# Poly(ethylene imine)-*g*-chitosan using EX-810 as a spacer for nonviral gene delivery vectors

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**Abstract:** Polyelectrolyte complexes have been widely studied as gene carriers in recent years. In this study, poly (ethylene imine) was grafted onto chitosan (PEI-*g*-CHI) as a nonviral gene carrier in order to improve the water solubility as well as the inherent transfection efficiency of chitosan. We present a novel method to conjugate the amine or hydroxyl groups of chitosan (CHI) and the amine groups of PEI through opening the epoxide rings of ethylene glycol diglycidyl ether (EX-810), which also brings the merits as mentioned in PEGylation chemistry. The degree of substitution of PEI onto CHI was characterized by NMR. The preliminarily cellular mechanisms, from the cellular entry to the endosomal release, were investigated by the correlations among the physicochemical properties of the DNA-polymer complexes, the buffering capacity of the modified polymer, the cytotoxicity, and the efficiency of the transgene expression. The cytotoxicity assayed by MTT shows that cell viability of PEI-*g*-CHI is higher than CHI especially noticeable at high concentrations using human kidney 293T cells. The efficiency of transgene expression and the amount of intracellular plasmid were monitored using green fluorescent protein (GFP) and visualized by fluorescence microscopy. The transfection efficiency of PEI-*g*-CHI/DNA polyplex is significantly better than CHI/DNA polyplex when using the weight ratios higher than 2.5. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res 88A: 1058–1068, 2009

**Key words:** chitosan; poly(ethylene imine); polyplex; transfection; cytotoxicity

## INTRODUCTION

Basically, nonviral gene delivery vectors can be divided into two classes: lipid-based transfection particles named lipoplexes and polymer-based transfection complexes named polyplexes. A number of positively charged polymers have been developed that can interact with the negative phosphate groups of naked DNA, condensing DNA into compact complexes. Chitosan, (CHI)[1–3] and poly(ethylene imine), (PEI)[4–7] are two attractive cationic polymers that have been widely studied. CHI is a biodegradable, nontoxic, and biocompatible polysaccharide composed of β(1, 4)-linked D-glucosamine and partly *N*-acetyl-D-glucosamine. Considerable attention has been given to chitosan-based materials and their applications in the field of tissue engineering[8,9] and drug delivery systems, especially as nonviral vectors for gene therapy[1–3,10–15] because of their bearing positive charges which can be complexed with negatively charged DNA. Chitosan can effectively bind DNA and protect it from nuclease degradation.[16,17] The transfection efficiency of chitosan polyplexes depends on the pH of the culture medium, the presence of serum, the ratio of chitosan to plasmid, the molecular mass, and degree of deacetylation of chitosan.[18] Treatment of chitosan/DNA polyplexes with polyethylene glycol (PEGylation) improves stability in storage and allows PEGylated polyplexes to be lyophilized without loss of transfection ability.[2,19,20] In addition, chitosan has been modified as gene vectors specifically to hepatocytes by lactosylation[11] or galactosylated chitosan-graft-polyethylenglycol.[20,21] Ligands can be conjugated to the polyplexes to stimulate receptor-mediated endocytosis and potentially

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to target a specific cell/tissue. To improve the low transfection efficiency of using CHI as a nonviral gene vector is always an issue to be discussed. In contrast, PEI behaves as a golden standard for nonviral gene vectors in clinical studies. The high transfection efficiency of PEI has been postulated to relate to its buffering capacity, which leads to the accumulation of protons brought in by endosomal ATPase and an influx of chloride anions, triggering endosome swelling and disruption, followed by the release of DNA into cytoplasm.[22] However, toxicity is one of the main concerns for PEI to be used in gene delivery, and increases with an increase in molecular weight.[23,24] Moreover, PEI is a nondegradable polymer and will accumulate in the body especially for high molecular weight, leading to an unknown risk for long-term use. Several approaches have been explored to reduce the cytotoxicity of PEI such as PEGylation of PEI,[25–29] and crosslinking a low molecular weight of PEI with cleavable cross-linkers, such as disulfide bonds,[30] biocompatible and degradable polyesters,[31,32] or polysaccharides.[33,34] The same strategy used to incorporate specific ligands to CHI has also been adapted to PEI to enhance the internalization with targeted cells.[4,32,35–40]

The chemical combination of chitosan and PEI used as a nonviral gene vector has been reported, recently.[41–43] In this study, we hypothesize that the conjugation of low molecular weight PEI onto CHI as one part can maximize their promising characters but minimize their disadvantages for a nonviral gene vector. PEI may improve the transfection efficiency but compromise with an increase in cytotoxicity of CHI. Therefore, we further incorporate EX-810 as a coupling agent that contains small segments of polyethylene glycol (~10 repeating units of ethylene oxide), hoping such modification can compensate for the PEI-*g*-CHI cytotoxicity due to the introduction of PEI. Besides the basic physico-chemical characterization, the cytotoxicity and preliminary gene transfection efficiency of the polyplexes prepared from nascent chitosan or PEI-*g*-CHI with pEGFP-$C_1$ plasmid at the various weight ratios were relatively compared.

## MATERIALS AND METHODS

### Materials

Chitosan was obtained from Fluca (Neu-Ulm, Germany). The degree of deacetylation was 90% as determined by $^1$H NMR, in $D_2O$ with 2% DCl as a solvent. The viscosity-averaged molecular weight was $3.30 \times 10^5$ g/mol as measured at 30°C in 0.2*M* acetic acid/0.1*M* sodium acetate. The branched poly(ethylene imine) with the reported molecular weight of 600 and 25,000 Da from Polyscience (Warrington, PA), and Poly(ethylene glycol) diglycidyl ether, EX-810 ($f_w$ = 512 g/mol) and ethidium bromide, from Sigma (St. Louis, MO) were purchased, respectively. The reporter gene pEGFP-$C_1$ was purchased from Clontech (Palo Alto, CA). Fetal bovine serum (FBS) was acquired from Biological industries (Beit Haemek, Israel). Agarose and DMEM reagent were purchased from Invitrogen (Carlsbad, CA).

