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Controlled complexation of plasmid DNA with cationic polymers: Effect of surfactant on the complexation and stability of the complexes

Marjukka Ikonen, Lasse Murtomäki*, Kyösti Kontturi

Helsinki University of Technology, Laboratory of Physical Chemistry and Electrochemistry, PO Box 6100, FI-02015 TKK, Finland

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ABSTRACT

The aggregation of the cationic polymer-plasmid DNA complexes of two commonly used polymers, polyethyleneimine (PEI) and poly-L-lysine (PLL) were systematically compared. The complexation was studied in 5% glucose solution at 25 °C using dynamic light scattering and isothermal titration calorimetry. The aggregation of the complexes was controlled by addition of the surfactant polyoxyethylene stearate (POES). The stability of the complexes was evaluated using dextran sulphate (DS) as relaxing agent. The relaxation of the complexes in the presence of DS was studied using agarose gel electrophoresis. This study elucidates the role of surfactant in controlling the size of the PEI/pDNA complex and reveals the differences of the two polymers as complexing agents.

1. Introduction

Recently, various viral and non-viral gene delivery vehicles have been developed for the use of gene therapy. Because of the safety concerns associated with viral vectors, such as their toxicity and potential for generating a strong immune response, non-viral gene delivery systems have gained increasing interest [1,2]. In addition to safety issues, the advantages of the non-viral gene delivery vehicles include the ease modification of their structure and low cost. Despite the advantages, a lot of research is still needed on non-viral vectors before they can be utilised in clinical applications as their efficiency is much lower compared to the viral vectors [2,3].

Commonly used non-viral vectors are usually classified into two types: polymeric delivery systems and liposomal delivery systems. In liposomes, DNA molecules are either entrapped in the aqueous cavity of the liposomes or are bound on their surface. Although promising results have been gained using cationic liposomes [4], the transfection efficiency of liposomal carriers is still very low.

Various polymeric carriers have been tested for gene delivery purposes. Their structures usually include protonable amines, the number and pK_a of which is different in each carrier [5]. Two of the most commonly used polycations are poly-L-lysine (PLL) and polyethyleneimine (PEI). Both of these cationic polymers form small toroidal complexes with DNA [6]. However, *in vitro* studies have shown that compared to PLL, the transfection efficiency of PEI is much higher [7,8]. The higher efficiency in gene transfer has been explained by its ability to buffer endosomes [3,7]. The buffering capacity of PEI results from its unique structure: only a part of the amino groups in PEI are protonated at physiological pH.

One major problem when using polymeric carriers is that the complexes tend to aggregate in aqueous solutions. The aggregation behaviour is influenced by the conditions in which the polycation-DNA complexes are prepared. Generally the aggregates grow bigger in higher ionic strength. Because of this, the aggregation is more severe under physiological conditions whereas in glucose solution the complexes aggregate to a lesser extent. Also, PEI/DNA complexes formulated in 5% glucose have been shown to be more efficient in vivo than complexes prepared in 150 mM sodium chloride [9]. It has been proposed that the difference in efficiency is due to the difference in their aggregation behaviour in different media. Nevertheless, the aggregation phenomenon exists even in 5% glucose, because of which detailed knowledge on the phenomenon is crucial when improving the efficiency of gene carriers.

In order to reach higher efficiency in gene delivery, the size of the DNA complexes formed using synthetic polymers has to be controlled. Much research has already been done concerning the control of the size of the complexes and the prevention of the aggregation process. In most of the studies, the attempts to control the aggregation behaviour of polycations have either involved the right choice of environment and protocols of complexing [10,11] or the covalent attachment of protecting groups [3,12]. In the work of Lee et al. [13] aggregation of complexes was prevented by

* Corresponding author. Tel.: +358 9 4512575; fax: +358 9 4512580.
E-mail address: Lasse.Murtomaki@tkk.fi (L. Murtomäki).

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electrostatic attachment of cationic fusogenic peptides conjugated with poly(ethylene) glycol to negatively charged PEI/DNA complexes. Also, biomaterial systems for controlled delivery of DNA have been used to overcome the aggregation problem [14,15]. Recently, it was proposed that the aggregation could also be prevented by surfactants [16]. Sharma et al. showed that the addition of the surfactant polyoxyethylene inhibits the aggregation process of PEI-DNA complex during 24 h storage. Control of aggregation is not only important during the storage of the gene medicine, but more importantly, controlling the size and charge of the complex is crucial when delivering the vector into a cell.

