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Biophysical Chemistry 119 (2006) 282 – 294

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

The Na^+ binding channel of human coagulation proteases: Novel insights on the structure and allosteric modulation revealed by molecular surface analysis

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Received 21 July 2005; received in revised form 30 September 2005; accepted 1 October 2005
Available online 9 November 2005

Abstract

Thrombovascular diseases result from imbalanced haemostasis and comprise important health problems in the aging population worldwide. The activity of enzymes pertaining to the coagulation cascade of mammalians exhibit several control mechanisms in order to maintain a proper balance between bleeding and thrombosis. For instance, human coagulation serine proteases carrying a F225 or Y225 are allosteric modulated by the binding of Na^+ in a water-filled channel connected to the primary specificity pocket (S1 subsite) of these enzymes. We have characterized the structure, topography and lipophilicity of this channel in the ligand-free fast (sodium-bound) and slow (sodium-free) forms of thrombin, in the sole available structure of activated protein C and in several structures of the coagulation factors VIIa, IXa and Xa, differing in the nature of the bound inhibitor and in the occupancy of exosite-I as well as the Ca^{2+} and Na^+ binding sites. Opposite to thrombin, the aqueous channels in all other coagulation enzymes sheltering a Na^+ binding site do not have an aperture on the enzyme surface opposite to the S1 subsite entrance. In these enzymes, the lack of the three-residue insertion in loop 1 (183–189) as found in thrombin allied to compensatory mutations in the positions 187–185 and 222 effects a constriction in the water-filled channel that ends up by segregating the ion binding site from the S1 subsite. We also disclosed major topographical changes on the thrombin's surface upon sodium release and transition to the slow form that culminate in the narrowing of the S1 subsite entrance and, strikingly, in the loss of communication between the primary specificity pocket and the exosite-I. Such observation is in accordance with existing experimental data demonstrating thermodynamic linkage between these distant regions on the thrombin surface. Conformational changes in F34, L40, R73 and T74 were the main responsible for this effect. A path by which these changes in the vicinity of exosite-I could be transmitted to the S1 subsite and, consequently, to the sodium binding site is proposed.

Keywords: Allostery; Na^+ binding; Molecular modeling; Thrombin; Coagulation factors; Serine proteases

1. Introduction

The physiologic response to a vascular lesion entails a number of sequential enzymatic steps catalyzed by distinct serine proteases, known as the coagulation cascade [1–3]. Vein or artery rupture triggers the formation of a calcium- and phospholipid-dependent complex between the plasmatic protein known as activated factor VII (FVIIa) and tissue factor (TF). In the next step, the tenase complex activates factor X and the resultant activated factor X (FXa) subsequently associates with activated factor V (FVa) on the surface of TF-bearing cells to form a prothrombinase complex. As the name suggests, the later converts the inactive circulating thrombin precursor, prothrombin, into its active form. Significantly, the tenase complex is also able to process a small amount of factor IX and the activated factor IX (FIXa) can diffuse to the surface of lesion-recruited platelets and activate FX with out being significantly

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doi:10.1016/j.bpc.2005.10.001

inhibited by TFPI or ATIII. In the final step of the coagulation cascade, the release of fibrinopeptides A and B by the action of thrombin over fibrinogen α and β chains, respectively, leads to the formation of fibrin monomers. An important regulatory pathway of the coagulation process results from the inactivation of factors Va and VIIIa by activated Protein C (aPC). The formation of the later is catalyzed by a complex between thrombin and thrombomodulin, a transmembrane protein from the endothelium.

Thrombin is a multifunctional protease, central to the coagulation process in mammals and other vertebrates. As such, thrombin must be under very tight control and consequently must interact with different substrates and molecular modulators. This diversity of functional interactions is also present in other serine proteases operating in the coagulation cascade, such as aPC and factors VIIa, IXa and Xa. Overall, efficiency of the coagulation system requires a rapid and localized response at the injury site that can be readily terminated. This is accomplished through a plethora of regulatory mechanisms among which sodium mediated allostery has emerged as the simplest and most intriguing [4]. The physiological role of sodium as a thrombin allosteric modulator was disclosed short after the characterization of the Na^+ dependent conformational change and activation of the enzyme was reported [5]. The fast form that is generated after Na^+ binding is considered procoagulant primarily because it cleaves fibrinogen with higher specificity than Protein C. Oppositely, the Na^+-free, slow form, is thought to be anticoagulant due to its higher specificity for Protein C in comparison to fibrinogen.

