Effect of Polyethylene Glycol on Gene Delivery of Polyethylenimine

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Polyethylene glycol (PEG) has been coupled to many cationic polymers such as polyethylenimine (PEI) to improve the stability and transfection efficiency. We prepared PEG-grafted PEI with different lengths and amounts of PEG and used these graft copolymers as nonviral gene vectors. We measured the complex size and zeta-potential of polymer–DNA complexes in the presence of salt to estimate the stability of polymer–DNA complexes. We also investigated the cytotoxicity and transfection efficiency in C3 cells. In the case of graft copolymers, the stability of polymer–DNA complexes and transfection efficiency were affected by the graft length and amount of PEG side chain. PEG side chains stabilize the polymer–DNA complexes in the presence of salt, but the longer PEG side chains also interrupt the gene delivery in the cells due to the more efficient steric hindrance by longer PEG side chains, and therefore the transfection efficiency is decreased. Short PEG side chains with molecular weight of 350 kDa stabilized the polymer–DNA complexes without decreased transfection efficiency.

Key words polyethylenimine; polyethylene glycol; nonviral vector; gene expression; vector stability

Nonviral delivery systems based on DNA complexes with polycations have recently generated siginificant interest in gene delivery systems. In the gene delivery system using nonviral vectors, polyethylenimine (PEI) was shown to be a useful carrier capable of condensing and delivering DNA *in vitro* and *in vivo*.[1—3] PEI forms polymer–DNA complexes that are stable against a aggregation in physiological buffer conditions and has a strong buffering capacity at almost any pH owing to the numerous number of primary, secondary and tertiary amino groups,[4] resulting in the potentially excellent safety profile of such complexes. However, there are still many issues to be dealt with such as the transfection efficiency, aggregation and half-life in the bloodstream, biocompatibility with less cytotoxicity, and solubility problems due to charge neutralization. To overcome such problems, some research groups proposed the use of cationic carriers with block or graft copolymer architecture consisting of polycationic polymers linked to a nonionic water-soluble polymer such as polyethylene glycol (PEG).[5—7] PEG has been used in many drug delivery systems because of its high solubility in water, nonimmunogenicity, and improved biocompatibility.[8] In gene delivery systems, PEG has also been coupled to polycationic polymers[9] or liposomes[10] to improve the solubility of complexes and transfection efficiency. It is also possible to incorporate receptor-binding moieties such as antibodies into PEI to target certain tissues,[11,12] including tumors.[13] However, to the best of our knowledge, there has been no systematic study on the effect of the length of the PEG side chain on gene delivery and the stability of cationic polymer–DNA complexes with salt.

In the current work, we studied the effect of the length of the PEG side chain on gene delivery and the stability of cationic polymer–DNA complexes. We prepared graft copolymers with different graft lengths and amotunts of PEG by the novel coupling reaction of epoxide and amine. We observed the change in the size and zeta-potential of polymer–DNA complexes based on the length and amount of PEG side chains. We also investigated the relationship between the transfection efficiency and cytotoxicity of graft copolymers and the structure of PEG side chains. From these results, we elucidated the effect of the length of the PEG side chain and the amount of PEG on the gene delivery process in cells.

MATERIALS AND METHODS

Chemicals Methoxy-PEG (MPEG, M_n 350, 750, 1900), epichlorohydrin, sodium hydride, branched PEI (25 kDa), and other reagents for graft copolymer synthesis were obtained from the Sigma-Aldrich Chemical (St. Louis, MO, U.S.A.).

Cell Line The C3 tumor cell line, derived from murine fibroblasts of C57BL/6 origin by human papillomavirus (HPV) infection, was grown in 2-mercaptoethanol. All the cells were maintained at 37 °C in a humidified, 5% CO_2 incubator.[14]

Plasmid DNA Plasmid pCMVluc, coding for the Photinus pyralis luciferase gene under the control of the cytomegalovirus immediate-early enhancer/promoter (Promega, Madison, WI, U.S.A.), has been described by Plank *et al.*[15] For further purification, plasmid pCMVluc solution was subjected to double CsCl steps and linear gradient centrifugation.[16]

Synthesis of Epoxide Terminated MPEG Epoxide-terminated MPEG was synthesized as follows.[17] Sodium hydride 1.37 g and epichlorohydrin 5.31 g were added to 200 ml of dry tetrahydrofuran (THF) under an argon atmosphere. Then MPEG 10 g (Mn 350, 750, 1900) was added at 40 °C and stirred for 2 h. After the reaction, the mixture was poured into diethyl ether and the supernatant was recovered by centrifugation (3000 rpm). The supernatant was evaporated to remove residual diethyl ether and the product was dried *in vacuo*.

Synthesis of PEI-Grafted PEG The epoxide-terminated MPEG and PEI (25 kDa) at the different weight ratios were added to THF 5 ml, and stirred at 50 °C for 50 h, and the reaction mixture was poured into diethyl ether, followed by

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collection of a viscous yellow product. The product was purified by dialysis against water using the Spectra/Por dialysis membrane (MWCO 3500, Spectrum, Los Angeles, CA, U.S.A.). The viscous product was dissolved in distilled water and the solution was dialyzed for 1 d and dried *in vacuo* for 1 d. As the PEG derivatives used were small in molecular weight, unreacted ones were efficiently removed from the mixture.

Characterization of PEI-Grafted PEG The structures of the graft copolymer were characterized using proton nuclear magnetic resonance (NMR) spectroscopy (Bruker-AMX-500 NMR spectrometer, Bruker). For NMR experiment, we prepared the polymer solutions (0.1 g/ml) with D_2O. The weight ratio of PEI and PEG in the graft copolymer sample was determined from the peak area of the protons of PEG and PEI in the ^{1}H-NMR spectra.

Thermal analysis of the graft copolymer was carried out under a nitrogen atmosphere using the DuPont Thermal Analyst 2010 model differential scanning calorimeter (DSC). All the measurements were carried out at a heating rate of 10 °C/min under a nitrogen atmosphere.

