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Structural and functional characterization of Latrodectins I and II, low molecular weight peptidic components from *Latrodectus hesperus* venom

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Latrodectins (Ltds) are low molecular weight proteins of around 70 amino acids (6-8kDa), with an acidic isoelectric point and a high content of disulfide bonds that have been detected within black widow spider venom (*Latrodectus* spp). They have been isolated in small amounts from the venomous cocktail of black widow species but have not been characterized in deep detail. They usually appear as an omnipresent component in latrotoxin (LTXs) preparations. These LTXs are the most characteristic high molecular weight proteins (110-140kDa) within *Latrodectus spp* venom, whose toxic activity relies in the formation of pores through presynaptic neural membranes. However, Ltds natural biological function is not yet known. The scarce available data suggest that they are essential to increase the neurotoxic activity of LTXs by increasing their binding affinity for the membrane. The association between these two groups of proteins (Ltds and LTXs) has driven some authors to propose their mutual assembly into a *latrotoxin-macromolecular-complex*. In order to contribute to sort out the specific role of Ltds in black widow spider venom, we have cloned the highly expressed LtdI and II proteins from *L. hesperus* in a suitable vector for their production in the yeast *P. pastoris*. The proteins were successfully purified to homogeneity in milligram amounts through several chromatographic steps. They did not show neither antimicrobial nor antifungal activities. Their six cysteine residues were forming disulfide bonds, as supported by mass spectrometry data. Circular dichroism characterization was consistent with thermostable and fully folded globular peptides, showing high α-helical content. Production of Ltds labelled with ^{15}N and ^{13}C isotopes allowed using NMR to determine their three-dimensional structures with atomic resolution.

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