### Preparation of PEI-*g*-CHI

Branched PEI ($M_w$ 600 Da) was grafted onto chitosan ($M_w$ 330 KDa) through a short PEG linker ($M_w \sim 440$ Da) with terminal epoxide rings. Briefly, 1:1 molar ratio of PEI:EX-810 was dissolved in 100 mL of double deionized (DD) water and reacted at room temperature for 3 h, and then 2 molar ratio of CHI was added into the solution, which was adjusted by acetic acid until complete dissolution of CHI occurred. The reaction mixture was left at room temperature for 24 h and the resultant PEI-*g*-CHI was purified by dialysis against DD water with a use of Spectra/Pro 2 membrane (MWCO = 6000–8000, Spectrum, Houston, TX) for three days to remove the un-reacted PEI and EX-810 and then freeze-dried for another day. High resolution $^1$H, $^{13}$C NMR experiments were recorded on a Varian, Mercury plus-400 spectrometer in $D_2O$ with 2% of DCl. FTIR spectra were performed with the use of a Perkin–Elmer system 2000. The dried CHI or PEI-*g*-CHI samples were ground with KBr powder and pressed into pellets for FTIR measurement. Sixty-four scans were signal-averaged at a resolution of 4 $cm^{-1}$.

### Titration of PEI-*g*-CHI

Potentiometric titrations (PT) were carried out with a PC-controlled system assembled with a 702 SM Titroprocessor, a 728 stirrer, and a PT-100 combination pH electrode (Metrohm). The pH electrode was calibrated using two standard buffer solutions. About 10 mg of a sample was added into 10 mL of 150 m*M* NaCl solutions and adjusted to pH = 12 using 0.0876*M* NaOH for complete deprotonation before titration. The titration was performed by addition of 0.0894*M* HCl solution under an atmosphere of $CO_2$-free $N_2$ at 25.0 ± 0.1°C.

### Preparation of pEGFP-$C_1$ plasmid

pEGFP-$C_1$ plasmid driven by a cytomegalovirus promoter was introduced into Escherichia coli strain DH5α (Gibco-BRL, Gaithersbury, MD), and purified by Viogene Plasmids Maxi Kits (Viogene, Sunnyvale, CA). Purity of plasmid DNA was certified by the absorbance ratio at $OD_{260}/OD_{280}$, and by distinctive bands of DNA fragments at corresponding base pairs in gel electrophoresis after restriction enzyme treatment of DNA. Plasmid DNA was stored at −20°C until used.

### Preparation of polymer/plasmid polyplex

CHI or PEI-*g*-CHI was dissolved to a concentration of 1 mg/mL in 5 m*M* acetic acid solution under gentle heating and the pH of the stock solution was adjusted to 5.5. Various amounts of polymers were made by dilution with 0.2 *M* sodium acetate at pH 5.5 to the volume of 50 μL. A total of 6 μg of plasmid DNA was dissolved in 50 μL of 50 m*M* of sodium sulfate solution. After heating to 55°C, equal volume of the chitosan and the pEGFP solutions were mixed and immediately vortexed at maximum speed for 30 s. Since the DNA concentration in polyplexes was fixed, manipulating the polymer concentrations could be used to adjust weight ratios between polymers and the plasmid DNA.

### Characterization of polymer/plasmid polyplex

#### Agarose gel electrophoresis

The DNA binding ability of polyplexes was evaluated by agarose gel electrophoresis using 0.8% agarose in TAE with ethidium bromide (1 μg/mL). The polymer/plasmid polyplexes were prepared at w/w rations of 0.5, 1.5, 2.5, 3.5, and 4.5 as the procedure stated above. The stability of the polyplexes was also performed in 10% FBS condition by gel electrophoresis. Gels were run at 100 V for 50 min and DNA retention was visualized under UV illumination.

PicoGreen assay was also carried out to analyze the free plasmid DNA quantitatively as reported recently.[12,16] Briefly, PicoGreen reagent (200X) was diluted to 200-fold in TE buffer before the experiment. About 50 μL of the diluted PicoGreen stock solution was mixed with the same volume of blank solution or polyplex solution prepared at various weight ratios. Fluorescence was measured with an FLx800T spectrofluorometer (BioTek, Tustin, CA) at room temperature. Excitation and emission wavelengths were set at 480 and 520 nm, respectively. Values were represented as relative fluorescence (%) to the value of naked plasmid DNA.

#### Particle size and zeta potential

The volume of each sample was 1.2 mL, containing a final DNA concentration of 10 μg/mL. Size and zeta potential of polyplexes were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS instrument (Marlvern, Worcestershire, UK). The zeta potential values were calculated using the Smoluchowski equation $\mu = \xi\varepsilon/\upsilon$, where $\mu$ is the electrophoretic mobility, $\xi$ the zeta potential, $\varepsilon$ the electric permittivity of the dispersion media, and $\upsilon$ the viscosity. Polystyrene nanospheres (220 ± 6 nm and −50 mV; Duke Scientific) were used to verify the performance of the instrument. CONTIN was used to fit the data and each data was averaged from three measurements.

The size and morphology of polyplexes were also observed using TEM (JEM-2000 EXII, JEOL, Japan). A carbon-coated 200-mesh copper specimen grid (Agar Scientific Ltd. Essex, UK) was glow-discharged for 1.5 min. One drop of the polyplex was deposited on the grid and left to stand for 1.5 min, after which time, any excess fluid was removed with filter paper. The grids were allowed to dry for a further 24 h at room temperature and were then examined with the electron microscope.

