157f Development and in Vitro Evaluation of a Peg-Insulin Conjugate Protein for the Treatment of Diabetes Mellitus

Anthony D. Tuesca and Anthony M. Lowman

In recent years, a significant number of therapeutic proteins and peptides have been developed for the treatment of diseases. A vast majority has significantly lower stability than other small molecule therapeutics and requires delivery directly to the bloodstream. In the case of diabetes, insulin must be delivered subcutaneously up to four times daily. Such a demanding dosing regimen often increases the amount of non-compliance in diabetic patients. The oral delivery of insulin would present a more convenient and less stressful method of delivery. However, it continues to be an elusive goal primarily due to its proteolytic degradation in the upper digestive system.

One proposed mechanism for the oral delivery of insulin is through the use of a pH responsive copolymer hydrogel of poly(ethylene glycol) (PEG) chains grafted on poly(methacrylic acid) (PMAA), designated P(MAA-g-EG). Insulin is sequestered into the polymer network through favorable thermodynamic interactions. In acidic environments the copolymer collapses due to hydrogen bonds between the protonated PMAA and pendant PEG chains. The collapsed hydrogel prevents insulin from diffusing out of the network and degradative enzymes from diffusing in. This collapsed hydrogel has been shown to effectively protect insulin through simulated gastric fluid in vitro. In neutral environments the hydrogel swells due to ionic repulsion of the negatively charged, deprotonated PMAA chains. Once swollen, insulin can easily diffuse out of the polymer. Our lab has demonstrated efficient loading of biologically active insulin (>90%). Additionally, the rate of release of insulin from the hydrogel was very low in acidic media (pH 2.2) and increased greatly in less acidic environments similar to that of the lower GI tract (pH 6.4). This polymer could potentially allow insulin to be carried through the stomach and upper intestines, where enzyme degradation occurs most rapidly, then release it in the intestines where it can be absorbed. This mechanism has been shown to effectively reduce blood glucose levels and increase plasma insulin levels in rats in vivo.

A major limitation of the P(MAA-g-EG) hydrogel mechanism is that once released, the transport of insulin across the epithelial wall of the intestine into the blood is very slow. There is still enough enzyme activity in the lower intestine that if transport does not occur quickly, a significant amount will be digested or cleared, reducing the bioavailability of the insulin dosage. In order to cross the intestinal wall, insulin must adhere to a mucus layer on the apical epithelium, then be transported via a trans-cellular (active transport) or para-cellular (tight junctions) mechanism. The covalent attachment of PEG to insulin, or PEGylation, has been shown to reduce enzymatic recognition of insulin, clearance by the immune system, and the immunogenic response properties by reducing self-association. Further, PEG has been shown to improve the mucoadhesion of materials through hydrophobic interactions. PEGylation of insulin may provide an increased circulation time in the lower intestine as well as increased transport across the epithelial wall due to high local concentrations at the mucus layer.

In this research, the conjugation of PEG to insulin is performed. Characterization of each step of the process is performed using differential scanning calorimetry (DSC), high performance liquid chromatography (HPLC), and electrospray mass spectrometry (MS). The samples are purified through dialysis and cation exchange chromatography. The bioactivity of PEGylated insulin is determined using ELISA. The in vitro uptake and release of the PEGylated insulin from various different P(MAA-g-EG) hydrogels is also performed. The hydrogels are prepared by free radical solution UV-polymerization under varying reaction environments to control the polymer network characteristics. These results are compared with those of native bovine insulin. Future work will involve in vivo studies of the PEGylated insulin loaded into the hydrogel in rats.