STRUCTURE AND FUNCTION OF FIBRINOGEN BβN-DOMAINS

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Fibrinogen is a polyfunctional plasma protein involved in various physiological and pathological processes through the interaction of its multiple domains with different ligands and cell receptors. Among fibrinogen domains, two BβN-domains are formed by the N-terminal portions of its two Bβ chains including amino acid residues Bβ1-64. Although their folding status is not well understood and the recombinant disulfide-linked (Bβ1-66)₂ fragment corresponding to a pair of these domains was found to be unfolded, some data suggest that these domains may be folded in the parent molecule. In contrast, their major functional properties are well established. Removal of fibrinopeptides B (amino acid residues Bβ1-14) from these domains upon fibrinogen to fibrin conversion results in the exposure of multiple binding sites in fibrin βN-domains (residues β15-64). These sites provide interaction of the βN-domains with different proteins and cells and their participation in various processes including fibrin assembly, fibrin-dependent angiogenesis, and fibrin-dependent leukocyte transmigration and thereby inflammation. The objective of this review is to summarize the current view of the structure and function of these domains in fibrinogen and fibrin and their role in the above-mentioned processes.

*K e y w o r d s: fibrinogen, fibrin βN-domains, heparin, VE-cadherin, VLDL receptor.*

Introduction

Fibrinogen is the critical component of the haemostatic system. Its major function is to polymerize upon conversion into fibrin to form a fibrin clot, which is the major constituent of a blood clot. Blood clots seal damaged vasculature thereby preventing blood loss. Thrombin-mediated conversion of fibrinogen to fibrin results in the exposure of multiple binding sites that enable its interaction with different proteins and cell types and subsequent participation of fibrin in fibrinolysis, wound healing, atherogenesis, tumorigenesis, and other important physiological and pathological processes. This polyfunctional character of the fibrinogen molecule is connected with its multidomain structure in which each fibrin(ogen) domain or combination thereof may participate in certain interactions and thereby carry out certain functions. Studying the structure and function of individual fibrinogen domains is an important step towards a more comprehensive understanding of how this polyfunctional molecule participates in multiple processes. This review summarizes the major findings about the structure and function of the fibrinogen BβN-domains.

Structure of fibrinogen BβN-domains

The fibrinogen molecule consists of two identical subunits, each of which is composed of three non-identical polypeptide chains, Aα, Bβ, and γ, linked together by 28 disulfide bonds [1] (Fig., *A*). The letters "A" and "B" in the Aα and Bβ chains designate the N-terminal fibrinopeptides A and B, respectively, that are removed by thrombin upon conversion of fibrinogen into fibrin [1, 2]. The N-terminal portions of all six fibrinogen chains come together to form the central E region while the C-terminal portions of these chains form two identical terminal D regions [2]. These regions are arranged in the molecule linearly in a D-E-D manner. All of these chains are folded into at least 20 distinct
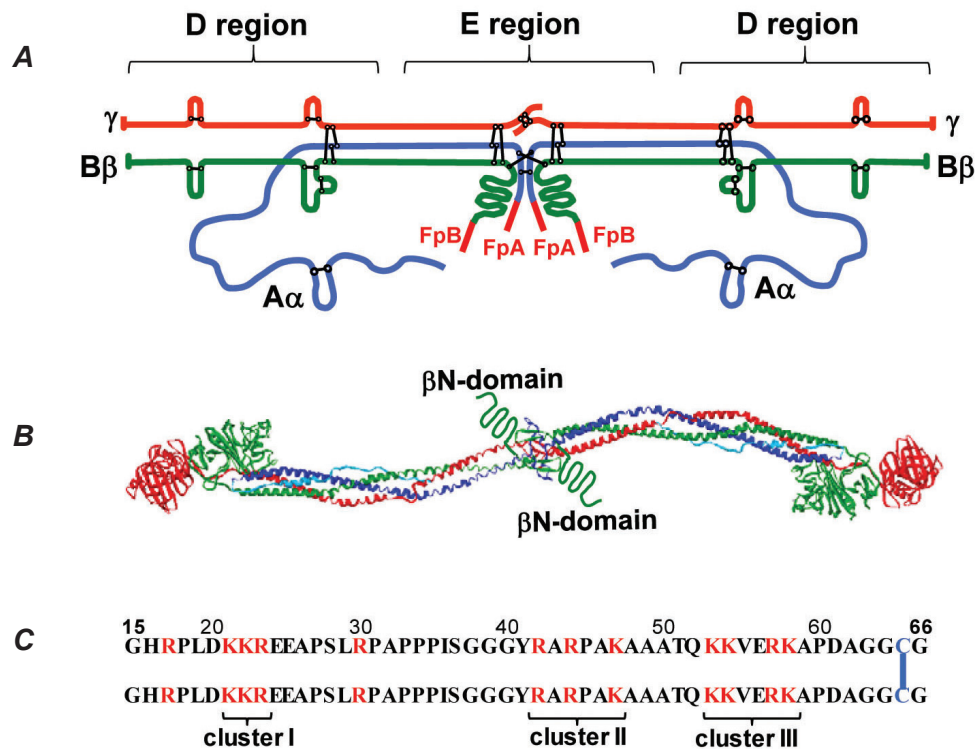
*Structures of the fibrinogen molecule and fibrin βN-domains. (**A**) Polypeptide chain composition of fibrinogen. The individual chains, Aα, Bβ and γ, are colored in blue, green, and red, respectively; fibrinopeptides A and B (FpA and FpB, respectively) are in red; disulfide bonds are represented by black bars. (**B**) Ribbon diagram of the fibrinogen molecule based on its crystal structure [14]. Fibrin βN-domains, whose location is arbitrary, are shown schematically by two green wavy lines. (**C**) The amino acid sequence of the dimeric disulfide-linked (β15-66)$_2$ fragment corresponding to a pair of fibrin βN-domains. The disulfide bond Cys65-Cys65 is shown schematically by a blue vertical line. Positively charged Lys (K) and Arg (R) amino acid residues are shown in red, and the three clusters (I, II, III) of positively charged residues are indicated*