### Cell line experiments

#### Cell culture

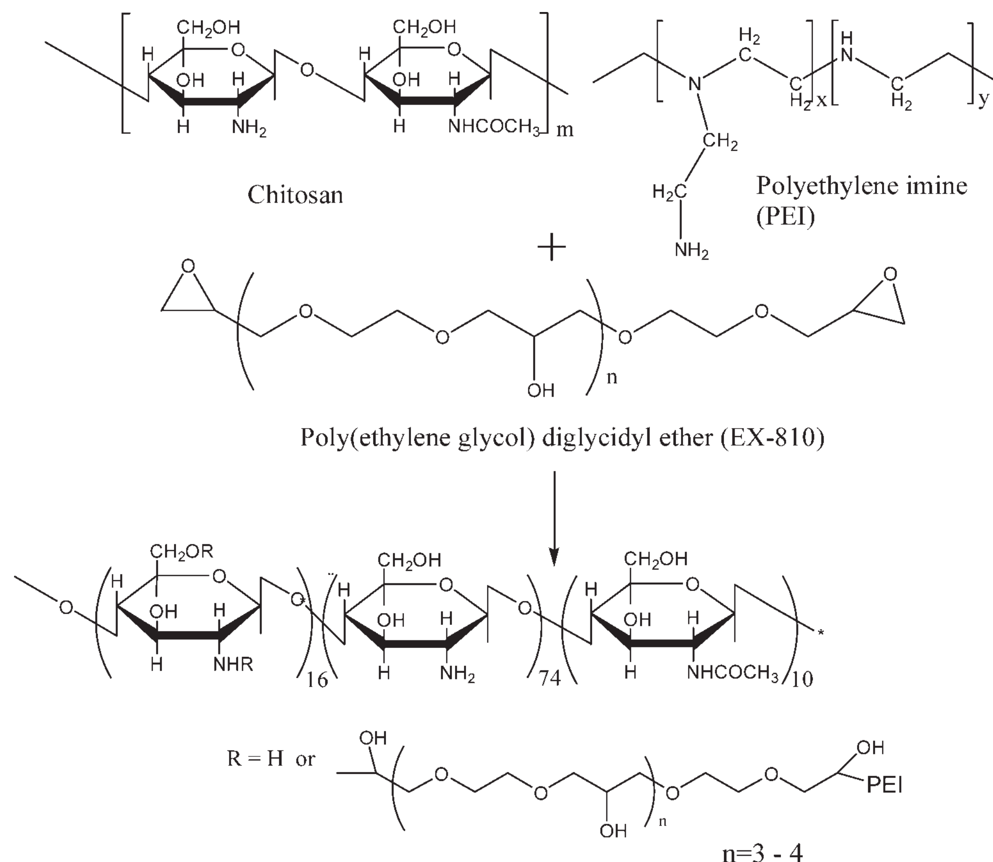
293T cells, a human kidney cell line kindly offered by Dr. Cheng at Biotechnology department of Kaohsiung Medical University in Taiwan, were cultivated in Dulbecco's modified essential medium (DMEM, Sigma) supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in humidified environment of 5% $CO_2$. The medium was replenished every other day and cells were sub-cultured after reaching confluences.

#### Cytotoxicity

293T cells were seeded in 96-well tissue culture plates at a density of $1.5 \times 10^4$ cells per well and incubated for 24 h prior to addition of the filtered polymers or polyplexes. The cytotoxicity of polymers was evaluated by determining the viability of the macrophages after incubation with the various concentrations of polymers (5–1000 μg of polymer/mL) and incubated for additional 24 h. The various weight ratios of polymer/DNA from 0.5 to 4.5 were prepared in the same conditions as the gene transfection studies in the latter section, in which the cells were incubated without using FBS. After addition of polyplexes into cells in DMEM with no FBS for 6 h, the complete medium was replaced and incubated for additional 24 h. The number of viable cells was determined by the estimation of their mitochondrial reductase activity using the tetrazolium-based colorimetric method (MTT conversion test).[44] This assay depends on the cellular reductive capacity to metabolize the yellow water-soluble tetrazolium salt, 3-(4, 5-dimethylthiazol-2-yl)-3,5-diphenyl tetrazolium bromide dye (MTT), to the water-insoluble blue formazan product. At the end of the incubation period with polymers or polyplexes, cells were incubated with 50 μL of a MTT solution (2 mg/mL) for 3 h at 37°C. One hundred microliters of dimethyl sulfoxide (DMSO) were then added in order to dissolve the formazan crystals. The UV absorbance of the soluble formazan crystals was measured spectrophotometrically at 490 nm. Cell viability was expressed as the ratio between the amount of formazan determined for cells treated with the different polymer or polyplex suspensions and for control nontreated cells.

#### In vitro gene expression

293T cells were seeded at a density of $1 \times 10^5$/well in 6-well plates and incubated in DMEM medium containing 10% FBS for 24 h before the addition of a polyplex. When the 293T cells were at 50–70% confluence, the culture medium was replaced with 2 mL of DMEM without 10% FBS.




**Scheme 1.** Synthesis of poly(ethylene imine) grafted chitosan (PEI-*g*-CHI) using EX-810 as a coupling reagent.

Polyplexes prepared by mixing 4 μg of pEGFP-$C_1$ and various amounts of polymers in 500 μL of serum-free DMEM medium were immediately added into the wells and incubated for 6 h. After that the cells received 2 mL of complete medium and were incubated for 72 h post transfection. The transfection efficiency was directly visualized under a fluorescence microscope (Nikon, TE 2000U, Tokyo, Japan).

After being observed by a fluorescence microscope, the quantitative EGFP expression was monitored by a fluorescence plate reader (BioTek FLx800T, Tustin, CA) to determine the transfection efficiency in 293T cells. To measure GFP transfection by the fluorescence plate reader, cells were washed with 1 mL of PBS (twice), and 1 mL of lysis buffer (0.1*M* Tris-HCl, 2 m*M* EDTA, and 0.1% TritonX-100, pH 7.8) as a protocol stated in Promega. An excitation wavelength of 488 nm was used with the fluorescence emission measured at 525 nm.

