ELSEVIER

Biophysical Chemistry 107 (2004) 273–281

Biophysical Chemistry

www.elsevier.com/locate/bpc

Studies of interaction between poly(allylamine hydrochloride) and double helix DNA by spectral methods

Yinglin Zhou, Yuanzong Li*

The Key Laboratory of Bioorganic Chemistry and Molecular Engineering, Department of Chemical Biology, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, PR China

Received 9 August 2003; received in revised form 20 September 2003; accepted 20 September 2003

Abstract

DNA interaction with cationic polyelectrolytes promises to be a versatile and effective synthetic transfection agent. This paper presents the study on interaction between a simple artificial cationic polymer, poly(allylamine hydrochloride) (PAA), and herring sperm DNA (hsDNA) using several spectroscopic methods, including light scattering, microscopic FTIR-, CD-spectroscopy and so on. The results show that PAA interacts with DNA through both the phosphate groups and the nitrogenous bases of DNA. The formation of DNA/PAA complex may change the microenvironment of double helix of DNA from B- to C-form and the great changes in DNA morphology occur when N:P ratio is near to 1.0. At the same time, the spectroscopic changes of ethidium bromide (EB) on its binding to DNA are utilized to study the interaction between PAA and DNA. Reversion of the maximum absorption wavelength (ν_{max}), reduction of induced circular dichroism and decrease in fluorescence intensity of DNA–EB on addition of PAA indicate that the formation of the complex between DNA and PAA is not in favor of the interaction between DNA and EB. The binding constant of EB and the number of binding sites per nucleotide decrease with increase in the concentrations of PAA, indicating noncompetitive inhibition of EB binding to DNA in the presence of PAA. It is also proved that the formation of the DNA/PAA complex is influenced by pH value and ionic strength.

Keywords: Poly(allylamine hydrochloride); DNA; Interaction; Conformation; Spectroscopy

1. Introduction

High density of negatively charged phosphate groups of the double helix provides the ability of DNA to form rather stable complexes with synthetic polycations [1,2]. Some of them were successfully used for increasing the efficiency of transformation of cells by the plasmids and for the protection of DNA from splitting by cell nucleases [3–5], which make the DNA/polycation complexes currently as very promising systems in genetic engineering and gene therapy [6–8]. Although their transfection efficiency remains relatively low up to now in comparison with viral-based vectors [9], the advantage of using synthetic polycations for self-assembly with DNA is based on an easy, cheap, safe and reproducible synthesis of such materials [10–12]. It seems evident that accumulation of data on structure and physicochemical

*Corresponding author. Tel.: +86-10-6275-7954; fax: +86-10-6275-1708.

E-mail address: yli@chem.pku.edu.cn (Y. Li).

0301-4622/04/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2003.09.009

characteristics of DNA complexes with polycations, and correlation of these data with transfection efficiency give us a way to reveal the algorithms for choosing among the great varieties of cationic polymers, those of them possessing the highest DNA-delivering potential, and purposeful synthesis of such cationic polymer. For a better understanding, the study of the interaction of such polycations with the genomic species DNA is much needed.

Poly(allylamine hydrochloride) (PAA) is a linear cationic polyelectrolyte with primary amine groups on the chain. Until now, the investigation on the interaction between DNA and PAA is just a focus on the formation of multilayer assemblies between DNA and PAA through layer-by-layer technique [13,14]. In our previous work, we found that the interaction between DNA and PAA can greatly enhance the light scattering intensity of PAA itself, which has been developed to determine micro-nucleic acids [15]. In this study, we examine in detail the interaction of PAA with DNA using IR-, CD, UV- and fluorescence spectroscopy, which is helpful to clarify the mechanism of the polycation effects on the structure and physical properties of DNA. EB, a phenanthridine fluorescence dye, binds to DNA through intercalation [16]. The interaction of EB with DNA is associated with a red-shift of ν_{max}, induced circular dichroism and enhancement of its fluorescence intensity. Here, the spectroscopic changes of EB on its binding to DNA are utilized to study the interaction between DNA and PAA. The results show that PAA interacts with DNA through both the phosphate groups and the nitrogenous bases of DNA forming a hydrophobic complex, thus changing the conformation of DNA from B- to C-form, so the binding state of EB and DNA is changed. At the same time, the effect of pH and ionic strength on the formation of DNA/PAA complex is investigated through fluorescence analysis.

2. Experimental

2.1. Chemicals

Stock solution of DNA was prepared at 0–4 °C by dissolving commercially purchased herring sperm DNA (hsDNA, Beijing Jingke Reagent Company, Beijing, China) in doubly deionized water. The concentration of DNA solution was determined from UV absorption at 260 nm using a molar extinction coefficient, 6600 $M^{-1}\,cm^{-1}$.

