

Role for heparan sulfate proteoglycan in thrombin-induced calcium transients and nitric oxide production in aortic endothelial cells

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Activation of protease-activated receptors (PAR), a sub-family consisting of four members (PAR-1 to PAR-4) of seven-transmembrane-spanning G protein-coupled receptors, plays a significant role in vascular physiology and pathophysiology. The multifunctional endolytic serine protease thrombin is known to activate PAR, especially PAR-1 in vascular endothelial cells, and thrombin hence activates intracellular signals (1). In intact vascular endothelial cells, thrombin causes calcium transients and release of nitric oxide (NO), PGI₂, ADP, ATP, von Willebrand factor and tissue plasminogen activator (1), while in injured vessels thrombin plays additional pathological roles such as the proliferation of smooth muscle cells (2).

Whereas the mechanism of activation of PAR by thrombin is well known (1), it was not clear how the extracellular thrombin remains in the proximity of the cells to exert its enzyme activities. In this issue of *Thrombosis and Haemostasis*, Kimura and Oike (3) propose a role for heparan sulfate proteoglycan (HSPG) by providing evidence that binding of thrombin to HSPG is essential for thrombin-induced PAR-activation in bovine aortic endothelial cells (BAEC).

HSPG are ubiquitously expressed on cell surfaces and throughout the extracellular matrix (ECM) of all mammalian tissues (4), with three subfamilies; the membrane-spanning proteoglycans (PG), the glycosphosphatidylinositol (GPI)-linked proteoglycans, and the secreted ECM proteoglycans. HSPG, which is abundantly expressed in the endothelium, occurs as a PG, consisting of two or three linear polysaccharide heparan sulfate (HS) side chains with heparin-like regions, attached to specific serine residues of the protein core in close proximity to cell surfaces or ECM proteins. The HS chains of HSPG can have extraordinary structural diversity, which allows HS to interact with a wide range of functionally diverse proteins (5). In the endothelium, HSPG play a significant role in the binding of fibroblast growth factor (6) and in mechanosensation (7).

Since thrombin has a heparin-binding site, thrombin can bind to the heparin-like regions of HSPG and Kimura and Oike (3) report that this binding plays a significant role in thrombin-induced calcium transients and NO production in BAEC (Fig. 1).

They showed complete inhibition of thrombin-induced calcium transients in BAEC that had been preincubated with heparinase III (Fig. 1A), which causes enzymatic degradation of the polysaccharide chains of HSPG. The thrombin-induced calcium transients were also completely inhibited when the heparin-binding exosite of thrombin (exosite 2) was masked by pre-incubation of thrombin with heparin (Fig. 1B). They also showed that pretreatment of BAEC with heparinase III (Fig. 1A) or treatment of BAEC with a pre-incubated thrombin-heparin mixture (Fig. 1B) strongly suppressed thrombin-induced NO production. In contrast, ATP-induced calcium transients (8) or NO production (9), were not affected in BAEC pretreated with heparinase III. Since thrombin- (10) and ATP-induced (11) calcium responses are mediated via IP₃, the results of Kimura and Oike (3) show that heparinase III-induced removal of the polysaccharide chains of HSPG does not affect the cellular calcium responsiveness to IP₃ in BAEC. Therefore, they state that the polysaccharide chains of HSPG do not play a role in the intracellular signaling pathways from IP₃ to NO production but do play a role in activation of PARs (Fig. 1).

