

Complexation between DNA and Peptides with Precisely Controlled Charge Density and Distribution*

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Abstract Using three designed peptides with precisely-controlled charge density and three types of DNAs with different length and flexibility, the effect of charge density on the formation of PEC was studied. Highly charged (KKKK)₅ interacts strongly with 21 bp dsDNA to form large complex, followed by precipitation; while the medium charged (KGKG)₅ only form complex with 21 bp dsDNA at proper +/- charge ratios; and no prominent complex between weakly charged (KGGG)₅ and 21 bp dsDNA is observed at the same conditions. Similar trend is observed when the peptides form complex with 2000 bp DNA or 21nt ssDNA. It is also found that the complex formed by adding peptide to DNA is in random coil conformation, but the complex prepared by the inverse order is in molten globule state. Re-dissolution of the complex occurs only when DNA is added to peptides with similar or shorter length.

Keywords: Peptide; Charge density; DNA; Polyelectrolyte complex.

INTRODUCTION

Polyelectrolyte complexes (PECs) have important applications in various fields, such as flocculation, coating, lubrication and modification of surface using layer-by-layer technique^[1–3]. Recently, PECs have also been considered as the carrier systems of great potential for drugs, proteins or DNA. A variety of polycations and cationic lipids have been employed as non-viral vectors for the neutralization and encapsulation of negatively charged DNA or siRNA^[4–8]. Moreover, many important biomacromolecules, such as polypeptides, glycosaminoglycans and DNA, are polyelectrolytes. A number of crucial life processes are determined by the electrostatic interactions between the charged macromolecules. The investigation of the complexation between peptide and DNA are fundamental to understand many life processes such as molecular recognition, protein-nucleotide interaction and DNA compaction^[9–12].

The main driving force for the formation of PECs is the significant gain in entropy due to the release of small molecular counterions^[13]. The structures and properties of PECs are controlled by charge density, ionic strength, pH, solvent quality, chain topology, mixing order and so on^[14–19]. Among all the parameters, the charge density is a pivotal factor since it determines the maximum amount of counterions that could be released upon complexation, even though the actual release of counterions also depends on the external parameters, such as salt concentration and pH, if the polyelectrolyte is weakly charged.

Two approaches are generally used to tune the charge density of polyelectrolyte. One approach is to control the density of the charged groups during synthesis or by chemical modification afterwards. Dautzenberg and

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Jaeger^[20] found that the cationic random copolymers with weaker charge density exhibited decreased level of aggregation when forming complex with sodium poly(styrene sulfonate), but the resulting structure was highly swollen. Mita *et al.*^[21] explored the interactions between DNA and ionic polymers ($-\text{[N}^+(\text{CH}_3)_2(\text{CH}_2)_m\text{N}^+(\text{CH}_3)_2(\text{CH}_2)_n\text{]}-$, with $m-n$ being 3-3, 6-6, and 6-10.). They found 3-3 ionic polymers bind cooperatively with DNA while 6-6 and 6-10 ionic polymers bind with DNA noncooperatively. Zelikin and coworkers^[22] synthesized a series of n , n -ionenes of different charge densities ($n = 3, 4, 6$ and 10) as polycation to bind with DNA. It was concluded that the charge density of polycation dominated the selective binding of DNA with n , n -ionenes. The other approach to adjust the charge density of the polyelectrolyte is to change the degree of ionization by varying pH or adding salt. For instance, it was observed that the electrostatic attraction dominated the binding of surfactant to polyion beyond a critical degree of neutralization^[23]. The ionization of weak polyelectrolytes could be tuned by pH. It has been reported that pH can substantially affect the property of the polyelectrolyte complex or the distance between layers in layer-by-layer if weak polyelectrolyte is involved^[24-26]. In both of the methods above, the charge density is generally controlled at macromolecular or segmental level. It is not easy to obtain the polyelectrolyte with precisely controlled charge distribution at fixed chain length, especially for that of the synthetic polyelectrolyte which possesses polydispersed size distribution in nature.

Peptide is a type of biocompatible polymers which can consist of 20 amino acids of different properties. It is easy to prepare peptides with medium length, monodispersity and desired sequences *via* solid-phase peptide synthesis. The incorporation of charged amino acids, *e.g.*, lysine, arginine, aspartate or glutamate, renders peptide a model polyelectrolyte or polyampholyte with precisely-controlled charge density or charge distribution at molecular (monomer) level. For instance, Bysell and coworkers^[27] used monodisperse peptides containing alanine and lysine in a series of repeated patterns to study the effect of charge density on the interaction between cationic peptides and oppositely charged microgels. Chen and colleagues^[28] studied the interaction of a polyampholyte peptide containing lysine and glutamate with oligonucleotide.

