ELSEVIER

Biophysical Chemistry 112 (2004) 141–145

Biophysical Chemistry

www.elsevier.com/locate/bpc

Deciphering the physiological pathway of clotting of fibrinogen in blood plasma

Laszlo Lorand*

Department of Cell and Molecular Biology, Northwestern University Medical School, Tarry Bldg. 8-711, 303 E. Chicago Avenue, Chicago, IL 60611, United States

Received 20 May 2004; received in revised form 26 May 2004; accepted 26 May 2004
Available online 28 August 2004

Abstract

I have been fortunate to have benefited over the years from the friendship and advice of John Ferry in our research to decipher the physiological reactions and regulatory events involved in the clotting of fibrinogen in blood. The article is a tribute to the memory of this creative scientist and remarkable individual.

Keywords: Fibrinogen; Thrombin; Factor XIII

I knew of Ferry's work on fibrinogen and fibrin well before I came to the United States, having included in my doctoral dissertation references to several of his papers [1]. But it was not until April 14, 1954 that we first met in Atlantic City—50 years almost exactly to the day of writing these words—at a symposium arranged by the American Society of Biological Chemists on the Chemistry of Prothrombin and Fibrinogen. The transactions were published in Physiological Reviews. While other lectures dealt with the biochemistry of prothrombin and thrombin, Ferry and I were asked to discuss the reactions of fibrinogen. My talk on the Interaction of Thrombin and Fibrinogen [2] was followed by his presentation on the Polymerization of Fibrinogen [3].

The decade preceding the Atlantic City meeting produced important insights into the mysteries of the clotting process. Laki showed that some—not yet unidentified—catalytic transformation of fibrinogen by thrombin made it possible for the fibrin particles to assemble into a clot [4]. Furthermore, the observation that the viscosity of fibrin in solution with 5 M urea was indistinguishable from that of the starting protein [5] indicated that thrombin action would not significantly alter the hydrodynamic properties of the parent protein, a conclusion supported by a comparison of sedimentation rates of fibrinogen and fibrin [6]. Then, the finding that repeated cycles of disassembly and reassembly could be carried out with fibrin, merely by adding and removing urea, provided conclusive evidence for the reversible nature of this step in blood coagulation [5,7,8].

In his lecture, Ferry described the production of intermediate polymers prior to clot formation [3] and suggested calling the thrombin-modified fibrinogen species (i.e. the fibrin monomer) activated fibrinogen, while reserving the fibrin name only for the finished clot. I would have liked to refer to the process of the reversible association of fibrin units as assembly or aggregation rather than polymerization, hoping to apply the latter name to the last phase of clotting whereby a urea-insoluble structure is generated by covalent linkages in the assembly. That would have avoided denoting the reactions of fibrin stabilization by the somewhat inaccurate term crosslinking for which, as John advised me, ligation would be more appropriate [9]. Also, as an adjective, unligated sounds better than non-

* Tel.: +1 312 503 0591; fax: +1 312 503 7912.
E-mail address: l-lorand@northwestern.edu.

0301-4622/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2004.05.013

crosslinked. Looking back, it seems that we both lost on the field of nomenclature.

Inasmuch as the solubility of fibrin clots in urea could not be reconciled with the contemporary notion that thrombin was an oxidase [10], other ideas had to be explored. Though earlier publications (contradicted by some) suggested that thrombin might be a protease, a definitive answer was lacking until our results with the Sanger procedure showed that fibrinogen and fibrin carried different end groups. Thrombin action creates new N-termini of Gly in strict proportion to the amount of fibrin formed in the clotting mixture, with simultaneous loss of some of the end groups originally present in the parent protein [11,12]. Sherry et al. [13] presented evidence at the Atlantic City meeting that thrombin was a trypsin-like enzyme with preferential specificity for Arg substrates. From this, it could be deduced, as amply verified by later studies, that thrombin cleavage occurred at Arg–Gly sites in fibrinogen.

Key to the unmasking of the self-assembly sites on the fibrinogen molecule is the specific removal of N-terminal peptides to which, because of their uniqueness, I gave the name: *fibrinopeptide* [14,15]. Ferry was pleased to see that clotting, measured as the gel point, set in long before the full release of *fibrinopeptides*.

Following our first meeting in Atlantic City, John continued to take interest in our research aimed at reconstructing the framework of physiological reactions and regulatory events that constitute the final phase of the clotting of fibrinogen in blood plasma. In vertebrates, this last step of the coagulation cascade is catalyzed by the thrombin and Ca^{2+}-activated factor XIII (denoted as A_2* or $XIII_a$ in Fig. 1 [16–18]). We visited with each other several times and communicated by mail. As the letters below illustrate, an enormous generosity of spirit shines through his writings.

