Characterization and Synthesis of a pH Responsive Polymeric Drug Delivery System

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ABSTRACT

In this study, we report the computation analysis and synthesis of folate functionalized poly(styrene-alt-maleic anhydride), PSMA. Folate was attached to the pH responsive SMA polymer via a biodegradable linker 2,4-diaminobutyric acid, DABA. To examine the linearity of the folate functionalized polymers, computational studies were used. Specifically, trimers were chosen to be the polymer length and the DFT -B3LYP/6-31G basis set was selected as the level of theory. The synthesis was also tested in multi-step experimental procedures. The structures were characterized using IR and NMR.

Keywords: folate receptor, drug delivery, pH-responsive, amphiphilic co-polymers, computational studies

1 INTRODUCTION

Drug delivery systems capable of unloading drugs in response to pH changes have received much attention in recent years.

Poly(styrene-alt-maleic anhydride), PSMA, is an amphiphilic alternating copolymer that self-assembles into nanotubes in aqueous solution, physiological-pH environments [1]. Its self-assembled structure with a 2nm hydrophobic inner diameter is stable in neutral pH solution, but collapses when pH is increased or decreased. These unique features of PSMA make it a great candidate delivery vehicle for controlled release of drugs into cancer cells. Increased specificity of the system could be achieved by folate conjugation. Folic acid has extremely high affinity (K_D~10M) towards its receptors which are over-expressed in certain types of cancers[2-4]. Indeed, folate-conjugated nanotubes could potentially transport small, highly toxic, hydrophobic chemotherapy agents safely through the body to be released selectively inside target cells. Once the drug is unloaded, the biocompatible SMA, folate, and linker groups would clear through the excretory system.

The rigid linearity of SMA in neutral, aqueous solution is essential to the formation of its nanostructure. Previous work has shown that the linearity of SMA in different chemical conditions can be well described using density functional theory (DFT) computational methods [5]. The relationship allows the linearity of a chain segment to be used as a proxy measure for supramolecular association, which would be difficult and costly to model directly. In the present work the geometry of functionalized SMA chains is optimized by DFT and their linearity used to determine its likelihood for inclusion in SMA nanotubes.

The synthesis was conducted in our lab. 2,4-diaminobutyric acid (DABA), a hydrophilic molecule, was attached to the maleic acid segments on SMA polymers as a linker between SMA and folic acid. The structures were purified in each step and characterized using IR and NMR.

2 SIMULATION METHODS

The SMA models used in this research were taken from existing files optimized to DFT-B3LYP/6-31G** by Malardier-Jugroot [5]. All other models were prepared and optimized using GaussView 09 computational chemistry visualization software [6]. To build and optimize the folate acid, 2,4-diaminobutyric acid (2,4-DABA) and diamino-poly(ethylene glycol) (DAPEG) folate derivatives, each dihedral optimization was carried out using a scan in energy about the dihedral angle at the semi-empirical PM3 level of theory [7,8], then each completed molecule was then re-optimized to the DFT-B3LYP/6-31G level of theory.

The trimer was the parsimonious choice for polymer length because it is both the basic repeating unit of PSMA and sufficient to demonstrate conformation linearity. Although each SMA subunit contains four chiral centres, chirality does not affect overall conformation and so one stereoisomer was chosen as representative of all. Both optimized folate derivatives were substituted onto each carboxyl of the central SMA subunit, creating four candidate molecules. Each molecule was again optimized stepwise to PM6, DFT-B3LYP/3-21G, DFT-B3LYP/6-31G [9,10] using High Performance Computing Virtual Laboratory (HPCVL) resources. PSMA quadrimer/2,4-DABA folate derivative conjoints were similarly produced for simulation at pH 3.

Conformation linearity was determined by inspection of the optimization result visualizations in GaussView 09. The software was also used to measure angles between the main polymer chain endpoints and the bonding site α-carbon to give a quantitative indication of deviation from
the linearity of unmodified PSMA. System energy calculations were taken from the optimization .log files.

3 EXPERIMENTAL METHOD

3.1 Materials

13% poly(styrene-alt-maleic anhydride) was purchased from Sigma Aldrich and freeze dried into white powder. Boc-2,4-diaminobutyric acid (DABA), N-hydroxy-succimide (NHS), dicyclohexylcarbodiimide (DCC), and folic acids were purchased from Sigma Aldrich and were used directly without further processing.

3.2 Synthesis of folic-conjugate

First, the carboxyl groups on PSMA were allowed to react with boc-protected linker boc-2,4-diaminobutyric acid (DABA). 13% PSMA, freeze dried prior to use, was dissolved in DMSO. DCC/NHS were added as coupling reagents along with boc-2,4-diaminobutyric acid at a 1:10 ratio. The reaction was carried out at room temperature over night. The crude product was dialysed against water for three days to remove DMSO solvent and the product freeze dried.

The boc protecting group was removed by trifluoroacetic acid and dichloromethane TFA/DCM at 30 degrees for 6 hours. The reaction was washed with triethylamine (TEA), dichloromethane (DCM) and recrystallized in water. The product was then filtered, air dried and characterized by $^1$H NMR.

