

Poly(1-vinylimidazole) Prospects in Gene Delivery

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Electronic Supplementary Information

Abstract Polymeric amines are being studied intensively as components of systems for gene delivery in genetic engineering and gene therapy of genetic disorders, including cancer. Despite remarkable achievements in the field, polymeric amines, such as polyethyleneimine, show some disadvantages. Strong interaction between the amine-containing polymer and nucleic acid hampers the release of nucleic acid in the cell cytoplasm. Amine groups can interact with the cell membrane which results in cell death. These limitations of polymeric amines stimulated an investigation of new structures for gene delivery. Imidazole-containing polymers have attracted attention as lesser basic substances, while they are able to interact with polymeric acids. Further development of imidazole-based gene delivery agents requires knowledge about some fundamental aspects of interaction between nucleic acids, and polymeric imidazoles. In this work, we studied the complexation of poly(1-vinylimidazole) and oligomeric DNA. We found that the number of active sites capable of binding with negatively charged phosphate groups is comparable with the number of protonated imidazole units in the case of high molecular weight polymer. The increase in polymer charge by 1-bromopropane quaternizing 1%–5% imidazole units or by decreasing the pH to 6.5–7 considerably increased the ability of poly(1-vinylimidazole) to interact with oligonucleotides. The pH sensitivity of this interaction is interesting for cancer gene therapy because the tumours have a lowered intercellular pH (stable oligonucleotide complex) and a higher extracellular pH which can lead to complex dissociation. Minimal critical length for complexation of quaternized poly(1-vinylimidazole) and DNA is below eight units which corresponds to polymers with amine groups. Fluorescence-tagged poly(1-vinylimidazole) samples were obtained and their potential for monitoring the polymer and polymer-oligonucleotide complex internalization into living cells was demonstrated.

Keywords Polymeric amines; Oligonucleotides; Fluorescence; Poly(1-vinylimidazole); Gene delivery

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INTRODUCTION

Synthetic polyamines are promising molecules as nonviral vectors after polyethyleneimine (PEI) has found wide acceptance as an effective transfection reagent. Unfortunately, the fact that the effectiveness of PEI is coupled with unacceptable toxicity has necessitated the search for an optimal polymer vector. A potential candidate must form a stable polyion complex (polyplex) with nucleic acids at physiological pH *ca.* 7.4, transfer the cargo through the cell membrane, and disrupt the endosomal compartment to release DNA/RNA into cytosol (endosomal escape) due to a high buffering capacity of the polymer species at endosomal pH 5.0–5.5. In the case of polyamines, buffering capacity results from protonable amino nitrogen atoms that behave as so-called “proton sponge”.^[1] On the other hand, it is also worth considering that higher concentrations of cationic charge in polycationic macromolecules will lead to greater cytotoxicity.^[2]

Many systems have been explored to find potential alternatives to PEI. Weak cationic polymers are of interest because they may demonstrate quite sophisticated complexation resulting from a weak interaction with DNA. For instance, poly(ethylene oxide)-block-poly(4-vinylpyridine) and circular plasmid DNA C8592 produced narrow-disperse nanorings with a perimeter of micrometers, which are difficult to prepare by existing methods.^[3]

Imidazole-containing polymers show lesser basicity compared to polymers bearing aliphatic amine groups, while pyridinic nitrogen is active in the interactions with metal cations and in complexation with acids giving interpolymer complexes.^[4] Polymeric derivatives of 1-vinylimidazole (VI), 4-vinylimidazole (4VI), and histidine (His) have been explored as gene delivery agents (Schemes S1–S7 in electronic supplementary information, ESI).^[5–18] In the context of poly(1-vinylimidazole) (PVI), agarose gel electrophoresis experiments, reported in an earlier work,^[5] demonstrated that it did not form polyion complex with DNA (salmon testis deoxyribonucleic acid sodium salt) at physiological pH 7.4

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when the VI/phosphate molar ratios were ≤ 40 . Most of the DNA migrated into the positive pole of the gel. In case of a covalent bioconjugate of PVI and peptide Boc-Cys-Trp-OMe, the formation of polyplex with RNA (from yeast *Torula utilis*, MW = 5–8 kDa) was observed at pH 5.5.^[6] Since the product was amphiphilic, the molecules self-assembled into micelles that aggregated in water. Using the FITC-tagged bioconjugate (FITC-Peptide-PVI, where FITC-fluorescein isothiocyanate, Scheme S2 in ESI), it was shown that these micelles were able to enter the cytosol of Chinese hamster ovary cells and could serve as a bio-imaging probe.

It is noteworthy that poly(4-vinylimidazole) (P4VI) readily formed the P4VI/DNA (pDNA, pGL3-control) complex in phosphate-buffered saline at pH 6.7–7.0 for P4VI/DNA weight ratios greater than 1.^[7] P4VI demonstrated transfection activity *in vitro* as effectively as PEI, but at lower cytotoxicity. Transfection *in vivo* appeared to be at least as effective using P4VI as with PEI.^[8]

A simple modification of PVI with Zn^{2+} ions drastically improved its properties for gene delivery.^[9] The chelated PVI having VI/Zn ratio of 50 was able to complex almost all DNA (pGL3-control) at VI/phosphate ratio over 16 and pH 7.4 during agarose gel electrophoresis. The transfection of luciferase gene to HepG2 cells by the PVI-Zn/DNA complexes showed outstanding gene expression without significant cytotoxicity. A specific role of Zn^{2+} ions in transfection was reported earlier.^[10] The authors studied ternary Zn^{2+} /methylated PVI/pDNA complexes (Scheme S3 in ESI) and concluded that in the case of pDNA (pGL3-control) that contained a nuclear protein (NF- κB) binding sequence, Zn^{2+} ions activated NF- κB , thereby enhancing the nuclear translocation of pDNA.

