Effect of Poly(ethylene glycol) Grafting on Polyethylenimine as a Gene Transfer Vector *in vitro*

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To evaluate the non-ionic polymer, poly(ethylene glycol) (PEG), as a component in cationic copolymers for non-viral gene delivery systems, PEG was coupled to polyethylenimine (PEI). We present the effects of different degrees and shapes of pegylation of PEI on cytotoxicity, water solubility and transfection efficiency. This work reports the synthesis and characterization of a series of cationic copolymers on the basis of the conjugates of PEI with PEG. The modified molecules were significantly less toxic than the original polymer. Moreover, the chemical modification led to enhancement of their solubility. The comparison of pegylated PEIs with different degrees of derivation showed that all the polymers tested reached comparable levels of transgene expression to that of native PEI. As assessed by agarose gel electrophoresis, even highly substituted PEI derivatives were still able to form polyionic complexes with DNA. However, aside from an increase in solubility and retention of the ability to condense DNA, methoxy-PEG-modified PEIs resulted in a significant decrease in the transfection activity of the DNA complexes. In fact, the efficiency of the copolymer was compromised even at a low degree of modification suggesting that the PEG action resulting from its shape is important for efficient gene transfer. The mode of PEG grafting and the degree of modification influenced the transfection efficiency of PEI.

Keywords: Polyethylenimine, Poly(ethylene glycol), Cytotoxicity, Gene delivery.

Introduction

Genes are attractive candidates for therapeutic agents, and the development of efficient delivery systems for gene therapy has been a major challenge in the practical application of therapeutic genes. Non-viral gene transfer vectors based on DNA complexes with polycations have recently generated very significant interest in gene delivery applications. Among the large number of cationic polymers developed, polyethylenimine (PEI) was shown to be a useful carrier capable of condensing and delivering DNA *in vitro* and *in vivo*.[1-3] This polymer spontaneously forms polyionic complexes with DNA as a result of cooperative electrostatic interactions between the ammonium groups of the polycation and phosphate groups of the DNA.[4] However, there are still many issues to be dealt with such as the gene transfer potency, the half-life in the blood stream, and the biocompatibility deserving less cytotoxicity. Moreover, the polyionic complexes have a solubility problem due to the charge neutralization.

To overcome the solubility problem, cationic carriers with block or graft copolymer architecture consisting of polycationic polymers linked to a non-ionic water-soluble polymer PEG have recently been proposed.[4-15] The complexes formed with DNA remained in dispersion due to the effect of the nonionic PEG chains that are soluble in water. The cationic copolymers were mixed with DNA to produce stable complexes dispersed in aqueous media that can be expected to have high gene expression activity. However, there is relatively little data on the transfection activity of plasmid DNA incorporated into these systems *in vitro*. Furthermore, these systems have not been compared with commercially available transfection agents, such as PEI, dendrimers, or cationic liposomes.[6,12]

This polyether polymer appears to be worth exploring as a component in cationic copolymers for gene transfer. This study was undertaken to prepare a series of cationic copolymers by grafting PEI with PEG and in order to investigate the effects of different degrees and modes of pegylation on cytotoxicity, water solubility, and transfection efficiency.

Experimental Section

Materials. Methoxy-PEG (Mn 550), PEG bis(carboxyl methyl) ether (Mn 600), PEI (25 kDa) and other reagents for copolymer synthesis were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA).

Methods

Synthesis of (M)PEG-grafted PEI. General procedure for carboxylic acid formation. The methoxy-PEG-carboxylic acid was synthesized as follows (Figure 1). In a three-necked 250 mL flask under argon atmosphere was placed 1.20 g (50 mmol) of sodium hydride and 50 mL of dry tetrahydrofuran (THF) and then 2.75 g (5 mmol, 2.52 mL) of methoxy-PEG (MPEG) was dropped in at room temperature and stirred for 4 h. 8.35 g (75 mmol, 8.31 mL) of ethyl bromoacetate was added to this mixture and stirred at room temperature for 48 h. The solution was evaporated under a reduced pressure to

