Investigation of DNA Spectral Conformational Changes and Polymer Buffering Capacity in Relation to Transfection Efficiency of DNA/Polymer Complexes

Jong Yuh Cherng

Department of Chemistry and Biochemistry, National Chung Cheng University, 168, University Rd. Chia-Yi 621, Taiwan, R.O.C.

Received, June 16, 2009; Revised, September 10, 2009, Accepted, November 20, 2009, Published, November 20, 2009.

ABSTRACT - Purpose The relation between transfection efficiency of DNA/polymer complexes and DNA conformational alterations is investigated. The buffering capacity of several synthetic polymers is also studied and related to their performance in transfection efficiency. **Methods** The cationic polymer/DNA interaction was evaluated by measuring the alteration of DNA secondary structures in solution before and after the addition of polymers using the ATR-FTIR technique. The degree of protonation in aqueous solutions of cationic polymers depends upon pH and the structures. A polymer capable of protonation acts like a proton sponge and reacts with H^+ when titrated with HCl. This characteristic was evaluated in relation to transfection efficiency because the capacity would help the release of endocytotic DNA from endosome/lysosome and subsequent gene expression. **Results** IR results show that the antisymmetric $PO2^-$ vibration of DNA (at 1224 cm^{-1}) shifts toward lower frequencies when complexed with PEI or PLLys (these polymers are able to transfect DNA), but shifts toward higher frequencies or no alterations in the presence of PDAMA or dextran (these polymers are poor in DNA transfection). Interestingly, the polymers with the best performance in transfection efficiency are in this order: PEI>PDMAEMA>PLLys>PDAMA>dextran which is in the same order as their polymer buffering capacity. These facts indicate that polymers possessing better buffering capacity should result in higher transfection efficiency. Also, we have demonstrated in this paper that the alteration in the antisymmetric PO_2^- stretching vibration in the IR spectra is sensitive to the binding of cationic polymers with DNA and the transfection efficiency of the formed complexes. These findings are useful for the developing polymer-based gene delivery systems with better in vitro and in vivo performance.
Novelty of the Work: this work establishes a rational relation between transfection efficiency of DNA/polymer complexes and DNA conformational alterations. Also, the buffering capacity of polymers is found to be closely related to their performance in transfection efficiency.

INTRODUCTION

Presently, a lot of attention is directed to the new development on gene delivery (1-3). Numbers of different cationic polymers are under investigation for delivering DNA (4,5). When DNA is complexed with cationic polymers, the resulting polymer/DNA complexes (polyplexes) are able to transfect eukaryotic cells both in vitro and in vivo (6-8). For DNA transfection, current examples of cationic polymers are based on the backbone structure of poly-L-lysine (PLLys) (9), poly 2-(dimethylamino)ethyl methacrylate (PDMAEMA) (10,11) , including membrane disruptive peptide-linked poly 2-(dimethylamino)ethyl methylamino-ethyl methacrylate (PDAMA) (12) and polyethylenimine (PEI) (13,14). Although these polymers are able to assist DNA to transfect cells, their transfection activities differ widely. The tranfection efficiency of formed polyplexes is known to be dependent on the size, zeta-potential of polyplexes, transfection medium (e.g. the presence of serum proteins), polyplex formulation (type and molecular weight of polymer) and polymer/DNA ratios (15-17) and other factors yet to be determined.

Fourier transformed IR spectroscopy with attenuated total reflectance technique has been used to characterize the secondary structure of DNA and to assess DNA interactions with drugs, organic chemicals and cationic metal ions in aqueous solution (18-20).

Correspondence Author: Jong Yuh Cherng; E-mail address: jycherng@yahoo.com

Recently, there have been studies made of the relationship of DNA with cationic lipid/DNA complexes and their effect on the hydration state of cationic lipid/DNA complexes (21,22). However, only a few attempts of characterizing the nature of cationic polymer/DNA complexation in aqueous solutions by IR spectroscopy and correlating their transfection efficiency have been made (23, 24). Moreover, in order to mimic polymer/DNA complexes within the acidic endosomal/lysosomal compartments of cells, the buffering capacity of the polymers was performed in this study in a milieu of decreasing pH and in relation to their transfection efficiency.

MATERIALS AND METHODS

Materials

Calf thymus DNA sodium salt type I (highly polymerized, 7% Na content) and poly-L-lysine hydrobromide (PLLys) (Mw=70-150 kDa) were purchased from Sigma Chemical Co. Plasmid DNA used for transfection studies was prepared and purified as previously reported (15). Polyethylenimine (PEI) (Mw = 25 kDa) was obtained from Fluka. Poly (2-dimethylamino)ethyl methacrylate (PDMAEMA) was prepared by a radical polymerization of 2-(dimethylamino) ethyl methacrylate essentially as described previously (Mw = 360 kDa)(15). Poly 2-(2-dimethylamino-ethyl)-methyl-amino) ethyl methacrylate (PDAMA) was synthesized via a radical polymerization essentially as described (Mw = 19 kDa); a sample was graciously provided by Utrecht University, The Netherlands (12). Dextran (Mw=222 kDa) was obtained from Fluka and used as supplied. All solutions were adjusted to the desired pH before complexing DNA with the appropriate polymers (see also Scheme 1).

