The Role of γA/γ′ Fibrinogen in Plasma Factor XIII Activation*

Maia Moaddel‡‡, Lisa A. Falls¶¶, and David H. Farrell**

From the ‡Department of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033 and ¶Department of Oral Molecular Biology, School of Dentistry, Oregon Health Sciences University, Portland, Oregon 97201

Factor XIII zymogen activation is a complex series of events that involve fibrinogen acting in several different roles. This report focuses on the role of fibrinogen as a cofactor in factor XIII activation by thrombin. We demonstrate that fibrinogen has two distinct activities that lead to an increased rate of factor XIII activation. First, the thrombin proteolytic activity is increased by fibrin. The cleavage rates of both a small chromogenic substrate and the factor XIII activation peptide are increased in the presence of either the major fibrin isoform, γA/γ′ fibrin, or a minor variant form, γA/γ′ fibrin. This enhancement of thrombin activity by fibrin is independent of fibrin polymerization and requires only cleavage of the fibrinopeptides. Subsequently, γA/γ′ fibrinogen accelerates plasma factor XIII activation by a non-proteolytic mechanism. This increased rate of activation results in a slightly more rapid cross-linking of fibrin γA and γ′ chains and a significantly more rapid cross-linking of fibrin α chain multimers. Together, these results show that although both forms of fibrin increase the rate of activation peptide cleavage by thrombin, γA/γ′ fibrinogen also increases the rate of factor XIII activation in a non-proteolytic manner. A revised model of factor XIII activation is presented below.

Plasma coagulation factor XIII (EC 2.3.2.13) is the zymogen precursor to the active transglutaminase factor XIIIa. Plasma factor XIII consists of two α subunits (Mr ~83,000) and two β subunits (Mr ~80,000) with the stoichiometry α2β2, whereas a form of factor XIII stored in platelets lacks the β subunits (1–4). In the final stages of blood coagulation, thrombin activates plasma factor XIII by cleaving an activation peptide (Mr ~4000) from the amino terminus of each α subunit (3). In the presence of calcium, active factor XIII (factor XIIIa) then catalyzes the formation of isopeptide bonds in polymerized fibrin strands between adjacent glutamine/lysine side chains (5). Cross-linking occurs in the α and γ chains of fibrin but not in the β chains, and it results in the formation of γ–γ dimers, γ chain multimers, α chain multimers, and complexes between α and γ chains (6). Cross-linked fibrin shows increased stability (7) and increased proteolytic resistance to fibrinolytic enzymes (8).

Factor XIII activation by thrombin is a multistep process that is modulated by calcium and by the presence of fibrinogen. An explicit model of factor XIII activation has been proposed (9) in which the initial activation step is thrombin cleavage of the activation peptide from the α subunit, which is then referred to as the α′ subunit. However, the α2β2 complex is not catalytically active. In the presence of high concentrations of calcium, i.e. >10 mM, the α2β2 complex dissociates into the α2 and β2 subunits. Fibrin facilitates this dissociation step such that it occurs at physiologic calcium concentrations (~1.5 mM), thereby increasing the rate of factor XIII activation under physiologic conditions. Finally, a conformational change occurs in the α2β2 subunit to unmask the active site cysteine to form active α2β2. This model is shown in Scheme 1.

A variant form of the fibrinogen γ chain, the γ′ chain (or γB chain), is found in about 5–15% of normal human plasma fibrinogen (10, 11). This variant chain arises by alternative mRNA processing (12, 13) and is present as a heterodimer with the more common γ (or γA) chain in γA/γ′ fibrinogen. This processing event is apparently conserved across several different species (Table I), although the relative amounts of γA/γ′ fibrinogen and γA/γ′ fibrinogen vary among species. In addition, the presence of tyrosine O-sulfate at position 418 in the γ′ chain is also conserved in humans and rats (14–16); however, the human γ′ chain is also sulfated at Tyr-422. Human γA/γ′ fibrin is more extensively cross-linked by factor XIIIa than is γA/γ′ fibrin, resulting in fibrin clots with increased fibrinolytic resistance (17). Furthermore, the level of γA/γ′ fibrinogen in plasma is an independent risk factor for coronary artery disease. However, the mechanism responsible for the more extensive cross-linking in γA/γ′ fibrinogen is not clear because binding interactions occur between both γA/γ′ fibrinogen and thrombin (18) and γA/γ′ fibrinogen and the b subunit of factor XIII (19).

