Abstract

Peritoneal adhesions are pathological fibrous structures that connect together organ and membrane at sites where the peritoneum is damaged. Post-operative adhesions forming after abdominal surgery have a very high occurrence and can develop painful and morbid complications. Numerous strategies aiming at adhesion prevention were developed, but no clinical treatment resulted in a complete inhibition of adhesion formation. One of the most investigated targets is the deposition and persistence of fibrin at injury sites, which was shown to be an important triggering mechanism for the maturation of adhesion into permanent fibrous structures. The fibrinolytic system is strongly involved in persistence of fibrin in the peritoneum, and plasminogen activators are its main regulators. Tissue-type plasminogen activator (tPA) is a clinically used fibrinolytic agent that showed some of the most efficient effects on adhesion prevention.

The fibrin A and B knob sequences are peptides found at the N-terminus of the α and β chains of fibrinogen. Upon exposure, knob sequences trigger the polymerization of fibrin monomers. A variant of the A-knob sequence, GPRP, was shown to inhibit fibrin polymerization efficiently, when present as a free peptide.

The aim of this project is to design and produce a chimeric protein consisting of a domain-deleted variant of tPA with a GPRP knob sequence fused to its N-terminus. The hypothesis is that the addition of the knob sequence on tPA will result in an enhanced therapeutic potential for adhesion prevention, with a synergic effect on fibrin targeting and degradation.

Cloning and expression of the chimeric protein in *E.Coli* resulted in the formation of insoluble inactive aggregates called inclusion bodies. A renaturation procedure was design to recover enzymatically active proteins from inclusion bodies. This process involved the solubilization of insoluble aggregates in high concentrations of a chaotrope, the refolding of proteins by dilution in an optimized renaturation buffer, and the purification of protein by a chromatographic technique. The resulting chimeric protein showed a high enzymatic activity, thus validating the production process.