Agarose Gel Electrophoresis of PEI-g-PEG To demonstrate the interaction between positively charged PEI-grafted PEG and negatively charged plasmid DNA, a gel retardation assay was performed by electrophoresis. Complexes between PEI-grafted PEG and plasmid DNA were formed at various N/P ratios, ranging from 1 to 5. The complexes were incubated for 30 min at 37 °C and subsequently run on a 0.8% agarose gel. The retardation of their complexes was visualized by ethidium bromide staining.

Measurement of Particle Size and Zeta-Potential of PEI-Grafted PEG/DNA Complexes PEI homopolymer and PEI-grafted PEG dissolved in deionized water were added to deionized water containing a fixed amount of plasmid DNA (20 μg/ml), and NaCl was added to each polymer–DNA complex (final concentration of NaCl 10 mM), and the size of the complex was measured. We also measured the complex size 30 min and 1 h after NaCl addition. The size of the complex in aqueous solution was determined by dynamic light scattering (Brookhaven Instruments Corporation, Holtsville, NY, U.S.A.). Every measurement was carried out in duplicate every 2 min.

For estimation of the surface charge, PEI-grafted PEG/DNA complexes were diluted in 10 mM NaCl aqueous solution and the zeta-potential was measured immediately using dynamic light scattering (Brookhaven Instruments). The data represent the means of at least three measurements.

Cytotoxicity Assay C3 cells were grown in 96-well plates at an initial density of 1×10^4 cells/well in 0.2 ml of growth medium and were incubated in a 5% environment of CO_2 for 24 h. The growth medium was replaced with a new growth medium containing polymer–DNA complexes. After 24-h incubation, the metabolic activity of C3 cells in each well was measured using the thiazolyl blue (3-[4,5-dimethylthiazol-2-y]-2,5-diphenyltetrazolium bromide), (MTT) assay.[18]

Transfection and Determination of Reporter Activity One day before transfection, 3×10^4 cells/well were seeded in 24-well plates (Costar, Corning, NY, U.S.A.) so that they could reach 60—70% confluence during transfection. All experiments were done in triplicate. For transfection, the desired amount of PEI-grafted PEG was added to 1 μg of DNA and allowed to incubate at room temperature in a microcentrifuge tube for 10—15 min. During this incubation, the medium in the cell wells was replaced with 400 μl of complete Iscove's modified Dulbecco's medium including 8% serum, and the cells were allowed to adjust to the new conditions for at least 20 min at 37 °C in a 5% CO_2 incubator. After incubation of the transfection solution, the solutions were adjusted to 100 μl of 150 mM NaCl. The entire 100 μl was added to the cell well and allowed to incubate at 37 °C in a 5% CO_2 atmosphere for 2—4 h. As a positive control, transfection using the PEI homopolymer was performed.

Cells were harvested 24 h after transfection, washed in PBS, and lysed with 250 mM Tris–C1(pH 7.8)/1 mM DTT by means of three freeze-thaw cycles. The cell lysates were centrifuged for 15 min at 12000 rpm to pellet debris. Luciferase light units were recorded (Biolumat LB 9500, Berthold, Paris, France) from an aliquot of the supernatants (20 μl/total 40 μl) with 30-s integration after automatic injection of the luciferin solution.

RESULTS

Synthesis of PEI-Grafted PEG PEI-grafted PEG copolymers with different graft lengths and graft frequencies were synthesized by conjugating PEI with small molecular weight epoxide-terminated MPEG. The grafting reaction was performed by the reaction of the epoxide group of epoxide-terminated MPEG and amino groups of PEI (Fig. 1).[19] In comparison with the PEG coupling reaction proposed by other research groups, our coupling reaction based on the reaction of epoxide and amine is simpler and there is no byproduct such as hydrogen chloride. This reaction might also be applied to the coupling reaction of receptors such as antibodies to PEI for targeted gene delivery.

The epoxide-terminated MPEGs with different molecular weights were prepared from the reaction of epichlorohydrin with different molecular weight MPEGs (M_n 350, 750, 1900). The graft frequency of PEG side chains was controlled by adjusting the weight ratio of the epoxide-terminated MPEG and PEI in the feed for the coupling reaction. Table 1 shows the weight ratio of PEG to PEI and the graft frequency of PEG to PEI in the graft copolymers. For brevity, PEI-PEG graft copolymers are designated in this paper as *m–n*, where *m* indicates the molecular weight of MPEG in the epoxide-terminated MPEG and *n* indicates the relative weight ratio (%) of PEG to PEI in the graft copolymer. For example, 350-4 means that MPEG (M_n 350) was grafted onto PEI and the content of the PEG side chains was 4 weight percent of the PEI main chain. The weight ratio of PEI and PEG units in the graft copolymer and the graft frequency of PEG were calculated from the peak area corresponding to the protons of the PEG and PEI units. The 350-80 and 750-84 have same content of PEG in weight in the graft copolymer but the length of the PEG side chain is different. By investigating the two graft copolymers containing the same weight-content of PEG but have different molecular weights, the effect of the length of the PEG side chain on the properties of graft copolymers could be also investigated.