Though some early experiments suggested that fibrin stabilization might exert a profound influence on the physical properties of the clot network [7], a systematic investigation of the issue was undertaken only when we learnt how to manipulate the crosslinking/ligation/spotwelding reaction by specifically blocking it at the monomeric stage without interfering with the assembly of fibrin particles, i.e. clotting, as such [19,20]. This work was carried out in collaboration with Lyle Mockros, Wes Roberts and Linus Shen [21–23] (see also Ref. [24]), and I also co-authored a couple of articles with Ferry and his coworkers [25,26]. At some point, John offered a postdoctoral position to Wes Roberts. Wes owned a small aircraft and I knew that he was a good pilot; nevertheless, I recall some anxious moments waiting for them to emerge from the clouds here at a nearby airfield.

The April 20, 1998 letter (Fig. 2) was in response to a series of studies showing that the C-terminal γ chain segments in fibrin assemblies are much better substrates for factor $XIII_a$ than the same sites in fibrinogen [27–29] (see also Ref. [24]). However, when the fibrinogen molecules—though still in solution—are aligned to form linear assemblies either through complex formation with the thrombin-modified central domain (*E fragments*) of the protein as template or with a synthetic double-headed *(GlyProArgPro)polyethyleneglycol* ligand as an alternating partner, the reaction rate is essentially as fast as seen with the clotted fibrin substrate. The first three residues of the ligand represent N-terminal sequences in fibrin (numbered for fibrinogen as *Aα res17–19*, where res17 is the new Gly

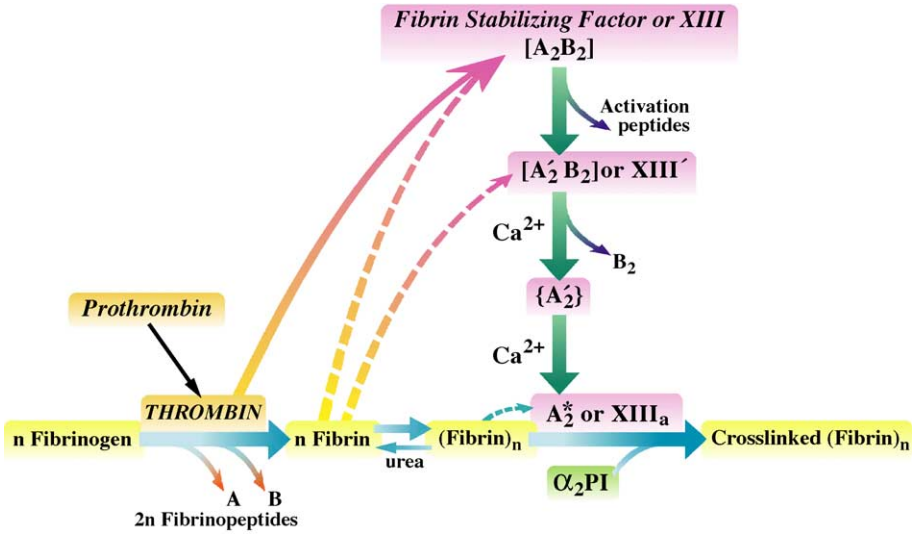

Fig. 1. Outline for the physiological pathway of clotting of fibrinogen in blood plasma. Thrombin controls the rate of conversion of fibrinogen to fibrin as well as the rate of activation of the fibrin stabilizing factor (factor XIII). This coagulation factor is the precursor of the enzyme (A_2* or $XIII_a$) that catalyzes the covalent crosslinking or ligation of the clot structure by N^{ϵ}(γ-glutamyl)lysine bonds. The broken arrows illustrate feed-forward regulations by fibrin [16–18].

UNIVERSITY OF
WISCONSIN
MADISON

April 20,1998

Professor Laszlo Lorand
Department of Cell and
Molecular Biology
Northwestern University Medical School
Searle Building 4-555
303 East Chicago Avenue
Chicago, Illinois 60611-3008

Dear Laszlo:

I was delighted to receive your letter and your fascinating papers from PNAS. I was extremely impressed by your ingenious design of alternating copolymers (to use polymer language) of fibrinogen and polyethylene glycol doubly functionalized with GPRP. Then long polymers formed from fragment E (des-AB) and fibrinogen are beautiful. I will be very interested to read the papers from Esther Ang's thesis that are now in preparation.

I am eager to know if clots can be formed from the fibrinogen-fragment E alternating copolymer at higher concentrations, and whether creep or stress relaxation studies of unligated clots would indicate a slow spontaneous dissociation and reassociation of junctions, as seems to happen in ordinary fine fibrin clots.

