Cryoprecipitation of Fibrin-Fibrinogen Complexes Induced by the Cold-insoluble Globulin of Plasma

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A cold-insoluble precipitate that formed from normal plasma treated with small amounts of thrombin was comprised of three main components: cold-insoluble globulin (Clg), fibrin, and fibrinogen. Its composition appeared to be the same as that of a pathologic plasma precipitate termed "cryofibrinogen." We examined the roles played by each of these components in generating such a precipitate. Fibrinogen from a preparation containing a high proportion of molecules with intact Aa chains (fraction 1-4) was coupled to Sepharose 4B; columns of this material were compared before and after conversion to fibrin with respect to their Clg binding capacity. Fibrinogen columns displayed significant Clg binding at 4°C but very little at 22°C; fibrin columns bound significant quantities of Clg at both temperatures. Under standardized conditions of pH (7.3 ± 0.1), ionic strength (0.2-0.25), and temperature (2°C), Clg or fibrinogen (fraction 1-2 or 1-4) alone or mixtures of Clg and fibrinogen did not form cold-insoluble precipitates. This was true as well of soluble complexes of fibrin and fibrinogen, even when saturated with fibrin, except when Clg was included in the mixture. In contrast, fibrin-fibrinogen complexes produced from molecules lacking the COOH-terminal region of the Aα chain (fraction I-8 or I-9) failed to precipitate in the cold in the presence of Clg, thus indicating that this region must be present for Clg-induced precipitation to occur. We conclude that each component of "cryofibrinogen" plays an important role in its formation. Fibrinogen, which is well known to form soluble complexes with fibrin, is necessary for maintaining fibrin in a soluble state prior to complexing and precipitation with Clg. There is an absolute requirement for both Clg and fibrin in this reaction, since precipitation does not take place in their absence. Considerations of the Clg:fibrin-fibrinogen ratios in precipitating mixtures suggest that Clg acts as a nucleus with multiple binding sites for participating fibrin-fibrinogen complexes.

In citrated, oxalated, or EDTA plasma from certain individuals there develops a cold-insoluble precipitate that can be dissolved, at least in part, by rewarming. This pathologic precipitate, popularly termed "cryofibrinogen," can be distinguished from another type of fibrinogen-enriched plasma precipitate, termed the "heparin-precipitable fraction," formation of which is induced in the cold from either normal or pathologic plasma by the addition of heparin or related mucopolysaccharides.1 Both types of precipitate differ qualitatively from "cryoglobulin," since this precipitate forms from either serum or plasma and in general lacks detectable fibrinogen-related protein.2,3

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Cryofibrinogenemia is a common finding often observed in conditions complicated by thromboembolic disease.\textsuperscript{9} The phenomenon can be produced in normal plasma after the direct addition of small amounts of thrombin or thrombinlike enzymes, or following the infusion of agents that enhance fibrin formation.\textsuperscript{10-12} Such observations imply that fibrin, as well as fibrinogen, is a component of “cryofibrinogen.” This notion has drawn support from the determination that the fibrinopeptide content of such plasma precipitates is deficient\textsuperscript{10,11} and from numerous investigations that have demonstrated complexes of fibrin and fibrinogen in the circulation.\textsuperscript{13-17}

There is at least one other nonfibrinogen component of some, perhaps all, “cryofibrinogen” precipitates; this protein is termed the cold-insoluble globulin of plasma (CIg). Its presence in this type of precipitate was first clearly recognized during studies of “cryofibrinogen” from a patient with a chronic intravascular coagulation syndrome secondary to ovarian carcinoma.\textsuperscript{7} This discovery was followed by investigations directed at its purification,\textsuperscript{18-20} elucidation of its physical, biochemical, and immunochemical properties,\textsuperscript{21-23} studies of its interactions with other plasma proteins,\textsuperscript{19,24-27} and determination of its cellular origin and function.\textsuperscript{28} Available data indicate that CIg has an affinity for both fibrin and fibrinogen,\textsuperscript{7,18,25-29} one that is particularly evident in the cold.

Our recent studies revealed the central importance of CIg in the formation of heparin-induced cold-precipitable complexes.\textsuperscript{27} Nevertheless, the role played by CIg in the formation of “cryofibrinogen” has not yet been clarified. In the present investigation we carried out experiments designed to explore that subject.

