A model of inversion of DNA charge by a positive polymer: fractionization of the polymer charge

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Charge inversion of a DNA double helix by an oppositely charged flexible polyelectrolyte (PE) is considered. We assume that, in the neutral state of the DNA-PE complex, each of the DNA charges is locally compensated by a PE charge. When an additional PE molecule is adsorbed by DNA, its charge gets fractionized into monomer charges of defects (tails and arches) on the background of the perfectly neutralized DNA. These charges spread all over the DNA eliminating the self-energy of PE. This fractionization mechanism leads to a substantial inversion of the DNA charge, a phenomenon which is widely used for gene delivery.

Inversion of the negative charge of a DNA double helix by its complexation with a positive polyelectrolyte (PE) is used for the gene delivery. The positive charge of DNA-PE complex facilitates DNA contact with a typically negative cell membrane making penetration into the cell hundreds times more likely. Charge inversion of DNA-PE complexes was confirmed recently by electrophoresis. If at a given concentration of long DNA helices one increases concentration of shorter PE molecules, at some critical point the electrophoretic mobility of a DNA-PE complex changes sign from negative to positive. Intuitively, one can think that when a PE completely neutralizes a DNA double helix new molecules of PE do not attach to DNA. Indeed, the Poisson-Boltzmann approximation for description of screening of a DNA by any counterions including PE does not lead to charge inversion. Counterintuitive phenomenon of charge inversion of a macroion by oppositely charged PE has attracted significant attention. It can be explained if one takes into account that the surface potential of already neutralized DNA is locally affected by a new approaching PE molecule, or in other words, taking into account correlations between PE molecules. Due to repulsive interaction between PE molecules a new PE molecule pushes aside already adsorbed on DNA surface molecules and creates on the surface an oppositely charged image of itself. The image attract the new PE molecule leading to charge inversion. This phenomenon is similar to attraction of a charge to a neutral metal. For quantitative consideration charges of DNA are often assumed to be smeared and to form uniformly charged cylinders. This approach ignores interference between chemical structure of DNA surface and of PE and clearly is not fully satisfactory. In this paper, we consider effects of discreteness and configuration of charges of the DNA double helix. In this case, we suggest an explanation of charge inversion based on "fractionization" of charge of PE molecules. It turns out to be even simpler and more visual than for smeared charges of DNA.

Negative elementary charges of DNA phosphates are situated along the two spirals at the exterior of both helices. When unfolded, each spiral is an one-dimensional lattice of such charges, with the lattice constant \( a = 6.7 \text{"Å"} \). Let us consider a toy model of a PE as a freely jointed chain of \( Z \) small +e monomers. The elastic energy cost for bending the PE is neglected in this model, so that one can concentrate on the electrostatic aspect of the problem. To maximize the role of discreteness of DNA charge we assume that the PE bond length \( b \) is exactly equal to the distance \( a \) between negative charges of a spiral. (The case when these lengths are different is discussed in the end of the paper). We assume that minimal distance, \( d \), between a PE charge and a charge of DNA is much smaller than \( a \). Then PE molecules can attach to a DNA charge spiral in such a way that every charge of a spiral is locally compensated by a PE charge and, therefore, DNA is completely neutralized. The case of \( Z = 3 \) is shown in Fig. 1a. The neutralization is so perfect that it is difficult to imagine how another PE molecule can be attached to DNA.

![FIG. 1. The origin of charge fractionization in PE adsorption. a) One of spirals of negative charges of DNA (empty circles) is completely neutralized by positive PE molecules with \( Z = 3 \) (their monomers are shown by solid circles). A new PE molecule is approaching DNA. b) The new PE molecule is "digested" by DNA. Its charge is split in +e charges of Z defects. They are tails and an arch (center).](image)
In this paper, we would like to discuss the fractionization mechanism which brings an additional PE to the neutralized DNA and leads to charge inversion. Fig. 1 shows how this mechanism works for the case of \( Z = 3 \). When a new PE comes to the DNA double helix which is already neutralized by PE, it creates a place for itself or, in other words, the oppositely charged image in the following way. Let us choose \( Z \)-already-absorbed PE molecules, which are situated far from each other. In each of them we detach one PE monomer from DNA surface. This leads to formation of positive defects (tails and arches) and negative vacancies on DNA. To create an image for a new PE let us shift adsorbed PE molecules along DNA in such a way that all \( Z \) vacancies join together and form a large vacancy of a length \( Z \). A new PE molecule is accommodated in this vacancy. As a result of consumption of this molecule \( Z \) defect + e charges appear on the top of completely neutralized spiral (see Fig. 1).

