

DOI 10.36074/grail-of-science.26.08.2022.17

## ALLOSTERIC REGULATION OF THE BLOOD CLOTTING CASCADE

**Chernyshenko Volodymyr**

Doctor of Biological Sciences, Head of the department,  
Protein structure and function department  
*Palladin Institute of Biochemistry of NAS of Ukraine, Ukraine*

**Korolova Daria**

Senior scientist  
Protein structure and function department  
*Palladin Institute of Biochemistry of NAS of Ukraine, Ukraine*

**Verevka Serhij**

Doctor of Biological Sciences, Professor, Head of the Department of Biochemistry  
*SI "O.S.Kolomiychenko Institute of Otolaryngology, NAMSU", Kyiv, Ukraine*

**Summary.** *Recognition of functional partners is a pivotal factor in the regulation of protein interactions. The areas of direct contact between complementary molecules that interact according to Koshland's "key - lock" scheme deserve special attention. The relevance of the study of this kind of interactions is obvious. In the case of the simplest serine proteinases the increased affinity of the enzyme to a certain area of the target protein is ensured by the synchronous interaction of the binding and allosteric sub-sites with amino acid residues of the target protein, that are adequate by ligand specificity and placed in an optimal conformation. The purpose of this work is to clarify the compliance of the components of the blood clotting cascade with this rule. Comparison of the primary sequences of sites of activation cleavage, reactive centers of serpins and sites of proteolytic inactivation testifies in favor of this assumption.*

**Keywords:** *blood coagulation system, allosteric regulation, serpins.*

Among the various transformations that protein molecules undergo during their existence, from biosynthesis on ribosomes to proteasomal cleavage to amino acids, a special place belongs to activation transformations. The vast majority of functionally active proteins are synthesized in the form of inactive precursors - proenzymes, profactors, proforms, etc. On the proper stage of protein processing, an inactive precursor undergoes transformations that lead to the appearance of functional activity. There are at least three distinct types of activation transformations. The first of them, mediated by chemical modification of the protein molecule, is inherent mainly to intracellular processes. A typical example of such activation is the phosphorylation of various tyrosinekinases involved in the regulation of many intracellular processes. For extracellular processes, two other types of activation processes are more characteristic - associative and proteolytic ones. Associative activation is due to the formation of active forms as a result of complex formation with a certain protein or non-

protein component. Instead, proteolytic activation is caused by the cleavage of one or more specific bonds by the proteinase-activator (PA) in the protein precursor. This leads to conformational changes and the appearance of this or that activity in the activated protein. A characteristic feature of PAs is high selectivity for limited number of cleavage. This feature is due to the mutual complementarity of the active center of the PA and the site of activation cleavage of the protein pro-form. The most famous PAs are trypsin-like serine proteinases. Like other proteins, they are also synthesized as inactive precursors that undergo one or another type of activation. Activated enzymes can be activators of other pro-forms, forming a kind of activation cascades. In the case of the simplest serine proteinases the recognition and selective cleavage in protein pro-forms is a consequence of the synchronous interaction of the binding and allosteric sub-sites of the enzyme with the corresponding residues of the activation cleavage region of the protein pro-form [1]. According to the Schechter-Berger nomenclature, the binding and allosteric sub-sites of the active center correspond to the S1- and S2'-sites of the zone of direct contact of the enzyme and the corresponding amino acid residues correspond to the P1- and P2'-residues, respectively (Fig. 1).

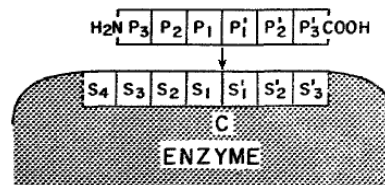


Fig. 1. Placement of the polypeptide chain at the interaction with enzyme according to Schechter-Berger nomination [2]. The arrow indicates the peptide bond that is being cleaved

Similarly, high affinity of the simplest serine proteins to the reactive centers of protein inhibitors of proteinases is ensured [1]. In terms of ligand specificity, the positively charged amino acid residues of lysine and arginine correspond to the binding site of trypsin (E.C. 3.4.21.1), and both positively charged and hydrophobic residues correspond to the allosteric site.

This work is devoted to clarifying the extent of this regularity to the activation cascade of the blood coagulation system, which is mediated by the sequence of activation cleavages in the group of trypsin-like proteinases, which undergo sequential activation and are in complex and ambiguous interactions with each other and with other components of the hemostasis system ( Fig. 2).