**MATERIALS AND METHODS**

**Preparation of fresh plasma, fibrinogen subfractions, and CIg.** Single-donor human blood was collected and mixed in polystyrene tubes with 0.02 vol\% sodium citrate solution containing Kunitz pancreatic trypsin inhibitor (Trasylol, FBA Pharmaceuticals, New York, N.Y.), 500 kallikrein inhibitor units/ml final concentration. Plasma was prepared at 4°C by centrifugation at 2000 g for 20 min, pooled with that from one or two other donors, and used immediately. In these experiments EDTA was also added to the citrated plasma (10 mM final concentration).

Fibrinogen subfractions 1-2, 1-4, 1-8, and 1-9 were prepared from outdated acid citrate dextrose (ACD) plasma as described by Mosesson and co-workers.\textsuperscript{30,31} The thrombin coagulability of each fraction was 95\% or more. Fibrinogen from fractions 1-2 or 1-4 was rendered free of detectable CIg (<0.2 CIg by electroimmunoassay\textsuperscript{32}) by DEAE-cellulose chromatography at room temperature on 0.9 x 30 cm columns, employing a combined pH and phosphate gradient.\textsuperscript{18,27,13} Fibrinogen fractions 1-8 or 1-9 had very low levels of CIg (<0.3\%) and required no further treatment. Fibrinogen preparations were judged to be free of detectable fibrin if, upon Sepharose 4B molecular exclusion (Fig. 1), they emerged in a single symmetrical peak and if a negative result was obtained in the “ethanol gelation” test.\textsuperscript{13} Any preparation giving evidence of fibrin by either or both criteria was further subjected to affinity chromatography at room temperature on a fibrinogen affinity column\textsuperscript{34} as recently described\textsuperscript{27} in order to render it free of fibrin by the criteria outlined above.

Human CIg was prepared by a modification\textsuperscript{20} of the original method.\textsuperscript{18} The concentration of purified fibrinogen or CIg was determined spectrophotometrically by assuming absorption coefficients (A\textsubscript{280}) at 280 nm of 15.2\textsuperscript{23} and 12.8\textsuperscript{18} respectively. For calculating the molar content of CIg or fibrin-fibrinogen in any mixture, the following molecular weights were assumed:\textsuperscript{21,36} fraction 1-2 or 1-4, 330,000; fraction 1-8 or 1-9, 270,000; CIg, 450,000 daltons.

**Radioactive labeling procedures.** Fraction 1-4 or purified CIg was radioiodinated with \textsuperscript{125}I (carrier free, New England Nuclear, Boston, Mass.) by the IC\textsubscript{1} method of McFarlane.\textsuperscript{37} Assuming that labeling efficiency was 50\%, the iodine content of the labeled protein was less than 0.5
Fig. 1. Molecular exclusion chromatography on Sepharose 4B of 8 mg Clg-free fibrinogen (fraction 1-4) (--), 12 mg thrombin-treated Clg-free fibrinogen (--), and 15 mg thrombin-treated fibrinogen fraction 1-8 (-- - -). As assessed by the appearance of visible fibrin threads, 1-8 sample shown was "saturated" with fibrin; 1-4 sample was not. Each sample chromatographed separately under same conditions on 2 x 82 cm column. Elution buffer was 0.05 M Tris-HCl, 0.3 M NaCl, pH 8.3; flow rate, 20 ml/hr; fraction of 0.5 ± 0.1 ml were collected. Vo, void volume.

atoms/molecule. After the labeling procedure, the radioiodinated samples were diluted with equal amounts of "cold" protein as suggested by Krohn et al.38 and extensive dialysis against 0.3 M NaCl was carried out. No changes were observed in the gel sieving properties of material that had been iodinated compared with those of unlabeled material. After the final dialysis, more than 98% of the radioactivity was precipitable with trichloroacetic acid (10% w/v). The labeled preparations were stored at −20°C.