Another way to enhance the stability of PVI/DNA polyplexes is the quaternization of imidazole nitrogens with alkyl halides.^[11–13] In particular, butylated PVI (PVI-Bu) sample was demonstrated to serve as anchor groups to retain DNA (from salmon testis).^[11] The increased hydrophobic/hydrophilic incline of these PVI-Bu/DNA complexes at endosomal pH enhanced membrane disruptive activity. The gene delivery efficiency of these PVI-alkyl/DNA complexes was found to be dependent on the length and density of the alkyl chains. Little gene expression was observed at any +/- ratio for the DNA complexes with PVI-Bu possessing a lower density (7 mol%) of butylated imidazole groups. In the case of PVI-Bu with a higher density (38 mol%) of butylated imidazole groups, the DNA complexes mediated remarkable gene expression even with a +/- ratio of 4. It is noteworthy that the DNA complexes with PVI_m-Bu possessing a middle density (23 mol%) of butylated imidazole groups showed little gene expression at the +/- ratio of 4, while at 12 their activity even slightly surpassed that of the 38 mol% sample. The effect of the alkyl chain length of PVI-R on gene expression was assessed for different R groups, namely methyl, ethyl, butyl, and octyl (density = 20 mol%). PVI-Me and PVI-Et mediated higher gene expression than PVI-Bu at lower +/- ratios did. Especially, the gene expression mediated by the PVI-Et/DNA complexes did not depend on the +/- ratio. PVI-Oc showed little gene expression because of very strong retention of DNA and significant

cytotoxicity.

Reaction of PVI with 2-bromoethylamine hydrobromide to form 3-(2-aminoethyl)imidazolium moieties was studied in an earlier work.^[5] The resulting PVI-NH₂ ($M_n \approx 8.1$ kDa) contained only 2.5 mol% of aminoethyl groups but it was proven to be sufficient to retain DNA (from salmon testis) as observed from the agarose gel retardation assay and exhibited membrane disruptive ability at endosomal pH.

The way of quaternization was improved in Ref. [15] where alkylation with various *t*-Boc-protected bromoalkylamines was used to not only impart a permanent charge onto the polymer species, but also incorporate primary amine groups followed by attaching folic acid moieties (Scheme S5 in ESI), with the degrees of quaternization as 15 mol% and 30 mol%. All the prepared imidazolium copolymers bound DNA at nitrogen/phosphorus (N/P) ratios of 4. Folic acid conjugation did not influence the DNA binding affinity of the copolymers, but played a dramatic role in gene delivery to HeLa cells *in vitro*. The non-functionalized imidazolium copolymers delivered gWiz-Luc inefficiently, while introduction of folic acid into the aminopropylated PAPVIM15-FA showed a 250-fold increase in luciferase expression.

In the case of PVI carboxymethylated with iodoacetic acid, the obtained polyampholyte (Scheme S4 in ESI) showed no significant interaction with DNA (salmon testis) during agarose gel retardation assay.^[14] Nevertheless, its complexation with PEI and DNA (gWiz-Luc plasmid encoding the firefly luciferase gene) gave a system that combined the gene expression efficiency of PEI/DNA binary complexes with increased cell viability.

A copolymer of VI and *N*-methylvinylamine (P(M245-V60)-Fh, Scheme S6 in ESI) containing 19% of VI units showed a very good transfection efficiency for HeLa cells with the use of various plasmids.^[17]

The utility of imidazole-containing polymers as an initial basis for construction of biocompatible gene delivery vehicles was also exemplified by non-vinyl polymers such as polyhistidine modified with gluconic acid (G-pHis) (Scheme S7 in ESI).^[18] The protein nature of the polymer promised good potential for biocompatibility, while gluconic grafts made the compound soluble in water at pH > 6.0. G-pHis was able to retard DNA (plasmid DNA pCMV-*b*-gal) completely at DNA/G-pHis ratio of 1:3 (*W:W*), indicating that this electrolyte complex was neutrally charged. A charge balance at this ratio indicated that only 17% of the histidine side chains were involved in complexation with DNA, leaving the remaining imidazole groups to act as a proton sponge.

This short literature analysis shows undoubted efficiency of imidazole groups in gene delivery constructions, but some issues should be clarified. The question of how imidazole-containing polymer interacts with nucleic acid at various pH and/or quaternization degrees remains unknown. A simple system with *ca.* 20-mer oligonucleotide is an adequate model compared to long chain DNA where the interactions are complicated with potential supramolecular effects. On the other hand, acid-base properties of PVI (the most well-known imidazole containing polymer) are not characterized properly as the reported titration data^[19] do not contain dependence of PVI protonation degree on pH. This work de-

scribes the interaction of DNA oligonucleotides with PVI depending on polymer molecular weight and charge. The charge was adjusted by changing pH or by PVI quaternization with 1-bromopropane or fluorescent alkylation agent.

EXPERIMENTAL

Reagents

Methanol, *n*-hexane, 1,4-dioxane, acetone, potassium hydroxide, dimethylformamide (DMF), 1-bromopropane, KH_2PO_4 , $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, $0.1 \text{ mol} \cdot \text{L}^{-1}$ HCl, KCl, trifluoroacetic acid, and azobisisobutyronitrile (AIBN) were of reagent grade (Sigma-Aldrich, Fisher, or Acros Chemicals). Deuterium oxide (D_2O , 99.8 atom D%) was purchased from Sigma-Aldrich. DMF was dried with anhydrous CuSO_4 and 3 Å molecular sieves, followed by distillation. AIBN was recrystallized from ethanol. Poly(1-vinylimidazole) (PVI) and its fractions were prepared according to the protocol reported earlier.^[20] The molecular weights (MWs) of the fractions were determined by viscosity measurement using parameters obtained from the literature.^[21] MW of the unfractionated PVI was 35 kDa, while PVI of other MWs were obtained from fractionated samples. PVI samples are abbreviated to PVI-XX, where XX indicates molecular weight (kDa); PVI-n means unfractionated sample. PEI ($M_w = 30000\text{--}40000$) was obtained from SERVA Fine Biochemica (Heidelberg).