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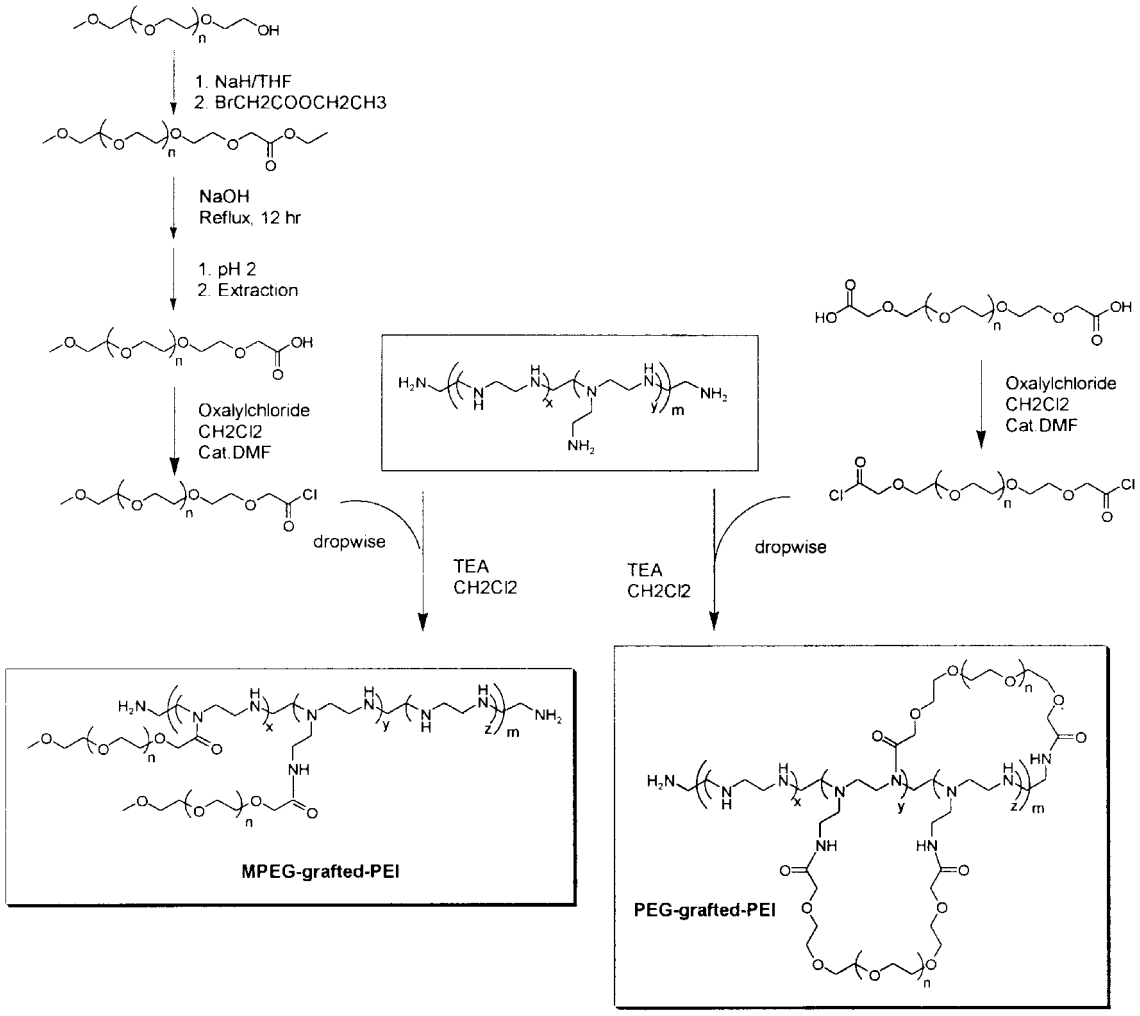

Figure 1. Scheme of the Synthesis of Cationic Copolymer. The ratio of PEG and PEI in the copolymer sample was determined from ^{1}H NMR spectra using integral values obtained for the -CH_2CH_2O- protons of PEG and -CH_2CH_2NH- protons of PEI.

remove almost all of THF and then poured into hexane to precipitate ethyl MPEG acetate, which was recovered by centrifuge (3000 rpm). The precipitate was evaporated to remove residual ethyl bromoacetate. The ethyl MPEG acetate was dissolved in 50 mL of 1N sodium hydroxide. After overnight in reflux, pH of the mixture was adjusted to 2. The MPEG carboxylic acid was extracted into chloroform. The organic extract was purified by washing with water and evaporated to give the MPEG carboxylic acid.

General Procedure for Acid Chloride Formation. For example, the MPEG acid chloride for 1 : 1 MPEG-grafted PEI was obtained as follows. Oxalyl chloride (0.64 mmol) was added dropwise to a solution of 70.0 mg (0.13 mmol) of MPEG and 2 drops of N,N-dimethylformamide (DMF) in 2 mL of dichloromethane (DCM) under Ar atmosphere. The reaction was allowed to reach completion for 2 h at room temperature. The excess oxalyl chloride was removed on the rotary evaporator by a stripping procedure with several portions of chloroform to give MPEG acid chloride as yellow oil that was used without any further purification. Acid chlorides for other copolymers were synthesized similarly except in scale. PEG bis(carboxyl methyl) ether was also used to activate carboxyl group.