A stock solution of polymer PAA (average MW 15 000, Aldrich Chem. Co.) was prepared by dissolving PAA in doubly deionized water. Its working solution was 1.0×10^{-2} M. If there is no specific explanation, the concentration of PAA means that of amine group. Ethidium bromide (EB, Beijing Huamei Reagent Company, Beijing, China) was used as received.

Tris–HCl buffer solution was used to control the acidity of the reaction systems. All reagents were of analytical reagent grade without further purification, and doubly deionized water was used throughout.

2.2. Spectral measurements

The formation of DNA/PAA complex was investigated by measuring changes in light scattering intensity using a F-4500 fluorescence spectroscopy (Hitachi). DNA (40 μM) in Tris–HCl (10 mM, pH 7.0) was incubated in a cuvette and the intensity of light scattering ($\lambda_{ex}=\lambda_{em}=600$ nm) was set to zero. PAA was added at 3 μM increments and the changes in scattering intensity monitored.

Microscopic FTIR-spectra of DNA complex with PAA, as well as the spectra of DNA and PAA were taken on Nicolet MAGNA-IR 750 (Nicolet) at room temperature. Solid hsDNA and PAA were used directly to obtain microscopic FTIR spectrum, while the samples of DNA/PAA complex were prepared as thin films by volatilizing the appropriate concentrated solutions on glass slides. Then we obtained the micro-solid powder of these samples from these films for the microscopic FTIR analysis.

CD-spectra were recorded with a 1-cm path length cuvette using JOBIN YVON-SPEX CD 6 (France Groupe Instruments S.A.) at ambient temperature and subtracted from the spectrum of buffer alone. Each measurement was the average of two repeated scans and the measured CD signals were converted to $\Delta\varepsilon$.

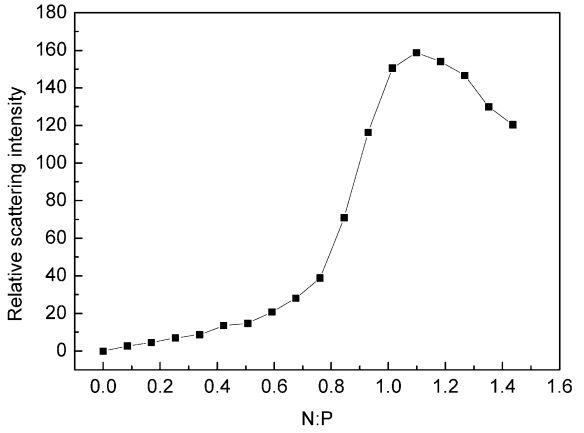

Fig. 1. Effect of the N:P ratio on the light scattering intensity of hsDNA–PAA system in pH 7.0, 10 mM Tris–HCl buffer; hsDNA, 40.0 μM.

The absorption spectra were recorded on a Hewlett Packard 8453 spectrophotometer (Agilent). The fluorescence intensities were measured using F-4500 fluorescence spectrophotometer (Hitachi) with a quartz cuvette (1×1 cm^2). A certain volume of PAA solution (total concentration of amine groups was 0.001 M) was added step by step into the solution of the complex (DNA–EB). The fluorescence intensity was measured at 595 nm with excitation wavelength at 535 nm. Then we observed the changes of the fluorescence intensity.

3. Results and discussion

3.1. The formation of DNA/PAA complex measured by light scattering

The formation of the DNA/PAA complex was investigated by measuring changes in light scattering intensity using a fluorometer. As we can see in Fig. 1, with the addition of PAA, the intensity of scattering light increases up to N:P (molar ratio of polymer amino groups to DNA phosphates) 1.0, with particularly steep rise between N:P 0.8 and 1.0. Above N:P 1.0 there was a decrease of the light scattering intensity. These data suggest few changes in DNA morphology at low N:P values, with self-assembly of discrete nanoparticles occurring between N:P 0.8 and 1.0 [17]. Through our previous work by dynamic light scattering [15], we prove that large particle is formed between DNA and PAA. The average hydrodynamic radius for the complexes formed is 39.4 ± 0.4 nm when the N:P is 1.0, which is much larger than that of either PAA or DNA.