Prior to entering the cell, DNA complexes may interact with various extracellular matrix components. Extracellular matrices contain sulphated proteoglycans consisting of a core protein covalently linked to one or more sulphated glycosaminoglycans (GAGs): heparin, heparin sulphate, dermatan sulphate, chondroitin sulphate or keratin sulphate [17]. These negatively charged GAGs may bind to the positively charged DNA complexes on its way to the cell. GAGs have been shown to have a dual role in gene delivery. While membrane associated GAGs have been suggested to mediate the binding and delivery of cationic complexes to the cell [18], many studies have also shown that extracellular GAGs can actually decrease the transfection efficiency of the non-viral carrier by blocking the access of the DNA complexes to the target cell [19–21]. If DNA is released from the complex upon interaction with GAGs, GAGs may be internalised into the cell with the free carrier instead of DNA [19,22]. Because of this, release of DNA by GAGs in the extracellular matrix is not desirable when designing new gene carriers.

In this study, the aggregation of the cationic polymer-DNA complexes of two commonly used polymers, PEI and PLL were systematically compared. The complexation was studied in 5% glucose solution at 25 °C using dynamic light scattering and isothermal titration calorimetry. The aggregation of the complexes was controlled by addition of the surfactant polyoxyethylene stearate (POES). The stability of the complexes in the presence of GAGs was evaluated using dextran sulphate (DS) as relaxing agent. The relaxation of the complexes was studied using agarose gel electrophoresis. DS is a highly charged polyanion and because of its resemblance with heparin, it can be used as a model molecule for extracellular matrix GAGs. This study elucidates the role of surfactant in controlling the size of the PEI/DNA complex and reveals the differences of the two polymers as complexing agents.

2. Materials and methods

2.1. Materials

The plasmid DNA, 750-kDa PEI and poly-(L-lysine) hydrobromide (PLL) were kind gifts from Centre for Drug Research (CDR) at University of Helsinki. DS sodium salt from *Leuconostoc* ssp. was obtained from Fluka. Glucose was from Merck and polyoxyethylene (100) stearate from Sigma–Aldrich. All solutions were prepared using MQ® water and they were filtered through 0.2 μm membrane prior to use.

2.2. Preparation of the complexes

Stock solutions of 0.4 mg/ml pDNA, 0.015 mg/ml PEI, 0.05 mg/ml PLL and 0.5% POES were prepared in 5% glucose solution. The polycation-pDNA complexation was done by rapidly adding an appropriate amount of the polycation to 0.02 mg of pDNA and diluting the solution to a total volume of 1.05 ml using 5% glucose solution. After this, the solution was stirred with a magnetic stirrer for one minute. The experiments with the surfactant POES were done in the similar manner except that the surfactant was added before the final dilution of the solution. Thus in every experiment the amount of DNA and the total volume were kept constant while the amounts of the polycations and the surfactant were varied.

2.3. Size and zeta potential measurements

The particle sizes and zeta potentials of the polycation-pDNA complexes formed at various ratios were determined using dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments). The values reported are *z*-average diameter values, which is the mean diameter determined from the sum of light scattered. The measurements were done in duplicate.

2.4. Isothermal titration calorimetry (ITC)

The ITC experiments were carried out using an isothermal titration calorimeter (Microcal VP-ITC, USA) at 25 °C and analyzed with Origin 5.0 software (Microcal, USA). All samples were degassed before use. The volume of the cell was 1.44 ml and the stirring rate used was 450 rpm. The injections were added at 4 min time intervals. The raw calorimetric data were corrected for the heats of dilution by subtracting blank titrations from the binding data. To study the interactions of the cationic polymer and pDNA, 5 μl injections of 2 mM cationic polymer solution (with respect to nitrogen atoms) were added to 0.086 mM pDNA solution (with respect to phosphates). To study the protonation of the polycation PEI, 10 μl injections of 0.2 M hydrochloric acid were added to 14 mM solution of PEI. In the case of protonation of PEI, a corresponding titration was also performed manually to measure the pH values for each data point.

2.5. pH titration

In the beginning of the pH titration, the pH of 14 mM PEI solution (volume 4 ml) was adjusted to pH 11 using 5 M NaOH. 20 μl injections of 0.2 M HCl were added to the PEI solution and the pH was measured.