Herein, we designed three peptides with different charge densities and studied their behavior of forming complex with DNA. The sequences of the peptides are shown in Table 1. The first peptide, denoted as (KKKK)₅, consists of 20 lysines (K), each lysine carrying one positive charge at pH = 7.4. The second and the third peptides, denoted as (KGKG)₅ and (KGGG)₅, respectively, are the same as (KKKK)₅ in length, but only half or ¼ in charge numbers. We choose glycine (G) as the neutral group to adjust the charge density. Glycine is an achiral amino acid with a minimal side chain of one hydrogen only. It can fit in either hydrophilic or hydrophobic environment, eliminating the formation of secondary structures or the extra interactions such as hydrophobic attraction upon complexation. All the peptides are in random coil conformation in aqueous solutions; and no secondary structure is observed (CD spectra not shown). Three types of DNAs are used as the polyanions: double-stranded oligonucleotides of 21 base pairs long (21 bp dsDNA), single-stranded oligonucleotides consisting of 21 nucleotides (21 nt ssDNA), and salmon tests DNA of 2000 bp long. The sequences of the oligonucleotides are also shown in Table 1. The complexation between peptides and DNAs was investigated by laser light scattering (LLS), atomic force microscopy (AFM) and UV absorption.

Table 1. Sequence of the peptides and oligonucleotides

Peptide/Oligonucleotide name	Sequence
(KKKK) ₅	Ac-KKKK KKKK KKKK KKKK KKKK-amide
(KGKG) ₅	Ac-KGKG KGKG KGKG KGKG KGKG-amide
(KGGG) ₅	Ac-KGGG KGGG KGGG KGGG KGGG-amide
21 nt -1	5'-CTT ACG CTG AGT ACT TCG ATT-3'
21 nt -2	5'-AAT CGA AGT ACT CAG CGT AAG-3'

EXPERIMENTAL

Materials

Synthetic peptides with designed sequence (purity > 99%) were purchased from GL Biochem. Ltd. (Shanghai, China) and used as received. Two single-stranded oligonucleotides (21 nt-1 and 21 nt-2, purity > 99%) with complementary base sequence were obtained from Invitrogen (Shanghai, China). One of them was used as the 21 nt ssDNA. Salmon testes DNA (2000 bp, named as salmon DNA for short), Trizma[®] base, and EDTA were purchased from Sigma (St Louis, US) and used as received. The double-stranded oligonucleotide (21 bp dsDNA) was obtained by annealing the two complementary single chains (21 nt-1 and 21 nt-2) at the same molar ratio in $1 \times \text{TE}$ (10 mmol/L tris base, 1 mmol/L EDTA) buffer at 95 °C for 5 min, followed by slow cooling to room temperature. The stock solutions of the peptides or salmon DNA were obtained by dissolving the powder sample with TE buffer. The resulting solutions were diluted by TE into desired concentrations.

Laser Light Scattering (LLS)

The equipment information and basic LLS principles are described elsewhere^[29, 30]. The complexes at different +/- ratios (+/-, the molar charge ratio, stands for the number of positive charge from all the peptides to the number of negative charge from all the DNA) were prepared by mixing peptide and DNA solution together. The mixture was vortexed for 30 s, and then stayed for 30 min before the measurement. We prepared the complexes by adding peptide to DNA at lower +/- ratios, and DNA to peptide at higher +/- ratios.

Atomic Force Microscopy (AFM)

The images were taken in tapping mode using a Nanoscope III microscope equipped with a 110 μm scanner (Veeco. Instrument Inc.). Imaging was performed in air with a FESP tip (Bruker instruments Inc.). The resonant frequency of the cantilever is about 70 kHz. 10 μL of the complex solution was deposited on a fresh mica surface using a pipette; the excess solution was blotted away with a strip of filter paper. The sample was washed with Milli-Q water (Millipore, USA) twice to remove salts. It was then air-dried for one day before AFM measurements.

UV Spectroscopy

For UV measurements, 21 bp dsDNA ($c = 1.0 \times 10^{-4}$ g/mL) was titrated into peptide ($c = 1.0 \times 10^{-5}$ g/mL) solution drop by drop to form complex. The UV absorbance of the complex sample was scanned from 220 nm to 350 nm on a Shimadzu 2101 UV/Vis spectrometer at 25 °C. The sample of DNA added into TE buffer with the same volume was used as the reference to monitor the increase of DNA concentration.

RESULTS AND DISCUSSION

Complex between Peptides and 21 bp dsDNA

According to the polyelectrolyte theory^[13], the strength of the electrostatic interaction (the effective charge density of a linear polyelectrolyte) can be estimated by the Coulomb coupling strength parameter, $\Gamma = \lambda_B/l_0$, where λ_B is the Bjerrum length $\lambda_B = e^2/(4\pi\epsilon_0\epsilon k_B T)$ with e , ϵ_0 and ϵ being the element charge, vacuum permittivity and relative dielectric constant respectively; and l_0 is the distance between neighboring charged groups. For water at room temperature, λ_B roughly equals to 0.7 nm. When all the charged groups are treated as point charges fixed on the backbone, the l_0 of (KKKK)₅, (KGKG)₅, and (KGGG)₅ is around 0.5, 0.9, and 1.8 nm, respectively. The Γ s of (KKKK)₅, (KGKG)₅ and (KGGG)₅ are calculated to be 1.6, 0.8 and 0.4, respectively. The Γ of dsDNA is around 4.1, far higher than those of all the peptides.

