

IDENTIFICATION, SYNTHESIS AND PURIFICATION OF A DISCONTINUOUS DIEPITOPE FROM THE ANATOXIN TsNTxP OF *Tityus serrulatus* SCORPION VENOM

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A non-toxic but immunogenic protein, TsNTxP, was purified from *Tityus serrulatus* venom, the principal species related to scorpionism in Brazil. A serum raised against this non-toxic protein was capable to neutralize *in vitro* and *in vivo* the main toxins of scorpion venom. In this work we have used the Spot-method of multiple peptide synthesis to prepare continuous and discontinuous epitopes in the TsNTxP sequence to later produce more potent and specific antivenoms. Twenty overlapping continuous pentadecapeptides frameshifted by two residues (X_{15}) and 153 discontinuous octadecapeptides ($(X)_8$ -Gly-Gly- $(X)_8$) covering the complete amino acid sequence of TsNTxP were synthesized on cellulose membranes. At the end of the synthesis, the peptides remain covalently bound to the membrane and are simultaneously assayed for antibody reactivity. Anti-TsNTxP antibodies bound to the membrane peptides, revealed one diepitope containing the N- and C-terminus regions (GREGYPADGGGLPDSVKI) corresponding to residues 1-8 and 47-54 of TsNTxP. This diepitope was synthesized by the F-moc technique and purified in a C18 semi-preparative column using the AKTA system. The purified peak had its mass confirmed by mass spectrometry ES-TOF.

Key words: SPOT, peptide synthesis, *Tityus serrulatus*, TsNTxP, diepitope
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