)2γγ'.

We have investigated the possibility that the difference in the amount of fibrinogen present in peak 1 as compared to peak 2 might result from a difference in the catabolic rates of these two molecular species. Fibrinogen was purified from two normal volunteers. Peaks 1 and 2 were separated and labeled with 131I and 125I, respectively. Simultaneous metabolic studies were then performed in each volunteer using autologous radiolabeled peak 1 and peak 2 fibrinogen. In addition, chromatography of plasma and of fibrinogen purified from plasma obtained after the injection of the radio-labeled proteins was performed to evaluate the possibility that one of the peaks might be an in vivo metabolite of the other.

MATERIALS AND METHODS

Fibrinogen Purification

Fibrinogen was purified from anticoagulant citrate dextrose solution U.S.P. (Fenwal Inc., Ashland, Me.) plasma obtained from two healthy donors by the glycine precipitation method of Kazal et al. modified by the addition of epsilon-aminocaproic acid to a final concentration of 0.1 M in plasma and buffers. The concentration of fibrinogen present in the purified material was measured spectrophotometrically at 280 nm, using A280 of 13.9, the figure obtained by Kazal et al. The recovery of fibrinogen was greater than 60%, and the purified fibrinogen was more than 95% clottable. All studies were performed after informed consent was obtained from both volunteers, and the protocol for these studies was approved by the university's human experimentation committee.

Purification of Peak 1 and Peak 2 Fibrinogen

Purified fibrinogen was dialyzed extensively against starting buffer at 4°C, and 150 mg of this fibrinogen was chromatographed on DEAE-cellulose (Schwarz/Mann Div., Becton, Dickinson and Co., Orangeburg, N.Y.) at 4°C in a column measuring 1.5 x 20 cm using a continuous concave gradient from starting buffer (0.005 M H3PO4, 0.039 M Tris, pH 8.6) to final buffer (0.5 M H3PO4, 0.5 M Tris, pH 4.1). The fractions were read at 280 nm in a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio), and peaks 1 and 2 were pooled separately and rechromatographed as in the previous step. Peaks 1 and 2 were again pooled separately, and both peaks were concentrated by dialysis against 25% polyethylene glycol.

Metabolic Studies

Peak 1 and peak 2 were labeled with 131I and 125I, respectively, by the iodine monochloride method of McFarlane modified as previously described. The labeled proteins were then chromatographed on Sephadex G-100 (Pharmacia Fine Chemicals Inc., Piscataway, N.J.) to remove unbound iodine. Each labeled protein contained less than 0.5 atom of iodine per molecule. The clottability of labeled peak 1 and peak 2 fibrinogen was determined by the method of Regoecci and ranged from 95% to 96%. Prior to
injection, the labeled proteins were passed through G2 0.22 μm Millipore filters. Approximately 10 μCi each of autologous radiolabeled peak 1 and peak 2 fibrinogen were injected into the antecubital vein of the respective volunteer at zero time. At 15 min following the infusion and on each day for the subsequent 5 days, 9-ml samples of blood were obtained. The catabolic rates of radiolabeled peak 1 and peak 2 were calculated from the plasma disappearance curves by the method of Matthews.

Chromatographic Studies of Radiolabeled Peak 1 and Peak 2 Fibrinogen

Prior to the infusion studies, aliquots of radiolabeled peak 1 and peak 2 fibrinogen were admixed with 2 ml of plasma and chromatographed on DEAE-cellulose. Fibrinogen was also purified from 15 ml of plasma obtained at 24 hrs and 40 hr in subject K.G. and from 100 ml of plasma obtained from each subject 6 days after the injections of the radiolabeled proteins. The purified fibrinogens were chromatographed on DEAE-cellulose as described above. Absorbance at 280 nm, 131I and 125I radioactivities, and pH were measured on column effluents. In one volunteer, R.J., blood was also obtained 24 hr after injection, the separated plasma was chromatographed on DEAE-cellulose, and 131I and 125I radioactivities were measured in the eluates.

Electrophoretic Studies

Peak 1 and peak 2 fibrinogen were reduced with β-mercaptoethanol and run in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).1 Densitometric scans of the gels stained with Coomassie blue were made with a Gilford gel scanner.

Sialic Acid Content

The sialic acid content of peak 1 and peak 2 fibrinogen was measured after acid hydrolysis by the thiobarbituric acid method of Warren.9

RESULTS

Normal fibrinogen eluted in the characteristic two major peaks, peak 1 and peak 2, when chromatographed on DEAE-cellulose under conditions as previously defined (Fig. 1). In addition, 131I-labeled peak 1 and 125I-labeled peak 2 mixed with 2 ml of normal plasma and chromatographed on DEAE-cellulose eluted in well separated peaks with minimal overlapping of radioactivity (Fig. 2).