Figure 1(a) compares the normalized excess scattered intensity (the intensity has been extrapolated to zero angle) of the complex formed by 21 bp dsDNA interacting with (KKKK)₅, (KGKG)₅ and (KGGG)₅, separately. The sharp increase in the excess scattered intensity directly indicates the formation of complex, because the intensity is roughly proportional to the sixth power of the particle size. As expected, the peptide with higher charge density has the tendency to form complex at a higher level of aggregation. When adding peptide to DNA (open symbols in Fig. 1a), the onset of the intensity occurs at +/- = 0.8 in the case of (KKKK)₅ (Fig. 1a, square

symbols). Further increasing the amount of $(\text{KKKK})_5$ to $+/- > 1.1$ leads to the formation of extremely strong aggregates which precipitate accordingly. In the case of $(\text{KGKG})_5$, the excess scattered intensity stays constant at low $+/-$ ratios but exhibits a dramatic increase at $+/- = 2.3$ (Figure 1a, triangle symbols), indicating that the heavy complexation between $(\text{KGKG})_5$ and 21 bp dsDNA occurs only at $+/-$ ratios far more than unity. Precipitation is also formed with increasing $+/-$ ratio, similar to the behavior of $(\text{KKKK})_5$. As for $(\text{KGGG})_5$, whose charge density is further reduced, no significant complex forms at the studied $+/-$ ratios. As shown in Fig. 1(a) (circle symbols), the excess scattered intensity of $(\text{KGGG})_5$ /21bp dsDNA solution is horizontal. Weak complexes with extremely small size might form at certain $+/-$ ratios, but the change in the scattered intensity is within the experimental error and cannot be detected.

