HETEROLOGOUS EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF AN ANTIOPHIDIC PROTEIN FROM *DIDELPHIS MARSUPIALIS*

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In a previous work we cloned the cDNA coding for DM43, a snake venom metalloproteinase inhibitor isolated from the serum of the opossum *Didelphis marsupialis*, and showed that its deduced amino acid sequence was homologous both to oprin (an antihaemorragic protein isolated from *Didelphis virginiana* serum) and to alpha1B-glycoprotein, a member of the immunoglobulin supergene family. In the present study, we have used specific oligonucleotides to clone the cDNAs coding for the first (D1) or the second (D2) domains of DM43 in the prokaryotic expression plasmid pET102D/TOPO (Invitrogen). The identities of the cloned cDNAs were confirmed by DNA sequencing. To increase the solubility, the recombinant eukaryotic proteins were expressed as a fusion protein of D1 (or D2) and thioredoxin in BL21 Star (DE3) competent *E. coli* cells. The recombinant proteins were isolated by HisTrap FF crude Kit (GE Healthcare) and their partial amino acid sequences were confirmed by mass spectrometry. Preliminary results indicate that both D1 or D2 are able to inhibit the fibrinogenolytic activity of snake venom metalloproteinases, meaning that these domains fullfill the minimum structural requirements enabling the inhibitor to function. Attempts to clone the third domain of DM43 and the five domains of DM64 (an antimyotoxic protein) are still in progress.

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