General Coupling Procedure. For example, as for 1:1 MPEG-grafted PEI, MPEG acid chloride synthesized above (0.13 mmol) diluted in a small amount of dry DCM and then was added drop by drop to the solution of 70 mg (0.0028 mmol) of PEI and 35 mL (0.26 mmol) of triethylamine (TEA) in 3 mL of dry DCM at room temperature under Ar atmosphere. The reaction was allowed overnight to reach completion. The DCM was evaporated and the yellowish viscous product was obtained. Figure 1 represented the synthesis scheme of cationic copolymer.

Purification of Grafted Copolymers. The polymers were purified by dialysis against water using the Spectra/Por dialysis membrane (MWCO 3400, Spectrum, Los Angeles, CA). After evaporation of DCM, the viscous product was solubilized in distilled water. Each product was dialyzed for 1 day and lyophilized before use for analysis and assay. As the PEG derivatives used were small in molecular weight, uncoupled ones were efficiently removed from the mixture.

Ratio of PEG and PEI. The ratio of PEG and PEI in the

copolymer sample was determined from ^{1}H NMR spectra using integral values obtained from the number of -CH_2CH_2O- protons of PEG and -CH_2CH_2N- protons of PEI. Prior to NMR measurements all copolymers were purified from admixtures of non-conjugated PEG by dialysis (MWCO 3400) with distilled water and freeze-drying.

Water Solubility Test. The aqueous solubility of the plasmid DNA/carrier complex was determined by following method. The pSV-β-gal plasmid DNA was mixed with the carrier in 1 mL of HEPES buffer (20 mM, 0.15 M NaCl, pH 7.4) with final concentration of 50 μg/mL in 1.5 centrifuge tubes. After incubation for 30 min. at room temperature, the tubes were centrifuged for 5 min. at 10000 rpm. The supernatant was taken and its absorbance at 260 nm was measured for the determination of the content of DNA remaining in the solution. The DNA sample without a carrier was used as a control.

Agarose Gel Electrophoresis of Polyplexes. Solutions of cationic copolymers and DNA were prepared in distilled water and mixed each other to obtain a desired N/P ratio (charge ratio of total nitrogen in cationic copolymers to phosphate groups in DNA). Various amounts of cationic copolymer, ranging from N/P ratio of 0.1 to 16, were added to 1 μg of plasmid DNA and the mixtures were incubated for 30 min. at room temperature. Polyplexes were formed in 20 mM Hepes buffer, pH 7.4, containing 0.15 M NaCl. The sample buffer for gel electrophoresis was added to each sample, and it was electrophoresed on 1% (w/v) agarose gel for 90 min. at 100 V. TBE (45 mM Tris-Borate, 1 mM EDTA, pH 8.0) solution was used as electrophoresis buffer. The gel was stained with ethidium bromide (0.5 μg/mL) for 30 min. and illuminated with an ultraviolet (UV) illuminator to show the location of the DNA.

Cell Lines and Cell Cultures. NIH3T3 cells, a mouse embryonic cell line, were maintained in DMEM medium supplemented with 4.5 g/L glucose and 10% FBS at 37 ºC in a 5% CO_2 incubator. Cells were normally grown in 25 cm^2 polystyrene tissue culture flasks until they became approximately 70% confluent as assessed by light microscopy. The cells were then trypsinized with appropriate amount of 0.25% trypsin solution for 5-10 min, followed by addition of FBS-containing media in order to stop trypsin reaction. The detached cells were collected and then counted by hemacytometer. For the transfection and cytotoxicity study, the cells were seeded at a density of 10×10^4 cells/well, 600 μL/well, in 24-well flat-bottomed microassay plates and incubated for 24 h before the addition of either the plasmid DNA/carrier complex or the carrier only.