2.6. Agarose gel electrophoresis

Complexes were prepared at nitrogen/phosphate (N/P) ratio of 3 for the agarose gel electrophoresis. To study the stability of the complexes in the presence of GAGs, DS was added to the complexes at a three-fold charge excess. After 2 h, 20 μl of complex solution containing 1 μg of pDNA was loaded to 0.9% agarose gel in Tris-acetate-EDTA buffer (TAE) pH 8.0. The samples were electrophoresed at 80 V for 2 h (Sub-cell GT, BIO-RAD), after which the gel was stained in 0.5 μg/ml ethidium bromide solution and photographed on an ultraviolet transilluminator.

3. Results and discussion

3.1. Size and zeta potential

Size and zeta potential of the polycation-pDNA complexes were measured at various N/P molar ratios (Fig. 1). As can be seen from Fig. 1, the average size of both PEI/DNA and PLL/DNA complexes is approximately 150 nm at the lowest N/P ratios, whereas at the highest N/P ratios the size is a bit less, approximately 120 nm. The corresponding zeta potentials are negative at low N/P ratio, approximately −30 mV for both polycation-DNA complexes, and positive at high N/P ratio, being 13 mV for the PEI/DNA complex and 25 mV for the PLL/DNA complex. Fig. 1 shows also that the crossing point from the negative to the positive zeta potential is very sharp for

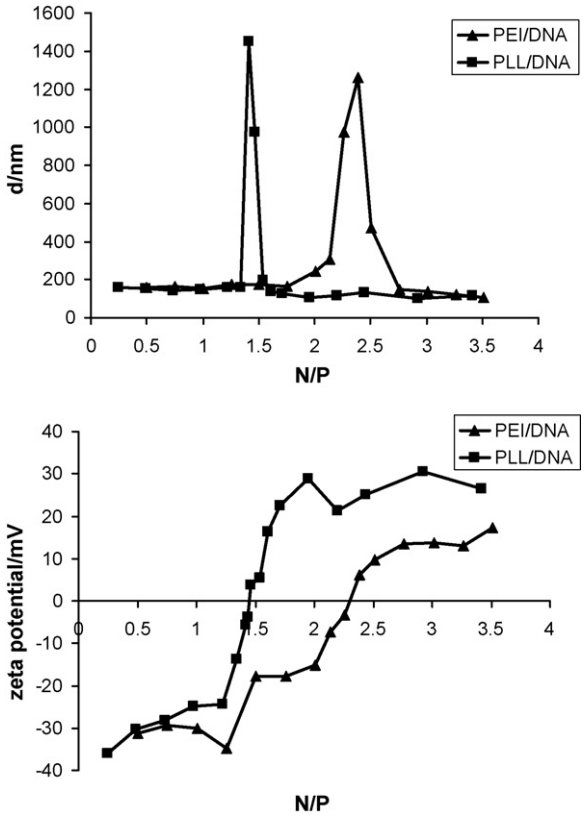

Fig. 1. Size and zeta potential of PEI/DNA and PLL/DNA complexes as a function of N/P ratio.

both polycation/DNA complexes. For PEI the change from negative to positive occurs at N/P 2.4 and for PLL at N/P 1.5. At N/P ratios close to zero zeta potential both polycation/DNA complexes aggregate significantly and the size of the complexes increases up to 1400 nm. While the size of the complexes remains constant over time at very low and high N/P ratios, near the charge neutral state the aggregation behaviour is time dependent.

Our data is consistent with previous studies: the complexes formed in 5% glucose solution are small and stable at most of N/P ratios [9,23]. However, compared to previous studies, in this study the complexation process at various N/P ratios was examined more in detail. Thus the results reveal a phenomenon that has not been reported earlier: the aggregation behaviour of the cationic polymer/DNA complexes also exists in glucose solution and it takes place at the N/P ratio where the complexes have no net charge. For PLL this is at N/P ratio 1.5 and for PEI 2.4. The ratio for PLL differs from unity probably because some of the primary amines might not be protonated. Deviation from the ratio 1 has been reported earlier also [6]. The ratio where PEI/DNA complex has no net charge is not close to unity because of the structure of PEI: in addition to primary amines, there are also secondary and tertiary amines and thus only a fraction of the nitrogens are protonated.

