EXPRESSION, PURIFICATION AND CRYSTALLIZATION TRIALS OF MESOTHELIN

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Mesothelin is a glycoprotein present in normal mesothelial cells and in a variety of tumor cells, including mesothelioma, ovarian cancer and pancreatic cancer. It is expressed as a 69-kDa precursor protein that is cleaved in megakaryocyte potentiating factor (31-kDa) and mesothelin (40-kDa). The first is released to the medium and the second is glycosylphosphatidylinositol-ancored to the membrane. Mesothelin is a differentiation antigen considered a promising candidate for target therapy of cancer and a potential tumor marker. Even though mesothelin's biological function is not known yet, anti-mesothelin immunotoxins with antitumor activity have been developed and DNA vaccines studies encoding mesothelin peptides have been carried out. The gene region corresponding to mesothelin was PCR amplified from a human leukocyte library and cloned into modified pET28a vector. Mesothelin (E296-G580) was expressed in *Escherichia coli* BL21(DE3) with a N-terminal His_6-tag. Only small amounts of protein remained soluble while the majority was present in the inclusion bodies. The soluble form was purified by affinity chromatography and gel filtration. The insoluble form was denatured, purified by affinity chromatography and refolded by dialysis. Biophysical characterization was performed for both samples, showing similar results and indicating that the refolded protein is in its native conformation. The refolded protein has been used for crystallization trials after the removal of the His_6-tag with TEV protease.

Mesothelin, refolding, biophysical characterization, crystallization.

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