Cell Viability Assay. Evaluation of cytotoxicity was performed by the MTT assay.[13,15] MTT was dissolved in 1 × PBS at 2 mg/mL, filtered through 0.22 μm polycarbonate membrane filters to sterilize and remove a small amount of insoluble residue present in some batches of MTT. At the end of the transfection experiment, the transfection mixture was replaced with 100 mL of fresh medium, containing 10 % FBS. Twenty six microliters of 2 mg/mL MTT solution in 1 × PBS was then added. Plates were incubated for additional 4 hr at 37 ºC in a tissue culture incubator, then MTT-containing medium was aspirated off and 150 μL of DMSO was added to dissolve the formazan crystal formed by living cells. Absorbance was measured at 570 nm using a microplate reader at the end of 1 hr after adding dimethyl sulfoxide (DMSO). The cell viability (%) was calculated by the following equation;

$$\text{Cell viability (\%)} = (OD_{570(\text{sample})}/OD_{570(\text{control})}) \times 100$$

where the $OD_{570(\text{sample})}$ represents the measurement from the wells treated with various plasmid DNA/carrier complexes and the $OD_{570(\text{control})}$ represents the measurement from the wells treated with PBS buffer only.

Transfection Experiments. The *in vitro* transfection experi- ment was performed as follows. All reagent used in transfection experiments were sterilized by filtering through 0.22 μm polycarbonate membrane filters, except for pSV-β-gal through 0.45 μm one because of its size over 300 nm. Plasmid DNA/carrier complexes were prepared by mixing 2 μg of plasmid DNA with various amounts of carrier in 100 μL serum-free cell culture medium, and incubated for 30 min at room temperature. Medium from each well of the 24-well plate was replaced with 600 μL of transfection mixture and cell culture medium in the presence of serum. The cells were then incubated for 48 h at 37 ºC in 5% CO_2 incubator.

Cell Transfection Assay. The β-galactosidase activity in transfected cells was determined spectrophotometrically at 420 nm using *o*-nitrophenyl-β-D-galactopyranoside (ONPG). Briefly, the growth medium was removed from the cells in 24-well plates to be assayed and the cells were washed twice with 1 × PBS carefully in order to prevent cell detachment. The cells were lysed by adding 120 μL of 1 × reporter lysis buffer and incubating for 30 min. at room temperature with rocking the plate in the middle of the incubation period followed by scraping the cells. Then 100 μL of ONPG (1.33 mg/mL) in 2 × assay buffer was added to the lysed cells and the cells were incubated at 37 ºC for 4 hr. The reaction was terminated by adding 300 μL of 1 M sodium carbonate solution to each well and the absorbance at 420 nm was read with the microplate reader for the β-galactosidase activity.

Results

Synthesis of Cationic Block Copolymers. Polycation grafted copolymers were synthesized by conjugating PEI with small molecular weight PEG derivatives in different degrees. They were synthesized by grafting PEG chains to the amino groups of PEI. The scheme for the synthesis of polycationic graft copolymers is presented in Figure 1.

According to this scheme, carboxylated-PEG was chosen for the preparation of surface charge-masked PEI. All the coupling reactions were performed by converting the carboxyl group into the corresponding acid chloride. Each acid chloride was prepared using 2 equivalent oxalyl chloride and a catalytic amount of DMF in DCM. The excess of oxalyl chloride was removed on the rotary evaporator by a stripping procedure with chloroform. The amide bond was then

Table 1. Characteristics of the synthesized cationic copolymers

Copolymer name[a]	Reacting polymers[b]		Molecular characteristics of the synthesized copolymers				
	PEG, Da	PEI, kDa	PEG[c]	N[d]	PEG : PEI[e]	Modification degree[f], %	Molecular mass[f], kDa
1 : 1 PEG-g-PEI	600	25	40	501	0.97	13.8	49.3
3 : 1 PEG-g-PEI	600	25	133	315	3.20	45.8	105
1 : 1 MPEG-g-PEI	550	25	45	536	0.99	7.75	49.7
3 : 1 MPEG-g-PEI	550	25	127	454	2.79	21.9	94.8

[a]The nomenclature for PEG and PEI copolymers accounts for the ratio of PEG : PEI. [b]Molecular masses of the reacting polymers are presented as provided by the manufacturers. [c]The number of PEG. [d]The number of amino group in PEI. [e]As determined by ^{1}H NMR analysis of the copolymer samples. [f]Calculated based on ^{1}H NMR data assuming that all polyether chains in the copolymer samples are linked to the PEI.

