2066 *Macromolecules* **2003,** *36,* 2066−2071

Conformational Changes of Aliphatic Ionenes in Water-Salt Solutions as a Factor Controlling Stability of Their Complexes with Calf Thymus DNA

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Received August 20, 2002; Revised Manuscript Received December 19, 2002

ABSTRACT: Fractions of symmetrical X,X-ionenes ($X = 3$, 6 and 10) with degrees of polymerization DP = 30−250 and nonsymmetrical $2,Y$-ionenes ($Y = 4$, 6, 8, and 10), DP = 4−30, were used as positively charged partners of calf thymus DNA in polyelectrolyte complexes (PEC). The determined values of salt concentration, C^*_{salt}, corresponding to the complete dissociation of PEC increased with the lengthening of ionenes of both families. C^*_{NaCl} for PEC formed by relatively short $2,Y$-ionenes of the same DP increased markedly with Y decrease. However the impact of this factor was substantially diminished upon lengthening of the $2,Y$-ionenes. For the long-chained X,X-ionenes a more than 3-fold increase in X resulted only in moderate decrease in C^*_{NaCl}. These finding suggest that the effective charge density of different ionenes releasing from PEC at relatively high C^*_{salt} becomes comparable owing to their pronounced compaction. This assumption was supported by the results of treatment of the experimental data using the developed model of PEC dissociation in water−salt media, which established the conformations of the releasing chains as slightly more swollen than θ-coil for 3,3-ionenes, θ-coil for 6,6-ionenes, and close to globule for 10,10-ionenes. The findings appear to be of particular interest to gain a better insight into the mechanism of DNA interaction with positively charged counterpart in vivo and in vitro.

Introduction

Polyelectrolyte complexes (PEC) are the products of coupled reactions between oppositely charged polyions. The cooperative electrostatic interaction between the partners endows PEC with a specific set of new technologically useful properties,[1,2] in particular the ability to undergo reversible phase transitions in narrow range of pH and/or ionic strength.[3] Not surprisingly, PEC have been extensively studied and found applications in various fields of both pure and applied science.[1−3] Complexes formed by polycation and DNA (or oligonucleotide) as a negatively charged counterpart are of special interest for these PEC hold much promise as gene delivery vehicles, for reviews see refs 4−6. Recently a close correlation between efficiency of cell transfection by DNA-containing PEC with aliphatic ionenes and tolerance of the PEC for the external salt has been established.[7] It suggests that elucidation of factors controlling stability of PEC of this family merits detailed consideration.

The integrity of DNA-containing PEC in water−salt solutions is a function of macromolecular characteristics of the polycation.[8,9] Thus, lengthening of poly(N-ethyl-4-vinylpyridinium) bromide (PEVP) chains led to expected stabilization of PEC against destructive action of external salt.[8] This trend was also observed with growth of the number of positively charged N-ethylpyridinium groups in partly alkylated PEVP chains, i.e., with increase of linear charge density.[9] However, the results of similar experiments with aliphatic 2,4-ionene and 2,8-ionene (Scheme 1) as DNA partners published by us as a communication[10] revealed that the behavior of PEC could be different and far from trivial. Even though the lengthening of both ionenes also caused a pronounced increase in PEC stability, on achievement of a certain DP the difference in stability of PEC with these ionenes vanished. Thus, the statement that linear charge density of polycation is a factor controlling PEC stability is sound but not universally true. This fact might have far-reaching implications both in development of self-adjusted gene delivery polyelectrolyte systems and for elaboration of bioseparation techniques based on PEC.

Being encouraged by above finding, we aimed to elucidate the reasons of the revealed diminishing of the role of ionenes linear charge density in PEC stability. Of special interest was to clarify whether this feature is inherent in different polycations with relatively low charge density or it is a peculiarity unique to ionenes studied in ref 10.

The data obtained in the current work appear to be of particular interest to gain better insight into the mechanism of DNA interaction with positively charged counterpart in vivo and in vitro, in particular under physiological conditions.

Experimental Part

Reagents. Ethidium bromide (EB), NaCl, and Tris buffer were purchased from Sigma. Concentration of EB in solutions was determined spectroscopically[11] (extinction coefficient $\epsilon_{480} = 5600$ L mol^{-1} cm^{-1}). In all experiments twice distilled and additionally purified by Milli-Q (Millipore) water was used.