## RESULTS AND DISCUSSION

### Synthesis and characterization of PEI-*g*-CHI

The synthesis of PEI-*g*-CHI depicted in Scheme 1 involves the reaction of epoxide rings of EX-810 with the amine groups of PEI at one end followed by the reaction with the amine or hydroxyl groups of CHI on the other end. The confirmation of PEI grafted onto CHI was evaluated by $^{1}H$ and $^{13}C$ NMR as shown in Figures 1 and 2, respectively. The ratio of the peak area between 1.98 ppm due to the three protons on acetyl amide groups of CHI and 4.01 ppm attributed to methine proton due to the opening of epoxide rings is used to calculate the substitution degree of PEI-grafted onto CHI (~0.16). As mentioned in the experimental section, the degree of deacetylation of CHI was 90%; therefore, the percentage of CHI amine groups left in PEI-*g*-ChI was 74%, as noted in Scheme 1. Besides the characteristic peaks of CHI, the $^{13}C$ NMR spectrum of PEI-*g*-CHI as seen in Figure 2 reveals a peak at 70 ppm attributed to EX-810 and multiple peaks at 45 ~ 50 ppm due to the PEI segments. The other evidence of successful PEI-grafted onto CHI was also seen in FTIR spectra, that present the reduced hydroxyl or amine stretching of CHI at ~3400 $cm^{-1}$ and an increasing $CH_2$ stretching peak at ~2900 $cm^{-1}$ because of introduction of PEI and EX-810 (Supplement 1). The water solubility of PEI-*g*-CHI was improved as compared to nascent CHI. Molecular weight of PEI-*g*-CHI was traced by gel permeation chromatography (GPC). Indeed it can be seen

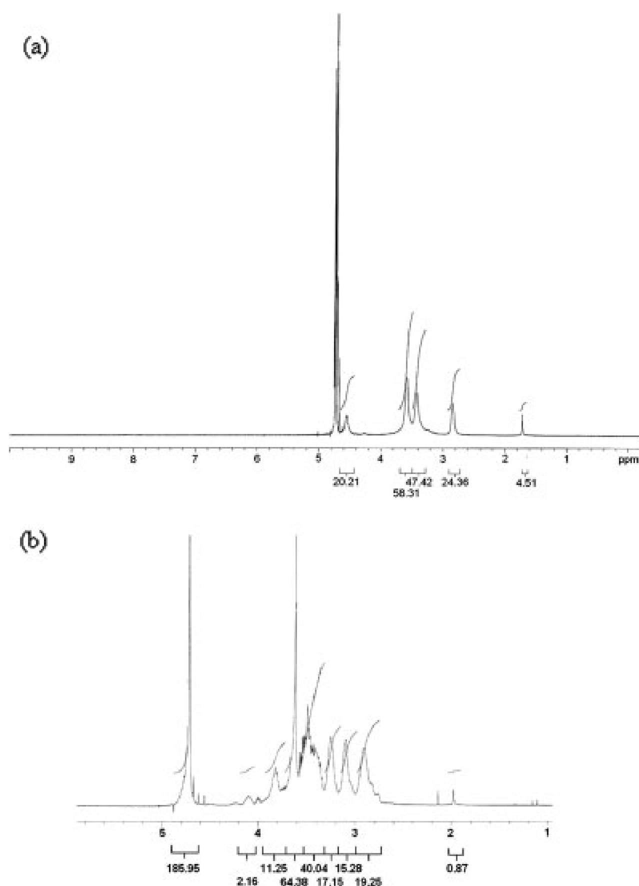



**Figure 1.** $^1$H NMR spectra of Chitosan (a), and PEI-g-CHI (b).

that the molecular weight distribution of PEI-*g*-CHI shifts into the shorter retention time, explaining an increase in the molecular weight after grafting PEI onto CHI as compared to nascent CHI (Supplement 2). The acid/base titration profiles carried out for PEI, CHI, and PEI-*g*-CHI are shown in Figure 3, indicating that PEI has the best buffer capacity and slightly depends on the molecular weight. The titration profile of PEI-*g*-CHI is similar to that of nascent CHI with a considerably better buffer capacity due to a higher amount of protons needed to change the pH from 8 to 5.5, which has been defined as a measurement of buffer capacity by Tseng et al.[45]

### Preparation and characterization of polymer/pEGFP-C$_1$ polyplexes

By changing polymer weight and keeping DNA weight as a constant, the various weight ratios of polyplexes were obtained.[46] The DNA protection from digestion was prepared by forming polyplexes with cationic polymers. Electrophoretic mobility analysis of CHI and PEI-*g*-CHI/DNA polyplexes was tested in the absence or presence of 10%FBS. Figure 4(a) shows the DNA binding ability with polymers in the different weight ratios. DNA can be complexed with PEI-*g*-CHI at a weigh ratio of 1.5 because the exposed DNA in the supernatant maintains a steady concentration determined by Picogreen. The DNA binding ability between CHI and PEI-*g*-CHI is similar in the absence of 10% FBS, whereas it is better for PEI-*g*-CHI when 10% FBS is present, as illustrated in Figure 4(b). Less than 20% of the naked DNA is traced in the supernatant of PEI-*g*-CHI polyplexes. The concentration of the naked DNA in a supernatant does not decrease with an increase in the weight ratio between polymers and DNA. Both of the polyplexes prepared from CHI or from PEI-*g*-CHI show pronouncedly encap-

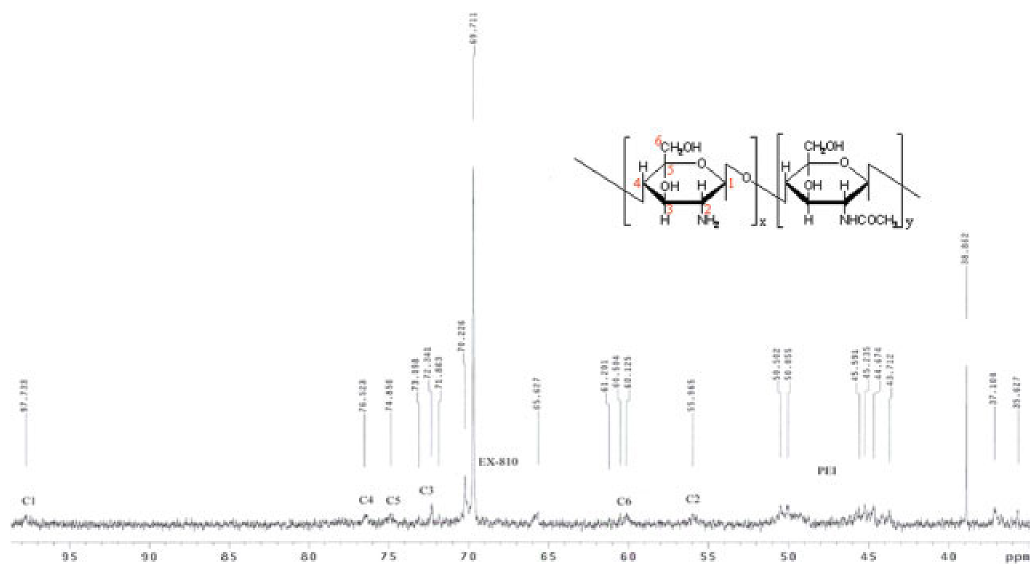



**Figure 2.** $^{13}$C NMR spectrum of PEI-*g*-CHI showing the characteristic peaks of PEI, EX-810, and CHI. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

