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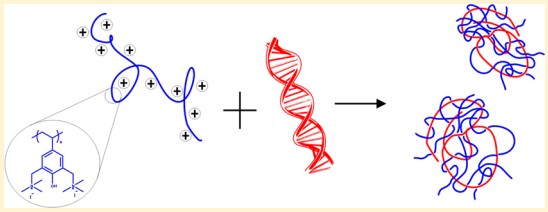
Quaternized Poly[3,5-bis(dimethylaminomethylene)hydroxystyrene]/DNA Complexes: Structure Formation as a Function of Solution Ionic Strength

Fotini Delisavva, Grigoris Mountrichas, and Stergios Pispas*

Theoretical and Physical Chemistry Institute, National Hellenic Research Foundation, 48 Vass. Constantinou Ave., 11635 Athens, Greece

Supporting Information

ABSTRACT: The formation of complexes between the cationic polyelectrolyte poly[3,5-bis(dimethylaminomethylene)hydroxystyrene] (Q-N-PHOS), bearing two cationic sites per repeating unit, and DNA molecules in aqueous solutions is investigated at pH 7 and at various salt (NaCl) concentrations and DNA/polymer ratios. The structural characteristics of the polyplexes at different DNA/polymer ratios were characterized in terms of mass, size, and charge using static, dynamic, and electrophoretic light scattering and fluorescence spectroscopy. The results indicate that the complexes have a loose spherical morphology with size in the range of 45−100 nm depending on polymer to DNA ratio and ionic strength of the solution. Most interestingly, it is found that the polyplexes' response to changes in the ionic strength of the surrounding solution after complexation depends strongly on the initial solution ionic strength during complex formation. This also points to differences in the nanostructures of polyplexes formed at different ionic strengths.



INTRODUCTION

Gene therapy is a modern pathway toward demanding disease treatment.[1,2] The most common procedure of gene therapy involves using DNA that encodes a functional, therapeutic gene in order to replace a mutated gene. Toward this end, synthetic polymers have been proposed as delivery agents of DNA. However, the elucidation of the mechanisms related to both complex formation and delivery process, as well as their interrelationships, is still a scientific demand. The use of synthetic polymers offers a number of advantages in comparison with systems as viral vectors, like easy, cheap, and reproducible synthesis of such materials.[3] The proposed polymeric delivery systems are mainly based on the electrostatic complexation of polymeric chains with DNA. In terms of the complexation process, a number of polycations have been studied as candidates for gene therapy nanosystems. Polycations tend to form compact complexes with DNA, named polyplexes, due to electrostatic bonds between negatively charged DNA phosphate groups and positively charged groups of the polycations.

However, beyond the successful complexation of the polycation with DNA, a set of appropriate physicochemical properties of polyplexes are also crucial toward using a polycation as delivery system for gene therapy. As far as the physicochemical properties investigations are concerned, interactions like hydrogen bonding and effect of salinity or temperature, as well as hydrophobic or van der Waals interactions, can play a significant role and therefore have to be studied in detail. The structure and physicochemical properties of polyplexes are affected by a wide gamut of parameters. Indicatively, the ratio of cationic and anionic charged groups, the concentration of the macromolecules, and the salinity of the solution can dramatically affect the structure and solution behavior of the nanosystem.[4−9]

Several groups have focused their efforts on studing and understanding the formation and properties of polyplexes. In particular, polyplexes formed by various types of linear cationic polymeric materials containing amino functional groups have been used to condense DNA, such as poly(ethyleneimine),[7,10] chitosan,[11] poly-L-lysine,[8] poly(amine-*co*-esters),[12] poly(amido-amines),[13] (poly[2-(dimethylamino)ethyl methacrylate],[8,14] and (poly(DMAEA-*co*-BA)phosphazene).[15] The above-mentioned examples are indicative of the huge interest concerning polymeric gene delivery systems. Moreover, the already presented results indicate also the complexity of such self-assembled macromolecular systems. Literature data point out that the formation mechanism, the stability, the size, and several other characteristics of polymer−DNA complexes are affected by the chemical structure of the cationic polymers as well as a number of physicochemical parameters. In order to stabilize and protect the polymer−DNA complexes, the well-known method of pegylation has been proposed by a number of research groups.[16−20] However, there are still a number of questions that have to be answered and a number of parameters that have not been fully explored yet.