3.2. Microscopic FTIR-spectra of DNA/PAA complex

Fig. 2 demonstrates microscopic FTIR-spectra of hsDNA, PAA and hsDNA complexes with PAA at the N:P ratio of 1.0. The main absorption bands of hsDNA are at: 1063 cm^{-1}, corresponding to the symmetric stretching vibration of the phosphate groups; 1222 cm^{-1}, the antisymmetric stretching vibration of the phosphate group; 1017 cm^{-1}, the vibration of C=N of ribose; 1693 cm^{-1}, the vibrations of C_6=O of guanine and C_4=O of thymine [18]. PAA itself has no absorption in the above absorption bands. As we can see, PAA induces the dramatic changes in the DNA spectrum. Changes are apparent in the shift of the characteristic absorption bands of the phosphate groups and bases (the absorption bands at 1063, 1222, 1693 cm^{-1} shift to 1064, 1225, 1656 cm^{-1}, separately), which are evidences of PAA interaction with both the phosphate groups and the nitrogenous bases of DNA. Moreover, the interac-

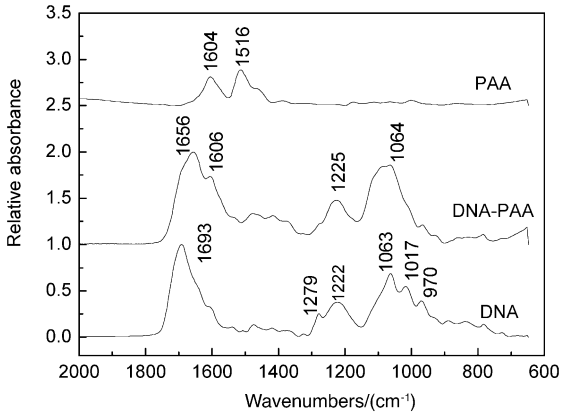

Fig. 2. Microscopic FTIR-spectra of hsDNA (bottom), PAA (top) and a mixture of hsDNA and PAA (middle), N:P=1.0.

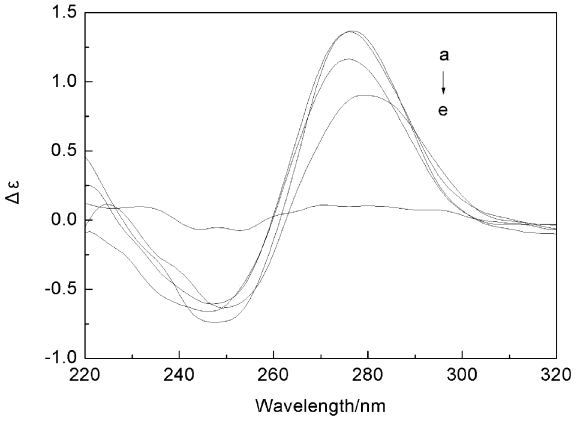

Fig. 3. CD-spectra of 80.0 μM hsDNA with different concentration of PAA, from (a) to (e), N:P ratios are: 0; 0.25; 0.50; 0.75 and 1.0 in pH 7.0, 10 mM Tris–HCl buffer solution.

tion of PAA with DNA alters the B-conformation of DNA, which can be seen from the disappearance of B-form marker bands at 1017 cm^{-1}.

3.3. Circular dichroism study of DNA/PAA complex

The CD-spectra in the UV range can be used to monitor the conformational transition of DNA [19–21]. In the absence of PAA, the CD spectrum of hsDNA was of typical B-form, which exhibited a positive Cotton effect at 276 nm and a negative Cotton effect at 248 nm as shown in Fig. 3a. The spectra of hsDNA change with the addition of PAA to the DNA solution. As we can see, at the lower N:P ratio such as 0.25, the addition of PAA has little effect on the positive peak, but reduces the negative peak. When the N:P ratio reaches 0.5, the positive peak decreases in height without the shift of the peak position. With further increasing of N:P ratio, the height of the positive peak decreases further, accompanied with a red shift of both the positive and the negative peaks, indicating a transition of the secondary DNA structure from B- to C-form [22,23]. Change in the intensity of the CD peak at 278 nm is associated with alteration of hydration of the helix in the vicinity of phosphate or ionic concentrations of the ribose ring [23,24]. It would be reasonable to suggest that replacement of sodium ion by PAA monomer would lead to a change in hydration near the phosphate group of the DNA helix since the alkyl chain of PAA is quite hydrophobic. It was found that at and above 1:1 N:P ratios the solution turned turbid and was thereby not amenable for spectral acquisition, which may be due to the aggregation of a large number of hydrophobic DNA/PAA complex. The CD experiment proves that the conformational transition of DNA is happened at the higher N:P ratio near to 1.0, which is consistent with the conclusion obtained by light scattering.