domains which were originally identified by differential scanning calorimetry [3-6], and then confirmed by numerous X-ray studies [7-14] (Fig., *B*). The X-ray studies established the 3D structure of human, bovine, and chicken fibrinogen molecules [9-11, 14]. However, the C-terminal portions of the Aα chains and N-terminal portions of the Bβ chains corresponding to the fibrinogen αC- and BβN-domains were not visible in the electron density maps [14], raising questions about the folding status of these regions in the fibrinogen molecule. While the 3D structures of bovine and human fibrinogen αC-domains had been established by NMR studies and molecular modelling [15-17], that of the BβN-domains remained unclear.

Each of the two fibrinogen BβN-domains includes Bβ chain residues β1-64 attached to the bulk of the molecule through a βCys65-αCys36 disulfide bond. According to the proposed nomenclature [2],

in fibrin, in which fibrinopeptides B are removed by thrombin, these domains encompass residues β15-64 and are called βN-domains. The crystal structure of human and bovine fibrinogens and their E fragments, whose overall folds are similar [14], revealed that these two disulfide bonds are located in the central region of the molecule very close to each other. Based on these findings, we prepared a recombinant dimeric (Bβ1-66)$_2$ fragment in which two identical Bβ1-66 chains, each corresponding to the BβN-domain, are linked together through a Cys65-Cys65 disulfide bond (Fig., *C*) and which mimics the dimeric arrangement of the BβN-domains in the fibrinogen molecule [18]. We also prepared a recombinant dimeric (β15-66)$_2$ fragment by treatment of (Bβ1-66)$_2$ with thrombin, which removes fibrinopeptides B [18]. This fragment corresponds to a pair of fibrin βN-domains. Our experiments performed using circular dichroism and differential scanning

calorimetry techniques revealed no folded structures in both dimeric fragments indicating that they are unordered in solution [18]. However, this does not mean that the BβN-domains are unordered in the fibrinogen molecule. In this connection, secondary structure prediction and computer modelling of the structure of the β1-55 and β15-55 sequences encompassing most of the fibrinogen BβN- and fibrin βN-domains, respectively, revealed a possibility of secondary and tertiary structure formation by these regions [19]. Namely, it was predicted that Bβ6-13, β19-26, and β43-55 portions of the BβN-domain may form α-helices that could be folded into certain tertiary structures and removal of fibrinopeptide B from this domain may result in a dramatic change in conformation [19]. In agreement, the results of computer modelling of the Bβ1-60 and β15-60 regions and limited proteolysis of the Bβ42-43 peptide bond performed by another group [20] also predict some folded conformations in the (B)βN-domains.