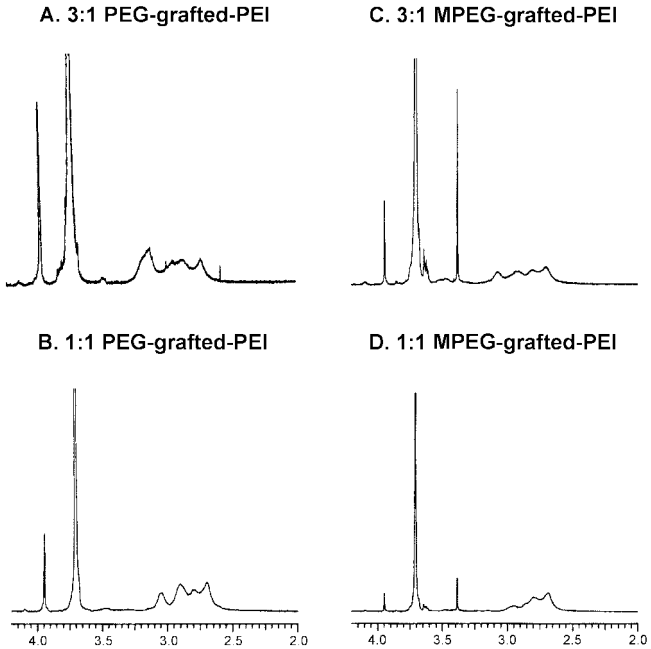

Figure 2. ^{1}H NMR Spectra of the Synthesized (M)PEG-grafted PEIs. ^{1}H NMR spectra was obtained in D_2O. The relative peak intensity of PEG to that of PEI in A and C is much higher than that in B and D, respectively.

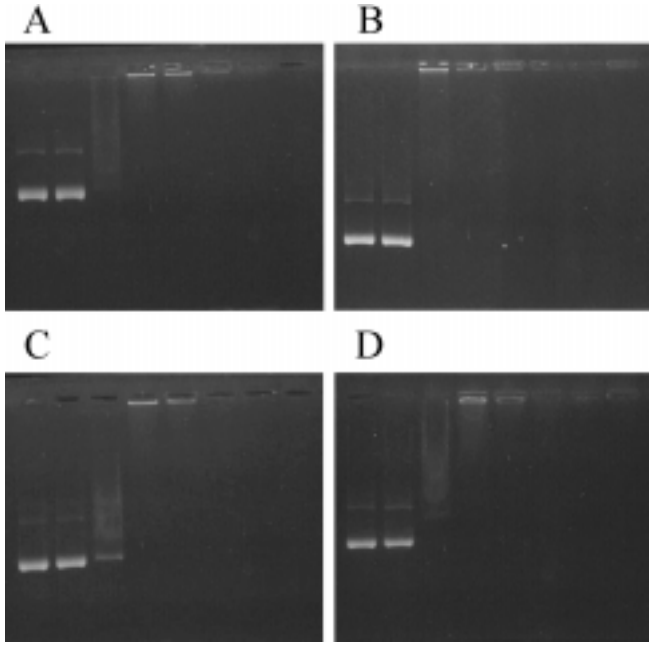

Figure 3. Electrophoretic Mobility Shift Assay using the Synthesized Cationic Copolymers. Complete retardation in all cationic polymers was achieved at and above 4 : 1 charge ratio of cationic copolymer/pSV-β-gal. Various amounts of each cationic copolymer, ranging from N/P ratio 0.1 to 16, were added to 1 μg of plasmid DNA. Lane 1 indicates pSV-β-gal without cationic polymer as control. Lane 2-8 indicates polyplexes at N/P ratio 0.1, 0.5, 1.0, 2.0, 4.0, 8.0, and 16, respectively. (A) MPEG-PEI 1 : 1, (B) MPEG-PEI 3 : 1, (C) PEG-PEI 1 : 1, and (D) PEG-PEI 3 : 1.

formed by reacting a selected amount of acid chloride with the amino groups in the presence of TEA in dry DCM.

The methoxy-PEG was prepared from PEG monomethyl ether by alkoxidation with sodium hydride in THF, followed by reaction with excess ethyl bromoacetate and then hydrolysis with sodium hydroxide. The formation of MPEG carboxylic acid was identified from the ^{1}H NMR spectra.

The nomenclatures and the characteristics of the cationic copolymers synthesized are presented in Table 1. The ratios of the PEG and PEI chains in the copolymer samples were determined from ^{1}H NMR spectra (Figure 2) by comparing the integral values obtained from the numbers of the -CH_2-CH_2O- protons of PEG and -CH_2CH_2N- protons of PEI. For the copolymer samples used in this work, PEG-grafted PEI and MPEG-grafted PEI, the molecular masses were calculated using the ^{1}H NMR analysis of the contents of PEG and PEI in these copolymers. According to the NMR data, PEG-grafted PEIs contain an average of 40 and 133 PEG segments per polycationic chain, respectively. MPEG-grafted PEIs have the average of 45 and 127 MPEG segments, respectively. The averaged values of 40 and 133 PEG chains in PEG-grafted PEIs correspond to the degrees of modification 13.8% and 45.8%, respectively. For MPEG-grafted PEIs, these copolymers also have similar degrees of modification varying from 7.75% to 21.9%, which correspond to the average value of 45 and 127 MPEG chains, respectively.

