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Poly(ethylenimine)-grafted-poly[(aspartic acid)-co-lysine] : A Non-viral Polymer with Potential for DNA Delivery

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Abstract: A biodegradable gene transfer vector, poly(ethylenimine)-grafted-poly[(aspartic acid)-co-lysine] has been developed by thermal polycondensation of aspartic acid and lysine, and branch poly(ethylenimine) (Mw less than 600) was grafted to the backbone. The polymer was characterized by ^{1}H NMR. It appeared lower cytotoxity compared to poly(ethylenimine) (25KDa), which was quantified by MTT assay. Electrophoresis indicated that the polymer could retardate DNA at N/P ratio 1.2-1.8 (w/w). Transfection efficiency of the complexes was studied in NT2 cell lines. It was 1.5 fold higher than molecular weight PEI (Mw = 25KDa).

Keywords: Poly(ethylenimine)-grafted-goly[(aspartic acid)-co-lysine], biodegradable, cytotoxicity, transfection efficiency.

Gene therapy has been progressively developed with the hope that it will be an integral part of medical modalities in the future. Viral vectors, while efficient in gene transfer *in vivo*, pose a safety concern unlikely to abate soon, rendering synthetic carriers attractive alternatives. Among existing synthetic vectors, polycation has been extensively studied for gene delivery. However, their toxicity and biocompatibility *in vivo* still leave much to be desired. Biodegradable polycations are now emerging as a new generation of synthetic carriers. Biodegradable polymers, such as low molecular weight PEI and difunctional PEG copolymers connected with biodegradable linkage, polyphosphoester have been reported recently to mediate gene transfection *in vitro* and *in vivo*[1,2]. Here we report a new biodegradable polymeric carrier, namely poly(ethylenimine)-grafted-poly[(aspartic acid)-co-lysine] (PEI-ASP-co-LY) by thermal polycondensation of aspartic acid and lysine under reduced pressure, and low molecular weight of branch PEI act as side chain. The polymer, synthesized in high molecular weights, binds DNA effectively, degrades over days under PBS buffer and enzymatic solution condition, and shows remarkably low cytotoxicity.

Experimental

The synthetic route of poly(ethylenimine)-grafted-poly[(aspartic acid)-co-lysine] is shown in **Scheme 1**.

The polymer **1** was prepared according to the literature 3. Reacting polymer **1** with 30% excess of PEI in DMSO using Et_3N as a catalyst under nitrogen atmosphere yielded

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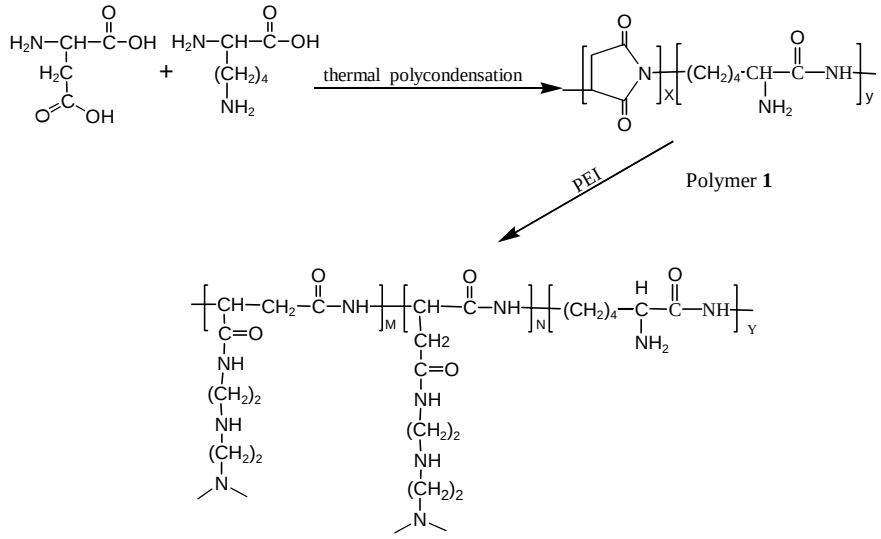
intermediate polymer **2**. Polymer **2** was precipitated in cold ethyl ether, dialysis for four days (dialysis tube will cut less than 10 KDa molecular weight), and lyophilize for two days.

Scheme 1 The synthetic route of PEI-ASP-co-LY



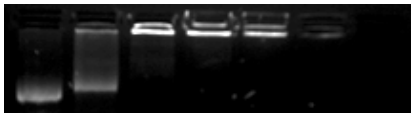
Results and Discussion

The structure of polymer **1** was confirmed by ^{1}H NMR (D_2O, 400MHz). In ^{1}H NMR spectrum, the peaks of δ 5.3 ppm (-NH-CO-**CH**-CH_2-CO-NH-) and δ 3.25 ppm (-NH-CO-CH-**CH_2**-CO-NH-) were assigned to ASP residue, δ 3.8 ppm (-CH_2-**CH**(NH_2)-CO-NH-) and δ 1.2-1.9 ppm(-CH_2-(-**CH_2)$_3$**-CH(NH_2)-), δ 2.9 ppm (-CO-NH-**CH_2**-(-CH_2)$_3$-) were assigned to LYS residue. The spectrum showed that the molecular ratio of ASP/PLY in polymer **1** was 1:1. For polymer **2**, after four days dialysis and lyophilize ^{1}H NMR showed that the peaks at δ 2.4-3.1 ppm were assigned PEI (-CH_2).