Preparation of fibrin-containing solutions. Solutions of fibrinogen containing soluble fibrin (fibrin-fibrinogen) were prepared from fibrinogen (5-7 mg/ml) to which had been added human thrombin (lot H-1, Bureau of Biologics, FDA) at room temperature (22°C ± 1°C) at a final concentration of 0.02-0.08 US units/ml. Sodium phosphate (0.05 or 0.10 M)/NaCl (0.05 or 0.1 M) solutions buffered at 7.3 ± 0.1 (ionic strength I = 0.2-0.25) were used. Under such conditions visible fibrin strands first appeared after incubation for 25-40 min, an occurrence that we assumed corresponded to saturation of the system with fibrin. Thrombin action was inhibited at selected time intervals by the addition of hirudin (Pentapharm, Basel, Switzerland) at a final concentration (in units of activity) ten times that of the thrombin. Solid clot formation did not take place in hirudin-treated samples. If hirudin was added after the first appearance of fibrin threads, these threads were subsequently removed by centrifugation at room temperature. For certain experiments, 125I-fibrinogen was added prior to thrombin addition, whereas 125I-Clg was added after the addition of thrombin (25-100 μl/ml solution, 1-5 mg protein/ml). Molecular exclusion chromatography on Sepharose 4B was used to confirm the presence (or absence) of fibrinogen-fibrin aggregates emerging before the symmetrical parent fibrinogen peak. Typical analyses of thrombin-treated fibrinogen fractions 1-4 and 1-8 are shown in Fig. 1. In general, the longer the period of incubation with thrombin, the greater the quantity of aggregates found.

Plasma containing soluble fibrin was produced from citrated plasma containing EDTA by allowing thrombin, at final concentrations of 0.06-0.1 US units/ml, to act for a period corresponding to about one-half that of the expected visual "clotting time" before the addition of hirudin at the same relative concentration described above. This plasma was then cooled to 4°C; the cold-precipitated fraction was collected by centrifugation and then extracted at 4°C with 0.05 M Tris-HCl, 0.15 M NaCl buffer, pH 7.4. Following this, the precipitate was solubilized in a solution of 1% sodium dodecyl sulfate and 8 M urea containing 5 mM sodium phosphate buffered at pH 7.

Procedures for monitoring cryoprecipitation. Clg or thrombin-treated fibrinogen solutions, or both, in various combinations, were mixed in 1-ml cuvettes at initial volumes of less than 0.2 ml. These mixtures were then adjusted to their final volume of 1 ml with 0.05 M sodium phosphate-0.1 M NaCl buffer, pH 7.3 ± 0.1 (I = 0.2), precooled to 4°C. The cuvettes were placed in a Gilford model 240 spectrophotometer, the jacketed chamber of which was maintained at 2°C, and the formation of precipitate was followed by periodic readings at 350 nm. The precipitate that formed
was opaque and appeared gel-like; maximum readings developed after overnight incubation and were stable for several hours thereafter. The absorbance readings were highly reproducible for any given set of conditions but were not necessarily a linear function of the Clg:fibrin-fibrinogen ratio.

Quantitative assessments of cryoprecipitation were made as follows: (a) The cold-insoluble precipitate that formed from mixtures to which had been added \(^{125}\text{I-Clg}\) or \(^{125}\text{I}-\text{fibrinogen}\) was collected by centrifugation at 2°C (10,000 g for 10 min), washed once with ice-cold buffer (0.01 M Tris-HCl, 0.15 M NaCl, pH 7), and solubilized in 1 ml 0.1 M NaOH. The radioactivity of the precipitate was counted in a Picker Autowell II gamma counter (Picker Nuclear, Cleveland, Ohio). (b) Alternatively or in addition, measurements of the absorbance at 280 nm were made both before cooling and after removal of the precipitate that had formed in the cold (typically, after 3 hr incubation at 2°C). The difference in absorbance was used to calculate the amount of protein precipitated.

Fibrinogen and fibrin affinity chromatography. Fraction I-4 fibrinogen or fibrin coupled to Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.) was prepared as described by Heene and Matthias. For affinity chromatography, columns of 0.9 × 25 cm were prepared, and stepwise elution was carried out at a flow rate of 16 ml/hr with the following buffers: buffer A, 0.05 M Tris-phosphate, 0.1 M NaCl, 0.025 M \(\epsilon\)-aminocaproic acid (\(\epsilon\)-ACA), pH 7.6; buffer B, 0.05 M Tris-phosphate, 1 M NaCl, 0.025 M \(\epsilon\)-ACA, pH 4.1; buffer C, 0.05 M Tris-phosphate, 0.5 M NaCl, 6 M urea, 0.025 M \(\epsilon\)-ACA, pH 4.1. Samples of \(^{125}\text{I-Clg}\) (2 mg protein) were applied in 10 ml buffer A; 4-ml fractions were collected.

