The conversion of fibrinogen to fibrin: A brief history of some key events

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Abstract

The conversion of fibrinogen to fibrin is a process that has long fascinated an army of researchers. In this brief review some early break-through observations are noted and a few later unexpected results described.

Introduction

Quite apart from its physiological importance, the transformation of fibrinogen to fibrin on its own has held a legion of researchers in thrall. The rapid conversion of these molecules from sol to gel after a brush with a catalytic trace of thrombin is truly mesmerizing. How does it happen?

That limited proteolysis was the trigger was suggested more than a century ago, but it wasn't until 1937 (only 80 years ago!) that the notion became generally accepted when papain, a demonstrated protease (unlike thrombin at the time), was shown to catalyze the reaction [1]. A decade later a constellation of experiments by several investigators revealed the fundamental details. First, Elemer Mihalyi performed elegant analytical studies that showed that the electrophoretic mobilities of fibrinogen and fibrin dispersed in urea were different, the former being more negatively charged [2]. Shortly thereafter, Koloman Laki found that when fibrinogen was treated with thrombin at low pH, no gel formed [3]. Finally, in a joint publication, the laboratories of Kenneth Bailey and Lazlo Lorand reported that the amino-terminals of fibrinogen and fibrin were different [4], in particular new glycine endgroups appearing presumably as the result of peptide material being released from fibrinogen by the action of thrombin. All these findings were summed up in a now classic 1952 paper by John Ferry in which he posited that the removal of some negatively charged patches on fibrinogen led to an intermediate that spontaneously polymerized in an overlapping fashion to yield a two-molecule thick protofibril [5].

$$F \xrightarrow{T} f \rightleftarrows f_n \longrightarrow \text{Fibrin}$$

A contentious decade followed during which it was shown that fibrin is further stabilized by the action of factor XIII introducing covalent cross-links between the constituent fibrin units, the contributors and combatants in that fracas being too numerous to list here. The main argument had to with the nature of the bonds between the individual units. For a while, one group contended that papain was able to catalyze both steps, i.e., the initial proteolysis-dependent conversion to fibrin and the subsequent covalent stabilization as well [6,7], leading them to suppose that factor XIII must be a transpeptidase operating on the amino groups exposed by the action of thrombin [8]. This was an interesting if misleading thought that we will return to forthwith. In fact, it turned out that factor XIII was incorporating covalent cross-links between particular lysine and glutamine side chains [9,10].

During the second half of the 20th century, enormous energy and resources were brought to bear on the details of fibrin formation, including electron microscopy that revealed the trinodular structure of fibrinogen [11,12], quantitative end-group analyses that showed

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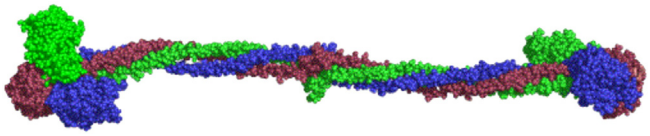

Fig. 1. Hypothetical model of protofibrinogen from a protochordate (sea squirt, *Ciona intestinalis*).

the existence of six polypeptide chains [13], a host of amino acid sequencing studies (again, too many participants to list), and eventually a flood of cloning and DNA sequencing studies. The climax, in my view, came with the determination of crystal structures, first of fibrinogen fragments [14–17] and then of the native (or close to native) protein [18,19].

During this period there were also countless studies of clinical importance, many of which aided in the understanding of the structural and mechanistic aspects of fibrin formation, including the identification of large numbers of variant human fibrinogens [20], as well as comparative studies aimed at understanding how and when the thrombin-fibrinogen system got started [21]. And there were many insightful studies with knock-out mice [22], as well as constant prodding and poking of fibrinogen and fibrin by a host of immunobiologists.

Watching all of this unfold over the past 58 years has been a genuine privilege. And with all respect to the many others who made this field their major undertaking (a quick check of PubMed lists more than 70,000 reports dealing either with fibrinogen or fibrin or both), I'd like to note here a few latter day and unanticipated observations of my own.

