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Suppression and promotion of DNA charge inversion by mixing counterions

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In the present study, we report the suppression and promotion of DNA charge inversion by mixing quadrivalent counterion (spermine) with mono-, di- and trivalent counterions by dynamic light scattering (DLS) and single molecule electrophoresis (SME) methods. We find that the electrophoretic mobility of DNA in spermine solution decreases in the presence of monovalent sodium ions and divalent magnesium ions. It means that the charge neutralization of DNA by the quadrivalent counterion is suppressed when adding extra mono- or divalent counterions. More specifically, at high concentration of spermine, the positive mobility can switch back to a negative value by adding mono- and divalent counterions. Thus, charge neutralization and inversion of DNA by quadrivalent counterions is suppressed in the mono- and divalent ion solution. However, the scenario changes dramatically when we add trivalent ions into the solution of DNA and spermine. In the case, the charge neutralization and inversion of DNA is promoted rather than suppressed by mixing with trivalent ions. The negative electrophoretic mobility can promote to a positive value, which corresponds to the charge inversion, by trivalent counterions. Thus trivalent and quadrivalent counterions work cooperatively in DNA charge neutralization and inversion. This promotion also happens when highly positively charged chitosan is introduced in the solution. We explain the observation by the counterions complexation that is related with DNA condensation, which is supported by the images of atomic force microscopy (AFM).

1. Introduction

DNA is an important biological polyelectrolyte that is highly charged in solution. The highly charged and stiff polymer can be condensed into compact structures by multivalent ions and many other condensing agents. The understanding of DNA condensation is not only important for study of fundamental biological process such as chromosome compacting, but also for developing new gene carriers in therapeutic applications. However, the underlying microscopic mechanism still needs further exploration. The process is generally considered to be related with the neutralization or, more likely, over compensation of DNA electric charge. Over compensation or charge inversion occur when the charge of counterions surrounding of DNA skeleton is more than the polyelectrolyte itself [1]. Overcharging is a counterintuitive phenomenon and cannot be described by the mean filed Poisson-Boltzman theory, which generally applies for the ions atmosphere surrounding polyelectrolytes.

In recent years, there has been a significant research effort aimed at understanding the general aspects of this counterintuitive phenomenon theoretically and experimentally. In the mean while, some numerical methods have been used to discuss ion distribution around the polyelectrolyte by Monte Carlo simulation [2,3,4] and by solving the boundary problem of PB equation [5,6,7,8]. For example, Aksimentiev group observed that reversal of the DNA’s electrical charge is different from the reversal of the DNA’s electrophoretic mobility [9,10]. The electrophoretic mobility results from a complex interplay of electrostatics and hydrodynamics and is related with electro-osmotic flow [11]. In fact, DNA charge inversion is closely related with its condensation and compaction. The complexation and aggregation of DNA by condensing agents is driven by screened electrostatic interactions, and is promoted by both the increase in the entropy of the system due to the release of counterions and by the “charge fractionalization” mechanism suggested by Shklovskii [12]. Charge fractionalization occurs when an additional polyelectrolyte molecule is absorbed by an almost neutral complex. The presence of these additional charges increases the conformational entropy of the adsorbed chains. Moreover, the charges on the free polyelectrolyte that were close together are now spread all over the complex. Noteworthy, fractionalization leads to a substantial overcharging (or charge inversion) of the complex, i.e. more charges are needed to neutralize the complex. In this case, there is a so called strongly correlation liquid (SCL) of counterions at the polyelectrolyte surface [13]. Since DNA is not a uniformly charged cylinder, the mechanism of its charge inversion still needs further investigation [10].

Charge inversion has also been directly observed by experiments. It has been shown that a charged silica surface inverts charge sign in trivalent and quadrivalent electrolyte solution [14, 15]. While for the case of DNA, its charge only
inverts in quadrivalent countiones solution. For example, Besteman group directly measured DNA charge reversal induced by multivalent countiones by dynamic light scattering (DLS) and magnetic tweezers (MT) [16]. The phenomenon is confirmed in our previous investigation [17]. In biological systems, overcharge of peptide and reentrant condensation of proteins are also directly observed by different experimental methods [18, 19, 20], and the complicated interaction among multivalent countiones and the biomolecules is the underlying origin.

