Novel pH-Sensitive Dendrimer Nanoparticles for Targeted Imaging

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Abstract:

The high detection sensitivity of optical contrast agents and the low autofluorescence of biological tissue in the near-infrared range enable noninvasive imaging of molecular conditions and processes. In particular, optical probes can be functionalized to target the molecular signatures of cancer cells in order to image tumors. Reducing background fluorescence would improve the contrast between normal and pathogenic tissue. We report progress on a novel dendrimer nanoparticle with potential of hydrogen (pH)-sensitive fluorescence activation in the acidic lysosomes of cancer cells for improved tumor imaging with reduced background fluorescence in normal tissue.

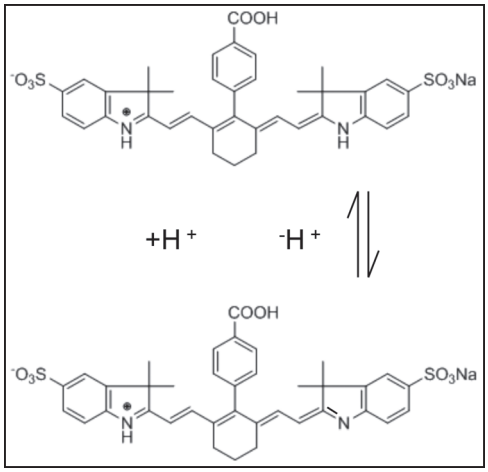

Figure 1: Structure of dye with pH-sensitive fluorescence (Absorbance maximum at 520 nm and 760 nm; Emission maximum of protonated fluorescent species at 780 nm).

Introduction:

Polyamidoamine (PAMAM) dendrimers are tree-like polymer nanoparticles with tunable pharmacokinetics and multifunctionality. Many recent studies report the design and synthesis of novel dendrimer carriers with imaging, targeting, and therapeutic moieties. It has been demonstrated that PAMAM dendrimers are delivered to acidic lysosomes after cell internalization [1]. Functionalization of the dendrimer with a pH-sensitive fluorescent dye yields an imaging probe that fluoresces after cell internalization and trafficking to the acidic lysosome (Figure 1). If further functionalized with a tumor-targeting peptide, the nanoparticle would fluoresce only when delivered to and internalized by a cancer cell [2]. Thus, fluorescence background signal would be reduced in normal tissue resulting in greater contrast.

Methods:

Synthesis of Dye-Dendrimer Conjugates. Generation 4 PAMAM dendrimers, which each possess 64 terminal amine groups, were reacted with 100 equivalents of acid-functionalized pH-sensitive fluorescent dye in the presence of activating reagents commonly used to couple amino acids. Specifically, hydroxybenzotriazole (HOBT) and O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) were used to activate the dendrimer and dye in the presence of the organic base N,N-diisopropylethylamine in dimethyl sulfoxide. The reaction was carried out overnight with vigorous mixing. The crude product was purified by size exclusion chromatography using a PD-10 column. The same method was performed with a pH-insensitive dye to synthesize a control nanoparticle.

Results and Discussion:

We have demonstrated pH-sensitive fluorescence on a dendrimer scaffold. The fluorescence of the pH-sensitive dye-dendrimer conjugate achieved greatest intensity in acidic buffer as previously observed with the free dye

(Figure 2). The wavelengths at which the dye-dendrimer conjugate had maximum absorbance and emission were shifted compared to those of the free dye (Figure 3). This red shift, or increase in wavelength, is due to changes in the electron system from conjugation to the dendrimer.

The large excess of dye was sufficient to saturate the reactive terminal amines of the dendrimer. Thus, no amine groups remained for conjugation to a targeting moiety. However, we found that using a smaller dye-to-dendrimer ratio resulted in an unexpected transformation of the spectral properties of the dye. A transformation was also observed when other organic dyes were subjected to the same reaction conditions. In particular, pH-insensitive dyes were transformed into pH-sensitive dyes (Figure 4).