IR spectroscopic studies of DNA/polymer complexes

Attenuated total reflectance (ATR) IR spectroscopy was used to explore the structural characterization of cationic polymer/DNA complexes in solutions. 10 mM of an acetate buffer (pH 5) and 10 mM of Tris buffer solutions of pH 7.4 and 9 were selected to avoid possible disturbance of the phosphate vibrations of DNA in the IR spectra. Stock solutions of DNA (2 mg/ml) and different polymer solutions were prepared in buffers and then adjusted to the desired pH with HCl or NaOH. The complexation of DNA with the polymer was carried out by adding 400 µl of the polymer into 400 µl of DNA solution. After mixing for 5 seconds, the resulting dispersions were incubated at room temperature for one-hour to complete complexation. Next, DNA/polymer samples were spread onto the ZnSe trough of ATR crystal so as to cover the ZnSe surface. The cationic polymer/DNA weight ratios (r) used in the experiments were selected at 4/1, 2/1, 1/1, 1/2, 1/20, and 1/40 to cover the range of forming smaller particles in the transfection studies and were no cytotoxicity observed. The IR spectra were obtained using a BioRad FTS6000 FTIR spectrometer equipped with a MID-IR DTGS detector and KBr beam splitter under a nitrogen purge. ATR-IR spectra were recorded after 1024 scans with settings of 2 cm^{-1} resolution, 5 kHz scan speed, and 2 cm^{-1} optic aperture from a 10-reflection, 45^{o}-incidence angle ZnSe horizontal crystal. Spectra were collected and manipulated with Bio-Rad Win-IR Pro software. A flat base line in the region of 2300-1850 cm^{-1} was obtained after buffer subtraction. Also peaks in the region from 3800 to 3700 cm^{-1} were minimized in order to have a good water vapor subtraction before spectral variations in DNA absorbance were compared. For obtaining spectral data, measurements were carried out at least in triplicate and were within ±1 cm^{-1} in wavenumber deviation.

Titration behavior of polymers with acid

The ability of cationic polymers to be protonated and carrying positive charges over the pH range from 12 to 2 was determined by titration with acids. A greater buffering capability of a polymer indicates less [H^{+}] in solution and less changes in pH values during HCl titration. Briefly, each polymer (1 mg/ml) was dissolved in 150 mM NaCl and titrated to pH11 with 1N NaOH. 10 ml of each solution was titrated with increasing volume of 0.1 N HCl and pH values were recorded with a pH meter (pH/mV/Temp meter 6171). To compare the titration behavior of polymers alone with the behavior of polymer/plasmid DNA complexes, 0.1 mg polymer (e.g. PEI) was mixed with 0.1 mg DNA to form complexes in 150 mM NaCl (10 ml) and titrated to pH 11. The polymer/DNA complexes were then titrated with 0.01N HCl.

Transfection studies and β-galactosidase assay

COS-7 cells were used to evaluate the transfection efficiency of the polymer/DNA complexes. The cells were seeded in a 96-well plate (1×10^4 cells per well) under complete DMEM and incubated for 24 hours before transfection. The polymer/DNA complexes (volume 200 μL) were then added to these cells. The DNA concentration was kept constant at 5 μg/mL and the amounts of polymer/DNA were expressed as mass ratios. After 1 hour at 37°C, the transfection mixture was removed and replaced with complete DMEM for additional 48 hours. Then a histochemical staining X-Gal assay was used to evaluate the transfection efficiency of the delivered gene (expression of β-galactosidase).

Transfection results (mean ± SEM of 3 experiments) expressed as relative transfection efficiency were normalized to the number of transfected cells found after incubation of the cells with a positive control (PDMAEMA /plasmid DNA complexes; optimal ratio(r = 4/1) at 20 and 5 μg/ml, respectively). The optimal ratio of PLLys/DNA and PDAMA/DNA complexes for their transfection is also at r = 4/1. The PEI/DNA complexes show an optimum transfection at r = 2/1. The ratio of dextran and DNA in experiments is at 4/1. The zeta potential of PDMAEMA, PDAMA and PLLys in complexes with DNA at their optimal ratios in transfection is circa +16mV and the zeta potential of PEI/DNA complexes is around +20 mV. The dextran has a neutral charge and naked DNA has the zeta potential of -20mV (measured by Malvern instrument Zetasizer Nano ZS)

RESULTS

IR spectra of DNA in aqueous buffers at various pH

The infrared spectrum of DNA in the region of 1800-800 cm^{-1} contains a variety of information on the conformational arrangement. Polymers with amido or amine groups can interact with DNA via electrostatic attractions after their protonation. However, the nitrogen base region (1700-1500 cm^{-1}) in the DNA spectrum overlaps with the amine signals of the polymers, requiring spectra between 1300 and 800 cm^{-1} to be designated for monitoring this possible interaction. Typical IR spectra of DNA (2 mg/ml) in acetate (pH 5) and Tris buffer (7.4 and 9) are shown in Figure 1A. The secondary conformation of DNA in aqueous solution clearly remains in B-form in the pH range 5 to 9 because the assignments of DNA at 1224, 1086, 1056, and 969cm^{-1} were observed. The absorption bands at 1224 and 1086cm^{-1} are known to be the antisymmetric and symmetric stretching vibration of the phosphate groups (PO_2^-), respectively. IR absorption at 1056cm^{-1} is typically assigned to the vibration of ribose (C-C sugar) and absorption at 969cm^{-1} is as an indication of the existence of DNA. The band at 1224 cm^{-1} is a marker of B-form DNA (25). The bands at 938, 894, and 836 cm^{-1} are also signals of the B-form DNA (26) but they are not prominent in the ATR spectra, making it difficult to observe possible conformational alterations after complexating with polymers. A transition from B- to C- form (all belong to B-family structures) was observed by circular dichroism as we previously reported (27-29) but the addition of polycation polymer, in many cases, could not lead to any significant changes in CD spectra and DNA remains in the B-form. (28,30,31). However, the antisymmetric vibration of PO_2^- (1224 cm^{-1}) exhibits a greater change either in band position or intensity before and after DNA condensation (32). In this study, the sensitive band at 1224 cm^{-1} is used to evaluate the changes before and after complexation with polymers as it is chosen to monitor the changes for the interaction of DNA with liposomes (33, 22). No significant changes in the DNA symmetric vibration of PO_2^- at 1086 cm^{-1} were observed either in the presence of polymers or liposomes.