In the present work, a novel high charge density cationic polyelectrolyte, namely quaternized poly[3,5-bis(dimethylaminomethylene)hydroxystyrene], has been studied as a potential candidate for DNA delivery nanosystems. The focus is placed on the physicochemical properties of polyelectrolyte/DNA complexes which were studied as a function of polymer to DNA ratio

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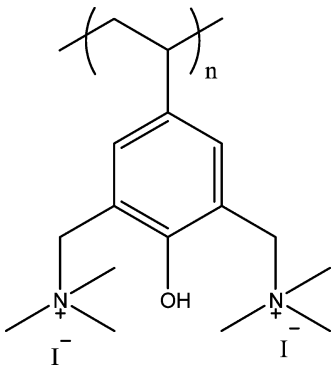
utilized, solution ionic strength and in terms of polyplex solubility and stability. A number of complementary techniques were used toward understanding the structure and properties of the polyplexes, while the obtained results are discussed in view of potential applications.

EXPERIMENTAL SECTION

Materials. Deoxyribonucleic acid sodium salt from fish sperm was purchased from Acros (molecular weight 50 000–100 000 Da, less than 5% protein). An average base molecular weight (308.95) was used in order to calculate the phosphate content in solution based on the DNA concentration. All other chemicals were purchased from Aldrich.

The well-defined linear flexible polyelectrolyte quaternized poly-[3,5-bis(dimethylaminomethylene)hydroxystyrene] (Q-N-PHOS-1), bearing two cationic sites on each repeating unit, was used (Scheme 1). The synthesis of the particular polyelectrolyte has been described in detail previously.[21] Molecular weight of Q-N-PHOS-1 was calculated to be 46 kDa based on the molecular weight of the precursor polymers and the extent of functionalization reactions. Previous investigations on the solution behavior of such cationic polyelectrolytes have shown aggregation effects due to the inherent hydrophobicity of the backbone.[21] The aggregates have a hydrodynamic radius R_h = 76 nm and a ζ-potential equal to +65 mV. They are formed by a small number of polyelectrolyte chains (calculated aggregation numbers, N_w, from light scattering are equal to 8 in 0.01 M NaCl aqueous solutions).

Scheme 1. Molecular Structure of Q-N-PHOS-1 Cationic Polyelectrolyte



Preparation of Polymer/DNA Complexes. Q-N-PHOS-1 was dissolved in 35 mL of phosphate buffer solution (pH = 7, salinity 0.01 M) to give a concentration of 2×10^{-3} g/mL. In the same way, DNA was dissolved in 40 mL of phosphate buffer solution (pH = 7, salinity 0.01 M) to give a concentration of 1×10^{-3} g/mL. The complexes were formed by a fast addition (less than 3 s) of the different volumes of polymer solution into constant volume of aqueous DNA solution under vigorous stirring. The addition was performed in separate vials in order to prepare complexes with different nitrogen/phosphate (N/P) molar ratios. The formed mixed solutions were left to stand for 5 min before the addition of proper amounts of buffer solution in order to reach the final desired total concentration (e.g., DNA concentration was kept constant and polymer concentration was varied). The formed solutions were allowed for 24 h for full equilibration before subjected to light scattering measurements. The formation of complexes was also studied by monitoring the solution just after their preparation (for fluorescence measurements). The formed complexes have N/P ratios within the range of 0.06–5.2; however, partial precipitation was observed for solutions with N/P ratio higher than 0.5. Additionally, the effect of increasing salinity has been studied in these initial solutions by addition of NaCl. A concentrated (1 M) NaCl solution was added dropwise to the solutions of the polyplexes in order to vary salt concentration up to 0.5 M. The physicochemical measurement of the solutions with varying salinity was performed directly after their preparation (within half an hour). A second series of solutions was prepared, where the pH and the salinity of samples were tuned in order to be similar with physiological conditions (pH = 7.4, initial ionic strength 0.154 M). The salinity of these solutions was also varied systematically by addition of 1 M NaCl solution as described in the previous case (final ionic strength up 0.5 M). It is generally known that the mixing protocol affects the properties of the resulting polyelectrolyte complexes.[22,23] However, we chose this mixing protocol because it gave the more well-defined complexes in terms of size and the most extended region of soluble complexes.

Methods. *Monitoring Complexation with Ethidium Bromide.* In order to investigate the complexation ability of the cationic polymer with DNA, the fluorescence of ethidium bromide has been studied in solutions with various N/P ratios. In particular, an initial solution of DNA 1×10^{-3} g/mL was prepared followed by addition of ethidium bromide ([EB] = [P]/4 = 8×10^{-6} M). Subsequently, the DNA aqueous solution was titrated using a concentrated polymer solution, up to a N/P ratio equal to 50. The titration was followed by fluorescence spectroscopy. A double-grating excitation and a single-grating emission spectrofluorometer, Fluorolog-3 Jobin Yvon-Spex spectrofluorometer (model GL3-21), was used (excitation at 535 nm, monitoring of the emission at 600 nm).[24]

