Electronic Supplementary Material (ESI) for Molecular BioSystems

Transfection activity and mechanism of pDNA-complexes based on the hybrid of low-generation PAMAM and branched PEI-1.8k

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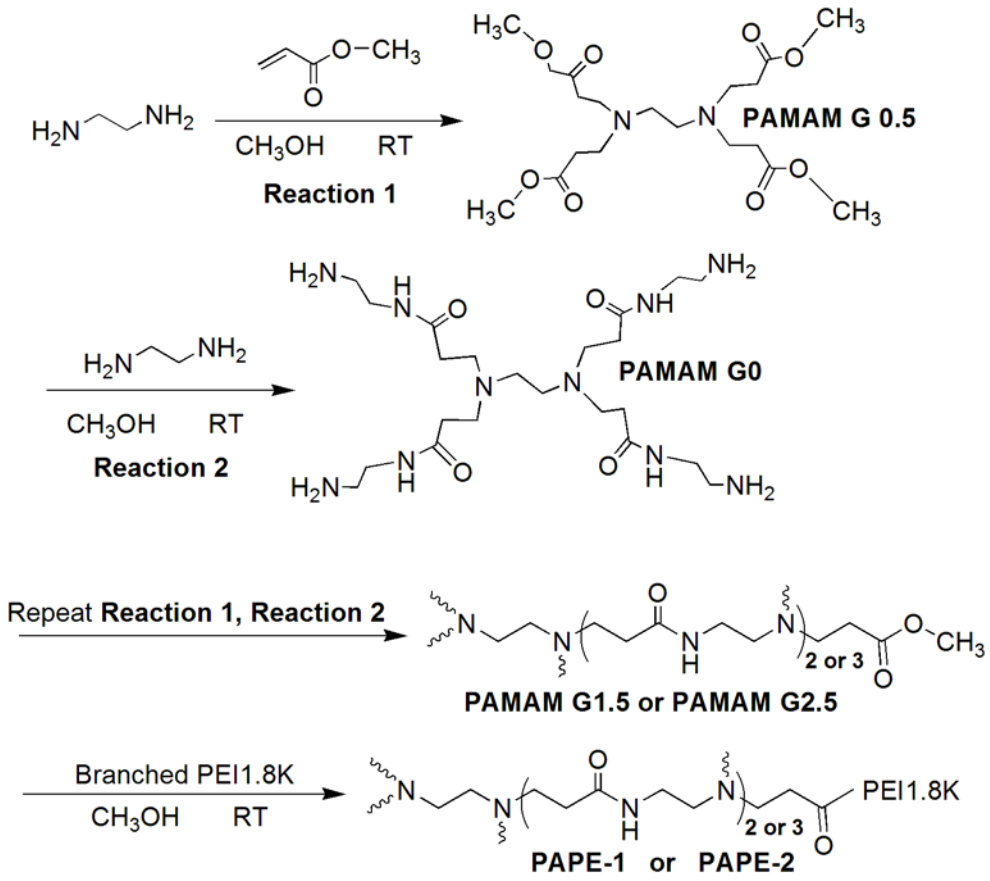
SUPPLEMENTARY DATA

1. Synthesis procedures of PAPEs



Scheme 1. Synthesis procedures of PAPEs.

2. Characterization of PAMAM G1.5 and PAMAM G 2.5

PAMAM G1.5 and PAMAM G2.5 dendrimers with EDA as the core were prepared according to the procedures reported by Tomalia and Wu.[1,2] After purification, they were characterized by FTIR and ^{1}H NMR. As given in Fig. S1, the peaks at about 3305 cm^{-1} (NH), 2953 and 2830 cm^{-1} (CH stretch), 1736 cm^{-1} (C=O), 1257 cm^{-1} (C-O), 1650 and 1540 cm^{-1}

(-CONH-), 1437 cm^{-1} (CH bending) and 1200 cm^{-1} (C-N) are all the characteristics of PAMAM dendrimers. The ^1H NMR spectra of PAMAM dendrimers were shown in Fig. S2. Taking G1.5 for example, Peaks in the range 3.74-3.81, 3.28-3.48, 2.45- 2.76, 2.83-2.98, 2.53-2.58 and 2.45-2.53 ppm were assigned to the protons of $–COOCH_3$, $-CONHCH_2-$, $-NHCH_2CH_2CO-$, $-CH_2CH_2N<$, $-NHCH_2CH_2COO-$ and $-NHCH_2CH_2CONH-$, respectively. Similar ^1H NMR spectra were observed in PAMAM G2.5 dendrimer. The results of FTIR and ^1H NMR spectra confirmed the successful synthesis of PAMAMG1.5 and G2.5 dendrimer.