Agarose Gel Electrophoresis To demonstrate the interaction between the positively charged PEI-grafted PEG and

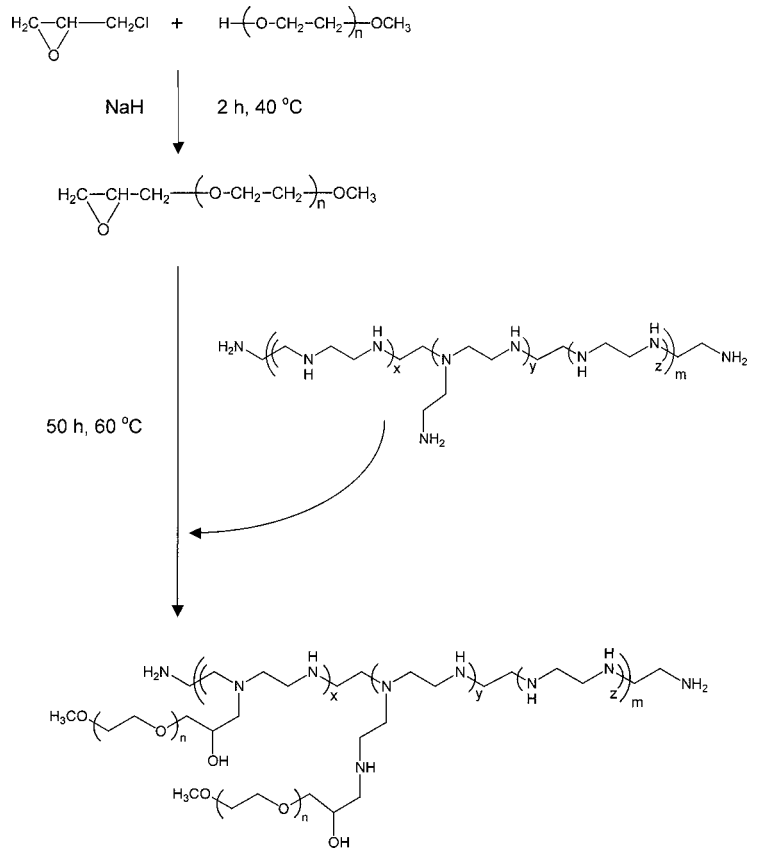

Fig. 1. Scheme of the Synthesis of PEI-Grafted PEG

Epichlorohydrin and MPEG are reacted in THF solvent at 40 °C for 2 h to obtain the epoxide-terminated MPEG. The epoxide-terminated MPEG was reacted with PEI 25 kDa at 60 °C for 50 h. The product was precipitated in diethyl ether and dried *in vacuo*. The graft copolymer was purified by dialysis against water.

Table 1. Characteristics of the Synthesized PEI-Grafted PEG Copolymers

Copolymer[a)]	Reaction reagent[b)]		Molecular characteristics of synthesized copolymers			
	PEI (kDa)	MPEG (Da)	Number of MPEG[c)]	PEG : PEI weight ratio[d)]	Modification degree (%)[d)]	Molecular weight (kDa)[d)]
350-4	25	350	3	0.04	0.3	26.1
350-13	25	350	9	0.13	1.5	28.1
350-80	25	350	57	0.80	9.8	45.0
750-6	25	750	2	0.06	0.3	26.5
750-30	25	750	10	0.30	1.7	32.5
750-84	25	750	28	0.84	4.8	46.0
1900-15	25	1900	2	0.15	0.3	28.8
1900-198	25	1900	26	1.98	4.5	74.4

a) The nomenclature for PEI-grafted PEG copolymers accounts for the molecular weights and amount of MPEG. *b*) Molecular masses of the reacting polymers are presented as provided by manufacturers. *c*) As determined by ^{1}H-NMR analysis of the copolymer samples. *d*) Calculated based on ^{1}H-NMR data assuming that all MPEG chains in the copolymer samples are linked to PEI.

the negatively charged plasmid DNA, a gel retardation assay was performed using electrophoresis. Figure 2 shows the electrophoretic mobility shift assay for the PEI homopolymer and 350, 750, and 1900 series graft copolymers. The plasmid DNA is observed from the bottom to top of lane 1 in each set. Complete retardation of complexes was observed when the graft copolymer and plasmid DNA were compacted at a very low N/P ratio, above 1, for all cases of the PEI homopolymer and graft copolymers. This result indicates that the PEG side chains have no significant effect on the complex formation between the PEI main chains and the anionic plasmid DNA by ionic interaction.

Complex Size and Measurements DNA and PEI-grafted PEG were mixed at the optimum polymer–DNA weight ratio for best transfection and NaCl salt was added into the aqueous solutions of polymer–DNA complexes. The effective diameter of the complex was then measured over time are to investigate the effect of salt on the size of the polymer–DNA complexes. Figure 3 shows the size of the complexes formed by the 350, 750, and 1900 series graft copolymers and the plasmid DNA in the presence of NaCl salt.

Immediately after the NaCl salt was added to the aqueous solution of the polymer–DNA complexes, the complex size

