MAPPING THE STRUCTURAL REQUIREMENTS OF BIOLOGICAL ACTIVITY IN THE ANTIOPHIDIC PROTEIN DM43

M. R. O. Trugilho[1], S. L. G. Rocha[1,2], I. R. León[1], G. B. Domont[2], J. Perales[1], A. G. C. Neves-Ferreira[1]
[1]Lab. Toxinologia, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, RJ, Brazil; [2]Lab. Química de Proteínas/ Unidade Proteômica, Dept. Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil; Email: monique@ioc.fiocruz.br

DM43 isolated from *Didelphis aurita* serum inhibits the hemorrhagic effect and the proteolytic activity of snake venom metalloproteinases (SVMPs). Upon SVMP addition, DM43 homodimer dissociates and one subunit binds to one SVMP. The 43 kDa monomer is composed of three immunoglobulin-like domains, two of which are glycosylated. To investigate the structure-function relationship in DM43, its domains were separately cloned into pET102D/TOPO vector, in fusion with thioredoxin. The third domain of DM43 was also cloned into pET101D/TOPO vector (without thioredoxin). All domains were expressed in BL21 Star (DE3) competent *E. coli* cells as inclusion bodies. After purification and dialysis refolding, CD spectra in the far UV region indicated the presence of folded recombinants presenting both alpha-helix and beta-sheet. Their identities were confirmed by polyclonal antibodies against serum DM43 and by MS/MS. None of the domains, either isolated or in combination, were able to inhibit the proteolytic activity of jararhagin. Thioredoxin-fused domains were readily digested by this SVMP. These results may indicate that glycosylation is functionally important for DM43 and/or that a multidomain native conformation is necessary for the biological activity of the inhibitor. To better understand its structural requirements, serum DM43 was studied after proteolytic degradation. Under native conditions, limited digestion of DM43 by trypsin or chymotrypsin generated various fragments, but no active peptide against SVMP was obtained. Extensive digestion of denatured DM43 by Lys-C endopeptidase produced smaller peptides. Even though not neutralizing the proteolytic activity of jararhagin, three DM43 Lys-C peptides were able to bind to SVMP. After sequencing by MS/MS, each peptide was located in one DM43 domain. Taken together, these results seem to reinforce the participation of all three domains of DM43 in the interaction with SVMPs.

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