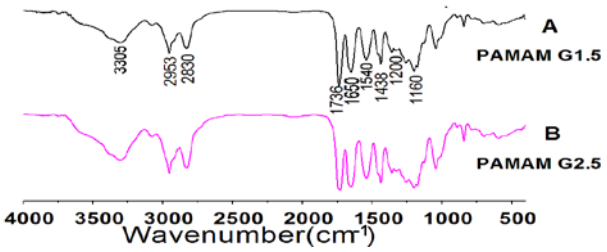


Fig. S1. The IR spectra of (A) PAMAM G1.5, (B) PAMAM G2.5

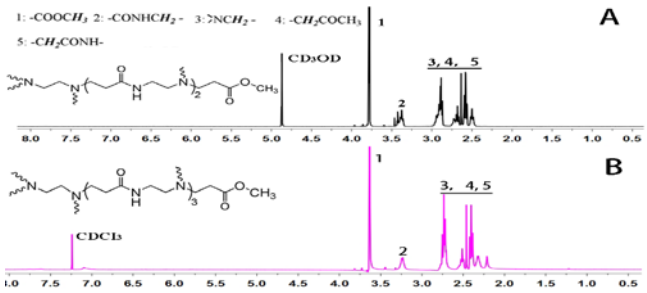


Fig. S2. The ^1H NMR spectra of (A) PAMAM G1.5, (B) PAMAM G2.5

3. Free PAPE effects

Previous reports have proved that at N/P=3-4, almost all DNA is complexed with PEI.[3-6] At N/P = 6 not all PEI is bound in the complexe and some free PEI exists.[5,7] However, at N/P>4 the transfection efficiency still increased continually. For example, PEI-25k complexes at N/P=10 showed its highest efficiency. Yue et al. preformed a gene transfection in the absence of serum with PEI complexes prepared at N/P=10, which has a N/P=3 portion corresponding to complexed PEI fraction plus a N/P=7 portion corresponding to the free PEI. In comparison

with PEI complexes at the N/P= 3, the transfection efficiency in the presence of free PEI was greatly increased.[8] This fact clearly suggested that addition of free PEI would improve transfection in the absence of serum. In our study, the similar result was also obtained. In the Fig. 4A, the transfection efficiency of PAPE complexes at N/P=25 was higher than that at N/P=13. The amount of free PAPE in the complexes at N/P=25 was larger than that at N/P=13. Likewise, as shown in Fig. 4B, the transfection efficiency of PAPE complexes at N/P= 75 or 90 was higher than that of at N/P=45. Therefore, these indirectly proved that addition of free PAPE would also improve the transfection efficiency in absence/presence of serum. In addition, to directly confirm free PAPE in the complexes could improve the transfection efficiency of PAPE/pDA complexes in the presence of serum, we performed a cell transfection with a combination of PAPE-2 complexes fixed at the N/P ratio of 25 and varying amount of free PAPE-2 which was added into complexes at the time of transfection. The total amount of PAPE-2 in each combination was equal to complexes prepared at N/P ratios of 75 and 90, respectively. As shown in Figure S3, transfection efficiency of PAPE-2 complexes at N/P=25 in the presence of different amount of free PAPE-2 was significantly higher than that of PAPE-2 complexes at N/P=25 alone ($P < 0.01$, n=3), but was lower than that of corresponding control. These results indicated free PAPE-2 in the complexes could make some contribution to the improved efficiency in the presence of serum.

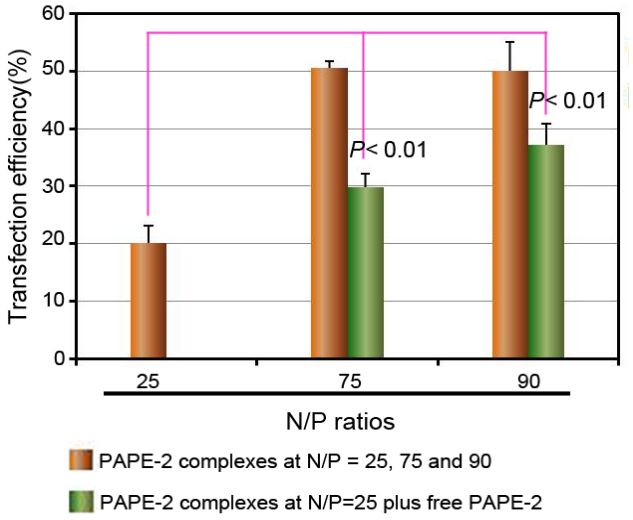


Fig. S3. The transfection efficiencies of PAPE-2 complexes at the N/P ratio of 25 in the presence of various amount of free PAPE-2. PAPE-2 complexes at N/P = 25, 75 and 90 were used as controls.

References

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