The N/P ratio of 2.4 for PEI/DNA complexes with no net charge is consistent with the N/P ratios reported in the literature. In the study of Choosakoonkriang et al. [24] it was recently reported that the change from negative to positive zeta potential occurs between N/P ratios 2 and 4. Also, DeRouchey et al. have reported that neutral particles are formed at N/P ratio of 2.5 [25], which is very close to the value we determined. In the work of Boeckle et al. [26] the role of purification was studied and the purified PEI/DNA complexes had the same final N/P ratio of 2.5 independent of the amount of PEI used in the complexation. When the complexation behaviour

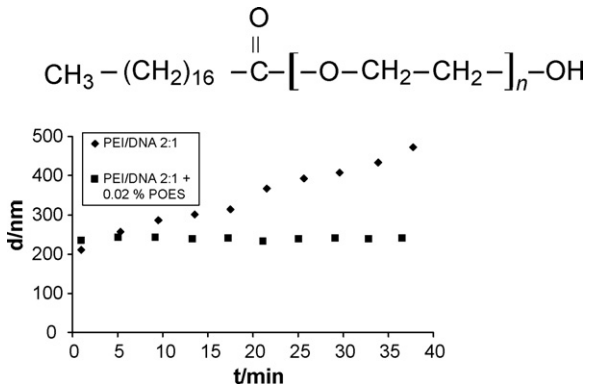

Fig. 2. Size of PEI/DNA complexes. The effect of 0.02% POES. The molecular structure of POES is shown in the insert.

of various cationic polymers has been studied earlier, PLL has been shown to aggregate more readily than PEI [6]. Our results support this only partly, as the aggregation phenomenon was observed with both polycations. PLL/DNA complexes, however, were observed to be somewhat more polydisperse than PEI/DNA complexes. Nevertheless, the different behaviour of the polycations was revealed, when the effect of the surfactant POES was studied.

3.2. The effect of surfactant

Sharma et al. [16] have reported that the aggregation process of PEI/DNA complexes can be inhibited by the surfactant POES. We examined the effect of POES on the aggregation more in detail over wide range of N/P ratios and compared the aggregation of PEI/DNA complex to that of PLL/DNA complex. Our results confirm the findings of Sharma et al.: At a given N/P ratio the aggregation of the PEI/DNA complexes is reduced notably and the size of the complexes remains constant over time. Fig. 2 shows the effect of 0.02% POES at N/P ratio 2.

The effectiveness of POES in stabilising the PEI/DNA complex has been explained by its dual hydrophobic–hydrophilic nature [16]. It has been proposed that the long hydrophilic chain group of the surfactant sterically prevents the complexes from approaching each other while the hydrophobic tail interacts with the hydrophobic parts of the PEI/DNA complexes.

However, the addition of POES does not prevent the aggregation over the whole range of N/P ratios studied. Instead, as shown in Fig. 3, the addition of POES shifts the aggregation process to a lower N/P ratio. The effect of POES concentration on the aggregation of the complexes is clear: the higher the POES concentration is, the more the aggregation peak is shifted to the direction of lower N/P ratios.

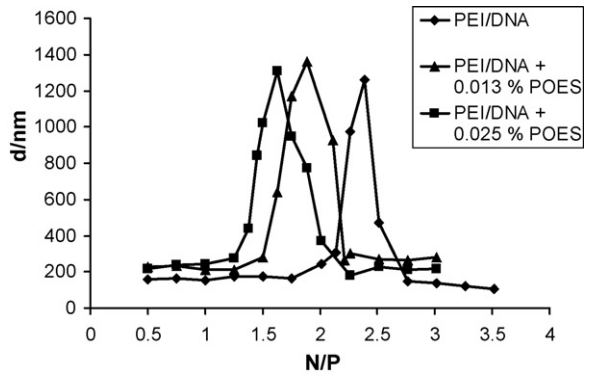

Fig. 3. Size of PEI/DNA complexes as a function of N/P ratio. The effect of POES.

Table 1
pK_a values of PEI and the effect of POES on pK_a

	α	pK$_a$
PEI	0.419	9.08
PEI + 0.013% POES	0.531	9.27
PEI + 0.025% POES	0.613	9.42

POES affects not only the size of the complexes, but also the zeta potential. The point of zero zeta potential is also shifted to the lower N/P ratios (data not shown). The highest diameter of the complexes was measured to correspond to the zeta potential closest to zero. The effect of POES concentration was studied systematically at two concentrations: 0.013% and 0.025%. The further increase of the POES concentration resulted in a very polydisperse system and thus the size distribution of these samples could not be measured reliably.

