Anticoagulant Function of a 24-Kd Fragment Isolated From Human Fibrinogen Aα Chains

By Herbert K.F. Lau

A fibrinogen fragment obtained by limited-plasmin proteolysis has been isolated and purified to apparent homogeneity by gel filtrations. This fragment, denoted as 24-Kd fragment, has an apparent M, $\approx$24,000 and contains an N-terminal sequence of met-glu-leu-glu-arg-pro-gly-gly-asn-glu-ile. The fragment contains a large number of acidic amino acid residues, and its amino acid composition is similar to several fibrinogen Aα chains degradation fragments isolated previously. It corresponds to a peptide of the fibrinogen Aα chains, the N-terminal of which starts at a Met-240. This peptide delays thrombin plasma clotting time. It does not bind calcium ions and does not inhibit thrombin’s amidolytic activity. It binds to immobilized fibrin but not fibrinogen. It also inhibits the polymerization of desAA and desAABB fibrin monomers by simultaneously decreasing the maximum rate and the maximum level of the polymerization reaction. However, the initial lag period of this reaction is not affected by the fragment. © 1993 by The American Society of Hematology.

FIBRINOGEN is composed of three pairs of nonidentical chains (Aα, Bβ, γ). It is roughly trinodal in shape, and consists of a central disulphide-bonded domain formed by the amino terminals of all six chains, and two flanking regions consisting of the carboxy terminal of β and γ chains. The carboxy terminal of the α chains may also interact with each other to form a distinct domain, which lies close to the central disulphide knot, and is linked by flexible protease-susceptible α chains to the rest of the molecule.1,2 On thrombin activation, the N-terminal 16 amino acids of Aα chains (fibrinopeptides A) are rapidly removed, and fibrin monomers form end-to-end protofibrils. Further action of thrombin is slow and releases the N-terminal 14 amino acids of Bβ chains fibrinopeptides B), resulting in lateral aggregation of the fibrin monomers. It is believed that a binding site on fibrin monomer is exposed after fibrinopeptides A are removed, which can be occupied by a complementary site on the carboxy terminal end of the γ chains. When fibrinopeptides B are removed, another set of binding sites is shown on the fibrin monomer that binds a second set of complementary sites,3,4 the nature and location of which have not been determined.

The carboxy terminal ends of Aα chains have been implicated in fibrin monomer polymerization.5–10 They have been postulated to either maintain a polymerization site on the adjacent amino terminals of α chains.11 to be responsible for the branching of the fibrin monomers,3 to act as the complementary sites that bind the fibrin monomers after fibrinopeptides B have been released.12 These studies made use of different preparations of partially degraded fibrinogen molecules whose full chemical structures were not determined. We have prepared a plasmin degradation fragment of fibrinogen to apparent homogeneity. It has an apparent M, $\approx$ 24,000 by gel filtration and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and constitutes approximately the middle two thirds of fibrinogen α chains. We have used this degradation fragment to provide direct evidence, for the first time, that this region of the fibrinogen Aα chains is important for fibrin monomer polymerization.

MATERIALS AND METHODS

Human fibrinogen was purchased from either Kabi Diagnostica (Grade L; Uppsala, Sweden) or from the American Diagnostica (Grade L; Greenwich, CT) and were $\sim$95% clottable. Bovine thrombin was a product of Merck (Germany). Lysine, ε-amino caproic acid, cyanogen bromide (CNBr), diisopropyl fluorophosphosphate (DFP), agarose and molecular weight markers for SDS-PAGE phosphorylase b (97,400), bovine serum albumin (BSA; 66,000), ovalbumin (45,000), trypsinogen (24,000), and α-lactoglobulin (18,400), were from Sigma (St Louis, MO). Sephacryl S-200, Sepharose 4B, and gel filtration calibration kit containing Blue dextran 2000, BSA, ovalbumin, and chymotrypsinogen A were products of Pharmacia (Uppsala, Sweden). BioGel P-30 was a product of BioRad (Rochmond, CA). Chemicals used in SDS-PAGE were obtained from Kodak (Rochester, NY). Fibrinogen fragments D and E, and antiserum against each fragment were purchased from Diagnostica Stago (Asnieres, France). 4CaCl₂ and carrier-free Na¹²⁵ io-dide were obtained from Amersham (Buckinghamshire, England), Urokinase was obtained from Green Cross (Osaka, Japan) or American Diagnostic as a mixture of two-chained high- and low-molecular weight urokinase forms. Human gelatin-plasminogen was prepared from fresh frozen plasma according to Deutsch and Mertz,13 except that 10 mmol/L benzamidine-HCl and 0.1 mg/mL of aprotinin were added to plasma before lysine-Sepharose chromatography, and Sephacryl S-200 was used to remove the trailing lipoprotein fractions of the product of the affinity chromatography.