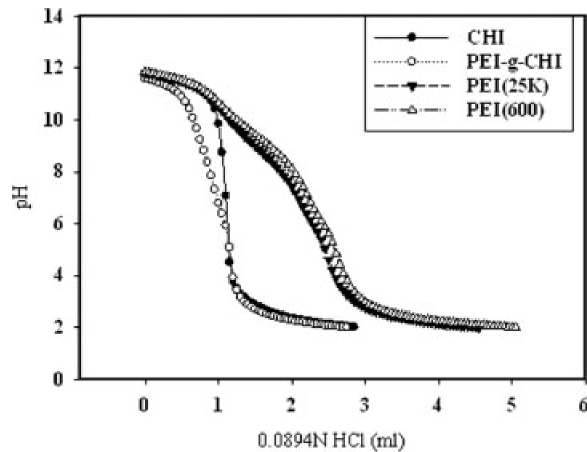
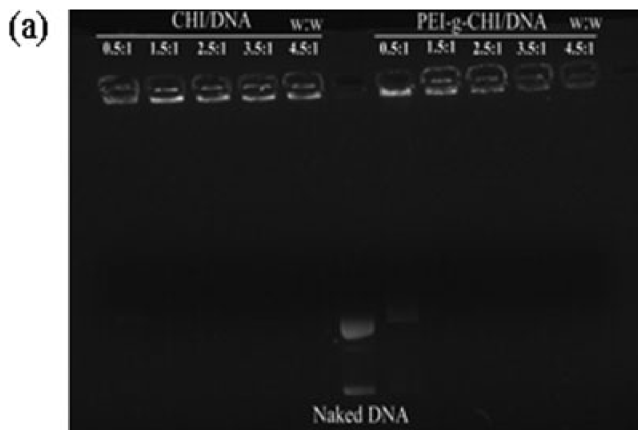


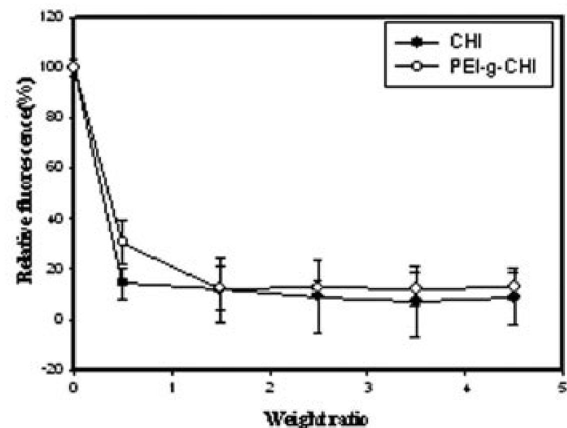

**Figure 3.** Titration curves of branched PEI ($M_w$ = 600 and 25,000 g/mol), CHI, and PEI-*g*-CHI.

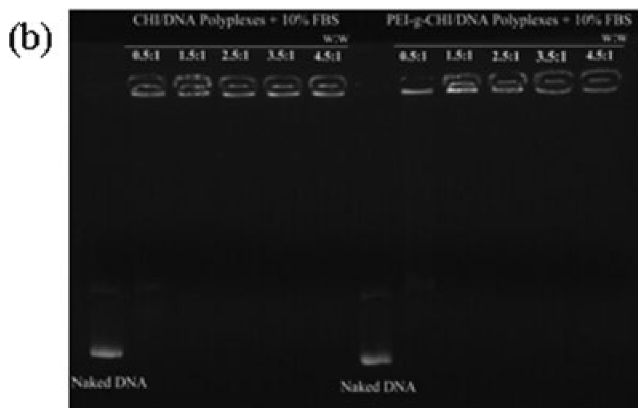
sulation ability to DNA because no free DNA is visualized in agarose gel electrophoresis and the DNA concentration in a supernatant keeps as a constant value. The similar results have been reported in CHI with 90% deacetylation formed polyplexes in either PBS or a complete cell culture medium containing 10% FBS.[47] The DNA encapsulating efficiency generally increases with the use of the high molecular weight of CHI. Because the molecular weight of CHI used in this study is relatively high (330K Da), the impact of PEI introduction on the DNA encapsulation is trivial. In the future, we may choose the lower molecular weight of CHI to check this hypothesis.

### Particle size and zeta potential

As shown in Table I, the particle size and zeta potential of CHI/DNA polyplexes slightly increases with the increase in the weight ratio. However, PEI-*g*-CHI/DNA, results in nanoparticles with the sizes from 200 to 265 nm and zeta potentials in the range of 20 ∼ 26 mV, independent of the weight ratios of polyplexes. The positive values of zeta potential suggest that the plasmid DNA is well encapsulated inside the polymers. The surface morphologies and relative sizes of PEI-*g*-CHI/DNA polyplexes were also visualized by TEM (Fig. 5). When compared



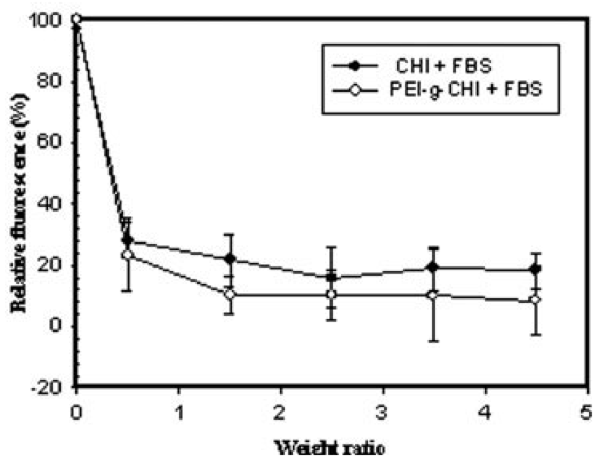




**Figure 4.** Agarose gel electrophoresis and Picogreen assay to test DNA retention in polyplexes prepared at different weight ratios (a) and in the presence of 10% FBS (b).