Agarose Gel Electrophoresis of Polyplexes. Varying amounts of each cationic copolymer, ranging from N/P ratios of 0.1 to 16, were added to 1 μg of plasmid DNA (Figure 3). Lane 1 indicates pSV-β-gal without cationic polymer as control. Lanes 2-8 indicate polyplexes at N/P ratios of 0.1, 0.5, 1.0, 2.0, 4.0, 8.0, and 16, respectively. Complete retardation in all cationic polymers was achieved at and above a 4 : 1 charge ratio of cationic copolymer / pSV-β-gal.

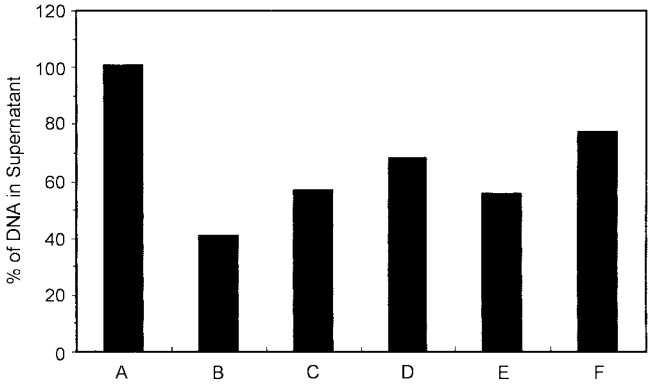

Figure 4. Water solubility test. DNA only (A), PEI (B), MPEG-PEI, 1 : 1 (C), 3 : 1 (D), PEG-PEI, 1 : 1 (E), and 3 : 1 (F).

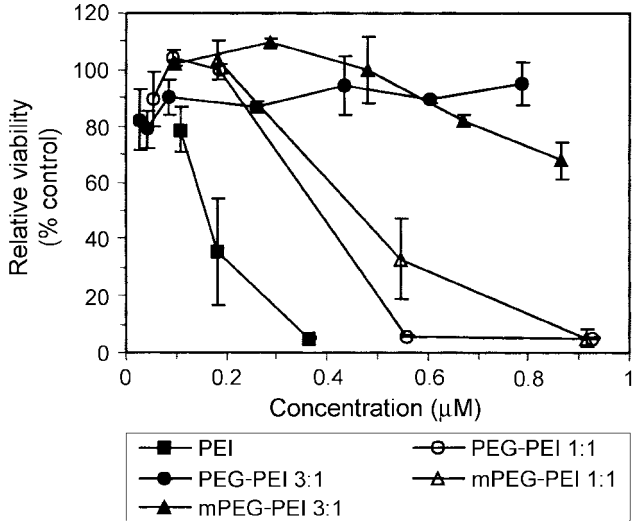

Figure 5. Comparison of cytotoxicity of (M)PEG-grafted PEIs. The gene carriers were incubated for 4 h with NIH/3T3 cells at 37 ºC in a 5% CO_2 incubator, and cell viability was determined using MTT assay method.

Water Solubility. As discussed earlier, an increase in the degree of charge neutralization of the DNA often results in extensive condensation and the separation of the DNA phase in the form of insoluble compact structures. Figure 4 shows that complexation between pSV-β-gal and PEI at a final DNA concentration of 50 μg/mL led to the formation of fine precipitates that sedimented upon centrifugation. On the other hand, water solubility of the complexes increased 1.5-2 fold by grafting PEG. When the 3 : 1 PEG-grafted PEI was used to make a complexe with pSV-β-gal, nearly 78% of DNA remained in the supernatant after centrifugation. So the attached PEG groups on PEI appear to prevent the pSV-β-gal/carrier complex from making fine precipitates and becoming insoluble.

Cytotoxicity of PEG-grafted PEIs. This work reports the effects of different degrees of pegylation of PEI on cytotoxicity by using a tetrazolium-based MTT colorimetric assay (Figure 5). NIH3T3 cells were incubated for 48 h with increasing amounts of polymers in the presence of serum.

With the PEI system, cell survival decreases by approximately 95% when the amount of DNA increases to 0.6 μM per well. On the other hand, modified PEI with PEG showed low cytotoxicity compared to the non-modified PEI. When the level of pegylation was increased to a 3 : 1 ratio, PEG-grafted PEI exhibited only very slight cytotoxicity on NIH/3T3 cells even at high concentrations. It showed above 80% cell viability for 1 μM of polymer. MPEG-grafted PEI also showed a comparably low cytotoxicity. This result led us to conclude that the PEG-modified PEI was significantly less toxic and that its biocompatibility increased with increasing degree of pegylation.