Unfortunately I won't be attending the Academy meeting this month. Although in reasonably good health, I find travel rather strenuous. Barbara and I moved to a small apartment a year and a half ago and are enjoying a quiet life there. I go to my office about twice a week. I haven't kept up adequately with the progress of science but am eagerly interested in current developments.

With very best wishes,

Sincerely,

John D. Ferry

Fig. 2. As far as I am aware, the interesting question posed by John in the second paragraph of this letter has not yet been answered.

endgroup produced by thrombin) which—as studies with a *mutant fibrinogen (Fibrinogen Detroit, Aα19Arg→Ser)* first revealed—are needed for polymerization [30]. These experiments [27,28] proved that, on the pathway of clot formation, the alignment of fibrin particles, i.e. *polymerization* takes place prior to the factor $XIII_a$-catalyzed event and that, because of this, the rate of the enzyme-driven crosslinking/ligation/spotwelding reaction is greatly accelerated (Fig. 1).

The August 26, 1998 letter (Fig. 3) was preceded 2 days earlier by a handwritten note and on the following day by a long typewritten letter to Esther (Ang) Ryan who was in the process of preparing her doctoral research for publication. These studies on the structural origins of clot rheology were very detailed [31,32] (also, see Ref. [24]) and it probably took a great deal of effort by John, already well into retirement, to analyze her experimental data. I think it is a tremendous tribute to Ferry as a scientist that he was willing to provide meticulous critique and most helpful recommendations to a young colleague, like Esther, who sought his advice but whom he never met.

The last paragraph of this August 26 letter, addressed to me in Woods Hole, brought back to mind a lovely day

UNIVERSITY OF
WISCONSIN
MADISON

August 26, 1998

Professor Laszlo Lorand
Marine Biological Laboratory
7 MBL Street
Woods Hole, MA 02543-1015

Dear Laszlo:

Thank you for sending the earlier draft of the Ryan manuscript with John Weisel. I did receive the final manuscript from John at about the same time. I was unable to respond for a few days because of some other matters and hope this delay has not been serious. I am enclosing a copy of a letter to Dr. Ryan that combines expressions of admiration with a few minor comments,. I regret that some of these involve small changes in some figures.

I am tremendously impressed by what you have accomplished in this systematic investigation. Accurate information on branch points and their importance, use of the inhibitors to vary the degree of ligation with quantitative control, and viscoelastic measurements correlated within the presence of various ligated species deduced from electrophoretic analysis, show clearly what is contributing to clot elasticity. The important role of the α chain is certainly striking. In my past thinking, it has always been somewhat of an orphan.

I had not heard of diCera and the role of chloride ion. The Cleveland meeting must have been interesting. I saw Mike Mosesson afterwards, also Dr. Michio Matsuda from Japan.

I hope you are having a good summer and am glad to know that you are still sailing. Barbara recently reworked a sketch of the Eel Pond to produce a nice colored painting with MBL and WHOI in the background. (Her art activities have been somewhat restricted since we moved into an apartment.) Best wishes to you and Joyce from both of us.

Sincerely yours,

John D. Ferry

Fig. 3. The underlined sentence in the third paragraph of the letter refers to an earlier note in which I called an interesting paper to Ferry's attention. After receiving his answer, I promptly forwarded this article [33] to John.

years earlier when John and Barbara came to see us at our summer house there. He enjoyed sailing in Vineyard Sound in my 18-ft Knockabout, cramped quarters for a tall person like John. He was happy to be back on Cape Cod, recalling his association with the Oceanographic Institution during the war, where he worked on a project for improving the antifouling properties of bottom paints for battle ships when not engaged in research on fibrin at Harvard.

References

[1] L. Lorand, Biochemical and biophysical studies of the clotting of blood. Doctoral dissertation, University of Leeds, England (1951).
[2] L. Lorand, Interaction of thrombin and fibrinogen, Physiol. Rev. 34 (1954) 742–752.
[3] J.D. Ferry, Polymerization of fibrinogen, Physiol. Rev. 34 (1954) 760–763.
[4] K. Laki, W.F.H.M. Mommaerts, Transition of fibrinogen to fibrin as a two-step reaction, Nature 156 (1945) 664.