In the process of investigating the mechanism responsible for the more extensive cross-linking in γA/γ′ fibrin, we discovered that both forms of fibrin increase the rate of factor XIII activation peptide cleavage by thrombin and that the γA/γ′ form of fibrinogen subsequently increases the rate of factor XIII activation to a much greater extent than γA/γ′ fibrinogen. This leads to more rapid cross-linking of γA/γ′ fibrin by factor XIIIa,

---

* This work was supported in part by Student Awards from the American Heart Association, Pennsylvania Affiliate (to M. M. and L. A. F.), Grant-in-aid for Research S98695P from the American Heart Association, Pennsylvania Affiliate (to D. H. F.), Grant-in-aid for Research 9950012N from the American Heart Association, Pennsylvania Affiliate (to D. H. F.), and National Institutes of Health Grants R29HL53997 and K02HL04215 from the NHLBI (to D. H. F.).

‡ Present address: Diabetes Branch-Experimental Diabetes, Metabolism, and Nutrition Section, NIDDK, National Institutes of Health, Bethesda, MD 20892.

¶ Present address: Center for Hemostasis and Thrombosis Research, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215.

** To whom correspondence should be addressed. Tel.: 503-494-8602; Fax: 503-494-8918; E-mail: farrelld@ohsu.edu.

1 R. S. Lovely, L. A. Falls, H. A. Al-Mondhiry, C. E. Chambers, H. Ni, and D. H. Farrell, manuscript in preparation.

2 R. S. Lovely, M. Moaddel, A. Stafford, J. I. Weitz, and D. H. Farrell, manuscript in preparation.
in particular, leading to the formation of α chain multimers. These findings not only provide a mechanistic explanation for the more extensive cross-linking observed in γA/γ' fibrin clots but also necessitate a reevaluation of the current model of factor XIII activation.

**EXPERIMENTAL PROCEDURES**

Proteins—γA/γA and γA/γ' fibrinogens were purified from plasminogen-free human fibrinogen (Calbiochem) using DEAE-cellulose as described previously (17, 20). In some experiments, the two forms of fibrinogen were further purified using a glycine-L-proline-L-arginine-L-proline-t-cysteine-agarose affinity resin as described previously (21).

Purified plasma factor XIII was generously provided by Dr. Michael W. Mosesson (Blood Center of Southeastern Wisconsin, Milwaukee, WI), or it was obtained from Enzyme Research Laboratories (South Bend, IN). Purified α-thrombin was generously provided by Dr. John W. Fenton, II (New York State Department of Health, Albany, NY) and Dr. Walter Kiesel (University of New Mexico, Albuquerque, NM). Purified γ-thrombin was obtained from Hematologic Technologies, Inc. (Essex Junction, VT).

**Thrombin Activity Assay**—Thrombin activity was quantitated by the hydrolysis of tosyl-glycyl-prolyl-arginine-4-nitranilide (Chromozym™ TH) according to the manufacturer’s protocol (Roche Molecular Biochemicals). Briefly, 9 nM α-thrombin and 0.177 mM tosyl-glycyl-prolyl-arginine-4-nitranilide were incubated in 227 mM NaCl, 50 mM Tris, pH 8.3, 1 mg/ml bovine serum albumin, 0.01% sodium azide at room temperature in the presence or absence of various concentrations of γA/γ fibrinogen or γA/γ' fibrinogen with 1 mM GPRP to prevent fibrin polymerization. In some experiments, α-thrombin, fibrinogen, and GPRP were incubated together for 1 h at room temperature before the addition of tosyl-glycyl-prolyl-arginine-4-nitranilide to convert the fibrinogen to fibrin. The absorbance at 405 nm was measured spectrophotometrically.