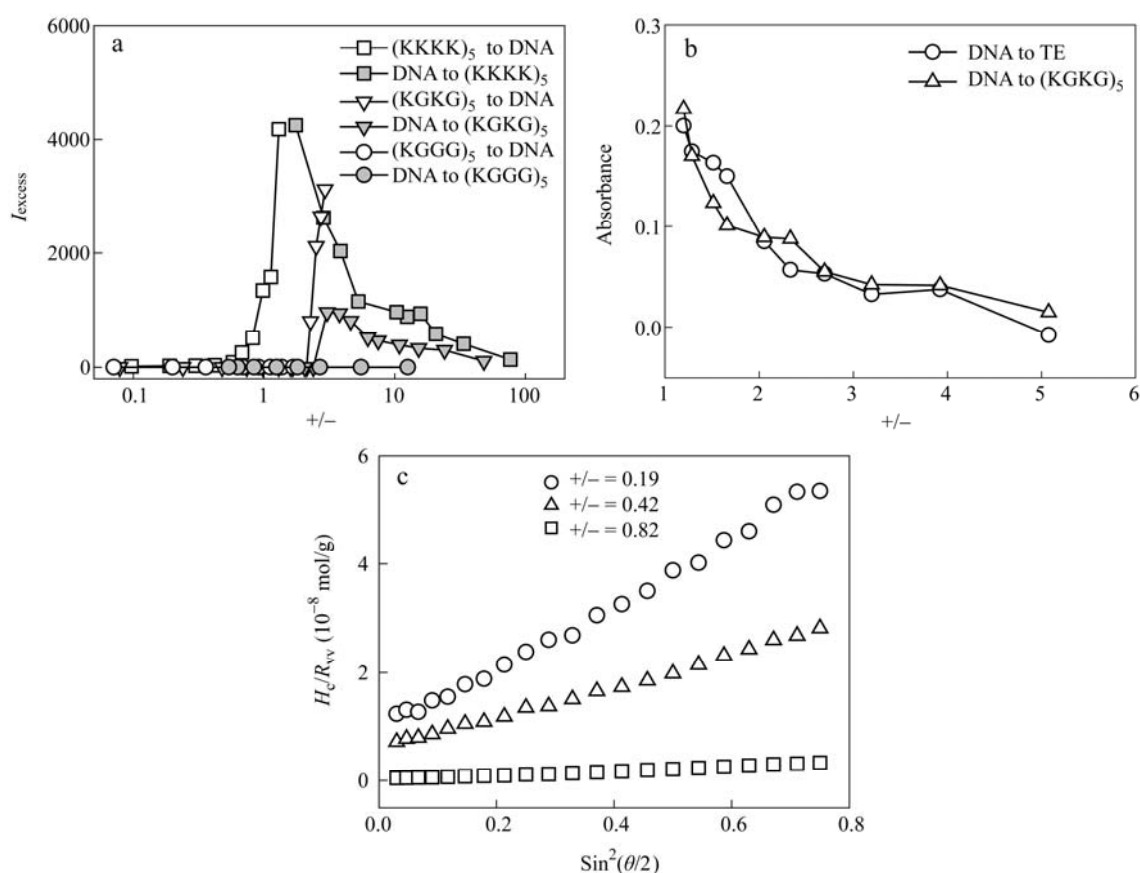


Fig. 1 (a) Excess scattered intensity of the complex formed by $(\text{KKKK})_5$ (square), $(\text{KGKG})_5$ (triangle) and $(\text{KGGG})_5$ (circle) with 21bp dsDNA, separately, in TE buffer (Open symbols: peptide is added to 21bp dsDNA, $c(\text{DNA}) = 1.0 \times 10^{-5}$ g/mL, $c(\text{peptide}) = 1.0 \times 10^{-4}$ g/mL; Filled symbols: 21 bp dsDNA is added to peptide, $c(\text{DNA}) = 1.0 \times 10^{-4}$ g/mL, $c(\text{peptide}) = 1.0 \times 10^{-5}$ g/mL); (b) UV absorbance of the complex prepared by adding 21 bp dsDNA into $(\text{KGKG})_5$ in TE buffer at 260 nm and (c) Selected angular dependence plots of the $(\text{KKKK})_5$ -21bp dsDNA complex at different $+/-$ ratios

The trend is similar when the complex is prepared by adding DNA to $(\text{KKKK})_5$ or $(\text{KGGG})_5$. $(\text{KKKK})_5$ forms strong complex with 21 bp dsDNA; and the precipitates are observed at $+/-$ ratio close to 1 (filled symbols, Fig. 1a). No complex is detected in the studied $+/-$ ratio range when $(\text{KGGG})_5$ is mixed with 21 bp dsDNA. However, the complex formed by adding 21 bp dsDNA to $(\text{KGKG})_5$ is different from that prepared in the inverse mixing order. The excess scattered intensity keeps increasing and reaches maximum at $+/- = 3.8$ (Fig. 1a, filled triangle symbols). Further adding DNA leads to a decrease in the intensity; at $+/- < 2.3$, the excess scattered intensity levels off and is similar to that without formation of any complex. The decrease in the

scattered intensity could be caused by the precipitation of the complex (phase separation or coacervation^[10, 15, 30, 31], which decreases the concentration of the complex in the supernatant) or by the dissociation of the complex. To test which hypothesis aforesaid is right, we measured the UV absorbance of the complex at different $+/-$ ratios. The complex is prepared by following the same procedure in LLS experiments. The sample prepared by adding the same amount of DNA into pure TE buffer is used as the reference. The UV absorbance at 260 nm indicates that the amount of DNA after forming complex at each of the studied $+/-$ ratio is similar to that of the reference at the same conditions (Fig. 1b), suggesting that the decrease in scattered intensity in Fig. 1(a) is caused by the dissociation of the complex (as there is no loss of DNA in the supernatant). However, no dissociation of the complex is observed for the complex at the same $+/-$ ratio but prepared by adding (KGKG)₅ to DNA (open triangle symbols in Fig. 1a). In other words, the morphology of the complex at the same $+/-$ ratio but prepared in different mixing orders could be dramatically different if the charge density of the polyelectrolyte decreases to a certain range.

Since both the peptide and DNA have nearly the same dn/dc values in TE, which is $\sim 0.17 \text{ mL/g}$ ^[32], we can estimate the $M_{w, \text{app}}$ (footnote “app” represents apparent.) of the complex with fair accuracy from the angular dependence of the excess scattered intensity (Selected plots are shown in Fig. 1c). The plots of the $M_{w, \text{app}}$ (data not shown) at different charge ratios exhibit curves similar to those of the excess scattered intensity (Fig. 1a). Clearly, peptide with higher charge density results in the DNA complex with larger molar mass at the same charge ratios.