3.4. Absorption spectroscopy of EB–DNA–PAA

The absorption spectrum (Fig. 4) of 30.0 μM EB at pH 7.0 in 0.01 M Tris–HCl buffer shows a single monomeric peak at 480 nm, indicating the weak aggregating tendency of the dye [25]. Upon adding of 120 μM DNA to the solution of EB, spectrum (a) changes to (b) with the absorption peak red-shifted from 480 to 500 nm and the decreased absorbance. With increasing PAA concentration, spectrum (b) changes from (c) to (f) with progressive reversion of the ν_{max} to shorter wavelength and the increase of the absorption. This gradual reversion of the ν_{max} indicates that the EB interaction with DNA is weakened by

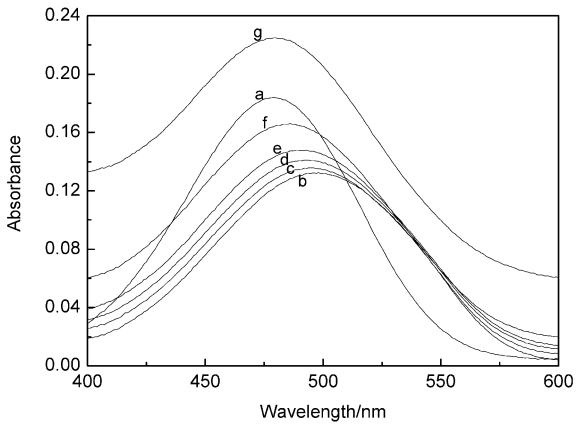

Fig. 4. Absorption spectra of 30.0 μM EB in pH 7.0 Tris–HCl buffer (a); in the presence of 120.0 μM hsDNA (b); (c)–(g) are of the solution (b) in the presence of increasing PAA 15, 30, 45, 60, 90 and 120 μM, respectively.

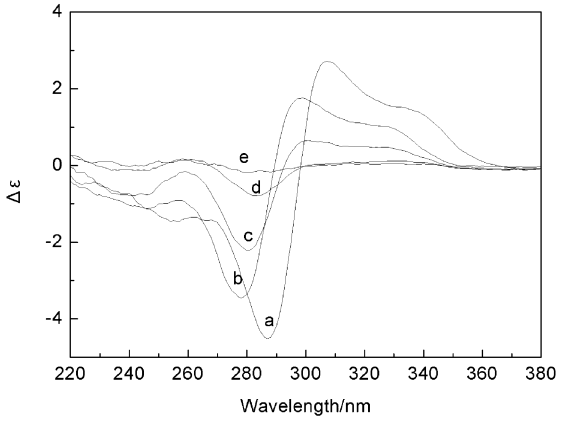

Fig. 5. Induced CD-spectra of EB–hsDNA (EB 20.0 μM, EB:hsDNA = 1:4) on the addition of PAA from (a) to (e), N:P ratios are: 0; 0.25; 0.50; 0.75 and 1.0 in pH 7.0, 10 mM Tris–HCl buffer solution.

PAA. As shown in Fig. 4, when the N:P ratio is 1.0, the baseline of spectrum increases greatly, which is also due to the formation of hydrophobic DNA/PAA complex.

3.5. Induced circular dichroism of EB–DNA–PAA

It is considered that EB itself does not display CD signal, but the CD signal can be induced based on the interaction of EB with DNA. Fig. 5 shows that the CD spectrum of EB–hsDNA exhibits a strong positive band at 307 nm and a negative band at 287 nm (Fig. 5a) in pH 7.0 Tris–HCl buffer. The maximum band at 307 nm is the characteristic one for intercalating agents [26]. However, with the addition of PAA to the solution, the induced CD spectrum of EB changes greatly (Fig. 5b–e), indicating that the environment of EB is changed due to PAA interaction with hs-DNA. When the N:P ratio of PAA to DNA is 0.25, both the positive and the negative bands shift to shorter wavelength (to 299 and 278 nm, respectively) with the sharp decrease of induced dichroism. But when the N:P ratio of PAA to DNA is increased to 0.5, both the positive and the negative bands show 2 nm bathochromic shift (to 301 and 280 nm) with the further decrease of $\Delta\varepsilon$. The positive band disappears when the N:P ratio reaches 0.75. And when the N:P ratio is 1.0, the induced CD spectrum of EB disappeared and almost no CD signal was found. Compared with the CD-spectra of DNA in the presence of PAA (Fig. 3) at the same N:P ratio, the change of the spectra in Fig. 5 is more apparent in the presence of EB. The spectral change in Fig. 5 is probably due to several factors, such as the change of the amount of DNA intercalated with EB and the conformational change of DNA caused by PAA.