FAM and ROX 3'-tagged DNA oligonucleotides were purchased from Evrogen JSC, Russia. The 21-mer FAM-DNA sequence was GATCTCATCAGGGTACTCCTT, 4-20-mer FAM-DNA was $(\text{TCAG})_n$, and 20-mer ROX-DNA sequence was TCAGTGCAGACTTGACCTAG. The yeast *Saccharomyces cerevisiae*, parent-type strain W303-1B (*MATa ade2-1 his3-11, 15 trp1-1 leu2-3112 ura3-1 [rho⁺]*) was procured from the collection of the Siberian Institute of Plant Physiology and Biochemistry (SIPPB SB RAS).

Equipment

^1H -NMR spectra were recorded on a DPX 400 Bruker instrument in D_2O at 400 MHz. Electrophoresis experiments were performed on a Mini-Sub (7 cm × 10 cm) Cell GT System (Bio-Rad Laboratories, Inc.) with an ELF-4 power supply (DNA-Technology LLC) and TCP-20LC transilluminator (Vilber Lourmat) operated at 254 nm. The interaction of oligonucleotides with polymers was estimated *via* size exclusion chromatography (SEC) using a Milichrom A02 chromatograph (JSC Econova) with 2 mm × 75 mm column filled with SRT SEC-100 5 μm phase (Sepax Technologies, Inc.). The apparatus was operated at 30 °C using $50 \text{ mmol} \cdot \text{L}^{-1}$ phosphate buffer with KCl $100 \text{ mmol} \cdot \text{L}^{-1}$ at

pH = 6.5, 7, and 7.4; the flow rate of the mobile phase was set at $0.03 \text{ mL} \cdot \text{min}^{-1}$ (pressure 0.32 MPa), whereas the injection volume of the sample solution was 2 μL. Zeta-potential (ζ) and hydrodynamic radius (R_h) were measured at 25 °C with a Zetasizer Nano-ZS ZEN3600 (Malvern Instruments Ltd., UK) device. A Motic AE-31T microscope equipped with a fluorescence attachment and Moticam Pro 205A camera was used for visual observation of the yeast cells.

Synthesis of Polymers

Quaternized poly(1-vinylimidazole)

Solutions of PVI and 1-bromopropane in DMF were mixed at required ratios to obtain the polymer concentration of $0.1 \text{ g} \cdot \text{mL}^{-1}$. After incubated in the dark for five days at room temperature, the polymer was precipitated and washed with acetone, followed by reprecipitation from a methanol solution with acetone:*n*-hexane = 9:1 (*V*:*V*) and drying in vacuum. Degree of quaternization (*Q*) was analyzed from ^1H -NMR data (Fig. 1) with the signals of methyl group (0.8–0.9 ppm) and the signals of methylene groups of the main chain and methylene group at methyl group (1.3–2.8 ppm). The detailed experimental data are presented in Table 1.

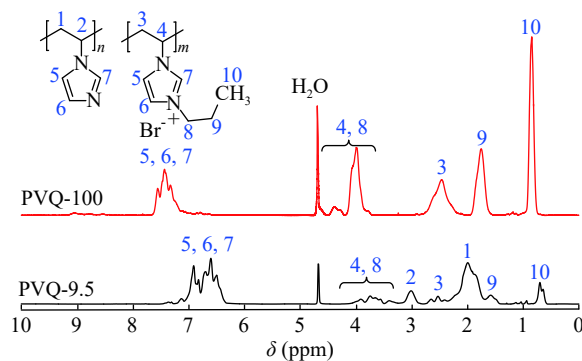


Fig. 1 ^1H -NMR spectra of PVQ-9.5 and PVQ-100 in D_2O

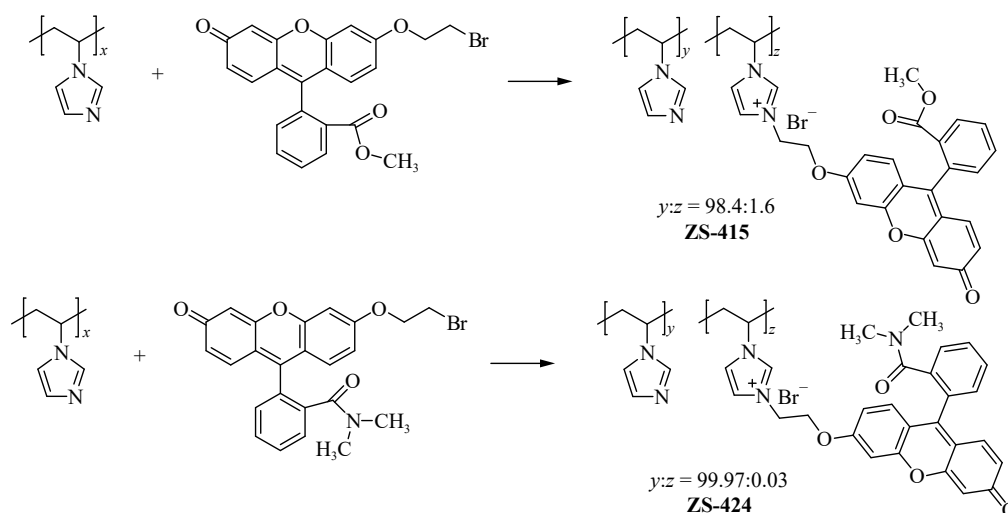
Fluorescent tagged poly(1-vinylimidazole)

Fluorescent moieties were introduced into PVI chain by the reaction with bromoalkyl derivative of fluorescein fluorophore (Scheme 1).