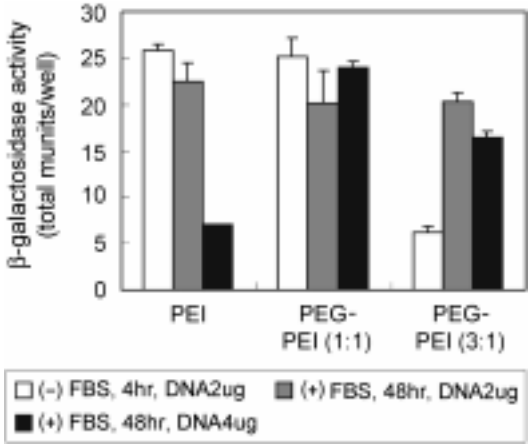

Figure 6. Transfection efficiency of PEG-PEI into NIH3T3 cells. Each value is the mean ± SD of three different experiments.

Transfection Efficiency of (M)PEG-grafted PEI. The effect of pegylation of PEI on the level of gene expression was investigated. Complexes formed between plasmid DNA and cationic copolymers were assessed for their *in vitro* transfection activity using a transient expression of galactosidase reporter gene.

A series of experiments were performed in the presence or absence of serum. Following 4 hr incubation in the absence of serum, the transfection efficiency of PEG-polyplexes decreased with an increase in PEG content although the decrease was minimal for 1 : 1 PEG-polyplexes. On the other hand, the pegylation did not affect the transfection efficiency of the complex for 48 hr transfection in the presence of serum. In addition, the pegylation increased the transfection efficiency of the complex at a higher DNA concentration (4 μg DNA). The results are demonstrated in Figure 6. What is

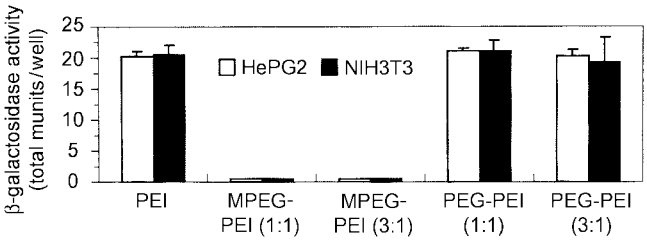

Figure 7. Transfection efficiency of (M)PEG-grafted PEIs. The concentration of pSV-β-gal was 2 μg per well of 24 well plate. The polyplexes of each gene carrier were incubated for 48 h in the presence of serum at 37 ºC in a 5% CO_2 incubator.

important is that, as for MPEG-grafted PEI, both polymers – 1 : 1 MPEG-grafted PEI and 3 : 1 MPEG-grafted PEI – showed no transfection efficiency (Figure 7). This indicates the importance of the PEG action resulting from its shape of graft for efficient gene transfer (see also below).

Influence of PEG Coupling Mode on Transfection Efficiency. To study the influence of pegylation on the transfection efficiency of native PEI, we synthesized several PEG-PEI derivatives according to the protocol described above. Transfection with polyplexes was performed at N/P ratios 5, 7 and 9. Agarose gel electrophoresis of polyplexes was carried out in parallel to the *in vitro* assays to verify that DNA was completely retarded by interaction with the polymer (Figure 3). Figure 7 summarizes the transfection efficiencies of the synthetic graft copolymers. It should be noted that as for MPEG-grafted PEIs, both 1 : 1 MPEG-grafted PEI and 3 : 1 MPEG-grafted PEI showed much reduced transfection efficiency. As assessed by agarose gel retardation assay, this phenomenon is not considered to result from a partial complexation of the DNA with highly modified polymer. It may be due to a decreased affinity of the polyplex for the cell surface leading to low transfection efficiency.

Discussion

The ultimate goal of designing synthetic gene delivery vehicles is to build multimolecular DNA/vector assemblies that are safe and efficient enough to be used in human therapy. We designed a new series of synthetic vectors using the approach of studying whether appropriate chemical modification of cytotoxic polymers can reduce their toxicity and, at the same time, retain their capability to efficiently condense DNA rather than synthesizing new types of polymers. The choice of PEI among chemicals from a catalogue (as opposed to the design and synthesis of a new molecule) was based on the hypothesis that the surface charge of PEI resulting in cytotoxicity could be masked at the same time as retaining its capability as a protonation reservoir of a molecule below neutrality leading to its transfection efficiency.[16] So we hypothesized that, by reducing the density of primary amino groups and introducing amphiphilic groups, such polymers could be detoxified and their ability to mediate transfection of eukaryotic cells could be enhanced. This relationship may be the basis for the design of new synthetic vectors, although the cellular mechanism underlying their action has yet to be analyzed.