Investigation on the Presence of Hydrophobic Nanodomains within the Polyplexes. The existence of hydrophobic nanodomains within the polymer–DNA complexes has been investigated through fluorescence spectroscopy (Fluorolog-3, model FL3-21, Jobin Yvon-Spex), using pyrene molecules as probes. Pyrene is typically used as a probe of hydrophobic domains in aqueous solutions due to changes of its fluorescence spectra in microenvironments of different hydrophobicity. In particular, fixed volumes of pyrene solution in acetone were introduced with a micropipet in glass vials, followed by evaporation of acetone. Subsequently, an appropriate volume of the solutions containing the polyplexes was introduced in the vial and the solution was allowed to stand for 24 h with occasional stirring. The emission fluorescence spectra of the solutions were recorded upon excitation at 335 nm. Emission spectra were recorded in the region of 350–500 nm, using an integration time of 0.5 s. Slit openings of 1 nm were used for both the excitation and the emission beams. The I_1/I_3 ratio is a characteristic parameter indicative of the polarity of the environment around pyrene, where I_1 and I_3 are the intensities of the first and the third peaks of pyrene fluorescence spectra at 372 and 383 nm, respectively.

Zeta Potential Measurements. Electrophoretic light scattering measurements were performed at 25 °C on a ZetaPlus Analyzer (Brookhaven Instruments Corp.) equipped with a 35 mW solid-state laser, operating at λ = 660 nm. ζ-potential values determined using the Smolukowski equation relating the ionic mobilities with surface charge. Final measurements are the average of the 10 repeated ones with an error better than ±3 mV.

Dynamic and Static Light Scattering. Light-scattering measurements were conducted on an ALV/CGS-3 compact

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goniometer system (ALVGmbH), equipped with a ALV-5000/EPP multi-τ digital correlator with 288 channels and an ALV/LSE-5003 light-scattering electronics unit for stepper motor drive and limit switch control. A JDS Uniphase 22 mW He−Ne laser (λ_0 = 632.8 nm) was used as the light source. Measurements were performed in the angular range from 45° to 135°. Solutions were filtered through 0.45 μm hydrophilic PTFE filters (Millex-LCR from Millipore) before light-scattering measurements. Measurements were made at the angular range of 30−150°.

The autocorrelation functions from DLS were analyzed by the constrained regularized CONTIN method in order to obtain distributions of relaxation rates. The decay rates provided distributions of the apparent diffusion coefficient ($D = \Gamma/q^2$), where q is the magnitude of the scattering vector. The apparent hydrodynamic radii were calculated using the Stokes−Einstein equation

$$R_h = kT/6\pi\eta D \quad (1)$$

where k is the Boltzmann constant, η is the viscosity of water at temperature T, and D is the diffusion coefficient at a fixed concentration. The polydispersity of the particle sizes was given as the μ_2/Γ^2 from the cumulants method, where Γ is the average relaxation rate, and μ_2 is its second moment.

Static light scattering data were evaluated via Zimm plots. The values of the radii of gyration, R_g, were obtained from the Zimm plots, which can be described by the following equation

$$\left(\frac{KC}{R_{vv}(q)}\right)_{c\to 0} \cong \frac{1}{M_w}\left(1 + \frac{1}{3}R_g{}^2q^2\right) \quad (2)$$

where $R_{vv}(q)$ is known as the Rayleigh ratio, $K = 4\pi^2n^2(dn/dC)^2/(N_A\lambda_0{}^4)$, and $q = (4\pi n/\lambda_0)\sin(\theta/2)$, with N_A, dn/dC, n, and λ_0 being the Avogadro number, the specific refractive index increment, the solvent refractive index, and the wavelength of the light in vacuum, respectively. Toluene was used as the calibration standard for obtaining absolute values for the scattered intensity.

RESULTS AND DISCUSSION

The successful complexation of the polymeric macromolecules with DNA has been confirmed through fluorescence measurements of DNA complexes solutions which were titrated with the Q-N-PHOS polymer, using ethidium bromide as the fluorescence probe. The above-mentioned probe tends to form loose complexes with DNA, but it exists as isolated molecules when other (macro)molecules replace it from the complexes. The fluorescence intensity of the probe depends on the status of the probe.[24] The changes of the fluorescence intensity through the titration of the polymer are shown in Figure 1. A rapid decrease of the ethidium bromide fluorescence was recorded upon increasing the content of Q-N-PHOS-1 at N/P ratio equal to 1. The above result clearly indicates the successful complexation between DNA and Q-N-PHOS polyelectrolyte. Moreover, it demonstrates that almost all the added cationic sites of the macromolecules tend to form complexes with the DNA's anionic sites.

