Synthesis and Characterization of a Charge-Reversal Photo-Active Amphiphile

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Statement of Purpose: Though nonviral gene delivery vectors (e.g., cationic amphiphiles and dendrimers) are currently being used for *in vitro* cell transfection and clinical gene therapy trials, overall transfection activity is still low compared to that of traditional viral vectors. (Huang L. Acad Press. 1999;7-15.) By engineering new vectors which overcome the extra- and intracellular barriers that impede transfection, such as endosomal escape and DNA release, one can improve delivery and take advantage of nonviral vectors' enhanced safety and biocompatibility features. (Kay MA. Proc Natl Acad Sci. 1997;94:12744-12746.) Cationic amphiphiles begin to address this problem by forming liposomes that electrostatically interact with anionic DNA and facilitate delivery into the cell by endocytosis, but these structures do not readily release DNA. We hypothesize that the preparation of an amphiphile which undergoes a net charge-reversal will allow us to better control liposome destabilization, and ultimately DNA release. We have previously reported such an amphiphile that transforms via an enzymatic reaction. (Prata CAH. JACS. 2004;126: 12196-12197.)

In this work we report a new photo-active charge-reversal system. An *ortho*-nitrobenzyl derivative has been chosen as our photolabile group due to its well characterized absorption maximum (near 365 nm) and facile synthesis. (Kikuchi Y. Langmuir. 2008;24:13084-13095.) We have designed and synthesized a cationic amphiphile which releases DNA upon photolysis of the terminal 1-(2-nitrophenyl)ethyl moieties (Figure 1). Along with characterizing the photolysis kinetics of our compounds, we have also evaluated the liposome destabilization effects as a prelude to *in vitro* studies.

Methods: Synthesis: An amphiphile with a peptide lysylglycylglycyl (KGG) head group, two C_{10} alkyl chains, and 1-(2-nitrophenyl)ethyl terminal groups was prepared. All chemical compositions were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance and mass spectrometry.

Irradiation studies: Decaging studies were performed on 50 µM samples (in 2 mL) of caged alkyl chains using long wave UV irradiation (365 nm, 23 W; Spectroline) and analyzed by liquid chromatography mass spectrometry (Agilent) to monitor the cleavage kinetics of the terminal photo-group. Liposomes were formed by sonication and extrusion to create a monodisperse population (approximately 100 nm diameter). Liposome destabilization was evaluated by measurement of the size and particle counts of liposomes in solution using dynamic light scattering (DLS; Brookhaven Instruments). Liposomes were irradiated for up to 20 min (xenon lamp, >300 nm, 15 W; Newport) and DLS measurements were compared to those of samples unexposed to light. Also,

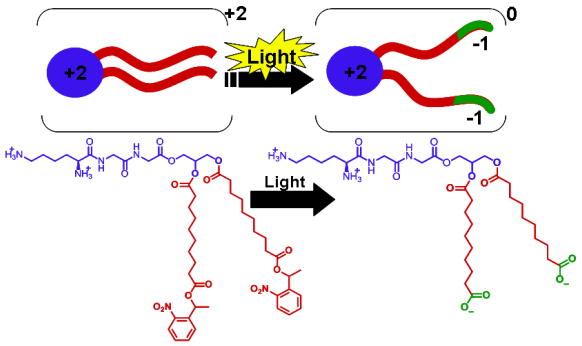

Figure 1. Illustration of the charge-reversal effect that occurs upon irradiation of the photo-active amphiphile.

lipoplexes were formed by incubating calf thymus DNA (Sigma) with the liposomes for 15 min (N/P = 20:1). The lipoplexes were irradiated and their destabilization was evaluated by DLS.

Results: Photo-decaging studies showed that 27.6% of chains in solution had their terminal group cleaved after 1 min of UV irradiation and 54.2% cleavage had occurred by 3 min. When irradiated, liposome particles showed an increase in diameter and a decrease in counts over time (Figure 2). This trend demonstrates that as decaging of the amphiphiles occur, individual liposomes destabilize and coalesce into larger particles. The control sample with no light exposure over the same time shows little change in size or counts. Lipoplexes also showed this destabilizing trend, as they exhibited an increase in diameter of 136.2 nm and a 67% decrease in counts after 20 min of irradiation.

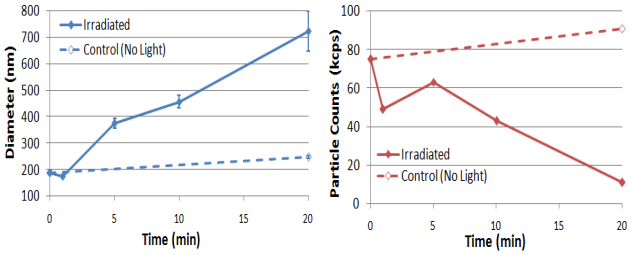

Figure 2. Liposome diameter (left) and particle counts (right) for both irradiated and control (no light) samples over time.

Conclusions: Our data show that UV irradiation can efficiently decage the *ortho*-nitrobenzyl group in under 5 min and that this photolysis leads to liposome destabilization. These data suggest that this new amphiphile will be a good candidate for further DNA binding and release studies and future gene transfection research.