Since the aggregation process of PEI/DNA complexes is not prevented over the whole range of N/P ratios, the steric hindrance by the long hydrophilic chains of POES [16] does not fully explain the phenomenon. Instead, the surfactant has to have an effect not only on the interactions of the complexes, but also on the interactions of PEI and pDNA and the way in which the complexes are formed. Since the zeta potential at a given N/P ratio is significantly lower when the surfactant is present, the surfactant affects the electrostatic forces that drive the formation of the complexes. In other words, the surfactant changes the pK_a value of PEI. Because of its structure, PEI does not have a single pK_a value. However, as the size of the aggregates grow largest at the N/P ratio where the zeta potential of the complex is zero, the peaks observed in the size-N/P curve can be related to the apparent pK_a of the polycation:$PCH^+ \rightleftarrows P\,C + H^+$

$$K_{app} = \frac{[PC][H^+]}{[PCH^+]} = \frac{(1-\alpha)[H^+]}{\alpha} \quad (1)$$

where $[H^+]$ is the concentration of protons measured using a pH-meter, being 6.03×10^{-10} mol dm^{-3} for PEI. The N/P ratio of the peak is equal to $1/\alpha$ where α describes the protonated fraction of the polycation. As seen from Table 1, the addition of POES affects the extent of PEI's protonation increasing its pK_a value significantly. Because of the structure of PEI, these values should not be interpreted as the determination of pK_a as such, but rather in terms the effect of the surfactant on the protonation of the polycation.

The shift in the pK_a of PEI can be explained in terms of the change in the Gibbs energy. As the surfactant molecule is in the vicinity of the polycation, the shielding effect of the like charges is increased and the distance between the charges is altered. As the surfactant screens the charges, a greater fraction of the polycation can be protonated. From Coulomb's law, the change in the Gibbs energy as the change in distance between the charges is

$$\Delta G = -\frac{e^2 N_A}{4\pi\varepsilon_0\varepsilon}\left(\frac{1}{r_1} - \frac{1}{r_2}\right) \quad (2)$$

where e is the elementary charge, N_A Avogadro constant, ε_0 vacuum permittivity, ε the permittivity of water, and r_1 and r_2 are the separation distances of the charges. On the other hand, the Gibbs energy is

$$\Delta G = -RT \ln K_a \quad (3)$$

And the change in the Gibbs energy

$$\Delta(\Delta G) = -RT \ln \frac{K_{a,1}}{K_{a,2}} \quad (4)$$

which can be rewritten as

$$\Delta(\Delta G) = 2.303RT\Delta pK_a \quad (5)$$

Combining the Eqs. (2) and (5) gives

$$\Delta(\Delta G) = -\frac{e^2 N_A}{4\pi\varepsilon_0\varepsilon}\left(\frac{1}{r_1} - \frac{1}{r_2}\right) = 2.303RT\Delta pK_a \quad (6)$$

Assuming the distance between the charges of the polycation is 5 Å without the surfactant, the measured pK_a change of 0.2 units by the addition of the surfactant results in r_2 = 7.4 Å. Likewise, if the separation distance of the charges is 10 Å, r_2 = 27.9 Å. Thus, it is evident that very small changes in the distance between the charges shift the pK_a value significantly.

In the literature, the pK_a values for polycations have been determined in order to estimate their buffering capacity. Due to the structure of PEI, ranges of pH values where PEI shows buffering capacity have been reported instead of one single pK_a value. The reported pH ranges are wide and cover almost the entire pH range [6,27] making the comparison to our work difficult. In the work of Choosakoonkriang et al. [24], however, the pK_a of PEIs with different molecular weight was determined from the maximum of the buffer capacity curve and a value of 8.3 was reported for 750 kDa PEI. This value has been obtained using acid titration and it is somewhat lower than the pK_a we determined for PEI without the surfactant POES.

To further elucidate the role of POES, the protonation of PEI was studied by means of pH titration. Fig. 4 shows the titration curve for PEI. The simulated curve was calculated using a simplified model, which assumes that PEI has only one pK_a value. In addition, the dissolution of carbon dioxide was included in the simulation. The equations used to calculate the simulated curve can be found in Supporting information. Interestingly, the shape of the measured titration curve does not provide any information on the different protonable groups of PEI. Nevertheless, it is consistent with that reported in the literature [27]. The difference between the measured titration curve and the simulated one shows that PEI must have more than one protonable group. Compared to conventional pH titration, acid titration performed using ITC is more powerful in revealing the distinct protonable groups in PEI as can be seen from Fig. 5. Fig. 5 shows the calorimetric data measured using ITC combined with manually measured pH data. Also, it can be seen that the best buffering capacity of PEI at the pH range studied is between pH 5 and 8. The buffering capacity of PEI at this pH range is important as it has been proposed that the high transfection efficiency of polymers is due to their ability to buffer pH at the range from pH 5 to 7 [6].