Not only the excess scattered intensity and $M_{w, \text{app}}$, the size of the complex also changes with the $+/-$ ratio. Since the peptide and 21 bp dsDNA are smaller in size, the diffusive mode attributed to the complex is dominant in the system before precipitation (Selected size distributions of the complex are shown in Fig. 2c.). The size of the complex can be determined with enough accuracy. Figures 2(a) and 2(b) show the apparent radius of gyration $R_{g, \text{app}}$ and the hydrodynamic radius $R_{h, \text{app}}$ of the complex formed between (KKKK)₅ and 21 bp dsDNA at different $+/-$ ratios. When adding (KKKK)₅ into DNA, both $R_{g, \text{app}}$ and $R_{h, \text{app}}$ exhibit a slight drop at early $+/-$ ratios, and then increase monotonously (Fig. 2a). The R_g/R_h ratio, a practical parameter to describe the conformation of the complex in solution, is almost constant at around 1.3 during the titration process. It has been established in literature that the R_g/R_h ratio is close to 1.5 for random coils; but it is only 0.775 for solid spheres^[33]. The value of 1.3 suggests that the conformation of the complex is relatively loose. The complex prepared in the inverse mixing order, however, shows different conformation. As shown in Fig. 2(b), with the addition of 21 bp dsDNA, the $R_{h, \text{app}}$ increases at a much faster rate than the $R_{g, \text{app}}$. The calculated R_g/R_h ratio is 0.7 at the beginning and becomes even smaller (around 0.5) during the titration process. It indicates that the complex forms a dense spherical conformation when the amount of DNA is low, and it then becomes like a molten globule (the chain density in the centre core of the complex is larger than that of the outer surface) with further increase in DNA. The R_g/R_h ratio of a molten globule is far below 0.7^[34, 35].

The prominent difference of the complexation is caused by the mixing order. The chain length of 21 bp dsDNA and (KKKK)₅ are similar. However, the Coulomb coupling strength parameter of DNA ($\Gamma = 4.1$) is much higher than that of (KKKK)₅ ($\Gamma = 1.6$). When DNA is added to (KKKK)₅, the freshly added DNA molecules are able to neutralize more than one surrounding (KKKK)₅ molecule. Overcharge (charge inversion) of the complex is also easier because the electrostatic repulsive force among (KKKK)₅ molecules is weaker than the attractive force between (KKKK)₅ and DNA. The overcharged complex behaves similarly like (KKKK)₅ with further addition of DNA. Therefore, large and dense complex is formed. The situation is reversed when (KKKK)₅ is added to DNA, the freshly added (KKKK)₅ molecules cannot fully neutralize the negative charges of DNA; and the strong electrostatic repulsion among DNA molecules also prevents further binding of more DNA chains. Therefore, the complex prepared in this mixing order is small in size and loose in conformation.

In the case of (KGKG)₅, the evolutionary change of the size and the conformation of the complex exhibits nearly the same trend as that of (KKKK)₅ (data not shown). However, for the complexes formed by (KGKG)₅, no size information can be obtained because the complexation is too weak.

