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.1 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.2,3 In the case of factor Xa, this activation is sufficient for neutralization of enzyme function.2 The heparin-assisted activation of antithrombin is of clinical relevance as heparinoids retain center stage in anticoagulant therapy.3 However, the extracellular environment is of clinical relevance as heparinoids retain center stage in anticoagulant therapy.5 In the case of factor Xa, this activation is sufficient for neutralization of enzyme function.2 The heparin-assisted activation of antithrombin is of clinical relevance as heparinoids retain center stage in anticoagulant therapy.3 However, the extracellular environment is of clinical relevance as heparinoids retain center stage in anticoagulant therapy.5 In the case of factor Xa, this activation is sufficient for neutralization of enzyme function.2 The heparin-assisted activation of antithrombin is of clinical relevance as heparinoids retain center stage in anticoagulant therapy.3 However, the extracellular environment is of clinical relevance as heparinoids retain center stage in anticoagulant therapy.5 In the case of factor Xa, this activation is sufficient for neutralization of enzyme function.2 The heparin-assisted activation of antithrombin is of clinical relevance as heparinoids retain center stage in anticoagulant therapy.3 However, the extracellular environment is of clinical relevance as heparinoids retain center stage in anticoagulant therapy.5 In the case of factor Xa, this activation is sufficient for neutralization of enzyme function.2 The heparin-assisted activation of antithrombin is of clinical relevance as heparinoids retain center stage in anticoagulant therapy.3 However, the extracellular environment is of clinical relevance as heparinoids retain center stage in anticoagulant therapy.5 In the case of factor Xa, this activation is sufficient for neutralization of enzyme function.2 The heparin-assisted activation of antithrombin is of clinical relevance as heparinoids retain center stage in anticoagulant therapy.3 However, the extracellular environment is of clinical relevance as heparinoids retain center stage in anticoagulant therapy.5 In the case of factor Xa, this activation is sufficient for neutralization of enzyme function.2

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 Wagner4 bring attention to the fact that water transfer influences a key regulatory reaction in the blood coagulation cascade. By osmotically stressing the solution, they demonstrate that the oversulfated CS, CSE, present in versican has an anticoagulant activity linked to water transfer. Importantly, the amount of oversulfated versican in advanced type IV atherosclerosis lesions is reduced significantly compared with healthy aorta, presaging an increased thrombogenic risk due to reduction of antithrombin activity. As a result, the reduction of oversulfated versican in atherosclerosis lesions reduces the availability of cofactor for antithrombin activation and, in addition, alters the homeostatic water balance of the extracellular milieu by compromising all interactions linked to water release. An interesting new paradigm emerges from these studies: GAGs may regulate protein-protein interactions in the blood coagulation cascade by altering water homeostasis in the extracellular matrix.

The role of water in macromolecular interactions has long captivated the interest of physical chemists and has helped shape our mechanistic understanding of protein folding, ligand binding and linkage.5 The Figure illustrates how water influences the energetic balance of a protein-protein interaction. When two proteins come together to form a complex, some of the waters on their hydration shell are released into the bulk solvent. The amount of water bound to the complex is less than the sum of the water content of the free proteins, meaning that formation of the complex is linked to a net release of water from the hydration shells to the bulk solvent. This transfer occurs unnoticed under standard investigations of the interaction of the two proteins. To reveal it, one should perturb the water content of the system using a cosolute (eg, polyethylene glycol) that “stresses” the osmotic balance of the solution (Figure). When the cosolute is added to the system at high enough concentrations, the amount of free water available for protein hydration decreases. The cosolute forces the proteins to partially dehydrate and indirectly drives the equilibrium toward formation of the complex that bears a smaller number of bound waters in its hydration shell. This is a consequence of thermodynamic stability and the linkage between water binding and complex formation causes a rebalancing of the equilibrium resulting in an apparent increase in affinity.5 The number of waters exchanged in the formation of the complex can be calculated from measurements of the perturbed equilibrium constant as a function of cosolute concentration.

McGee and Wagner4 bring attention to the fact that water transfer influences a key regulatory reaction in the blood coagulation cascade. By osmotically stressing the solution, they conclude that about 2,000 water molecules must be released when antithrombin inhibits factor Xa in the presence of CSE. This is a massive water exchange that offers plenty of regulatory potential of the antithrombin-factor Xa interaction by any factor that can alter the water activity in the extracellular environment. Because of the large amounts of versican and oversulfated CS present in healthy arteries, it is conceivable that CS itself may regulate water homeostasis in the vascular bed. CS may function as the cosolute in the Figure, in addition to directly promoting antithrombin activation for factor Xa inhibition. Hence, the decrease of versican containing CSE documented in atheroscle-
Schematic representation of the role of water in protein-protein interactions. Two proteins (left) form a complex by releasing the waters (yellow balls) absorbed on their surface of recognition. The water transfer in the reaction affects the energetics of the binding equilibrium. When a cosolute (green) is added to the solution (right), some of the waters (yellow and blue balls) in the bulk solvent are sequestered. Thermodynamic stability drives the equilibrium toward the state with reduced number of water molecules bound, which is the protein-protein complex. 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).

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 Wagner4 exceeds by at least one order of magnitude that measured for typical protein-protein interactions and may reflect the peculiar mechanism of enzyme inactivation by serpins.1,2 Future experiments on the water balance associated with the protease-zymogen interactions in the blood coagulation cascade will be necessary to test the procoagulant role of water suggested by McGee and Wagner.4

Our current view of blood coagulation is centered on the proteins that define the cascade of enzymatic reactions leading to clot formation.6 Ca2+,7 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,7 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,8 although the clinical association between hypernatremia and thrombosis has been known for almost 20 years.9 Almost all of the prothrombin mutations associated with a bleeding phenotype are now known to disrupt the Na+ site.10 The role of Cl− as a coagulation factor is therefore hard to dispute. The results reported by McGee and Wagner4 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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References

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