The fact that the surfactant affects not only the size and stability, but also the zeta potential of the PEI/DNA complexes, is important, since the charge of the complexes partly determines their efficiency in transfection. In *in vitro* studies, most cationic carriers have shown an optimal transfection efficiency when they have

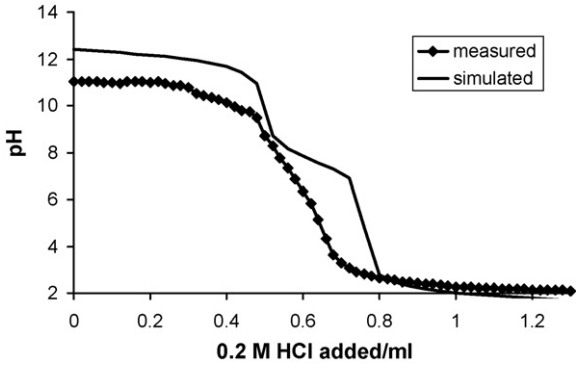

Fig. 4. Measured and simulated titration curves for PEI.

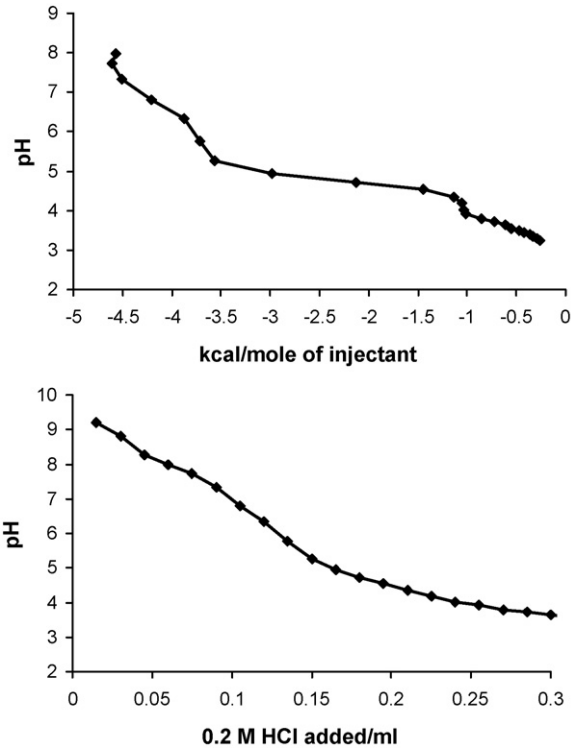

Fig. 5. pH as a function of change in enthalpy and ml of HCl.

a positive charge [24,28]. This has been explained by the binding of complexes to anionic proteoglycans on the cell surface [18]. The positive charge is, however, a problem when DNA complexes are in the blood circulation as they induce erythrocyte aggregation and interact with various plasma components [23]. It has been shown that it is the free PEI, not the PEI/DNA complexes themselves, which causes the erythrocyte aggregation [26]. Also, *in vivo* studies have shown that complexes closest to neutral in charge are the most effective in transfection [29]. In addition, even though the binding of positive charged complexes to the negative charged proteoglycans on the cell surface may help in transfection, this binding may also inhibit cation mediated gene transfer in many cases [21]. Thus for *in vivo* transfection, an ideal polycation/DNA complex is small and uncharged. With the help of the surfactant POES it is possible to control the size and charge of the PEI/DNA complex when designing optimal carriers. Furthermore, the use of POES as stabilising agent may help to reduce the amount of PEI in the complexes and thus cytotoxicity of the complex.

The effect of POES on the complexation of PLL and DNA is shown in Fig. 6. Unlike the PEI/DNA complexation, the PLL/DNA complexation was almost unaffected by the addition of POES. The disability of the surfactant to inhibit the aggregation process of the PLL/DNA complexation can be explained by the structural difference of the polycations and the complexes formed. PEI has three different ionisable groups, primary, secondary and tertiary amines whereas PLL possesses only primary amines.