**Factor XIIIa Cleavage/Activity Assay**—To quantitate the rate of factor XIII cleavage by thrombin and activation, factor XIII (43 nM) was incubated for 1 h at room temperature in the presence or absence of γA/γ fibrinogen, γA/γ' fibrinogen, or a mixture of the two, maintaining the total fibrinogen concentration at 86 nM. 1 mM CaCl₂ and 1 NIH unit/ml α-thrombin or 0.33 μM γ-thrombin were then added, and the thrombin was inactivated at the indicated times with 0.5 mM PPACK. Factor XIIIa activity was measured by the incorporation of 5-(biotinamido)pentylamine into immobilized N,N'-dimethylcasein and detected at 405 nm by the hydrolysis of p-nitrophenyl phosphate after incubation with streptavidin-conjugated alkaline phosphatase. The results show more rapid activation of factor XIII by α-thrombin in the presence of γA/γ fibrinogen.

**RESULTS**

**Factor XIII Is Activated by Thrombin More Rapidly in the Presence of γA/γ' Fibrinogen**—To determine the mechanism behind the increased cross-linking and fibrinolytic resistance of γA/γ' fibrin clots (17), especially in light of previous reports that γA/γ' fibrinogen binds to both thrombin (18) and factor XIII (19), we tested the hypothesis that γA/γ' fibrinogen affects the rate of factor XIII activation. Fig. 1 shows that the activation formation, 43 nM factor XIII was incubated for 1 h at room temperature in the presence of γA/γ fibrinogen or γA/γ' fibrinogen ranging from 0 to 600 nM. Clotting was initiated by the addition of 1 mM CaCl₂ and 1 NIH unit/ml α-thrombin, and the reaction was stopped after 6 min with electrophoresis sample buffer. The cross-linked fibrin chains were resolved by electrophoresis in 7% polyacrylamide gels after reduction and quantitated by scanning the silver-stained bands. The data are expressed as the ratio of optical density of the fibrin chain bands divided by the optical density of the α-fibrinogen affecting the concentration of the bands scanned in these assays (data not shown). For quantitation of α-multimers, 43 nM factor XIII was incubated for 1 h at room temperature in the presence of γA/γ fibrinogen or γA/γ' fibrinogen ranging from 0 to 600 nM. 1 mM CaCl₂ and 1 NIH unit/ml α-thrombin were then added, and the reaction was stopped after 16 min with electrophoresis sample buffer. The cross-linked fibrin chains were resolved by electrophoresis in 7% polyacrylamide gels following reduction and quantitated by scanning the silver-stained bands. The data are expressed as the ratio of optical density of the α chain multimer bands divided by the optical density of the β chain bands.

**Fig. 1. Factor XIII activation kinetics by α-thrombin in the presence or absence of fibrinogen.** 43 nM factor XIII and 1 mM GPRP were incubated for 1 h at room temperature in the presence or absence (●) of 86 nM γA/γ fibrinogen (●), 86 nM γA/γ fibrinogen (○), or a 1:1 mixture (▲) of the two (86 nM total). 1 mM CaCl₂ and 1 NIH unit/ml α-thrombin were then added, and the thrombin was inactivated at the indicated times with 0.5 mM PPACK. Factor XIIIa activity was measured by the incorporation of 5-(biotinamido)pentylamine into immobilized N,N’-dimethylcasein and detected at 405 nm by the hydrolysis of p-nitrophenyl phosphate after incubation with streptavidin-conjugated alkaline phosphatase. The results show more rapid activation of factor XIII by α-thrombin in the presence of γA/γ fibrinogen.

The cleaved α chains were resolved by electrophoresis in 6% polyacrylamide gels after reduction (22) and quantitated by scanning the silver-stained bands. The data are presented as the optical density of the bands, expressed in arbitrary units relative to the background density. The linearity of this method was verified empirically for the concentration of the bands that were scanned in these assays (data not shown). Factor XIIIa activity was measured by the incorporation of 5-(biotinamido)pentylamine (Pierce) into immobilized N,N’-dimethylcasein (Sigma) and detected at 405 nm by the hydrolysis of p-nitrophenyl phosphate after incubation with streptavidin-conjugated alkaline phosphatase (23).

