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ORIGINAL CONTRIBUTION

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Complexation of well-controlled low-molecular weight polyelectrolytes with antisense oligonucleotides

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Abstract The influences of polymer-related properties such as molecular weight, charge density, counter ion, and hydrophilic block on the complexation of polyelectrolytes and a fluorescein-labeled oligonucleotide (ON) were investigated. A series of well-defined and well-controlled 2-(*N,N*-dimethylamino)ethyl methacrylate (DMAEMA) polymers and block copolymers were prepared using living anionic and radical polymerization methods. Fluorescence measurement was used to reveal the effects of polymer molecular weight, charge density, and counter ion type on the complexation. PolyDMAEMA samples having double molecular weights of the chosen oligonucleotide gave the optimal complexation performance. Kinetic studies showed that high-molecular weight/high-charge density polymer samples produced very stable complexes. The fully charged polyDMAEMA displayed the strongest binding with the ON. These complexes were therefore less sensitive to the changes in the environment. PolyDMAEMA–DMSQ samples had slightly higher complexation ability than polyDMAEMA–MCQ (DMSQ: dimethylsulfate quat; MCQ: methylchloride quat). Both poly(DMAEMA-b-HEMA) and poly(DMAEMA–MCQ-b-PEG) block copolymers showed good complexation ability and steric stability [HEMA: 2-hydroxyethyl methacrylate; PEG: poly(ethylene glycol)]. PEG, but not HEMA block, enhanced the effectiveness of polyDMAEMA–MCQ binding with the ON.

Keywords Polyelectrolytes · Complexation · Antisense oligonucleotides · Polymer vector · Delivery · Living polymerization

Introduction

Since the first use of antigene therapy in 1978 by Zamecnik and Stephenson [1], synthetic antisense oligonucleotides have become very attractive as highly selective nucleic acid drugs for inhibiting the expression of disease-causing genes. However, there are several barriers for an oligonucleotide (ON) to reach the cytoplasm or nucleus of target cells to perform the inhibitory functions, including [1] low cellular uptake due to relative large size, negative charge, and hydrophilicity [2]; short lifetime due to rapid degradation by nuclease; and [3] lack of targeting ability. Suitable vector/carrier systems are essential for an efficient delivery of ONs into target cells. Cationic polymers, normally used for gene delivery, were explored for this purpose because of their ability to bind ONs by electrostatic interaction and their multi-functionality.

Great efforts have been undertaken to improve cellular uptake, degradation resistance, and specificity for

cationic polymeric ON delivery. Several cationic polymers have been developed for this purpose, including poly(L-lysine) [2–5], polyethylenimine (PEI) [6, 7], and poly[2-(dimethylamino)ethyl methacrylate] (polyDMAEMA) [8–10]. The use of cationic polymers as carrier, results in high affinity to negatively charged membranes and high uptake rates. Unfortunately, increasing positive surface charge also yields high toxicity at both cellular and systemic levels. To overcome these drawbacks, cationic–hydrophilic copolymers have currently been developed to improve polymer biocompatibility and to prolong blood circulation time. Such polymer systems include polyspermine-poly(ethylene oxide) (PS-PEO) [11], poly(L-lysine)-PEO [12], poly[(trimethylamino) ethyl methacrylate chloride]–poly[*N*-(2-hydroxypropyl) methacrylamide] [poly(TMAEMA-b-HPMA)] [13].

A challenge associated with these polymer systems is the control of polymer molecular weight and charge density. ONs are usually 10–30 nucleotides in length and have molar mass in the range of ~3,000–9,000 g/mol. Most cationic polymers used for antisense delivery, on the other hand, have very high-molecular weights. The complexation between an ON with a cationic (co)polymer can be quite problematic due to the large size differences between the two classes of macromolecules. Another significant drawback is that the used polymers have very broad molecular weight distributions. To overcome these problems, Stolnik et al. [14] have recently conducted a study on using well-defined cationic polymers derived from polyDMAEMA, poly(ethylene glycol) (PEG), and poly(ethylene glycol methacrylate) (PEGMA) for antisense DNA binding. These polymers were prepared by a group transfer polymerization (GTP) technique.

2-(Dimethylamino)ethyl methacrylate-based polyelectrolytes are particularly attractive for delivery applications of gene and antisense oligonucleotides as PolyDMAEMA-based block copolymers can be cross-linked to form nanospheres with cross-linked shell, which can act as nanoreactors and drug delivery vehicles [15]. Because of this, there is a significant need for examining the properties of polyDMAEMA (such as molecular weight, charge density and block composition) on the complexation with antisense DNAs and the delivery of these molecules into cells. For example, it needs to be determined what cationic charge level is adequate to neutralize ONs and also to provide optimal residual charge for interactions with cell membranes without causing much cytotoxicity.