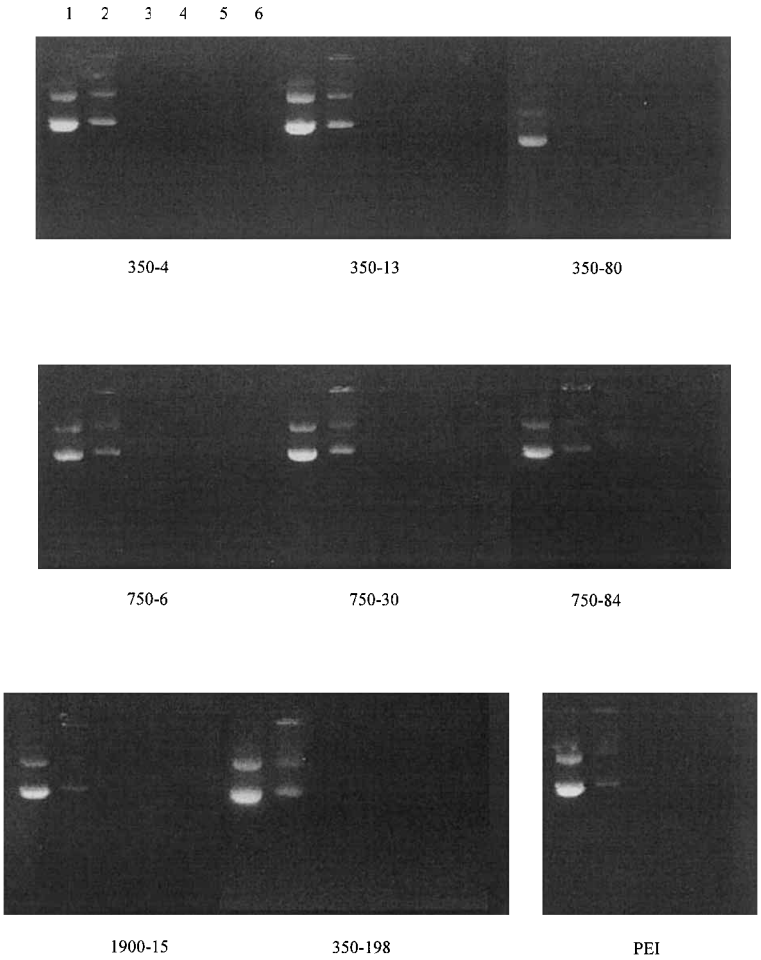

Fig. 2. Agarose Gel Electrophoresis of pCMVLuc (Lane 1) and Its Complexes with Various PEI-Grafted PEG Copolymers at Different N/P Ratios: 1 (Lane 2), 2 (Lane 3), 3 (Lane 4), 4 (Lane 5), 5 (Lane 6)

of 350 series graft copolymers was about 130 nm for all cases. However, with time the complex size increased and the range of size increase was different for each copolymer. The complex size of PEI 350-4 and 350-13 gradually increased with time, reaching about 400—500 nm 60 min after NaCl salt addition; this phenomenon might be attributed to NaCl salt aggregation into the polymer–DNA complexes due to the interaction of NaCl and the PEI main chain. However, in contrast to PEI, 350-4 and 350-13, the complex size of PEI 350-80 was 160 nm at the moment of salt addition and maintained the initial size over time. The change in the complex size for the 750 series graft copolymers with time after salt addition (Fig. 3b) was similar to that for the 350 series. The change in the complex size for the 1900 series graft copolymers with time after salt addition is shown in Fig. 3c, but the result was different from those for 350 and 750 series graft copolymers. The complex sizes of PEI 1900-15 and 1900-198 were about 100 nm without NaCl salt and did not change with time in the presence of NaCl salt. In the case of the 1900 series graft copolymers, the size of polymer–DNA complexes was not affected by the amount of PEG side chains in the graft copolymer.

Zeta-Potential Measurements To investigate the effect of surface charge on complex size, we measured the zeta-potential of complexes. Figure 4 shows the zeta-potential of polymer–DNA complexes for 350, 750, and 1900 series graft copolymers. In the case of the PEI homopolymer, the zeta-potential of the polymer–DNA complex at an N/P ratio of 10 was about 20 mV. In the case of the 350 series graft copolymers, the surface charge of polymer–DNA complexes gradually decreased with increasing amounts of PEG side chain, and the minimum level of zeta potential was around 10 mV for PEI 350-80. For the 750 series graft copolymers, the extent of decrease in surface charge was much larger than that for the 350 series graft copolymers, and the zeta-potential was about 4 mV for PEI 750-84, which contains the same amount of PEG as 350-80. In comparison with the 350 and 750 series graft copolymers, the surface charge of the 1900 series graft copolymers abruptly decreased to the saturated value of 2 mV with a smaller amount of PEG side chain.

Cytotoxicity Test To evaluate the cytotoxicities of PEI and graft copolymers, the colorimetric assay was used (Fig. 5). Cells incubated with PEI at an N/P ratio of 10 showed approximately 30% cell viability and decreased with increasing N/P ratio. In the case of the 350 series graft copolymers, cell viability was enhanced by grafting PEG side chains onto PEI, and cells incubated with PEI 350-80 at an N/P ratio of 10 had maximum level of cell viability of around 55%. With the increase in the molecular weight of PEG side chains (750, 1900), cell viability shows a remarkable increment compared with short PEG side chains with molecular weight of 350 kDa. In the case of the 1900 series graft copolymers,