**TABLE I**
**Particle Size and Zeta Potential of Polyplexes**

| Polyplex | Weight Ratio | Particle Size (nm) | PDI | Zeta Potential (mV) |
|---|---|---|---|---|
| CHI(330K)/DNA | 0.5 | 184.9 ± 1.2 | 0.18 ± 0.03 | 20.6 ± 1.4 |
| | 1.5 | 236.1 ± 4.8 | 0.21 ± 0.02 | 24.3 ± 0.3 |
| | 2.5 | 241.9 ± 2.8 | 0.23 ± 0.01 | 22.8 ± 0.5 |
| | 3.5 | 273.1 ± 2.2 | 0.31 ± 0.01 | 29.3 ± 0.9 |
| | 4.5 | 293.9 ± 1.6 | 0.35 ± 0.01 | 30.2 ± 1.3 |
| PEI-*g*-CHI/DNA | 0.5 | 265.3 ± 2.8 | 0.23 ± 0.01 | 26.0 ± 0.2 |
| | 1.5 | 228.2 ± 13.6 | 0.31 ± 0.03 | 22.4 ± 0.9 |
| | 2.5 | 199.8 ± 2.9 | 0.24 ± 0.01 | 25.8 ± 0.5 |
| | 3.5 | 196.6 ± 6.0 | 0.21 ± 0.02 | 20.4 ± 1.3 |
| | 4.5 | 255.4 ± 45.9 | 0.31 ± 0.06 | 21.7 ± 2.8 |

TEM micrographs to those reported in CHI/pEGFP,[3] PEI-*g*-CHI/pEGFP polyplexes show the better regular spherical shape. The average sizes of the PEI-*g*-CHI/pEGFP polyplexes are 164.7 ± 25.5 nm, 172.7 ± 18.3 nm, 74.1 ± 6.7 nm, 57.5 ± 7.2 nm, and 72.8 ± 4.5 nm, respectively, to PEI-*g*-CHI/DNA at the weight ratios of 0.5, 1.5, 2.5, 3.5, and 4.5. It is clearly seen that the particle size decreases dramatically at a

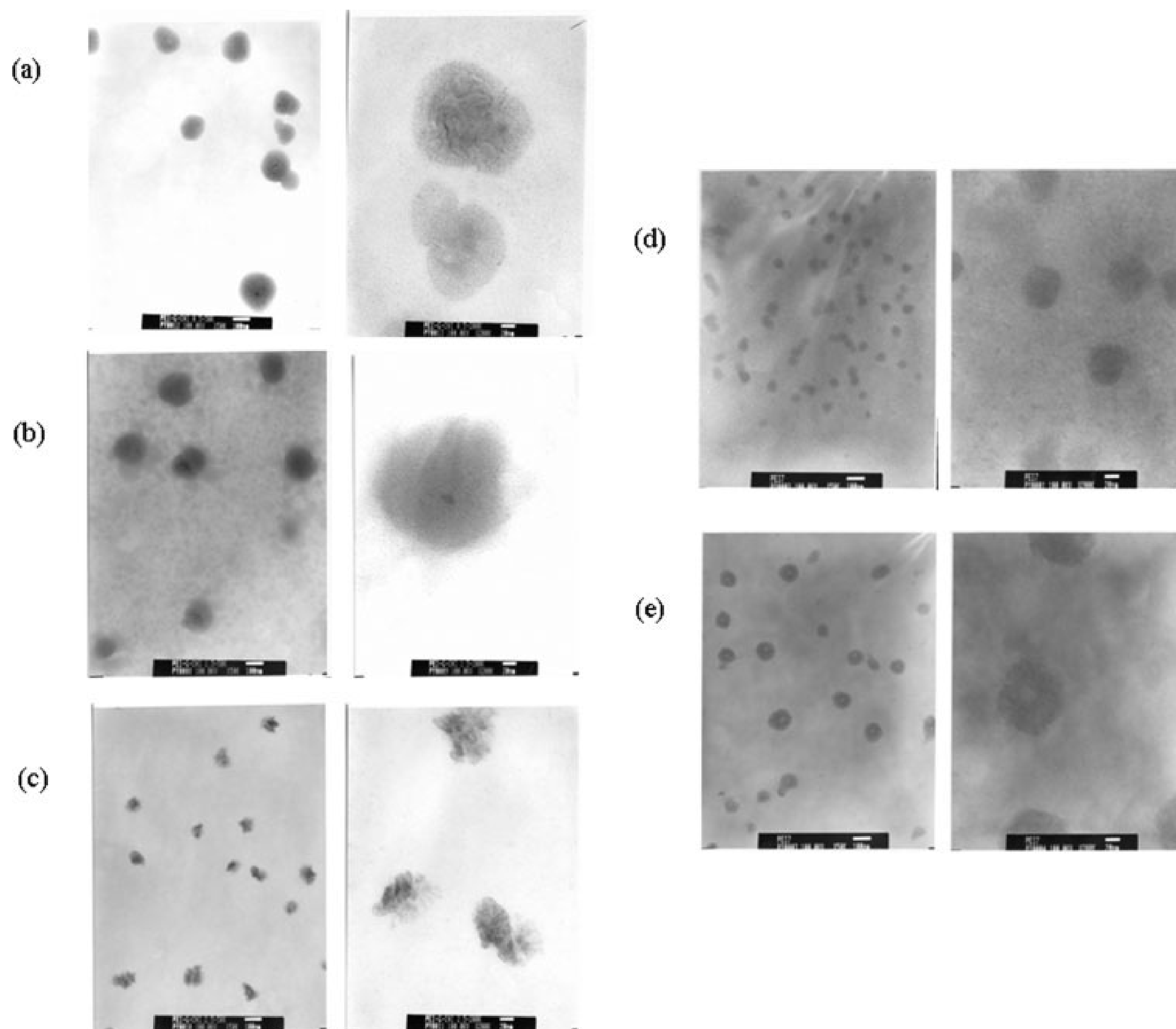



**Figure 5.** TEM micrographs for the PEI-*g*-CHI/DNA polyplexes at the weight ratios of 0.5 (a), 1.5 (b), 2.5 (c), 3.5 (d), and 4.5 (e) using two magnifications. The white bars on the bottom of each panel represent 20 and 100 nm for right and left panels, respectively.