To establish these structure-performance relationships, it is essential to synthesize well-defined and well-controlled polymers and to evaluate their ability to complex with ONs. In our previous work, we reported the successful preparations of well-defined and well-controlled DMAEMA-based (co)polymer samples by living polymerization methods, such as living anionic polymerization (LAP) [16] and atom transfer radical polymerization (ATRP) [17–19]. Very recently, Armes group directly synthesized poly(trimethylamino)ethyl methacrylate chloride (polyDMAEMA-MCQ) in proton media by ATRP [20]. These advances in polymerization technologies make it possible to prepare well-defined well-controlled polyelectrolytes for antisense binding studies.

Many methods can be used to study the complexation of cationic polymers and nucleic acids such as gel electrophoresis, density gradient analysis, and fluorescence-based assays. Among these methods, those using fluorescently labeled ONs are outstanding because it can not only be conveniently used to investigate the association of polymer with nucleic acid in vitro, but can also be applied to study the dissociation of such complexes in the cytoplasm of cells [8–10].

In this study, we synthesized a series of well-defined low-molecular-weight cationic polyDMAEMA having narrow molecular weight distributions and controlled charge densities by both living anionic polymerization (LAP) and ATRP methods. Fully charged polyDMAEMA quats were prepared by a sequential quaternization of polyDMAEMA samples, as well as by a direct ATRP. Hydrophilic cationic block copolymers of polyDMAEMA-b-HEMA and polyDMAEMA–MCQ-b-PEG were also prepared by ATRP, to evaluate the effects of block-related factors on the polymer/ON complex properties [HEMA 2-hydroxyethyl methacrylate; MCQ methyl chloride quat; PEG poly(ethylene glycol)]. The complexation of these polymers with a 5′-fluorescein-labeled oligonucleotide was investigated by fluorescence spectroscopy. The influences of molecular weight, charge density, counter ion type, and block copolymers on the complexation were systematically evaluated. The complexation kinetics of the polymers having different molecular weights was also examined.

Experimental

Materials

2-(Dimethylamino)ethyl methacrylate (DMAEMA), was supplied by Aldrich and distilled over CaH_2 prior to use. 2-Hydroxyethyl methacrylate (HEMA) was supplied by Aldrich and dissolved in water (25% vol of monomer). The solution was washed four times with an equal volume of hexane. NaCl was added to salt out the monomer. Addition of excess NaCl salt to HEMA monomer solution significantly suppressed hydrogen bonding between HEMA monomer and H_2O, and thus decreased solubility of HEMA in water. The monomer was dried over $MgSO_4$ and distilled under vacuum prior to use. Methoxy oligo(ethylene glycol), 2-bromoisobutyryl bromide, trichloroacetyl chloride, and triethyl-

amine, as initiator precursors, were used as received from Aldrich. Other reagents, such as Cu(I)Cl, $Cu(II)Cl_2$, 2,2′-bipyridine, dimethly sulfate $(CH_3)_2SO_4$, tetrahydrofuran (THF), methanol (MeOH), acetone, *N*,*N*′-dimethylformamide (DMF) and hexane were all from Aldrich and were used without further purification.

Preparation of cationic polymers

Synthesis of DMAEMA homopolymers

The DMAEMA homopolymers were synthesized by LAP as described elsewhere [16]. The molecular weight and molecular weight distribution of the polymers, summarized in Table 1, were characterized by gel permeation chromatography with polystyrene as standard and THF as solvent.

Synthesis of polyDMAEMA quaternary salts

PolyDMAEMA–DMSQ samples were prepared by quaternizing the polyDMAEMA samples synthesized by LAP with dimethyl sulfate $(CH_3)_2SO_4$. A typical quaternization procedure was as follows: 1 g of polyDMAEMA was dissolved in 10 ml of acetone at room temperature. A required molar ratio of dimethly sulfate was added to the solution dropwise and was stirred for 4 h. The polymer quat precipitate was isolated and dried in a vacuum oven. The degree of quaternization was measured by ^{1}H NMR (Bruker ARX-200) using D_2O as solvent. PolyDMAEMA-MCQ, donated by Professor S.P. Armes University of Sussex UK, was prepared by a direct atom transfer radical polymerization of DMAEMA–MCQ monomer, as described elsewhere [20]. The molecular weights and molecular weight distributions of these polymers are summarized in Table 1.