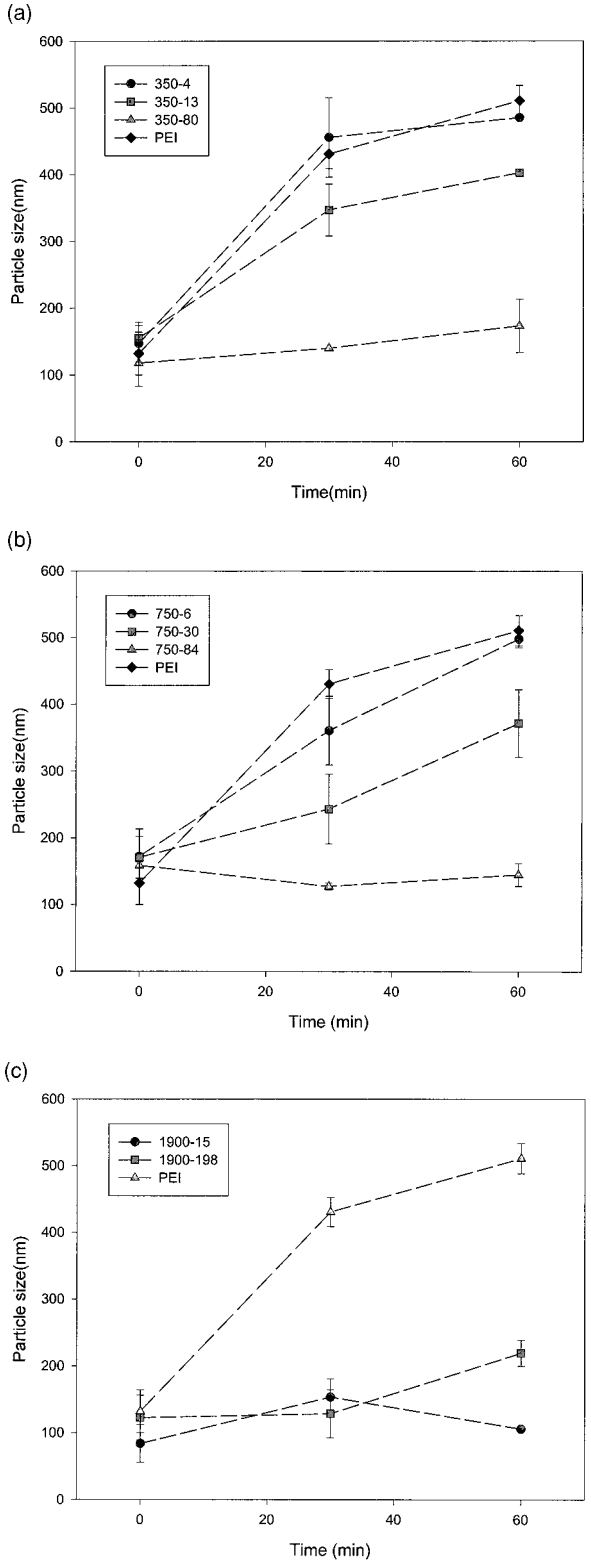

Fig. 3. Effective Diameters of the Complexes Formed between pCMVLuc Plasmid and (a) 350 Series, (b) 750 Series, and (c) 1900 Series Graft Copolymers as a Function of the Time after NaCl Salt Addition

Effective diameters of the complexes formed between the pCMVLuc plasmid and PEI homopolymer are shown for comparison. The measurements were carried out after 0 min, 30 min, and 60 min of complex formation.

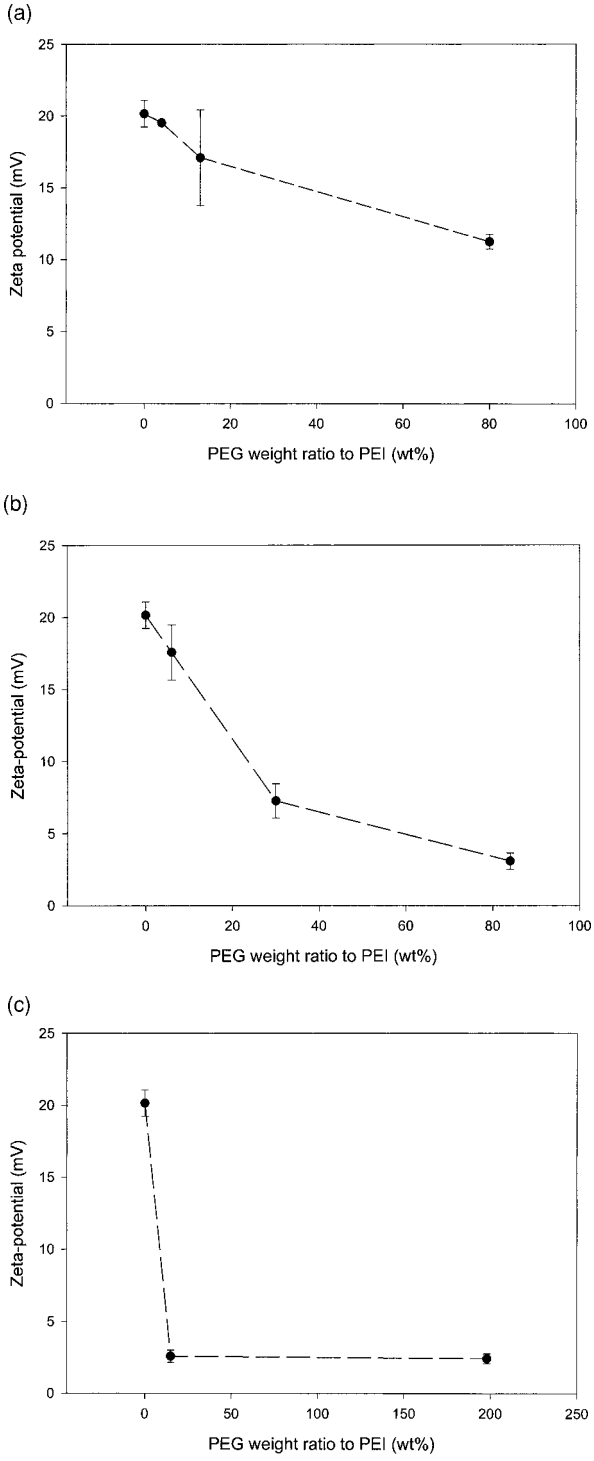

Fig. 4. Zeta-Potential of Polymer–DNA Complexes for (a) 350 Series, (b) 750 Series, and (c) 1900 Series Graft Copolymers as a Function of PEG Weight Ratio to PEI at a Fixed N/P Ratio of 10

cells showed more than 90% viability at an N/P ratio of 10 regardless of the amount of PEG side chains in graft copolymer. The longer PEG side chains also alleviate the diminution of cell viability with the increase in the N/P ratio.

Transfection Test The effect of PEGylation of PEI on the level of gene expression was investigated. First, we investigated the transfection efficiency of grafted copolymers with different PEG side chain lengths and then compared the transfection efficiency of grafted copolymer complexes with the same amount of PEG that had different molecular weights. Figure 6 shows the transfection efficiency of PEI-grafted PEG copolymers with different graft frequency in C3