Thrombin (E.C.3.4.21.5) deserves special attention. It is not only a key enzyme of the blood clotting system, but is also involved in a number of physiological and pathophysiological processes [4]. It has long been known about the existence of an additional binding site for hydrophobic molecules in this enzyme, which is located somewhere near the active center [5]. It is also known about the stimulation of the hydrolytic activity of thrombin by hydrophobic iso-propanol [6]. A comparison of the primary sequences of the sites of activation cleavage of the so-called proteinase-activated receptors clearly indicates that the ligand specificity of the S2'-site of thrombin meets the requirements for the hydrophobicity of the ligand [7]. In order to assess compliance with these requirements of other components of the blood clotting cascade, the primary sequences of the corresponding activation cleavage sites in the interval P1-...-P2' were analyzed (Table 1).

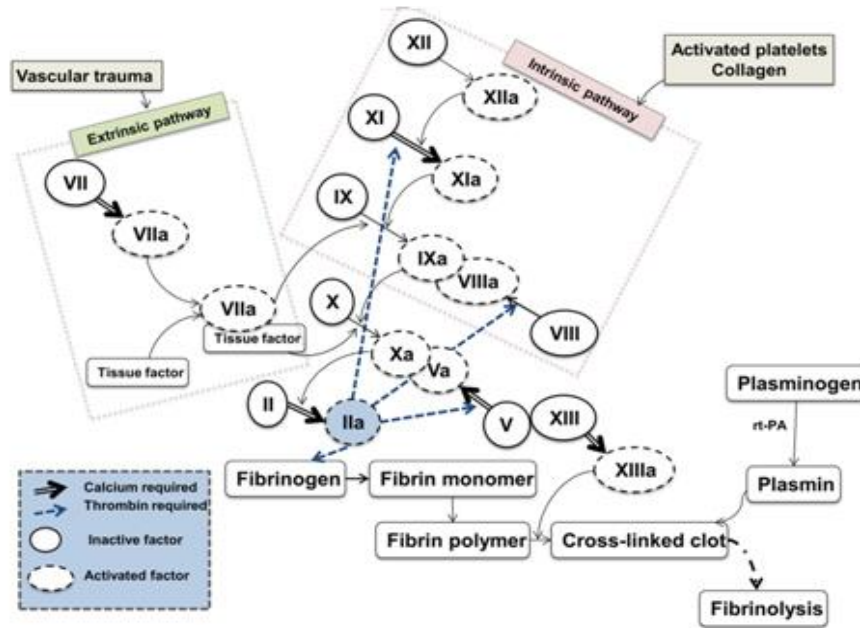


Fig. 2. The coagulation cascade showing the final conversion of fibrinogen to fibrin [3]

Table 1

P1-...-P2' sequences of activation cleavage sites of key factors of the blood coagulation cascade.

Factor	Activated form	Activators	P <sub>1</sub> -...-P <sub>2</sub> ' sequences of sites of activation cleavage
XI	XIa E.C.3.4.21.27	XIIa, IIa, XIa	-R <sub>369</sub> ↓I <sub>370</sub> -V <sub>371</sub> - [8]
VII проконвертин	VIIa E.C. 3.4.21.21	IIa, IXa, Xa, VIIa, XIIa, kallikrein, plasmin	-R <sub>152</sub> ↓I <sub>153</sub> -V <sub>154</sub> - [9]
IX Christmas factor	IXa E.C.3.4.21.22	XIa	-R <sub>145</sub> ↓A <sub>146</sub> -E <sub>147</sub> - -R <sub>180</sub> ↓V <sub>181</sub> -V <sub>182</sub> - [10]
VIII	VIIIa	IIa	-R <sub>1389</sub> ↓S <sub>1390</sub> -F <sub>1391</sub> - -R <sub>740</sub> ↓S <sub>741</sub> -F <sub>742</sub> - R <sub>372</sub> ↓S <sub>373</sub> -V <sub>374</sub> - [11]
X Stuart-Prower factor	Xa E.C.3.4.21.6	Under the influence of factors VIIa, VIIIa, IXa and tissue factor	-R <sub>194</sub> ↓I <sub>195</sub> -V <sub>196</sub> - [12]
V proaccelerin	Va	IIa	-R <sub>709</sub> ↓S <sub>710</sub> -F <sub>711</sub> - -R <sub>1018</sub> ↓T <sub>1019</sub> -F <sub>1020</sub> - -R <sub>1545</sub> ↓S <sub>1546</sub> -M <sub>1547</sub> - [13]
II prothrombin	IIa E.C.3.4.21.5	Xa, IIa	R <sub>273</sub> ↓T <sub>274</sub> -A <sub>275</sub> - R <sub>322</sub> ↓I <sub>323</sub> -V <sub>324</sub> - [14]
XIII	XIIIa transaminase E.C.2.3.2.13	IIa in the presence of fibrin and Ca <sup>2+</sup>	R <sub>37</sub> ↓G <sub>38</sub> -M <sub>39</sub> - [15]
Protein C	APC E.C.3.4.21.69	IIa in complex with thrombomodulin and phospholipid membrane.	R <sub>169</sub> ↓L <sub>170</sub> -I <sub>171</sub> [10]