Electrophoretic and related procedures. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed essentially as described by Weber and Osborn. The final gel polymerization mixture contained 0.05 M sodium phosphate buffer, pH 7, 0.1%, SDS, 4 M urea, and 5% acrylamide. Samples (1-2 mg/ml) were incubated for 2 hr at 37°C in a solution of 1%, SDS and 8 M urea containing 5 mM sodium phosphate and 14 mM dithiothreitol and then mixed with an equal volume of a solution of glycerol (50%, v/v). 0.1 M sodium phosphate buffer, pH 7, prior to application. Gels were stained with Coomassie Brilliant Blue as previously described. Densitometric scans of stained gels were made in a Gilford model 240 spectrophotometer equipped with a linear transport apparatus.

RESULTS

C1g-induced Cryoprecipitation of Thrombin-treated Plasma and Fibrin-Fibrinogen Solutions

Thrombin-treated plasma was cooled to 4°C and the precipitate that formed was examined by SDS-PAGE (Fig. 2). The major bands corresponded in anodal migration rate to those of C1g or fibrin-fibrinogen. Further studies were designed to explore the role played by these components in forming such a precipitate.

Fibrin-free fibrinogen (0.5–6.5 mg/ml) or purified C1g (0.05–0.6 mg/ml) solutions or mixtures of these (C1g:fibrinogen molar ratios 0.1–0.5) were incubated at 2°C. In no case did cryoprecipitation occur after overnight incubation periods or longer. This was true as well for thrombin-treated C1g-free fibrinogen solutions (at concentrations as high as 4.5 mg/ml), even those that were “saturated” with fibrin.

The addition of C1g to fibrin-containing solutions produced a much different result. Incubation of such mixtures at 2°C led to precipitation. The precipitates were at least partially and sometimes completely soluble after incubation at 37°C for 1–2 hr. First, the influence of C1g concentration on this phenomenon was examined (Fig. 3). As assessed by turbidimetry, cryoprecipitation in this experiment was evident at a C1g:fibrin-fibrinogen ratio ≥ 0.075. There was an increase of both the rate and the amount of precipitation as the level of C1g was raised. A plot of the \(^{125}\text{I-fibrin-fibrinogen radioactivity}\) in the precipitate versus
the Clg:fibrin-fibrinogen ratio indicated that the amount of precipitation was related directly to the Clg content (Fig. 4). Similarly, a plot of the amount of protein precipitated, as determined by differential absorbance measurements at 280 nm, versus the Clg:fibrin-fibrinogen ratio also suggested the same conclusion (data not shown).

Studies using $^{125}\text{I}$-Clg instead of $^{125}\text{I}$-fibrinogen in such mixtures (Fig. 4) provided confirmation that increases in precipitation of solutions of fibrin, fibrinogen, and Clg were associated with parallel increases in the amount of Clg incorporated in the precipitated fraction. However, they also indicated that Clg incorporation in the precipitate was incomplete. The amount of Clg radioactivity incorporated at any given Clg concentration amounted to only a minor proportion (2.6\(^{\%}\)–3.3\(^{\%}\)) of the total radioactivity that had been added. Further-
more, densitometric scans of SDS-PAGE gels of these cryoprecipitated fractions showed that the Clg:fibrin-fibrinogen ratio in the precipitate was considerably lower than it had been in the original solution. For example, at a Clg:fibrin-fibrinogen molar ratio of 0.1 Clg amounted to ~12% of the protein in the original mixture, yet at any given Clg:fibrin-fibrinogen molar ratio in this experiment the Clg content in the precipitate was 1.2%-4% of the total protein.

The linearity of the relationship between Clg content and the amount of precipitation is also well documented by data from an experiment in which the influence of the fibrin:fibrinogen ratio upon Clg-induced cryoprecipitation was evaluated (Fig. 5). Fibrinogen solutions that had been exposed to the action of thrombin for varying periods of time were incubated with several concentrations of Clg. The amount of precipitation that took place at any given Clg level increased with the length of time that thrombin had been incubated with the fibrinogen solution, clearly showing that Clg-induced cryoprecipitation of fibrinogen/fibrin mixtures was a function of both the fibrin content and the Clg level.
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Fig. 6. CIg-induced precipitation as function of fibrin-fibrinogen concentration. CIg was added to fibrin-fibrinogen fraction 1-4 containing 125I-labeled fibrinogen. Final CIg:fibrin-fibrinogen molar ratio was 0.075. Radioactivity (cpm) precipitated after 3 hr incubation at 2°C plotted against final concentration of fibrin-fibrinogen [fibrinogen].

