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Some comments on John Ferry's most enduring paper

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Abstract

In this brief memoir, I reflect on the great insight John Ferry exhibited in his extremely influential 1952 paper on the mechanism of fibrinogen being changed into fibrin.

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1. Introduction

Arguably, John Ferry's most enduring and influential paper in the area of fibrinogen–fibrin appeared in July, 1952 (a mere 6 weeks after its submission to the Proceedings of the National Academy of Science) [1]. It was only four pages long and contained no illustrations. The heart of the paper was a simple rendering:

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

Although no new data were presented, the paper was based on an enormous amount of physicochemical experimentation conducted on fibrinogen and solublized fibrin during the previous decade in several laboratories including the Ferry lab at the University of Wisconsin.

The timely appearance of the paper, however, was doubtless tied to a series of biochemical reports that had appeared in 1950 and 1951. In one of these, Koloman Laki [2] had shown that the action of thrombin could be separated from the polymerization phase in that fibrinogen treated with thrombin at pH 5 did not clot, but upon neutralization to pH 7 instantaneously gelled. In another key experiment, Elemer Mihalyi [3] had shown that fibrinogen and fibrin dissolved in urea solutions had measurably different isoelectric points, fibrinogen having more negative charge at neutral pH than fibrin under the same conditions. Finally, it was found by both Kenneth Bailey and Lazlo Lorand and their respective colleagues that thrombin effected a change in the amino-terminal groups of fibrinogen and fibrin [4]. Taken in aggregate, these studies showed that thrombin removed one or more negatively charged groups from the starting fibrinogen molecule. That proteolysis might be involved in the "activation" of fibrinogen had long been suspected.

The demonstration of limited proteolysis provided the last key in a general understanding of how the soluble protein fibrinogen is transformed into the insoluble polymeric gel called fibrin, capping the tremendous amount of physicochemical experimentation that had already been performed on fibrinogen and fibrin in different solution environments.

In the 1952 paper, John Ferry laid it all out clearly and simply. Thrombin modified the fibrinogen in such a way, he conjectured, that it exposed a region of positive charge on the sides of an elongated molecule, leading to intermolecular associations with negatively charged regions near the ends of neighboring molecules in a kind of staggered overlap. The intermediate polymer grew progressively by the same process until it reached a critical length, after

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which the intermediate polymers themselves began to associate in bundles to form the fibrin strands. A direct result of the underlying process was that the intermediate polymers–which he called protofibrils–were two molecules thick. The noncovalent forces involved in these associations were presumed to be dipole–dipole interactions, hydrogen bonds, and nonpolar interactions.

How did Ferry come to these conclusions? To start, his idea that the polymerization occurred in two stages was based on findings from several laboratories that fibrin was dissociated to different degrees by different solvents. Thus, fibrin dissolved in 3.5 M urea had the same size and shape as fibrinogen under the same conditions and must be monomeric; this is the molecular species denoted by f in Ferry's equation. If the urea concentration were only 1.0 M, however, a larger intermediate polymer was evident, denoted by f_n in the equation. The same kind of intermediate polymer could be demonstrated in various polyhydric alcohol solutions including 0.5 M hexamethyleneglycol (HMG) [5]. The two kinds of intermediate, one a monomer and the other an oligomer, were at the heart of his reasoning.

That the intermediate polymer had a rigorously defined width double that of fibrinogen had already been suggested by light-scattering studies on "fine"—as opposed to "coarse"—fibrin gels [6]. Even earlier, Ferry's lab had shown that flow birefringence experiments combined with results from sedimentation studies showed that the intermediate polymers were in agreement with a model that was two molecules thick [7]. The notion that the oligomeric units were staggered, and not just connected end-to-end, was in part based on light-scattering experiments in Ferry's lab that had not yet been published (they appeared later that year [8]). These latter reinforced the conclusion that the widths (actually, cross-sectional areas) of the intermediate polymers were always double that of fibrinogen, whereas the lengths were, on the average, about 10 times greater. Almost intuitively, Ferry felt that a staggered overlap was the best explanation. Still, there was no real experimental evidence that the units were not perfectly aligned side-by-side and then further polymerized end-to-end, a scheme diagrammed earlier by Steiner and Laki [6]. Ferry thought an overlapping scheme was simpler and more likely.

Interestingly, although it was already known that negatively stained fibrin fibers have a distinct periodicity of 230 Å when observed in the electron microscope [9], this was not a part of Ferry's reasoning about the staggered overlap. This was partly due to the fact that the length of the fibrinogen molecule was not accurately known at the time; Ferry had quoted a value of 600 Å. Nor was it known that fibrinogen is a symmetrical covalent dimer. If Ferry had known that the true value of its length was about twice the periodicity observed in fibrin (450 Å), he almost certainly would have made the connection, although, as we shall see, even when all the data are available, preconceived notions can induce temporary blindness.

2. Some challenges

In this regard, not all investigators appreciated the beauty of Ferry's staggered overlap proposal. Seven years later, Hall and Slayter [10] published electron micrographs of metal-shadowed fibrinogen molecules showing a trinodular structure with a length of 475±25 Å. They also showed elegant pictures of polymerizing molecules and a negative-stained fully matured fiber with the same banding pattern of 230 Å that Hall [9] had published earlier. Remarkably, they rejected Ferry's suggestion of a staggered overlap, interpreting their own micrographs of polymerizing fibrin as favoring a strict end-to-end interaction. They attributed the 230 Å spacing to a squeezing together of the individual units to half their original lengths!