3.6. Fluorescence studying of the formation of DNA/PAA complex by EB

The effect of PAA on the fluorescence of EB–DNA is represented in Fig. 6. Curve (a) shows the change in fluorescence of 40.0 μM DNA and 10.0 μM EB with added PAA in increasing concentration in pH 7.0 Tris–HCl buffer. The fluorescence intensity of the system decreases progressively with increasing PAA, but the slope drastically changes when the concentration of PAA reaches 40 μM; at this concentration the fluorescence intensity drops to 48% of its initial value. Further increase of the concentration of PAA up to 100 μM changes the fluorescence intensity sluggishly. This significant quenching of the fluorescence also indicates that the formation of the DNA/PAA complex is not in favor of the interaction between DNA and EB.

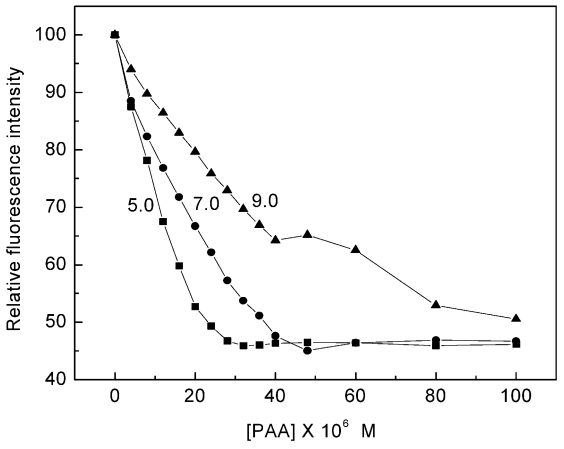

Fig. 6. Effect of added PAA on the fluorescence intensity of EB–DNA containing 10.0 μM EB and 40.0 μM DNA in three different pH conditions.

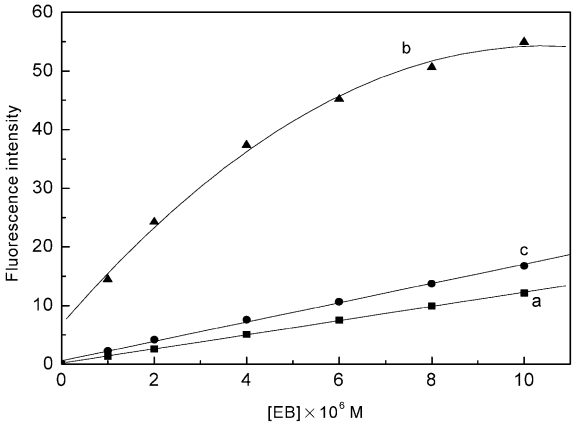

Fig. 7. The fluorescence intensity of EB alone vs. its concentration in 10 mM Tris–HCl, pH 7.0 (a); in the presence of 30.0 μM DNA (b); in the presence of both 30.0 μM DNA and 30.0 μM PAA (c).

The effect of PAA on the fluorescence intensity of EB in the presence of DNA has also been followed by adding increasing amounts of EB to a preformed DNA/PAA complex containing 30.0 μM DNA and 100.0 μM PAA. The result is represented in Fig. 7c and the fluorescence of an aqueous solution of EB itself of comparable concentration and that in the presence of 30.0 μM DNA alone are also shown in Fig. 7a and b. Evidently, the fluorescence in (c) is higher than that in (a) but is lower than that in (b), indicating that EB can still bind to DNA in the presence of a large amount of PAA and enhance its fluorescence intensity, but the fluorescence quantum yield is much lower than that in the presence of DNA alone. IR- and CD-spectra prove that the formation of the complex between DNA and PAA changes the conformation of secondary structure of DNA from B- to C-form. Compared with B-DNA (right-handed, 1 turn per 10.4 residues, rise per bp is 3.3–3.4 Å [19]), C-DNA consists of a non-integral helix with approximately 9.33 residues per turn in rise per bp 3.31 Å [27]. Maybe it is the minor change of the conformation of DNA that is not in favor of the stacking interaction between DNA base pair and planar EB molecule, which leads to the decrease of the fluorescence of EB in the presence of PAA.

The effect of PAA on the fluorescence of EB–DNA complex at the different pH medium is represented by curve (b) and (c) of Fig. 6. As we can see, in different pH medium, the ability of PAA to quench the fluorescence intensity of EB–DNA is different, which means the formation of the complex between DNA and PAA is pH-dependent. At pH 5.0, PAA can sharply decrease the fluorescence intensity of the system. When the concentration of PAA reaches 28 μM, the fluorescence drops to 47% of its initial value. The further increase of the concentration of PAA changes the fluorescence intensity little, indicating that at this concentration of PAA the binding of PAA to EB–DNA complex reaches the equilibrium. In neutral medium, although the quenching efficiency is still apparent, the slope of the curve is flatter than that in acidic medium. The concentration of PAA required for reaching the equilibrium system is 40 μM; at this concentration of PAA, the fluorescence intensity also drops to approximately 48% of its initial value. In alkaline medium, the efficiency of the quenching of PAA is the minimal. As we can see, even in the concentration of 100 μM PAA, the fluorescence intensity of the system still has 51% of its initial value. The interaction between DNA and PAA is mainly through the positively charged amine groups of PAA with the negatively charged phosphate of DNA. The total charge of the double helix of DNA under the pH region between 4 and 10 remains virtually unchanged since in this pH region the DNA secondary structure is not modified [28]. Therefore, pH dependence of the formation of the DNA/PAA complex is mediated by the charges carried on PAA. Evidently, the protonation of PAA will be in favor of the interaction between PAA and DNA leading to more efficient quenching of the fluorescence of EB.