In a related experiment (Fig. 6), the behavior of a solution of CIg and fibrin-fibrinogen, at a constant CIg:fibrin-fibrinogen ratio, was evaluated as a function of protein concentration. A linear relationship was found between the amount of precipitate forming and the final concentration of fibrin-fibrinogen (1.3–6.5 mg/ml).

Effect of CIg on Thrombin-treated Fibrinogen Fractions 1-8 and 1-9

Fibrinogen comprising fractions 1-8 and 1-9 differs from lower-solubility fibrinogen fractions (e.g., I-4) in that these fractions lack various portions of the molecule containing the COOH-terminal regions of Aα chains. As was the case for thrombin-treated CIg-free fibrinogen derived from fraction I-4 or I-2, thrombin-treated fractions I-8 and I-9 yielded positive results in the “ethanol gelation” test, revealed the presence of fibrin-fibrinogen aggregates by molecular exclusion chromatography (Fig. 1), and did not form a precipitate in the cold even when incubated at concentrations as high as 6 mg/ml. However, in sharp contrast to the behavior of CIg with fibrin-fibrinogen derived from fractions I-2 or I-4, even CIg:fibrin-fibrinogen molar ratios as high as 0.5 did not induce cryoprecipitation of fibrin-fibrinogen prepared from fractions I-8 or I-9. These findings clearly indicate that the presence of the COOH-terminal region of the Aα chain must be present for CIg-induced precipitation to occur.

Adsorption of CIg to Insolubilized Fibrinogen or Fibrin

Our recent studies on the heparin-precipitable fraction of plasma showed an affinity between fibrinogen and CIg that was evident in the cold. Our present experiments suggest that fibrin has a considerably greater affinity for CIg than does fibrinogen. To evaluate these binding affinities, we compared the adsorption of 125I CIg to insolubilized fibrinogen or fibrin (Fig. 7). A high proportion of 125I-CIg applied at 4°C in buffer A was bound to the fibrinogen as well as to the fibrin column (58% and 67%, respectively). Raising the column temperature
to room temperature resulted in almost complete desorption of fibrinogen-bound Clg. Under the same conditions, there was some release of fibrin-bound Clg, but it was not nearly as complete. Attempts to desorb the Clg remaining bound to the fibrin-Sepharose by increasing the molarity (1 M NaCl) and lowering the pH to 4.1 (buffer B) resulted in only minor additional release of Clg. Elution with a urea-containing buffer (buffer C) did, however, result in complete desorption of the remaining Clg.

**DISCUSSION**

Our present investigations provide new insights into the mechanism of "cryofibrinogen" formation, particularly with regard to the role played by each of the main components of the fraction: Clg, fibrin, and fibrinogen. Fibrin is essential for producing such a phenomenon, since precipitation of Clg-fibrinogen mixtures does not take place in the cold in the absence of fibrin; the amount of precipitate that forms can be increased by increasing the ratio of fibrin to fibrinogen (Fig. 5). The absolute necessity for fibrin in this system stands in sharp contrast to requirements for forming the heparin-precipitable fraction of plasma. In that case, although fibrin may be included as a component of the heparin-induced precipitate under certain circumstances, its presence is not required for precipitate induction.

Fibrinogen is also a component of the "cryofibrinogen" precipitate, as is clear from the thrombin coagulability of such fractions, but by itself it does not form cold-insoluble precipitates with Clg. However, fibrinogen serves to maintain fibrin in a soluble state. Thus when cryoprecipitation with Clg does
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take place fibrinogen becomes incorporated in the precipitate by virtue of the complexes it had formed with fibrin; since fibrinogen also has an appreciable affinity for CIg that is particularly evident in the cold (Fig. 7), it is probably included in the precipitate to some extent by virtue of this interaction as well as the one with fibrin.