Synthesis of poly(DMAEMA-b-HEMA)

The linear DMAEMA-b-HEMA diblock copolymers were synthesized by the ATRP of DMAEMA with polyHEMA as macroinitiator. In a typical experiment of the polyHEMA macroinitiator preparation, 15.3 mg (0.154 mmol) of Cu(I)Cl and 48.1 mg (0.308 mmol) of 2,2′-bipyridine were charged to a 10-ml round-bottom flask. The flask was sealed with a rubber septum, evacuated and back-filled with ultra-high pure nitrogen three times. An amount of 2 g (15.4 mmol) of HEMA and 2 ml of methanol, predegassed with bubbling argon for 30 min, were transferred to the reaction flask via a double-tipped needle under vacuum to form a 50% w/v solution. 62 µl (0.154 mmol) of oligomeric methoxy polyethylene glycol 2-bromoisobutanate (OEGBr) was also degassed and added to the flask by a micro syringe to initiate the polymerization. The OEGBr initiator was prepared following the procedure reported by Wang et al. [21]. The flask was then immersed into a water bath at room temperature. Excess methanol containing $CuCl_2$ was added to terminate the reaction. Methanol was evaporated, the mixture was dissolved in THF, and the monomer residue was removed by hexane precipitation. The copper catalyst was removed by passing the solution through a silica gel column.

Table 1 Properties of DMAEMA-based polyelectrolytes

Polymer HomopolyDMAEMA[a]	Method	M_n GPC	DP	M_w/M_nGPC
A1	LAP	4,440	28	1.04
A2	LAP	11,700	75	1.03
A3	LAP	16,400	104	1.12
A4	LAP	25,500	160	1.04
A5	LAP	45,950	290	1.04
PolyDMAEMA-MSQ				
QA1	LAP + Quaternization	7,580	28	1.04
QA2	LAP + Quaternization	19,970	75	1.03
QA3	LAP + Quaternization	28,000	104	1.12
QA4	LAP + Quaternization	42,800	160	1.04
QA5	LAP + Quaternization	78,430	290	1.04
PolyDMAEMA-MCQ[b]	Direct ATRP	19,880	100	1.25
Block copolymer				
PolyDMAEMA-b-HEMA[c]	ATRP	59,700	100/50 [d]	1.39
PolyDMAEMA–MCQ-b-PEO[b]	ATRP	24,870	100/50[d]	1.29

LAP living anionic polymerization; *ATRP* atom transfer radical polymerization

[a]Characterized by organic GPC using THF-2%(v/v) triethylamine as eluent at 25°C with polystyrene as standard

[b]Determined by aqueous GPC using 0.3 M Na_2SO_4-0.1% (w/v) NaN3 as eluent at 30°C with polyDMAEMA–MSQ as standard

[c]Determined by GPC using DMF-0.2 w/v% of LiBr as eluent at 50°C with polystyrene as standard

[d]Measured by ^{1}H NMR spectroscopy

In a typical experiment of the ATRP of DMAEMA, using polyHEMA macroinitiator, 12.59 mg (0.127 mmol) CuCl and 39.69 mg (0.254 mmol) 2,2′-bipyridine were charged to a 10-ml round bottom flask. The flask was sealed with a rubber septum, evacuated and back-filled with extra-pure nitrogen three times. Two grams (17.74 mmol) of DMAEMA, degassed with bubbling nitrogen for 30 min, was transferred to the flask by a double-tipped needle under vacuum. The mixture was stirred for 10 min. In a 10-ml pear-shaped ampoule, 1.47 g (0.127 mmol) polyHEMA (DP = 50, $M_w/M_n = 1.25$) was dissolved in 2 ml methanol and was degassed for 20 min. The prepared polyHEMA solution was then transferred into the flask via a double-tipped needle to start the polymerization. The reaction was terminated by adding excess $CuCl_2$/methanol solution. The mixture solution was then passed through a silica column to remove the copper catalyst. Methanol was evaporated. The concentrated copolymers were diluted by THF and then precipitated by excess hexane. The samples were vacuum dried for characterization.

Synthesis of polyDMAEMA–MCQ-b-PEG

PolyDMAEMA–MCQ-b-PEG block compolymers, prepared by atom transfer radical polymerization, were donated by Prof. Armes group at Sussex University, UK.

Oligonucleotides and buffers

The fluorescein-labeled oligonucleotide, denoted FDNA (15 nt) and used throughout the study, has the following sequence: 5′-FGCGGAGCGTGGCAGG-3′ (F: fluorescein). It was prepared by an automated DNA synthesis via cyanoethylphosphoramidite chemistry and supplied by Keck Biotechnology Resource Laboratory, Yale University. The raw FDNA was purified by reverse-phase high-performance liquid chromatography using a Beckman–Coulter HPLC System Gold equipped with an Agilent Zorbax ODS C_{18}column (4.5×250 mm, 5 μm) and a 168 Diode Array detector. Solvent A (0.1 M triethylammonium acetate pH 6.5) and Solvent B (pure acetonitrile) were used as mobile phase. An optimal separation was achieved by a gradient method (10% B for 10 min, 10–40% B in 65 min) at a flow rate of 0.5 ml/min. The main peak was found to have very strong absorption at both 260 nm (for DNA) and 491 nm (for fluorescein). The DNA within central two-thirds of peak width was collected and dried under vacuum. The concentration of purified FDNA was determined by ultraviolet spectroscopy. The molecular weight of FDNA was 5,007 g/mol.