The activating effects of Na^+ over the hydrolytic activities of the coagulation proteases, FXa, thrombin and aPC, has been known for many years [6–8]. The coagulation factors FVIIa and FIXa are suspected to shelter a Na^+ binding site based upon the Dang and Di Cera's rule [9]. This rule was elaborated after the canonical thrombin Na^+ binding site (Fig. 1) was identified crystallographically through rubidium substitution [5,10]. It states that serine proteases of the chymotrypsin family (clan S1A) carrying a phenylalanine or a tyrosine residue at position 225 can properly provide a Na^+ binding site whereas family members presenting P225 cannot. Indeed, it was demonstrated that FIXa displays a 4-fold enhancement in specificity for synthetic amide substrates upon Na^+ addition to the reaction media [11].

The specificity of coagulation serine proteases is primarily dictated by the complementary interactions between the major pocket in the substrate binding cleft (the S1 subsite) and the side chain of the residue N-terminal to the scissile bond (P1 residue). Nonetheless, these enzymes display on their surfaces other sites involved in substrate recognition such as the exosite-I in thrombin and the Ca^{2+} binding site found in aPC and coagulation factors VIIa, IXa and Xa. These sites are distributed in analogous regions of the serine protease COOH-terminal domain. Exosite-I is delineated by loops

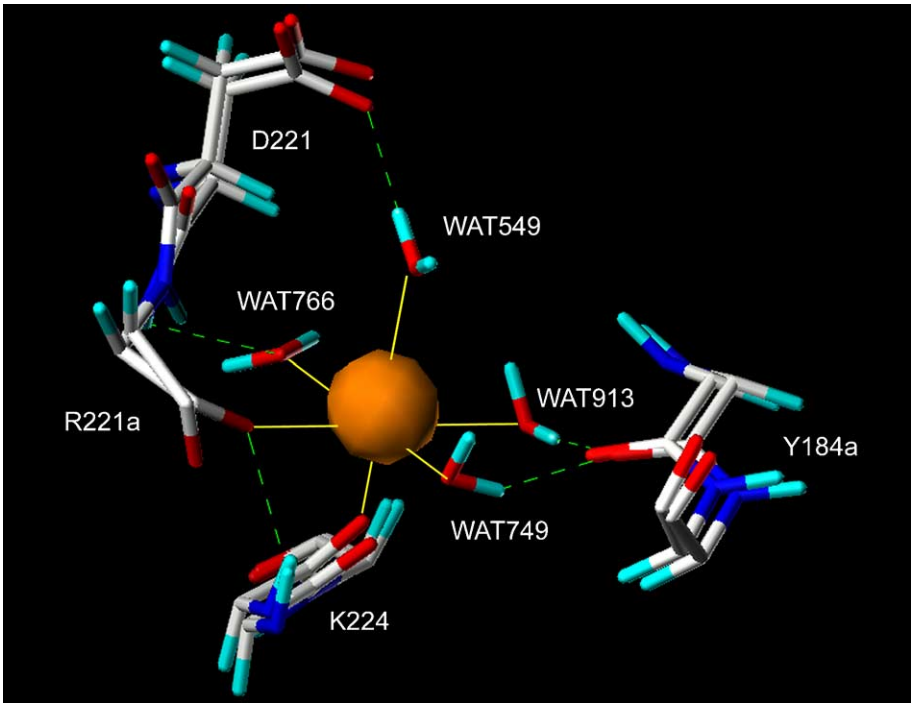

Fig. 1. Comparison of the Na^+ coordinating residues conformations in thrombin fast and slow forms. The Na^+ ion is plotted as an orange van der Waals sphere while protein residues are depicted as CPK-colored capped sticks. For the fast form, water molecules coordinating Na^+ are shown along with hydrogen bonding interactions (green dashed lines; 2 dashes per angstrom) and coordination bonds (yellow lines), which is composed of the carbonyl O atoms of residues 224 and 221a and four water molecules in thrombin or in the case of aPC and FXa by the carbonyl oxygen atoms of residues 185, 184a, 224 and 221a along with two water molecules. The site architecture is largely maintained upon sodium release by the thrombin fast form (pdb 1SG8) and transition to the slow form (pdb 1SGI). One important exception is the loop 1 backbone whose change in conformation culminating at residue R221a (depicted by deviations of 41.4° and 31.9° in ϕ and ψ torsion angles, respectively) moves the carbonyl oxygen atom in the slow form almost 1.0 Å away from its position relative to the fast form.