We found that the pH-sensitive dye transformation was dependent on the presence of excess amine groups. To avoid the dye transformation, the 100:1 dye-to-dendrimer ratio was used to decrease the amine-to-dye ratio. In addition, we made progress on synthesizing peptide-dye conjugates for coupling to modified dendrimers with greater control. Specifically, a portion of the amine groups of the dendrimer could be blocked with acetic anhydride to avoid the dye transformation by eliminating excess amine groups.

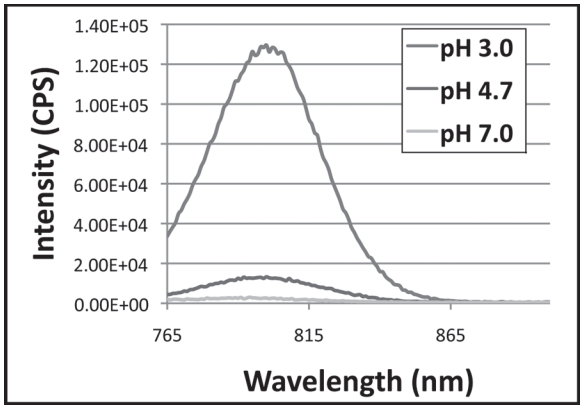

Figure 2: Fluorescence emission of pH-sensitive dye-dendrimer conjugates (Maximum emission at 800 nm).

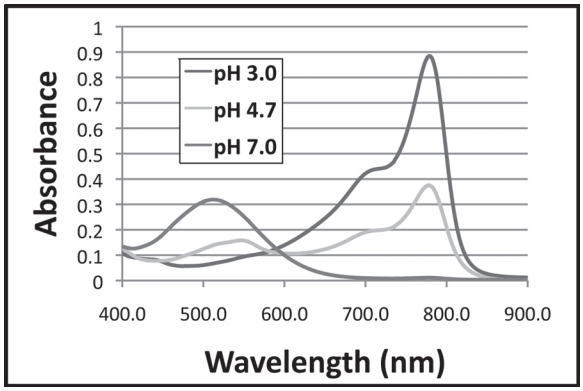

Figure 3: Absorption of pH-sensitive dye-dendrimer conjugates (Maximum absorbance at 510 nm and 780 nm).

Conclusions and Future Work:

Molecular probes that are pH-sensitive promise improved contrast and decreased background. A novel dendrimer nanoparticle with pH-sensitive fluorescence in the near-infrared range was synthesized and characterized. *In vitro* studies are in progress. In addition, an unexpected pH-sensitive dye transformation was observed and characterized.

Characterization of the products of the unexpected pH-sensitive dye transformation by nuclear magnetic resonance (NMR) and high resolution mass spectroscopy is ongoing. Future work includes functionalizing the nanoparticle with tumor-targeting peptides, particularly continuing to synthesize a peptide-dye conjugate for coupling to the dendrimer.

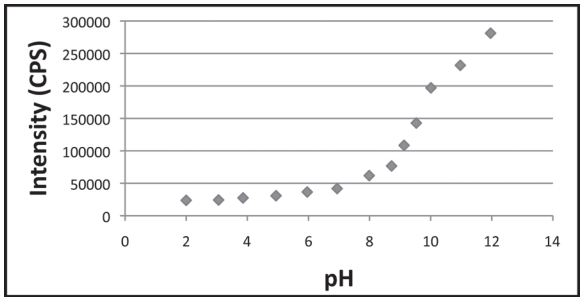

Figure 4: Fluorescence emission of pH-insensitive control dye after unexpected pH-sensitive transformation.

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References:

[1] Albertazzi, L. et al. Mol. Pharmaceutics, Vol. 7 (3), 680–688 (2010).

[2] Le, H. et al. Bioconjugate Chem., Vol. 22 (4), pp 777–784 (2011).