

Adhesives properties of a hemolin from *Lonomia obliqua* caterpillar bristles and mechanisms of activation of human factor X

Flores, M.P.A., Ramos, O.H., Chudzinski-Tavassi, A. M.
Laboratório de Bioquímica e Biofísica, Instituto Butantan, São Paulo, Brazil

A 45 kDa protein named Losac from *L. obliqua* bristles was reported as the first factor X (FX) activator isolated from a lepidopter. Multiple comparison of the deduced amino acid sequence revealed variable percentages of identity with cell adhesion molecules involved in development of the nervous system: HcHemolin (76%), Neuroglian (34%) from *D. melanogaster* and human L1-NCAM (26%). We studied these two properties. It was found that Losac exerts its function through a serine protease-like mechanism and also induces a proliferative and anti-apoptotic effect on HUVECs. The cDNA corresponding to mature Losac was sub-cloned into the pAE vector and used to transform *E. coli* BL21(DE3) cells. The recombinant protein was expressed in fusion to a minimal Nterminal His6-tag as a 48.6 kDa protein. rLosac is composed by four Ig domains. Based on chromogenic assays and FX fragmentation analysis by SDS-PAGE, rLosac was able to activate the FX. The fragmentation pattern observed is similar to that generated by RVV-X. Moreover, Losac was capable to cleavage the gamma-carboxyglutamic acid domainless FXa (des-FXa) which indicates that Losac binds to FX in a different mode that of the RVV-X/FX interaction. On the other hand, preliminary studies using a mouse thymus endothelial cell line (tEnd) treated with rLosac showed a protective effect in starvation conditions as measure by MTT method, in agreement with previous reports. The characteristic of this hemolin pointed a strong candidate that could explain the effect observed on immune responses in lepidopters. Further studies will be carried-out aiming a closer look at molecular features implicated in the FX activation activity.

Supported by Fapesp.