In line with the aforementioned results, the fluorescence data obtained using pyrene as the probe also indicate the absence of hydrophobic nanodomains within the polyplexes. In particular, during the formation of the polyplexes, the intensity ratio I_1/I_3, a characteristic ratio for the determination of hydrophobic regions in the solution, adopts values around 1.9 which are

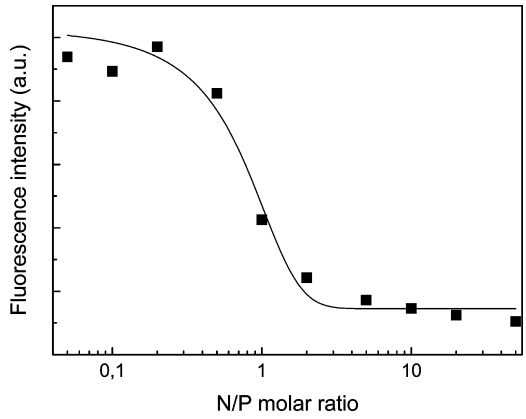

Figure 1. Fluorescence intensity of polyplexes−EB solutions vs the N/P ratio. The line is a guide to the eye.

typical for a polar (hydrophilic) environment around the probe molecules. Moreover, the observation of excimer also suggests the absence of extensive hydrophobic domains. Following the above-mentioned results, the absence of hydrophobic nanodomains indicates that the formed aggregates are formed solely by electrostatic interactions and not due to hydrophobic interactions. In addition electrostatic complexation, although it leads to macromolecular aggregation, it does not induce the formation of hydrophobic domains within the polyplexes. Interestingly, the same results are observed independent of the solution salinity and the N/P ratio utilized.

In order to extract more information for the DNA−polymer polyelectrolyte complexes, the ζ-potential of the nanosystems has been studied as a function of the N/P ratio (Figure 2).

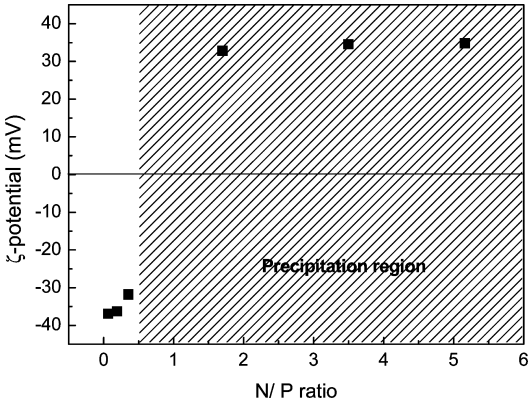

Figure 2. ζ-potential of the resulting complexes vs N/P ratio at 0.01 M ionic strength and pH = 7. Dashed area defines the precipitation region for the polyplexes. Data points within this area correspond to polyplexes in the supernatant solution.

Upon addition of Q-N-PHOS-1 to DNA solution, the charge increases passing from negative to positive values, following the stoichiometry utilized. The variation of the effective charge of the complexes formed at various N/P ratios demonstrates the successful complexation of the positively charged polyelectrolyte and the negatively charged DNA chains. A region of partial sedimentation can be observed for N/P values above 0.5. Due to partial sedimentation, the intensity of scattering light was very low and the solution at N/P = 0.6 could not give reliable measurements. Measurements of the supernatant solutions at N/P > 0.6 show the existence of positively charged particles. It is

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well-known that aggregates are electrostatically well stabilized when the ζ-potential adopts values higher than +30 or lower than −30. In the present case, the DNA/polymer nanoassembly is at the limit of this stability value, leading to partial precipitation of the sample for N/P > 0.6. Presumably, at and above this ratio the existing charges on the complex nanoparticles are not sufficient to keep the nanoassemblies in solution, and the majority of complexes precipitate. This is also connected to the fact that Q-N-PHOS-1 bares two cationic sites per monomeric unit and for charge ratio (N/P) higher than 0.6, the cationic sites in the system are more than the anionic sites. As a result, all DNA molecules must be completely bound to the polymer chains. The rest of the polymer chains which are not directly connected to DNA chains cause a second aggregation to the polyplexes (see below at kinetics) and the large particles sediment due to gravitational force. In addition, the hydrophobic backbone of the polymer can also play a significant role on the solubility and colloidal stability of the complexes. In this scenario, particles remaining in solution should be composed primarily of polyelectrolyte chains. Similar results were obtained for the case of pH = 7 and I = 0.154 M solutions.