After injection of autologous 131I-labeled peak 1 and 125I-labeled peak 2 into the respective normal volunteer, the plasma half-life of peak 1 was 84 hr in volunteer K.G. and 80 hr in subject R.J. and that of peak 2 was 82 and 76 hr, respectively (Fig. 3). The fractional catabolic rates of peak 1 and peak 2 expressed as percent of the plasma pool per day are shown in Table I. The fractional catabolic rate of peak 1 was nearly identical to that of peak 2 in each subject. The intravascular and extravascular distributions were also very similar (Table I).

From plasma obtained from subject R.J. 24 hr after infusion of the radiolabeled proteins and chromatographed on DEAE-cellulose, 131I radioactivity eluted in peak 1 and 125I radioactivity eluted in peak 2 (Fig. 4). Similar studies were performed with fibrinogen purified from subject K.G. at 40 hr and from both subjects on the sixth day postinfusion. While the purified fibrinogen eluted in two peaks, 131I radioactivity originating from infused 131I-labeled peak 1 was confined to peak 1, and 125I radioactivity originating from infused 125I-labeled peak 2 was confined to peak 2. The results for subject R.J. fibrinogen obtained on the sixth day postinfusion are shown in Fig. 5.
minimal overlapping of radioactivity was similar to that present between peak 1 and peak 2 prior to infusion.

The reduced peak 1 and peak 2 fibrinogen run in SDS-PAGE showed normal mobility of the Aα, Bβ, and γ chains.

Both peak 1 and peak 2 had 6.1 residues of sialic acid per molecule of fibrinogen.

**DISCUSSION**

Normal human fibrinogen demonstrates a stable heterogeneity when chromatographed on DEAE-cellulose. In this system, fibrinogen elutes in two major peaks, peak 1 and peak 2, which appear to differ only in the composition of their γ chains. This difference is observed in electrophoresis on either acid or alkaline urea polyacrylamide gels on which the γ chain of peak 1 migrates as a single band while that of peak 2 resolves into two bands of similar concentration. One of these bands has the same mobility as peak 1 γ chain and the second, γ', exhibits a more anodal migration. γ' makes up approximately 7%-8% of the total γ-chain composition of fibrinogen.

Additional heterogeneity of the γ chains of both human and bovine fibrinogens has been demonstrated by chromatography of the S-sulfo derivatives of these proteins on CM-cellulose. Under these conditions, the γ chains eluted in two fractions, γ-1 and γ-2. Similar findings were obtained when the carboxymethylated chains of human fibrinogen were analyzed on CM-cellulose. The γ-1 and γ-2 fractions of both bovine
and human fibrinogen differed in their carbohydrate content.\(^9\) In addition, a difference in amino acid composition has been demonstrated between bovine \(\gamma\)-1 and \(\gamma\)-2 chains.\(^9\) In contrast, peak 1 and peak 2 fibrinogen have the same sialic acid contents, and the \(\gamma\)-1 and \(\gamma\)-2 fractions comprise about one-half of the \(\gamma\)-chain population of peak 1 and of peak 2 fibrinogen. It would thus appear that the \(\gamma\)-1, \(\gamma\)-2 heterogeneity is different from and superimposed on the \(\gamma/\gamma\)' heterogeneity of peak 2.\(^2\)

Another type of heterogeneity of the fibrinogen molecule may result from in vivo metabolism. The \(A\alpha\) chain has been shown to lose its N-terminal alanine when mixed with normal serum producing the \(A\) \(Y\)-chain.\(^12\) Phosphorylated \(A\alpha\) chains (AP) have been dephosphorylated in vivo by alkaline phosphatase.\(^13\) Plasmin attacks fibrinogen initially at the C-terminal end of the \(A\alpha\) chain, causing alterations in the chromatographic, electrophoretic, and biologic properties of the molecule.\(^14\) In addition, these plasmin-induced fibrinogen derivatives have been shown to have increased catabolic rates as compared to the intact fibrinogen.\(^15\)

We studied the possibilities that the difference in concentration between peak 1 and peak 2 might be a reflection of a difference in their catabolic rates or that one peak might be an in vivo metabolic conversion product of the other. Our studies demonstrate that both peaks have similar catabolic rates and identical intravascular–extravascular distributions. In addition, the chromatographic studies involving plasma and fibrinogen purified during the metabolic studies failed to demonstrate any in vivo interconversion between peak 1 and peak 2.

It is possible that \(\gamma\) chain and \(\gamma\)' chain production are controlled by two separate genes or that one of the \(\gamma\) chains undergoes postsynthetic modification prior to its secretion into the peripheral circulation. Recent evidence derived from studies of fibrinogen Paris I do not support the two-gene hypothesis. Fibrinogen Paris I is characterized by the presence of a normal \(\gamma\) chain and a mutant \(\gamma\) chain called \(\gamma\) Paris I. Chromatographic and electrophoretic studies reveal the presence of \(\gamma\)' in the mutant \(\gamma\) chain.\(^16\) Since an identical mutation in two different genes is most unlikely, the best explanation for the origin of \(\gamma\)' is that it results from a postsynthetic modification that occurs prior to secretion of the protein into the peripheral circulation.

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