ZS-415. A solution of 6-oxy-(bromoethyl)-9-(2'-methoxycarbonyl) (9.38 mg, 0.0206 mmol) fluorescein^[22] in a mixture of 500 μL of 1,4-dioxane and 500 μL of DMF was added to a solution of PVI-21.6 (97.44 mg, 1.035 mmol) in 1500 μL of DMF. The mixture was kept at 60 °C for 20 h. The course of the reaction was followed with TLC on silica gel plates (acetone, $R_f = 0.6$ for the dye, $R_f = 0$ for the polymer). The polymer was precipitated with 30 mL of acetone, washed with acetone (15 mL × 5), reprecipitated from 1 mL

Table 1 Synthesis of quaternized PVI

Polymer	PVI (mg)	Br-Pr (mg)	DMF (mL)	<i>Q</i> (%) (from charging)	Yield (%)	<i>Q</i> (%) (NMR)
PVIQ-0.5	217.98	1.44	2.18	0.5	97.9	0.5
PVIQ-1	205.07	2.71	2.05	1.0	97.8	1.0
PVIQ-1.9	216.84	5.71	2.17	2.0	88.4	1.9
PVIQ-5.3	530.87	38.46	5.31	5.5	89.8	5.3
PVIQ-9.5	309.86	40.44	3.10	10.0	92.6	9.5
PVIQ-15	528.52	108.59	5.29	15.7	86.2	15.3
PVIQ-100	140.60	370.00	2.10	200.0	86.3	100.0



Scheme 1 Introducing fluorescent tags into PVI

of its DMF solution with acetone, and washed again with acetone (15 mL $\times$ 6). The resultant product was dried in vacuum with an oil pump for about 20 h at room temperature to yield 81 mg of bright yellow powder. Fluorescent tagging of the polymer was 1.6% from UV-Vis absorption data of which extinction coefficient (at 456 nm) of 6-oxy-(bromoethyl)-9-(2'-methoxycarbonyl) fluorescein in aqueous solution was taken into account.

ZS-424 was prepared according to the protocol described in an earlier report.^[23]

Potentiometry Titration of PVI and PEI

Potentiometry measurements were performed on a “Multi-test” ionometer using a combined pH-electrode in a temperature-controlled cell at (20 ± 0.02) °C. Solutions of $0.005 \text{ mol} \cdot \text{L}^{-1}$ were prepared for potentiometry titration. A solution of $0.1 \text{ mol} \cdot \text{L}^{-1}$ HCl was employed as a titrant. Protonation degree was calculated according to the method described earlier.^[24] Buffer capacity was calculated from the potentiometry data according to the International Union of Pure and Applied Chemistry (IUPAC) recommendations as a derivative of the added NaOH concentration versus pH (the number of moles of strong base required to change the pH by one unit when added to one liter of the solution).^[25]

Study of the Polymer Interaction with DNA Oligonucleotides

The interaction between DNA oligonucleotides and poly-

mers was investigated by electrophoresis on agarose gel. Complexes were prepared by mixing solutions of the polymer and oligonucleotide. The samples were incubated at room temperature for 30 min and placed into the wells of 1% agarose gel. Free oligonucleotide, as a control, was also loaded onto the gel. The gel running buffer was $40 \text{ mmol} \cdot \text{L}^{-1}$ Tris acetate (adjusted to pH 7.4) and $1 \text{ mmol} \cdot \text{L}^{-1}$ EDTA. A glycerol gel loading buffer was used (0.5% sodium dodecyl sulfate, $0.1 \text{ mol} \cdot \text{L}^{-1}$ EDTA (pH = 8), and 50% glycerol for 10 $\times$ reagent). The gel was run at 90 V for 20 min and the fluorophore-tagged oligonucleotide was visualized on a UV transilluminator.

Study of the Polymer-DNA Penetration into Model Yeast Cells

The cells of *S. cerevisiae* were maintained at 30 °C on YEPD medium (0.5% yeast extract, 1% peptone, 2% glucose). The cells were grown at 30 °C in 10 mL plastic vials with 2 mL of liquid YEPD. Cells in the logarithmic or stationary growth phase were used in the experiments. Penetration of the model DNA-polymer complex into the yeast cells was monitored by epifluorescent microscopy for 4 h after the addition of complex into the cell culture.

RESULTS AND DISCUSSION

Gel electrophoresis data (Fig. 2a) show the formation of PVI-n-DNA complex at N/P ratio 2000. Decrease of PVI-n

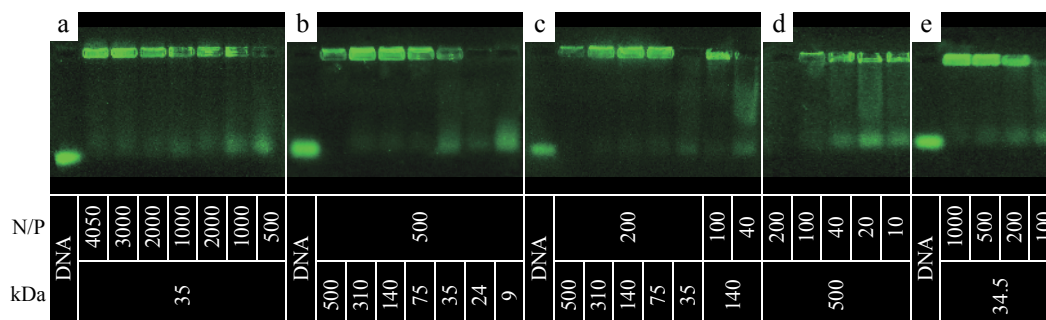


Fig. 2 Gel electrophoresis data for PVI complexes with 21-mer FAM-DNA at pH 7.4. N/P ratios are indicated in the first row and PVI molecular weight in the second row. DNA concentration was $5 \mu\text{mol} \cdot \text{L}^{-1}$ (calculating on DNA chains).