When more than one countiones exist in solution, the electrostatic interaction between DNA and countiones becomes more complicated. Previous study shows that increasing monovalent salt concentration hinders charge inversion by multivalent ions and even causes charge inversion to disappear entirely [21]. To understand the mechanism of charge inversion further, we explore DNA charge inversion by mixing countiones in solution. Surprisingly, we found that DNA charge inversion can not only be suppressed by mixing countiones, but also promoted by the mixing. We investigated the suppression and promotion of DNA charge inversion by mixing countiones by a synergistic use of different experimental approaches: Dynamic light scattering (DLS) [22], single molecule electrophoresis (SME) [23] and atomic force microscopy (AFM) [24]. To explain our experimental results, we develop a theoretical description by incorporating competing cation mechanism [10,25,26,31] with the strongly correlated theory of charge inversion [1,12]. It works at least qualitatively for the case of suppression, but fails to predict the promotion of charge inversion. Combining the data with the observation of AFM, we propose that the promotion of charge inversion is characterized by the countiones complexation, which is related with DNA condensation.

2 Experiments and materials

2.1 Materials

Double strands λ-phage DNA for DLS and SME was purchased from New England Biolabs company and didn’t go through purification when it is used. As received from the manufacturer, the concentration of λ-phage DNA stock solution is 500 ng/µl. The chemical compounds (sodium chlorid, magnesium chlorid, hexamine cobalt (III) chloride, spermidine, spermine tetrahydrochloride, and chitosan oligosaccharide lactate with average Mn=5000) were purchased from Sigma-Aldrich. Measurements were done in a Tris hydroxymethylaminomethane monovalent buffer at pH 8.0 (TRIS) with varying concentration of mixing countiones. The deionized water (18.2MΩ) was purified through the Milli-Q water purification system (Millipore corporation, American). All experiments were repeated at least twice to ensure consistent results while taking the standard deviation as the error bar.

2.2 Sample preparation and measurements

The electrophoresis-mobility measurements were carried out by using dynamic light scattering device of Malvern Zetasizer nano ZS90 equipped its patented M3-PALS technique. The laser source was a He-Ne gas laser (λ=633nm) and the light scattering by the avalanche photodiode mounted on the goniometer arm at to the direction of the incident radiation. The DNA molecules were diluted to a concentration of 1 ng/µl in a buffer solution containing 1mM TRIS, pH 8.0, and different concentration of NaCl, divalent (MgCl₂), trivalent (spermidine and cobalt hexamine) or quadrivalent (spermine) salts or chitosan. All measurements were carried out after 5 minutes incubation at room temperature. A 1 ml volume of DNA solution was placed in the folded capillary cells and put in the sample groove of the instrument. During the measurement, the groove temperature was kept at 25°C.

The atomic force microscopy imaging was performed in air with a multi-mode AFM with nanoscope controller (SPM-9600, Shimadzu, Kyoto, Japan) in the tapping-mode. All AFM images were captured at conventional ambient tapping mode, with scan speeds of ~2 Hz and data collection at 512x512 pixels. All the images were smoothed manually using off-line analysis software equipped with the microscope. Mica disks of diameter one centimetre attached to magnetic steel disks were used as substrates for DNA adsorption. All manipulations were carried out in 1mM TRIS (pH=8.0) with a final concentration of 1 ng/µl DNA and the specified concentration of countiones. The mixture was deposited onto freshly cleaved mica and incubated for 5 minutes at room temperature. Right after incubation, the samples were rinsed with a flow of 20 ml water for ten times, and then rapidly blown dried using a burst of compressed nitrogen.

The single molecule electrophoresis is based on an inverted fluorescence microscope (Nikon, TE-2000E) equipped with an oil immersion objective (Nikon, 100x, N.A=1.49) and a ND filter slider (Nikon, 330–385/460–490/510–550nm). A 100W high-pressure mercury lamp served as illumination. The intensified charge-coupled device (CCD) camera (512×512 pixels, Cascade II512) was used for video recording, which is used for analyzing the DNA electrophoresis mobility. NIS-Element D3.1 software was used to acquire the video and analyze the data. Electrophoretic-mobility measurements were carried out using a home-made electrophoresis slot (40mm×3mm×0.1mm) shown in Fig 1. The electrophoretic slot was made by bonding two glass slides sandwiched two layers of laser cut sealing membrane. Two platinum electrodes were put at the both end of the slot, and a voltage is applied between the electrodes for electrophoresis.

In the electrophoresis, the DNA was stained with YOYO-1 fluorescent dye at a dye-base-base ratio of 1:10 with TRIS buffer before using. The complex samples were incubated for 30min at room temperature in the dark before measurement. The electrophoresis mobility measurements were conducted in the TRIS buffer with different concentration of the mixing countiones. The final concentration of DNA was about 0.4 ng/µl.