Effect of PEI and PLLys ratios on IR spectra

The IR spectrum of PEI indicates that its absorbance bands at circa 1224cm^{-1} are distinguished from the DNA phosphate peak (Figure 1B). For PEI/DNA complexes, the optimal transfection efficiency was found at a PEI/DNA ratio of 2/1 to 1/1 where the smallest particles were formed. The typical IR spectra of PEI/DNA complexes at various pH (from 5 to 9) are shown in Figure 2A and show that DNA still remains in the B conformation in the presence of PEI as indicated by a band at 937 cm^{-1}. At low PEI/DNA ratios (r = 1/40) in pH 5, 7.4 and 9 buffers, the spectra of DNA in complexes were nearly identical to naked DNA and the antisymmetric phosphate band stands is close to 1224 cm^{-1} (Figure 2B). This indicates that there is fairly less interaction between DNA and PEI, which is in agreement with the evidence

of found large particles (>1μm). By increasing the PEI/DNA ratio to r =2/1, a shift of the antisymmetric phosphate band from 1224 to 1220 cm^{-1} was observed. The shift to a lower frequency at lower pH means an occurrence of a stronger interaction between PEI and DNA and smaller particles were formed. Also, this shift to a lower frequency suggests an increase of DNA hydration state (22, 34, 35). These alterations are all factors which result in the higher transfection efficiency of polyplexes.

The IR spectra of PLLys/DNA complexes at various pH are shown in Figure 3A. The PLLys/DNA complexes were studied at r =4/1 where smallest particles and their optimal transfection efficiency were achieved (36). When PLLys/DNA ratios increased from r =1/20 to r =4/1, a similar trend as in PEI was observed, i.e., a shift of the antisymmetric phosphate band at 1224 cm^{-1} to a lower frequency (1220 cm^{-1}, Figure 3A and 3B). This also means an increased occurrence of hydration around DNA PO_2^- groups when DNA is complexated with PLLys. At pH 7.4 and 9 with PLLys, DNA still remains in the B conformation (Figure 3A). The IR spectrum of DNA is also affected by the presence of cations (e.g. $Pb2^+$, $Cu2^+$, H^+) (18, 37), and as shown, it can also be affected by cationic polymers. With PEI or PLLys at pH 9, the DNA PO_2^- antisymmetric band was nearly identical to naked DNA and unchanged in position because the limited protonated positive charges of the polymer can barely interact with DNA (Figure 2B and Figure 3B). It has been previously reported by our group that the particle size of polymer/DNA complexes is dependent on pH and the polymer/DNA ratios (16). The particle size of complexes formed at pH 9 was larger and was responsible for the loss of transfection efficiency (15).

Although the shift of the $PO2^-$ antisymmetric band to lower frequencies was found after complexation, the presence of cationic polymers does not alter the secondary structure of DNA from the B conformation in buffer solutions. Previous studies have reported that DNA in solution appears to be B form in the presence of following milieu: DOTAP/cholesterol liposomes or DOTAP/DOPE liposomes (22) or cation ions or polymers (26,32,34,38), in spite of the different chemical structures of the interacted polycations, are in agreement with the findings of this study (Figure 2A, 3A).

Effect of PDAMA, PDMAEMA and dextran on IR spectra

For the complexes prepared at a PDAMA/DNA ratio of 4/1, the DNA absorptive band at 1224 cm^{-1} was not altered after complexation as shown in Figure 4. Interestingly, the formed PDAMA/DNA complexes were found in vitro to bear a very low activity in transfection. PDMAEMA, the polymer/DNA ratio at r =4/1 could bring out an optimal transfection and a small particle size as we have reported (16, 39). Since the position of the DNA antisymmetric phosphate band at 1224 cm^{-1} was found to overlap with the polymer absorbance peaks, the possible spectral change of DNA in the presence of PDMAEMA is difficult to analyze (vibration at 1234 cm^{-1} is assigned to PDMAEMA, Figure 4, line c). Nevertheless, the observation of the sugar vibration of DNA assigned at 894 cm^{-1} is another indication that the B form DNA is present in the complexes in a hydration state and its transfection ability is preserved. The spectrum of the dextran polymer with DNA shows an unchanged antisymmetric phosphate band at 1224 cm^{-1} as naked DNA (Figure 4, line a), which means that the interaction between DNA and the dextran polymer is minimal and consistent with very low transfection efficiency found in experiments.

The buffering capacity of PEI, PDMAEMA, PLLys, PDAMA, dextran and NaCl

In Figure 5, it shows that PEI possesses the best buffering capacity within the pH range. NaCl and the dextran polymer containing no amino groups in their structures demonstrate no buffering effect in the range of pH 5 to 8. The polymers with the best performance in buffering capacity are in this order: PEI > PDMAEMA > PLLys > PDAMA > dextran =NaCl. Interestingly, the same order was found for the transfection efficiency of these polymers (Figure 5 and Figure 6). This demonstrates that the higher transfection potential of a polymer shows, the better buffering capacity is to acids.

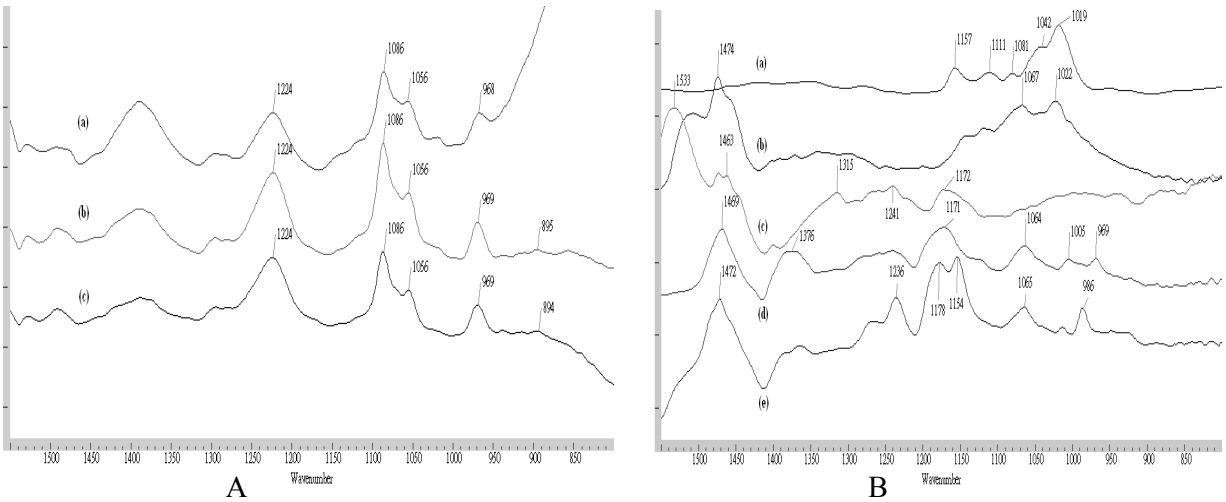

Figure 1. (A) DNA spectra at (a) pH 5, (b) pH 7.4, and (c) pH 9; **(B)** Spectra of (a) dextran, (b) PEI, (c) PLLys, (d) PDAMA and (e) PDMAEMA