This work reports the synthesis and characterization of a series of cationic copolymers on the basis of the conjugates of PEI with PEG. The initial objective for the development of cationic copolymers, such as PEG-based block or graft copolymers, was to increase solubility of the polycation-DNA complexes. This approach has been used to produce stable complexes dispersed in aqueous media that revealed high activity in regulating gene expression in *in vitro* and *in vivo* studies. Several systems have recently been described including PEG-b-polyspermine,[5,9] PEG-g-PEI,[4,9-11] PEG-b-poly(L-lysine),[6-8] PEG-b-poly(L-lysine) dendrimer,[12] poly(L-lysine) dendrimer-b-PEG-b-poly(L-lysine) dendrimer,[13] PEG-g-poly(L-lysine),[14] and lactose-PEG-grafted poly-L-lysine.[15] Dispersions of complexes of DNA in aqueous media were obtained using these copolymers.[4-15] However, little improvement in transfection activity of plasmid DNA was reported. Here, we suggest a good reference point for evaluating the effects of cationic polymer modification on gene transfer by considering different degrees and shapes of pegylation.

Pegylated PEI derivatives were synthesized. Above all, the modified molecules were significantly less toxic than the original polymer. It can also be seen that their biocompatibility increased with increasing degree of pegylation. Especially, it should be noted that using 4 μg of plasmid and PEG-grafted PEI at N/P ratio 7.0, the highest transfection activity was still obtained with little damage to cells. Owing to the lower toxicity of the modified PEI, it had an effective transfection activity even at higher concentration. On the contrary, PEI had a decreased transfection activity under the same conditions, 4 μg of plasmid and PEI at N/P ratio 7.4. Moreover, this chemical modification led to enhancement of its solubility. The improved solubility of the complexes formed by pegylated PEI is due to the effect of the hydrophilic PEG chain. In such systems, the ammonium groups of the PEI bind to the DNA phosphate groups, resulting in the formation of micelle-like species with a hydrophobic core from neutralized DNA and PEI chains and a hydrophilic corona from PEG segments.[17] Most likely, in the case of the pegylated PEI, incorporation of an excess of copolymer into the complex is prevented by steric repulsion of the PEG chains. In contrast, complexes formed by homopolymer PEI (25 kDa) at high N/P ratios contain a charged polycation 'corona' surrounding the neutralized polycation/DNA 'core'.[18] Interactions of the low molecular mass electrolytes and serum proteins with the polycation corona can affect the stability of these systems in aqueous dispersion.

It was also found that pegylation retained transfection efficiency comparable to non-modified PEI in cell transfection assays *in vitro*. The comparison of pegylated PEIs of different degrees of derivation showed that all synthetic polymers using PEG bis(carboxyl methyl) ether reached a high level of transgene expression, similar to that obtained with the PEI of 25 kDa. It should be noted that the result was obtained in the presence of serum. Considering the influence of pegylation on the transfection efficiency relating to the presence of serum, PEG modification may protect DNA complex from enzymatic attack. Of course, as assessed by gel retardation, even highly substituted PEI (with a ratio of 3 for the $-CH_2CH_2O-$ protons of PEG and $-CH_2CH_2N-$ protons of PEI) was still able to form complexes with DNA. However, aside from an increase in solubility and retention of the ability to condense with DNA, methoxy-PEG modification of PEI results in a significant decrease in the transfection activity of the DNA complexes. This result is in line with previous reports that the transfection activity of complexes with DNA of the PEG-grafted PEI decreased as the degree of grafting with PEG chains was increased.[4,9-11] Indeed, the activity of the copolymer was eliminated even at a low degree of modi-

fication.[19] The results show that PEG shape rather than modification degree is the significant factor that influences the transfection efficiency. As assessed by agarose gel retardation assay, this phenomenon is not due to a partial complexation of the DNA with highly modified polymer. The free linear PEG chains in methoxy-PEG grafted PEI are presumed to hinder the interaction of copolymer/DNA complexes with the cells.

In conclusion, the detoxification of cationic polymers seems to be a feasible approach to the development of new polymer-based synthetic gene delivery systems. This detoxification is meaningful because of its high water-solubility. However, it must be considered that pegylation of polymers used for gene transfer has some grafting limits in the mode of PEG conjugation and modification degree.

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