Polymers. Calf Thymus DNA. The Na salt of highly polymerized calf thymus DNA (~10000 base pairs) was purchased from Sigma and used without purification. The

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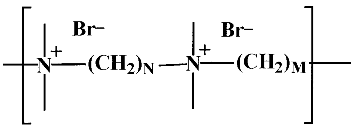
10.1021/ma021361x CCC: $25.00

Scheme 1



1) ***2,Y*-Ionenes:** N = **2**; M = **Y**;

2) ***X,X*-Ionenes:** N = M = **X**

purity of the DNA samples was verified by UV/vis spectroscopy and thermal denaturation profile of the sample. Concentration of DNA phosphate groups, [P] was determined by UV absorbance measurements[12] at 260 nm assuming molar extinction coefficient $\epsilon_{260} = 6500$ L mol^{-1} cm^{-1}.

Modified ionenes were synthesized by the Menshutkin reaction from *N,N,N′,N′*-tetramethylalkyldiamine and dibromoalkyles, subsequently modified by *p*-nitrotoluene bromide, fractionated by ion-exchange chromatography, and characterized as described in details elsewhere.[13] In all cases, the DP value signifies the number of charged groups per polymer chain.

Methods. Spectrophotometric measurements were performed using a Hitachi 150–20 spectrometer in water-thermostatic cell under permanent stirring.

The fluorescence intensity was measured using a Jobin-Yvon-3CS spectrofluorimeter in a quartz fluorescence cell at permanent stirring in TRIS buffer, pH 9.0, at 25 °C in a water-thermostated stirred cell holder. The excitation and emission wavelengths were 535 and 595 nm, respectively. In all experiments a DNA·EB complex of composition [P]/[EB] = 20/1, where [P] and [EB] are molar concentrations of the phosphate groups and the dye, respectively, was used. Solutions of polyelectrolyte complexes were prepared directly in the fluorimetric cell by mixing of definite volumes of stock solutions of the polymers. Fluorimetric titration was performed by successive addition of 4 M NaCl solution and measuring the intensity of fluorescence. Time interval between the additions was 5 min. The value of fluorescence intensity, *I*, was normalized by the intensity of fluorescence of DNA·EB solution at the same concentration of NaCl, I_0.

Results and Discussion

To emerge common features of dissociation of DNA-containing PEC in water–salt media, symmetrical *X,X*-ionenes (Scheme 1, part 2) with different numbers of methylene groups between charges along the chain (*X* = 3, 6 and 10) and various degrees of polymerization (DP), i.e., number of charged groups per polymer chain, have been synthesized and characterized.[13] Moreover, to consider in more details the role of charge density of relatively short polycations in PEC stabilization, the investigation of 2,*Y*-ionenes has been extended, so that the samples with *Y* = 4, 6, 8, and 10 were synthesized and studied as DNA partners.

The dissociation of PEC caused by addition of sodium chloride or different potassium halides was monitored by fluorescence quenching technique with the use of intercalating cationic dye ethidium bromide (EB). Upon intercalation into DNA double helix, a substantial increase in fluorescence intensity of the dye was observed. The electrostatic interaction of DNA with polycations resulted in competitive displacement of the dye to the solution followed by quenching of its fluorescence. The dissociation of PEC by the external salt allowed EB to intercalate in free sites of DNA registered by ignition of the fluorescence.[8]

To illustrate the assay of PEC dissociation, the fluorescence titration curves of DNA·EB solution and mixture of DNA·EB solution and solution of 2,8-ionene, DP = 15, with sodium chloride are represented in Figure 1. The solutions were prepared as it is described in the Experimental Part. The observed decrease of fluorescence intensity I_0 of the DNA·EB complex (curve 1) is caused by a changing of the environment of intercalated EB without expulsion of EB from the DNA structure. Though the addition of the salt is followed by a decrease of EB affinity to nucleic acid, the DNA·EB complex remains rather stable even at high ionic strength.[14] The negligible fluorescence intensity *I* of DNA·EB mixture with 2,8-ionene corresponding to the start point of curve 2 suggests that almost all EB molecules are squeezed out by the added polycation. Curve 2 has a sigmoid shape with right-hand branch reflecting the dissociation of DNA–2,8-ionene polyelectrolyte complex. The coincidence of both curves at $C^*_{\mathrm{NaCl}} = 0.2$ M indicates a complete liberation of the ionene from the complex and full intercalation of EB into the DNA double helix.