Figure 1. Single molecule electrophoresis setup

3 Result and discussion

3.1 The change of DNA electrophoresis mobility induced by mixing countiones

The double helix structure of the λ-DNA whose skeleton was...
surrounded by a lot of co-ions and counterions dissolved in the solution. The positive counterions and the negative co-ions in a ratio of 1:1 could coexist in the solutions. When the additional ions are added to solutions, they compete with each other in the vicinity around the polyelectrolyte, then became major origin of forces including attraction, repulsion and transformation entropy. Owing to the strong lateral repulsion, at the surface of polyelectrolyte, the counterions could form a SCL that resembled the Wigner crystal in the short-range order. The chemical potential of the liquid resulted in an extra and correlative electrophoresis mobility (1).

\[ \mu = (\varepsilon \zeta / 1.5 \eta) f(kr) \]

(1)

Where \( \varepsilon \) is the dielectric constant of the solvent, \( \zeta \) is the zeta potential, \( \eta \) is viscosity coefficient of the solvent, \( r \) is radius of spherical ion, \( f(kr) \) is the Henry function. The electrophoresis mobility could obtain in the DLS experiment, as shown in the Figs. 2–6 (a).

As mentioned above, shape of the homemade SME slot was a very narrow channel in which an electric field is applied. We assume the electric field is uniform since the width of channel is 0.1 mm. The DNA molecules in the channel move according to the electrostatic law. Under the condition of balance between electric field force and frictional resistance, the DNA molecule moved at a constant velocity \( \psi \). Then the DNA electrophoresis mobility, \( \mu \), was defined from this equation as the ratio of the velocity to the electric field strength \( \mu = \psi / E \), which can be measured directly. We use both DLS and SME methods to measure the electrophoresis mobility to obtain more consistent results. The SME results were shown in the Fig. 2–6 (b).

The DNA electrophoresis mobility with 1mM TRIS buffer containing spermine and sodium chloride are shown in Fig. 2. We can see that the mobility of DNA changed from negative to positive values with increasing spermine concentration. However, when additional sodium chloride was added to the solution, the mobility is entirely shifted to a lower value. Both DLS and SME data shows that DNA charge inversion happens when the concentration of spermine is more than about 0.5 mM. When the concentration of spermine is less than 0.5 mM, the DNA electrophoresis mobility become less negative with increasing quadrivalent-ion concentration, and decreasing monovalent-buffer concentration (0.5 mM, 1 mM). When the concentration of the spermine was up to 1.5 mM, the value of \( \mu \) correspondingly increased to 0.22 (in units of \( 10^{-5}\mathrm{cm}^2\mathrm{V}^{-1}\mathrm{s}^{-1} \), the same unit is used for mobility in the following and is omitted for clarity), which means that charge inversion occurs. When the monovalent ion was added to spermine-DNA solution, the value of \( \mu \) slightly decrease to 0.21 and 0.2 when the concentration of NaCl is 0.5 mM and 1 mM NaCl respectively, measured by DLS. We can see that the change of DNA electrophoresis mobility by adding monovalent counterions is small but tends to lower its value. Thus we can conclude that the charge neutralization and inversion of DNA by monovalent counterions is suppressed, which is consistent with previous study [14, 15].

To justify the conclusion further, we measured the DNA electrophoresis mobility with 1 mM TRIS buffer containing mixed counterion (NaCl) by SME. The data is shown in Fig. 2(b). The results are similar to those from DLS. In the experiment, NaCl solution of 0.5 mM and 1 mM was added to the spermine-DNA solution, and then the DNA mobility was measured by recording DNA migration video in fluorescent microscopy. Analysis shows that the mobility decreases when adding monovalent counterions. Here the suppression of charge neutralization and inversion of DNA by monovalent counterions was observed directly. The typical video file can be found in the supplementary materials. Although the experiment values of the electrophoresis mobility didn’t match each other strictly for the two methods, the trend of electrophoresis mobility is the same at the different concentration of spermine and monovalent.

![Figure 2](image2.png)

**Figure 2.** DNA molecule electrophoresis’ mobility as a function of the spermine concentration with monovalent ions. (a) DLS measurements for 0, 0.5 mM, 1 mM sodium chloride (black, red, green). (b) SME measurements for 0, 0.5 mM, 1 mM sodium chloride (black, red, green). The arrows indicate increasing concentration of the sodium chloride.