Thus, although there is no experimental evidence confirming these predictions, one can speculate that these domains may be folded in the intact molecules where their structure can be stabilized by interactions with the neighboring regions. In agreement with this speculation, a previous study showed interaction between fibrinogen αC-domains and BβN-domains in which fibrinopeptides B play a significant role [21]. Additional studies with the whole fibrinogen molecule and the E_1 fragment derived from the central region of fibrin are needed to test this conjecture.

Role of fibrinogen BβN-domains in fibrin assembly

The functional properties of the (B)βN-domains in fibrinogen and fibrin were extensively studied and some of their major functions were established. For example, their role in the fibrin assembly process is well known. Although fibrin polymerization can occur after the removal of only fibrinopeptides A [22], this type of fibrin has an altered structure and resistance to fibrinolysis [23-25]. Thus, the removal of fibrinopeptides B is important for formation of a normal fibrin clot [26].

It is now widely accepted that fibrin polymerization occurs through the interaction between two pairs of complementary polymerization sites called holes 'a' and 'b' and knobs 'A' and 'B', which are located in the C-terminal D and central E regions, respectively [27]. The 'a' and 'b' sites (holes) in the D regions are always exposed while the complementary 'A' and 'B' sites (knobs) in the E region are protected by fibrinopeptides A and B. Proteolytic removal of these fibrinopeptides with thrombin results in the exposure of the 'A' and 'B' knobs and subsequent 'A'-'a' and 'B'-'b' (knob-to-hole) interactions that lead to the formation of fibrin polymer [2, 27]. Since the two pairs of 'A' and B' knobs are located in the central E region and the complementary 'a' and 'b' holes are in the terminal D regions, this interaction is often called DD:E (or D:E:D) interaction in which the E region of one fibrin molecule interacts with the D regions of two neighboring fibrin molecules. The 'a' and 'b' holes are represented by so-called polymerization pockets, whose structure was well characterized by crystallographic studies [7, 8, 28], while the 'A' and 'B' knobs are represented by Gly-Pro-Arg and Gly-His-Arg sequences, respectively, exposed after removal of fibrinopeptides A and B from the fibrinogen Aα and Bβ chains [27, 29, 30]. Thus, fibrin βN-domains contain Gly-His-Arg knobs which are involved in the 'B'-'b' knob-to-hole interaction. However, this is not the only function of the βN-domains in the fibrin assembly process.

Fibrin assembly is a highly ordered process which occurs in two stages. It was shown by Blombäck and collaborators that fibrinopeptides A and B are removed by thrombin in a sequential manner and the sequential release of these fibrinopeptides results in sequential activation of two sets of polymerization sites [31]. In the first stage, removal of fibrinopeptides A results in 'A'-'a' interaction between the D:E:D regions and formation of two-stranded protofibrils [32]. In the second stage, protofibrils aggregate laterally resulting in thicker fibrils, which form a 3D fibrin network [32]. This process coincides with the removal of fibrinopeptides B. Further, it was shown that the release of fibrinopeptides B, which is very slow at the start of the reaction, is accelerated upon polymer formation [31, 33-35]. Although the exact molecular mechanism underlying such sequential cleavage of fibrinopeptides is not completely understood, it is accepted that non-substrate interaction of thrombin with fibrinogen, which occurs before proteolytic cleavage of fibrinopeptides, plays an important role in both stages. After establishing the crystal structure of thrombin in complex with the E fragment [13], which represents the central region of fibrin molecule, it became clear that fibrinopeptide A-containing portions of fibrinogen Aα chains are located in the vicinity of

two molecules of bound thrombin and, therefore, their fibrinopeptides A are better positioned than fibrinopeptides B to be cleaved first by thrombin [36]. Further, our experiments showed that the (Bβ1-66)$_2$ fragment, representing a pair of fibrinogen BβN-domains, interacts with the dimeric crosslinked D-D fragment with a greater than 10-fold higher affinity than with the monomeric D fragment (K_d of 13 μM vs 153 μM) [36], suggesting that such an interaction may play a role in the orientation of fibrinopeptides B towards the active site of thrombin. Indeed, molecular modeling of the fibrin protofibril with bound thrombin revealed that such an interaction may direct fibrinopeptides B towards active sites of two bound thrombin molecules for efficient cleavage [36]. This modeling result is in good agreement with previous findings that fibrinopeptide B release is accelerated upon polymer formation [31, 33-35]. Thus, fibrinogen BβN-domains not only donate knobs 'B' for 'B'-'b' interaction but also interact with the DD regions in protofibrils to accelerate the removal of fibrinopeptides B by thrombin.

