STRUCTURAL STUDIES ON JABURETOX: AN INTRINSICALLY DISORDERED POLYPEPTIDE DERIVED FROM JACKBEAN (Canavalia ensiformis) UREASE

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Ureases are metalloenzymes that catalyze the hydrolysis of urea. These proteins display biological properties unrelated to their enzymatic activity, such as antifungal and insecticidal activities. The toxicity of urease against insects involves its hydrolysis by insect digestive cathepsins and the release of an entomotoxic internal polypeptide of 91 amino acid residues. Jaburetox is a recombinant version of this polypeptide expressed in Escherichia coli, which besides of a broad spectrum of activity against insects, is also fungitoxic. Jaburetox was characterized as an Intrinsically Disordered Protein, whose structure elucidated by Nuclear Magnetic Resonance (NMR), presents an $\alpha$-helix motif in the N-terminal region and two turn-like structures, one in the central region and another near the C-terminal portion. In order to understand Jaburetox’s entomo- and fungitoxic modes of action, here our objectives were to study the structural properties of Jaburetox in presence of lipids mimicking membranes. Three different membrane models, large unilamellar vesicles (LUVs), bicelles and SDS micelles, were used to study changes in secondary structure of Jaburetox, applying CD and intrinsic fluorescence techniques. NMR was performed to obtain individual amino acids assignments in comparison to those of native Jaburetox, as a way to detect interaction with membranes. Fluorescence and CD experiments revealed a tendency of change in Jaburetox’s secondary structure in the presence of LUVs and bicelles and a significant change in presence of SDS micelles. These changes were confirmed by NMR using the superposition of two HSQC spectra. Even though some significant variations were detected at the C-terminal portion, the unfolded structure was maintained. These results suggest that the interaction of Jaburetox depends on the relative concentrations of lipids and the polypeptide and the nature of lipid membranes. Future work will include studies of Jaburetox interaction with lipids extracted from fungi and insect membranes.

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