![Figure 3](image3.png)

**Figure 3.** DNA molecule electrophoresis’ mobility \( \mu \) as a function of the spermine concentration with divalent ions. (a) DLS measurements for 0, 0.5 mM, 1 mM magnesium chloride (black, red, green). (b) SME measurements for 0, 0.5 mM, 1 mM magnesium chloride (black, red, green). The arrows indicate increasing concentration of the magnesium chloride.

In Fig. 3, we plot the electrophoretic mobility of DNA versus the concentration of spermine and spermie with MgCl2. We can see that the DNA electrophoresis mobility changes from a negative to a positive value with the concentration of spermine increasing.
However, for the mixed electrolyte solutions (spermine+MgCl₂), the DNA’s mobility curves shift downward entirely though the descending values at different concentrations vary. As shown in Fig. 2, the mobility of DNA becomes from negative to positive value with increasing spermine concentration when additional MgCl₂ exists in the solution. In this case, DNA charge inversion happened when the concentration of spermine was greater than about 0.5mM. When the concentration of the spermine was up to 0.7 mM, the value of μ correspondingly increased to 0.1, which means that charge of DNA-counterions complex now changes from negative to a positive value counter-intuitively. However, if the divalent ion was added to spermine-DNA solution, the value of μ correspondingly decreased to -0.008, -0.070 in the cases of spermine+0.5 mM MgCl₂, spermine+1 mM MgCl₂, respectively. Clearly, the positive mobility can be switched back to a negative value by adding divalent counterions. Once again, the charge neutralization and inversion of DNA is suppressed by adding divalent counterions. To confirm the suppression, we adopted the SME technology to measure DNA electrophoresis mobility at the different concentration of (spermine+magnesium chloride), shown in figure 3(b). The experimental result is systematically consistent with that of the DLS although there are some small deviations at some sampling points.

The suppressing effect for the DNA charge inversion depends on the concentration of magnesium chloride. From figure 2 and 3, we can see that the suppression grows with increasing the concentration of mono- or divalent ions. As pointed out in Ref. [15], the phenomenon happens because the monovalent and bivalent counterion was full of the strong correlation liquid to prevent the other ions entering inner DNA. Thus these low valance ions block the DNA charge neutralization and inversion.

When we add trivalent ions into the solution, some interesting phenomenon happens. Figure 4(a) displayed typical curves of the DNA electrophoretic mobility by DLS when adding hexamine cobalt (0, 0.25 mM, 0.5 mM) into solution of spermine and DNA. In the case, the electrophoretic mobility is promoted rather than suppressed by mixing with trivalent ions. The critical concentration of the spermine induced DNA charge inversion was about 0.5mM. When hexamine cobalt (0.25 mM, 0.5 mM) was added to the spermine solution, the critical value is much smaller than the one when there is only spermine in the solution. With increasing concentration of the hexamine cobalt, the charge inversion of DNA is promoted quickly. For example, the electrophoretic mobility of DNA is ~0.1 when the concentration of spermine is 0.1mM. When trivalent cobalt ions (0.25mM) is added to the solution, the mobility is promoted to ~0.04. Even more, when the concentration of trivalent cobalt ions is increased to 0.5mM, the electrophoretic mobility is promoted to 0.05, a positive value. In general, the electrophoretic mobility curve is shifted upward when trivalent ions is added to the system of DNA and quadrivalent ions. It is noteworthy that the promotion is almost insignificant at high spermine concentration (such as 1.5 mM) where the charge reversion is saturated. A similar measurement is accomplished by the SME setup. The data is shown in figure 4(b). In the experiment, the video of DNA migration in the channel is recorded to calculate its mobility where hexamine cobalt ions of different concentrations were mixed to the spermine solution. As we can see in figure 4(b), the results are similar to those by DLS. Therefore, we can draw a conclusion by the two methods that trivalent counterions in the presence of spermine in the electrolyte solution could promote the DNA charge neutralization and inversion. In this case, trivalent and quadrivalent counterions work cooperatively in the electrostatic interaction between DNA and counterions.

![Figure 4](image)

Figure 4. DNA molecule electrophoresis’ mobility μ as a function of the spermine concentration with tri-valent cobalt ions. (a) DLS measurements for 0, 0.25 mM, 0.5 mM of hexamine cobalt (black, red, green). (b) SME measurements for 0, 0.25 mM, 0.5 mM of hexamine cobalt (black, red, green). The arrows indicate increasing concentration of the hexamine cobalt.