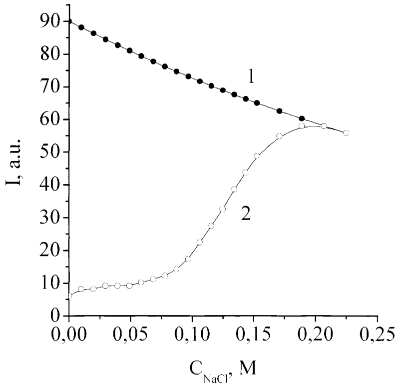


Figure 1. Dependence of fluorescence intensity I_0 of solution of DNA (1) and the intensity *I* of solution of DNA–2,4-ionene mixture with charge ratio $\varphi = [+]/[-] = 2.0$ (2) in the presence of ethidium bromide on NaCl concentration. [P] = 4×10^{-5} M, 0.01 M Tris buffer, pH 9.0, 25 °C.

To verify whether curve 2 of Figure 1 corresponds to equilibrium, portions of DNA·EB and an 2,8-ionene mixture of the same composition were prepared in NaCl solution and then diluted with different amounts of distilled water. Fluorescence intensity of the diluted portions coincided with *I* values measured by the salt titration at the same concentration of the components and ionic strength, at least in the studied region, [NaCl] > 0.05 M. It suggests that the right-hand branch of curve 2 reflects equilibrium process that can either be viewed as dissociation of PEC with increase in ionic strength of the solution[15,16] or association of the polyelectrolytes with its decrease.[17,18] Therefore, composition of the mixture should influence C^*_{NaCl} value according to the equilibrium of the dissociation/association. Yet in the range of the mixture composition, $\varphi \equiv [+]/[-] = 1.5–2.5$, this influence was rather weak (data not shown). Accordingly, the 2-fold excess of polycation was found to be optimal composition of the mixture. This composition provided the optimal signal response in the experiment and was utilized in all experiments described herein.

Fluorescence titration curves of this mixture (curve 2) and DNA·EB mixture with 2,4-ionene (curve 1) with the same DP = 15 are depicted in Figure 2 as a dependence of relative fluorescence intensity I/I_0 on the salt concentration. The curves slope steeply up on addition of definite amounts of salt proving a cooperative character of dissociation of DNA–2,*Y*-ionene polyelectrolyte complexes. These data are in accordance with

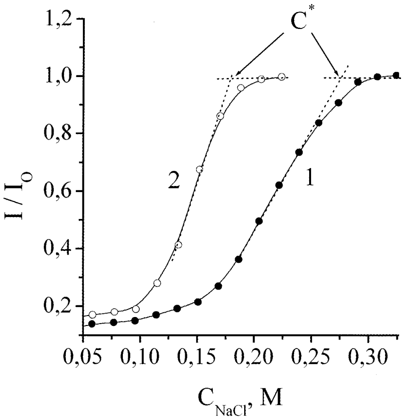


Figure 2. Dependence of relative fluorescence intensity I/I_0 on NaCl concentration in solutions of mixtures of DNA with 2,4-ionene (1) and 2,8-ionene (2), DP of the ionenes = 15. Other conditions are the same as in Figure 1.

the reported change of the binding constant of short polylysines with DNA upon increase in the ionic strength of solutions.[15] Values of the salt concentration, C^*_{NaCl}, corresponding to complete dissociation of PEC, were determined as shown in Figure 2 and defined as a quantitative measure of PEC tolerance for the added salt.

Formation of polyelectrolyte complex results in the liberation of a large amount of counterions from ionic atmospheres of the constituent polyions to the solution. Inasmuch as the interaction of the polyions is close to being athermic, the main driving force of PEC formation is an increase in entropy of the system due to the liberation of counterions.[15] Dissociation of PEC, i.e., the process reciprocal to the formation of the complex, inevitably results in entropically unfavorable reconstruction of the ionic atmospheres around the polyions. It is apparent that the dissociation proceeds at concentrations of the external salt equal or higher than the local concentrations of the ions in the ionic atmospheres of the polyions when the loss in entropy is compensate. The higher charge density of the polyion, the higher the electrostatic potential inside the polyelectrolyte coil and, hence, the higher the local ionic strength created by the counterions. Other things being equal, this should lead to higher C^*_{NaCl} values, which is the case (Figure 2).