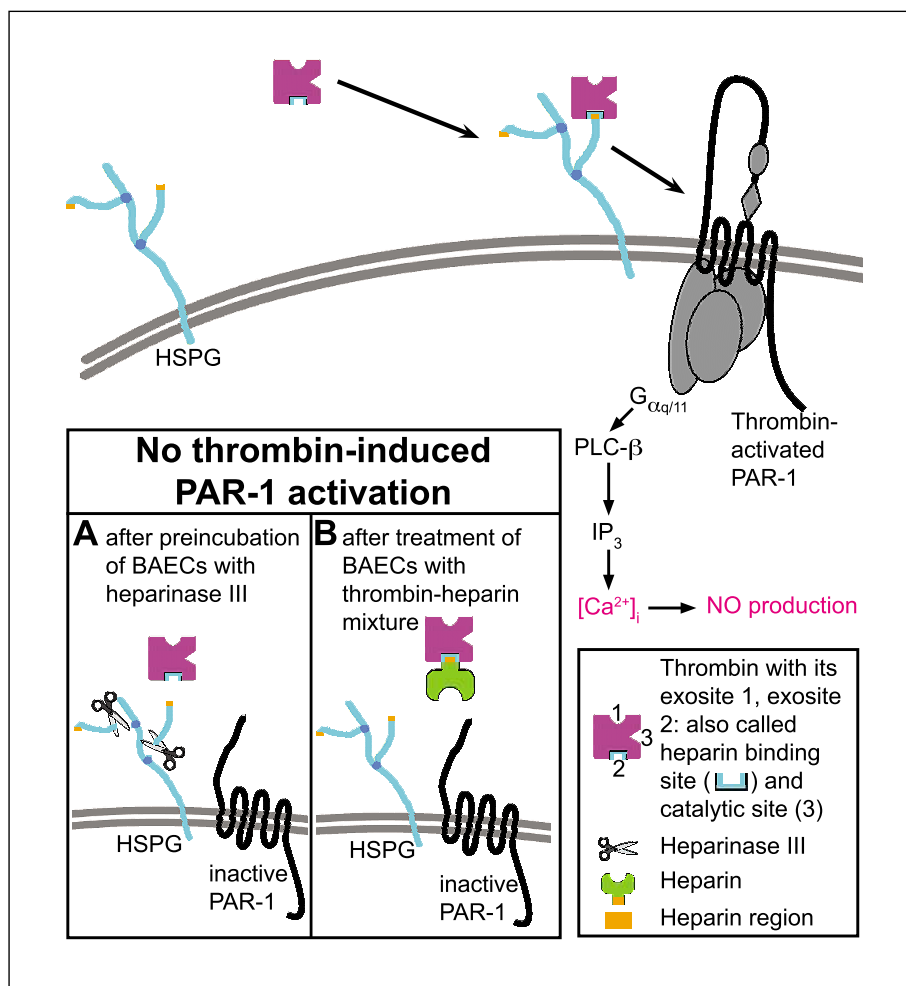
There is long-standing evidence that heparin-like components are involved in the enzymatic activities of thrombin. In 1973 Damus et al. (12) already proposed an involvement of anticoagulant active heparin-like components on the luminal surface of endothelial cells in the non-thrombogenic properties of blood vessels. From 1983 till 1986 Marcum et al. (13–18) managed to identify these heparin-like components. They extracted HS glycosaminoglycans with heparin-like activity from bovine vascular endothelial cells and examined the binding of the unique subset of anticoagulant active HSPG with the heparin-binding site of antithrombin (AT). Hatton et al. (19) found that heparinase treatment of the exposed subendothelium reduced the AT binding and also seemed to deplete the subendothelium of small PG-rich granules and basement membrane. In 1990, de Agostini et al. proposed a model in which the coagulatory activity is regulated by luminal and abluminal anticoagulant active HSPG (20). Later on, HSPG have been implicated in processes ranging from mechanical support to functions in cell adhesion,

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Figure 1: Binding of thrombin to HSPG is required for PAR-1 activation in bovine aortic endothelial cells (BAEC). Thrombin (purple) has different sites: an anion binding exosite 1 (number 1), which is responsible for substrate recognition, an exosite 2 (number 2), which is also called the heparin-binding site (blue), and a catalytic site, also called active site (number 3). The binding between a polysaccharide chain of heparan sulfate proteoglycan (HSPG) and the heparin-binding exosite of thrombin is required for PAR-1 activation and subsequently results in thrombin-induced calcium transients and nitric oxide (NO) production. Upon activation of PAR-1, thrombin recognizes the N-terminal exodomain of PAR-1. The interaction of thrombin with PAR-1 involves both the anion binding exosite 1, which recognizes the GYEQIP region of PAR (oval), and the catalytic site, which cleaves the PAR between receptor residues Arg 41 and Ser 42 (after the SFLLRN region of PAR [diamond]). Activation of PAR-1 is due to thrombin-induced PAR-1 cleavage, which unmasks a new N terminus, beginning with the sequence SFLLRN (diamond). This sequence functions as a tethered ligand, docking intramolecularly with the second extracellular loop of PAR-1, to result in receptor activation and subsequently in transmembrane signaling. A) When BAEC are pretreated with heparinase III, which causes enzymatic degradation of the polysaccharide chains of HSPG, thrombin's heparin-binding exosite cannot bind to the polysaccharide chain of HSPG and hence thrombin cannot activate PAR-1. B) Preincubation of thrombin with heparin, resulting in the masking of thrombin's heparin-binding exosite, inhibited the binding of thrombin to the polysaccharide chain of HSPG in BAEC.



motility, proliferation, differentiation, morphogenesis, and control of cell growth (21–24).

Studies during the last years have shown that the activity of many inflammatory factors is regulated by binding to HSPG in a HS-dependent manner (4, 25, 26). Based on the involvement of HSPG in a variety of pathophysiological processes (4, 5), they were also suggested as a potential target in cardiovascular disorders (27). Since HSPG modulate cell-ECM interactions (28–30), the binding of thrombin to HSPG might play a role in the thrombin-induced formation of interendothelial gaps resulting in breakdown of endothelial barrier integrity (31) and hence

play a role in tissue injury, inflammation and wound healing. Endothelial barrier integrity substantially depends on the cytoskeleton, which ensures actin stress fiber formation, and regulates cell shape and adhesion via actomyosin-driven contraction (31).

Besides the many organ-specific functions of HSPG, Kimura and Oike (3) now have provided evidence that the binding between the polysaccharide chain of HSPG and the heparin-binding exosite of thrombin plays a critical role in the localized actions of thrombin on aortic endothelial cells revealing another important role of HSPG in endothelial physiology and pathophysiology.

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