3.7. The effect of PAA on the binding of EB to DNA

The influence of PAA on the binding of EB to DNA has been studied. The binding constant (K) and the number of EB molecules intercalated to DNA (n) in the presence of PAA were calculated using Scatchard analysis [29,30]. The titration

curves of fluorescence intensity of a series of DNA/PAA complexes (N:P ratio is 0–1.67) were obtained by the addition of increasing concentration of EB in 0.01 M Tris–HCl buffer of pH 7.0. The fluorescence Scatchard plots were obtained for binding of EB to DNA in the absence (○) and in the presence (●) of various concentrations of PAA and are shown in Fig. 8. The values of *K* and *n* are listed in Table 1. As shown in Fig. 8 and Table 1, when N:P ratio is lower than 1.00, both the slope i.e. *K* and the intercept on the abscissa i.e. *n* (number of binding sites per nucleotide) decrease with increase in the concentrations of PAA, indicating noncompetitive inhibition of EB binding [28,31] in the presence of PAA. In fact, PAA progressively decreases the number of EB virtual sites on DNA, while the binding constants change little until N:P ratio = 0.67, meaning PAA cannot influence the binding constant at the lower N:P ratio. At higher ratios between N:P 0.67 and 1.0, the number of binding sites decreases further with the sharp decrease of the binding constant of EB to DNA. The decrease of the binding constant may be due to the change in DNA morphology when N:P ratio is near to 1.0, which is consistent with the conclusion obtained from the above experiments such as light scattering and CD-spectra. Further increase of PAA shows less influence on both the *K* and *n* values, meaning that at high PAA concentration there is still some binding which is rather independent of changes in the PAA concentration.

Table 1
Binding parameters for the effect of PAA on the fluorescence of EB in the presence of herring sperm DNA

Compound	N:P	$K\times10^{-5}$ (M^{-1})	*n*
PAA	0.00	10.08	0.076
	0.33	8.31	0.048
	0.67	8.11	0.025
	1.00	1.91	0.01
	1.67	1.24	0.008

3.8. Effect of the ionic strength on the stability of DNA/PAA complex

Fig. 9 represents the effect of ionic strength on the fluorescence quenching of EB–DNA caused by PAA. The relative fluorescence intensity is increasing while the concentration of NaCl increases in solution, indicating the dissociation of DNA/

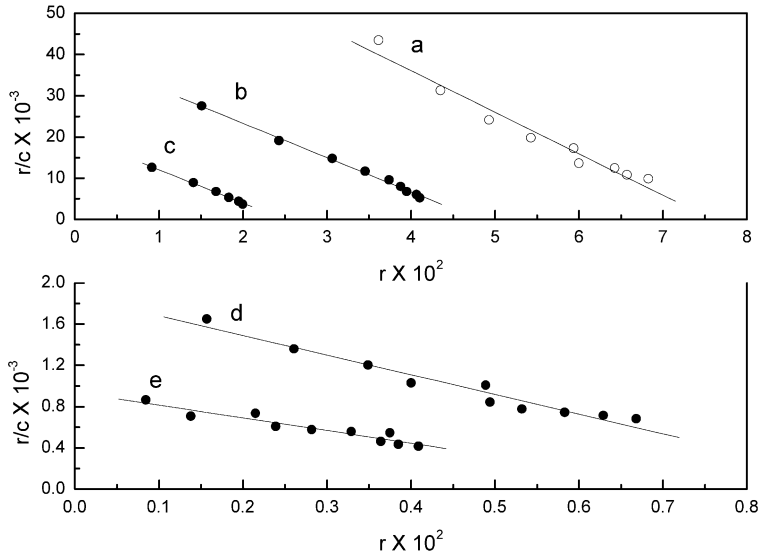

Fig. 8. Fluorescence Scatchard plots for the binding of EB (1.0–10.0 μM) to DNA (30.0 μM) in the absence (○) and the presence (●) of increasing concentration of PAA. N:P ratio increases in the order of 0.0 (a), 0.33 (b), 0.67 (c), 1.0 (d), 1.67 (e).