Figure 3 shows the dependence of C^*_{NaCl} determined from the fluorescence titration curves on the degree of polymerization of 2,4-ionene (curve 1) and 2,8-ionene (curve 2). As expected, the lengthening of the ionenes leads to a progressive shift of C^*_{NaCl} to higher values. The tolerance of PEC for the external salt increases with decrease in Y, i.e., with increase of linear charge density of relatively short ionenes, cf. left-hand branches of the curves. However, the impact of this factor on PEC stability is diminished on lengthening of the ionenes, and at DP ≈ 25, it does not work at all.

We have conducted analogous experiments using long chained X,X-ionenes (Figure 4). A general run of C^*_{NaCl} vs DP curves remains the same, yet they are significantly shifted to higher ionic strength as compared with the curves corresponding to 2,Y-ionenes (Figure 3). The latter is readily explained by markedly higher values of the DP of X,X-ionenes used. In contrast to 2,Y-ionenes, the difference in C^*_{NaCl} values remains virtually unchanged in all studied DP ranges and is only moderate despite more than a 3-fold variation in the X value. This observation suggests that the impact of linear charge density on the stability of PEC formed by long-chained ionenes appears only slightly.

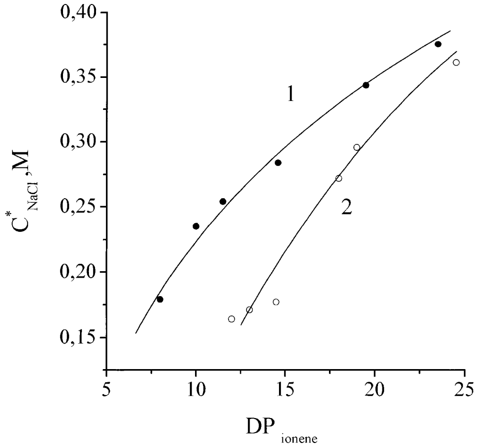


Figure 3. Dependence of C^*_{NaCl}, M on the degree of polymerization of the ionene in solutions of mixtures of DNA with 2,4-ionenes (1) and 2,8-ionenes (2). The conditions are in Figure 1.

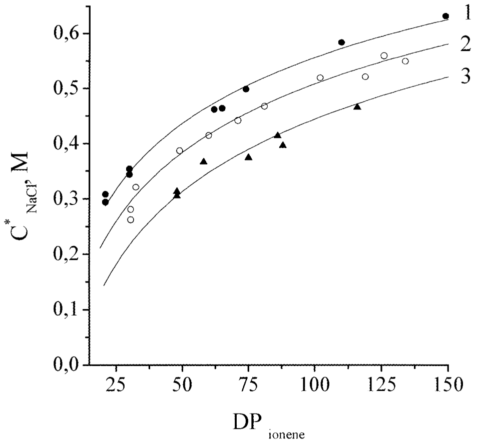


Figure 4. Dependence of C^*_{NaCl}, M on the degree of polymerization of the ionene in solutions of mixtures of DNA with 3,3-ionenes (1), 6,6-ionenes (2), and 10,10-ionenes (3). The conditions are as in Figure 1.

As an illustration of the important role of the ionenes DP in the influence of X (Y) on the stability of PEC, Figure 5 shows dependencies of C^*_{NaCl} determined for X,X-ionenes, DP = 100, and 2,Y-ionenes, DP = 15, on mean linear charge density ξ of the polycation. Parameter ξ was calculated according to formula $\xi = e^2/4\pi\epsilon\epsilon_0 kTb$, where e is a unit charge, ϵ and ϵ_0 are the electric permittivity of water and vacuum respectively, k is Boltzman's constant, T is absolute temperature, and b is the mean charge spacing of the fully unfolded chain. For the sake of convenience, the ξ range was limited by the values inherent in ionenes of both families, i.e., $\xi \cong 0.80$ ($X = 6$, $Y = 10$) and $\xi \cong 1.4$ ($X = 3$, $Y = 4$). In the case of the relatively short 2,Y-ionenes (curve 1) the growth of ξ value from 0.80 (2,10-ionene) to 0.93 (2,6-ionene) increased C^*_{NaCl} by 0.1 M or 57%, whereas the comparable change of ξ for the long-chained X,X-ionenes (curve 2) resulted in only 0.04 M NaCl shift or 8%.