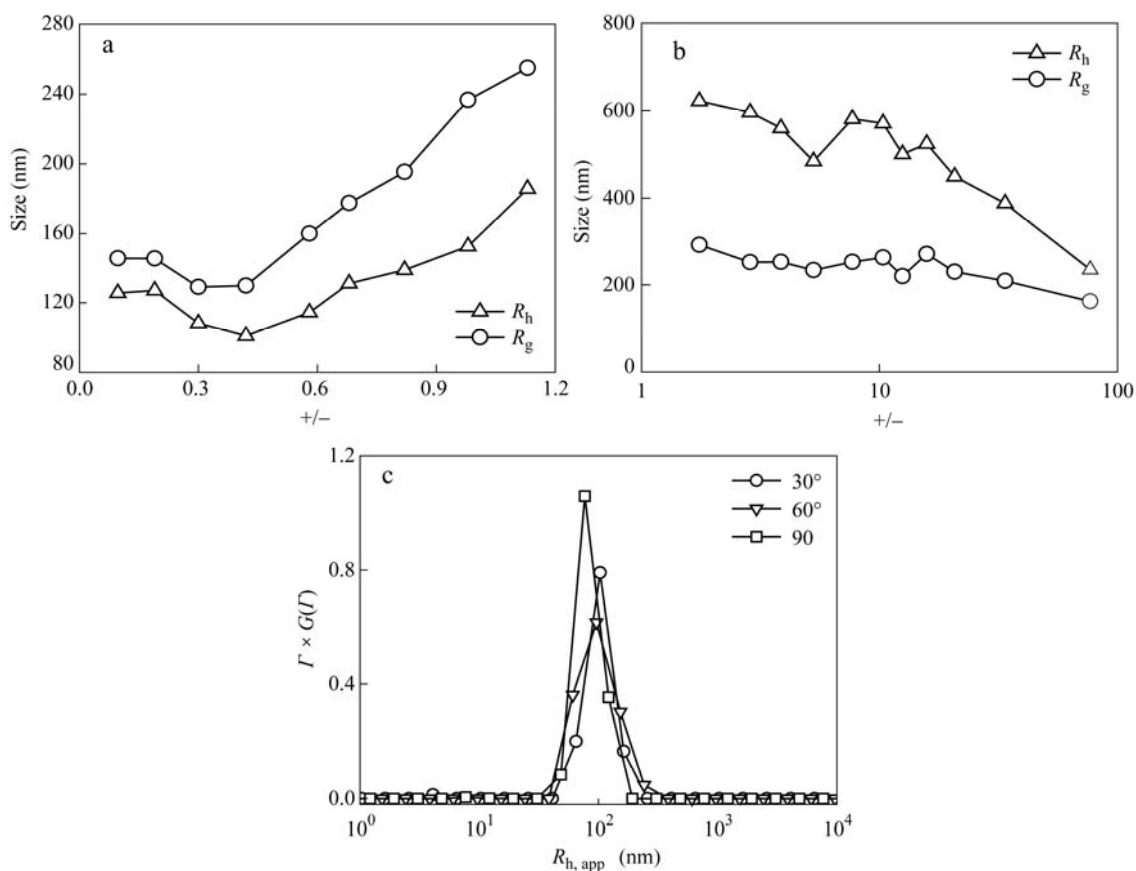


Fig. 2 Sizes of the (K4KKK)₅/21bp dsDNA complex in TE buffer during the titration process: (a) addition of (K4KKK)₅ to DNA, $c(\text{DNA}) = 1.0 \times 10^{-5}$ g/mL, $c(\text{K4KKK})_5 = 1.0 \times 10^{-4}$ g/mL; (b) addition of DNA to (K4KKK)₅, $c(\text{DNA}) = 1.0 \times 10^{-4}$ g/mL, $c(\text{K4KKK})_5 = 1.0 \times 10^{-5}$ g/mL and (c) selected $R_{h,app}$ distributions of (K4KKK)₅/21bp dsDNA complex at $+/- = 0.42$

To further clarify the charge density effect on the size and morphology of polyelectrolyte complex, we prepared the complex at $+/- = 3.0$ by adding known amount of 21 bp dsDNA into peptide in one batch. Figure 3(d) compares the size distribution of three complexes measured at 30°. Similar results are observed at other scattering angles. The peptide with higher charge density forms complex with 21 bp dsDNA more intensively at the same charge ratio and concentration. As indicated in Fig. 3(d), the complex formed by (K4KKK)₅ has the largest size ($R_{h,app} = 400$ nm) and a broad distribution; the $R_{h,app}$ of the complex formed by medium charged (KGKG)₅ is about 260 nm, but with relatively narrow distribution; weakest charged (KGGG)₅ could barely interact with 21 bp dsDNA, the size distribution is broad with the average $R_{h,app}$ about 40 nm. The $R_{g,app}$ of the complex is obtained by fitting of the form factors ($P(q)$) by spherical model (for the homogeneous spherical model for particle with $qR_g > 1$: $P(q) = 9(\sin(qR_g) - qR_g \cos(qR_g))^2 / (qR_g)^6$). The results show that the $R_{g,app}$ of the complex formed between 21 bp dsDNA and (K4KKK)₅ is about 240 nm, which is also larger than that of the complex formed between (KGKG)₅ and 21 bp dsDNA (~180 nm). The intensity of (KGGG)₅/DNA complex is too low to be analyzed. No form factor or $R_{g,app}$ is obtained.

The morphology of the complex determined by AFM confirms the LLS results. As shown in Fig. 3, the complex between (K4KKK)₅ and DNA has the largest size and highest height. The complexes cover almost the whole mica surface (Fig. 3a). (KGKG)₅/DNA complex are much lower in height and fewer in number (Fig. 3b). In the case of (KGGG)₅, only very few complexes with even lower height and smaller size are visible in the whole view field (Fig. 3c).

