HIGH PRESSURE REFOLDING OF BOTHROPS TOXIN I FROM INCLUSION BODIES IN *ESCHERICHIA COLI*

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Expression of recombinant proteins as inclusion bodies in *Escherichia coli* is one of the most efficient ways to produce recombinant proteins, as long as the inclusion body protein can be successfully refolded. High hydrostatic pressure (HHP) is emerging as a powerful tool to improve renaturation yields of renaturation of aggregated proteins. High hydrostatic pressure modulates protein–protein and protein–solvent interactions through volume changes. Protein states with lower volumes due to lower cavity spaces are favored by higher pressure. Moderate pressures (100–300 MPa) have been reported to be effective in dissociating protein oligomers and aggregates. Thus, under pressure, intermolecular hydrophobic interactions are disrupted, allowing the use of lower levels of chaotropic chemicals for aggregate dissolution. Bothrops toxin I (BthTX-I) is a Lys-49-fosfolipase A2 from the venom of *B. jaracussu*, whose molecular weight is 13,7kDa and 121 aminoacids, its PI is 8,2 Bth TX-I has 7 disulfide bridges and secondary structure formed basically for alfa helix. The present work aims the refolding of recombinant Bothrops toxin I expressed in *Escherichia coli* as inclusion bodies using HHP. In the current study we submitted inclusion bodies for 16 hs to HHP (3000psi) varying the redox shuffling agent, levels of guanidine hydrochloride and other additives in the refolding buffer. The best condition for obtainment of soluble Bothrops toxin I was 2 M guanidine hydrochloride, glutathione oxidized (GSH) and reduced (GSSG) with a proportion of 1:2 and concentration of 3 mM. HHP was a very efficient tool in the refolding of Bothrops toxin I from inclusion bodies, allowing production of a soluble protein.

Key words: Bothrops toxin I, refolding, inclusion bodies.
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