Before attaching folic acid to the polymer, its carboxylate group was activated by DCC and NHS. Folic acid was dissolved in DMSO and allowed to react with DCC/NHS at room temperature overnight. The product was filtered using 0.2 ul filter. The resulting activated folic-NHS was added dropwise to a solution of SMA-DABA in DMSO. The reaction was carried out at room temperature over night. The crude product was precipitated by water, filtered, and air dried. The dry product was characterized using $^1$H NMR in DMSO and IR via KBr pellet.

4 SIMULATION RESULTS

Limited curvature over the length of the backbones chain is likely to be overcome by normalizing conformation changes induced by the supramolecular structure and minimization of polar-nonpolar interfaces. Severe or repeated turns characteristic of SMA in extreme pH values demand a greater energy to linearize than the advantage of nanotube association can afford, as evidenced by the structural collapse in those conditions.

At pH 7, neither of the 2,4-DABA variants show the 90° bends known to disrupt nanotube association at high or low pH. The simulation result for conjugation on the #2 site is shown in figure 1 (typical). That molecular rigidity and linearity are maintained is a good indication that 2,4-DABA variant folate-conjugated PSMA could associate with unaltered PSMA into nanotubes in a neutral, aqueous environment.

The linearity of DAPEG linked conjugates was found to be bonding site dependent. In the #1 site, hydrogen bonding interactions with the PEG chain was found to disrupt SMA backbone linearity, as shown in Figure 2. On the #2 site, however, disruptive interactions were blocked by steric action and chain linearity was comparable to that seen with 2,4-DABA variants. Due to the potential requirement for site-specific synthesis, DAPEG variants were not included in further simulations.

Figure 1: (2,4-DABA) folic acid derivative conjoined to a PSMA trimer at the #2 carboxylic acid of the central trimer. Polymer backbone angle = 140°; conjugation on #1 COOH returns similar results (127°)

Figure 2: linking molecule bonding a folic acid derivative to a PSMA trimer at the #1 carboxylic acid of the central trimer. Polymer backbone angle = 86°; conjugation to the #2 site is more linear, 130°.

To estimate the normalizing effect longer chains would have on the linearity of the conjugation site, two layer ONIOM simulations of a nine subunit chain were carried out for 2,4-DABA vairant on each site. The longer chain
was found to straighten the overall chain by ~15° in each case, strengthening the argument that nanotube formation is possible with functionalized chains.

As seen in Figure 3, at low pH the folate-conjugated PSMA exhibits conformation changes identical to the unmodified polymer. Because this change is implicated in nanotube pH sensitivity, these simulations show that folate conjugated PSMA will retain pH responsiveness. Disruption of the SMA structure is a vital characteristic to support the its use as a drug delivery platform since lysosome acidity is the proposed release method.

Figure 3: 2,4-DABA variant folate-conjugated PSMA quadrimer bonded at the #2 carboxylic acid in pH 3 conditions. Each monomer forms a ~90° angle with its neighbour.

5 EXPERIMENTAL RESULTS

The complete reaction pathway is illustrated by Figure 4. PSMA was first coupled with DABA linker using the classic peptide bond forming DCC coupling reaction. Dialysis and freeze drying techniques were used to purify the product and remove the solvent. The product was dissolved in D$_2$O and characterized by NMR. The peaks at 3.44 and 3.38 ppm confirm the formation of amide bond. Unfortunately the amide peak around 3.3 ppm is masked by the presence of water. It is apparent that the spectrum originates from both SMA and DABA: the signal at 7.07 ppm corresponds to the styrene group on SMA while signals at 2.54 and 2.04 ppm correspond to protons adjacent to the amine group (H$_2$N-CH$_2$, H$_2$N-CH$_2$-CH$_2$) on the boc protected DABA linker.

The boc protecting group was then removed by TFA/DCM solvent and allowed to react with activated folic acid-NHS. The resulting product was characterized by NMR (Figure 5) and IR (Figure 6). The presence of folate is confirmed by peaks at 6.61 and 7.64 ppm, which correspond to its para-aminobezoic acid group, and the signal at 8.63 ppm, corresponding to its pteridine moiety proton.

In Figure 6, the loss of peaks 1850 cm$^{-1}$ and 1780 cm$^{-1}$ indicates the conversion of maleic anhydride groups; the strong peak at 1731 cm$^{-1}$ is produced by the remaining carboxylic acid. Furthermore, bands at 1626 and 1530 cm$^{-1}$ are attributed to the formation of amide C=O and N–H functionalities respectively, demonstrating the link between SMA and folic acid [7].

Figure 4: synthetic pathway to SMA-folic conjugates. a DCC/NHS with boc-DABA in DMSO, r.t over night; b. TFA/DCM wash with TEA/DCM and precipitate with water; c. DCC/NHS with Folic-NHS in DMSO, r.t, overnight

6 CONCLUSION AND FUTURE STUDIES

The current study examined and synthesized folate-SMA conjugates via a DABA linker. The computational results show that the modified SMA polymer will retain its ability to form nanotubes and respond to pH changes. The experimental procedures indicate that folic acid was successfully linked to SMA polymers through amide bonds. Future work will focus on characterization of the morphology and pH sensitivity of the drug vesicles using DLS, TEM, and AFM. After complete and detailed characterization of the functionalized drug carrier, hydrophobic drugs will be selected and tested in cell studies [11].
REFERENCES