Preparation of 24-Kd fragment. Human plasminogen (3.3 μmol) was activated by urokinase (800 U) in 20% glycerol in a total volume of 2 mL for 1 hour at 37°C. Human fibrinogen (141.2 μmol) in 10 mL of 0.1 mol/L sodium phosphate pH 7.4, was admixed with the plasmin prepared above (plasmin:fibrinogen = 2.3% mol/mol) for 30 minutes at 37°C, and the reaction was stopped by adding 10 mmol/L DFP. It was chromatographed in a Sephacryl S-200 column (2.6 × 85 cm), developed in 0.1 mol/L sodium phosphate, pH 7.4, at a flow rate of 15 mL/h. Fractions trailing the major protein peak were concentrated by ultrafiltration with an Amicon YM-5 membrane (Amicon, Beverly, MA). Concentrates from two runs were pooled and rechromatographed on a second Sephacryl S-200 column (1.6 × 100 cm) at a flow rate of 10 mL/h. Alternatively, the pooled sample was gel filtered in BioGel
P-30 under the same conditions. Successive Sephacryl S-200 column chromatographies appeared to yield purer fragment preparations. All column chromatographies were performed at 4°C.

Protein determination, amino acid analysis and N-terminal sequencing. Protein concentrations were determined according to Lowry et al.\textsuperscript{12} Using BSA as standards, or monitored by 280-nm absorbance. The molecular weights of fibrinogen, fibrin monomer, and plasminogen used were 340,000, 334,000 and 90,000, respectively. The amino acid composition of the 24-Kd fragment was obtained after hydrolysis in 6 mol/L HCl for 24 hours, and chromatographed in an LKB 4400 amino acid analyzer (Pharmacia, Uppsala, Sweden). Peptide sequencing was performed using a Porton gas-phase microsequencer (Porton, Taza, CA; model 2090) equipped with an on-line phenyl thiodyantoin (PTH)-amino acid analyzer\textsuperscript{12} after the protein was adsorbed on polyvinylidene difluoride membrane. This was performed by the Biotechnology Service Centre of the University of Toronto.

Immunologic methods. Hyperimmune antiserum against 24-Kd fragment were raised in a New Zealand white rabbit by multiple injections as described before.\textsuperscript{16} Double immunodiffusion was performed according to Ouchterlony and Nilsson,\textsuperscript{17} using antiserum against 24-Kd fragment, or commercially available antiserum against either fibrinogen fragment D or fragment E, and antigen concentrations between 0.1 and 1 mg/mL.

Inhibition of clotting by 24-Kd fragment. The anticoagulant activity of 24-Kd fragment was measured by the prolongation of plasma clotting time induced by 24-Kd fragment. This was performed by a thrombin time assay,\textsuperscript{16} by adding 0.2 mL of thrombin (3 NIH U/mL) to 1.0 mL normal pooled human plasma, which was premixed for 3 minutes at 37°C with 0.1 mL of various concentrations of 24-Kd fragment (5 × 10⁻⁷ to 5 × 10⁻⁶ mol/L) in 0.9% NaCl. The times needed to form a visible clot were then determined. An average of five determinations for control with 0.9% NaCl alone, and five determinations for each sample were taken, and the experiment was performed with two different preparations of 24-Kd fragment. The average time needed to clot plasma in the presence of 0.9% NaCl alone was 38 ± 3 seconds (n = 8).

Inhibition of thrombin by 24-Kd fragment. Inhibition of the amidolytic activity of thrombin was performed by measuring the residual enzyme activity after thrombin was reacted with 24-Kd fragment. Thrombin (final concentration 0.04 NIH U/mL) in 0.1 mL of 0.01 mol/L sodium phosphate, 0.13 mol/L NaCl, pH 7.4, buffer was first incubated with 0.1 mL of either buffer or 24-Kd fragment (final concentrations 5.04 × 10⁻⁴ mol/L and 2.5 × 10⁻⁴ mol/L) at 37°C for 15 minutes. Then, 0.7 mL of the phosphate buffer and 0.1 mL of 0.4 mmol/L H-D-Phe-Pip-Arg p-nitroanilide (S-2238; Kabi) were added and the chromophore released was followed at 405 nm.