Fibrin Cross-linking Assay—To quantitate the rate of γ-γ dimer formation, 43 nM factor XIII was incubated for 1 h at room temperature in the presence of γA/γ fibrinogen or γA/γ' fibrinogen ranging from 0 to 600 nM. Clotting was initiated by the addition of 1 mM CaCl₂ and 1 NIH unit/ml α-thrombin, and the reaction was stopped after 6 min with electrophoresis sample buffer. The cross-linked fibrin chains were resolved by electrophoresis in 7% polyacrylamide gels after reduction and quantitated by scanning the silver-stained bands. The data are expressed as the ratio of optical density of the α chain multimer bands divided by the optical density of the β chain bands.
tion rate of factor XIIIa by thrombin was significantly increased in the presence of γA/γ′ fibrinogen but only slightly increased in the presence of γA/γ fibrinogen. A 1:1 mixture of γA/γA and γA/γ′ fibrinogens, in which the total amount of fibrinogen was kept constant, was intermediate in its ability to enhance the activation of factor XIII, indicating that there was no inhibitory substance in the γA/γ fibrinogen preparation. These experiments show that γA/γ′ fibrinogen serves as a more active cofactor in factor XIII activation than does γA/γ fibrinogen.

However, although these results demonstrate the exposure of the catalytic site of factor XIIIa toward a small molecule substrate, namely 5-(biotinamido)pentylamine, they do not necessarily mean that factor XIIIa activity toward macromolecular substrates is increased. The rate of factor XIIIa-mediated cross-linking of two macromolecular substrates, the α and γ chains of fibrin, was therefore quantitated in both γA/γA and γA/γ′ fibrins. Fig. 2 shows that the amount of γ-γ dimers formed in fibrin with a fixed amount of factor XIII increased in a dose-dependent manner with increasing fibrinogen concentration and reached only a slightly higher plateau in γA/γ′ fibrin than in γA/γ fibrin. However, the amount of α chain multimers formed in γA/γ fibrin was much greater than in γA/γ fibrin (Fig. 3). The presence of α chain multimers is correlated with fibrinolytic resistance (25). These results demonstrate that factor XIII is activated by thrombin more rapidly in the presence of γA/γ′ fibrinogen, resulting in increased cross-linking of γA/γ′ fibrin.

To determine whether γA/γ′ fibrinogen or γA/γ fibrin is the active cofactor, the experiment was repeated using γ-thrombin. γ-Thrombin is an autoproteolytic product of α-thrombin that lacks the ability to cleave fibrinopeptides A and B to convert fibrinogen to fibrin; however, it maintains the ability to cleave factor XIII. Fig. 4 shows that γA/γ fibrinogen still serves as a cofactor for factor XIII activation under these conditions, indicating that its conversion to γA/γ′ fibrin is not required for cofactor activity.

**Fibrin Increases the Rate of Thrombin Cleavage of Factor XIII**—Although the data in Fig. 4 show that γA/γ′ fibrinogen does not require conversion to fibrin to serve as a cofactor for factor XIII activation, previous reports have shown that conversion to fibrin is required for fibrinogen to have the maximal cofactor activity for factor XIII activation (26). We therefore tested the hypothesis that fibrin may have a direct effect on thrombin proteolytic activity rather than on factor XIII itself. Fig. 5 shows that the amidolytic activity of α-thrombin is enhanced in the presence of either γA/γA or γA/γ′ fibrin compared to fibrinogen. When α-thrombin was preincubated with either γA/γA fibrinogen or γA/γ′ fibrinogen to form fibrin, its amidolytic activity toward a peptide substrate, tosyl-glycyl-prolyl-arginine-4-nitranilide, was significantly increased. However, although these results demonstrate an increase in thrombin’s ability to cleave a small peptide substrate, they do not necessarily mean that thrombin activity toward macromolecular substrates such as factor XIII is increased. Therefore, the...
cleavage rate of the factor XIII activation peptide was analyzed by polyacrylamide gel electrophoresis and quantitated by scanning densitometry (Fig. 6). The results show that fibrin increases the rate of α-thrombin cleavage of the factor XIII activation peptide, which is consistent with the fibrin effect on α-thrombin amidolytic activity. The rate of generation of the α₂-thrombin amidolytic activity was quantitated by the hydrolysis of tosyl-glycyl-prolyl-arginine-4-nitranilide at 405 nm by the hydrolysis of p-nitrophenyl phosphate after incubation with streptavidin-conjugated alkaline phosphatase. The results show a more rapid activation of factor XIII by γ-thrombin in the presence of γA/γ′ fibrinogen, indicating that γA/γ′ fibrinogen can enhance factor XIII activation without prior conversion to γA′ fibrin.