[5] L. Lorand, A study on the solubility of fibrin clots in urea, Hung. Acta Physiol. 1 (1948) 192–196.
[6] S.P. Shulman, P. Ehrlich, J.D. Ferry, An intermediate in the conversion of fibrinogen to fibrin, J. Am. Chem. Soc. 73 (1951) 1388–1389.
[7] L. Lorand, Fibrin clots, Nature 166 (1950) 694–696.
[8] L. Lorand, W.R. Middlebrook, The action of thrombin on fibrinogen, Biochem. J. 52 (1952) 196–199.
[9] L. Lorand, Fibrinoligase. The stabilizing factor system of blood plasma, Ann. N.Y. Acad. Sci. 202 (1972) 6–30.
[10] K. Laki, Transition of fibrinogen to fibrin, XVII Internatl. Physiol. Congr., Oxford, 21–25 July 1947, Abstracts, Oxford University Press, Oxford, UK, 1947, pp. 373–374.
[11] K. Bailey, F.R. Bettelheim, L. Lorand, W.R. Middlebrook, Action of thrombin in the clotting of fibrinogen, Nature 167 (1951) 233.
[12] L. Lorand, W.R. Middlebrook, Studies on fibrino-peptide, Biochim. Biophys. Acta 9 (1952) 581–582.
[13] S. Sherry, W. Troll, H. Glueck, Thrombin as a proteolytic enzyme, Physiol. Rev. 34 (1954) 736–741.
[14] L. Lorand, "Fibrino-peptide": new aspects of the fibrinogen–fibrin transformation, Nature 167 (1951) 992.
[15] L. Lorand, Fibrino-peptide, Biochem. J. 52 (1952) 200–203.
[16] L. Lorand, Sol Sherry lecture. Research on clot stabilization provides clues for improving thrombolytic therapies, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 2–9.
[17] L. Lorand, Factor XIII: structure, activation, and interactions with fibrinogen and fibrin, Ann. N.Y. Acad. Sci. 936 (2001) 291–311.
[18] L. Lorand, R.M. Graham, Transglutaminases: crosslinking enzymes with pleiotropic functions, Nat. Rev., Cell Mol. Biol. 4 (2003) 140–156.
[19] L. Lorand, A. Jacobsen, Specific inhibitors and the chemistry of fibrin polymerization, Biochemistry 3 (1964) 1939–1943.
[20] L. Lorand, H.H. Ong, Labeling of amine-acceptor cross-linking sites of fibrin by transpeptidation, Biochemistry 5 (1966) 1747–1753.
[21] W.W. Roberts, L. Lorand, L.F. Mockros, Viscoelastic properties of fibrin clots, Biorheology 10 (1973) 29–42.
[22] L.F. Mockros, W.W. Roberts, L. Lorand, Viscoelastic properties of ligation-inhibited fibrin clots, Biophys. Chem. 2 (1974) 164–169.
[23] L. Shen, L. Lorand, Contribution of fibrin stabilization to clot strength. Supplementation of factor XIII-deficient plasma with the purified zymogen, J. Clin. Invest. 71 (1983) 1336–1341.
[24] J.W. Weisel, The mechanical properties of fibrin for basic scientists and clinicians, Biophys. Chemist. 112 (2004) 267–276 (this issue).
[25] G.W. Nelb, C. Gerth, J.D. Ferry, L. Lorand, Rheology of fibrin clots: III. Shear creep and creep recovery of fine ligated and coarse unligated clots, Biophys. Chem. 5 (1976) 377–387.
[26] G.W. Kamykowski, D.F. Mosher, L. Lorand, J.D. Ferry, Modification of shear modulus and creep compliance of fibrin clots by fibronectin, Biophys. Chem. 13 (1981) 25–28.
[27] G.P. Samokhin, L. Lorand, Contact with the N-termini in the central E domain enhances the reactivities of the distal D domains of fibrin to factor $XIII_a$, J. Biol. Chem. 270 (1995) 21827–21832.
[28] Y. Veklich, E.K. Ang, L. Lorand, J.W. Weisel, The complementary aggregation sites of fibrin investigated through examination of polymers of fibrinogen with fragment E, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 1438–1442.
[29] L. Lorand, K.N. Parameswaran, S.N.P. Murthy, A double-headed GlyProArgPro ligand mimics the functions of the E domain of fibrin for promoting the end-to-end crosslinking of γ chains by factor $XIII_a$, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 537–541.
[30] B. Kudryk, B. Blombäck, M. Blombäck, Fibrinogen Detroit—an abnormal fibrinogen with non-functional NH_2-terminal polymerization domain, Thromb. Res. 9 (1976) 25–36.
[31] E.A. Ryan, L.F. Mockros, A.M. Stern, L. Lorand, Influence of a natural and a synthetic inhibitor of factor XIIIa on fibrin clot rheology, Biophys. J. 77 (1999) 2827–2836.
[32] E.A. Ryan, L.F. Mockros, J.W. Weisel, L. Lorand, Structural origins of fibrin clot rheology, Biophys. J. 77 (1999) 2813–2826.
[33] E. DiStasio, C. Nagaswami, J.W. Weisel, E. DiCera, Cl^- regulates the structure of fibrin clot, Biophys. J. 75 (1998) 1973–1979.