CIg is indispensable for induction of precipitation. Although its presence is certainly not a requirement for complex formation between fibrin and fibrinogen per se, solutions containing fibrin-fibrinogen complexes, even when "saturated" with fibrin, fail to form a precipitate in the cold unless CIg is also present in the mixture. The amount of precipitate that forms in the presence of CIg is dependent upon several variables, including protein concentration (Fig. 6), the relative proportion of fibrin in the mixture (Fig. 5), and the CIg: fibrin-fibrinogen molar ratio (Figs. 3, 5). We observed that the amount of CIg required for inducing precipitation of fibrin/fibrinogen mixtures is relatively low with respect to the total amount of fibrin-fibrinogen in the mixture (~ 0.05:1 or less). Since the fibrinogen/fibrin ratio at saturation is reported to be ~ 4:1,11,43 the ratio of CIg to fibrin in such mixtures is higher than that to fibrin-fibrinogen, but it still does not approach unity.

The estimated CIg:fibrin-fibrinogen ratio in the cryoprecipitated fraction is even lower, by a factor of 3 or more, than it is in the mixtures from which such precipitates are formed (see Fig. 4 and text). We did not directly measure the amount of fibrin in the precipitate, but since these cryofractions are usually soluble upon rewarming, we assume that the fibrinogen:fibrin ratio in the precipitate is not less than 4:1.11,43 Thus the ratio of CIg:fibrin in the precipitate remains considerably less than unity. Although several explanations are possible, these considerations suggest that CIg behaves as a nucleus with multiple binding sites for the fibrin-fibrinogen complexes that participate in cryoprecipitation.

The central role played by CIg in modifying the solubility of fibrin-fibrinogen complexes may be signaling an important underlying biologic role for this interaction, although this role still remains obscure. In any case, significant quantities of CIg contaminate purified fibrinogen preparations such as fraction I-419,27,28 unless this contaminant is specifically removed by chromatography; it therefore seems likely that the previously reported precipitability of fibrin-fibrinogen mixtures in the cold, whether observed in "purified" systems11,12 or in plasma or plasma subfractions10,12 was most likely directly related to the presence of significant amounts of CIg. In this regard, Ströder and Hörmann44 detected complexed forms of fibrinogen in fractions derived from human or bovine plasma that they believe represent disulfide-bridged oligomers. Since an electrophoretic band corresponding to CIg seems to be present in their fractions, it appears that additional evidence will be required to establish the nature of these complexes unambiguously.

Our present binding experiments with fibrin- and fibrinogen-Sepharose columns clearly demonstrate the affinity of CIg for fibrin both in the cold and at room temperature. Affinity of CIg for fibrinogen is unmistakably evident at 4°C but is weaker at room temperature, an observation that is consistent with our recent report on heparin-induced cryoprecipitation of CIg-fibrinogen mix-
tures\textsuperscript{27} and with observations reported by Ruoslahti and Vaheri.\textsuperscript{28} Stemberger and Hörnmann\textsuperscript{25} concluded that CIg had affinity only for fibrin. However, their results do not really conflict with our own, since they arrived at their conclusion on the basis of a somewhat different experiment. They observed that plasma CIg did not bind at all to a fibrinogen-Sepharose affinity column. The fact that fibrinogen molecules in plasma compete for CIg with those immobilized on the column would tend to minimize the possibility of demonstrating such binding affinity.

Fibrinogen subfractions I-8 and I-9 are comprised of catabolite molecules lacking portions containing the COOH-terminal region of the Aα chain.\textsuperscript{31,40,41} This region of the molecule also apparently contains the site(s) joined to CIg as a result of factor XIIIa-catalyzed cross-linking\textsuperscript{19} and those that account for binding and precipitation of fibrinogen with CIg in the presence of heparin.\textsuperscript{27} Our present experiments with these same subfractions extend those conclusions. Even though thrombin-treated fractions I-8 and I-9 formed fibrin-fibrinogen complexes (Fig. 1), they failed to form a cold-insoluble precipitate in the presence of CIg, thus proving in another way that the COOH-terminal region of the Aα chain is required for direct binding of fibrin or fibrinogen with CIg. Such conclusions do not, however, exclude the possibility that catabolized forms of fibrin or fibrinogen lacking this region of the Aα chain might still be incorporated indirectly in such precipitates by virtue of their ability to complex with fibrin or fibrinogen molecules that do contain it.

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