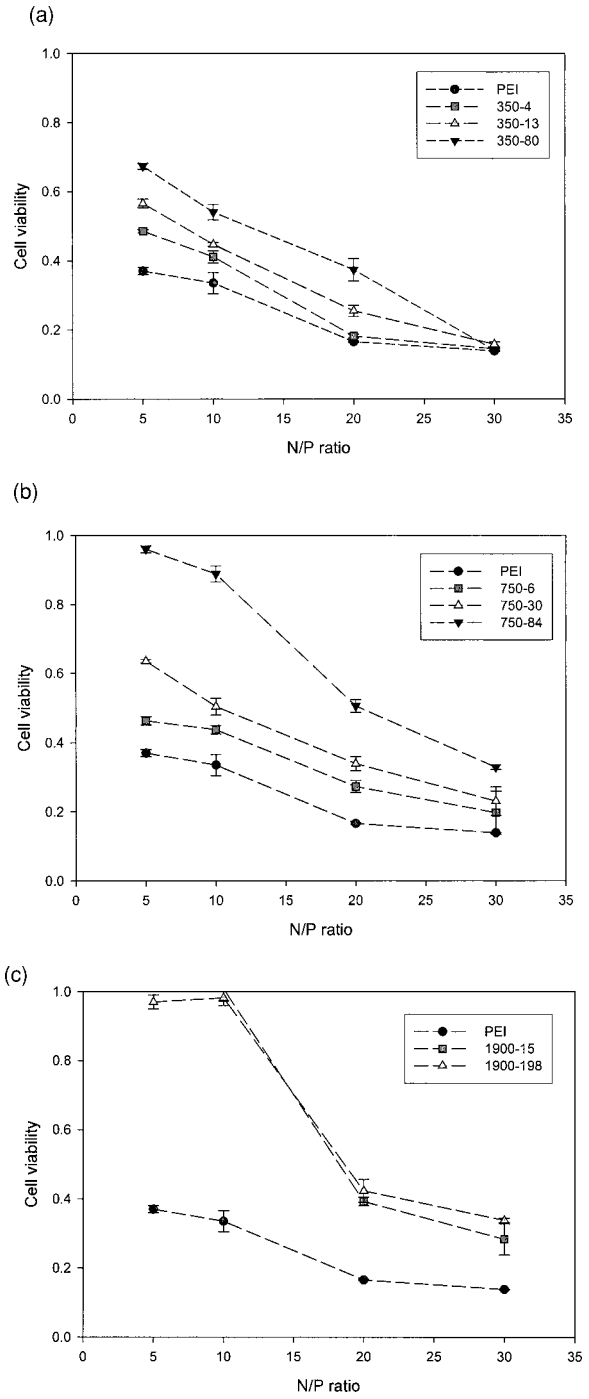

Fig. 5. Viability of C3 Cells as a Function of N/P Ratio of (a) 350 Series, (b) 750 Series, and (c) 1900 Series Graft Copolymers

Cell viability was determined using the MTT assay, and each assay was repeated at least three times to ensure reproducibility.

cells.

In the case of the PEI homopolymer, the transfection efficiency showed the maximum value at an N/P ratio of 15 and decreased with increasing N/P ratio, which is usual for the PEI homopolymer. The transfection efficiency of PEI 350-4, 350-13, and 350-80 showed nearly the same tendency as the PEI homopolymer, and the maximum transfection efficiency was also similar to that of the PEI homopolymer.

The changes in PEI and 750 series graft copolymers with increasing N/P ratio are shown in Fig. 6b. The transfection efficiency of PEI 750-6 showed a similar tendency to that of the PEI homopolymer, but the transfection efficiencies of PEI 750-30 and 750-84 were different tendency. For PEI 750-30, the transfection efficiency was maximal at an N/P ratio of 15 and decreased with increasing N/P ratio. The maximum value of the transfection efficiency of PEI 750-30 was slightly lower than that of the PEI homopolymer. The shift of the optimum N/P ratio from 10 to 15 might be attributed to the PEG side chain with molecular weight of 750 kDa. For PEI 750-84, the change in transfection efficiencies with various N/P ratios was much different from that of the PEI homopolymer. The transfection efficiency increased gradually with increasing N/P ratio and showed peak values above the N/P ratio of 25. The maximum value of the transfection efficiency of PEI 750-84 decreased by one order compared with that of the PEI homopolymer.

The changes in transfection efficiency of PEI and 1900 series graft copolymers with increasing N/P ratio are shown in Fig. 6c. The transfection efficiency of PEI 1900-15 and 1900-198 showed a different tendency compared with that of the PEI homopolymer. The PEI 1900-15-mediated gene expression was the highest at an N/P ratio of 15, which was the same N/P ratio as for the PEI homopolymer, and decreased with increasing N/P ratio. However, the maximum value of transfection efficiency decreased by one order in comparison with that of the PEI homopolymer. This result may be attributed to the longer PEG side chain in the PEI 1900-15 copolymer. Compared with PEI 350-13, the transfection efficiency of PEI 1900-15 was much lower than that of PEI 350-13. The transfection efficiency of the PEI 1900-198 complex increased gradually with increasing N/P ratio and showed the maximum value at 20, which was much lower than that of the PEI/DNA complex, and decreased with increasing N/P ratio.

DISCUSSION

It has been reported that hydrophilic polymers on the surface of liposomes or DNA/transferrin-PEI complexes can protect them from salt-induced aggregation.[20—23] In an analogous fashion, this study examined whether the PEG side chains sterically stabilize the polymer–DNA complexes in the presence of salt. The PEI 350, 750, and 1900 series graft copolymers that contained different amounts of PEG with different molecular weights (350, 750, 1900 kDa) had different polymer–DNA complexe sizes in the presence of NaCl salt (see Fig. 4). In the case of the PEI 350 and 750 series graft copolymers, the complex size was stable when the amount of PEG in the copolymer was about 80 weight percent. On the other hand, the complex size of the PEI 1900 series graft copolymers was stable for both 1900-15 and 1900-198. To stabilize the complex size, the PEG side chains might have to cover the surfaces of complexes. Because of the short length of the PEG side chains, in the PEI 350 and 750 graft copolymers they could cover the surface of complexes sufficiently only when the amount of PEG was large. In the case of the PEI 1900 series graft copolymers, the longer side chains of PEG might cover the surface of the complexes more easily and thus the complex size did not increase in the presence of NaCl salt in spite of the smaller amount of PEG compared with the PEI 350 and 750 series graft copolymers. Based on these results, we can assume that