3.3. Isothermal titration calorimetry

Microcalorimetric titrations of the polycations into DNA at 25 °C were performed in order to study the enthalpy changes associated to the complexation process and to determine the binding constant for the complexation. The ITC data for PEI/DNA complexation is shown in Fig. 7. However, the measured enthalpy changes were so

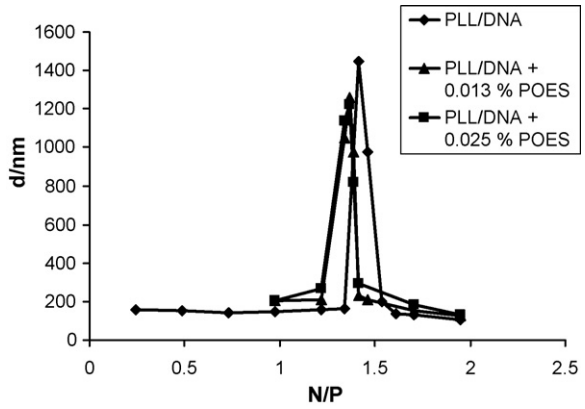

Fig. 6. Size of PLL/DNA complexes as a function of N/P ratio. The effect of POES.

small that the binding constant could not be determined reliably. Thermodynamic data determined using the one binding site model is also shown in the insert of Fig. 7.

As seen from Fig. 7, the enthalpy changes related to the process are minute: $\Delta H = -640$ cal mol^{-1} = -2678 J mol^{-1}, which is of the same magnitude as the thermal energy *RT*. For PLL, the change in enthalpy was so small that it could not be measured. As the change in entropy for the PEI/DNA complexation is 27 cal mol^{-1} K^{-1} and thus the term $T\Delta S$ is 8046 cal mol^{-1} = 33.6 kJ mol^{-1}, hence over 10-fold to the enthalpy contribution, the complexation process of pDNA with the polycation must be entropy driven. The results are also consistent with the values given in the literature for DNA binding with other polycations: The binding enthalpies determined for the complexation of DNA with (dimethylamino)ethyl methacrylate homopolymer varied from 0 to -6 kJ mol^{-1} of polymer, depending on the pH of the solution [30], whereas the binding enthalpies of the interaction of linear polyaminoamine polymer with DNA were from 0 to -3.5 kJ mol^{-1} [31]. Also binding enthalpies of the same magnitude, but endothermic have been observed. For the binding of DNA with trivalent cations cobalt hexamine and spermidine the enthalpies of binding ranged from 0 to 1 kcal mol^{-1} of cation [32] and enthalpies ranging from 0 to 0.6 kcal mol^{-1} of copolymer have been measured for the binding of DNA with a cationic

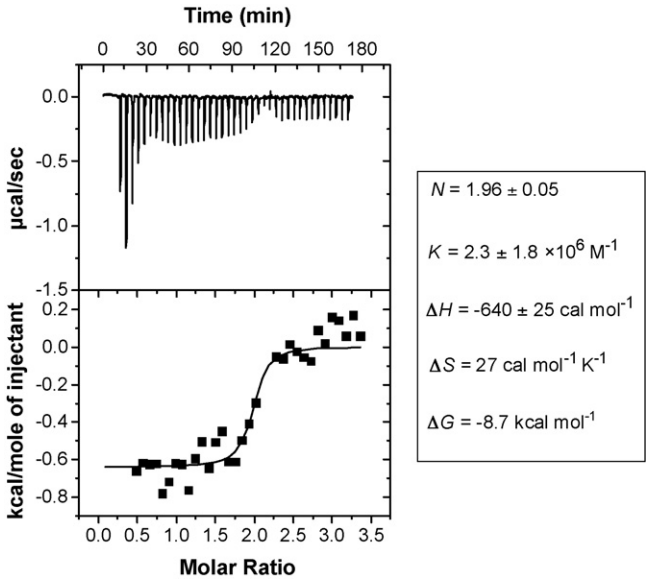

Fig. 7. ITC data for PEI/DNA complexation. Thermodynamic parameters for the binding of PEI to DNA are shown in the insert.

