Disagregin: a platelet aggregation inhibitor from the tick *Ornithodoros moubata*

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Ticks, hematophagous parasites, obtain blood by puncturing the skin of vertebrates. When a blood vessel is intruded, the extrinsic coagulation pathway is activated by excretion of tissue factor (TF) from damaged endothelial cells and the blood coagulation cascade starts to repair the vessel wall. TF forms a complex with Factor VIIa (FVIIa) that activates FX into FXa. Then, FXa, together with calcium, phospholipids and FV, converts prothrombin into thrombin. Subsequently, thrombin cleaves fibrinogen into fibrin and a stable clot is formed. Ticks secrete saliva containing several anticoagulant proteins to continue feeding on the host. Here, we studied a protein consisting of 60 amino acids with proposed anticoagulant properties: Disagregin[1], derived from salivary glands of the *Ornithodoros moubata*, a soft tick living in parts of Africa.
It is assumed that Disagregin blocks signaling of αIIbβ3 integrin receptor on platelets, resulting in less platelet aggregation and reduced fibrin levels. Commonly, αIIbβ3 antagonists contain an Arg-Gly-Asp (RGD) sequence for specific binding. In contrast, Disagregin contains an Arg-Glu-Asp (RED) sequence, suggesting a different mode of inhibitory action in platelet aggregation.

Aims

The aim of the study was to investigate the effect of Disagregin on platelet aggregation. Additionally, the purpose was to investigate whether the RED sequence is involved in αIIbβ3 integrin receptor binding.

Methods

Disagregin was synthesized by solid-phase peptide synthesis (SPPS) using tert-butyloxycarbonyl (Boc) chemistry and native chemical ligation (NCL). Light Transmission Aggregation (LTA) was performed in platelet-rich plasma (PRP) from healthy volunteers to assess the inhibitory effect of Disagregin.
To investigate the possible role of the RED sequence, an E15G Disagregin analogue (RGD) was synthesized and both proteins were studied by NMR experiments Next, flow cytometry was performed using Disagregin and the RGD analogue.

Results / Conclusions

Disagregin inhibited adenosinediphosphate (ADP)- and collagen-activated platelet aggregation in plasma with an IC50 = 99.1 nM and 63.8 nM, respectively. Interestingly, from a platelet extracellular vesicle (EV) release assay it appeared that Disagregin also reduced EV- release from Convulxin-activated platelets.
NMR data showed that E15G substitution does not lead to a substantial difference in overall protein folding compared to native Disagregin. Both proteins adopted a Bovine Pancreatic Trypsin Inhibitor (BPTI)-type structural fold in solution. Both Disagregin and the E15G analogue displayed two major different conformations in fast exchange (milliseconds time scale), which internal conformational changes are located relatively distant from the RGD loop. At the local structural level of the RGD loop, differences in chemical shift values suggest the presence of a salt-bridge between the end group of R41 and the side chain carboxylic group E15 in Disagregin, while this interaction appears not present in the E15G analogue.
Tick Disagregin seems to have evolved passed classical RGD binding and now uses an antagonistic style of binding.

References:

1. Karczewski J, Endris R, Connolly TM. Disagregin is a fibrinogen receptor antagonist lacking the Arg-Gly-Asp sequence from the tick, Ornithodoros moubata. J Biol Chem 1994; 269(9): 6702-8.