Preparation of polymer/oligonucleotide complexes

Cationic polymer/FDNA complexes with different molar ratios were prepared as follows: a 10 μg/ml purified FDNA solution was prepared by diluting the stock solution of purified FDNA with 20 mM HEPES buffer at pH 7.4 with 150 mM of NaCl. The polymer/FDNA dispersions of different molar ratios of amino units and nucleotides were prepared by keeping FDNA concentration at 10 μg/ml and adding different volumes of cationic polymer stock solution (1–10 μg/μl in HEPES buffer at pH 7.4 with 150 mM of NaCl). The polymer solutions were filtered with a 0.45-μm pore size filter before added to the FDNA solution to avoid dust particles. The polymer complex dispersions were vortexed for 10 s and incubated for 30 min at room temperature before use.

Measurements

NMR spectroscopy

Monomer conversion, degree of polymer quaternization, and copolymer block composition were determined by ^1H NMR (Bruker ARX-200) using either CDCl3, d-methanol or D_2O as solvents.

Gel permeation chromatography

Molecular weights (Mn and Mw, respectively) of polyDMAEMA were measured by GPC (Varian MicroPak column G1000, 3000, 7000 HXL) using THF-2%(v/v) triethylamine as eluent at 25°C with an RI detector. Narrow polystyrene standards were used for calibration. Quaternary polyDMAEMA and its block copolymers were characterized by aqueous GPC (Waters, Ultrahydrogel linear column 6–13 μm, four sets) using 0.3 M Na_2SO_4 −0.1% (w/v) NaN_3 as eluent at 30°C with an RI detector. Poly(DMAEMA-b-HEMA) block copolymers were characterized by Waters 2,690 liquid chromatography equipped with two Varian MicroPak columns (G2500 H8, 7000 HXL) and one Waters styragel HR 5E 78×300 mm linear column, and 2,410 refractive index detector at 50°C. DMF with 0.2 (w/v)% LiBr was used as solvent. Data were recorded and processed using the Windows based Millenium 2.0 software package.

Fluorescence measurements

The fluorescence of cationic polymer/FDNA complexes was measured with a Cary Eclipse Fluorescence Spectrophotometer (Varian) at an excitation wavelength of 490 nm and an emission wavelength of 520 nm. A ki-

netic study with up to 30 min of running time recorded the intensity data at 1-min intervals. Fluorescence measurements of each dispersion were done in duplicate.

Results and discussion

Figure 1 shows the relative intensity of fluorescence as a function of the molar ratio of amino units/nucleotides. The fluorescence of the FDNA solution in the absence of cationic polymer was set to 100%. Three definable regions can be identified. At low polymer/FDNA ratios, the fluorescence decreased as polyDMAEMA molecules were added into the FDNA solution. The relative intensity reached the lowest point at an amino/nucleotide ratio between 1 and 3, depending on the chain properties of polyDMAEMA. Further increase in the molar ratio resulted in the polyelectrolyte-assisted dispersion of the FDNA chains with the intensity over 100%. Similar results were observed and explained by van Rompaey et al. [9, 10]. The decrease in intensity at low amino/nucleotide molar ratios was attributed to self-quenching of the fluorescein-labeled FDNA. The increased fluorescence of the complexes at higher amino/nucleotide ratios was the result of the FDNA redistribution. In this report, we evaluated the effects of polymer molecular weight, charger density, counter ion type, and hydrophilic block on the formation of the complexes. The decrease of fluorescence at the low concentrations of polymer is likely due to self-quenching of the fluorophore. When the polymer concentration is low, many fluorophore-labeled DNA molecules (FDNAs) can bind the same polymer, which brings two fluorophores into physical proximity, resulting in fluorescence quenching [22]. When the polymer concentration increases, some of these closely located FDNA molecules are dissociated from the original polymer and bind to a newly added polymer. This redistribution process reverses the self-quenching, giving rise to the recovery of fluorescence.