Interaction of fibrinogen BβN-domains with heparin

A second important function of fibrinogen BβN-domains is their interaction with heparin. Heparin is a structural analogue of heparan sulfate found on the surface of most cells and in the extracellular matrix [37, 38]. Heparan sulfate-containing proteoglycans anchored to cell surfaces play an important role in various biological processes [37, 38]. Heparin and heparan sulfate both belong to the glycosaminoglycan family and are represented by a mixture of linear, highly sulfated, and negatively charged polysaccharide chains of different lengths [39, 40]. Since fibrin βN-domains contain several positively charged residues grouped into three positively charged clusters (Fig., *C*), it is not surprising that they interact with heparin and proteoglycans due to electrostatic attraction between opposite charges. Heparin, which was found in mast and some hematopoietic cells [41, 42], is widely used as an anticoagulant due to its ability to interact with antithrombin III and thrombin and to enhance thrombin inhibition by antithrombin III [42, 43]. At the same time, heparin forms a ternary complex with thrombin and fibrin [44, 45] in which thrombin is markedly protected from inactivation by heparin-antithrombin III while maintaining its activity toward fibrinogen [45-48]. Thus, interaction of fibrin with heparin plays an important role in

modulation of thrombin activity. It was also reported that the β15-42 sequence of fibrin representing a portion of the fibrin βN-domain mediates heparin-dependent binding of fibrin to endothelial cell surface proteoglycans [49]. The functional role of this binding remains to be elucidated.

Interaction of heparin with fibrinogen and fibrin was demonstrated in several studies [45, 50-53]. The first attempt to localize heparin-binding sites in fibrinogen was performed by Mohri et al. [54] who found two heparin-binding fragments derived from the fibrinogen D region. In agreement, Raut and Gaffney [52] reported that fibrinogen fragment D bound to heparin, while no binding was observed with fragment E. In contrast, Odrljin et al. [53] localized heparin-binding sites of fibrinogen and fibrin in the 1-42 and 15-42 portions of their Bβ and β chains, respectively. They also found that fibrin binds to heparin with a 3.5-fold higher affinity than does fibrinogen [53]. It should be noted that there are two major plasmin cleavage sites in the fibrinogen BβN-domain, at βArg42-Ala43 and βLys53-Lys54 [1], and that Bβ1-42 and β15-42 fragments are naturally occurring plasmin degradation products of fibrinogen and fibrin, respectively. These fragments can be easily prepared from plasmin digest of fibrin(ogen) [55] or synthesized. In addition, a specific protease III from the venom of the western diamondback rattlesnake purified in the laboratory of Dr. A. Budzynski cleaves only the β1-42 portions of fibrinogen Bβ chains resulting in so-called fibrinogen 325 [56-58]. Fibrinogen 325, as well as the Bβ1-42 and β15-42 fragments, have been used in experiments resulting in localization of the heparin-binding site to the fibrin(ogen) Bβ1-42 and β15-42 regions [53] and in other experiments described elsewhere in this review.

To clarify the contradictory results described above and to further localize the heparin-binding site in fibrino(gen), we performed a detailed study of the interaction of heparin with fibrinogen, fibrin, and their various fragments including the recombinant (Bβ1-66)$_2$ and (β15-66)$_2$ fragments corresponding to a pair of fibrinogen BβN- and fibrin βN-domains, respectively [59]. The results showed that the (B)βN-domains are the only domains in fibrin(ogen) that bind heparin, in agreement with the previous study [53]. However, in contrast to this study, which localized the heparin-binding site of fibrin to the β15-42 region [53], we found that the full-length βN-domain fragment, either monomeric or dimeric,