content to $N/P = 1000$ resulted in noticeable band of free DNA, and a considerable fraction of DNA was not complexed at $N/P = 500$. In the case of PVI-34.5 fraction (Fig. 2e), the complex existed at $N/P = 500$ and was partially complexed at $N/P = 200$. The 9 and 24 kDa PVI fractions did not form complexes at $N/P = 500$ (Fig. 2b). Our results revealed that the increment in PVI molecular weight gave rise to more stable complexes (Figs. 2b–2d). The 140 kDa polymer complexed completely with the DNA at $N/P = 100$ and the 500 kDa sample worked at $N/P = 20–40$.

PVI, in contrast to PEI and other polymers with aliphatic amine units, was considerably protonated at low pH values only. Potentiometry titration data (Fig. 3) show that PVI was 4% protonated at pH 7.4 and more than 20% protonation was observed at pH below 5.5. Based on gel electrophoresis and potentiometry data, the number of DNA chains per PVI chain in the complex and the ratio of protonated imidazole units to negative charges in DNA chains were calculated (Fig. 4). Despite the qualitative nature of the gel electrophoresis data, two inferences regarding the influence

of PVI chain length on the interaction with DNA could be arrived at. First, complexes based on short PVI chains contained considerably more than one PVI chain per DNA molecule and in the cases with polymerization degree over 1500 (140 kDa sample) there were more than one DNA chain coordinated with one PVI macromolecule. Secondly, complexation with long chain of PVI (310–500 kDa) proceeded at 1–2 ratio of protonated imidazole cycle to negative-charged DNA unit, whereas the decrease of PVI length resulted in excess of protonated VI units in the complex. We could therefore hypothesize that the interaction between negatively-charged DNA oligomer and PVI chain proceeded by a redistribution of protonated imidazole units along the polymer chain. This process was relatively easy in the case of long chains but was impossible for short chains, e.g. 35 kDa PVI contained 16 protonated units per polymeric chain which was lower than the DNA length. PVI units are inclined to association by hydrogen bonds between protonated and free VI units^[26] and these interactions are the probable reasons for aggregation of imidazole-containing poly-

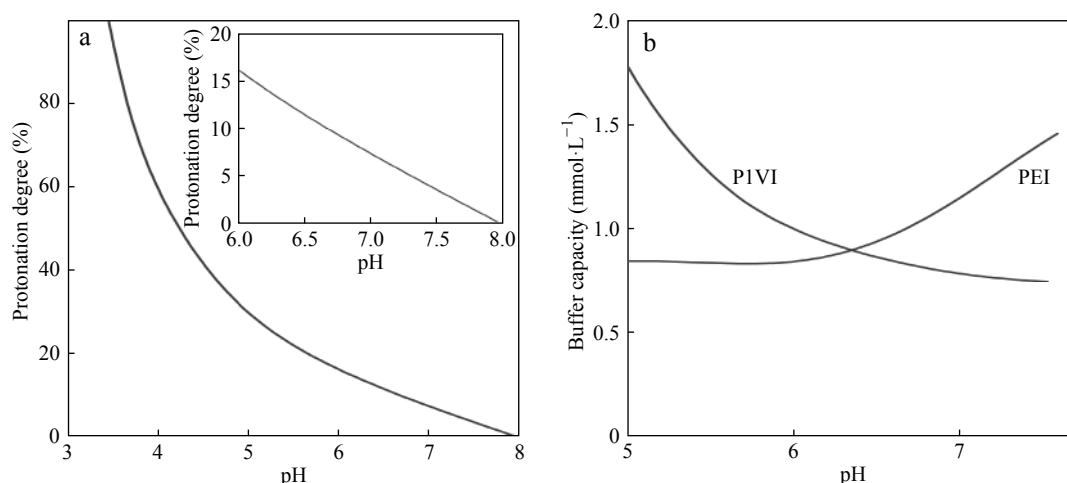


Fig. 3 Dependence of (a) protonation degree and (b) buffer capacity on pH. The data were obtained with PVI-n sample and results for other PVI sample did not show considerable difference.

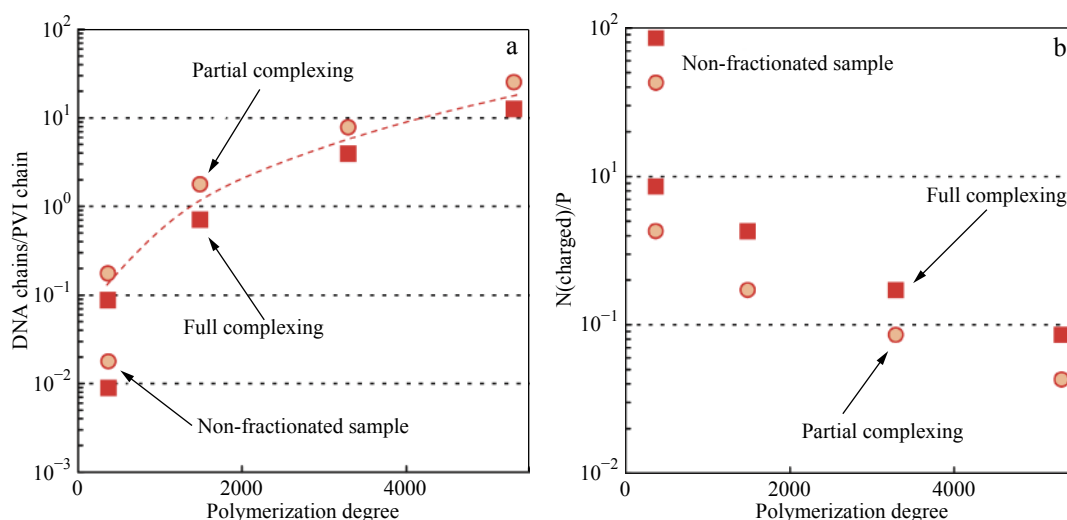


Fig. 4 Dependence of (a) number of DNA chains per PVI chain in the complex and (b) ratio of protonated imidazole units to negative charges in DNA chains on PVI polymerization degree at pH 7.4