Calcium binding. Solutions of 24-Kd fragment and fibrinogen were prepared free of Ca²⁺ ions before reacting with "Ca as described.\textsuperscript{17} Protein, 0.5 mg, in 1-mL solutions were equilibrium dialyzed in 10⁻⁴ mol/L to 10⁻⁵ mol/L of nonradioactive CaCl₂, to which were added a constant 20 μL of 77.7 μCi/g ⁴⁵Ca (25 Ci/g from Amersham). Triplicate 0.1-mL aliquots of inner and outer solutions after dialysis were then counted in a Beckman LS-250 liquid scintillation counter (Beckman, Fullerton, CA) after mixing with 10 mL Aqualos-2 (New England Nuclear, Boston, MA).

Binding of 24-Kd fragment to fibrinogen-bound and fibrin-bound Sepharose. Fibrinogen-bound and fibrin monomer-bound Sepharose 4B gels were prepared according to Heene and Matthias,\textsuperscript{16} and the amount of fibrinogen bound per milliliter of gel was estimated by noting the absorbance at 280 nm that was not bound after dialysis with 10 mL Aqualos-2 (New England Nuclear, Boston, MA).

Fig 1. The time course of plasmin digestion of fibrinogen. Plasmin was added to fibrinogen and aliquots of the reaction mixtures were removed into SDS-PAGE sample buffer, boiled for 2 minutes, and electrophoresed on 11% cross-linked gel. (Top) Nonreduced samples. (Bottom) Reduced samples. The lanes labeled with 0, 10, 20, 30, 40, and 60 represented samples taken at these different time points in minutes. The lane labeled as F was the product after the first Sephacryl S-200 column. The lane labeled as T was molecular weight marker trypsinogen. The lane labeled as S contained molecular weight markers BSA (468K), ovalbumin (45,000), and trypsinogen (24,000).
ANTICOAGULANT FIBRINOGEN FRAGMENT

Briefly, 0.98 mL of 24-Kd fragment in concentrations of 2.9 \times 10^{-4} mol/L to 2.4 \times 10^{-5} mol/L or 0.05 mol/L Tris/0.1 mol/L NaCl, pH 7.5, buffer alone was added to a cuvette. Twenty microliters of 6 mg/mL fibrin monomer in 0.02 mol/L acetic acid (final concentration 3.5 \times 10^{-7} mol/L) was then added and the spontaneous fibrin polymerization on dilution was followed spectrophotometrically at 350 nm. The maximum rate of polymerization was measured as the slope at the inflexion point of the sigmoidal curve.

Electrophoresis. SDS-PAGE was performed according to Laemmli. To monitor the progress of fibrinogen degradation by plasmin, reaction mixtures containing \sim 5 \mu g proteins, at 0, 10, 20, 30, 40, and 60 minutes after proteolysis, were boiled in the SDS buffer and electrophoresed in 11% cross-linked gel.

RESULTS

Preparation of 24-Kd fragment. The time course of fibrinogen degradation by plasmin was followed by SDS-PAGE and is shown in Fig 1. A fibrinogen fragment of apparent Mₙ \approx 24,000 appeared after 10 minutes of incubation and remained as a prominent band throughout the time course. This fragment was isolated by gel filtrations using the reaction mixture obtained after 30 minutes of proteolysis. Occasionally a fibrinogen sample would produce one major and several minor peptides of similar size under this condition and the sample would not be used for isolation of the fragment. The degradation products were separated by Sephacryl S-200 (Fig 2A). Fractions 123 to 136 contained relatively small degradation products. They were concentrated, pooled with fractions of a similar column run, and rechromatographed on a smaller Sephacryl S-200 column (Fig 2B). A single protein peak could be eluted whose migration on the gel filtration medium was similar to that of chymotrypsinogen. The peak fractions 52 to 64 displayed on SDS-PAGE a stained band of Mₙ \approx 24,000. Fractions 55 to 60 were pooled and used as 24-Kd fragment in subsequent experiments.