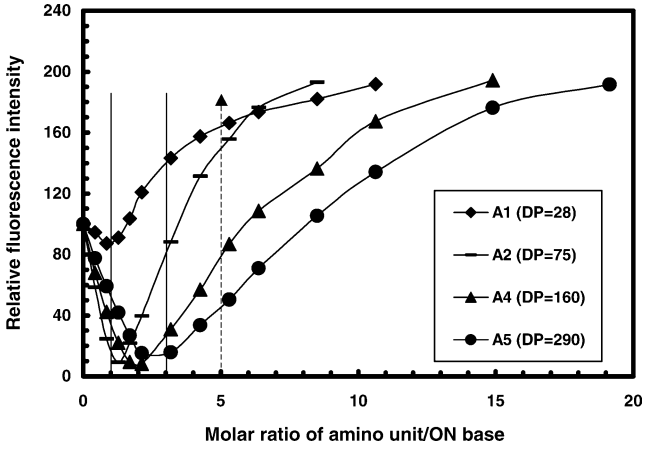


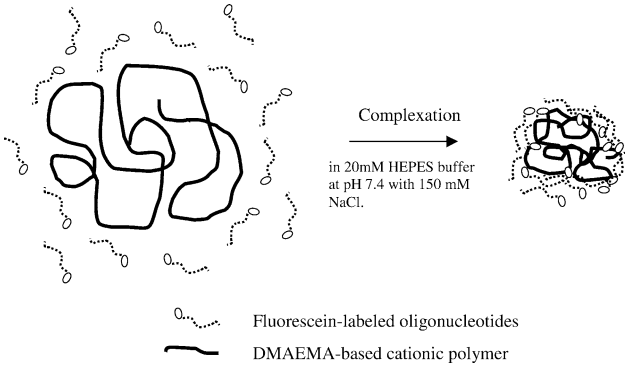
Fig. 1 The relative fluorescence intensity versus the amino/base molar ratio: The effects of polymer molecular weight on the complexation of polyDMAEMA with FDNA. (*filled diamond*) Sample A1 (DP = 28); (*continuous line*) Sample A2 (DP = 75); (*filled triangle*) Sample A4 (DP = 160); (*filled circle*) Sample A5 (DP = 290). The fluorescence of the FDNA solution without polymer addition was considered 100%

Effect of polymer molecular weight

Figure 1 shows the fluorescence intensity of the complexes formed with different molecular weights (the degree of polymerization or the average number of amino units per polyDMAEMA chain: DP = 28–290) of polyDMAEMA. The molecular weight of the cationic polymer clearly influenced the formation of the complexes. Sample A1, which has a molecular weight (Mn = 4,440 g/mol; DP = 28) close to that of the FDNA (Mn = 5,007 g/mol, DP = 15), clearly lacked complexation ability. The lowest relative intensity of fluorescence was 90% at the molar ratio of one and this small level of self-quenching suggested that there was very limited number of FDNA molecules bound onto each polyDMAEMA molecule. Doubling the polyDMAEMA molecular weight (Sample A2: Mn = 11,700, DP = 75) decreased the fluorescence intensity dramatically. The lowest intensity reached 10% at the molar ratio of 1.3.

Further increase in the polyDMAEMA molecular weight (Sample A4: DP = 160, and Sample A5: DP = 290) did not improve the complexation ability. The lowest intensity of 9% for A4 and that of 15% for A5 were observed at the molar ratios of about two. The high amino/nucleotide ratio indicated that more polymer materials were required to complex the same amount of FDNA with higher molecular weight polyDMAEMA. This may be caused by a diffusion/shielding effect. PolyDMAEMA chains were in a form of expanded coils in aqueous media due to the protonated amino groups. FDNA molecules should readily complex with the amino groups at the coil surface. Upon complexation, the shell of the coil collapsed and formed a shield for the amino groups at the core. It became difficult for FDNA to penetrate the shell due to diffusion limitations, as illustrated in Scheme 1.

The molecular weight of polyDMAEMA also had significant effects on the redistribution of FDNA chains at high amino/nucleotide ratios. For example, at the ratio of five in Fig. 1, the relative intensity of Sample A1 was 165%, while those of A2, A4, and A5 were 150, 80, and 45%, respectively. This was likely due to different levels of difficulty for FDNA to dissociate from the complexes formed with these polymers. In the high-molecular weight polyDMAEMA complex systems, the FDNA chains experienced a higher level of difficulty to dissociate from original complexes in order to bind newly added polyDMAEMA chains as most FDNA



Scheme 1 The diffusion/shielding effect of high-molecular weight polyDMAEMA on the complexation