has higher affinity to heparin than the β15-42 fragment [59]. This finding indicates that the third positively charged cluster of fibrin βN-domain (Fig., *C*) is also involved in heparin binding. We also found that removal of fibrinopeptides B from fibrinogen BβN-domains results in a 3-fold increase of heparin affinity to these domains (the K_d value is decreased from 228 to 72 nM) [59], in accord with the previous finding [53]. Further, we found that the affinity of the dimeric (β15-66)$_2$ fragment to heparin is much higher than that of the monomeric β15-64 fragment (the determined K_d values were 66 nM for (β15-66)$_2$ and 7.1 µM for β15-64) and is comparable to that of fibrin and the E$_1$ fragment (K_d = 72 and 70 nM, respectively) [59]. These findings suggest that the heparin-binding site in fibrin(ogen) is formed by a pair of the (B)βN-domains. They also indicate that the dimeric (Bβ1-66)$_2$ and (β15-66)$_2$ fragments mimic well the heparin-binding properties of fibrinogen and fibrin, respectively.

Thus, the results of the studies described above revealed the following facts. First, the major, and probably the only, physiologically relevant heparin-binding site in fibrinogen is located in its central E region, namely, in its BβN-domains. Second, the dimeric arrangement of these domains is critical for formation of the fully active heparin-binding site in both fibrinogen and fibrin. Third, conversion of fibrinogen to fibrin results in a 3-fold increase in the affinity of these domains to heparin. Finally, all three positively charged clusters of Lys/Arg residues in the (B)βN-domains (Fig., *C*) are involved in the interaction with heparin. The individual Lys and Arg residues that are critical for binding of heparin to fibrin(ogen) remain to be established.

Interaction of fibrin βN-domains with vascular endothelial (VE)-cadherin and its role in fibrin-dependent angiogenesis

A third important function of fibrin βN-domains is their interaction with vascular endothelial (VE)-cadherin, a member of the cadherin family of homophilic cell-cell adhesion receptors with a typical modular structure that includes five homologous extracellular domains, as well as cytoplasmic and transmembrane domains [60]. This interaction promotes angiogenesis (formation of new blood vessels) [61], which plays an important role in wound healing, tissue repair, tumorigenesis, and some cardiovascular diseases [62-65]. Although the ability of fibrin gel to support capillary growth was reported several decades ago [66, 67], this function has been studied more recently and described in detail by Dr. J. Martinez and collaborators [61]. First, they studied the formation of capillary tubes by endothelial cells sandwiched between two fibrin gels under serum-free conditions and observed maximal tube formation with fibrin desAABB, minimal tube formation with fibrin desAA, and complete absence of tube formation with fibrin 325 desAA, which lacks the N-terminal β15-42 sequence [68]. They also found that the addition of the β15-42 fragment significantly reduced the number and length of the tubes [68]. Based on these findings, they suggested that the β15-42 sequence of fibrin interacts with a component on the endothelial surface and this interaction plays a fundamental role in the induction of endothelial capillary tube formation [68]. Next, they demonstrated that a monoclonal antibody against human VE-cadherin inhibited formation of capillary tubes by endothelial cells sandwiched between fibrin gels [69]. Finally, they identified endothelial cell VE-cadherin as a receptor for fibrin which interacts with its β15-42 sequence and demonstrated that this interaction requires removal of fibrinopeptides B to expose this sequence [70]. It should be noted that although the βN-domains of fibrin are involved in the 'B'-'b' interaction, experiments with the monoclonal antibody T2G1 recognizing the fibrin β15-21 sequence showed that about 14% of these sequences are recognized by this antibody [71]. This finding indicates that at least part of fibrin βN-domains in fibrin polymers are available for interaction with VE-cadherin and other ligands and receptors.

VE-cadherin represents membrane-anchored adhesive molecules located at the endothelial intercellular junctions [72]. They are anchored to the cytoskeleton through their cytoplasmic domains while their extracellular portions, each consisting of five homologous domains, are involved in homophilic interaction with VE-cadherins of neighboring molecules thereby mediating cell-cell interaction and contributing to the integrity of the endothelium [72, 73]. Fibrin was the first heterophilic ligand identified for VE-cadherin [70]. Thus, the discovered heterophilic interaction of VE-cadherin with fibrin, which promotes fibrin-dependent angiogenesis [61], expands the functional properties of VE-cadherin beyond its adhesive function.