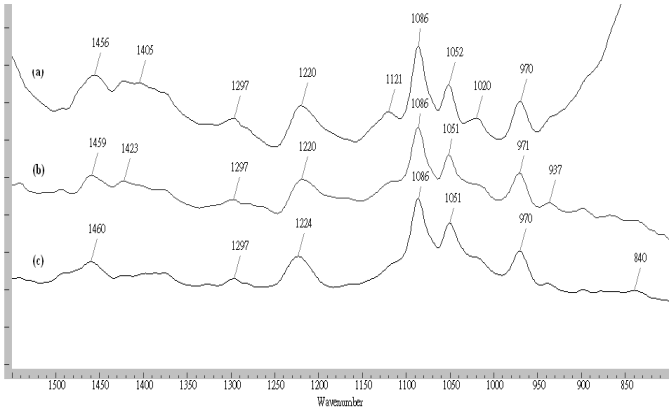

Figure 2A

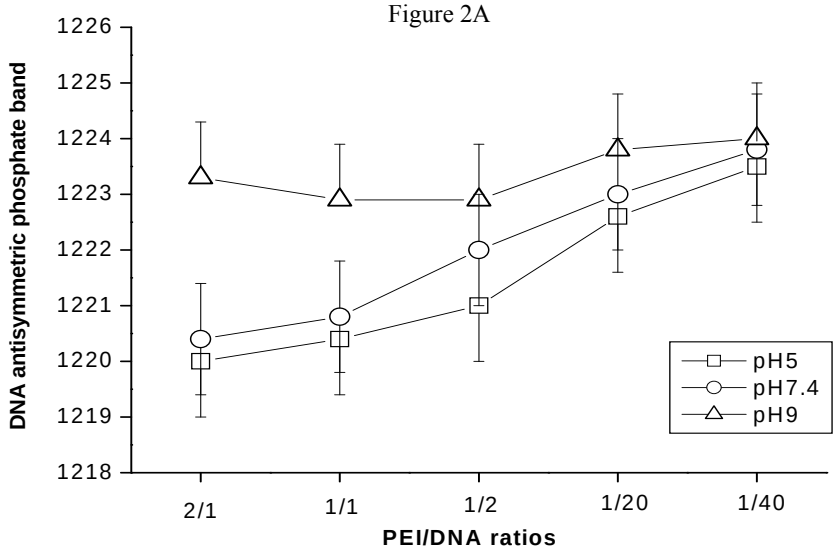

Figure 2B

Figure 2. (A) Spectra of PEI/DNA complexes at polymer/DNA ratio (r) of 2/1 at (a) pH 5, (b) pH 7.4, and (c) pH 9; **(B)** Shifting of DNA antisymmetric PO_2^- vibrations at different PEI/DNA ratios and pH.