mers.^[27] These aggregates could mimic a large “macromolecule” which interacted with DNA in the case of short PVI samples. A similar behavior of PVI was observed during its interaction with siliceous nanoparticles^[20] where the critical point was also near 100 kDa molecular weight. Compared with the fraction of the same average molecular weight, the lower complexing tendency of non-fractionated PVI sample was explainable with the presence of very short chains in the non-fractionated sample.

Quaternization of PVI increased its ability to interact with long-chain DNA at 18%–38% of modified units.^[11] We found (Fig. 5) that a small amount (0.5%–2%) of modified imidazole units could increase the PVI complexation with DNA oligonucleotides considerably. The complexation at N/P = 200 for the modified units was comparable with the results for unmodified PVI at N/P = 1000. PVI containing 5.3% of quaternized units was able to completely associate with DNA at N/P = 100 while the completely modified polymer (PV18-PQ15) exhibited total complexation at N/P = 10. The change of oligonucleotide length from 24 to 4 (Fig. 5e) showed full interaction for 8-mer DNA and absence of complexation for 4-mer DNA. This result agrees well with experiments on critical length of DNA to interact with polymeric amines.^[28] It is interesting that at the same N/P ratio, complexes with longer DNA showed larger spots in the direction of the negative electrode. The spot with 8-mer DNA was concentrated near the starting well. This indicated that short oligonucleotides interacted with PVI by “all-or-nothing” scheme,^[29–31] thus resulting in some PVI chains almost completely complexed.

In the case of normal cells, intracellular pH (pHi) and ex-

tracellular pH (pHe) remain mostly within 7.0–7.2 and 7.3–7.4, respectively.^[32] Cancer cells consume higher amounts of glucose than normal cells mainly through aerobic glycolysis, converting most of the incoming glucose to lactate even under normoxic conditions, which is referred to as the “Warburg effect”.^[33] This metabolic pattern results in an alkaline pHi (7.2–7.4) and lower pHe (6.5–7.1) in the cancer tissue.^[34] This fact inspired us to study the stability of PVI-DNA complexes at pH 6.5 and 7. The gel electrophoresis data (Fig. 6) show significant increase in complex stability. The 35 kDa non-fractionated polymer gave stable complex at N/P = 2000 (pH 7.4), 200 (pH 7), and 40 (pH 6.5). This result agreed with ionization degrees of 4.3%, 11.5%, and 16.2%, respectively, at the same pH values. Buffer capacity of PVI (Fig. 3b) was higher than the value of PEI in acidic zone, which could enhance the endosomal escape in imidazole-containing systems.

Recently, we have synthesized PVI tagged with fluorescein moiety (ZS-424, 0.03% of the tag) and in this work, we obtained ZS-415 sample which contained 1.6% of the modified units.^[23] The dye conjugation was achieved through quaternization of the azole cycle so that the tagged PVI samples contained the corresponding amount of positively charged units. With the use of ROX-DNA (red fluorescence) the polymer and DNA could be simultaneously observed. Gel electrophoresis data (Fig. 7) confirm the increase of complex stability at small content of positive charge on the PVI chain and incomplete complexation was observed at N/P = 1000, 200, and 40 for pure PVI, ZS-424, and ZS-415 samples, respectively.

Light scattering data (Fig. 8) show aggregation of PVI

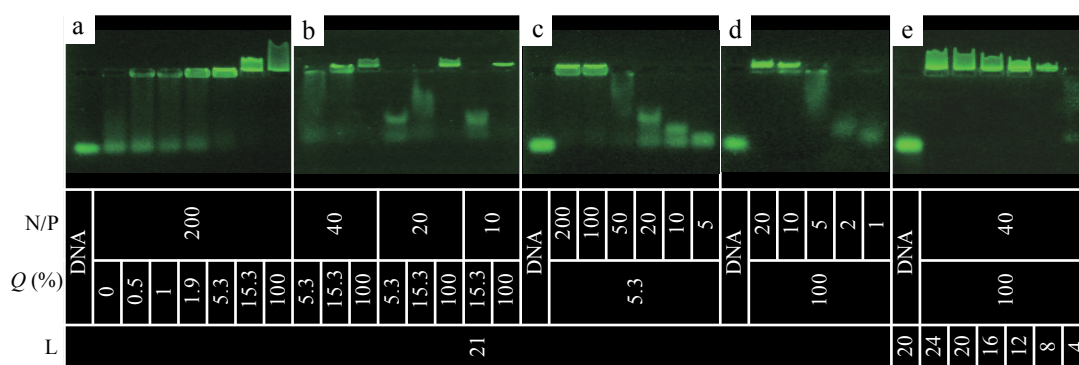


Fig. 5 Gel electrophoresis data for FAM-DNA complexes with PVI and quaternized PVI at pH 7.4. The bottom row represents the number of nucleotides in DNA, the top row represents N/P ratio, and quaternization degree is presented in the middle row. DNA concentration was $5 \mu\text{mol}\cdot\text{L}^{-1}$ (calculating on DNA chains).

