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A photo-degradable gene delivery system for enhanced nuclear gene transcription

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ABSTRACT

There currently exists a significant gap in our understanding of how the detailed chemical characteristics of polycation gene carriers influence their delivery performances in overcoming an important cellular-level transport barrier, i.e., intranuclear gene transcription. In this study, a UV-degradable gene carrier material (ENE4-1) was synthesized by crosslinking low molecular weight branched polyethylenimine (bPEI-2k) molecules using UV-cleavable *o*-nitrobenzyl urethane (NBU) as the linker molecule. NBU degrades upon exposure to mild UV irradiation. Therefore, this UV-degradable carrier allows us to control the chemical characteristics of the polymer/DNA complex (polyplex) particles at desired locations within the intracellular environment. By using this photolytic DNA carrier, we found that the exact timing of the UV degradation significantly influences the gene transfection efficiencies of ENE4-1/DNA(pGL2) polyplexes in HeLa cells. Interestingly, even if the polyplexes were UV-degraded at different intracellular locations/times, their nuclear entry efficiency was not influenced by the location/timing of UV degradation. The UV treatment did not influence the size or binding strength of the polyplexes. However, we confirmed that the degradation of the carrier molecules impacts the chemical characteristics of the polyplexes (it produces carbamic acid and nitrosobenzyl aldehyde groups on ENE4-1). We believe that these anionic acid groups enhance the interaction of the polyplexes with nuclear transcription proteins and thus the final gene expression levels; this effect was found to occur, even though UV irradiation itself has a general effect of reducing transfection efficiencies. Excess (uncomplexed) ENE4-1 polymers appear to not play any role in the UV-enhanced gene transcription phenomenon.

1. Introduction

Polymer-based gene carriers, though promising as safer alternatives to viral gene carriers, have yet been limited in large part due to their poor transfection performance. Future design and development of better polycation gene carriers will be greatly facilitated by an improved understanding of the relationship between the polycation chemistry and performance mechanism. Prior studies over the years have revealed many useful information along these lines. However, the current state of knowledge in this area is still tentative and insufficient to serve the purpose [1,2].

In particular, an important gap exists in the lack of a discussion and explanation of seemingly inconsistent data regarding the exact optimal timing and location of DNA release from polycation/DNA complexes (polyplexes) during the post-internalization (i.e., post-endocytosis) intracellular trafficking pathway [2]. It was originally the objective of the present study to establish the correlations between various molecular parameters of polyplexes (such as polyplex size and compactness) and their performances in the above-mentioned delivery aspects (post-endosomal trafficking, and timely release of DNA) and ultimately in the expression of the delivered gene. Specifically, we sought to answer the following question: What is exactly the role that a polycation plays in promoting the nuclear import of DNA? In order to address this problem, we developed a UV-degradable DNA carrier. We thought that by using this photolytic DNA carrier it would be possible to control the precise location of the (partial or complete) disintegration of

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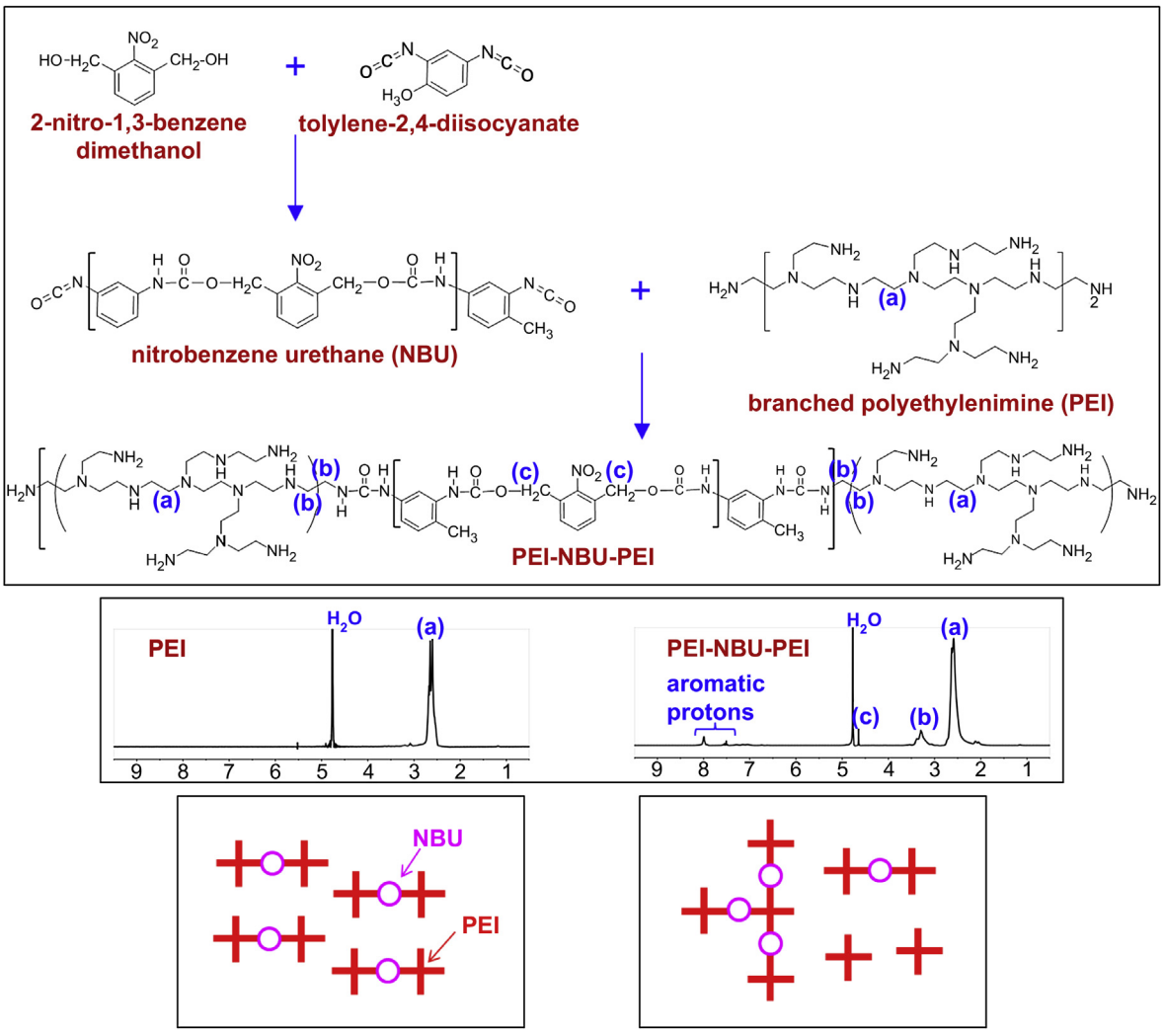