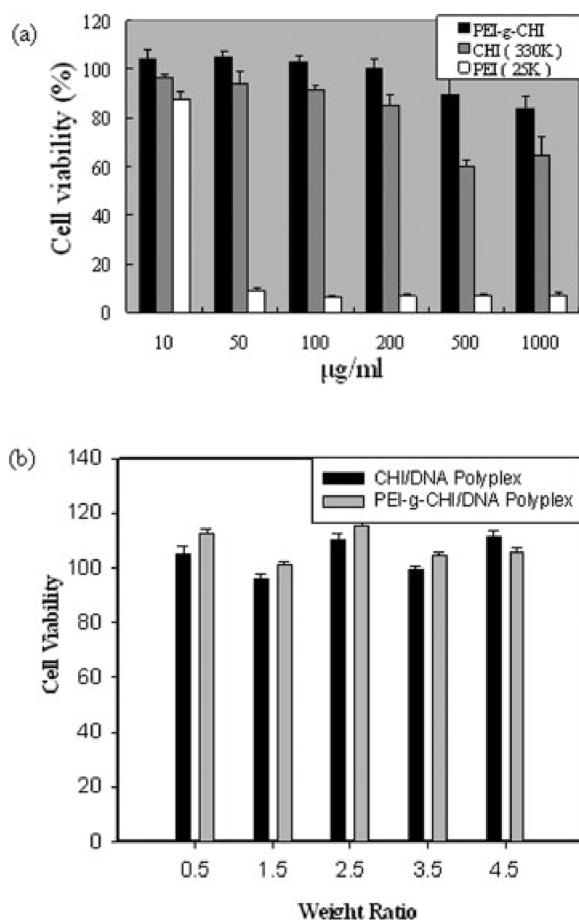



**Figure 6.** Cellular cytotoxicity induced by PEI-*g*-CHI, CHI, and PEI at various concentrations (a); the polymer/DNA polyplex at the various weight ratios without FBS (b). Cytotoxicity was assayed by the MTT method, and expressed as the percentage of cell viability by setting the untreated cells as 100%.

weight ratio of 2.5. In this condition, the PEI-*g*-CHI molecules complex and condense DNA into a more compact structure with a less deviation of particle size. The small values of particle size were also obtained in polyplexes prepared at the weight ratios of 3.5 and 4.5. There is a size discrepancy measured by TEM and DLS as tabulated in Table I. The particle sizes measured by TEM are almost one half of the values determined by DLS. This may be because of the fact that the particle sizes measured by DLS are done in a fully hydrated state in solution and those for TEM measurements have been dried at room temperature for 24 h after dropping the prepared polyplexes onto carbon-coated copper meshes. Because PEI-*g*-CHI has the better water solubility, the degree of swelling becomes pronounced with an increase in the PEI-*g*-CHI composition in the polyplexes. Hence, the larger particle size was observed at the weight ratio of 4.5 than those at 2.5 and 3.5. The similar result in size discrepancy in TEM and DLS has been reported in the nanoparticles prepared from cross-linked chitosan-*N*-poly (ethylene glycol).[48] Particle size measured by TEM varies in the range of 4–24 nm, but in the swollen state, the average particle size increases into the range of 50–120 nm depending on the molecular weight of chitosan and the ratio of cross-linking agent used.

## Cytotoxicity

The cell viability was tested in 293T cells because the transgene expression of a gene vector is a function of the corresponding toxicity of polymers. As can be seen in Figure 6(a), all polymers exhibit minimal toxicity at the low concentration of 10 μg/mL but PEI increases the toxicity dramatically at the concentration of 50 μg/mL. Although there is an increase in toxicity with the increases in concentrations, the toxicity of PEI-*g*-CHI is less than CHI at all tested concentrations. This situation indicates that the cause in the cytotoxicity due to the moiety of PEI can be compromised with the introduction of PEG segments. When cytotoxicity of the polyplexes was tested at the various weight ratios in the absence of FBS, both CHI and PEI-*g*-CHI formed polyplexes showed low cytotoxicity as indicated in Figure 6(b).

## Transfection efficiency of polymer/pEGFP-$C_1$

The relative gene transfection efficiency of CHI/DNA and PEI-*g*-CHI/DNA polyplexes at the various weight ratios was studied. As seen in Figure 7, the relative transfection efficiency of the CHI/DNA polyplexes slightly increases with the increase in the weight ratios. In contrast, the PEI-*g*-CHI/DNA polyplexes show a similar efficiency of transgene expression at the weight ratios of 0.5 and 1.5 and the trans-

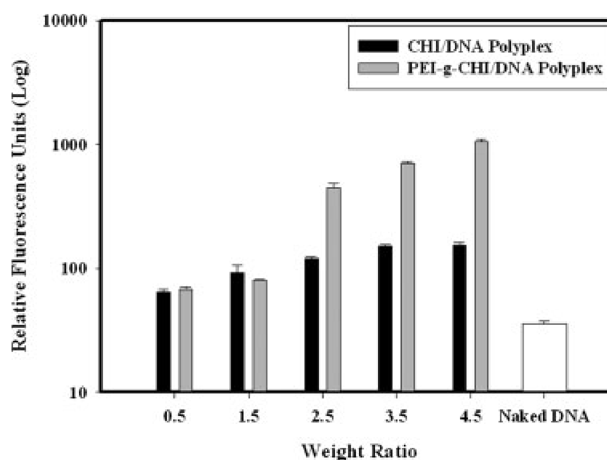



**Figure 7.** Gene transfection efficiency of CHI/DNA and PEI-*g*-CHI/DNA polyplexes at the various weight ratios. Polyplexes were incubated with 293T cells for 6 h and the fluorescence intensity was measured after 72 h postincubation.