Taken together with the factor XIII activation experiments, these results show that the previously reported fibrin enhancement of factor XIII activation is because of two distinct components. 1) Enhanced α-thrombin proteolytic activity by both γA/γ fibrinogen and γA/γ′ fibrinogen leads to a more rapid cleavage of the factor XIII activation peptide; and 2) γA/γ′ fibrinogen serves as a cofactor in factor XIII activation.

**DISCUSSION**

The activation of plasma factor XIII is a complex process involving several different steps and cofactors. The current model of factor XIII α₂β₂ activation (27) begins with cleavage of the activation peptide from the α₂ subunits by thrombin. Subsequently, the activation peptide is released to yield the cleaved but inactive α₂β₂. The dissociation of α₂β₂ to yield α₂ dimers and β₂ dimers is facilitated by calcium and fibrin. The α₂ dimer then undergoes a calcium-dependent structural change that exposes the active site cysteine residue to yield active α₂⁺.

Fibrinogen serves several roles in this activation process. First, it acts as a carrier protein for unactivated α₂β₂ zymogen (19); second, it serves as a cofactor in the activation of α₂β₂ (28, 29); and finally, fibrinogen acts as a substrate for the activated α₂⁺ (30, 31). There is also an increase in substrate availability caused by fibrin polymerization. This increase is because of the juxtaposition of fibrin monomers in polymerized fibrins, such that particular glutamine/lysine side chains become properly oriented to serve as substrates for factor XIIIa cross-linking (29). In this report, we demonstrated that a minor isoform of fibrinogen, γA/γ′ fibrinogen, facilitates activation of α₂⁺ to a greater extent than does the major isoform, γA/γ fibrinogen. A simple interpretation of these findings is that γA/γ′ fibrinogen is the underlying active cofactor seen in previous studies (9) that used unfractionated fibrinogen during factor XIII activation. By inference from other studies (32), γA/γ′ fibrinogen could serve to facilitate dissociation of α₂⁺ from β₂ because γA/γ′ fibrinogen binds to the β₂ subunits (19).

However, another possibility is that γA/γ′ fibrinogen facilitates dissociation of the activation peptides. It has been assumed in previous studies that the release of the activation peptide coincides with thrombin cleavage; however, there are reasons to question this assumption. Several previous studies that measured the activation peptide release (9, 26, 33) used perchloric acid precipitation to separate α₂⁺ from the activation peptide. This technique is likely to underestimate the amount of cleaved but undissociated activation peptide if the cleaved peptide is still bound noncovalently to α₂. In fact, the crystal
structure of thrombin-cleaved \( \alpha_2^\text{a} \) reveals that the activation peptide is still bound after cleavage and blocks the active site cysteine (34). Therefore, the release of the activation peptide may occur much later in the activation process than previously assumed. As a consequence, it is possible that \( \gamma A/\gamma' \) fibrinogen may accelerate factor XIII activation by facilitating dissociation of the activation peptide from the cleaved \( \alpha_2^\text{a} \beta_2 \). Experiments to determine whether \( \gamma A/\gamma' \) fibrin facilitates dissociation of the activation peptide and/or facilitates dissociation of \( \alpha_2^\text{a} \) from \( \beta_2 \) have been hampered by the fact that free \( \beta_2 \) subunits of factor XIII form an insoluble precipitate in the presence of \( \gamma A/\gamma' \) fibrinogen (19).

