Tethering PEG density on polyplex micelles and stealthiness in blood circulation

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ABSTRACT SUMMARY

Gene carrier, prepared by polyanion complexation of plasmid DNA (pDNA) and poly(ethylene glycol) (PEG)-polycation block copolymer, which spontaneously forms a core-shell structured nanoparticle (polyplex micelle), is promising character toward systemic application because its PEG palisade can prevent non-specific interaction with blood components. To prolong blood circulation, PEG tethering density σ should play a central role; nevertheless it has never been precisely estimated. Hereby, we demonstrated the σ estimation using our recent finding for detailed core geometry; pDNA is packaged according to highly regulated folding scheme with $n$-times folding in quantized manner. σ of polyplex micelles, prepared from block copolymer of PEG-b-poly(L-lysine) (PEG-PLys) of different PLys degree of polymerization (PLys DP), was found to increase as PLys DP decrease. This PEG crowdedness plays a major contribution in determining packaging structure of pDNA within the polyplex micelles. Furthermore, it played a critical role for prolonging blood circulation period.

INTRODUCTION

Development of safe and efficient gene delivery system is a key technology to establish gene therapy. We have developed polyplex micelles as a promising delivery system for systemic gene delivery. The polyplex micelles are constructed through electrostatic interaction spontaneously forming distinct core (condensed pDNA)-shell (PEG palisade) architecture (Fig. 1). A crucial issue here is the packaging of pDNA into the polyplex micelles with protective PEG palisade because this offers basic requirements for delivery, including protection from reticulo endothelial system (RES) capture triggered by protein opsonization, enzymatic digestion as well as cellular uptake. In particular, PEG palisade should demonstrate crucial role in systemic application because it may determine the blood circulation period. However, a key physical property of the palisade, tethering density (σ), has never been estimated for the polyplex micelles. Indeed, this was difficult because precise geometry of polyplex micelle core was not understood. Recently, we found unique folding mechanism of rigid double-stranded DNA: DNA is folded with local double-strand dissociation,1 and form rod-shaped structures with highly regulated folding scheme. pDNA is packaged as bundle of DNA strands with quantized-length rods (Fig. 1a), being $1/2(n+1)$ of the original pDNA contour length by folding $n$ times (Fig. 1b).2 Using this model, we estimated σ for polyplex micelles from PEG-PLys with different PLys DP because packaging structure as well as gene transfer efficiency are affected by the PLys DP.3 Finally, circulation period in the blood were investigated for these polyplex micelles to clarify correlation with PEG density.

EXPERIMENTAL METHODS

PEG-PLys with fixed PEG molecular weight (PEG $M_w$) of 12k, and different PLys DP 20, 39, and 70 (verified by $^1$H-NMR) was prepared by the ring-opening polymerization of Nε-trifluoroacetyl-L-
lysine-N-carboxyanhydride with α-methoxy-ω-amino PEG as initiator, followed by TFA deprotection using basic treatment. All polymers have molecular weight distributions (Mw/Mn) of around 1.05 confirmed from GPC results. Polyplex micelles were prepared by mixing PEG-PLys into pDNA (pBR322, DNA base pair = 4361) solution at N/P ratio 2 (N/P ratio = molar ratio between amines (N) in PLys segment and phosphates (P) in nucleotides). The polyplex micelles rod structures were characterized by (1) transmission electron microscopy (TEM), and (2) Cryo-TEM. Number of binding polymer was determined from analytical ultracentrifuge measurement. Blood circulation period was estimated using in vivo confocal laser scanning microscopy by monitoring fluorescence of Cy5-labeled pDNA loaded polyplex micelles at vein of ear lobe.

RESULTS AND DISCUSSION

Packaging structure of pDNA within the polyplex micelles were investigated by TEM for series of PLys DP and found that the average rod length shifted shorter by increasing PLys DP. All the samples showed specific length distribution that is characteristic of quantized folding model (Fig. 1), which enables to find the folding number as well as the fraction of rods at every n (f_n). Surface area A at every folding number n (A_n) was firstly calculated and then number-average surface area <A> was obtained by considering the fraction of rods at every n (<A> = Σ (f_n A_n)). Finally, number-averaged tethering PEG density <σ> was obtained by dividing the number of tethered PEG strands by <A>. We found that <σ> was increased with decreasing PLys DP (Table 1). The average tethered PEG height was found from cryo-TEM images, showing that height for the polyplex micelles of PLys DP 70 was almost equal to the height of unperturbed random coil (mushroom) for PEG 12k, which is 9.4 nm, while the other two were slightly higher (Table 1). This indicated that polyplex micelle of PLys 70 was still in mushroom conformation, while the others were already in mushroom to brush transition regime.

The blood circulation period exhibited prolonged for the polyplex micelles of shorter PLys DP having denser PEG palisades (Fig. 2). These results suggested that regulating PEG crowdedness, which can be obtained by changing polycations segment length, will provide essential contribution to fabricate gene carriers for systemic use.

CONCLUSION

PEG density for pDNA polyplex micelles has been successfully estimated for the first time based on the quantized folding scheme of pDNA within rod-shaped polyplex micelle. An increased PEG tethering density was found with decreasing PLys DP. The tethered PEG conformation was also modulated by PLys DP, which might influence blood circulation profile as well.

REFERENCES


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Table 1. PEG shell height and PEG density of the polyplex micelles.

<table>
<thead>
<tr>
<th>PLys DP</th>
<th>PEG height (nm)</th>
<th>Binding number of polymer</th>
<th>σ (chain/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>12.4 ± 1.6</td>
<td>436 ± 31</td>
<td>0.075</td>
</tr>
<tr>
<td>39</td>
<td>10.9 ± 1.8</td>
<td>258 ± 10</td>
<td>0.051</td>
</tr>
<tr>
<td>70</td>
<td>9.6 ± 2.0</td>
<td>168 ± 2</td>
<td>0.038</td>
</tr>
</tbody>
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