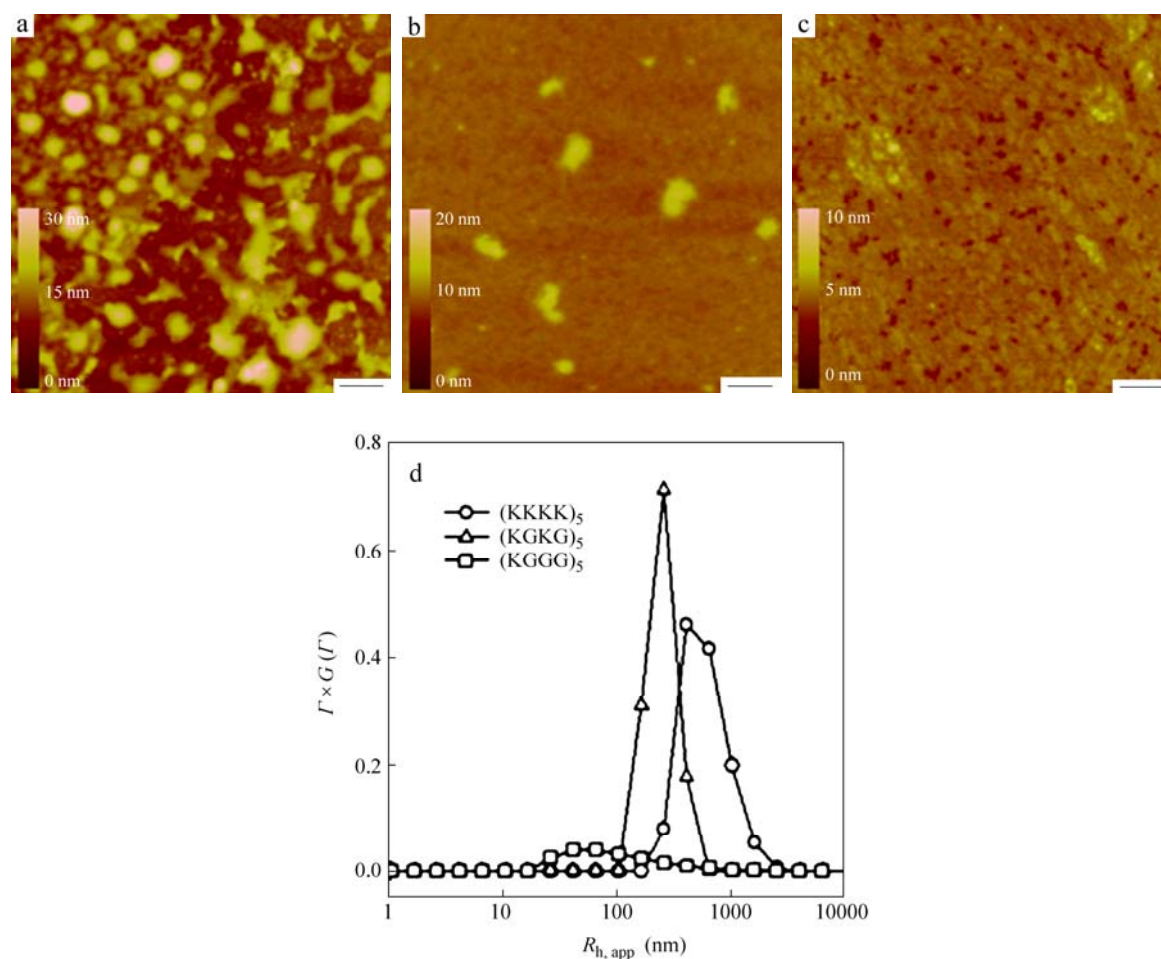


Fig. 3 AFM images of the complex formed by adding 21 bp dsDNA into (KKKK)₅ (a), (KGKG)₅ (b) and (KGGG)₅ (c), separately, at $\pm = 3.0$. $c(\text{peptide}) = c(\text{DNA}) = 1.0 \times 10^{-5}$ g/mL (Scar bar: 200 nm.); (d) size distribution of the complex formed by (KKKK)₅, (KGKG)₅ and (KGGG)₅ interacting with 21 bp dsDNA, separately, in TE buffer (The DNA is added into peptide in one batch to reach $\pm = 3.0$. $c(\text{peptide}) = c(\text{DNA}) = 1.0 \times 10^{-5}$ g/mL, scattering angle: 30° .)

Complexation between Peptides and ssDNA or Salmon dsDNA

We also choose 21 nt ssDNA and 2000 bp salmon DNA to study the effect of charge density on the polyelectrolyte complexation. ssDNA has a flexible chain conformation and a half charge density compared with dsDNA; while salmon DNA has a relatively large $M_{w,app}$ and a random coil conformation in aqueous solution^[36]. Figure 4(a) compares the excess scattered intensity of the complex formed by ssDNA with the three peptides at 90° . The data at other scattering angles exhibit the same trends. Only (KKKK)₅, which has the highest charge density, can form complex with ssDNA during the titration process. The behavior is similar to the complexation with 21 bp dsDNA (Fig. 1a, square symbols). At the same conditions, (KGKG)₅ forms complex with 21 bp dsDNA (Fig. 1a, triangle symbols) but not with ssDNA of the same contour length. This further demonstrates that the charge density plays a key role during the polyelectrolyte complexation.

Figure 4(b) shows the excess scattered intensity of the complex between salmon DNA and peptides at 90° . The data at other scattering angles exhibit the same trends. The degree of complexation is significantly improved with increasing DNA length. When adding (KKKK)₅ to salmon DNA, the onset of the intensity occurs at the $\pm$ ratio smaller than that in the case of 21 bp dsDNA (Fig. 1a, square symbols); and the precipitates are observed at $\pm$ ratio around 0.6, instead of 1.1. For (KGKG)₅, no dissociation of the complex, but precipitation is observed

when the amount of DNA reaches a certain level. Interestingly, the dissociation is observed when a large amount of salmon DNA is added to (KGGG)₅, the peptide with even lower charge density. Kabanov and coworkers proposed that the short guest polyelectrolyte in the complex can redistribute with the addition of fresh and oppositely charged host polyelectrolyte (longer length). Thus the stoichiometric PEC can re-dissolve when the amount of host polyelectrolyte is more than unity^[37]. Our study on DNA complex clearly indicates that the charge density of the guest polyelectrolyte has to be lower than that of the host polyelectrolyte, especially when the host polyelectrolyte is long.