In our revised model of factor XIII activation (Fig. 7), factor XIII is depicted as two elliptical \( \alpha \) subunits and two rectangular \( \beta \) subunits after a model proposed by Carrell et al. (35), and \( \gamma A/\gamma' \) fibrin monomers are depicted as elongated trinodular structures after a model proposed by Hall and Slayter (36). Factor XIII is assumed to be bound initially to two molecules of \( \gamma A/\gamma' \) fibrinogen. The rationale for this belief comes both from the assumption \( \text{a priori} \) that each of the two \( \beta \) subunits of factor XIII could potentially bind one molecule of \( \gamma A/\gamma' \) fibrinogen and from sedimentation equilibrium studies that show a complex of plasma factor XIII with two molecules of \( \gamma A/\gamma' \) fibrinogen.4

After fibrin formation, the factor XIII activation peptide is cleaved by thrombin at an increased rate but remains associated with the factor XIII \( \alpha_2^\text{a} \) subunit. Dissociation of the activation peptide and/or \( \beta \) subunits is accelerated by \( \gamma A/\gamma' \) fibrin and, the active thrombin remains bound to the fibrin clot. The \( \beta \) subunits dissociate from the \( \gamma' \) chain, and the activated \( \alpha_2^\text{a} \) dimer then binds to a different site than does the \( \alpha_2 \beta_2 \) zymogen to catalyze the cross-linking of fibrin.

The lack of \( \beta_2 \) subunits in platelet factor XIII (1–4) is intriguing from a physiologic viewpoint. Activation of platelet factor XIII is not influenced by fibrin (40), suggesting the specific interactions between \( \gamma A/\gamma' \) fibrin and the \( \beta \) subunits in plasma factor XIII (19)4 are required for the accelerative effect of \( \gamma A/\gamma' \) fibrin on \( \alpha_2^\text{a} \beta_2 \) activation. Without the \( \beta \) subunits, there is no need to have \( \gamma A/\gamma' \) fibrin for platelet factor XIII activation. Coincidentally, platelets also lack \( \gamma A/\gamma' \) fibrinogen (41). Plasma factor XIII may have therefore evolved to be activated less rapidly than platelet factor XIII, regulated by the presence of \( \gamma A/\gamma' \) fibrin. The physiologic significance of this is unclear at present, but it may contribute to the extensive fibrin cross-

---

4 Moaddel, M., Farrell, D. H., Daugherty, M. A., and Fried, M. G. (2000) Biochemistry 39, 6698–6705.
linking mediated by platelet factor XIIIa in platelet-rich clots, particularly the cross-linking of α2-antiplasmin to fibrin (42).

Acknowledgments—We thank Dr. Michael W. Mosesson for providing purified factor XIII and Drs. John W. Fenton, II and Walter Kisiel for providing purified human α-thrombin.