Chemical and immunologic properties of 24-Kd fragment. The amino acid composition of 24-Kd fragment was obtained by acid hydrolysis followed by ion exchange chromatography. This fragment contained an unusually large number of hydrophilic amino acids. Its amino acid composition was compared with that of an Mₙ \approx 24,000 plasmic fibrinogen degradation fragment obtained by Mihalyi and is presented in Table 1. For comparison, the amino acid compositions of several fibrinogen degradation products of similar size obtained by other methods are also included in the table. The amino acid compositions of all peptides were normalized against that of Mihalyi by assuming that 21.7 residues of aspartate were found in each of them. There appears to be close agreement among most of the published data for these degradative fragments, variously known as fragment A,24,25 fragment H,26 and cyanogen bromide fragments ‘Fcb-3’27 and ‘Hi2-Ala.’28 The amino acid sequence analysis of our degradative fragment showed an amino terminal sequence of met-glu-leu-glut-leu-glu-arg-pro-gly-gly-asn-glu-ile. This corresponds to a sequence of amino acids from fibrinogen Aa chains, starting at residue Met-240. This material will be denoted as 24-Kd fragment.

Inhibition of fibrin polymerization by 24-Kd fragment. DesAABB and desAA fibrin monomers were prepared according to Belitser et al.\textsuperscript{19} using, respectively, thrombin or snake coagulant Atroxin (Bathrops atrox) (Sigma). The inhibition of fibrin monomer polymerization was performed according to Budzynski et al.\textsuperscript{21} Twelve milliliters of the digest was loaded on to a Sephacryl S-200 column. From right to left are shown fractions 52 (containing very faint bands), 54, 56, 58, 60, 62, 64, and molecular weight markers phosphorylase b, BSA, trypsinogen, and a-lactalbumin.

In Fig 2. (A) Gel filtration of fibrinogen after 30 minutes of plasmin digestion. Twelve milliliters of the digest was loaded on to a Sephacryl S-200 column (2.6 \times 85 cm) and chromatographed in 0.1 mol/L Na phosphate pH 7.4 buffer at a flow rate of 15 mL/h. The elution was monitored by 280-nm absorbance and 2-mL fractions were collected. The arrows on top of the figure indicate the migration of different gel filtration molecular weight markers. The bar indicates fractions that were pooled with similar column eluates from a different preparation. (B) Three milliliters of pooled and concentrated samples from Sephacryl S-200 were rechromatographed on a smaller Sephacryl S-200 column (1.6 \times 100 cm). The bar indicates samples to be used as 24-Kd fragment. (Inset of B) Ten percent nonreduced SDS-PAGE of the fractions from the Sephacryl S-200 column. From right to left are shown fractions 52 (containing very faint bands), 54, 56, 58, 60, 62, 64, and molecular weight markers phosphorylase b, BSA, trypsinogen, and a-lactalbumin.
Table 1. Amino Acid Composition of 24-Kd Fragment and Seven Fibrinogen Fragments of Similar Sizes

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The amino acid compositions of all peptides were normalized against an M, 24,000 plasminolytic fragment of fibrinogen that represents the sequence 240 to 491 of Aa chains. The peptide referred to under (1) was from Mihalyi; (2) Hessel; (3) Takagi and Kawai; (4) Takagi and Doolittle; (5) Gollwitzer and Timpl; (6) Harfenist and Canfield; (7) Watt et al.; and (8) Sobel et al.

The antiserum against 24-Kd fragment forms a single precipitin arc with 24-Kd fragment and it cross-reacted completely with fibrinogen (inset of Fig 3). However, the antiserum did not cross-react with either fragment D or fragment E (inset of Fig 3). On the other hand, antiserum against either fragment D or fragment E did not cross-react with 24-Kd fragment at a concentration of 1 mg/mL (data not shown). These results indicated that 24-Kd fragment contained little, if any, contaminating fragment D or E.

Anticlotting activity of 24-Kd fragment. Increasing concentrations of 24-Kd fragment were found to prolong plasma clotting time up to 65% of control plasma (Fig 3). Two different preparations of 24-Kd fragment were used for these experiments and essentially the same results were obtained. To understand how this anticoagulation was brought on by this fragment, inhibition of the enzymatic activity of thrombin by 24-Kd fragment, binding of Ca$^{2+}$ ions necessary for fibrin cross-linking, and inhibition of fibrin monomer polymerization were studied.

Inhibition of thrombin amidolytic activity. The 24-Kd fragment at concentrations ranging from $5.04 \times 10^{-8}$ mol/L to $2.5 \times 10^{-8}$ mol/L was not able to inhibit the amidolytic activity of thrombin. The absorbance at 405 nm was 0.24 in the beginning as was at the end of the incubations in the presence of various concentrations of 24-Kd fragment (data not shown).