To clarify the molecular mechanism of heterophilic interaction between fibrin and VE-cadherin, we expressed the VE-cadherin fragment corre-

sponding to four extracellular N-terminal domains of VE-cadherin, VE-cad(1-4) fragment, and a number of βN-domain fragments containing the β15-42 sequence, and tested interactions between them [18]. The experiments demonstrated that only the dimeric (β15-66)$_2$ fragment bound to the VE-cad(1-4) fragment with high affinity (K_d = 80 nM), while the monomeric β15-42-containing fragments exhibited no binding at concentrations up to 400 nM [18]. As in the case with heparin-binding [59] described above, the affinity of (β15-66)$_2$ to VE-cad(1-4) was very similar to that of fibrin to VE-cad(1-4) *(K_d = 80 and 69 nM, respectively)* [18], indicating that the dimeric (β15-66)$_2$ completely preserves the VE-cadherin-binding properties of fibrin βN-domains. Further, we mutated several residues in the N-terminal portions of the dimeric (β15-66)$_2$ fragment and found that His16 and Arg17 are critical for the high affinity binding to occur [18]. We also localized the complementary fibrin-binding site within the third extracellular domain of VE-cadherin [74].

Thus, the studies described above showed that interaction of fibrin with endothelial cell VE-cadherin promotes capillary tube formation, i.e. angiogenesis, which occurs during normal wound healing or neovascularization during tumor growth and metastasis [61, 68]. This interaction requires the removal of fibrinopeptides B from fibrinogen and occurs through a pair of fibrin βN-domains and the third extracellular domain of VE-cadherin [18, 70, 74]. The N-terminal His16 and Arg17 residues of fibrin βN-domains are critical for the interaction [18]. However, one should also consider that other βN-domain residues may be involved in this interaction. This possibility needs to be investigated. Additional studies are also required to further clarify exactly how the interaction of fibrin with VE-cadherin promotes angiogenesis.

It should be noted that interaction of fibrin βN-domains with VE-cadherin was also implicated in fibrin-dependent inflammation. Namely, it was proposed that the interaction of the fibrin degradation product E$_1$ fragment, which contains these domains, with VE-cadherin promotes transendothelial migration of leukocytes and thereby inflammation, and the β15-42 fragment significantly reduces leukocyte transmigration by inhibiting this interaction [75, 76]. We later confirmed the inhibitory properties of the β15-42 fragment on leukocyte transmigration and found that the dimeric version of this fragment, (β15-44)$_2$, is a superior inhibitor over β15-42 and exhibits significant anti-inflammatory properties and

cardioprotective effect in *in vivo* mouse models [77]. However, our recent study [78] revealed that β15-42 cannot inhibit the E$_1$ fragment-VE-cadherin interaction due to its very low affinity for VE-cadherin. Its inhibitory effect on leukocyte transmigration most likely occurs through a putative endothelial receptor which remains to be identified.

Interaction of fibrin βN-domains with the very low density lipoprotein (VLDL) receptor and its role in fibrin-dependent inflammation

Another important interaction of fibrin βN-domains, which is involved in the inflammatory response, was discovered more recently. We found that fibrin and some of its degradation products interact with the very low density lipoprotein (VLDL) receptor on endothelial cells and that this interaction promotes fibrin-dependent leukocyte transmigration [79, 80]. We also found that the fibrin-VLDL receptor interaction occurs through fibrin βN-domains [79] and all three positively charged clusters of these domains (Fig., *C*) are involved in this interaction [81]. The complementary fibrin-binding site was localized to the second and third cysteine-reach (CR) domains of the VLDL receptor while the presence of its fourth CR domain, although not required for the binding, increases the affinity of this binding by about 2-fold [82]. The structure of the fibrin-binding fragment of the VLDL receptor, VLDLR(2-4), containing these three CR domains, has been established by NMR [83]. Finally, we clarified the molecular mechanism by which the fibrin-VLDL receptor interaction promotes leukocyte transmigration [78]. This mechanism includes interaction of fibrin with the VLDL receptor located on endothelial cells which triggers the VLDL receptor-dependent pathway of leukocyte transmigration inside the cells resulting in inhibition of the Src kinase Fyn [78]. The inhibition of Fyn prevents inhibition of GTPase protein RoA, which in the active state increases endothelial junction permeability [84, 85] resulting in increased leukocyte transmigration.

