Intracellular trafficking and activity of histone-mimetic gene delivery vehicles

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Statement of Purpose: Effective gene transfer strategies would fundamentally alter healthcare, and depend upon our ability to create materials that can chaperone DNA transport and release within a diverse biological environment. A critical problem in non-viral gene therapy is how to trigger nucleus-specific DNA release: current electrostatic DNA packaging techniques condense DNA to prohibit DNase access, but inhibit transcription in the process. We have created a new class of DNA packaging materials that are designed to promote efficient transcription within the nucleus by interacting with histone effector proteins. These materials contain peptides that are comprised of histone tail sequences known to signal nuclear import and transcriptional activation. In this work, we have explored the gene transfer activity of polyplexes containing trimethylated H3 histone tail peptides ($H3K4Me_3$) that are known to interact with two nucleus-specific effector complexes that initiate transcriptional activation. Our hypothesis is that the peptides within these polyplexes will interact with natural histone effectors within the nucleus, stimulating polyplex unpackaging and transcriptional activation.
Methods: Polyplexes were self-assembled from the green fluorescent protein (GFP)-expression gWiz plasmid DNA (Genlantis) and the indicated mixtures of 25 kDa polyethylenimine (Sigma) or cysteine-terminated H3 N-terminal tail peptides (ARTKQTARKSTGGKAPRKQ LASKAARKSGC), either with or without trimethylation at the K4 position. Peptides were synthesized by Fmoc solid phase synthesis, according to standard procedures. The gWiz plasmid was amplified in *E. coli* and purified with the Qiagen Giga Kit, according to the manufacturer's protocols. The size and compactness of the polyplexes were characterized by dynamic light scattering (DLS) and agarose gel electrophoresis, respectively. Subsequently, the gene transfer activity of the polyplexes was analyzed in NIH/3T3 and CHO cells. Cells were transfected with polyplexes containing the H3-derived peptides or PEI or the indicated mixtures, and transfection was assessed by fluorescence microscopy and flow cytometry, according to standard protocols. Endosomal trafficking routes were analyzed by pretreatment of cells with inhibitors associated with the indicated endocytic pathways, followed by standard cellular transfection. To determine whether the trimethylated peptides were stimulatating rapid transcription within cellular nuclei, polyplexes were microinjected into NIH/3T3 cells and the rate and efficiency of gene expression were analyzed via time-lapse fluorescence microscopy. A mass action kinetic model was used to correlate the overall measured gene expression kinetics with kinetic parameters associated with polyplex unpackaging and transcriptional activation.
Results: The H3K4 and $H3K4Me_3$ peptides formed tightly packaged, nuclease-inaccessible, polyplexes that were approximately 100 nm in diameter over a range of N:P charge ratios. When used to transfect cells, these polyplexes exhibited limited gene transfer activity, which was attributed to their inability to escape the endosome. Thus, the H3-based peptides were combined with PEI to form hybrid polyplexes, based on the hypothesis that PEI's known capacity to escape the endosome might facilitate the activity of the H3 peptides. When combined, PEI and the H3 peptides exhibited synergistic activities for cellular transfection, where the hybrid materials were capable of transfecting approximately twice the number of cells as an optimized formulation of PEI-DNA polyplexes. To determine the mechanisms underlying the differences in the activities of these materials, subcellular trafficking studies were completed in which cells were pretreated with various endocytic inhibitors and then transfected. Transfections performed following pretreatment with chlorpromazine or wortmannin (associated with clathrin-mediated uptake) demonstrated that the H3-based materials strongly disfavored the clathrin pathway, as gene expression levels increased following inhibitor treatment. Transfections performed following pretreatment with filipin 3 (associated with caveolar uptake) demonstrated that the H3-based materials strongly favored caveolar uptake. In contrast, similar studies with PEI-based polyplexes demonstrated a weaker preference for caveolar uptake, suggesting that alternate trafficking patterns exhibited by the formulations might underlie their differences in activity. To further explore the possibility of that the trimethylated motifs were stimulating nuclear unpackaging and activation, polyplexes were microinjected into NIH/3T3 cells and the kinetics of gene expression were monitored and analyzed by the use of a first-order mass action model. These studies demonstrated that polyplex unpackaging was significantly more rapid in the presence of the trimethylated H3 motif.
Conclusions: We demonstrate that the H3-based materials initiate gene expression more rapidly than traditionally packaged polyplexes or polyplexes made from non-methylated H3 histone tail peptides when introduced directly into the nuclei of cells. Furthermore, a simple mass action kinetic model demonstrates that the enhanced activity of the $H3K4Me_3$-containing polyplexes results from their more rapid unpackaging/activation. We have used immunocytochemical staining to show that the $H3K4Me_3$–containing polyplexes co-localize more efficiently that the non-methylated polyplexes with elements of the transcriptional machinery, presumably as a result of their specific interactions with resident nuclear proteins. When the $H3K4Me_3$ peptides are combined with materials that promote endosomal release, the histone tail peptides comprise highly efficient and non-cytotoxic gene delivery vehicles.