To confirm the discovery, we added a different trivalent ion, spermidine, into the spermine and DNA solution to measure DNA electrophoresis mobility. The result from both DLS and SME is shown in figure 5. We can see the significant promoting effect for electrophoresis’ mobility of DNA. In DLS experiment, for example, at 0.7 mM concentration of spermine, the electrophoresis’ mobility of complex is promoted from 0.1 to 0.2 when spermidine of 0.5 mM is introduced to the solution. On the other hand, the experiment data of SME shown in figure 5(b) indicates the electrophoresis’ mobility change from -0.07 to 0.05 when spermidine of 0.5mM was added to the spermine (0.3 mM) and DNA solution. Therefore, the promotion of charge neutralization and inversion is not limited to some specific chemical agents, in contrast, results from the general electrostatic interaction in solution.

![Figure 5](image)

Figure 5. DNA molecule electrophoresis’ mobility μ as a function of the spermine concentration with tri-valent spermidine. (a) DLS measurements for 0, 0.25 mM, 0.5 mM of spermidine (black, red, green) (b) SME measurements for 0, 0.25 mM, 0.5 mM of spermidine (black, red, green). The arrows indicate increasing concentration of the spermidine.
Figure 6. DNA molecule electrophoresis' mobility $\mu$ as a function of the spermine concentration with chitosan. (a) DLS measurements for 0, 0.01 mM, 0.05 mM of chitosan (black, red, green). (b) SME measurements for 0, 0.01 mM, 0.05 mM of chitosan (black, red, green). The arrows indicate increasing concentration of the chitosan.

To test the generality of the suppression and promotion of charge inversion, we use chitosan to further experiments. Chitosan, a cationic polysaccharide obtained by alkaline N-deacetylation of chitin, is one of the most widely utilized polysaccharides [27]. It is a non-toxic biodegradable polymer with low immunogenicity. These characteristics make chitosan an excellent candidate for various biomedical applications such as drug delivery, tissue engineering, and gene delivery [28]. It can be used for gene delivery system because positively charged chitosan can be complexed with negatively charged DNA [29, 30]. Figure 6 shows the electrophoretic mobility as a function of the spermine concentration in the presence of chitosan (0, 0.01 and 0.05 mM) by DLS and SME.

As can be seen, there are significant changes of the electrophoretic mobility of DNA when chitosan of 0.01 mM and 0.05 mM are introduced to the solution, and they had some noticeable features. At the low concentration of the spermine (0.1 mM), when added chitosan into the spermine, the mobility switches from negative to positive value quickly, which means that the charge inversion can occur at the very early stage with the help of chitosan. And with increasing concentration of the spermine (or concentration of the chitosan), the electrophoresis' mobility continues to increase and finally reach a saturation value. The mobility is very sensitive to the addition of chitosan, which is probably related with its huge positive charge in solution. Among all the agents of NaCl, MgCl$_2$, spermidine, hexammine cobalt and chitosan, chitosan has the most significant influence of DNA charge neutralization and inversion.

3.2 DNA morphologies induced by mixing counterions

We noted that the suppression and promotion of DNA charge inversion are related with the capability of compacting DNA of the additional counterions. For example, it has been shown that DNA molecules cannot be condensed or compacted by mono- and divalent counterions. However, they can be easily condensed or compacted in the presence of trivalent and quadrivalent ions. Specifically, trivalent ions can induce DNA condensation, but cannot lead up to its charge inversion. To elucidate their relation between DNA condensation and charge inversion, we image the systems of DNA and counterions by atomic force microscopy (AFM), shown in figure 7 and 8. Figure 7(a) displays the morphology of DNA on mica surface with MgCl$_2$ concentration of 1 mM. We can see that DNA is freely extended on mica. Figure 7(d) shows the images of DNA on mica surface with the spermine concentration of 10 µM. In these cases, DNA segments wrap around the core to form a flower structure. When NaCl (0.5, 1 mM) or MgCl$_2$ (0.5, 1 mM) were added to spermine (10 µM)-DNA solution, the flower patterns shrink loosely and the sizes are almost the same as in 10 µM spermine solution only, occasionally looks even larger, shown in figure 7(b), (c) and (e),(f).