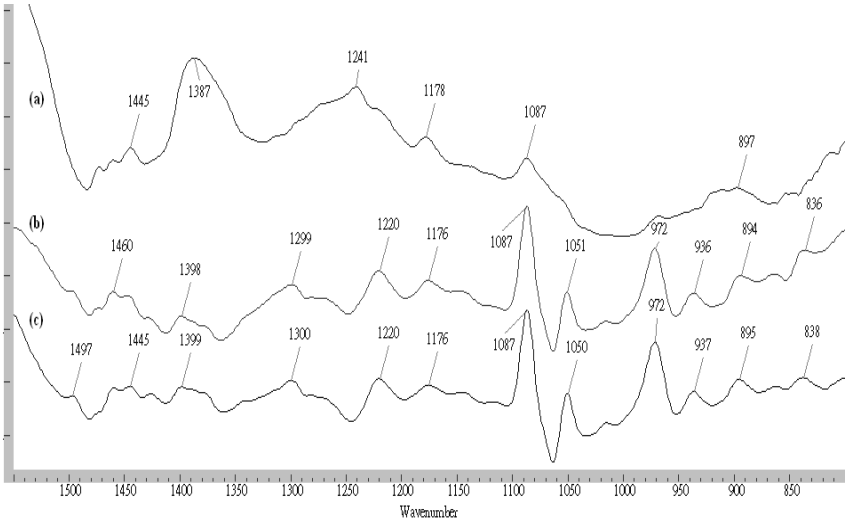

Figure 3A

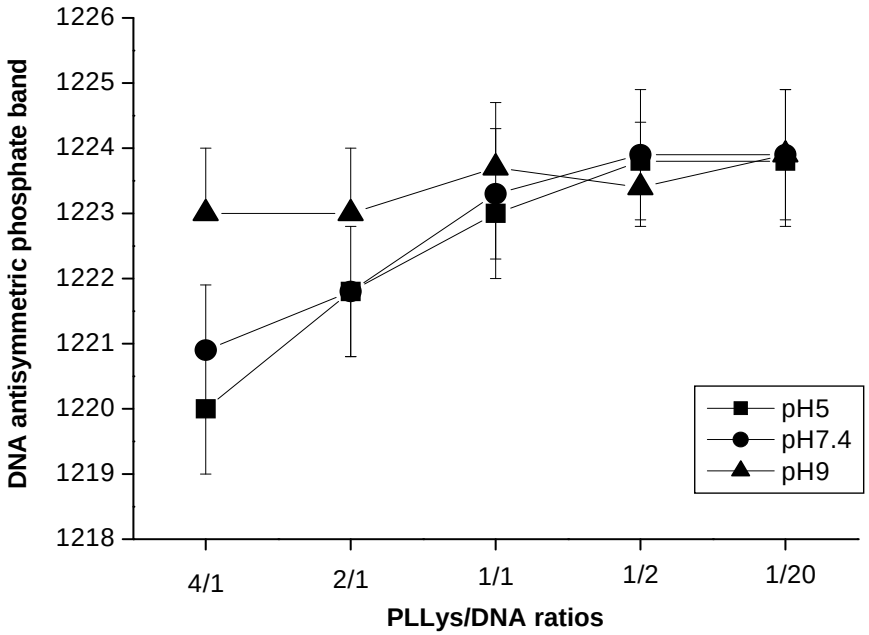

Figure 3B

Figure 3. (A) Spectra of PLLys/DNA complexes at r = 4/1 at (a) pH 5, (b) pH 7.4, and (c) pH 9; **(B)** Shifting of DNA antisymmetric PO_2^- vibrations at different PLLys/DNA ratios and pH.

DISCUSSION

Correlation between the alterations of DNA spectra in the presence of polymers and their transfection efficiency

The relative transfection efficiency of PEI and PLLys with respect to the reference polymer PDMAEMA is about 1.3 and 0.5, respectively (Figure 6). The antisymmetric PO_2^- vibration of DNA shifts to a lower frequency from 1224 cm^{-1} to 1220 cm^{-1} in the presence of PEI or PLLys, which is attributable to the electrostatic interactions between cationic polymers and DNA, yielding a hydration effect in the region of DNA phosphate groups (21, 24). Moreover, the formed small particles of polymer/DNA complexes that result from strong electrostatic interaction and hydration effect could make these complexes better for transfection into cells.

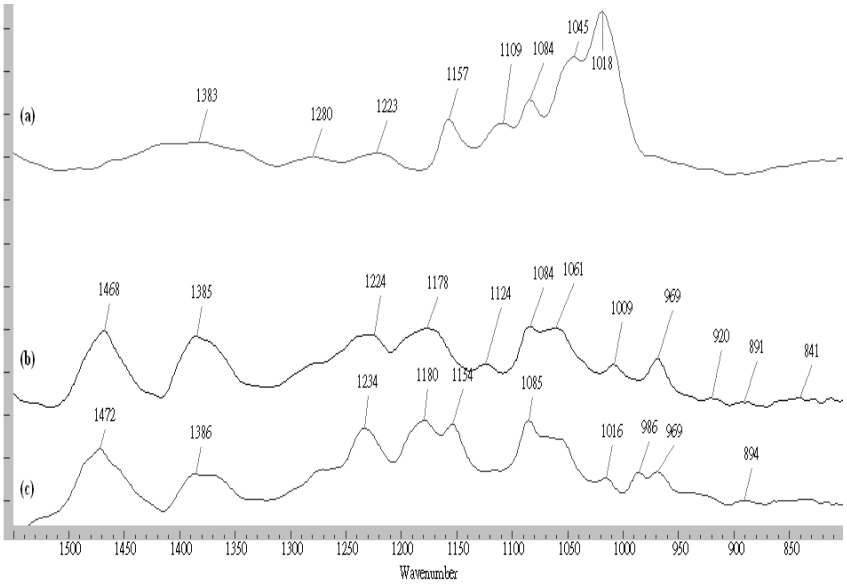

Figure 4. Spectra of (a) dextran/DNA, (b) PDAMA/DNA, and (c) PDMAEMA/DNA in pH 5 (r = 4/1).

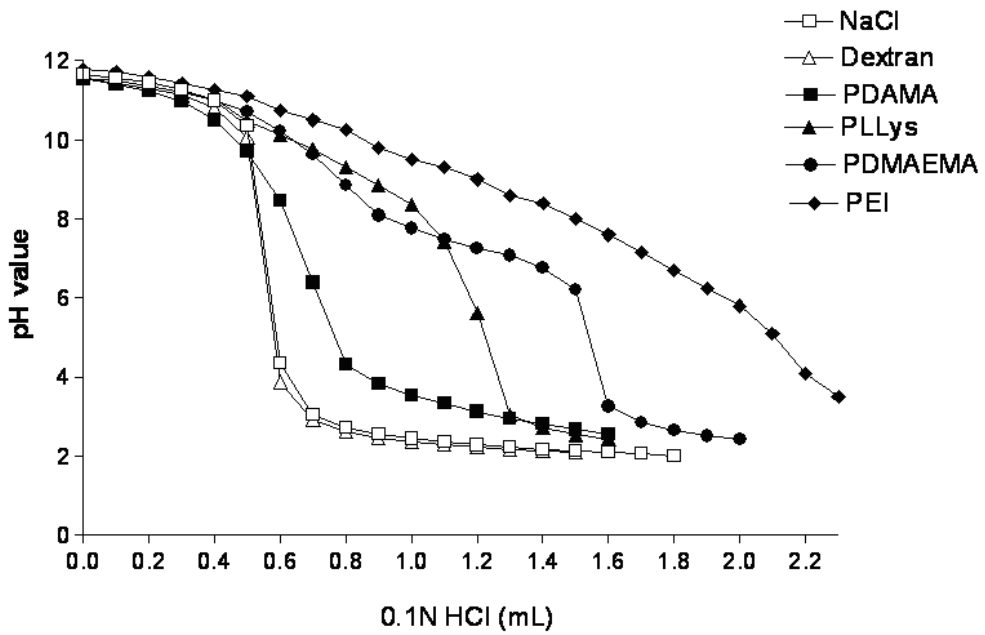

Figure 5. The buffering capacity of polymers of PEI, PLLys, PDAMA, PDMAEMA, dextran and NaCl in titration with acids HCl (0.1N).