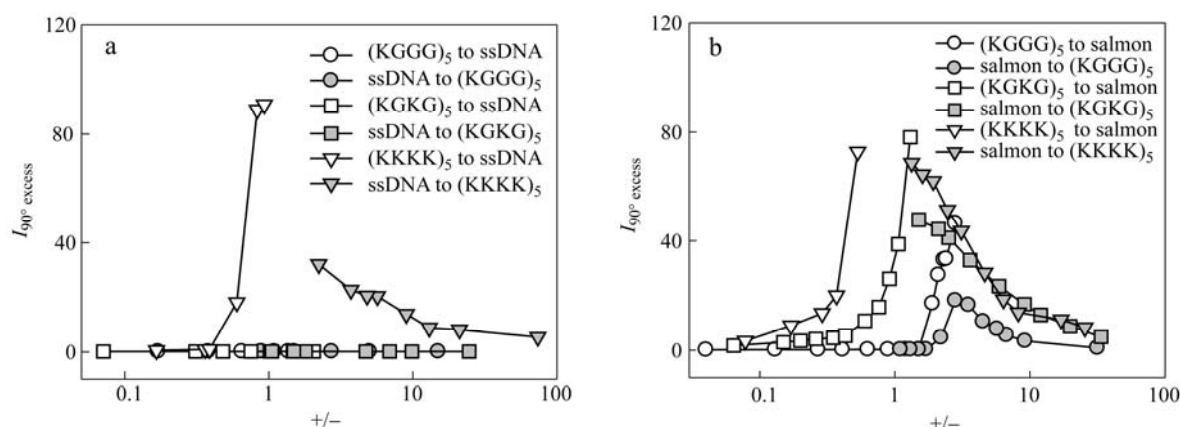


Fig. 4 (a) Excess scattered intensity of peptides/21nt ssDNA complex at whole +/- range (Open symbols: peptide to 21 nt ssDNA, $c(\text{DNA}) = 1.0 \times 10^{-5}$ g/mL, $c(\text{peptide}) = 1.0 \times 10^{-4}$ g/mL; Filled symbols: 21 nt ssDNA to peptide, $c(\text{DNA}) = 1.0 \times 10^{-4}$ g/mL, $c(\text{peptide}) = 1.0 \times 10^{-5}$ g/mL.) and (b) Excess scattered intensity of the complex formed between salmon DNA and the peptides (Open symbols: adding peptide to salmon DNA, $c(\text{salmon}) = 1.0 \times 10^{-5}$ g/mL, $c(\text{peptide}) = 1.0 \times 10^{-4}$ g/mL; Filled symbols: adding salmon DNA to peptide, $c(\text{salmon}) = 1.0 \times 10^{-4}$ g/mL, $c(\text{peptide}) = 1.0 \times 10^{-5}$ g/mL.)

Table 2 summarizes the results of the complex formed by salmon DNA, 21 bp dsDNA and 21 nt ssDNA interacting with the peptides of different charge densities. The general trend is that the polyelectrolyte with higher charge density is susceptible to form larger complex, followed by precipitation, at lower charge ratios. It is independent of the mixing order. No significant complex is observed when the charge density of one or both of the polyelectrolytes decreases to a certain level. Dissociation (or re-dissolution) of the complex occurs only when the polyelectrolyte with higher charge density is added to the polyelectrolyte with lower charge density and similar or shorter length.

Table 2. Results on the complexes between DNAs with peptides

	Salmon DNA		21 bp dsDNA		21 nt ssDNA	
	Order A	Order B	Order A	Order B	Order A	Order B
(KKKK) ₅	P > 0.6	P < 1.9	P > 1.2	P < 1.7	P > 1.0	P < 2.2
(KGKG) ₅	P > 1.3	P < 1.5	P > 1.3	R	N/A	N/A
(KGGG) ₅	P > 2.8	R	N/A	N/A	N/A	N/A

Order A: adding peptide to DNA; Order B: adding DNA to peptide;

P stands for precipitation; R is abbreviated for re-dissolution; N/A: no complexation is observed.

CONCLUSIONS

Charge density plays a key role in polyelectrolyte complexation. Peptides provide a platform to design polyelectrolyte with precisely-controlled charge density and charge distribution. The complexation between three types of DNA and designed peptides were investigated using LLS, AFM and UV. Our study demonstrated that the peptide with higher charge density is prone to form complex of larger size with DNA. Lowering the charge density of peptide to a certain level, no obvious complexation is observed. Complex with different

morphologies such as loose complex, solid sphere or molten globule were obtained by tuning not only the charge density of peptide/DNA but also the mixing order. Dissociation (or re-dissolution) of the complex occurs only when the polyelectrolyte with higher charge density is added to the polyelectrolyte with lower charge density and similar or shorter length. This study offers more insights into designing peptide materials for gene delivery system based on polyelectrolyte complexation.

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