Structure-function relationships of von Willebrand factor

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Summary

As soon as the endothelium is compromised, platelets are recruited on the exposed subendothelial structures. Initially, VWF is bridging the subendothelial collagen and the platelets, supported by its multiple subunits, each with a binding site for GP Ib and for collagen (A1 and A3 domains). Interestingly, both VWF and its receptor circulate in the bloodstream simultaneously, but they only interact if pathologically high shear forces are present and/or VWF is immobilised. Present theories about the regulation of this interaction hypothesise conformational changes at different scales (affinity or accessibility of the binding site) and/or emphasise the control of multimer size (avidity).

We have investigated the possible regulatory role of the D'D3 region, which is suggested by the fact, that the monoclonal antibody 1C1E7 binds to here, but enhances the GP Ib -- VWF interaction. Cross-blocking studies with monoclonal antibodies revealed the proximity of the D'D3 and A1 domains in solution, but not when immobilized. Ristocetin induced platelet agglutination studies with VWF deletion mutants lacking the D'D3 region demonstrated increased reactivity, suggesting that D'D3 is limiting the accessibility of A1. We have found, that 1C1E7 and the A1 domain has a similar sequence --- possibly binding to D'D3 --- that suggests that 1C1E7 may disrupt the binding of D'D3 and A1 leaving the latter unblocked.

For avidity related questions, we have improved electrophoretic techniques of VWF multimer analysis and developed a new computerized procedure to accurately describe the size of multimers. We have compared the new and present methods, and demonstrated the accuracy and the utility of our methods. Finally, we have established a system, where platelets are substituted with similar sized corpuscules bearing functionally active GP Ibα. This aids the study of large scale conformational changes by modelling the drag forces exerted on VWF by platelets in flow.

Keywords: von Willebrand factor, shear, conformation, VWF multimer analysis
Tárgyszavak: von Willebrand faktor, nyíró erő, konformáció változás, VWF multimer analízis