This effectively looks as cutting of the new PE molecule into \( Z \) individual monomers and spreading them out along the spiral. In other words, charge inversion of DNA happens by fractionization of the PE molecule charge. Of course, none of the chemical bonds is really cut, and this phenomenon is solely due to the correlated distribution of PE molecules, which avoid each other at the DNA spiral. In this sense, fractionization we are talking about is similar to what happens in fractional quantum Hall effect \( \text{[2]} \) or in the polyacetylene \( \text{[3]} \), where many-electron correlations result in fractionization of the electron charge.

Fractionization is driven by elimination of the self-energy of free PE in solution. By the self-energy we mean the energy of repulsive interactions of \( Z \) positive charges of the PE molecule in extended conformation which it has in the solution. In the fractionized state, charges of monomers are far from each other and practically do not interact, so that the PE self-energy is eliminated and, therefore, gained.

Let us now calculate the net inverted charge using this fractionization mechanism. We denote the linear charge density of the inverted (positive) net charge of the double helix DNA by \( \eta^* \). The chemical potential of the PE absorbed at the spiral is

\[
\mu_s = Z k_B T \ln(\eta^*/\eta_0) + Z e \psi(0)
\]

(1)

The first term in the right hand side of Eq. (1) is the chemical potential of the one-dimensional gas of defects \((-\eta_0 \simeq 0.6 e/\text{Å} \) is the bare charge density of DNA). We used expression for the chemical potential of an ideal gas because the Coulomb interaction between defects at the a distance of a few \( a \) is much smaller than \( k_B T \) (\( a \simeq l_B \), where \( l_B = e^2/D k_B T \simeq 7 \text{Å} \) is the Bjerrum length.) The second term in the right hand side of Eq. (1) is the repulsion energy of the new PE from the inverted charge of the DNA. In this term, \( \psi(0) \) is the averaged surface potential of the DNA helix. We assume in this paper that the net charge of DNA is screened by a monovalent salt at the screening length \( r_s \), which is much larger than \( a \).

Then \( \psi(0) \) can be calculated as the surface potential of a cylinder with radius of DNA helix \( R \) and linear density of charge \( \eta^* \)

\[
\psi(0) \simeq \frac{2 \eta^*}{D} \ln \left[ \frac{r_s + R}{R} \right].
\]

(2)

To find \( \eta^* \) in the equilibrium state, one has to equate the chemical potential of the absorbed PE molecules with that of the free PE in the solution. The later one can be calculated as following. Due to the repulsive Coulomb interaction between monomers, a free PE in the solution has an extended shape to minimize its energy. Therefore, the chemical potential of a free PE in solution can be written as the self-energy of a rigid rod with the length \( Na \) and the linear charge density \( e/a \)

\[
\mu_0 = (Z e^2/D a) \ln(L/a),
\]

(3)

where \( L = \min(r_s, Za) \) and \( D \) is the dielectric constant of water. We have assumed that the concentration of PE in the solution is large enough so that its translational entropy can be neglected. In this sense, we are calculating the maximum possible charge inversion. If the PE molecule is long \( (Z \gg 1) \) this limit is reached at a concentration of PE which is exponentially small \(~\exp(-Z)\).

Equating the chemical potentials of Eqs. (2) and (3), one has

\[
\psi(0) = (e/D a) \ln(L/a) + (k_B T/e) \ln(\eta_0/\eta^*)
\]

(4)

One can interpret the right hand side as a “correlation” voltage (provided by the total free energy gain in fractionization of PE charge) that (over-)charges the DNA to the potential \( \psi(0) \).