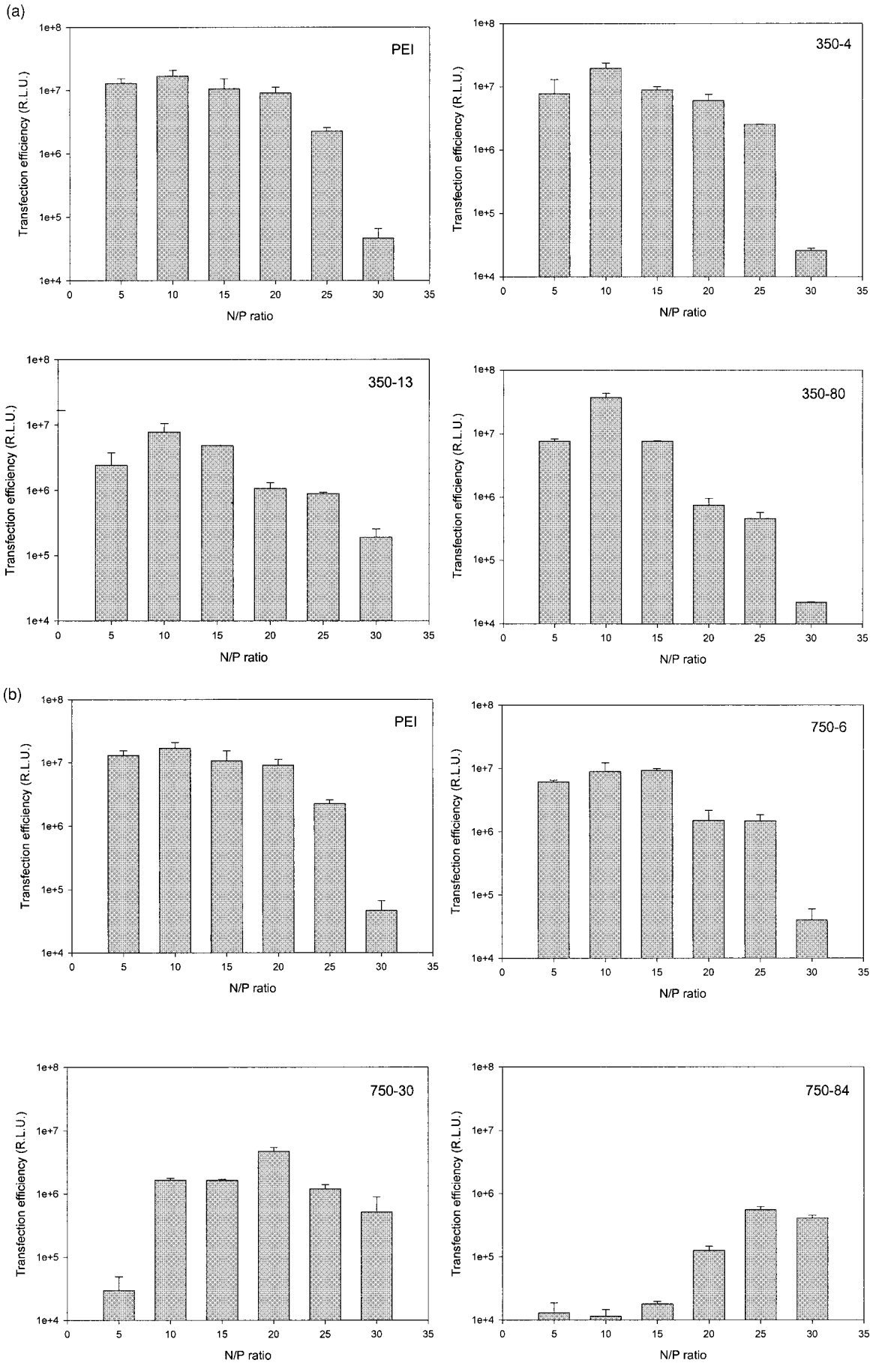
(a)
PEI
350-4
350-13
350-80
(b)
PEI
750-6
750-30
750-84
Transfection efficiency (R.L.U.)
N/P ratio
1e+8
1e+7
1e+6
1e+5
1e+4
0
5
10
15
20
25
30
35

