

PROBING FIBRINOGEN STRUCTURE: INSIGHTS FROM LIMITED PROTEOLYSIS, PEPTIDES, AND MONOCLONAL ANTIBODIES

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Blood plays a critical role in transporting various substances throughout our body and must remain in its liquid state to perform this task effectively. However, when blood vessels are damaged, it becomes imperative to prevent blood loss by initiating a localized transition of blood into a solid state, a process known as blood clot formation. The system responsible for maintaining blood in a liquid state while facilitating coagulation upon injury is known as the hemostasis system [1].

The coagulation link of the hemostasis system contains a large number of proteins. When a blood vessel is damaged, a series of reactions begin, leading to the conversion of the soluble glycoprotein fibrinogen into fibrin. Fibrin has the ability to associate with other fibrin molecules, resulting in the formation of a framework for a blood clot [1, 2]. Considering the significant importance of fibrinogen for the functioning of the hemostasis system, researching its structure and function is a current issue in the fields of biology and medicine.

Fibrinogen is a glycoprotein with a molecular weight of approximately 340 kDa. The fibrinogen molecule consists of two subunits, each containing three polypeptide chains: A α (molecular weight 67.6 kDa), B β (with a mass of 54.8 kDa), and γ (46.4 kDa) [2, 3]. The N-terminal amino acid residues of the polypeptide chains of fibrinogen are located in the central region of the molecule, which is called the E region. In each subunit of the fibrinogen molecule, the C-terminal regions of the B β and γ chains are structurally and functionally combined into two peripheral D regions [2]. The sequences of the A α -chain from residues 221 to 610 form the α C regions. Each α C region includes the α C-connector (A α 221–391) and the α C domain (A α 392–610). α C-domains connect through electrostatic bonds with the central E-region of the molecule and with each other [4]. Despite the rather detailed information about the structure of the fibrinogen molecule, the features of the construction of individual regions and their importance in the performance of fibrinogen functions have not been studied enough.

A wide range of approaches and molecular tools can be employed for investigating the structure and functions of proteins. These include monoclonal antibodies and their Fab fragments, polypeptide sequences that mimic specific regions of the fibrinogen molecule, and highly specific proteases.

The aim of this study was to analyze the prospects of using these various methods in investigating fibrinogen structure and function.

Antibodies and Fab-fragments. Since antibodies are specific to a small sequence of amino acid residues (or a small region of the molecule), monoclonal antibodies can be used to investigate the importance of a specific site on fibrinogen (Figure). For example, using the 1-5A antibodies, which are specific to the C-terminal region of the A α -chain of fibrinogen (A α 509–610), the importance of these regions in the lateral association of protofibrils was established [5]. The study indicated that the presence of various antibodies, each specific to a relatively small region of the fibrinogen molecule (A α 105–206), could cause different effects on the functioning of the molecule. This can be explained by the conformational changes that occur when the antibodies bind to these closely situated sites on the fibrinogen molecule [6]. However, this approach has its drawbacks, primarily related to the size of the antibody molecule. Binding of immunoglobulin to fibrinogen, with a mass of approximately 150–170 kDa, can impose significant steric restrictions on interactions with other molecules of

the hemostasis system. This limitation is somewhat alleviated by the use of Fab fragments. These fragments of antibody molecules, while retaining their specificity for certain epitopes of fibrinogen, have much smaller sizes, typically within 50 kDa.

Peptides. An important tool in studying the role of individual sites of the fibrinogen molecule in its functioning are polypeptides, which in their composition repeat the sequence of amino acid residues of functionally important fragments of the molecule (Figure). Thus, using small peptides, was discovered the important role of the hinge sites of the coiled-coil region of the fibrin(ogen) molecule in the process of fibrin polymerization. It has been demonstrated that synthetic peptide $\text{A}\alpha 195\text{--}205$ inhibits the stage of fibrin protofibril formation, while peptide $\gamma 69\text{--}77$ inhibits the lateral association of protofibrils [7]. The use of small molecules helps minimize steric constraints that may arise from their binding to the fibrin(ogen) molecule. However, accurately assessing the importance of specific protein regions using this approach can be quite challenging. Even relatively large polypeptides typically exist in a disordered state in solution, contributing to their unique interaction characteristics with other molecules, thus complicating the analysis of the obtained data.