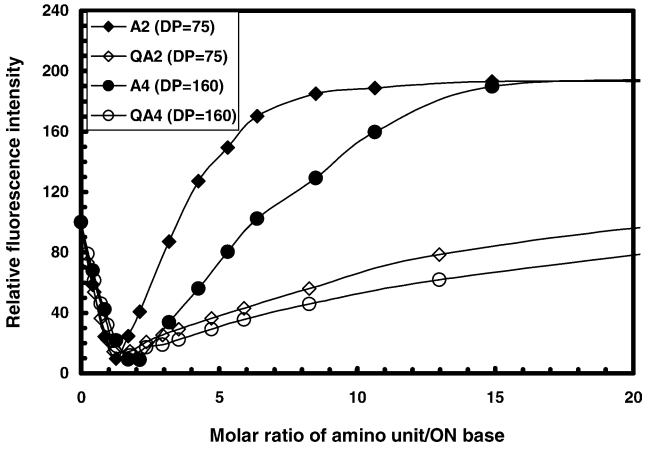


Fig. 2 The effect of charge density on the complexation of polyDMAEMA quats with FDNA. (*filled diamond*) polyDMAEMA (A2: DP = 75) versus (*diamond*) polyDMAEMA–DMSQ (QA2: DP = 75); and (*filled circle*) polyDMAEMA (A4: DP = 160) versus (*open circle*) polyDMAEMA–DMSQ (QA4: DP = 160)

molecules were trapped or partially trapped inside the large complexes. In other words, the high-molecular weight complexes were more stable than their low-molecular weight counterparts.

Effect of charge density and counter ion type

The objective of using fully charged polyDMAEMA for a complexation study is to establish the quantitative relationships between charge ratio and complexation performance. A fully charged polyDMAEMA sample is expected to have high complexation ability because of its high-charge density. Figure 2 shows the comparison for two polyDMAEMA samples of different molecular weights. Samples QA2 and QA4 are the dimethylsulfate quats of A2 and A4. It is of interest to observe that there was no significant improvement, if any, in complexation ability with polymers of higher charge density. The

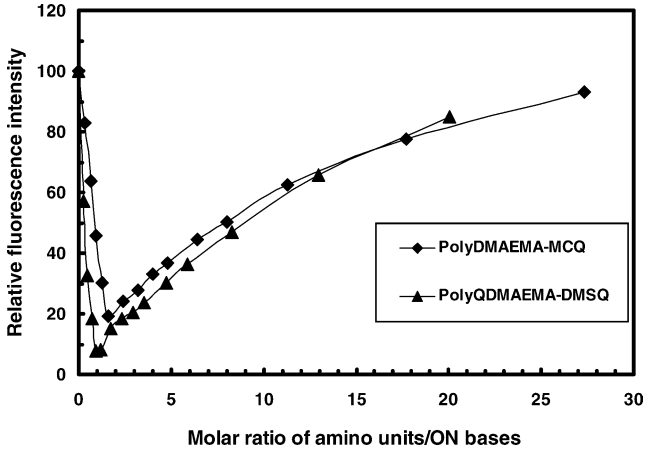


Fig. 3 The effect of counter ion on the complexation of polyDMAEMA quat with FDNA. (*filled diamond*) polyDMAEMA–MCQ versus (*filled triangle*) polyDMAEMA–DMSQ

lowest intensities were at the same level, and the optimum amino/nucleotide ratios were also similar. However, the intensity recovery portions were very different between the quat and original samples. The quat complexes, once formed, were not sensitive to the addition of fresh quat polymers, indicating strong binding between FDNA and quats. From these experiments, it appeared difficult for such complexes to release FDNA chains.

Figure 3 shows the comparison of polyDMAEMA quats with two different types of counter ion. PolyDMAEMA–DMSQ was quaternized with dimethyl sulfate (counter ion: $CH_3SO_4^-$), while PolyDMAEMA–MCQ was quaternized with hydrochloride (counter ion: Cl^-). The former had slightly higher complexation ability than the latter.

Effect of hydrophilic block

The use of hydrophilic block such as PEG, OEGMA, and PHPMA, for improving biocompatibility of cationic polymer/DNA complexes is essential for safe delivery of the nucleic acid. In this work, poly(DMAEMA-b-HEMA) versus polyDMAEMA as well as polyDMAEMA–MCQ-b-PEG versus polyDMAEMA–MCQ were compared to assess the effects of the hydrophilic blocks on the complexation performance. Figure 4 shows the results of poly(DMAEMA-b-HEMA). The block copolymer appeared to be as good as the homopolymer, if not any better, in terms of the lowest intensity value. The optimum amino/nucleotide ratio of the copolymer was slightly higher than that of the homopolymer. However, the hydrophilic block significantly affected the intensity recovery and therefore, increased the stability of the complexes. The block increased the molecular weight of the total polymer, which may have made it more difficult for FDNA to redistribute. Figure 5 shows the compar-