Fig. 6

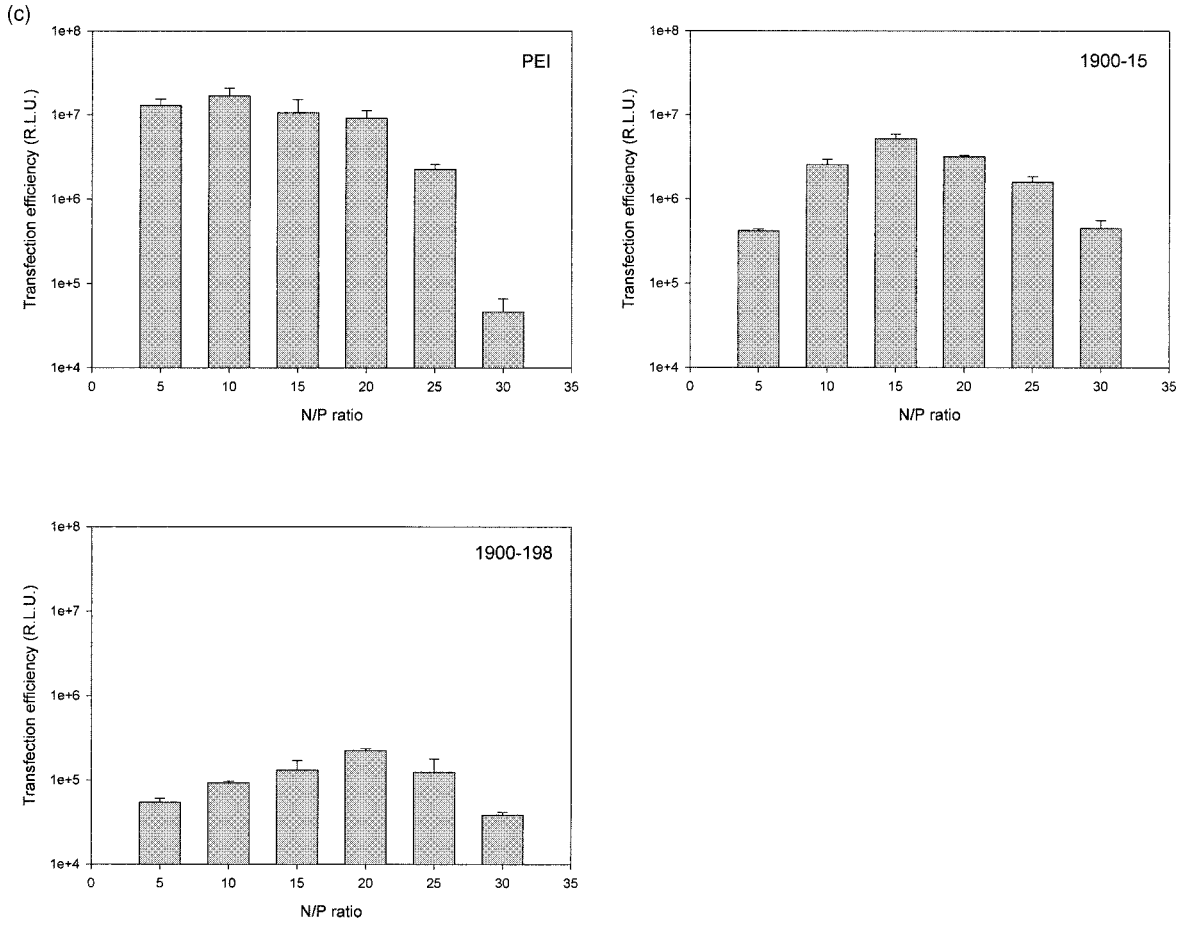

Fig. 6. Transfection of C3 Cells with the pCMVLuc Plasmid Formulated with (a) 350 Series, (b)750 Series, and (c)1900 Series Graft Copolymers at Various N/P Ratios

Transfection of the pCMVLuc plasmid formulated with the PEI homopolymer is shown for comparison. Transfection was carried out on C3 cells at pCMVLuc 1 μg per well for 24 h at 37 °C. After transfection, the cells were harvested for the measurement of luciferase activity.

the PEG side chains allow the polymer–DNA complexes to be protected from aggregation in the presence of NaCl salt, the longer PEG side chains could cover the surface of complexes more effectively, and thus the graft copolymers containing longer PEG side chains show greater stability against aggregation.

To elucidate the effect of the length of the PEG side chain on the surface coverage, chain we measured the zeta-potential for polymer–DNA complexes (see Fig. 5). In the case of the PEI 350 and 750 series graft copolymers, a larger amount of PEG side chain is required to decrease the surface charge. However, in the case of the PEI 1900 series graft copolymers, less PEG side chain is required to show a similar effect on the surface charge. From the zeta-potential results, we confirmed that the longer PEG side chain decreases the surface charge of polymer–DNA complexes more easily and this phenomenon might be attributed to more effective surface coverage by longer PEG side chains. In the case of short PEG side chains, a much larger amount of PEG is required to obtain effective surface coverage.

The cytotoxicity of graft copolymers was also affected by the length of PEG side chains. The decrement in cytotoxicity of the PEI 350 series graft copolymers was much smaller than that of the 750 and 1900 series, and this might be related to the surface charge of polymer–DNA complexes. In the case of the PEI 350 series graft copolymers, the relatively high surface charge of polymer–DNA complexes could induce considerable damage to cells, and thus the PEI 350 series graft copolymers showed relatively higher cytotoxicity compared with the PEI 750 and 1900 series graft copolymers.

PEG side chains can also affect the gene delivery of polymer–DNA complexes to cells. In an *in vitro* transfection test, we investigated the effect of the length and amount of PEG side chains on transfection efficiency (see Fig. 6). In the transfection test, the length of the PEG side chain was the dominant factor in transfection efficiency. When the PEG side chain was short such as molecular of weight 350 kDa, it had little effect on the transfection efficiency. However, with the increase in PEG side chain length, the effect became more dominant on transfection efficiency, which suggests that the longer PEG side chains have some effect on the process of gene delivery to the cells. In the previous paragraph, we suggested that the surface of the polymer–DNA complex might be covered by longer PEG side chains more effectively than by short ones. In the process of entry of polymer–DNA complex into the cell, the PEG side chains covering the surface can hinder the interaction between the polymer–DNA complex and cell membrane sterically. Therefore the polymer–DNA complex cannot enter the cell easily, and

the transfection efficiency might be lower in comparison with that of the PEI homopolymer.[24] It is possible that the longer PEG side chains could hinder the interaction of complexes and cell membrane more easily. Therefore the longer the PEG side chains, the lower the gene transfer efficiency of graft copolymers. From the complex size measurement, zeta-potential measurement, and transfection test for grafted copolymer complexes, we can assume that the grafting of short PEG side chains prevents the polymer–DNA complex from the aggregation in the presence of salt without any reduction in the transfection efficiency compared with that of the PEI homopolymer.

It is expected that the gene delivery of PEI-grafted PEG might be affected by the behavior of PEG side chains and the chain mobility of graft copolymers, which are controlled by the length and amount of PEG side chains. The behavior of PEG side chains may be related to the interaction between the polymer–DNA complexes and the cell membrane in the process of cellular uptake. For practical application, PEG could be coupled to the various cationic polymers such as PEI, poly-l-lysine (PLL), and liposomes. In each case, our research results might serve as a guide for designing PEG-conjugated cationic polymers that have higher transfection efficiency and good stability in the serum.

CONCLUSION

In this paper, we investigated the effect of PEG side chains on the stability of PEI/DNA complexes, cytotoxicity, and transfection efficiency. The stability and cytotoxicity of complexes are enhanced with increasing graft length and amount of PEG side chain, and the enhancement is marked for longer PEG side chains. However, the longer PEG side chains also interrupt the gene delivery to the cells due to the more efficient steric hindrance by longer PEG side chains, and thus the transfection efficiency diminishes as PEG side chains grow longer.

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