The dimensions of the complexes were determined using dynamic light scattering for polyplexes prepared in a low ionic strength environment (I = 0.01 M and pH = 7) and at physiological conditions (I = 0.154 M and pH = 7.4). In the low ionic strength series of complexes, the formed polyplexes are rather polydisperse having polydispersity index (PDI) values equal to or larger than 0.2. It has to be noted that the PDI determined for the net polymer solutions is also ca. 0.2, due to the presence of polymer aggregates, and this polydispersity should contribute to the increased polydispersity of the complexes. The mean hydrodynamic radius, R_h, increases from 45 to 62 nm at N/P up to 0.1 and 0.4, respectively. However, for N/P ratio higher than 0.6, where partial precipitation is observed, decreased values of R_h are observed, obviously due to precipitation of the larger aggregates. Therefore, the remaining soluble complexes were measured to have R_h in the range of about 25 nm (Figure 3). It should be mentioned that R_h values of the polyplexes are lower than the value for Q-N-PHOS aggregates, indicating a more compact structure for the resulting Q-N-PHOS/DNA complexes, compared to the initial polyelectrolyte aggregates. Additional information regarding the physicochemical characteristics of the polyplexes can be extracted by studying the intensity of the scattered light which is directly related to the mass of the aggregates (Figure 3). In particular, the measured intensity decreases by addition of the polyelectrolyte. A possible explanation could be that the formed complexes are larger in size but looser in terms of structure. Another relative possibility is that partial disaggregation of the initial polymer aggregates or polymer/DNA complexes is taking place due to complexation with DNA. This scenario may also explain the precipitation of the complexes at larger N/P ratios due to the bridging action of loose Q-N-PHOS chains on the initially formed polyplexes. Moreover, the shape of the complexes was determined to be spherical with a rather loose structure, as was extracted by the R_g/R_h ratio which was found to be in the range of 1.2−1.4 for the polyplexes studied. Unfortunately, a more quantitative assessment of the static light scattering results could not be made, due to the inherent difficulties encountered in these systems. The main problem is that the specific refractive index, dn/dc, cannot be determined since the solutions of the polyplexes are turbid in the concentration range needed for dn/dc measurements. Therefore, the apparent mass, $M_{w,app}$, of the complexes cannot be evaluated accurately. This is also due to the fact that calculation of the dn/dc of the mixed system based on the

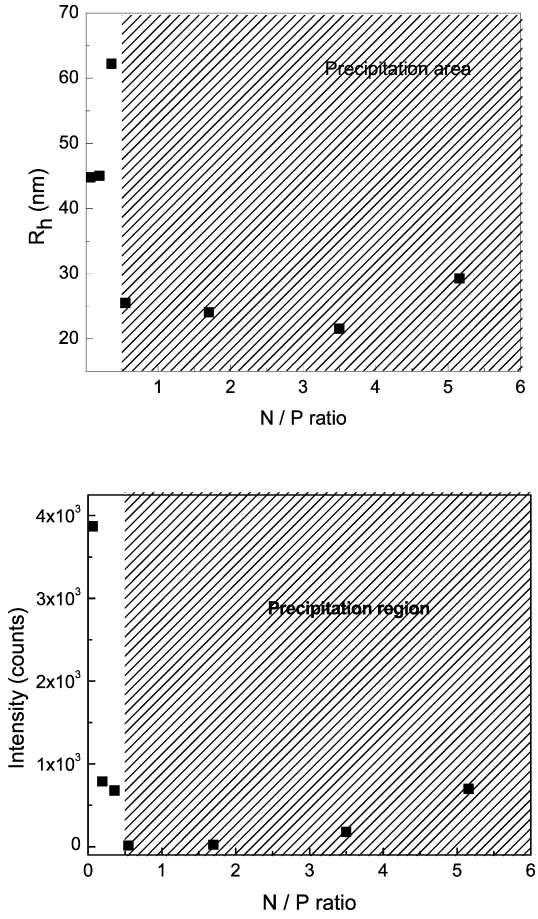

Figure 3. R_h (upper) and scattered light intensity (lower) vs N/P ratio (I = 0.01 M, pH = 7). Dashed area indicates precipitation region. Data points within this area correspond to polyplexes in the supernatant solution.

dn/dc values of the individual components is not accurate, since after complexation the chemical nature of the components changes.[21] However, a rough estimation of the complexes molecular weight is given in Supporting Information (Figure S2).

Light-scattering data on the polyplexes formed under physiological conditions (I = 0.154 M and pH = 7) are shown in Figure 4. Even though the same trends were observed, the formed complexes have a lower polydispersity (about 0.1), while the R_h is in the range of 60−100 nm. The scattered intensity is much higher, indicating an increased mass of the complexes, compared to the case of lower ionic strength. Moreover, the R_g/R_h is in the range of 0.9−1.4, which lies closer to the expectations of spherical aggregates.

It seems that the polyplexes prepared under different ionic strength conditions have different sizes, mass, and compactness and this should be a result of the difference of the screening effects, due to the presence of NaCl electrolyte, toward electrostatic interactions that affect the formation and structure of the complexes.