To the first approximation, one can neglect the entropic term in the right hand side of Eq. (4) and easily get a solution for the net charge density

\[
\eta^* \simeq \frac{e}{2a} \ln\frac{\ln(L/a)}{\ln((r_s + R)/R)}.
\]

(5)

Now one can check that this solution is consistent with the assumption that the entropic term can be neglected by substituting it back into Eq. (4). Of course, \( \eta^* \) is positive indicating that the bare DNA charge is inverted. Knowing \( \eta^* \) and using \( |\eta_0| = 0.6 e/\text{Å} \simeq 3.9 e/a \) the charge inversion ratio can be calculated

\[
\left| \frac{\eta^*}{\eta_0} \right| = 0.13 \frac{\ln(L/a)}{\ln((r_s + R)/R)}.
\]

(6)

For DNA \( R = 10 \text{ Å} \) and \( a = 6.7 \text{ Å} \), so that at \( r_s \geq 10 \text{ Å} \) the ratio of logarithms can be only slightly larger than unity. Thus, the charge inversion ratio created by fractionization is limited by 20%. Up to such point we indeed can neglect Coulomb interactions between defects in the chemical potential of the gas of defects (the first term in the right hand side of Eq. (4)).

We emphasize that it would be incorrect to conclude that fractionization discussed above is a strictly
one-dimensional phenomenon, similarly to the well-known cases of one-dimensional density wave\textsuperscript{13} and polyacetylene.\textsuperscript{14} It is easy to see that our fractionization mechanism applies equally well for a two-dimensional surface with discrete charges which form a square lattice with the same lattice constant as the PE bond length $c$. Indeed, one can view Fig. 1 as a cross-section of this lattice and all previous arguments about the role of tails and arches can be carried over to this case. There are, however, small modifications of the analytic formulæ for charge inversion. Defects with $+e$ charges form now a two-dimensional gas with concentration $\sigma^*/e$, where $\sigma^*$ is the net positive surface charge density playing the role of $\eta^*$. The chemical potential of this gas is $k_B T \ln(a^2 \sigma^*/e)$. The surface potential is now $\psi(0) = 2\pi \sigma^* r_s / D$. The balance of the chemical potential of PE molecules adsorbed at the surface with that of a free PE in the solution now reads

$$2\pi \sigma^* r_s / D = (e/D a) \ln(L/a) + (k_B T / e) \ln(e/a^2 \sigma^*) \quad (7)$$

and the solution, for $a \simeq l_B$, within a numerical factor, is

$$\sigma^* \simeq (e/\pi a r_s) \ln(r_s/a) . \quad (8)$$

One can see that, for $a \simeq l_B$, in the free energy gained by fractionization of the PE molecule charge, the entropy contribution is comparable to the self-energy, in contrary with the one-dimensional case, where the entropic term can be neglected. This is obviously due to a higher number of degrees of freedom which a two-dimensional surface provides to the gas of defects. If $r_s \gg a$, the fractionization induced charge inversion ratio for the two-dimensional case is smaller than for DNA:

$$\left| \frac{\sigma^*}{e/a^2} \right| = \frac{a}{r_s} \ln \frac{r_s}{a} . \quad (9)$$

An important role of elimination of the self-energy for adsorption of a flexible PE on an oppositely uniformly charged surface was previously emphasized in Refs. 13-14.

Until now we considered adsorption of linear charged molecules (PE) both on one- and two-dimensional lattices of the background charge. It is interesting to note that the fractionization mechanism works for molecules of other shapes, too. Let us, for example, consider dendrimers (star-like branching molecules with a large number of monovalent positive charges on their periphery), which were also shown to invert the charge of DNA\textsuperscript{15}. Dendrimers with charges $Z=4, 8$ can easily compensate a compact group of nearest $Z$ charges of both DNA helices. If a DNA double helix is totally covered and neutralized by such dendrimers an additional dendrimer can still be adsorbed on DNA because one monomer of each of $Z$ already adsorbed dendrimers can be raised above the DNA surface. As in the case of linear molecules, this leads to fractionization of the dendrimer charge and to the gain of its self-energy.

Returning to DNA-PE complexes we would like to remind the reader about additional mechanisms of charge inversion beside the fractionization mechanism. So far, to clearly demonstrate the role of the fractionization mechanism in charge inversion, we worked with the case when distance between charges of PE, $b$, is equal to the distance between charges of an unfolded DNA spiral, $a$. One can show that if $b$ varies in the vicinity of $a$, the point $b = a$ is the local minimum of the charge inversion ratio. Away from $b = a$ point, interaction of a long PE molecule with a spiral of DNA charges can be calculated neglecting discreteness of PE and DNA charges, i.e. assuming that the charge is uniformly distributed along both the PE molecule and the DNA spiral.