Proteases. Another approach in the study of proteins is limited proteolysis. By using specific proteases, unique forms of macromolecules can be obtained. Studying the changes that occur due to such modifications allows us to gain insights into the role of the cleavable areas in the functioning of the protein [8–10] and also get additional information about the protein structure [11, 12]. For example, using proteases from the culture medium of *Bacillus thuringiensis* and the venom of *Gloydius halys halys* we separated the functional role of different parts of fibrin(ogen) αC -region in fibrin polymerization, platelet aggregation and endothelial cells vitality [10] (Figure). One of the advantages of this approach is the ability to obtain not only high-molecular-weight protein forms but also small molecule fragments, which can be used as peptide mimetics. However, when proteases act on protein molecules, a change in the primary structure of the molecule is observed. This will lead to changes in certain molecular parameters, such as conformational changes and stability.

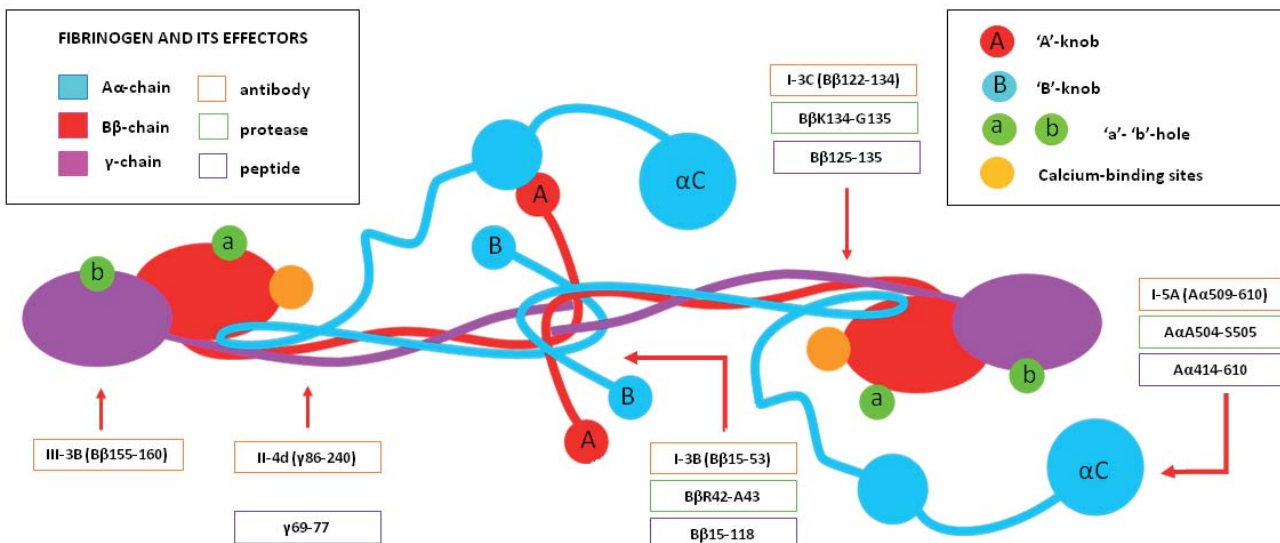


Fig. Scheme of fibrinogen molecule with some effectors (antibody, protease, peptide) targeted to the distinct parts of the molecule. Modified after: [13]

I-3C antibody targeted to 122-134 residue of B β -chain of fibrinogen [14]; B β K134-G135 is a peptide bond that is cleaved during the formation of TSK-fragment of fibrinogen [15]; B β 125-135 is a synthetic peptide [16]; I-5A antibody targeted to 509-610 residue of A α -chain of fibrinogen [17]; A α A504-S505 — is a peptide bond that is cleaved by protease II from the culture medium of *Bacillus thuringiensis* [18]; A α A414-610 is a peptide generated by protease from the venom of *Gloydius halys halys* [19]; I-3B antibody targeted to 15-53 residue of B β -chain of fibrinogen [20]; B β R42-A43 is a peptide bond that is cleaved by protease II from the venom of *Echis multisquamatis* [21]; B β 15-118 is a synthetic peptide [22]; II-4d antibody targeted to 86-240 residue of γ -chain of fibrinogen [23]; γ 69-77 is a synthetic peptide [7]; III-3B antibody targeted to 155-160 residue of B β -chain of fibrinogen [24].

Conclusions. Thus, it is important to acknowledge that the described approaches in protein research each have their own advantages and disadvantages, and scientists may choose them based on specific research objectives. However, for more reliable information about the characteristics of protein structure and function, a comprehensive approach is necessary, utilizing multiple scientific tools simultaneously.

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