Binding of $^{45}$Ca to 24-Kd fragment. The 24-Kd fragment did not bind calcium ions under our experimental conditions. One-half milligram of 24-Kd fragment in 1 mL bound ~5% of the input radioactive $^{45}$Ca in the presence of unlabeled, 1, 5, 10, 30, 70, 100, 500, and 1,000 \mu mol/L of CaCl$_2$. This amount of bound radioactivity was found to be
the same as the background without 24-Kd fragment. As a control to show that calcium binding could be determined under our experimental conditions, the same amount of fibrinogen was found to bind, respectively, 5.3%, 8.0%, and 9.7% of the input 45Ca, in the presence of 100, 10, and 1 µmol/L unlabeled CaCl₂.

Binding of 24-Kd fragment to fibrinogen and fibrin monomer. To study the interactions between 24-Kd fragment and fibrinogen or fibrin monomers produced by thrombin, 24-Kd fragment was allowed to bind with fibrinogen or fibrin monomer immobilized on Sepharose 4B gels. It can be seen in Fig 4 that 24-Kd fragment was able to bind fibrin-Sepharose, but not fibrinogen-Sepharose. This experiment has been performed on three occasions with three different preparations of 24-Kd fragment. The inset of Fig 4B shows SDS-PAGE pattern of the materials bound by fibrin-Sepharose, which consisted essentially of 24-Kd fragment.

Inhibition of fibrin polymerization. Fibrin monomer polymerization was inhibited in the presence of 24-Kd fragment. As shown in Fig 5A, increasing concentrations of 24-Kd fragment decreased both the maximum level of polymerization and the maximum rate of polymerization. The polymerization of desAA fibrin was similarly inhibited by 24-Kd fragment (data not shown). The decrease of the maximum level of polymerization was 55% of the control level at a 24-Kd fragment:fibrin ratio of 4:1 (Fig 5A). Similarly, the decrease of the maximum rates of polymerization for both types of fibrin monomers at the same 24-Kd fragment:fibrin ratio reached 54% of the control values obtained in the absence of 24-Kd fragment (Fig 5B). On the other hand, there was no significant difference in the initial lag times in the absence or presence of 24-Kd fragment (Fig 5A). This indicated that the process(es) responsible for changes in the lag period may be different from the process(es) that gives rise to changes in maximum rate and maximum level of polymerization, and that 24-Kd fragment only affected the latter ones.

To further understand the effect of 24-Kd fragment on fibrin monomer polymerization, the maximum rates of polymerization of 5 × 10⁻⁷ mol/L to 2 × 10⁻⁸ mol/L desAABB fibrin were measured in the presence or absence of 4 × 10⁻⁸ mol/L of 24-Kd fragment. As shown in Fig 6, the maximum rates of polymerization were reduced in the presence of 24-Kd fragment for all concentrations of fibrin used. This would suggest that 24-Kd fragment might have functioned to reduce the concentrations of fibrin-monomer molecules available for the polymerization reaction.

DISCUSSION

A fibrinogen fragment was isolated from a mixture of degradation products after partial plasmin digestion. It was purified by directly separating the mixture on Sephacryl S-200. The molecules with slower mobility were rechromatographed to yield an apparently homogeneous preparation of peptides having Mₐ ≈ 24,000 on gel filtration, and in reduced and nonreduced SDS-PAGE. N-terminal sequence analysis indicates that this peptide is part of fibrinogen Aα chains, starting at Aα chain amino acid Met-240. The fragment represents the middle part of Aα chains and contains approximately one third of its total length toward the carboxyl terminal end. Plasmic degradation fragments of fibrinogen having similar molecular weights had been isolated before, and the amino acid composition of 24-Kd fragment appeared to be similar to them (Table I). The fragment isolated by Mihalyi against which all the other fragments have been normalized was reported to compose...
of Aa 240 to 491. To precipitate the larger degradation molecules, these previous preparations had all used a heating or acidification step before isolation. Our 24-Kd fragment has not been subjected to such treatments. It has an identical N-terminal sequence as that reported for fragment H, although the molecular weight of fragment H was reported to be 20,000. This discrepancy in size could be a reflection of the difference in the methods used for molecular weight determination, or that fragment H was a truncated form of the 24-Kd fragment.