↓ - зв'язок активаційного розщеплення.

As follows from the data presented in Table 1, the P1 positions of the activation cleavage sites are occupied exclusively by arginine, as it should be for hydrolysis by trypsin-like enzymes. On the other hand, in P2'-positions there is an almost total dominance of hydrophobic amino acid residues. Therefore, we can confidently talk about the participation of allosteric S2'-regions of the components of the blood coagulation system in the recognition and cleavage of the sites of activation cleavage of inactive factors of the blood coagulation cascade.

The sequence of reactive centers of key inhibitors of the blood coagulation system is subject to the same pattern (Fig.3, Tabl.2).

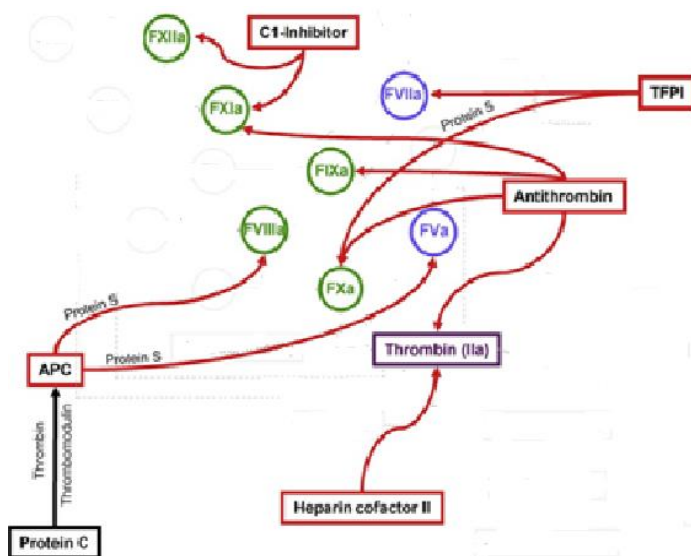


Fig. 3. Schematic diagram of the natural inhibitors of the blood coagulation pathways [16].

Table 2

P1-...-P2' sequences of reactive centers loops (RSL) of inhibitors of key factors of the blood coagulation cascade.

Inhibitor	Blood coagulation factors that undergo inhibition	P1-...-P2' sequences of RSL
α <sub>1</sub> -інгібітор протеїназ	IIa, IXa, XIa, XIIa, APC	M <sub>358</sub> ↓S <sub>359</sub> -I <sub>360</sub> [17]
антитромбін III	IIa, IXa, Xa, XIa, XIIa	R <sub>393</sub> ↓S <sub>394</sub> -L <sub>395</sub> [18]
C1-inhibitor	XIa, XIIa	R <sub>444</sub> ↓T <sub>445</sub> -L <sub>446</sub> [19]
Tissue factor pathway inhibitor (TFPI)	VIIa, Xa	K <sub>36</sub> ↓A <sub>37</sub> I <sub>38</sub> R <sub>107</sub> ↓G <sub>108</sub> Y <sub>109</sub> [20]

↓ - the reactive center of the inhibitor.

