A putative platelet aggregation inhibitor from *Haementeria depressa* leech: Cloning and Expression

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The saliva of haematophage animals has substances that maintain the blood fluidity during the feeding process, including molecules affecting haemostatic processes. Our group has recently identified several compounds and determined the profile of transcripts and proteins from *H.depressa* leech salivary complex through biochemistry, transcriptomic and proteomic analysis. In this tissue were detected some clones similar to an inhibitor of collagen-induced platelet aggregation from *H.officinallis* leech (LAPP). The purpose of this work is to clone a transcript of salivary complex from the *H.depressa* cDNA library which presented identity to LAPP and to express and purify the recombinant protein for future comparative studies.

Among the isoforms of the LAPP_like produced by the *H.depressa* leech salivary complexes, we decided initially to study that one presenting the higher similarity to this inhibitor, this transcript (L02F02_pGEM11Zf) was cloned in a expression vector (pAE) between the *Bam*H I and *Hin*d III sites and its nucleotide sequence was confirmed showing 93% of similarity with LAPP sequence, all conserved Cys residues and the 3D structure prediction also showed homology to that inhibitor. The recombinant protein was purified by Ni-Sepharose chromatography from the expression in *E.coli* (BL21DE3) soluble fraction and analyzed by SDS-PAGE. Further, the N-terminal sequencing will also be confirmed.

The *H.depressa* leech produces some interest compounds and among then, in this work, we were able to express and purify a putative recombinant platelet aggregation inhibitor and we intend to characterize this new compound by comparative analysis with LAPP.

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