Different factors could be accounted for to explain the revealed diminishing of the role of X (Y) values in the PEC tolerance to dissociation. The topological complementarity of the interacting chains is evidently important for pairs of stiff-chained polyelectrolytes or the ones with fixed conformation having limited if any flexibility

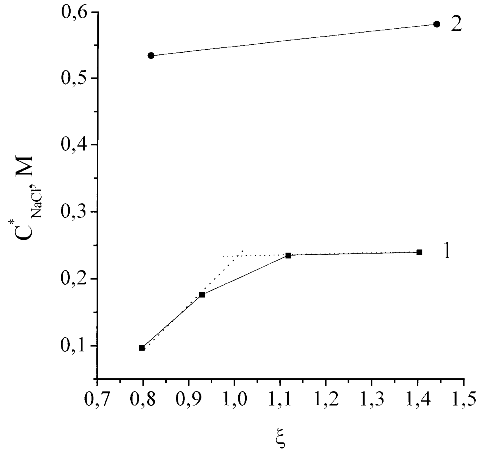


Figure 5. Dependence of C^*_{NaCl}, M determined in solutions of mixtures of DNA with 2,Y-ionenes, DP = 15 (1), and X,X-ionenes, DP = 100 (2), on the mean linear charge density of the polycations. The conditions are as in Figure 1.

to tune adjust and form optimal contacts. In contrast to rigid DNA double helix, aliphatic ionenes are rather flexible. Accordingly, the complementarity could noticeably influence PEC stability only if the dissociation occurs at relatively low ionic strength, otherwise a shielding of the charges of the releasing ionenes by the external salt would result in their folding. Hydrophobic interactions between the polyions can also contribute to the stability of PEC, yet the data presented in Figure 4 show that this factor does not dominate over the electrostatic interactions of the polyelectrolytes. Thus, stability of PEC decreases with increase in X, i.e., with increase in hydrophobicity of the chains. What seems to be the most deserving approach to explain the data presented is the elimination of the difference in charge density of different ionenes releasing from PEC formed by long-chained ionenes.

PEC dissociation is controlled by the properties of the shorter constituent of the complex,[2] in particular DP and charge density of the polycation in DNA-containing PEC.[8-10] As to DP, it is evidently independent of the environment. The same is true for the linear charge density. However, it appears to be only the hypothetical case of fully stretched chain when its charge density is solely dictated by *X (Y)* values of the ionenes. Inasmuch as the dissociation is caused by addition of salt, the size of the polycation coil in the moment of its release from PEC is inevitably lesser than in salt free solution due to electrostatic shielding of the positively charged groups by added counterions.[19] Thus, effective charge density of the releasing chain is higher than the linear charge density. The higher the DP of the polycation the higher the C^*_{NaCl} value. Accordingly, the releasing chains are more compact, and hence, the effective charge density for different ionenes becomes comparable. The conformational changes of the ionenes with high *X (Y)* are favored by the presence of long flexible sequences of methylene groups in the chains. Moreover, it seems plausible that intramolecular hydrophobic interactions, particularly pronounced in the latter case, also facilitate the compaction of the ionene chains.[20] Using this line of reasoning, it is appropriate to assume that difference between effective charge densities of various ionenes of the same DP becomes lesser and lesser with gradual increase of DP, which is the case (Figure 3).

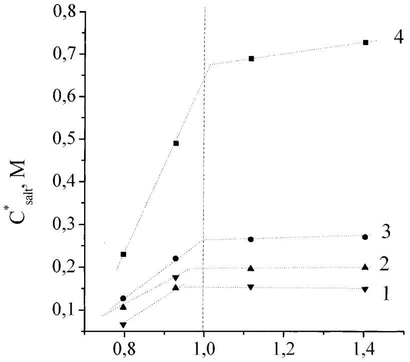


Figure 6. Dependence of C^*_{KX}, M determined in solutions of mixtures of DNA with 2,Y-ionenes, DP = 15, in solutions of different potassium halides: I^- (1), Br^- (2), Cl^- (3), and F^- (4) on the mean linear charge density of the polycations. The conditions are as in Figure 1.

Thus, the external salt might produce a 2-fold effect on PEC stability acting in the opposite directions. On one hand, it weakens the interpolyelectrolyte interactions screening the charges in the ion pairs and hence, inducing PEC dissociation, and on the other, the compaction of the flexible polycation results in growth of its effective charge density stabilizing PEC against destructive action of the external salt.