REFERENCES

1. Bohn, H. (1972) Ann. N. Y. Acad. Sci. 202, 256–272
2. Chung, S. I. (1972) Ann. N. Y. Acad. Sci. 202, 240–255
3. Schwartz, M. L., Pizzo, S. V., Hill, R. L., and McKee, P. A. (1971) J. Biol. Chem. 246, 5851–5854
4. Schwartz, M. L., Pizzo, S. V., Hill, R. L., and McKee, P. A. (1973) J. Biol. Chem. 248, 1395–1407
5. Chen, R., and Doolittle, R. F. (1969) Proc. Natl. Acad. Sci. U. S. A. 63, 420–427
6. Siebenlist, K. R., and Mosesson, M. W. (1994) J. Biol. Chem. 269, 28414–28419
7. Lorand, L., Jeong, J.-M., Radek, J. T., and Wilson, J. (1993) Methods Enzymol. 222, 32–35
8. Crede, R. B., Curtis, C. G., and Lorand, L. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 4234–4237
9. Lorand, L., Parameswaran, K. N., and Murthy, S. N. P. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 537–541
10. Lewis, S. D., Janus, T. J., Lorand, L., and Shafer, J. A. (1983) Biochemistry 22, 6269–6272
11. Wolfenstein-Todel, C., and Mosesson, M. W. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5069–5073
12. Chung, D. W., and Davie, E. W. (1984) Biochemistry 23, 4232–4236
13. Fornace, A. J., Cummings, D. E., Comeau, C. M., Kant, J. A., and Crabtree, G. R. (1984) J. Biol. Chem. 259, 12826–12830
14. Farrell, D. H., Mulvihill, E. R., Huang, S., Chung, D. W., and Davie, E. W. (1991) Biochemistry 30, 9414–9420
15. Hogg, P. J., and Jackson, C. M. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 3619–3623
16. Weitz, J. I., Hudoba, M., Massel, D., Maraganore, J., and Hirsh, J. (1990) J. Clin. Invest. 86, 385–391
17. Hogg, P. J., and Jackson, C. M. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 3619–3623
18. Ikematsu, S., McDonagh, R. P., Reisner, H. M., Skrzynia, C., and McDonagh, J. (1981) J. Lab. Clin. Med. 97, 662–676
19. Chung, S. I., Lewis, M. S., and Folk, J. E. (1974) J. Biol. Chem. 249, 940–950
20. Finlayson, J. S., and Mosesson, M. W. (1963) Biochemistry 2, 42–46
21. Farrell, D. H., and Thiagarajan, P. (1994) J. Biol. Chem. 269, 226–231
22. Laemmli, U. K. (1970) Nature 227, 680–685
23. Slaughter, T. F., Acheson, K. E., Lai, T.-S., and Greenberg, C. S. (1992) Anal. Biochem. 205, 166–171
24. Lewis, K. B., Teller, D. C., Fry, J., Lasser, G. W., and Bishop, P. D. (1997) Biochemistry 36, 995–1002
25. Schwartz, M. L., Pizzo, S. V., Hill, R. L., and McKee, P. A. (1971) J. Clin. Invest. 50, 1506–1513
26. Lewis, S. D., Janus, T. J., Lorand, L., and Shafer, J. A. (1985) Biochemistry 24, 6772–6777
27. Lorand, L., Jeong, J.-M., Wilson, J., and Lorand, L. (1993) Methods Enzymol. 222, 32–35
28. Crede, R. B., Curtis, C. G., and Lorand, L. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 4234–4237
29. Lorand, L., Parameswaran, K. N., and Murthy, S. N. P. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 537–541
30. Laki, K., and Lorand, L. (1948) Science 108, 280
31. Lorand, L., Jeong, J.-M., Radek, J. T., and Wilson, J. (1961) J. Biol. Chem. 236, 2634–2643
32. Radek, J. T., Jeong, J.-M., Wilson, J., and Lorand, L. (1993) Biochemistry 32, 3527–3534
33. Hornyak, T. J., and Shafer, J. A. (1992) Biochemistry 31, 423–429
34. Yee, V. C., Pedersen, L. C., Bishop, P. D., Stenkamp, R. E., and Teller, D. C. (1995) Thromb. Res. 78, 389–397
35. Carrell, N. A., Erickson, H. P., and McDonagh, J. (1949) J. Biol. Chem. 264, 551–556
36. Hall, C. E., and Slayter, H. S. (1959) J. Biophys. Biochem. Cytol. 5, 11–16
37. Ikematsu, S., McDonagh, R. P., Reisner, H. M., Skrzynia, C., and McDonagh, J. (1981) J. Lab. Clin. Med. 97, 662–676
38. Chung, S. I., Lewis, M. S., and Folk, J. E. (1974) J. Biol. Chem. 249, 940–950
39. Kunicki, T. J., Newman, P. J., Amrani, D. L., and Mosesson, M. W. (1985) Blood 66, 808–815
40. Reed, G. L., Matsueda, G. R., and Haber, E. (1992) Thromb. Haemostasis 68, 315–320