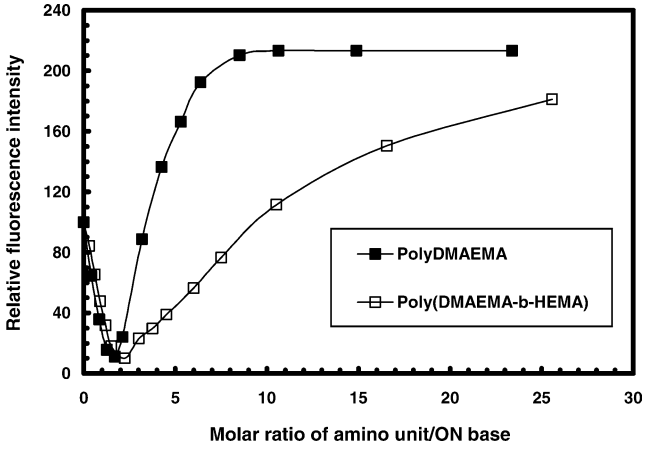


Fig. 4 The effect of polyHEMA block on the complexation of polyDMAEMA with FDNA. (*filled square*) polyDMAEMA versus (*open square*) poly(DMAEMA–b-HEMA)

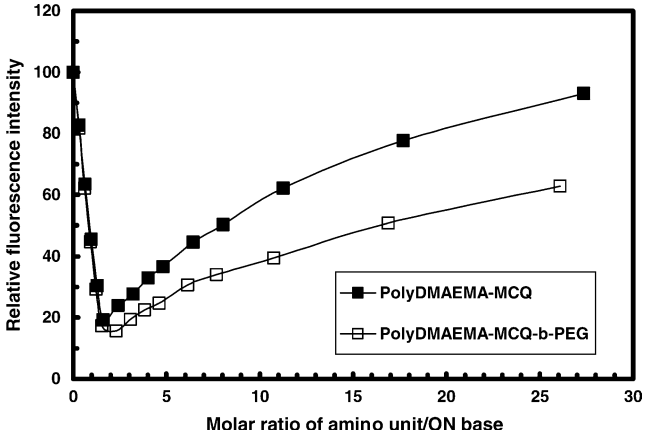


Fig. 5 The effect of PEG block on the complexation of polyDMAEMA–MCQ with FDNA. (*filled square*) polyDMAEMA–MCQ versus (*open square*) polyDMAEMA–MCQ-b-PEG

ison between polyDMAEMA–MCQ and its block copolymer with PEG (i.e., polyDMAEMA–MCQ-b-PEG). The PEG block appeared to slightly enhance the complexation and yielded a slightly smaller value of the lowest intensity. The effect of PEG on the recovery portion of the profile was similar to that of polyHEMA. It also increased the complex stability and created an additional barrier for the release of FDNA.

Complexation kinetics

Oligonucleotide-polymer complex formation in a buffer solution is a time-dependent process of chain diffusion and charge interaction. In this work, a kinetic study on complexation was carried out with an auto-kinetic fluorescence spectroscopy. To study the kinetics of complexation, polyDMAEMA/FDNA complexes with

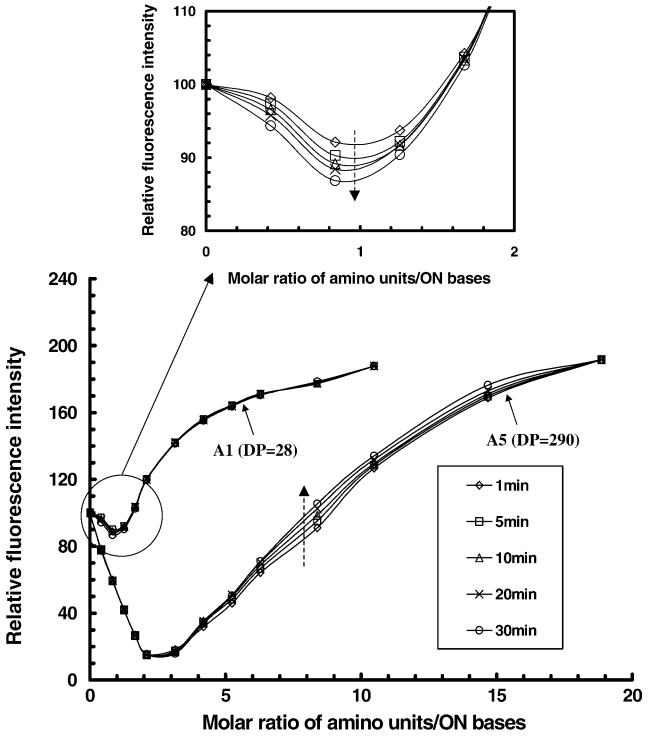
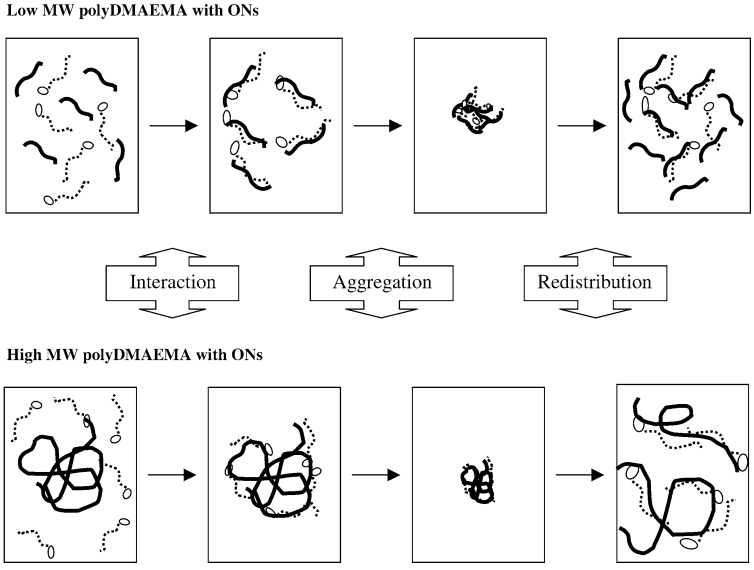


