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HIGH- PRESSURE REFOLDING OF AGGREGATED FUSION PROTEINS : ENDOSTATIN-PROAPOPTOTIC PEPTIDES WITH ENHANCED ANTIANGIOGENIC ACTIVITY

Chura-Chambi, R.M.[1]; Affonso, R.[1], Gênova, L. and Morganti, L.[1]

[1]Centro de Biotecnologia, Instituto de Pesquisas Energéticas e Nucleares, CNEN/SP, Brasil
email: rosachura@yahoo.com

Endostatin is a specific inhibitor of endothelial cell proliferation, migration and a potent angiogenesis inhibitor. The obtainment of bioactive recombinant endostatin (ES), from *E. coli* inclusion bodies (IB) using traditional refolding processes has proved to be challenging, because ES solubilized under denaturing condition invariably aggregates when the chaotropic agent are withdrawn. This aggregation can lead to loss of biological activity and can be harmful for therapeutic uses. Recent studies have shown that high hydrostatic pressure (HHP) is an attractive alternative to traditional methods of protein disaggregation and refolding. HHP induces disruption of protein aggregates and subsequent refolding in a single process step, without the need for high concentrations of chaotropic agents, improving renaturation yields with reduced chemical costs. HHP facilitates the preparation of proteins for structural and functional studies and also for applications in the biotechnology industry. In the present study we report the expression and refolding by HHP of fusion proteins composed of two functional domains: endostatin, which presents affinity for activated endothelial cells, and a second domain composed by a short peptide, corresponding to the minimal sequence required to promote apoptosis. The fusion proteins, expressed in *E. coli* BL21(DE3) in aggregated and insoluble cytoplasmic inclusion bodies, were submitted to HHP (200MPa or 2kbar) combined with nondenaturing concentrations of guanidine hydrochloride (GdnHCl) and a redox pair, in order to refold. All proteins were successfully refolded and characterized by SDS-PAGE and western blotting. *In vitro* cell proliferation assays on C-PAE cells demonstrated that the refolded proteins were fully active. The fusion proteins demonstrated to be more potent than wt endostatin, with 38.1% increased growth inhibition effect on the proliferating C-PAE endothelial cells. We demonstrated that high hydrostatic pressure can successfully reconstitute endostatin and its fusion proteins, obtaining soluble and stable proteins with biological activity from insoluble aggregates. The biological assay has shown that the fusion proteins presented enhanced antiangiogenic activity when compared to the wild type endostatin.