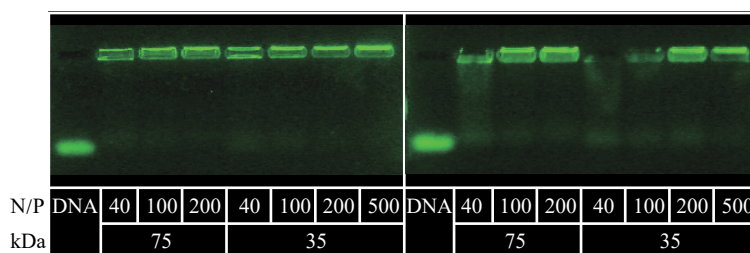


Fig. 6 Gel electrophoresis data for PVI complexes with 21-mer FAM-DNA at pH 6.5 (left) and 7.0 (right). N/P ratio is presented in the first row and PVI molecular weight in the second row. DNA concentration was $5 \mu\text{mol}\cdot\text{L}^{-1}$ (calculating on DNA chains).

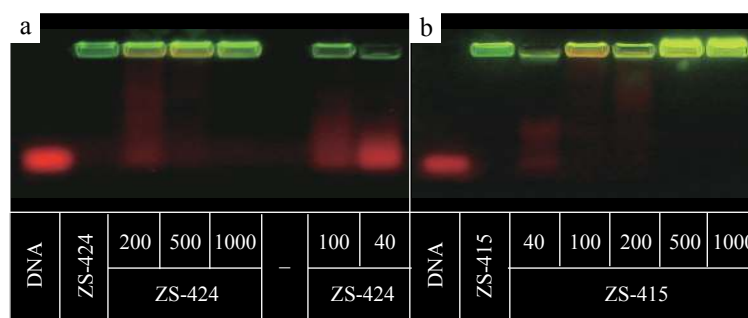


Fig. 7 Gel electrophoresis data for 20-mer ROX-DNA (red fluorescence) complexes with fluoPVI (green fluorescence) at pH 7.4. The first row represents N/P ratio. DNA concentration was $5 \mu\text{mol}\cdot\text{L}^{-1}$ (calculating on DNA chains). ZS-424 and ZS-415 had 0.03% and 1.6% of the modified units, respectively.

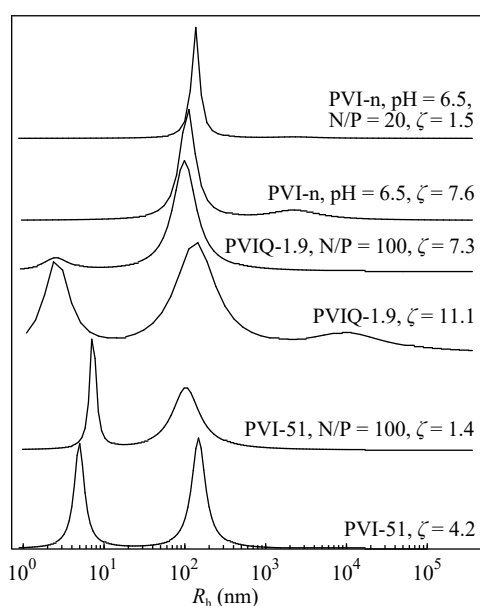


Fig. 8 Representative dynamic light scattering (DLS) data (intensity% versus R_h and ζ potential, mV) for the polymers and polyplexes with GATCTCATCAGGGTACTCCTT 21-mer DNA. The polymer concentration was $2 \text{ g}\cdot\text{L}^{-1}$, pH = 7.4 except two top lines.

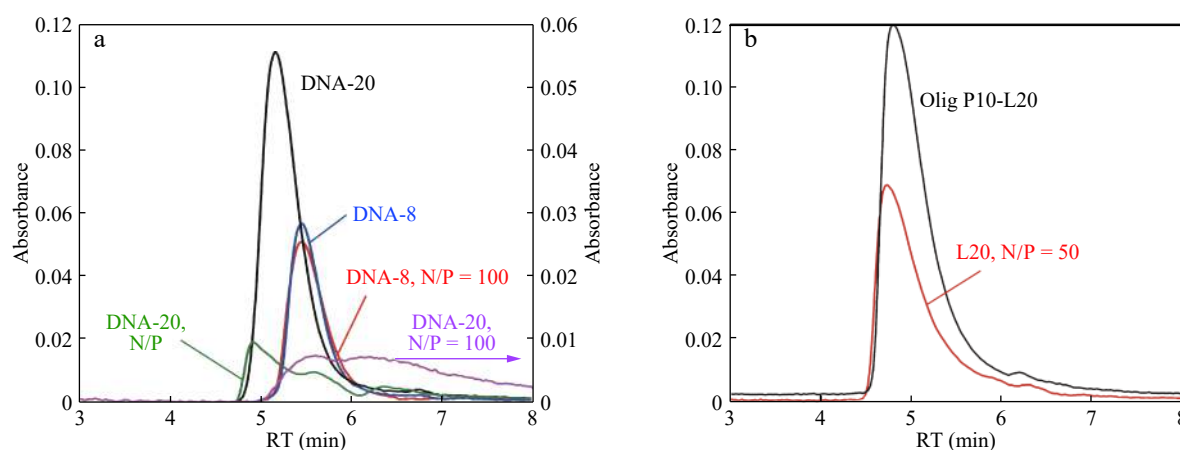


Fig. 9 SEC data for complexes of (a) PV18-PQ15 (100% quaternization) and (b) PVI (35 kDa) with oligonucleotides at different N/P ratios. Concentrations of oligonucleotides was $5 \mu\text{mol}\cdot\text{L}^{-1}$. Concentration of PV18-PQ15 (PVI): (a) $8.66 \mu\text{mol}\cdot\text{L}^{-1}$ (for L20, N/P = 100), $1.73 \mu\text{mol}\cdot\text{L}^{-1}$ (for L20, N/P = 20), $3.50 \mu\text{mol}\cdot\text{L}^{-1}$ (for L8, N/P = 100), (b) $9.99 \mu\text{mol}\cdot\text{L}^{-1}$. Detection at 260 nm. PVI and PV18-PQ15 do not have noticeable absorbance at 260 nm and according to detection at 220 nm, their chromatographic peak is placed at RT = 7 min.

macromolecules into 100–200 nm particles and coordination with DNA oligonucleotides did not influence considerably the particle size because of the large excess of PVI. Although ζ potential decreased in the presence of DNA confirming coordination, its value was still positive, which is favorable to penetration into cells.

