A critical regulatory pathway of the blood coagulation cascade is provided by antithrombin, a serine protease inhibitor (serpin) that specifically shuts down the activity of factor Xa and thrombin. Much of what we know about the mechanisms of antithrombin function has come from studies using the cofactor heparin, that allosterically activates antithrombin for optimal presentation to the target protease. In the case of factor Xa, this activation is sufficient for neutralization of enzyme function. The heparin-assisted activation of antithrombin is of clinical relevance as heparinoids retain center stage in anticoagulant therapy. However, the extracellular environment is of clinical relevance as heparinoids retain center stage in anticoagulant therapy. The extracellular milieu of antithrombin functions in vivo is populated by a variety of glycosaminoglycans (GAGs) like heparin sulfate, dermatan sulfate and chondroitin sulfate (CS). GAGs have anticoagulant activity, but they often lack the heparin pentasaccharide unit required for antithrombin activation, which has raised important questions about their precise mechanism of action.

In the extracellular matrix, GAGs are anchored to proteoglycans and feature different degrees of sulfation that can affect both ion distribution and the water content of the environment. Versican is the most abundant proteoglycan present in the arteries and uses long chains of CS as GAG appendices. Because of the ionic nature of GAGs and their spatial arrangement in the extracellular matrix, several possible mechanisms may be at the basis of their anticoagulant activity. Electrostatic forces generated by the sulfated components of GAGs may steer antithrombin and target proteases to facilitate productive collision. Furthermore, the spatial arrangement of the long GAG chains may “crowd” the extracellular environment and facilitate protein-protein interactions by simply reducing the effective volume available for diffusion. Another possibility is that the variability in pattern of sulfation of GAGs may affect the water balance of the extracellular environment by sequestering solvent that could otherwise abundantly hydrate protein surfaces.

In an article appearing in this issue of the Journal, McGee and Wagner bring attention to the fact that water is available at http://www.atvbaha.org DOI: 10.1161/01.ATV.0000090960.74185.DB
rotic plaques not only deprives the antithrombin pathway of a precious cofactor, but it also reduces the cosolute needed to maintain water activity at the physiological levels. The reduction in CSE increases the free water in the vascular bed and compromises formation of the antithrombin-factor Xa complex. This translates into an increased risk of thrombosis, which is obviously in line with the progression of atherosclerosis.

When the water activity increases due to the drop in versican containing CSE, all interactions linked to water release are weakened, including those that promote coagulation. It is conceivable that the reaction of prothrombin with the prothrombinase complex, the cleavage of fibrinogen by thrombin and fibrin polymerization are also linked to water release. Why is then the reduction of versican containing CSE on atherosclerosis plaques a thrombotic rather than a bleeding risk factor? The answer to this question must await information on the water transfer of other reactions in the coagulation cascade. The number of waters released on formation of the factor Xa-antithrombin-CSE complex measured by McGee and Wagner exceeds by at least one order of magnitude that of the factor Xa-antithrombin-CSE complex measured by McGee and Wagner. Addition of the cosolute indirectly favors the interaction of the two proteins by affecting the water activity. The mechanism depicted in the Figure explains the water-linked enhancement of the antithrombin-factor Xa interaction caused by CS acting as the cosolute (green).

Our current view of blood coagulation is centered on the proteins that define the cascade of enzymatic reactions leading to clot formation. Ca\(^{2+}\) has been included in the list of coagulation factors by virtue of its important role in promoting the assembly of enzyme complexes. The role of other physiologically important variables, like Na\(^+\) and Cl\(^-\), has been more difficult to dissect experimentally and has been overlooked for decades. Cl\(^-\) has a striking inhibitory effect on the structure of the fibrin clot, which has been confused for over 50 years with an ionic strength effect. Likewise, the remarkable procoagulant effect of Na\(^+\) on thrombin function has been unraveled only recently, although the clinical association between hypernatremia and thrombosis has been known for almost 20 years. Almost all of the prothrombin mutations associated with a bleeding phenotype are now known to disrupt the Na\(^+\) site. The role of Na\(^+\) as a coagulation factor is therefore hard to dispute. The results reported by McGee and Wagner come as a timely reminder of the role played by local variables in the control of blood coagulation and how easy it is to overlook them. As for Cl\(^-\) and Na\(^+\), elucidation of the role of water poses challenging experimental tests, but will likewise regale us with new and important insights into how blood clotting proteases work.

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