Noteworthy is the fact that Hackl et al. has reported a shift of the DNA antisymmetric PO_2^- band to a higher frequency (from 1224 cm^{-1}) in the presence of bivalent ions (such as Ca^{2+}) (38, 25). These co-precipitates of bivalent ions have an effect on the dehydration of DNA resulting in a very low transfection activity. Since the zeta potential of studied complexes is similar (from +16 to +20mV, except the dextran) and only non-specific interaction to the studied cells is discussed, the effect of cell surface charge with the counter-charge complexes is minimal. In a series of polycationic polymers (based on the same framework in structure), the higher molecular weight of a polymer has, the better transfection efficiency would be (40).

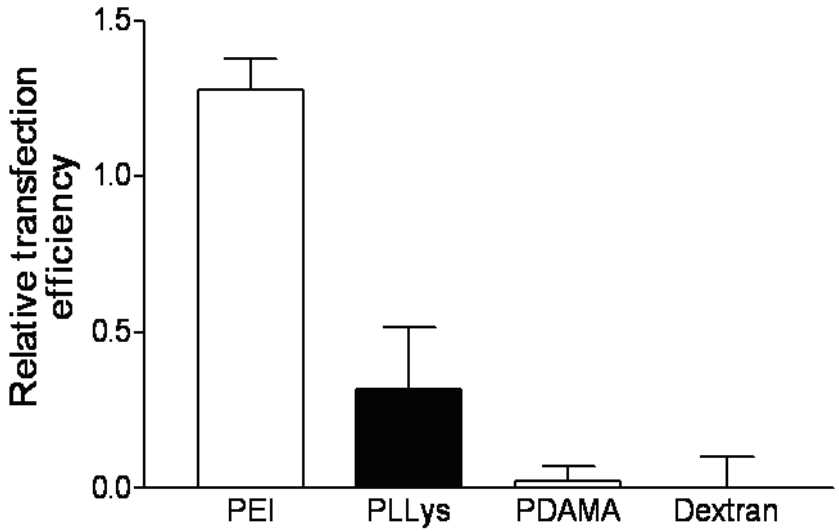

Figure 6. Relative transfection efficiency (normalized to PDMAEMA as reference) of PEI, PLLys, PDAMA, dextran, in complexation with plasmid DNA.

Results show that the lowest Mw (19 kDa) PDAMA bears the worst transfection but the PEI in a similar Mw (25 kDa) showed the best transfection activities. This suggests that PDAMA is likely lack of the interaction to DNA antisymmetric $PO2^-$ group because the absorption peak at 1224 cm^{-1} remains unchanged. However, a possibility that a substantial increase in Mw of PDAMA resulting in a higher transfection activity can not be excluded.

Correlation between the buffering capacity of polymers and their potential in transfection efficiency

The transfection potential is found to be related to the polymers' buffering capacity although overlapping of polymer bands in the DNA region around 1224 cm^{-1} could occur (ex. PDMAEMA). Titration studies were performed to determine the buffering capacity of the studied polymers within the cellular endosomal/lysosomal compartments. Protonable polymers e.g., PEI with a plenty of amino groups in the structure could not only mediate the DNA binding and DNA condensation but also help endosomal release through their ability to act as a "proton sponge". Evidence shows that the retardation of pH change in lysosome vesicles by lowering the pH to around pH 5 leads to vesicle swelling, rupture and eventually DNA/polymer complexes are released from the entrapment of endosome/lysosome vesicles (41). Therefore, the buffering capacity of polymers is related to the prevention of DNA degradation in lysosomes and the ease of delivery of DNA into nucleus from lysosomes which results in better transfection activities.

Since dissociation of DNA and polymer must be occurred at nucleus for a successful transfection and negatively charged proteins would compete the binding to cationic polymers with DNA, electrophoretic studies were conducted to show DNA dissociation of PEI/DNA complexes in the presence of negatively charged albumin (at pH 5 and pH 8) (Figure 7). There is no free DNA band visible in lanes 2, 3 and 4 (Figure 7A, 7B) for PEI/DNA complexes. However, in the presence of various amounts of albumin, DNA bands and albumin bands were observed (lanes 5, 6 and 7) in comparison to only albumin (lane 8). These results suggest that DNA can be dissociated from complexes and provide good transfection potential even though a strong interaction occurs between PEI and DNA especially at pH 5.

Figure 7A (pH 5)

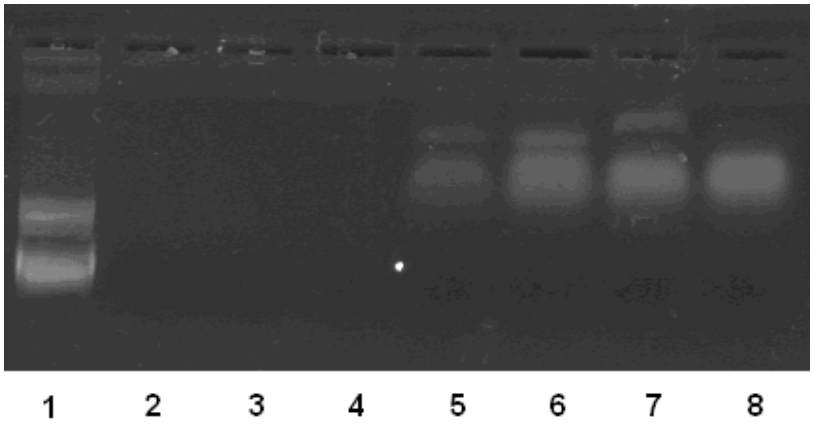

Figure 7B (pH 8)