Fibrinogen fragment D contains a major fibrin polymerization site and its contamination in our 24-Kd fragment preparations would have affected some of our results. Our 24-Kd fragment was found to be free of fragment D and fragment E, because these fragments were not found on SDS-PAGE and because an antiserum raised against 24-Kd fragment did not cross-react with either fragment D or E. Conversely, the antiserum against either fragment D or E also did not cross-react with 24-Kd fragment preparations. Furthermore, 24-Kd fragment inhibited fibrin polymerization in a very different manner as compared with fragment D (see later text).

A small portion of the 24-Kd fragment could bind fibrin immobilized on Sepharose but none of it was able to bind fibrinogen immobilized on Sepharose gel (Fig 4). This could be caused by a combination of low-affinity binding or less-than-optimal binding conditions. Fragment A, which has an amino acid composition similar to our 24-Kd fragment, had been found to possess no fibrin-binding capacity and did not inhibit fibrin polymerization. However, no biochemical characterization of the fragment A other than its $M_r$ of 21,000 had been reported in these studies. It could be that a shorter form of the peptide compared with 24-Kd fragment was used, or as alluded before, heating or acidification of the degradation mixture before chromatography might have altered the fragment's structure. These suggestions are in accord with the view that peptide conformation is very important as far as fibrin polymerization is concerned. This can be illustrated by the contradictory results with regard to the polymerization inhibitory activity of the carboxyl ends of $\gamma$ chains. It has been reported that the sequence $\gamma$ 374 to 411 of fragment D could inhibit fibrin polymerization, and this observation was collaborated in a $\gamma$ 374 to 396 peptide. However, the peptide $\gamma$ 374 to 411 was later found to be noninhibitory in another study, and no inhibitory activity in peptides $\gamma$ 303 to 356, $\gamma$ 357 to 373, and $\gamma$ 374 to 405 could be detected either singly or in combinations. Recently, fragment D and D dimers were found
to lose their fibrin-binding capacity and polymerization inhibitory activity on denaturation. Thus, it was proposed that either combination of the fragments used in the conflicting studies were different or that other parts of $\gamma$ chains may also be involved in the formation of these binding sites.

The anticoagulant activity of 24-Kd fragment could be a result of inhibition of thrombin activation of fibrinogen, a sequestration of calcium ions needed for fibrin polymerization, or a retardation of the polymerization reaction. Our results indicate that at least the amidolytic activity of thrombin is not affected by 24-Kd fragment. The carboxyl ends of $\alpha\alpha$ chains were thought to form a calcium binding site, but 24-Kd fragment did not appear to bind calcium ions, therefore eliminating the possibility that it takes up the metal ion necessary for polymerization. Therefore, it is likely that 24-Kd fragment interferes with fibrin monomer polymerization (Fig 5). This may in turn lead to inhibition of the plasma thrombin clotting time, although no binding of the fragment to either purified or plasma-fibrin clot has been shown yet. The underlying mechanism of how 24-Kd fragment could accomplish this appears to be complex. 

Both desAA and desAABB fibrin-monomer polymerizations were affected by 24-Kd fragment (Fig 5B), suggesting the probable interference of end-to-end association of monomers. The 24-Kd fragment did not appear to affect the initial lag phase of the fibrin monomer polymerization, but inhibited both the maximum rate and extent of this reaction (Fig 5A). This inhibition did not appear to be very potent because at a 4:1 molar ratio of 24-Kd fragment to fibrin monomers, only $\sim 45\%$ of the original maximum rate and maximum level of polymerization could be inhibited. This would suggest that 24-Kd fragment did not function as complementary binding sites for fibrin monomers per se, because stoichiometric complex between 24-Kd fragment and fibrin monomer would be more likely in this case (ie, 50% of maximum rate should have been reached at a 1:1 molar ratio between 24-Kd fragment and fibrin monomer in Fig 5B). Shen et al. and Medved et al. had reported that the C-terminal two-thirds of $\alpha\alpha$ chains do not form a polymerization site, but help to maintain one during the polymerization of fibrin monomers. In agreement with this was the finding that fibrinogen fragment X does not form interconnected fibrin networks. The results in these studies had been inferred by using fibrinogen preparations where various lengths of the C-terminal sequence of $\alpha\alpha$ chains were missing (fragment X). We have shown here that an isolated fragment from this part of the molecule directly interferes with polymerization of fibrin monomers.

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Anticoagulant function of a 24-Kd fragment isolated from human fibrinogen A alpha chains

HK Lau