Let us first assume that $b < a$ so that the PE molecule has larger linear charge density than the DNA spiral. Then PE molecules repel each other and form on DNA spiral a strongly correlated liquid where PE molecules alternate with vacant places. This liquid reminds a Wigner crystal. A new PE molecule pushes aside previously adsorbed ones or, in other words, attracts vacancies. This provides another mechanism of creation of image of an approaching PE molecule in addition to the defect formation mechanism. Therefore, the negative chemical potential of PE adsorbed on the spiral becomes larger in the absolute value and charge inversion increases. This is the same mechanism of Wigner-crystal-like correlations which was studied in Ref. 9,10,14. In the opposite case, $b > a$, when PE has a smaller linear charge density than a DNA spiral, more than one layer of PE is adsorbed on DNA to neutralize it. Polarization of the incomplete second layer by a new PE molecule results again in an additional to defect formation mechanism of attraction of this molecule to a neutralized DNA\textsuperscript{15}. This leads to larger charge inversion than in $b = a$ case.

Changing flexibility of a PE we can separate the role of fractionization. For example, for the absolutely rigid PE molecules defects can not appear and fractionization mechanism does not work. As a result at $b = a$, when the layer of PE neutralizing DNA is completely incompressible charge inversion vanishes (see a two-dimensional analog of this problem in Ref. 2). In a flexible PE, the fractionization mechanism adds charge inversion weakly dependent on $b$, making the minimal value of the charge inversion ratio at $b = a$ finite.

Wigner-crystal-like correlations play an important role in the discussed above case of DNA charge inversion by dendrimers, too. This happens when we deal with high generations of dendrimers which have a very large charge such as $32e$ or $64e$. Because of the three-dimensional architecture of their chemical bonds these molecules can not expand enough so that each charge of them reaches an opposite charge of DNA and compensates it. In other words, when projected to a DNA double helix, these high generation dendrimers have much larger linear density of charge than the double helix itself. Thus, large segments of the helix between adsorbed dendrimers remain negatively charged, and form a Wigner-Seitz cell...
around each dendrimer. This is how with growing charge of dendrimers the fractionization mechanism gets replaced by the mechanism of Wigner-crystal-like correlations. Qualitative difference between low and high generations of dendrimers has been clearly demonstrated experimentally.

Let us return to complexation of a DNA double helix with PE molecules with the matching bond length, \( b = a \), and discuss another mechanism of charge inversion, which is related to the discreteness of DNA charge and further increases the positive charge of DNA-PE complex. Let us consider a monomer tail of PE and explore whether some energy can be gained if the positive charge of this monomer moves down to the plane of DNA charges, approaches already neutralized negative charge of the DNA and shares it with the end monomer of the neighboring PE molecule in a way shown in Fig. 2. If these two end monomers may sit on opposite sides of the negative charge of DNA, the additional energy \( \frac{e^2}{2d} \) can be gained, where \( d \) is the distance of the closest approach of a PE monomer and a DNA charge. At a sufficiently small \( d \) this energy can be even larger than the gain per tail from elimination of the self-energy. In a DNA double helix, all the negative charges indeed are on the ridge above neighboring neutral atoms. Two sufficiently small monomers may fit into the large and small groves on both sides of the ridge. On the other hand if, because of sterical limitations, they can not be in the perfect opposition the energy gain is smaller and can even vanish.

In this paper, we have considered three mechanisms of charge inversion of DNA by a PE: fractionization of the PE charge, Wigner-crystal-like correlations and sharing of one DNA charge by two monomers of the PE. We showed that depending on properties of PE they can work in different combinations. In conclusion we emphasize that all these mechanisms are due to the fact that a new PE molecule rearranges already adsorbed PE in such away that its image or correlation hole strongly attracts this new PE molecule. None of these mechanisms can be described by the Poisson-Boltzmann theory because this theory uses the mean-field potential which does not depend on the position of the new PE molecule. Thus all three effects are based on correlations between different PE molecules. Fractionization of PE charge is the most visual realization of these correlations.

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