When the concentration of spermidine increases, the condensation grows gradually, shown in figure 8(a)-(c). When 10, 50 or 125 µM spermidine were added to spermine (10 µM)-DNA solution, the flower patterns compacted more tightly than the ones due to spermine or spermidine separately. We can see similar patterns but with increasing clustering as shown in figure 8(d)-(f). Most of these condensates are single layered, but containing more than one DNA molecules. It can be seen that the condensation patterns become much more apparent and more compact than those induced by the corresponding condensing agents separately.
4 Discussions and Conclusions

The physical mechanism of suppression and promotion of DNA charge inversion is needed to systematically explore further. The suppression mechanism is proposed in Ref. [15]. They thought that the monovalent and divalent counterions were full of the strong correlation liquid to prevent the other ions entering inner DNA. They proposed an explanation for the re-entrant condensation based on a new theory of screening of macroions by multivaneous cations, emphasizing the strong corrections of multivalent cations at the surface of DNA [31].

When more than one counterion is present in solution, the theory on the competitive electrostatic binding to polyelectrolytes is developed by Rouzina and Bloomfield in the framework of the non-linear Poisson-Boltzmann equation [25]. Unfortunately, the theory cannot be applied to our system directly since there is no charge inversion in PB theory. However, if the influence of strong correlation of counterions at the surface of a macron is included as a boundary condition, the modified PB theory can elucidate the charge inversion. For the current mixing couterions system, we can incorporate Rouzina and Bloomfield’s theory to SCL framework developed by Shklovskii group [11,12]. In the presence of competing cations of any valency, following the similar derivation of Ref. [31], we can have the general expression for the net linear charge density of DNA

\[ \eta^* = -\sum_i Z_i \Theta_i \frac{\eta_C}{2Z_i} \ln \left( \frac{N_{i0}/N_i}{1 + r_{i}/a} \right) \]

where \( Z_i \) is the valency of a counterion of sort i, \( \eta_C = e|\lambda_b| \), e is the elementary charge and \( \lambda_b \) is the Bjerrum length, \( N_j \) is cation concentration and \( N_{i0} \) is its concentration at the point of zero electrostatic potential, \( r_{i} \) is Debye screening length and a is the radius of DNA. \( Z_i \Theta_i \) in Eq.(2) is the fraction of charge neutralization, whose expression and derivation can be found in Ref.[31]. According Eq.(2), we can calculate the net charge density and electrophoretic mobility in the system of mixing counterions. For example, DNA mobility in 0.025mM spermine solution is about -0.45 and the DNA mobility in 1mM NaCl solution is about -2. However, the measured mobility is -0.58 when we mixed spermine (0.025mM) and NaCl (1mM) to DNA solution while the calculated value is about -1.6 in the same condition. For the case of mixing divalent MgCl\(_2\) (1mM) with spermine (0.025mM), the measured mobility is -0.57 and the calculated value is about -0.69. The charge neutralization is suppressed in both cases and the theory gives the correct trend. When additional hexammine cobalt (0.5mM) is introduced to the DNA and spermine (0.1mM) solution, the DNA mobility is promoted from -0.01 to 0.05 while the calculated value is still suppressed to -0.27. From these examples, we can see that the mixing theory works at least qualitatively for the case of suppression, but fails to explain the promotion of charge inversion. Therefore, we need a new mechanism to understand the phenomenon. It has been shown that DNA molecules cannot be condensed or compacted in the presence of trivalent and quadrivalent ions. Specifically, trivalent ions can induce DNA condensation, but cannot lead up to its charge inversion. Based on this observation, we propose the promotion mechanism as follows: different counterions work cooperatively if DNA is compacted or condensed, and interact with the polyelectrolyte respectively if DNA molecules are in extensible states in which the suppression mechanism is in effect. Thus trivalent and quadrivalent counterions work cooperatively in charge neutralization and inversion in company with DNA condensation. More study is needed for understand the physical mechanism of promotion of DNA charge inversion.

In summary, we found the phenomenon of suppression and promotion of DNA charge inversion by mixing quadrivalent counterion (spermine) with low valance counterions. It was shown that the electrophoretic mobility of DNA in spermine solution decreases in the presence of monovalent sodium ions and divalent magnesium ions. This means that the charge neutralization of DNA by the quadrivalent counterions is suppressed when adding extra mono- or divalent counterions. At high concentration of spermine, the electrophoretic mobility of DNA can increase to a positive value, which means that charge inversion occurs. However, the positive mobility can switch back to a negative value by adding mono- and divalent counterions. This means that the charge neutralization and inversion of DNA by quadrivalent counterions can be suppressed by mixing with mono- and divalent ions. Interestingly, the scenario changes dramatically when we add trivalent spermidine or hexammine cobalt into the solution of DNA and spermine. In that case, the charge neutralization and inversion of DNA is promoted rather than suppressed by the additional trivalent ions.

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