The Q-N-PHOS/DNA complexes formed at physiological conditions have been studied as a function of time, by light scattering, immediately after mixing of the components. A representative example is shown in Figure 5 for N/P = 4.8. R_h increases rather rapidly after the addition of Q-N-PHOS chains. After approximately 2 h, large particles with sizes larger than 1 μm are formed in solutions, due to a secondary aggregation process. No further aggregation is observed in the experimental

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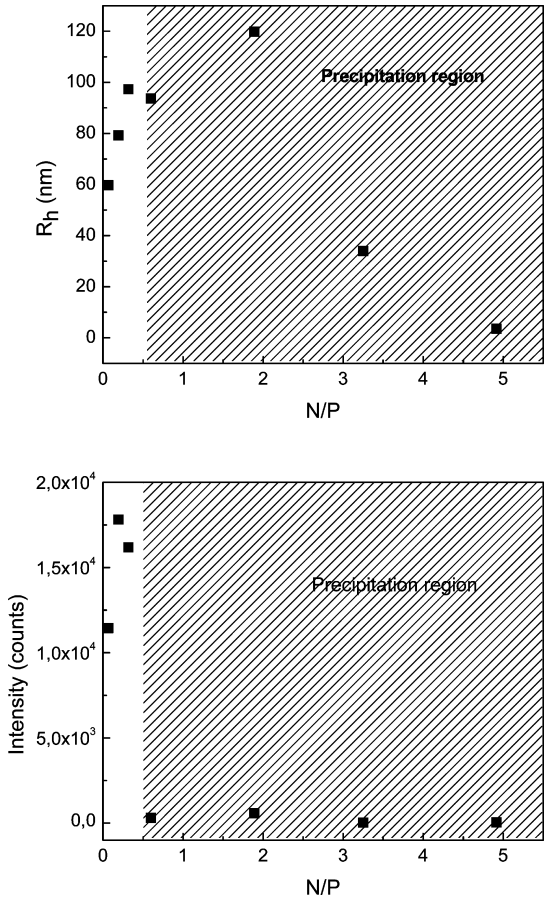

Figure 4. R_h (upper) and scattered light intensity (lower) vs N/P ratio (I = 0.154 M, pH = 7.4). Dashed area indicates the partial precipitation region. Data points within this area correspond to polyplexes in the supernatant solution.

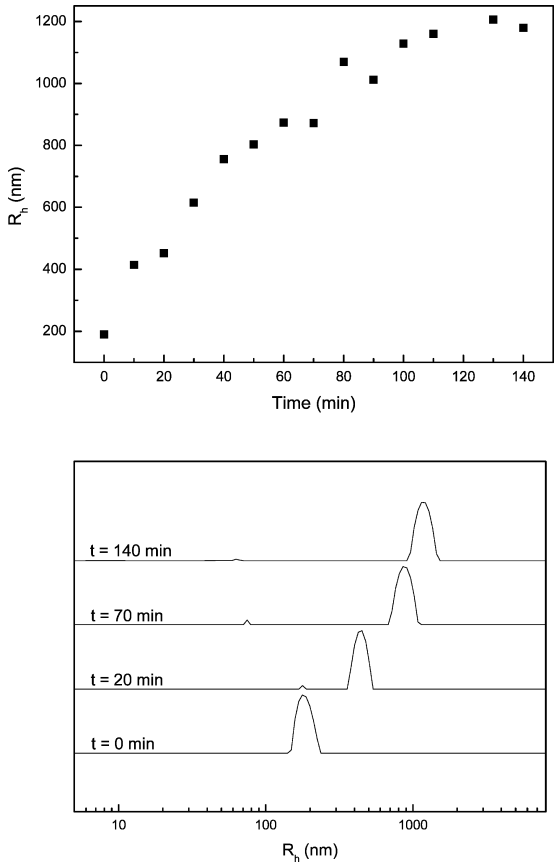

Figure 5. R_h vs time (upper) and CONTIN analysis for four time intervals (lower) for the Q-N-PHOS/DNA complexes after addition of polymer (N/P = 4.8) at physiological conditions (I = 0.154 M, pH = 7.4).

time frame. The results clearly demonstrate the formation of the large aggregates before precipitation is visible in the Q-N-PHOS/DNA mixed solutions. The large aggregates are not formed instantly after the addition of polymer chains but there is an incubation time during which a disaggregation/restructuring of the initially formed complexes may take place and this may be the reason for the secondary aggregation, as it was discussed before. It has to be noted that in the N/P range where precipitation is not observed, the kinetics of complex formation are very fast and they cannot be followed by the existing light-scattering experimental setup. Moreover, the initial size of the aggregates in solution (about 180 nm at experimental t = 0) is larger than the one recorded for the supernatant solution after aging. Therefore, most probably the large structures are formed predominantly due to a secondary aggregation of the initially formed polyplexes.