Poly (ethylenimine) is one of the efficient gene carriers with the highest cationic charge density potential. Branched PEI consists of primary, secondary and tertiary amines and is able to condense and delivery DNA[4,5]. High transfection efficiency of PEI, along with its cytotoxicity, strongly depends on the molecular weights. It is believed that PEI with a molecular weight higher than 25 KDa displays high transfection efficiency and cytotoxicity, while PEI with a molecular weight less than 2KDa shows almost no transfection efficiency but is less toxic[6].

Figure 1 PEI-ASP-co-LY readily formed complex with plasmid DNA in 5% glucose solution, as analyzed by gel electrophoresis **(Figure 1).** Complete binding of pRE-luc(N/P ratio 1.2-1.8, w/w).

Figure 1 Agarose gel electrophoresis of complexes of PEI-ASP-co-LY/DNA



W/w DNA 0.6/1 1.2/1 1.8/1 3/1 6/1 9/1

PEI-ASP-co-LY was designed to have nontoxic building blocks. The ultimate degradation products are expected to be α-amino acid and low molecular weight PEI, all with minimal toxicity profiles. Bioactivity evaluation of PEI-ASP-co-LY/pRE-luc complexes was performed with a cose 7 cell line. The cytotoxicity of PEI-ASP-co-LY was assessed in a 24-hour cell culture test in comparison with PEI (25KDa) and PEI(0.6KDa), using the MTT assay. As expected, PEI-ASP-co-LY and PEI (0.6KDa) have no significant change in cell morphology and proliferation rate was observed. In contrast, PEI (25KDa) exhibited much higher toxicity.

Figure 2 Cell toxicity of PEI-ASP-co-LY

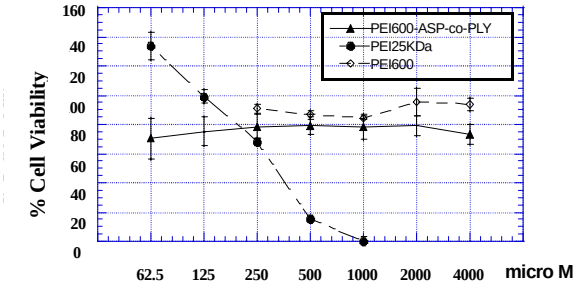


Figure 3 shows the relative pRE-luc activity of PEI-ASP-co-LY with different charge ratios between 7.5/1 and 20/1 to plasmid DNA and PEI with molecular weight of 0.6KDa, which is the same molecular weight of initial PEI used for the synthesis of PEI-ASP-co-LY, as a control. As expected after the low molecular weight of PEI conjugating to the co-polymer as a side chain, the transfection efficiency increased at the charge ratio at 7.5/1 up to 1540 fold higher than that of PEI600 with the initial molecular weight.

Figure 4 shows the he ASP-co-LY underwent degradation in PBS at 37°C. Because of two kinds of amino acids polycondensation in the backbone, the degradation kinetics of ASP-co-LY was followed by GPC analysis. The weight of PEI-ASP-co-LY dropped 27% after four days. In enzymatic solution, it degraded faster than in PBS buffer. The weight of ASP-co-LY was dropped from 32% in a 12 h in papain solution (1mg/mL, 37±0.1°C), and almost become small fragments after 24 h, and in trypsin enzymatic solution (1mg/mL, 37±0.1°C), the polymer got in small fragments after 12 h.

Figure 3 Transfection efficiency of PEI-ASP-co-LY/DNA

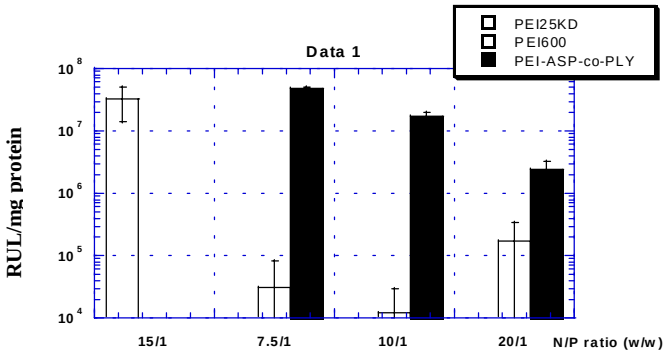
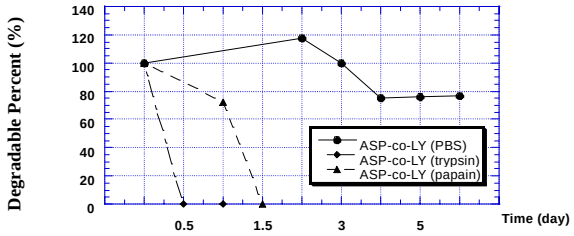


Figure 4 Degradability of ASP-co-LY in different solution



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