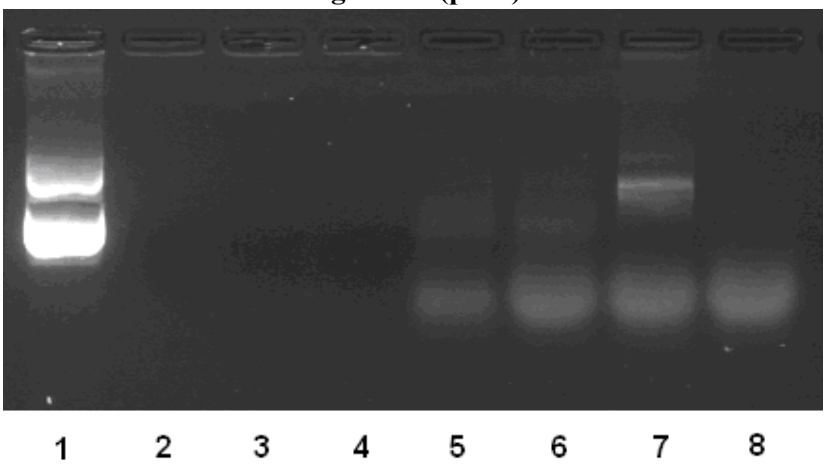

Figure 7. (A) electrophoresis (at pH 5) of naked DNA and PEI/DNA complexes (fixed at 10 μg/ml plasmid DNA) and electrophoresis of naked DNA and PEI/DNA complexes (fixed at 10 μg/ml plasmid DNA) at pH 8 **(B)**. lane1: naked DNA; lane 2: complexes (PEI/DNA r = 2:1); lane 3: complexes (PEI/DNA r = 1:1); lane 4: complexes (PEI/DNA r = 1:2); lane 5: complexes (PEI/DNA r = 2:1) + 2mg albumin; lane 6: complexes (PEI/DNA r = 2:1) + 6mg albumin; lane 7: DNA + albumin 6mg; lane 8: H2O + albumin 6mg.

CONCLUSION

In this paper, we have demonstrated the structure-function relationship in polymer/DNA complexes and the DNA antisymmetric PO_2^- vibration is sensitive to the binding of cationic polymers. The alterations in shifting frequencies could be used to interpret the performance in transfection after polymer complexation. Also, polymers that possess better buffering capacity have higher transfection efficiency. These findings could be very useful for the development of polymer-based gene delivery systems with better performance in vitro and in vivo.

REFERENCE

1. Hosseinkhani H, Hosseinkhani M. Biodegradable polymer-metal complexes for gene and drug delivery. Curr Drug Saf, 2009; 4:79-83.
2. Hosseinkhani H, Hosseinkhani M, Gabrielson NP, Pack DW, Khademhosseini A, Kobayashi H. DNA nanoparticles encapsulated in 3D tissue-engineered scaffolds enhance osteogenic differentiation of mesenchymal stem cells. J Biomed Mater Res A, 2008; 85:47-60.
3. Hosseinkhani H. DNA nanoparticles for gene delivery to cells and tissue. Int J Nanotechnology, 2006; 3:416-461.
4. Midoux P, Pichon C, Yaouanc JJ, Jaffrès PA. Chemical vectors for gene delivery: a current review

on polymers, peptides and lipids containing histidine or imidazole as nucleic acids carriers. Br J Pharmacol, 2009; 157:166-78.
5. Barteau B, Chèvre R, Letrou-Bonneval E, Labas R, Lambert O, Pitard B. Physicochemical parameters of non-viral vectors that govern transfection efficiency. Curr Gene Ther, 2008; 8 :313-23.
6. Hosseinkhani H, Azzam T, Tabata Y, Domb AJ. Dextran-spermine polycation: an efficient nonviral vector for in vitro and in vivo gene transfection. Gene Ther, 2004; 11:194-203.
7. Hsiue G.H, Chiang HZ, Wang CH, Juang TM. Nonviral gene carriers based on diblock copolymers of poly(2-ethyl-2-oxazoline) and linear polyethylenimine. Bioconjugate Chem, 2006; 17:781-786.
8. Roques C, Fattal E, Fromes Y. Comparison of toxicity and transfection efficiency of amphiphilic block copolymers and polycationic polymers in striated muscles. J Gene Med, 2009; 11:240-249.
9. Lühmann T, Rimann M, Bittermann AG, Hall H. Cellular uptake and intracellular pathways of PLL-g-PEG-DNA nanoparticles. Bioconjug Chem, 2008; 19:1907-1916.
10. Layman JM, Ramirez SM, Green MD, Long TE. Influence of polycation molecular weight on poly(2-dimethylaminoethyl methacrylate)-mediated DNA delivery in vitro. Biomacromolecules, 2009; 10:1244-1252.
11. Verbaan FJ, Klouwenberg PK, Steenis JH van, Snel CJ, Boerman O, Hennink WE, Storm G.. Application of poly(2-(dimethylamino)ethyl methacrylate)-based polyplexes for gene transfer into human ovarian carcinoma cells. Int J Pharm, 2005; 304:185-92.
12. Funhoff AM, Nostrum CF van, Koning GA, Schuurmans-Nieuwenbroek NM, Crommelin DJ, Hennink WE. Endosomal escape of polymeric gene delivery complexes is not always enhanced by polymers buffering at low pH. Biomacromolecules, 2004; 5:32-39.
13. Werth S, Urban-Klein B, Dai L, Hobel S, Grzelinski M, Bakowsky U, Czubayko F, Aigner A. A low molecular weight fraction of polyethylenimine (PEI) displays increased transfection efficiency of DNA and siRNA in fresh or lyophilized complexes. J Control Release, 2006; 112:257-270.
14. Jiang HL, Kim YK, Arote R, Jere D, Quan JS, Yu JH, Choi YJ, Nah JW, Cho MH, Cho CS. Mannosylated chitosan-graft-polyethylenimine as a gene carrier for Raw 264.7 cell targeting. Int J Pharm, 2009; 375:133-139.
15. Cherng JY, Wetering P van de, Talsma H, Crommelin DJ, Hennink WE. Effect of size and serum proteins on transfection efficiency of poly ((2-dimethylamino)ethyl methacrylate)-plasmid nanoparticles. Pharm Res, 1996; 13:1038-42.
16. Cherng JY, Talsma H, Verrijk R, Crommelin DJ, Hennink WE. The effect of formulation parameters on the size of poly((2-dimethylamino)- ethyl methacrylate)-plasmid complexes. Eur J Pharm Biopharm, 1999; 47:215-224.
17. Hosseinkhani H, Tabata Y. Ultrasound enhances in vivo tumor expression of plasmid DNA by PEG-introduced cationized dextran. J Control Release, 2005; 108:540– 556.
18. Tajmir-Riahi, HA, Ahmad R, Naoui M, Diamantoglou S. The effect of HCl on the solution structure of calf thymus DNA: a comparative study of DNA denaturation by proton and metal cations using Fourier transform IR difference spectroscopy. Biopolymers, 1995; 35:493-501.
19. Neault, JF, Naoui M, Manfait M, Tajmir-Riahi HA. Aspirin-DNA interaction studied by FTIR and laser Raman difference spectroscopy. FEBS Lett, 1996; 382:26-30.
20. Neault JF, Tajmir-Riahi HA. Structural analysis of DNA-chlorophyll complexes by Fourier transform infrared difference spectroscopy. Biophys J, 1999; 76:2177-2182.
21. Braun CS, Jas GS, Choosakoonkriang S, Koe GS, Smith JG.. The structure of DNA within cationic lipid/DNA complexes. Biophys J, 2003; 84:1114-1123.
22. Choosakoonkriang S, Wiethoff CM, Koe GS, Koe JG, Anchordoquy TJ, Middaugh CR. An infrared spectroscopic study of the effect of hydration on cationic lipid/DNA complexes. J Pharm Sci, 2003; 92:115-130.
23. Braun CS, Vetro JA, Tomalia DA, Koe GS, Koe JG, Middaugh CR. Structure/function relationships of polyamidoamine/DNA dendrimers as gene delivery vehicles. J Pharm Sci, 2005; 94:423-36.
24. Prevette LE, Lynch ML, Kizjakina K, Reineke TM. Correlation of amine number and pDNA binding mechanism for trehalose-based polycations. Langmuir, 2008; 24:8090-8101.
25. Hackl EV, Kornilova SV, Blagoi YP. DNA structural transitions induced by divalent metal ions in aqueous solutions. Int J Biol Macromol, 2005; 35:175–191.
26. Andrushchenko V, Leonenko Z, Cramb D, van de Sande H, Wieser H. Vibrational CD (VCD) and atomic force microscopy (AFM) study of DNA interaction with Cr^{3+} ions: VCD and AFM evidence of DNA condensation. Biopolymer, 2002; 61:243-260.
27. Cherng JY, Talsma H, Crommelin DJA, Hennink WE. Long Term Stability of Poly((2-dimethylamino)ethyl Methacrylate)-Based Gene Delivery Systems. Pharm Res, 1999; 16:1417-1423.
28. Kypr J, Kejnovska I, Renciuk D, Vorlickova M. Circular dichroism and conformational polymorphism of DNA. Nucleic Acids Research, 2009; 37:1713–1725.
29. van Dam L, Levitt MH. BII nucleotides in the B and C forms of natural-sequence polymeric DNA: a new model for the C form of DNA. J Mol Biol, 2000;