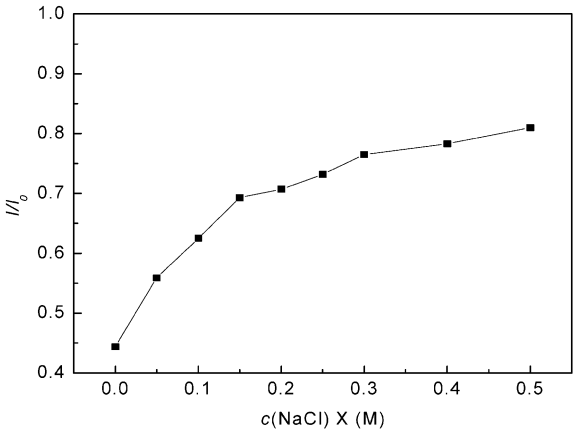

Fig. 9. Relative fluorescence intensity I/I_0 (I, the fluorescence intensity of EB–DNA–PAA solution; I_0, the fluorescence intensity of EB–DNA solution) on NaCl concentration in pH 7.0, 10 mM Tris–HCl buffer. EB, 10.0 μM; DNA, 40.0 μM; PAA, 40.0 μM.

PAA complex. Since the DNA/PAA complex is held together by strong cooperative electrostatic forces, due to increasing NaCl concentration (ionic strength) in the medium, the charges on DNA/PAA complex are screened, cooperative binding becomes weaker and dissociation of the complex occurs [7], which leads to the increase of the fluorescence intensity.

4. Conclusion

Present study shows that the complex between DNA and PAA is easily obtained when DNA is mixed with PAA, which might be useful for DNA transfection. On the basis of IR-spectra, we prove that PAA interacts with DNA through both the bases and the phosphate groups of DNA and the formation of PAA/DNA complex causes the change of the conformation of the DNA secondary structure to a certain extent at the N:P ratio near to 1.0, which is also proved by light scattering and CD-spectra. The formation of the DNA/PAA complex and their stability are influenced by pH and ionic strength, which is proved by the fluorescence quenching technique using fluorescence cationic dye EB as a probe.

Acknowledgments

This work is supported by the National Nature Science Foundation of China (Project No. 20275002). And we acknowledge the State Key Laboratory for Structural Chemistry of Unstable and Stable Species for the experiment of CD.

References

[1] I. Moret, J.E. Peris, V.M. Guillem, et al., Stability of PEI–DNA and DOTAP–DNA complexes: effect of alkaline pH, heparin and serum, J. Control. Release 76 (2001) 169–181.
[2] V.A. Izumrudov, M.V. Zhiryakova, Stability of DNA-containing interpolyelectrolyte complexes in water–salt solutions, Macromol. Chem. Phys. 200 (1999) 2533–2540.
[3] I.R.C. Hill, M.C. Garnett, F. Bignotti, S.S. Davis, Determination of protection from serum nuclease activity by DNA–polyelectrolyte complexes using an electrophoretic method, Anal. Biochem. 291 (2001) 62–68.
[4] K.W. Leong, H.Q. Mao, V.L. Truong-Le, K. Roy, S.M. Walsh, J.T. August, DNA-polycation nanospheres as non-viral gene delivery vehicles, J. Control. Release 53 (1998) 183–193.
[5] R. Kircheis, L. Wightman, E. Wagner, Design and gene delivery activity of modified polyethylenimines, Adv. Drug Delivery Rev. 53 (2001) 341–358.
[6] A.V. Kabanov, V.A. Kabanov, DNA complexes with polycations for the delivery of genetic material into cells, Bioconj. Chem. 6 (1995) 7–20.
[7] T. Reschel, C. Konak, D. Oupicky, L.W. Seymour, K. Ulbrich, Physical properties and in vitro transfection efficiency of gene delivery vectors based on the complexes of DNA with synthetic polycations, J. Control. Release 81 (2002) 201–217.
[8] D. Oupicky, C. Konak, K. Ulbrich, M.A. Wolfert, L.W. Seymour, DNA delivery systems based on complexes of DNA with synthetic polycations and their copolymers, J. Control. Release 65 (2000) 149–171.
[9] W.F. Anderson, Human gene therapy, Nature 392 (1998) 25–30.
[10] C.L. Gebhart, A.V. Kabanov, Perspectives on polymeric gene delivery, J. Bioact. Compat. Polym. 18 (2003) 147–166.
[11] M. Matsuura, Y. Yamazaki, M. Sugiyama, et al., Polycation liposome-mediated gene transfer in vivo, BBA-Biomembranes 1612 (2003) 136–143.
[12] C.H. Lee, Y.H. Ni, C.C. Chen, C.K. Chou, F.H. Chang, Synergistic effect of polyethylenimine and cationic liposomes in nucleic acid delivery to human cancer cells, BBA-Biomembranes 1611 (2003) 55–62.
[13] F. Caruso, E. Rodda, D.F. Furlong, K. Niikura, Y. Okahata, Quartz crystal microbalance study of DNA