The discovery of the fibrin-VLDL receptor-dependent pathway of leukocyte transmigration and thereby inflammation prompted us to search for efficient inhibitors of this pathway. Our search resulted in identification of two monoclonal antibodies, mAb 1H5 and mAb 1H10, which inhibited fibrin-VLDL receptor interaction and significantly reduced fibrin-dependent leukocyte transmigration [86]. These

monoclonal antibodies were prepared earlier against the VLDL receptor in the laboratory of Dr. D. Strickland [87]; however, their inhibitory functions towards the fibrin-VLDL receptor interaction were not tested. After establishing that the epitopes for these antibodies overlap with the fibrin-binding site of the VLDL receptor [86], we realized that they can be used for inhibition of the fibrin-VLDL receptor-dependent pathway. Indeed, in a mouse model of peritonitis, in which leukocyte infiltration into the peritoneum is stimulated by injection of the pro-inflammatory agent thioglycollate, both monoclonal antibodies inhibited this infiltration by almost 50%, indicating their significant anti-inflammatory properties [86]. In addition, both antibodies exhibited a significant cardioprotective effect in a mouse model of myocardial ischemia-reperfusion injury [86]. Thus, these two antibodies are potent inhibitors of the fibrin-induced VLDL receptor-dependent pathway of leukocyte transmigration.

In summary, although the structures of the BβN- and βN-domains in fibrinogen and fibrin are still unclear, one can consider the possibility that they are folded in the fibrinogen molecule due to interactions with the neighboring structures and may unfold upon conversion of fibrinogen to fibrin to expose their binding sites for interaction with their ligands and receptors. These domains are multifunctional, i.e. they are involved in various (patho)physiological processes. It is now well established that they participate in the fibrin assembly process, interact with heparin, VE-cadherin, and the VLDL receptor. Their interaction with heparin is involved in modulation of thrombin activity. In addition, they interact with cell surface proteoglycans whose identity and functions remain to be determined. Interaction of fibrin with VE-cadherin, which occurs exclusively through fibrin βN-domains, promotes fibrin-dependent angiogenesis. The exact molecular mechanism underlying this process remains to be established. Finally, interaction of fibrin with the VLDL receptor through its βN-domains triggers the fibrin-VLDL receptor-dependent pathway of leukocyte transmigration which is involved in the inflammatory response in normal and pathological states. The molecular mechanism of this pathway has been clarified; however, putative intermediates of this pathway remain to be identified. Importantly, the two monoclonal antibodies, mAb 1H5 and mAb 1H10, identified as efficient inhibitors of this pathway could possibly be developed as potent therapeutics for treatment of fibrin-dependent inflammation-related cardiovascular diseases.

СТРУКТУРА І ФУНКЦІЇ ВβN-ДОМЕНІВ ФІБРИНОГЕНУ

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Фібриноген – поліфункціональний протеїн плазми крові, що бере участь в різних фізіологічних і патологічних процесах шляхом взаємодії своїх численних доменів із різними лігандами і клітинними рецепторами. Серед доменів фібриногену, два ВβN-домени утворені N-кінцевими ділянками двох Вβ-ланцюгів, що включають амінокислотні залишки Вβ1-64. Хоча сталого уявлення про їхню конформацію немає, а експерименти з рекомбінантним димерним (Вβ1-66)₂ фрагментом, який відповідає парі цих доменів, не виявили у ньому впорядкованої структури, деякі дані дозволяють припустити, що ці домени у нативній молекулі можуть бути просторово впорядковані. Проте, їхні основні функціональні властивості вивчено досить добре. Відщеплення фібринопептидів В (амінокислотні послідовності Вβ1-14) від цих доменів у разі перетворення фібриногену у фібрин призводить до експозиції численних сайтів зв'язування у βN-доменах фібрину (послідовність β15-64). Ці сайти забезпечують взаємодію βN-доменів із різними протеїнами і клітинами, що обумовлює їхню участь у різних процесах, зокрема у самоскладанні фібрину, фібрин-залежному ангіогенезі, а також у фібрин-залежній трансміграції лейкоцитів у процесі запалення. Метою цього огляду є узагальнення сучасного уявлення про структуру та функції цих доменів фібриногену і фібрину та їхньої функціональної ролі.

К л ю ч о в і с л о в а: фібриноген, фібрин, βN-домени, гепарин, VE-кадгерин, VLDL-рецептор.

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