Effect of Solution Ionic Strength on the Structure of Preformed Polyplexes. In addition to the experiments described above, it was interesting to examine the effects of salt addition to preformed Q-N-PHOS/DNA polyplexes. In other words, it is interesting to see how different polyplexes (i.e., polyplexes formed under different solution ionic strength conditions) respond to a further increase of solution ionic strength. To this end, light-scattering measurements were performed on solutions of Q-N-PHOS/DNA polyplexes formed under different initial solution conditions. These studies were focused on soluble and colloidally stable polyplexes. The recorded changes in R_h and scattered intensity during titration with a 1 M NaCl of the complexes formed at I = 0.01 M, N/P = 0.4, and pH = 7.0 (up to I = 0.5 M) are shown in Figure 6. The obtained data show that, for ionic strength up to 0.2 M, the R_h of the complexes remains constant, and roughly equal to about 50 nm. This is followed by a rapid increase of the size (R_h increases up to 98 nm), and by a further increase of the solution ionic strength. A similar pattern is also recorded for the scattered intensity (Figure 6). Particularly, scattered intensity remains almost constant for a solution ionic strength of up to ~0.1 M and it then increases further and faster for I > 0.1 M. For I higher than 0.3 M, scattered intensity decreases again but up to I = 0.5 M it does not acquire the initial value. The presented results indicate that, in terms of structure, for I higher than 0.3 M the complexes become larger while their mass decreases; in other words, the complexes are becoming looser. This might indicate aggregation of the complexes at 0.1 < I < 0.3 M and at least a partial dissociation of the complexes for higher ionic strength. This should be a result of screening effects due to the presence of increasing low molecular weight electrolyte in the aqueous solutions. Interestingly, the PDI of the nanoaggregates gradually decreases from about 0.35 to about 0.1, indicating that by increasing solution ionic strength the complexes become more monodisperse in size. No precipitation of the complexes was observed even at the highest ionic strength utilized in the time frame of the experiments. It has to be noted that the same trend in the recorded data is also observed for other N/P ratios (Supporting Information, Figure S1).

The picture is somehow different when the initial polyplexes were prepared at I = 0.154 M, pH = 7.4, and at the same ratio

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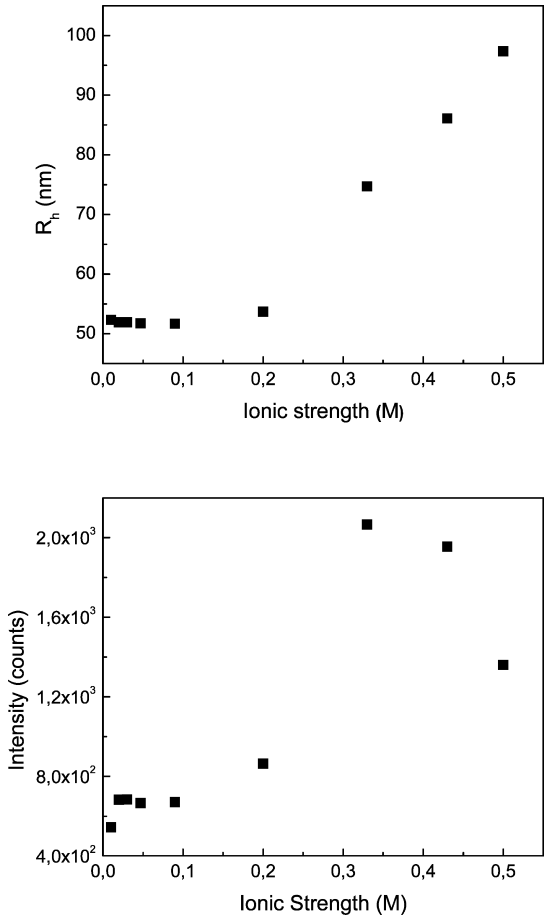

Figure 6. R_h (upper) and scattered light intensity (lower) vs increasing solution ionic strength for Q-N-PHOS/DNA complexes initially prepared at I = 0.01 M, N/P = 0.4, and pH = 7.