immobilization and hybridization for nucleic acid sensor development, Anal. Chem. 69 (1997) 2043–2049.
[14] X.D. Chen, J. Lang, M.H. Liu, Layer-by-layer assembly of DNA-dye complex films, Thin Solid Films 409 (2002) 227–232.
[15] Y.L. Zhou, Y.Z. Li, Determination of nucleic acid based on light scattering of a complex formed with poly(allylamine hydrochloride), Anal. Lett. 36 (2003) 2831–2843.
[16] J.-B. Lepecq, C. Paoletti, A fluorescent complex between ethidium bromide and nucleic acids—physical–chemical characterization, J. Mol. Biol. 27 (1967) 87–106.
[17] A.L. Parker, D. Oupicky, P.R. Dash, L.W. Seymour, Methodologies for monitoring nanoparticle formation by self-assembly of DNA with poly(L-lysine), Anal. Biochem. 302 (2002) 75–80.
[18] E.V. Hackl, S.V. Kornilova, L.E. Kapinos, et al., Study of Ca^{2+}, Mn^{2+} and Cu^{2+} binding to DNA in solution by means of IR spectroscopy, J. Mol. Struct. 408/409 (1997) 229–232.
[19] M.J.B. Tunis-Schneider, M.F. Maestre, Circular dichroism spectra of oriented and unoriented deoxyribonucleic acid films—a preliminary study, J. Mol. Biol. 52 (1970) 521–541.
[20] V.I. Ivanov, L.E. Minchenkova, A.K. Schyolkina, A.I. Poletayev, Different conformations of double-stranded nucleic acid in solution as revealed by circular dichroism, Biopolymers 12 (1973) 89–110.
[21] M.J. Carvlin, N. Datta-Gupta, R.J. Fiel, Circular dichroism spectroscopy of cationic porphyrin bound to DNA, Biochem. Biophys. Res. Commun. 108 (1982) 66–73.
[22] J.Y. Cherng, N.M.E. Schuurmans-Nieuwenbroek, W. Jiskoot, et al., Effect of DNA topology on the transfection efficiency of poly((2-dimethylamino)ethyl methacrylate)–plasmid complexes, J. Control. Release 60 (1999) 343–353.
[23] Z. Zhang, W. Huang, J. Tang, E. Wang, S. Dong, Conformational transition of DNA induced by cationic lipid vesicle in acidic solution: spectroscopy investigation, Biophys. Chem. 97 (2002) 7–16.
[24] Z.X. Wang, D.J. Liu, S.J. Dong, Voltammetric and spectroscopic studies on methyl green and cationic lipid bound to calf thymus DNA, Biophys. Chem. 87 (2000) 179–184.
[25] M.K. Pal, J.K. Ghosh, Spectroscopic probe of the competitive binding of ethidium bromide and neomycin to DNA, Spectrochim. Acta, Part A 51 (1995) 489–498.
[26] D.M. Gray, R.L. Ratliff, M.R. Vaughan, Circular dichroism spectroscopy of DNA, Methods Enzymol. 211 (1992) 389–406.
[27] D.A. Marvin, M. Spencer, M.H.F. Wilkins, The molecular configuration of deoxyribonucleic acid III. X-ray diffraction study of the C form of the lithium salt, J. Mol. Biol. 3 (1961) 547–565.
[28] J.-B. LePecq, Methods of Biochemical Analysis, vol. 20, Interscience Publishers, New York, London, Sydney, Toronto, 1971, pp. 41–86.
[29] M. Howe-Grant, K.C. Wu, W.R. Bauer, S.J. Lippard, Binding of platinum and palladium metallointercalation reagents and antitumor drugs to closed and open DNAs, Biochemistry 15 (1976) 4339–4346.
[30] K.G. Strothkamp, R.E. Strothkamp, Fluorescence measurements of ethidium binding to DNA, J. Chem. Educ. 71 (1994) 77–79.
[31] Z.M. Wang, H.K. Lin, Z.F. Zhou, et al., Copper (II) complexes with *N*,*N*′-dialkyl-1,10-phenanthroline-2,9-dimethanamine: synthesis, characterization, DNA-binding thermodynamical and kinetic studies, Bioorg. Med. Chem. 9 (2001) 2849–2855.