Fluorescence-quenching-resolved Spectroscopy of the *Schizolobium parahyba* Inhibitor in Complex with a-Chymotrypsin

Andrade1, E. L., Silva[1], A. J., Teles, R. C. L., Barbosa[2], J. A. R. G. and Freitas[1], S. M.

[1]*Department of Cell Biology, University of Brasília, Brasília-DF.* [2]Brazilian Synchrotron Light Laboratory, Campinas-SP. Email: adelsonjoel@gmail.com

The *S. parahyba* chymotrypsin inhibitor (SPCI) is a Kunitz type inhibitor suppressing the chymotrypsin activity through the stable binary complex. The fluorescence quenching of tryptophan residues can provide important information concerning the structure and dynamics of proteins in solution. The goal of this work is the resolution of heterogeneous fluorescence of multitryptophan-containing SPCI-chymotrypsin complex by fluorescence-quenching-resolved spectra (FQRS) method and fluorescence lifetime spectroscopy. The parameters were obtained by non-linear-squares analysis of Stern-Volmer plots using the iodide as a quencher. The FQRS method showed that the fluorescence emission of complex can be resolved into two spectral components (λ_{max} = 332 and 325 nm). The redder component is exposed (Stern-Volmer constant, k_{SV1}, of 10.48 M^{-1}) to the solvent and participates with 37% of the total fluorescence emission. The second is less accessible (k_{SV2} = 1.11 M^{-1}) and participates with 63%. The values of fluorescence lifetime obtained by the time-resolved method were: binary complex (τ_1 = 0.88 ns; 325 nm and τ_2 = 4.15 ns; 332 nm), α-chymotrypsin (τ_1 = 0.150 ns; 325 nm, τ_2 = 1.45 ns; 332 nm and τ_3 = 4.2 ns; 343 nm) and SPCI (τ_1 = 0.67 ns and τ_2 = 4.98 ns). These data show that SPCI-chymotrypsin interaction promotes conformational changes in the tryptophan environments of the enzyme as indicated by reducing from three to two spectral components. Moreover, these results suggest that the hydrophobic tryptophan environments were preserved, whereas exposed tryptophans reduced their accessibility to the solvent.

Key words: Kunitz inhibitor, fluorescence-quenching-revolved spectra.

Supported by CAPES, FINATEC, LNLS, FINEP and FAPDF.