INTERACTION OF BOWMAN-BIRK INHIBITOR PEPTIDES WITH LIPID BILAYER

Oliveira, S. A., Pinheiro, C. G. A., Joanitti, G. A. and Freitas S. M.
Universidade de Brasília, Depto de Biologia Celular, Brasília, Brazil. Email: sandrieleaires@gmail.com; nina@unb.br.

Protease inhibitors have potential for regulation of proteolytic activities in specific pathways. Bowman-Birk inhibitors (BBIs) are small proteins with seven disulfide bonds able to inhibit trypsin and chymotrypsin in monocotyledonous plants. In this work, we present the inhibitory activity of the peptides (pep1 and pep2) derived from a BBI and the fluorescence spectroscopy analysis of the interaction of the peptides with liposomes. Peptides were synthesized and their inhibitory activities were assayed by evaluating the serinoproteases activities using the chromogenic substrates. The enzymatic hydrolysis of the substrates, in the presence of the peptides, was evaluated by recording the absorbance at 410 nm. The residual activities of the enzymes were estimated considering the free enzyme activity to be 100%. Inhibition curves were obtained by plotting decreasing relative activities of the serinoproteases versus peptide concentration. Dissociation constants of the enzyme-peptide complexes, K_i, were determined from fitted inhibition curves using the Grafit program. A magnitude reduction in the order of ~ ten of the affinity to serinoproteases for both peptides (pep1, Ki = 7.9×10^{-6} M; pep2, Ki = 5.8×10^{-5} M) was observed compared with original BBI. The liposomes encapsulated with fluorescein were prepared by extrusion methodology and purified by molecular exclusion chromatography. The association of the peptides (50 to 500 µM) with liposomes was monitored by fluorescence emission spectra of leakage fluorescein from 450 to 650 nm (λ_{exc}=480 nm). The increase fluorescence intensity of the recorded emission bands in the presence of the peptides was less than 30%, indicating a low association of these molecules with membrane model.

Key words: Bowman-Birk, peptide-membrane interaction, protease inhibitor.

Supported by CNPq, CAPES, FAPDF