At the same time, it is worth noting several important details. The TFPI structure consists of three domains, each of which is a homologue of the Kunitz-type trypsin-kallikrein inhibitor. The reactive center of the first domain (K<sub>36</sub>↓A<sub>37</sub>I<sub>38</sub>) inactivates factor VIIa, and the reactive center of the second (R<sub>107</sub>↓G<sub>108</sub>Y<sub>109</sub>) - factor Xa. On the other hand, the third domain (R<sub>199</sub>↓A<sub>200</sub>N<sub>201</sub>) does not show an inhibitory effect [20]. α<sub>1</sub>-inhibitor of proteinases, antithrombin III and C1-inhibitor belong to the family of serpins. Unlike most families of protein inhibitors, the main form of their complex with proteinases is a covalent acyl-enzyme [21,22]. In addition, RSLs of serpins are mobile, passing through the "canonical conformation" that is optimal for interaction with the active center of

enzymes [23]. It is likely that this mobility provides a broad inhibitory specificity of the  $\alpha$ 1-inhibitor of proteinases in relation to blood clotting factors (Table 2). Placement of the methionine residue in the P1-position of this inhibitor, which is atypical for trypsin-like proteinases, does not become an obstacle for effective enzyme-inhibitor interaction due to the "blurring" of the ligand specificity of the S1-site when the allosteric site S2' is included in the process [24]. The data on the composition of the sites of inactivation cleavage of factors by activated protein C are also of interest (Table 3).

Table 3

**P1-...-P2' sequences of the sites of inactivation cleavage by activated protein C of activated factors Va and VIIIa.**

Factor	P1-...-P2' sequences of inactivation cleavage sites
Va	R <sub>306</sub> ↓M <sub>307</sub> -L <sub>308</sub> R <sub>506</sub> ↓G <sub>507</sub> -I <sub>508</sub> R <sub>679</sub> ↓K <sub>680</sub> -M <sub>681</sub> [13]
VIIIa	R <sub>336</sub> ↓M <sub>337</sub> -K <sub>338</sub> R <sub>562</sub> ↓G <sub>563</sub> -N <sub>564</sub> [11]

↓ - site of inactivation cleavage

As can be seen from the Table. 3 data, in the case of inactivation of the factor Va P2'-positions of all three inactivation cleavages are occupied exclusively by hydrophobic amino acids. In the case of factor VIIIa, these positions are occupied by positively charged lysine and neutral asparagine.

**Discussion.**

As follows from the above material, the pronounced dominance of hydrophobic amino acids in the P2'-positions of the areas of functional interaction of the components of the blood coagulation system indicates the participation of the allosteric areas of the corresponding proteinases in the regulation of the coagulation cascade of the hemostatic system. It is worth noting that most of these processes take place with the participation of third components (phospholipid membranes, cellular components, protein factors, fibrin, heparin, etc. Because of this, the substituents in the P2'-positions of functional cleavages are clearly worth more attention than the statement of "not-acidic acid" [25]. On the one hand, it contributes to the necessary conformational changes necessary to transfer certain components into a reactive state. On the other hand, the fixation of two components at the distance necessary for their interaction is functionally equivalent to the growth of their local concentration with a dramatic change in the dynamics of interaction. All this is a prerequisite for the regular and consistent course of processes of the blood clotting system.

**References:**

- [1] Verevka, S. (2022). Allosteric site of serine proteinases: location, functional role and manifestations in vitro. *Grail of Science.*, 12-13, 188-197. DOI: 10.36074/grail-of-science.29.04.2022.029
- [2] Schechter, I., Berger, A. (1967). On the size of the active site in proteinases. I. Papain. *Biochem. Biophys. Res. Commun.*, 27 (2), 157-162.
- [3] Kell, D., Pretorius, E. (2016) Proteins behaving badly. Substoichiometric molecular control and amplification of the initiation and nature of amyloid fibril formation: lessons from and for blood clotting. *Progr. Biophys. Mol. Biol.* –DOI:10.1016/j.pbiomolbio.2016.08.006
- [4] BrogaardLarsen, J., Hvas, A-M. Thrombin: A (2021). Pivotal Player in Hemostasis and Beyond. *SeminThrombHemost.*; 47 (7), 759-774. DOI: 10.1055/s-0041-1727116.

