Protein-polymer conjugates as therapeutics

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ABSTRACT SUMMARY

The targeted delivery of therapeutic agents for diseases such as cancer is challenging. Many well-described systems within the literature use targeting ligands conjugated to synthetic polymers. Here, we explore how, through controlled radical polymerization, the site-specific conjugation of synthetic polymers to known residues of proteins can allow us to develop protein-polymer conjugates with potential use in drug delivery applications. The synthesis of the protein-polymer conjugates was verified using various techniques such as gel electrophoresis and matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) whereas, the biophysical evaluation of the architectures formed by these conjugates was conducted with techniques such as atomic force microscopy (AFM) and transmission electron microscopy (TEM).

INTRODUCTION

The concept of using protein-polymer assemblies as drug delivery systems has existed for many years and has many potential advantages¹. In the context of diseases such as cancer, the use of proteins as targeting ligands provides enhanced specificity, which can translate into reduced adverse effects and greater therapeutic efficacy. Certain receptors are known to be over expressed on the surface of cancer cells and thus have gained significant interest for use as target sites. However, in order for a protein-polymer assembly to be effective for drug delivery it must retain its targeting ability post conjugation to a synthetic polymer. Previous studies have achieved the efficient synthesis of protein-polymer conjugates by conjugation to random amine residues on the protein². Although, this may provide high conjugation efficiency, there are several disadvantages, including the lack of conjugative control and the reproducibility of reactions. This, in turn, can lead to a reduction in the biological function of the ligand, thus reducing its targeting capacity.

Since the development of recombinant protein technologies, site-specific conjugation has become more common practice. The ability to engineer specific sites on proteins for conjugation means that these ligands have the potential to overcome issues such as loss in biological activity³. Herein, we have used atom-transfer radical polymerization (ATRP) as a technique to graft our polymers from the protein. We explore how thiol conjugation chemistries can be used for the attachment of polymer to a single free cysteine residue of a protein.

RESULTS AND DISCUSSION

ATRP initiator was synthesised with thiol end-group functionalities for subsequent site-specific conjugation. An example of their use in subsequent macroinitiator synthesis is as follows: initiator (30 mg, 0.0568 mmol) was dissolved in 15.0 mL of degassed phosphate buffered saline (PBS) (0.5 mM, pH 7.4) solution. Protein (0.0011 mmol, 75 mg) was added drop wise to this solution and the reaction left stirring at room temperature overnight. The macroinitiator was subsequently used for the Cu (II) Br₂/TPMA mediated ATRP polymerisation of poly (ethylene glycol methacrylate) based monomers (PEGMA). Briefly, macroinitiator (20 mg, 0.0003 mmol), PEGMA-246 (74 mg, 0.30 mmol), Cu (II) Br₂ (0.33 mg, 0.0015 mmol) and TPMA (0.43 mg, 0.0015 mmol) were dissolved in 300 µL PBS (pH 7.4). The mixture was placed on an ice bath and bubbled with argon for 15 minutes after which ascorbic acid (0.039 mg, 0.0002 mmol) was added to initiate the polymerisation. The reaction was stopped after 16 hrs and purified through dialysis membrane (MWCO 6-8,000) against deionised water. The macroinitiator was characterised using MALDI-TOF and high performance liquid chromatography (HPLC) where the molecular weight (MW) of native protein was used for comparison purposes. Synthesis of protein-polymer conjugates was confirmed using 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Ellmans assay and pyridyl disulphide assay. Critical micelle concentration (CMC) studies were performed using pyrene as a fluorescent probe. The hydrodynamic size of formed micelles was studied using dynamic light scattering (DLS), AFM and TEM.

EXPERIMENTAL METHODS

Polymer assembly was made with thiol end-group functionalities for subsequent site-specific conjugation. An example of their use in subsequent macroinitiator synthesis is as follows: initiator (30 mg, 0.0568 mmol) was dissolved in 15.0 mL of degassed phosphate buffered saline (PBS) (0.5 mM, pH 7.4) solution. Protein (0.0011 mmol, 75 mg) was added drop wise to this solution and the reaction left stirring at room temperature overnight. The macroinitiator was subsequently used for the Cu (II) Br₂/TPMA mediated ATRP polymerisation of poly (ethylene glycol methacrylate) based monomers (PEGMA). Briefly, macroinitiator (20 mg, 0.0003 mmol), PEGMA-246 (74 mg, 0.30 mmol), Cu (II) Br₂ (0.33 mg, 0.0015 mmol) and TPMA (0.43 mg, 0.0015 mmol) were dissolved in 300 µL PBS (pH 7.4). The mixture was placed on an ice bath and bubbled with argon for 15 minutes after which ascorbic acid (0.039 mg, 0.0002 mmol) was added to initiate the polymerisation. The reaction was stopped after 16 hrs and purified through dialysis membrane (MWCO 6-8,000) against deionised water. The macroinitiator was characterised using MALDI-TOF and high performance liquid chromatography (HPLC) where the molecular weight (MW) of native protein was used for comparison purposes. Synthesis of protein-polymer conjugates was confirmed using 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Ellmans assay and pyridyl disulfide assay. Critical micelle concentration (CMC) studies were performed using pyrene as a fluorescent probe. The hydrodynamic size of formed micelles was studied using dynamic light scattering (DLS), AFM and TEM.

REFERENCES


Once synthesis of the conjugates was confirmed, CMC studies were performed to identify the potential of the conjugates to form micelles in solution. Results demonstrated that all PEGMA-protein conjugates had CMCs which varied between 25-75µg/mL. Previously, it has been shown that AFM can be used to investigate the morphology of architectures formed by protein-polymer conjugates. The PEGMA-protein conjugates formed assemblies that appeared as spherical features in AFM images, consistent with the morphologies also observed in TEM studies. Some of the smallest features observed in the AFM were between 20-22 nm in diameter. Furthermore, mass distribution data from our DLS studies suggested hydrodynamic sizes of between 11-19 nm in diameter. The features measured by the different techniques are similar in size however; some measurements made in the AFM and TEM images can overestimated due to the sample preparation as well as the broadening of the features by the AFM tip.

**Table 1** A summary of the hydrodynamic size characterisation data for the different PEGMA-protein assemblies formed using AFM, TEM and DLS.

**CONCLUSION**

Here, we have outlined the use of a controlled polymerisation technique for the careful design of protein-polymer architectures for future investigation as drug delivery systems. We have demonstrated the ability to synthesize protein-polymer conjugates using a specific site on recombinant proteins and performed biophysical studies to identify the architectures formed.

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