Fig. 1. (Upper) Synthesis route for the preparation of a UV-degradable crosslinked polyethylenimine (PEI) material (named "ENE4-1"). Low molecular weight branched PEI (bPEI-2k) polymers were crosslinked with a UV-cleavable *o*-nitrobenzyl urethane (NBU) spacer. **(Middle)** ^{1}H NMR spectra of the bPEI-2k precursor and the PEI-NBU-PEI product in D_2O. In the synthesis of the ENE4-1 material, bPEI-2k was reacted with NBU in a 2:1 stoichiometric molar ratio. As the **bottom right** cartoon describes, the random nature of the coupling reaction results in a polydisperse product (rather than a monodisperse product such as shown in **bottom left** of the figure).

the polycation/DNA polyplex particles within the intracellular environment; thus, the effects of the location of the photo dissociation (i.e., cytosol, nucleus, or no degradation) on the nuclear localization and gene expression of the polyplexes could be studied.

Previously, several strategies for controlling the timing and location of the intracellular DNA release by external stimuli have been demonstrated by other researchers. Examples of these approaches include the use of such mechanisms as changes in pH [3], temperature [4] or redox potential [5–7], and UV [8] or IR [9] irradiation. In particular, photolytic DNA carriers allow to control the location/timing of the disintegration of the polyplexes within the intracellular environment without relying on inherent biochemical characteristics of intracellular compartments; using these photo-degradable gene delivery systems, it has been demonstrated that the intracellular degradation of the carrier material greatly enhances the delivery performance of DNA [8] (or siRNA [10]).

In many of these chemically degradable gene delivery systems, the chemical degradation not only impacts the physical strength of the polycation/DNA binding, but the chemical characteristics of the polyplexes too. For instance, the degradation of the UV-cleavable [8,11] polymers typically produces anionic (acid) groups on polyplexes that can alter the interaction of the polyplexes with intracellular proteins (e.g., transcription-related proteins present in the nucleus) and thus the final gene expressions levels. However, this aspect of the intracellular carrier degradation has not been investigated. In this regard, one remarkable recent finding is that when an anionic component (such as hyaluronic acid [12], heparin [13], poly(aspartic acid) [13], poly(γ-glutamic acid) [14], carboxylic acid pendant groups [15] or oligonucleotides [16]) is incorporated into the DNA/polycation complex, the transfection efficiency is increased by multiple orders of magnitude. A common explanation for this phenomenon has been that the anionic additive facilitates efficient release of DNA in the cell's nucleus and thus the gene transcription process, by loosening the binding between the polycation and DNA. However, the isolated effects of carrier's anionic groups on the individual steps of the intracellular trafficking processes (i.e., endosome escape, nuclear entry, and interaction/binding with transcription-related proteins) have not been determined.

In the present study, we developed a polyethylenimine(PEI)-based DNA carrier that is degradable upon exposure to mild UV irradiation (Fig. 1). This polycation carrier contains UV-cleavable *o*-