In 1962, a full decade after Ferry's prescient paper, Koloman Laki [11] published an article in the Scientific American in which he took issue with Hall and Slayter and their squeezed arrangement of units in fibrin. Instead, he showed a depiction of fibrin in which all the individual units were oriented with their long axes perpendicular to the fiber axis! The cross-striation of stained fibrin fibers, he said, was attributable to deposition of phosphotungstate between the side-by-side polymerized units. Not only was this arrangement contrary to a plethora of data indicating that the units in fibrin were aligned in the direction of the fiber axis, but it also contradicted light-scattering experiments from Laki's own lab that had shown that fibrin strands in "fine" clots were two molecules thick [6]!

Gradually, the Ferry suggestion came to be accepted, however, and by the early 1970s, it was the model of choice [12]. By this time, John Ferry had just completed the administrative phase of his career (he was chair of the Department of Chemistry at Wisconsin from 1959 to 1967), and he may not have even noticed that there had been a delay in the model's universal acceptance.

3. Synthetic peptide knobs

I first met John Ferry in the mid-1970s when he was visiting a cousin in La Jolla. We had already begun what was to be a long and enduring correspondence. In 1978, one of my graduate students, Andrew Laudano, synthesized a number of peptides corresponding to the amino-terminal sequences that occur at the newly exposed ends of fibrin. These peptides bound to both fibrinogen and fragments D, and some of them were very effective in blocking polymerization [13]. Moreover, in contrast to the hexamethylene glycol (HMG) that Ferry's group had used to arrest the association of protofibrils, the tetrapeptides were presumably blocking at the very first step of polymerization. On a molar basis, they were about a thousand times more effective than HMG.

By this time, John was well into the second experimental phase of his career. He was very interested in these

peptides, and he asked if we could spare him some, a request we were of course pleased to grant. His elegantly conceived viscoelastic experiment on the effects of these tetrapeptides on the creep and creep recovery of fibrin [14] were surprising to me. Not only could synthetic peptide knobs prevent polymerization; they could actually reverse gelation!

4. Confirmation from X-ray structures

Some of the predictions made in the 1952 Ferry paper remained to be borne out by X-ray structures completed 45 years later. As an example, in explanation of why fibrin did not polymerize much below pH 6, Ferry predicted that there must be a critical histidine residue near the ends of fibrinogen molecules. Indeed, X-ray structures of fragments D from fibrinogen showed that there is an invariant histidine at the seat of the binding pocket ("hole") into which "knobs" are inserted during protofibril formation [15,16]. If this histidine becomes protonated, the positively charged amino group of the knob's terminal glycine cannot be accommodated. In another prediction fulfilled, the positioning of these holes at the ends of the fibrinogen molecule turned out to be such that the insertion of pairs of knobs from the mid-regions of neighboring molecules could pin them together.

5. The Laboratory of Physical Chemistry at Harvard Medical School

As is doubtless discussed elsewhere in this volume, John Ferry's interest in proteins—as opposed to synthetic polymers—was stimulated while he was a Junior Fellow at Harvard in the late 1930s, during the tenure of which he had discovered the Laboratory of Physical Chemistry headed by E.J. Cohn at Harvard Medical School. It was while working in that laboratory during the Second World War where he had access to large amounts of fibrinogen freshly prepared from human plasma that he became immersed in the fibrinogen–fibrin area. The approaches that were being used in the Cohn lab included viscosity, analytical ultracentrifugation, light scattering, flow birefringence, and some electron microscopy.

In 1946, John left Harvard to join the faculty of the University of Wisconsin, where he was to stay for the rest of his long career. But the Cohn lab, which was unique and unorthodox in many ways, had obviously left its mark on him. By chance, some 11 years after John had left for Wisconsin, I arrived as a graduate student in that same fourth floor laboratory where he had been quartered (one of my advisers, J.L. Oncley, had taken his PhD at Wisconsin and was the person who recommended John Ferry for the faculty job).

To me, one of the fascinations of that quaint laboratory was that they seemed never to throw anything away. They also had an interesting custom regarding publications; most of them were double-numbered serially by particular project (Roman numerals) and cumulatively for the more general study (Arabic numerals). For example, one of John Ferry's last publications from the Cohn lab is Number 52 in the series "Studies on the Plasma Proteins," but number X (Roman numeral) in the particular project series [17]. Moreover, the lab had a meticulously maintained reprint collection of just about every paper of the lab that had ever been issued by members and often former members.

When Ferry moved to Wisconsin, he continued the double-numbering practice with his own lab, and not surprisingly many of those papers were soon in the collection at Harvard. That is how it happens that I possess a set of John Ferry's early Wisconsin work (I have numbers II and V–XII of the series titled "The Conversion of Fibrinogen to Fibrin," which appeared in the time period 1950–1953). Among them, but not numbered as a part of any series, is a covered reprint of the 1952 PNAS paper [1]. At this point, it is dog-eared and much written on. I had dug it out, along with the others, after being asked to prepare something for this memorial volume. Upon re-reading, I found it so remarkable, I decided it needed more than another perfunctory citation, and that is what has provoked these comments. It was a remarkable paper by a remarkable man.

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