STRUCTURAL ANALYSIS OF A BOWMAN-BIRK INHIBITOR IN TERNARY COMPLEX WITH TRYPSIN AND CHYMOTRYPSIN

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The black-eyed pea trypsin/chymotrypsin inhibitor (BTCI) is a Bowman-Birk type proteinase inhibitor that presents two different and independent reactive sites for trypsin and chymotrypsin. In this work, we present the three-dimensional structure of BTCI in ternary complex with trypsin and a-chymotrypsin and conformational analysis of BTCI in free form and in binary/ternary complex. Crystallization was achieved by screening with commercially available crystallization kits using the sitting-drop vapor-diffusion method. The X-ray diffraction datasets were collected at the MX1-D03B beamline of the Brazilian Synchrotron Light Laboratory (LNLS). The chymotrypsin-BTCI-trypsin ternary complex crystallized in space group *P1* with unit-cell parameters a = 49.48, b = 54.57 and c = 69.29 Å and a = 67.28, ß = 71.04 and γ = 73.55°. The crystal diffracted to a resolution of 1.70 Å and the refinement converged to a final working R_{cryst} and R_{free} factors of 0.218 and 0.171, respectively. The final quality of the model was checked by PROCHECK. In addition, the conformational changes of the BTCI complex were analyzed in free form and in association with chymotrypsin and/or trypsin using fluorescence lifetime technique (frequency domain). Fluorescence lifetime values are related with different substate and with conformational changes occurring during the association of the molecules.

Key words: BTCI, Bowman-Birk, proteinase inhibitor, crystallization, three-dimensional structure, fluorescence lifetime

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