304:541-561.
30. Zhou LH, Yang M, Zhou H, Zhang J, Li K, Xiang YZ, Wang N, Tian YF, Yu XQ. Novel reticular cyclen-based polymer as gene vector in DNA transfection. Chem Biol Drug Des 2009; 73:216–224.
31. Cui L, Chen D, Zhu L. Conformation transformation determined by different self-assembled phases in a DNA complex with cationic polyhedral oligomeric silsesquioxane lipid. ACSNano, 2008; 2:921-927.
32. Choosakoonkriang S, Weithoff CM, Anchordoquy TJ, Koe GS, Smith JG, Middaugh R. Infrared spectroscopic characterization of the interaction of cationic lipids with plasmid DNA. J Bio Chem, 2001; 276:8037-8043.
33. Bruni, P, Cingolani F, Iacussi M, Pierfederici F, Tosi G. The effect of bivalent metal ions on complexes DNA-liposomes: a FT-IR study. J Mol Struct, 2001; 565:237-245.
34. Choosakoonkriang S, Lobo BA, Koe GS, Koe JG, Middaugh CR. Biophysical characterization of PEI/DNA complexes. J Pharm Sci, 2003; 92:1710-1722.
35. Sukhorukov GB, Feigin LA, Montrel MM, Sukhorukov BI. X-ray and infrared study of Langmuir-Blodgett films of the complexes between nucleic acids and aliphatic amines. Thin Solid Films, 1995; 259:79-84.
36. van de Wetering P, Cherng JY, Talsma H, Hennink WE. Relation between transfection efficiency and cytotoxicity of poly(2-(dimethylamino)ethyl methacrylate)/plasmid complexes. J Control Release, 1997; 49:59-69.
37. Tajmir-Riahi HA, Naoui M, Ahmad R. The effects of Cu2+ and Pb2+ on the solution structure of calf thymus DNA: DNA condensation and denaturation studied by Fourier transform IR difference spectroscopy. Biopolymer, 1993; 33:1819-1827.
38. Hackl EV, Kornilova SV, Kapinos LE, Andrushchenko VV, Galkin VL, Grigoriev DN, Blagoi, YP. Study of Ca2+, Mn2+ and Cu2+ binding to DNA in solution by means of IR spectroscopy. J Molecular Structure, 1997; 408/409:229-232.
39. van de Wetering P, Cherng JY, Talsma H, Crommelin DJ, Hennink WE. 2-(Dimethylamino)ethyl methacrylate based (co)polymers as gene transfer agents. J Control Release, 1998; 53:145-53.
40. Shau MD, Tseng SJ, Yang TF, Cherng JY, Chin WK. Effect of molecular weight on the transfection efficiency of novel polyurethane as a biodegradable gene vector. J Biomed Mater Res A. 2006; 77:736-746.
41. Cristiano RJ. Targeted non-viral gene delivery for cancer gene therapy. Frontiers in Biosciecne, 1998; 3:1161-1170.