Stabilization of Complex with PKCα-Specific LPEI-Peptide Conjugate via Hydrophobic Interaction

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ABSTRACT SUMMARY

We demonstrate that linear poly (ethylenimine) (LPEI), as a potent candidate with high transfection ability for gene delivery, is grafted with hydrophobically modified cationic peptides that is the substrate of PKCα. Hydrophobically modified LPEI-peptide conjugate (LPEI-C10-peptide) could form a stable complex with pDNA through electrostatic and hydrophobic interactions between anionic DNA strand and cationic peptide substrate containing long alkyl chain group. Thus, we successfully demonstrated LPEI-C10-peptide as a stable and cancer-specific gene carrier. The novel approach described in this work can be expanded to conventional disease-site targeting system for more specific and safer gene therapy.

EXPERIMENTAL METHODS

PKCα-responsive peptide, FKKQGSFAKK, was prepared by standard Fmoc-chemistry as described previously1-3. To modify hydrophobic group on peptide substrate, 11-azidoundecanoic acid was synthesized with 11-bromoundecanoic acid by addition of 2 equiv of sodium azide for 1 day at room temperature in 1:1 DMF/DMSO solution. 11-azidoundecanoic acid was then reacted with peptide in the presence of coupling reagents. After complete reaction, peptide was cleaved from the resin and was purified by reverse-phase liquid chromatography equipped with an electrospray mass spectrometer for the detector.

The polymer was synthesized by following a two-step synthetic procedure. LPEI was dissolved in dry DMSO (30 mL) followed by addition of pyridine, 1,8-diazabicyclo[5,4,0]-7-undecene (DBU), and 5-chloro-1-pentyne. After the reaction, the obtained polymer (LPEI-pentyne), azido peptide substrate, copper (II) sulfate pentahydrate, and sodium ascorbate were dissolved in 808 μL of water/ethanol (1/1=v/v) and maintained at room temperature with continuous stirring for one day. After the reaction, the crude product was dialyzed against 0.05 N HCl and water by using dialysis membrane bag (MW cut off, 10,000), followed...
RESULTS AND DISCUSSION

We confirmed that LPEI-C_{10}-peptide conjugate was formed complex with pDNA from the results of gel electrophoresis and ethidium bromide exclusion (EtBr) assay. The result indicates that anionic pDNA was effectively associated with cationic peptide.

To gain an insight into the effect of hydrophobic interaction of LPEI-C_{10}-peptide conjugate, the cellular uptake property of complex was analyzed by a Tali™ Image-Based Cytometer using fluorescently labeled pDNA with the intercalating nucleic acid dye YOYO-1 iodide (Fig 1.). The uptake of LPEI-C_{10}(S) was higher than those of unmodified LPEI-C_{2}(A) and naked pDNA. The result indicates that the increased stability via hydrophobic interaction may enhance the cellular uptake of complex in the cell culture condition.

Moreover, the gene expression of negative control complexes [LPEI-C_{2}(A) and LPEI-C_{10}(A)] were kept low level, while the LPEI-C_{2}(S) and LPEI-C_{10}(S) complexes showed much higher expression irrespective of the N/P ratios (Fig 2.). This result clearly showed suppression of gene expression in negative control complexes and the PKCa-responsive gene expression in LPEI-C_{2}(S) and LPEI-C_{10}(S) complexes.

CONCLUSION

We demonstrated the abilities of hydrophobically modified LPEI-peptide conjugate as a proper gene carrier. The enhanced cellular internalization via hydrophobic association and the gene expression level of LPEI-C_{10}-peptide complex with pDNA responding to PKCa were much higher than PKCa-responsive carrier that we reported previously\textsuperscript{7}. Thus, hydrophobically modified LPEI-peptide conjugate is useful for highly cancer-specific therapy and imaging.

REFERENCES


ACKNOWLEDGMENTS

This work was financially supported by a grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.