Formation of complexes of PVI and quaternized PVI with DNA was confirmed with SEC data (Fig. 9). Changes on chromatograms are more pronounced in the case of quaternized PVI and free PVI shows a decrease by nearly half the intensity in the DNA band. This was in agreement with the hypothesis about the key role played by macromolecular aggregation in the formation of non-quaternized PVI-oligonucleotide complexes. Chromatographic separation induces destruction of the aggregates and thus decreases the complex stability.

Ability of the PVI-DNA complexes to penetrate into living cells was demonstrated with *S. cerevisiae* yeast cells (Fig. 10, Files a–d.avi in ESI). Bright yellow fluorescence was observed due to simultaneous emission from fluorescent tagged PVI and DNA molecules. Intensity of the fluorescence decreased during several seconds and became more reddish because of higher photostability of rhodamine dye (ROX tag) than that of the fluorescein moiety in ZS-415

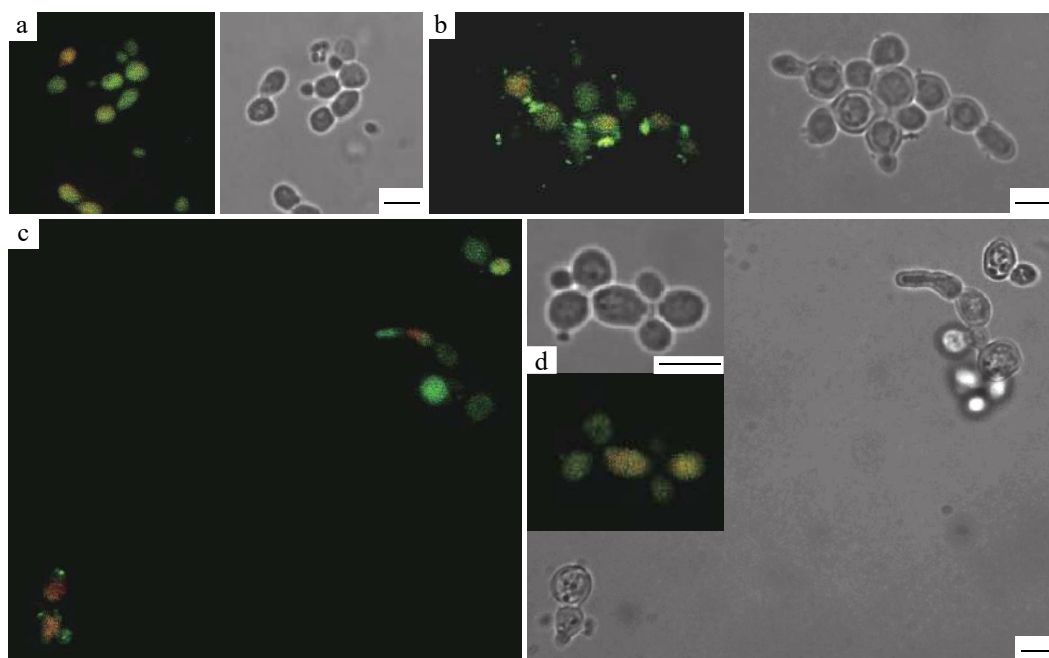


Fig. 10 Images of *S. cerevisiae* cells after incubation with 20-mer ROX-DNA (red emission) coordinated with fluorescent tagged polymer **ZS-415** (green emission). N/P ratio was 100. The polymer concentration in the cell culture was 0.022 g·L⁻¹. Video files demonstrating bleaching of the green tag are presented in ESI as files (a–d).avi. Scale bars represent 10 μm.

polymer. The fluorescent area was less than total cell size, which confirms complex penetration into cytoplasm. This preliminary data suggested that this polyplex could be explored for gene delivery applications in other types of cells including mammalian cells.

CONCLUSIONS

We have demonstrated that poly(1-vinylimidazole) could interact with oligonucleotides at physiological pH 7.4. The number of active sites capable of complexing with negatively charged phosphate groups was comparable with the number of protonated imidazole units in the case of high molecular weight polymer. The decrease of poly(1-vinylimidazole) molecular weight increased N/P ratio of the polymer-DNA complex, which possibly indicated the ability of the short-chain polymer to interact with oligonucleotides in the associated state only. Increase of the polymer charge by quaternization of 1%–5% imidazole units or by decreasing pH to 6.5–7 considerably increased the ability of poly(1-vinylimidazole) to interact with oligonucleotides. These observations confirmed a good potential of poly(1-vinylimidazole) as a basic structure for design of gene delivery systems. The observed pH sensitivity of the poly(1-vinylimidazole)-oligonucleotide complexes is interesting for cancer gene therapy because the tumors have a lowered intercellular pH (stable oligonucleotide complex) and elevated extracellular pH which impedes complexation. The results suggested that the obtained fluorescent tagged poly(1-vinylimidazole) samples were useful for monitoring the penetration of polymer and polymer-oligonucleotide complex into living cells.

Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at <http://dx.doi.org/10.1007/s10118-019-2240-1>.

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