One of these was a computer-based genomic study. I had long wondered how the fibrinogen molecule could have evolved, musing on the fact that the α, β and γ chains are homologous. Where did the protein get its start? Was the earliest fibrinogen a homotrimer?

In 2003 the complete genome of the sea squirt, *Ciona intestinalis*, was published. It was an observation of long standing that these creatures, like other protochordates, have a blood-like hemolymph that does not clot. The genomic sequence data were quickly scrutinized in search of any rudiments of a clotting system [23], and although those preliminary searches turned up numerous fibrinogen-related-domains (FREDs) of the sort that are found in most animals [24], we reported that "no full-length genes were found with potential for the coiled coils that are the hallmarks of fibrinogen" [23].

This conclusion turned out to be premature. A decade later, while combing through the sea squirt genome again, I realized that the automated sequence-splicing programs used by the NCBI (National Center for Biotechnological Information) had stitched together a series of open frames into a single gene that indeed looked like it could encode a protein closely related to vertebrate fibrinogen [25]. But when I tried to model the hypothetical structure, it defied many simple rules of how proteins fold. A closer examination of the DNA revealed that the computer programs had missed several important exons. In fact, there was a cluster of *three* genes, just as occurs in vertebrates [26]. Moreover, the distribution of cysteines was consistent with a covalent dimer composed of six chains ($\alpha_2\,\beta_2\,\gamma_2$).

But there were no obvious thrombin cleavage sites; there were holes but no knobs. It looked to be a protofibrinogen, the role of which remains to be explained in the non-clottable plasma (hemolymph) of the sea squirt. I have written elsewhere that for me it was "like an anthropologist stumbling across. a veritable 'Lucy' of molecular antiquity" [27]. When all the exons were assembled appropriately into the three different chain types, it was a simple matter to model the sea squirt protofibrinogen, the main difference compared with vertebrate fibrinogens being three globules at each end rather than two (Fig. 1).

Another recent result I found surprising came about because of cleaning out an old refrigerator and finding a long forgotten papain suspension. Would the enzyme still clot fibrinogen, I wondered? In fact, papain is remarkably durable, and a quick test showed how active it was (Fig. 2). It reminded me of those old reports from the 1960's that papain not only gelled fibrinogen but also stabilized the fibrin with covalent bonds [6,7]. Personally, I had always suspected that those early results were compromised by fibrinogen preparations being contaminated with factor XIII, an enzymatic activity that was subsequently found to be notoriously difficult to eliminate [28]. Given the many technological

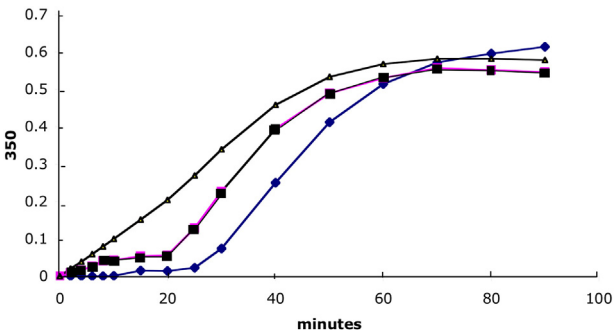

Fig. 2. Gelation of human fibrinogen by three dilutions of an old papain suspension.

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advances that had been introduced during the last half century, including SDS gels, I thought it would be an easy matter to resolve.

In fact, it took almost two years to realize that my suspicions were mistaken and that papain really does both cleave and transpeptidate. In the end, modern mass spectrometry revealed that the transpeptidation does not involve the glycine endgroups exposed by thrombin as had been hypothesized, but instead depends on an incidental cleavage at the other end of the molecule; in no way is it a parallel for what occurs with thrombin and factor XIII [29]. At the very least, the work explains those early results, misleading as they may have been, and removes an old mystery.

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