Fig. 6 The dependence of complexation kinetics on the polymer molecular weight. Samples A1 (DP = 28) versus A5 (DP = 290)

different molar ratios of amino/nucleotide were prepared with the change in fluorescence intensity recorded every minute for 30 min. Figure 6 shows profiles of the fluorescence intensity versus amino/nucleotide ratios at different time intervals for two polyDMAEMA samples. For the low-molecular weight sample (A1: DP = 28), the fluorescence intensity varied with time at low amino/nucleotide ratios, but not much at high amino/nucleotide ratios. At a ratio of one, the intensity of the A1/FDNA complex decreased from 92% in 1 min to 87% in 30 min. For the high-molecular weight sample (A5: DP = 290), the situation was the opposite; the time affected the performance at high amino/nucleotide ratios, but not much at low amino/nucleotide ratios. For example, at a ratio of eight, the fluorescence intensity of Sample A5 increased from 80% at 1 min to 100% at 30 min.

The possible explanation for this peculiar observation is as illustrated in Scheme 2. The complexation process can be divided into three definable steps. The first step is the binding between cationic polymer chains and anionic FDNA chains through ionic interactions to form complexes. This step is rapid and does not contribute to the reduction of fluorescence intensity because the complexes are still dispersed in the system. The second step is the aggregation of the complexes to form clusters/particles. The fluorescence intensity de-



Scheme 2 Three definable steps in the complexation of cationic polymers with anionic FDNA: the performance of high-molecular weight polymer versus low molecular weight polymer

creases as a result of the aggregation. The third step is the redistribution of FDNA into newly added cationic polymer chains and it occurs if an excess amount of polymer is added. This step is accompanied with a recovery of fluorescence intensity. For the low-molecular weight polyDMAEMA sample, the individual complexes are probably pairs of one FDNA chain with one polyDMAEMA chain. These small complexes aggregate through the translational diffusion of the pairs, which may be the rate-determining step. On the other hand, a high-molecular weight polyDMAEMA chain can host many FDNA chains. An individual FDNA chain is paired with a segment of the polyDMAEMA chain. The aggregation step is thus realized through the diffusion of the paired segments, which is faster than the translational diffusion. When an excess amount of polyDMAEMA is added, FDNA are redistributed. For a high-molecular weight polymer sample, this redistribution step is slower because of chain entanglements.

Conclusion

Using a 15-nt fluorescein-labeled oligonucleotide FDNA as a model oligonucleotide (ON), we examined the ON complexation behaviors of several well-defined DMAEMA-based cationic polymers of different molecular weights, charge densities, counter ion types, and block compositions. The following conclusions can be drawn from the study.

1. The polymer molecular weight had significant effects on the ON complexation. The polyDMAEMA with a molecular weight doubled that of the selected ON exhibited optimal complexation. However, the polymer sample with a molecular weight similar to the ON was not efficient in complexation. Samples of much higher molecular weights did not improve the efficiency but yielded stable complexes, and imposed diffusion limitations to the redistribution of FDNA.
2. Increasing the polymer charge density did not significantly change the optimal value of amino/nucleotide ratio and that of intensity reduction. The high-charge density provided strong interactions with the ON and resulted in stable complexes. The type of counter ions in polyDMAEMA quats played a minor role in complexation.
3. Both poly(DMAEMA-b-HEMA) and poly(DMAEMA–MCQ-b-PEG) block copolymers showed good complexation ability and some steric stability. PEG block improved polyDMAEMA–MCQ interactions with ON, while HEMA block did not show such a change.
4. The kinetic study of the complexation of FDNA and polyDMAEMA of varying molecular weights showed that low molecular weight samples experienced diffusion limitations at low amino/nucleotide ratios, while high-molecular weight samples demonstrated diffusion effects at high amino/base ratios.

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