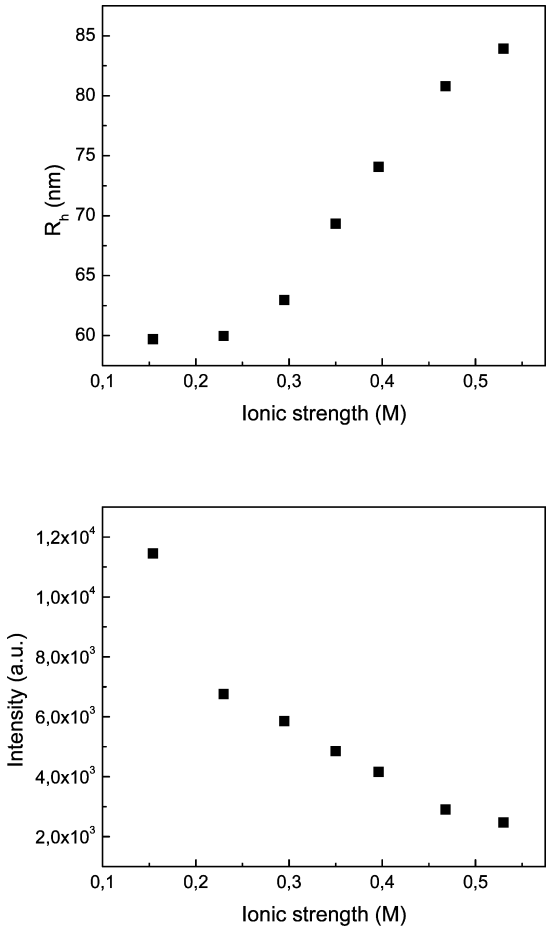

Figure 7. R_h (upper) and scattered light intensity (lower) vs increasing solution ionic strength for Q-N-PHOS/DNA complexes initially prepared at I = 0.154 M, N/P = 0.4, and pH = 7.4.

N/P = 0.4 (Figure 7). Light-scattering results of ionic strength dependence show that even though R_h follows the same pattern as before, the intensity is decreased upon increasing of ionic strength. R_h remains constant at ∼60 nm, up to I ∼ 0.23 M, while it increases up to 83 nm, as the solution ionic strength increases up to 0.5 M. At the same time, a strong decrease of the scattered intensity is observed (i.e., a decrease in the mass of the complexes) upon salt addition by more than 4 times in the same ionic strength range. The results probably indicate that as ionic strength increases the Q-N-PHOS/DNA polyplexes prepared at almost physiological conditions dissociate initially with not appreciable change in their size, while at higher ionic strengths the complexes' dissociation is accompanied by a swelling of the remaining nanostructures. This should happen because of the decrease of the electrostatic interactions between the cationic polyelectrolyte and DNA chains, as more salt is added to the system, in addition to an increased charge screening effect due to the increased initial salt concentration when complexes are prepared. In any case, this behavior indirectly shows that the nanostructure of Q-N-PHOS/DNA complexes depends on the solution ionic strength during their preparation, and these differences in structure result in different responsiveness toward variation of solution ionic strength after polyplex preparation. In other words there are a number of physicochemical parameters that can be utilized in order to tune the nanostructure and behavior of Q-N-PHOS/DNA complexes. These observations should generically correlate to the peculiar chemical structure of the Q-N-PHOS chains, that is, (i) the high charge density of the cationic polyelectrolyte (having two cationic charges per monomeric unit), a characteristic that may induce considerable chain stiffness, and (ii) a polyelectrolyte chain with considerable inherent hydrophobicity, due to the nature of the polymer backbone and the charged side groups. The results reported here may also have important consequences on the utilization of such polyplexes in gene delivery protocols, in terms of polyplex stability to precipitation and dissociation, as well as regarding the penetration properties of the particular polyplexes through cell membranes, which may be influenced by the hydrophobicity of the Q-N-PHOS polyelectrolyte.

CONCLUSIONS

In conclusion, the complexation of DNA with the cationic polymer quaternized poly[3,5-bis(dimethylaminomethylene)-hydroxystyrene] has been studied in terms of polyplex structure and physicochemical behavior. The results obtained from ethidium bromide assay, electrophoretic, and dynamic and static light scattering indicate that anionic sites of DNA can complex with the cationic sites of the synthetic Q-N-PHOS polymer. However, the above-mentioned complexation can lead to different nanostructures of the polyplexes depending on the N/P ratio and the ionic strength of the solution during polyplex preparation. Depending on the ratio between DNA and cationic polymer, the formed structures could be colloidally

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stable or precipitate due to a secondary aggregation mechanism. Moreover, upon increasing the salt concentration in solutions of preformed complexes, the polyplexes tend to change their structure in terms of size and mass. The results reported here demonstrate the importance of preparation protocol in the formation process and the nanostructure of the resulting complexes, as well as their responsiveness toward ionic strength variations. These observations can be valuable in the understanding of polyelectrolyte/nucleic acid complex formation, in elucidating the effects stemming from the chemical structure of the cationic polyelectrolyte, as well as in the construction of novel polyplexes for gene delivery.

■ ASSOCIATED CONTENT

Supporting Information

Data on the solution behavior of complexes versus ionic strength and the dependence of complexes apparent weight average molecular weight on the N/P ratio. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: pispas@eie.gr.

Notes

The authors declare no competing financial interest.

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