# Missing pieces in clotting puzzle described by UCSD scientists

## October 1, 1997

HOLD FOR NATURE EMBARGO: 2 P.M. EDT, Wednesday, October 1, 1997

Media Contact: Warren R. Froelich, (619) 534-8564, wfroelic@ucsd.edu

MISSING PIECES IN CLOTTING PUZZLE DESCRIBED BY UCSD SCIENTISTS

To loosely paraphrase Shylock, the lead character from Shakespeare's Merchant of Venice, bleeding is something we all do, regardless of heritage. So it is with clotting, a process that in the parlance of the theater, prevents bleeding from lowering the final curtain on us all.

Because of its significance to advanced life including humans, for more than a half century researchers have been trying to piece together the complex biological puzzle that results in clotting--the coagulation of blood at the site of a wound. Trouble is, the precise shape of the puzzle pieces has remained unknown.

Now, a scientific team from the University of California, San Diego has revealed in atomic detail the three-dimensional structure of one of the key pieces--a fragment of a protein called fibrinogen--by itself and when linked to a neighbor.

The research promises to clarify how clotting works and, conversely, what happens when it goes awry in ailments such as stroke and heart disease. The work also is allowing the visualization of precise structural consequences produced by genetically defective fibrinogen, defects that result in clotting disorders.

The article, published in the current issue of the British science journal Nature, was authored by Glen Spraggon, Stephen Everse and Russell Doolittle, researchers from UCSD's Center for Molecular Genetics.

Briefly, clotting is a complex biological process involving at least a dozen different proteins. In the final stage, fibrinogena large molecule that ordinarily circulates in the blood plasmacomes under attack by an enzyme called thrombin, which is released at the wound site. Provoked by thrombin, fibrinogen undergoes a remarkable transformation during which molecules spontaneously align themselves into long threadlike polymers called fibrin, which causes the blood around it to gel. A clot is formed.

To better understand how fibrinogen is transformed into fibrin during this final stage, for several decades scientists have been trying to learn its precise chemistry and structure.

An early model of fibrinogen was first proposed in 1959, when under an electron microscope, the molecule revealed itself as a series of small globules or nodes, connected to each other in a straight line. When split into its components, researchers identified two core fragments: fragment E, which corresponds to the molecule's central core; and a fragment D, two of which are positioned on either end of the molecule.

Further investigation suggested that thrombin acts to remove small bits of protein called fibrinopeptides--which otherwise form shields around structures called "knobs" located on the surface of fragment E. The newly exposed knobs then align with, and fit into, corresponding holes on the surfaces of the D-portions of other fibrinogen

molecules. Thus, the E-portion of each molecule pins two D-portions together from two different molecules. Combined, the chemical trio (the double-D with fragment E) forms, in essence, a single link in a polymer chain that's tethered together by similar links. The result is fibrin.

Although much has been learned by chemical studies and early imaging efforts, scientists realized they needed the three-dimensional structure of the key elements in particular, fragment D and Eto understand precisely how clotting is achieved.

In their study, the UCSD researchers describe for the first time the detailed three- dimensional structure of fragment D from human fibrinogen, at a resolution of 2.9 angstroms. A computer model of the fragment D structure, consisting of three polypeptide chains, bears a slight resemblance to a farmer's plow.

"To the trained eye, however, it's a thing of beauty," said Doolittle. "It shows for the first time how this molecule is packed together in a clot at atomic resolution. It means that all the inferences built up over a half century can now be checked."

The UCSD research also produced a three-dimensional model of the "double D" fragment, with a synthesized peptide bound as a "dummy knob."

"Even though we don't have fragment E, we have the legs of fragment E so to speak, that pin the molecule together," said Doolittle.

As described in the Nature article, the fibrinogen fragments were crystallized and their structures studied by X-ray crystallography with the aid of synchrotrons at Daresbury, England; Stanford University; and the Brookhaven National Laboratory. Further computer refinement and modeling resulted in colorful, three-dimensional images of the structures.

Although Doolittle traces the effort at UCSD to isolate pure crystals of fibrinogen back to 1976, he said work did not begin in earnest until October 1989. Not long thereafter, Everse joined the group as a graduate student. Following four years of painstaking work, the group finally obtained a pure crystal of fragment D, isolated from fibrinogen that had been prepared from blood plasma obtained from the San Diego Blood Bank.

The next phase was to construct a highly refined three-dimensional map of the crystal. To obtain the molecular snapshot they were seeking, the researchers used X-ray crystallography, an essential technique used to discern the atomic structures of complex molecules like proteins.

Much of the early data was collected by Everse with equipment available at UCSD. But it soon became apparent, however, that X-ray beams available from standard laboratory sources were limited and that other, more powerful and versatile sources of radiation would have to be found. For this, the group turned to synchrotrons.

By this time, Spraggon who was trained in crystallography at Oxford University had joined the group. The breakthrough came when he and Everse were able to spend a day at the Daresbury Synchrotron in England where X-rays of a different wavelength were available.

"From the data collected with one day's worth of synchrotron time in England," said Doolittle, "we could begin to see a real structure starting to emerge."

Even then, the results were not really good enough to obtain a high resolution image. But Spraggon then employed a novel averaging approach that greatly improved the picture. With that, the team was able to build an accurate model of the molecule.

Once the structure of fragment D was in hand, it was an easier job to get the structure of double-D.

Aside from answering fundamental questions about clotting, Doolittle said the fibrinogen fragments are also shedding light on the protein's evolution, something that's intrigued him since his days as a graduate student at Harvard many years ago.

It has long been hypothesized that the three chains that make up the molecule were identical in the beginning. But one of these (polypeptide) chains, the alpha, doesn't have a chemical "blob" on the end like the others. The UCSD team modeled in this hypothetical missing piece and found an exact space for it. The results are allowing the researchers to see how this "blob" must have appeared in the primordial fibrinogen molecule and how it was displaced.

Also contributing to the study were Marcia Riley-Callender and Leela Veerapandian, staff research associates in Doolittle's lab. Funding for the research was provided by the National Institutes of Health, and a postdoctoral fellowship to Stephen Everse from the American Heart Association.

(October 1, 1997)