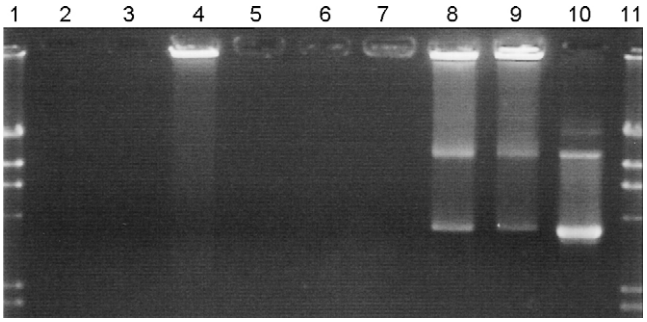

Fig. 8. Effect of dextran sulphate on the stability of PEI/DNA and PLL/DNA complexes. Marker (lanes 1 and 11), PEI/DNA (lane 2), PEI/DNA/POES (lane 3), PEI/DNA + DS (lane 4), PEI/DNA/POES + DS (lane 5), PLL/DNA (lane 6), PLL/DNA/POES (lane 7), PLL/DNA + DS (lane 8), PLL/DNA/POES + DS (lane 9), plasmid DNA alone (lane 10).

graft copolymer [33]. The binding entropy of 27 cal mol^{-1} K^{-1} is in accordance with the previously reported values for DNA binding with various polycations. In the entropy driven processes, the magnitude of 10 kcal mol^{-1} for $T\Delta S$ has been reported both for the complexation of DNA with trivalent cations [32] and with a cationic graft copolymer [33]. As the measured enthalpies are very small, reliable determination of the binding constant using ITC is very difficult. However, our ITC data did confirm the result obtained using dynamic light scattering: DNA is totally bound to PEI at N/P range 2–2.5.

3.4. Agarose gel electrophoresis

Plasmid DNA complexation and the stability of the complexes in the presence of DS was studied using gel electrophoresis. Only the free negatively charged pDNA is migrated in the electric field whereas fully complexed pDNA cannot be seen in the gel. Image of agarose gel electrophoresis of the studied complexes is shown in Fig. 8. Electrophoresis confirms the stabilising effect of POES on the PEI/DNA complexes: PEI/DNA complex is relaxed upon addition of DS (lane 4), while PEI/DNA complexes stabilised by POES do not show this relaxation (lane 5). The results complement the findings of Sharma et al. [16]: POES not only prevents the aggregation of PEI/DNA complexes, but also stabilises the complexes in the presence of extracellular GAGs. The low electrophoretic mobility of the pDNA from PEI/DNA complexes (lane 4) suggests that pDNA remains partly bound to the complex after relaxation. However, pDNA strands must be loose enough to interact with ethidium bromide. A fraction of pDNA is released from the complex and migrated in the gel seen as fluorescent marks.

PLL/DNA complexes, on the other hand, were not as resistant for the effect of DS. Our results are somewhat contradictory with the results reported in the literature: PEI complexes have shown to be less resistant to the effects of GAGs compared to PLL complexes [34,35]. The different results may be explained by the polydispersity of our PLL/DNA complexes. Greater polydispersity of PLL/DNA complexes compared to PEI/DNA complexes has been reported earlier also [6]. When the complex is more loosely structured, anionic GAGs may have better opportunities to displace pDNA from cationic carrier. Another reason for the discrepancy between our results and the ones reported in the literature could be that in our experiments, the complexes were formed in 5% glucose solution, whereas in the studies of Ruponen et al. [34] and Männistö et al. [35] the experiments were done in buffer solutions of higher ionic strength. In the study of Bertschinger et al. [36], it was shown that increasing ionic strength increases the release of DNA from PEI/DNA complexes. As ionic strength has a significant effect on complex formation and stability, it may also have an effect on the releasing effect of GAGs. Nevertheless, the interesting result is that POES does not have a stabilising effect on PLL/DNA complexes, which confirms our results from size and zeta potential measurements.

The release of pDNA from the complexes was also tested at pH 5.5 (not shown) to mimic endosomal conditions, but no significant differences compared with experiments carried out at pH 8.0 were detected. Hence, POES presumably prevents premature DNA release by endosomes prior to its transfer to the nucleus.

4. Conclusions

To conclude, this paper presents detailed study on the size and charge of the DNA complexes with two commonly used polycations PEI and PLL. The study also examines the effect of the surfactant POES on the complexation and elucidates the origin of the stabilising effect of the surfactant. In particular, the stability of the complexes in the presence of extracellular GAGs is evaluated. This area of research is important because knowledge on the interactions of surfactants with DNA complexes may help to adjust the size and charge of the gene carrier complexes to a desired value when designing new gene carriers.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.colsurfb.2008.05.012.

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