- [5] Berliner, L., Shen, J. (1977). Physical evidence for an apolar binding site near the catalytic center of human  $\alpha$ -thrombin. *Biochemistry*, 16 (21), 4662-4626.
- [6] Shvachko, L., Ribirev, V. (1988). Influence of isopropanol on the enzymatic activity and stability of thrombin. *Ukr. Biochem. Zhurn.*, 60 (3), 15-19. [In Russian].
- [7] Mihaylik, I., Verevka, S. (1999). Structural regularities of activationsplitting sites of thrombin receptors. *Ukr. Biochim. Zhurn.*, 71 (6), 111-112. [In Russian].
- [8] Fujikawa, K., Chung, D., Hendrickson, L., Davie, E. (1986). Amino acid sequence of human factor XI, a blood coagulation factor with four tandem repeats that are highly homologous with plasma prekallikrein. *Biochemistry.*, 25 (9), 2417-2424.
- [9] Herrmann, F., Wulff, K., Auerswald, G., Schulman, S. (2008). Factor VII deficiency: clinical manifestation of 717 subjects from Europe and Latin America with mutations in the factor 7 gene. *Haemophilia*, 15 (1), 267-280. DOI:10.1111/j.1365-2516.2008.01910.x
- [10] Foster, D., Yoshitake, S., Davie E. (1985). The nucleotide sequence of the gene for human protein C. *Proc. Natl. Acad. Sci. USA.*, 82 (14), 4673-4677.
- [11] Vehar, G., Keyt, B., Eaton D, Rodriguez H, et al. (1984). Structure of the human factor VIII. *Nature*, 312 (5992), 337-342.
- [12] Venkateswarlu D, Perera L, Darden T, Pedersen L. (2002). Structure and dynamics of zymogen human blood coagulation factor X. *Biophys. J.*, 82 (3), 1190-1206.
- [13] Kane, W., Davie, E. (1988). Blood coagulation factors V and VIII: structural and functional similarities and their relationship to hemorrhagic and thrombotic disorders. *Blood*, 71 (3), 539-555.
- [14] Mann, K, Elion, J., Butkowski, R., Downing, M, Nesheim, M. (1981). Prothrombin. *Meth. Enzymol.*, 80, 286-302.
- [15] Recheis, B., Osander, A., Haubenweller, S. et al. (2000). Chicken coagulation factor XIIIa is produced by the theca externa and stabilizes the ovavian follicular wall. *J. Biol. Chem.*, 275 (45), 35320-35327.
- [16] Cilia La Conte, A., Phillipou, H., Ariens, R. (2011). Role of fibrin structure in thrombosis and vascular disease. *Adv. Prot. Chem. And Struct. Biol.*, 83, 76-127.
- [17] Holmes, W., Nelles, L., Lijnen, H., Collen, D. (1987). Primary structure of human  $\alpha$ -2-antiplasmin, a serine proteinase inhibitor (serpin). *J. Biol. Chem.*, 262 (4), 1659-1664.
- [18] Moureu, L., Samama, J., Delarue, M., Choay, J., et al. (1990). Antithrombin III: structural and functional aspects. *Biochimie.*, 72 ( ), 599-608.
- [19] Grover, S., Mackman, N. (2022). Anticoagulant serpins: endogenous regulators of hemostasis and thrombosis. *Front Cardiovasc. Med.*, 3 (9), 878199. DOI: 10.3389/fcvm.2022.878199
- [20] Broze, G., Giran, T. (2012). Tissue factor pathway inhibitor: structure – function. *Front Biosci.*, 17, 262-280.
- [21] Enghild, J., Valnichkova, Z., Thodersen, J, Pizzo, S. (1994). Complex between serpins and inactive proteinases are not thermodynamically stable but are recognized by serpin receptors. *J. Biol. Chem.*, 269 (31), 29159-29166.
- [22] Patston, P., Gettins, P., Beechem, J., Shapira, M. (1991). Mechanism of serpin action: evidence that C1 inhibitor functions as a suicide substrate. *Biochemistry*, 30 (36), 8876-8882.
- [23] Lawrence, D., Olson, S., Pammaman, S., Ginsburg, D. (1994). Serpin reactive center loop mobility is required for inhibitor function, not for enzyme recognition. *J. Biol. Chem.*, 269 (44), 27657-27662.
- [24] Malezhyk, A., Voroshylova, N., Obernikhina, N. (2022). Allosteric modulation of primary specificity of serine proteinases. *Grail of Science*, 13-14, 231-233. DOI: 10.36074/grail-of-science.27.05.2022.041
- [25] Gallwitz, M., Enoksson, M., Thorpe, M, Hellman L (2012) The Extended Cleavage Specificity of Human Thrombin. *PLoS ONE* 7(2): e31756. doi:10.1371/journal.pone.0031756