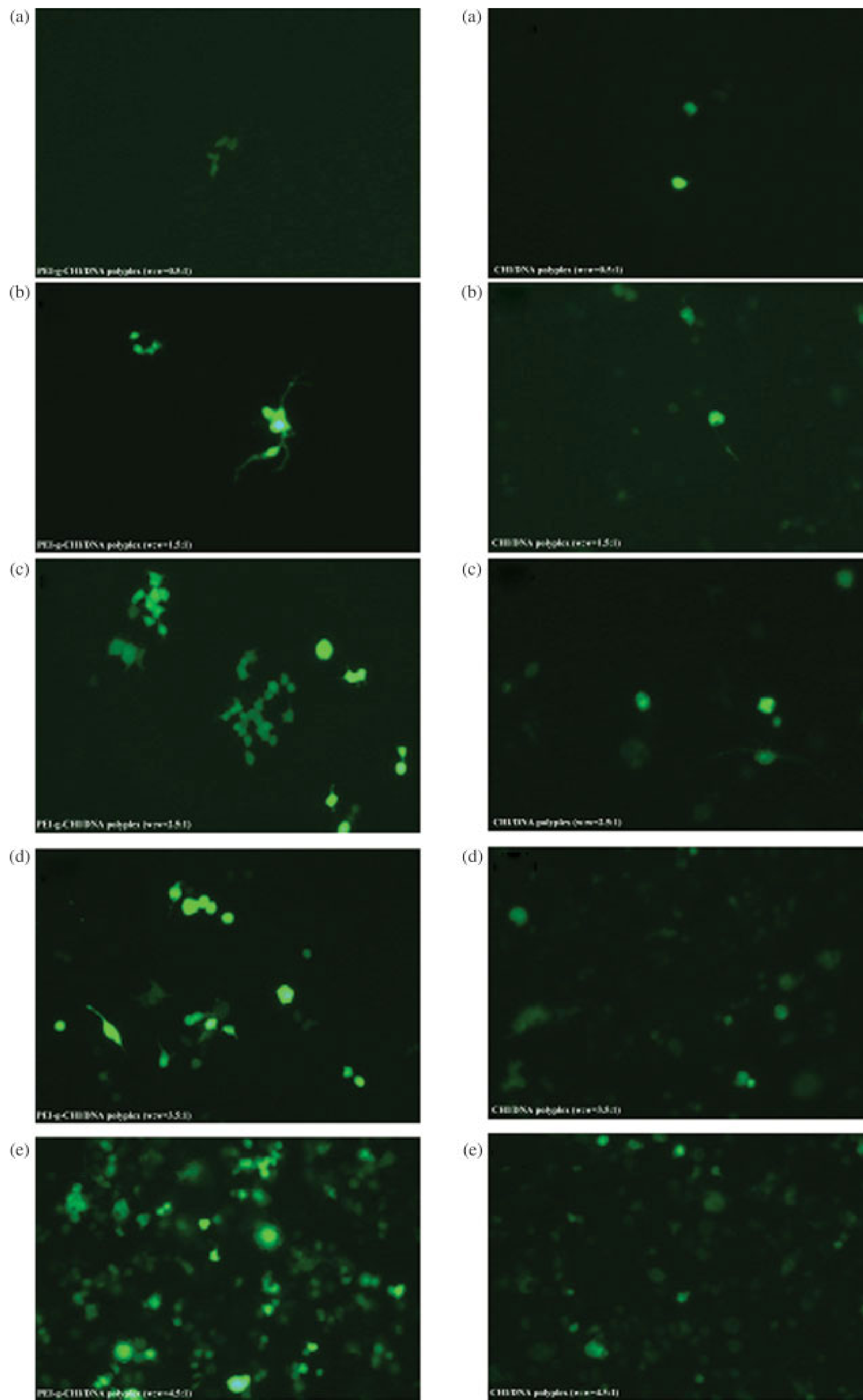



**Figure 8.** Fluorescence images for pEGFP-$C_1$ expression in 293T cells taken at the same time as Figure 7. CHI/DNA and PEI-*g*-CHI/DNA are on the right and left panels, respectively with the weight ratios of 0.5 (a), 1.5 (b), 2.5 (c), 3.5 (d), and 4.5 (e). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

fection efficiency dramatically increases at the weight ratios higher than 2.5 when compared to the CHI/DNA polyplexes. The fluorescence pictures of GFP expressed in 293T cells were also visualized in Figure 8. Obviously, the GFP expression in the PEI-*g*-CHI/DNA polyplexes increases with the increases in the weight ratios compared to the CHI/DNA polyplexes.

## CONCLUSIONS

PEI can be grafted onto CHI with the use of PEG as a spacer. The PEI-*g*-CHI/DNA polyplexes show less cytotoxicity than the CHI/DNA polyplexes. The size and zeta potential of both polyplexes where there are complete DNA binding are not significantly different. However, compared to TEM pictures reported for CHI/DNA,[3] the PEI-*g*-CHI/DNA polyplexes show the better compact structure. The polyplexes are stable for CHI or PEI-*g*-CHI incubated in PBS either with or without 10% FBS. The transfection efficiency slightly varies with the increase in the weight ratios in CHI/DNA polyplexes. However, a notable increasing transfection efficiency is obtained when using the weight ratio of PEI-*g*-CHI/DNA larger than 2.5. This may be due to the better compact between PEI-*g*-CHI and DNA, which results in the small particle size. Thus, grafting PEI onto CHI is an effective method to enhance transgene expression without an increase in cytotoxicity of PEI-*g*-CHI if the PEG segments are incorporated.

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