

Editorial Focus

Is Na⁺ a coagulation factor?

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Blood coagulation is founded upon the regulated equilibrium of active and inactive states of trypsin-like proteolytic enzymes. Protein-based cofactors, Ca²⁺, and a phospholipid surface were woven together with proteolytic enzymes into a response to vascular damage. In addition to these coagulation factors, the most abundant alkali metal in plasma – Na⁺ – has emerged as an important effector of protease activity that played a key role shaping the evolution of clotting enzymes and proteases in general (1).

Orthner and Kosow first identified Na⁺ activation in factor Xa (2) and thrombin (3). Steiner and Castellino discovered a

drastic Na⁺ activation in activated protein C (4), but reported that Na⁺ had no effect on the interaction of this enzyme with the physiologic target factor VIIIa (5). A defined role for Na⁺ in coagulation proteases arose when the kinetic mechanism of Na⁺ activation of thrombin was found to be allosteric (6), the Na⁺ binding site of thrombin was crystallographically identified (7), and Na⁺ was shown to be a requirement for cleavage of fibrinogen, but not for activation of the anticoagulant protein C (8). Subsequent studies demonstrated that Na⁺ promotes thrombin cleavage of PAR1, PAR3 and PAR4 (9), thereby facilitating prothrombotic and signaling functions of the enzyme. The procoagulant role of Na⁺ was later reinforced by the groups of Leung, Fay and Walsh with the observation that Na⁺ promotes activation of factors V (10), VIII (11) and XI (12) by thrombin.

Does Na⁺ have effects on other clotting factors similar to those observed in thrombin? One would expect similarities given common ancestral origin and similar Na⁺ binding environments. A necessary (yet not sufficient) structural determinant for Na⁺ binding to serine proteases was identified as the presence of Tyr or Phe at position 225 (13). A Pro residue at this position, as found in the majority of serine proteases, is sufficient to abrogate Na⁺ binding. Remarkably, all vitamin K dependent clotting proteases carry Tyr or Phe at position 225, raising the possibility that the procoagulant role of Na⁺ in thrombin can be compounded in the progression of the coagulation cascade by similar effects on factors Xa, IXa and VIIa.

In this issue of *Thrombosis and Haemostasis*, Gopalakrishna and Rezaie (see pages 936–41) (14) examine the effect of Na⁺ on the ability of factors IXa and VIIa to activate factor X in the intrinsic and extrinsic Xase complexes, respectively. The study follows up on the effect of Na⁺ on factor Xa in the prothrombinase complex (15) and completes the analysis of Na⁺-activated clotting factors in their physiologic interactions. The authors make an important observation: although factors IXa and VIIa possess structural prerequisites for Na⁺ binding (13), there is no effect of the cation on the activity of these proteases when assembled in their Xase complexes. Previous studies have shown that the activity of factor IXa toward small chromogenic substrates is en-

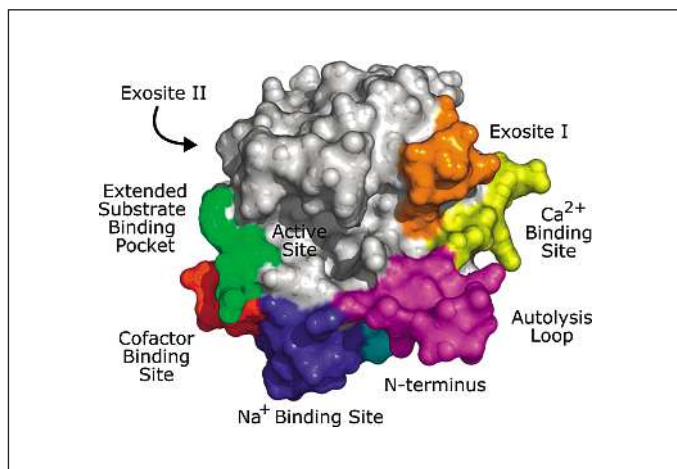


Figure 1: Surface representation of a typical clotting protease. Position of the Na⁺ binding site is optimal for regulation of proteolytic activity and allows allosteric communication between opposing faces of the protease domain. Two loops involved in Na⁺ coordination (blue) have connections with the extended binding pocket (green), cofactor binding site (red), autolysis loop (magenta), and N-terminus (cyan). This arrangement provides long range communication with the active site and exosite I (orange), which in many proteases contains a Ca²⁺ binding site (yellow). Cofactor binding likely evolved to exploit the structural link between the Na⁺ site and the active site to achieve control of catalytic activity. The structure used is Protein Data Bank entry 4HTC.

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hanced by Na⁺ binding (13, 16), as is the case for factor Xa (2, 15, 17–19), but the effect is more evident in the absence of Ca²⁺. However, the Na⁺ effect on the protease is abrogated upon binding of the respective cofactor, i.e. tissue factor for factor VIIa (20), factor VIIIa for factor IXa, and factor Va for factor Xa (15, 19).

The requirement for a cofactor in factors VIIa, IXa and Xa has reduced the role of Na⁺ as a procoagulant in these enzymes, perhaps through evolutionary transitions where the original effect of Na⁺ was replaced by specialized protein-protein interactions. The Na⁺ binding site is ideally positioned to relay communication between opposing faces of the protease domain (Fig. 1). The spatial proximity of the Na⁺ binding site and the locale for cofactor binding suggests that the cofactors may have hijacked the determinants for the long-range communication between the bound Na⁺ and the active site residues so eloquently revealed by the structural investigation of thrombin (21).

What is, then, the role of Na⁺ in blood coagulation? Na⁺ has no significant effect on factors VIIa, IXa and Xa bound to their cofactors, but is required by thrombin to ensure physiologically significant activation of fibrinogen and PAR1 (22), as well as to trigger the initial build-up of factors Va (10), VIIIa (11) and XIa

(12) necessary for the explosive generation of thrombin (23). Does that make Na⁺ a coagulation factor? A direct proof would be difficult to obtain. Unlike protein coagulation factors and Ca²⁺, Na⁺ is present in plasma at a concentration of 140 mM that would make removal by chelation problematic. Changes in the concentration of Na⁺ in the blood are quite common (24, 25). The resulting effects on blood coagulation have been examined only rarely (26, 27), but were found to be consistent with a procoagulant/prothrombotic effect of Na⁺. Significant support to the role of Na⁺ as a coagulation factor comes from the observation that several naturally occurring mutations of the prothrombin gene, like prothrombin Frankfurt (E146A) (28), Salakta (E146A) (29), Greenville (R187Q) (30), Scranton (K224T) (31), Copenhagen (A190V) (32) and Saint Denis (D221E) (33) affect residues responsible for Na⁺ binding (21) and are often associated with bleeding. In murine thrombin, the need to overcome the deleterious effect of such mutations has evolved in complete functional mimicry of the Na⁺ effect that locks the enzyme in a Na⁺-bound form (34). More studies are obviously needed to provide a conclusive answer, but evidence in favor of Na⁺ as a coagulation factor is quite compelling.

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