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Ligand Complexes of von Willebrand Factor Domain A1

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Von Willebrand factor (vWF) is a multimeric glycoprotein which mediates platlet adhesion at the site of vascular damage. VWF is a 250kD protein which forms multimers up to 10MD in size. VWF forms a bridge between collagen of the subendothelium and the platelet glycoprotein 1b complex under the high shear stress conditions of circulating blood. This process is tightly regulated so that vWF does not normally interact with circulating platelets and only does so at sites of vascular damage. VWF consists of four domain types which occur in the order: D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK.
The structure of the A1 domain (residues 475-709) of vWF, which is the focus of this study, has been solved to show that A1 consists of a central hydrophobic parallel β-sheet flanked by 2 amphipathic helices.[1] A disulphide bond (Cys509-Cys695) is present towards the N and C termini. A1 has an overall cuboid shape with distinct positively and negatively charged areas on the surface. The A1 domain has been shown to form complexes with the glycoprotein 1b complex (Gp1b) [2], heparin [3], collagen IV [4] and protein A from *Staphylococcus aureus* [5]. The structure of the A1 domain bound to the concave face of Gp1ba shows a conformational change occurs in the b switch of Gp1bα upon binding to A1. A continuous β sheet is formed between the β3 strand of A1 and the β switch of Gp1bα which is critical to the affinity of this interaction [2]. Heparin has been shown to competitively inhibit Gp1b binding vWF. The heparin binding site of vWF has been isolated to the A1 domain and scanning mutagenesis indicated 642KKKK645 and 569KDRKR573 as potential heparin binding sites.The residues Arg 632 and Lys599 were also shown to be important [3]. The A1 domain of vWF has been shown to bind microfibrillar collagen IV, but not fibrillar collagens which bind the A3 domain of vWF [4]. VWF has also been shown to bind the major surface protein, Protein A, of *S. aureus* [5]. Protein A contains five homologous extracellular domains each of which form a three-helix bundle. ELISA assays have shown that it is mainly the A1 domain of vWF which binds protein A.
We will present data obtained using various techniques, including crystalliastion, nuclear magnetic resonance and isothermal titration calorimetry, which analyse the interactions between domain A1 of vWF and its ligands.

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Tryptophan Rotamers as evidenced by X-ray, Fluorescence and Molecular Dynamics

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We have investigated the native-state dynamics of the 66-residue beta-barrel protein Bc-Csp L66E (PDB code 1HZB [1]), using parallel tempering simulations. Loop fluctuations were identified. Trp rotamer distributions were extracted. From high-temperature simulations, we predicted the lifetimes of the most important rotameric states. The results were compared with dead-end elimination calculations, fluorescence decay experiments and x-ray crystal structures. The results confirm the existence of two Trp conformations as observed with x-ray crystallography and provide an accurate description of the relative weights for this disordered residue.

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