MUTANTS OF DISBA-01, A RGD-DISINTEGRIN WITH POTENTIAL ANTITHROMBOTIC AND ANTIMETASTATIC ACTIVITIES

Gonçalves, D. F. C.[1]; Ramos, O.H.P.[2].;Selistre-de- Araújo, H. S[3]; Sousa, D.H. F.[1]

[1] Departamento de Química, UFSCAR, Brasil.;
[2] Laboratório de Bioquímica e Biofísica - Instituto Butantan, São Paulo;
[3]Departamento de Ciências Fisiológicas, UFSCAR, Brasil.

DisBa-01, a recombinant RGD-disintegrin still not isolated from *B. alternatus* venom, exhibits high affinity for $\alpha_{IIb}ß_3$ integrin, contributing for the inhibition of platelet aggregation (Ramos, 2005). DisBa-01 possesses also antithrombotic and antimetatatic activities *in vivo* (Ramos, 2005). In order to obtain shorter molecules maintaining activities of DisBa-01 two mutants are in study. Molecules called DisBa Δ(1-32) and DisBa Δ(1-36) should have 32 and 36 residues unless DisBa-01, respectively, in the N-terminal region. Oligonucleotides were constructed and the DNA's were amplified by PCR using pET28a-DisBa-01 vector as the template. PCR products were cloned into expression vector pET32a. Sequence analysis showed that the inserted target genes and its reading frames were completely correct. The recombinant plasmids were introduced in BL21(DE3) *E.coli* strain. The recombinant plasmids were induced with IPTG to express the proteins in fusion with thioredoxin. Proteins were expressed in a soluble form and with molecular mass coincident with prediction. Fusion proteins were purified by affinity chromatography in nickel resin and cleaved with enterokinase enzyme. Purification and cleavage steps were analyzed by SDS-PAGE and immunoblotting reaction using anti-Echistatin antibody. Purification experiments of DisBa Δ(1-32) and DisBa Δ(1-36) proteins are currently being realized. Proteins will be tested in platelets aggregation and cell adhesion experiments. Supported by Fapesp and CNPq