Solid Phase Synthesis of Thermally Responsive Dendritic Macromolecules for Drug Delivery Purposes

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Statement of Purpose: The current state of the art for actively targeted drug delivery involves the use of antibody or peptide functionalized dendrimers. Dendrimers are perfectly defined macromolecules that have distinct branching structures. Their tree-like architecture provides a dense surface full of end groups while having a relatively sparse interior. These properties of dendrimers give them the ability to load pharmaceuticals in the interior while targeting specific cells at the surface.

While most dendrimers are synthesized using small branching molecules, a subset of these molecules exists consisting of larger, polymer branches. These dendritic macromolecules have some variability between individual molecules, which arises from the fact that they are synthesized by linking polydisperse polymer chains. While dendritic polymers are not absolutely defined like their dendrimer counterparts, they still exhibit many of the same interesting properties such as large numbers of functionalizable end groups on the surface of the molecule and densely packed branches with a sparse core. By creating dendritic poly(n-isopropyl acrylamide) (pNIPAAm), we aim to combine the drug loading and targeted delivery aspects of dendritic macromolecules with the thermally responsive properties of pNIPAAm. We aim to eventually couple this technology with nanoshell technology which has been shown to heat up significantly under the presence of certain wavelengths of light. This combination will form a multifunctional hybrid system for targeted drug delivery.

One of the main challenges in making dendritic pNIPAAm is the synthesis of well defined linear pNIPAAm chains with functionalized end groups. This can be achieved using reversible addition-fragmentation chain transfer (RAFT) polymerization with well chosen chain transfer agents (CTAs). RAFT polymerization is a well established controlled "living" radical polymerization method that uses di- or trithiocarbonates as CTAs to control radical polymerization. These CTAs react with free radicals to create stable transition states for the actively polymerizing chains, thereby reducing the risk of termination reactions that cause large polydispersities.

Methods: The chain transfer agent (CTA), S,S'bis(α,α'-dimethyl-α''-acetic acid)trithiocarbonate (CTA1) was synthesized according to the procedure set forth by Lai et al. Briefly, acetone (6.62 mL, 0.1 mol), chloroform (7.26 mL, 0.1 mol), carbon disulfide (2.16 mL, 0.04 mol), and tetrabutylammonium hydrogen sulfate (0.24 g, 0.7 mmol) were mixed in 12 mL of mineral spirits in a 100 mL roundbottom flask. The reaction mixture was purged with nitrogen for five minutes and run in a water bath at room temperature. 50% NaOH was added dropwise over 90 minutes and the reaction was left to run overnight. 90 mL of water was then added, followed by 42 mL of 6N HCl. The reaction mixture was then purged under nitrogen for half an hour and filtered. The resulting product was recrystallized from acetone to yield 4 grams of product.

Polymerization was conducted as follows: A 45:1:0.5 ratio of NIPAAm:CTA1:AIBN was placed in a sealed 100 mL roundbottom flask equipped with a magnetic stir bar. The mixture was purged with Nitrogen and Nitrogen purged dioxane was added. The solution was reacted at 50^{o}C for 72 hours and was quenched by exposure to air. The pNIPAAm-co-AAc was precipitated into ether, filtered and dried under vacuum. The resulting solid was resuspended in nanopure water (18 MΩ) and dialyzed using 2500 MWCO cassettes (Pierce) overnight. The solution was then lyophilized to obtain the product.

Solid phase dendritic polymer synthesis was achieved by first conjugating the polymer to Wang resin via typical Wang resin attachment methods. After conjugation, the polymer was biotinylated using a maleimide-PEG-Biotin chain and reacted with streptavidin. The resulting branch was then reacted with a previously biotinylated polymer branch to create a generation two dendritic polymer. This process was repeated until generation 4 was achieved.

Results: Characterization of CTA1 was conducted using electrospray mass spectroscopy (Fig.1A). The linear pNIPAAm was characterized using proton NMR spectroscopy (Fig.1B).

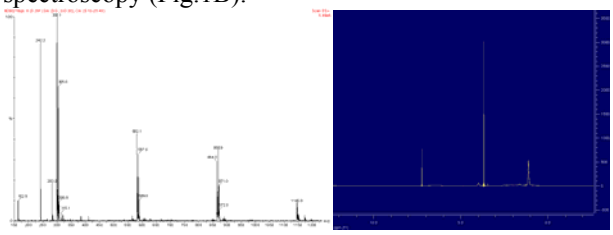

Fig.1: A) Electrospray mass spectrum of CTA1. B) pNIPAAm polymerized using CTA1

Linear pNIPAAm was further characterized using gel permeation chromatography. Results indicate a degree of polymerization of 23 with a polydispersity of 1.04. The lower critical solution temperature was also observed visually and noted to be at 34.2^{0}C at a pH of 7.2.

The Wang resin conjugated pNIPAAm gave a yield of 98%. Branching reactions with streptavidin and biotinylated pNIPAAm are ongoing.

Conclusions: The results show the ability to create well defined linear pNIPAAm chains. These chains have a carboxylic acid group on one end and a thiol on the other, allowing for easy conjugation with a variety of bioactive molecules. As such, they have been conjugated to Wang resin for solid state dendrimer synthesis. Future experiments will be done to test the LCST of these dendritic macromolecules as well as the effects of drug loading and functionalizing the surface with different targeting peptides on the behavior of the dendrimer.

References:

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