ABSTRACT

Haemostasis is an absolutely essential body defense system for normal life that impedes the disturbance of blood flow and the loss of blood and provides the repair of injured vasculature and tissue. Thrombus generation occurs when a vessel is damaged or inflamed. The complex process of thrombus generation is mediated by platelets, endothelial cells and coagulation factors. At the same time, the fibrinolysis system and physiological inhibitors are activated to inhibit the mechanism of clot generation. These two opposing phenomena contribute to a self-control mechanism of clot growth and thus, the process of thrombus creation is brief.

Von Willebrand factor (vWF) is a big multimeric glycoprotein responsible for stopping bleeding in case of vascular damage. vWF contains several functional domains that are arranged in the order D', D3, A1, A2, A3, D4, B1, B2, B3, C1, C2 and CK. The A1 domain contains a binding site for the platelet receptor GPIbα. GPIbα is essential for platelet adhesion to exposed tissues, where a discontinuity in the vascular endothelium is directing platelet aggregation and thrombus formation. The A2 domain contains a cleavage site specific for ADAMTS13 metalloprotease. This proteolysis is the predominant physiological for normal feedback of vWF and prevention of blood coagulation. The A1 domain binds to platelet receptor GPIbα inhibiting the cleavage of A2, while A2 prevents the formation of the A1-GPIbα complex. Recent data suggested an interaction between A1 and A2 domains, which inhibits the binding to the receptor GPIbα and platelet thrombus growth underscoring the antithrombotic potential. The study of the A1-A2 interaction could help to clarify the mechanism of complex formation and its role in haemostasis reactions.

The human genes encoding A1 and A2 domains were cloned and expressed to a bacterial system and the recombinant A1 and A2 proteins were overexpressed and purified in the absence of post-translational modifications occurring *in vivo*. The secondary structure of each protein and their mixture were analyzed by Circular Dichroism (CD) and the analysis indicated conformational changes. The binding of the two proteins was investigated indirectly by home made ELISA using an antibody against the A2 domain and the thermodynamic characteristics of the complex were studied by Isothermal Calorimetry (ITC). The interaction of A1 and A2 domains was also studied by Fluorescence Spectroscopy obtaining spectra at the wavelength of maximum absorption of tryptophan.

Molecular Dynamic Simulations were used to search for models of the A1-A2 complex and three probable model structures were identified. The critical residues of A2 domain participating to A1-A2 interaction were also located. Some of these residues at the first model were Glu^{1549} and Glu^{1554}, at the second model was Glu^{1640} and at the third model were Glu^{1511}, Glu^{1519}, Glu^{1522} και Asp^{1663}. By Site-Directed Mutagenesis A2 mutant proteins were generated for each model, where the residues Glu^{1549}, Glu^{1640} and Glu^{1511} were converted to alanines. The three A2 mutant proteins were expressed, purified and their binding to the wild type A1 domain was studied by Fluorescence Spectroscopy. This study of the A1-A2 complex characteristics may provide evidence for the establishment of the A2 domain as the lead compound for the design of new and potent antithrombotic factors against the vWF-GPIbα axis.