It is intriguing that substitution of 2,6-ionene for 2,4-ionene (DP = 15) does not result in any noticeable change of PEC stability. Curve 1 of Figure 5 consists of two practically linear parts with inflection point at ξ close to unity (shown by dotted line). In contrast to a significant stabilization of PEC with ξ increase at $\xi \leq 1$, further increase in ξ virtually does not influence C^*_{NaCl}. The observed disruption in PEC stabilization appears to be explicable on the basis of the polyelectrolyte theory of Manning.[21] According to Manning, at $\xi > 1$ a condensation of counterions onto the polyion from the bulk solution should occur to decrease the ξ value to unity. Thus, an increase of linear charge density of the polyelectrolyte above unity should not change its effective charge density. Accordingly, the dissociation of PEC formed by ionenes with $\xi > 1$ should proceed at similar values of ionic strength, which is the case (Figure 5, curve 1). This reasoning is supported by the data on electrophoretic mobility of the polyelectrolytes of various linear charge density in free solution and gel electrophoresis[22,23] for which the increase in ξ value was followed by increase in the mobility only to ξ value equal to unity. Further increase in charge density of the polyion resulted in little if any change in its mobility, as predicted by Manning.[24] Yet it was shown[15,25] that for the short-chained polyions the counterions' accumulation per structural charge of a macroion is relatively small questioning the applicability of the above explanation of data presented in Figure 5 and 6 on the basis of Manning theory. What seems to be a more reasonable explanation is the compaction of the ionene chains at high ionic strength as discussed previously. Indeed, the dissociation of PEC with 2,6-ionene already proceeds at relatively high ionic strength that can be sufficient for pronounced compaction of the chains providing similarity in effective charge density of 2,6-ionene and 2,4-ionene at PEC dissociation.

Interestingly, the same nonmonotonic changing of PEC stability formed by different 2,*Y*-ionenes with DP = 15 is observed when the dissociation is achieved by addition of various potassium halides (Figure 6). Although determined C^*_{Salt} values are significantly different, a general run of the curves remains the same.

In all cases, a decrease of Y from 10 to 6 results in the expected pronounced rise in PEC stability, but on further shortening of the distance between charges along the chain this tendency ceases to be true. Tolerance of PEC for the salts progressively reduces upon substitution of anions in the row F^-, Cl^-, Br^-, and I^-, and agrees well with the previously published data for another pair of oppositely charged polyelectrolytes.[8] Presented above row of potassium halides, destructive action is also consistent with their ability to cause the conformational changes of the ionenes.[19] This finding provides an added argument sustaining the hypothesis of the crucial influence of the conformation of the releasing polyelectrolyte chains on the value of the ionic strength at which the dissociation proceeds.

To further verify the above reasoning the experimental data were treated with the use of the developed and presented below model of PEC dissociation.

Model of PEC Dissociation in Water—Salt Media. The stability of PEC formed by oppositely charged longer polyelectrolyte (LM) and shorter polyelectrolyte (SM) is strongly dependent on DP of the SM component.[2] The total change in Gibbs energy on complexing between one LM chain and one SM chain is a linear function of the DP of the SM component both for complexes stabilized by electrostatic interactions and for hydrogen bonds.[26] The dissociation of PEC, in particular in water−salt media, is also dependent on DP of the shorter polyion, but this dependence does not obey a simple linear proportion between ΔG and DP.

In salt-free solutions or at low concentrations of external salt, PEC is stable and conformations of both constituting polyelectrolytes are agreed, so that the number of interchain salt bonds, n, is proportional to DP of SM (to simplify, n = DP). Let us consider the transformations caused by addition of salt to solution of PEC as consisting of two stages.

First Stage. The number of the interchain salt bonds decreases without release of SM to the solution, i.e., concentration of the external salt is lower than that needed for dissociation of PEC. In this case the n value changes from n = DP to $n = x$(DP) < DP.

Second Stage. SM chains appear in solution due to the destruction of the last x interchain salt bonds at an "intrinsic" salt concentration C (DP). The dissociation of PEC is considered to be an equilibrium process

$$(\mathrm{LM{-}SM})_x + x\mathrm{AB} = x\mathrm{LM{-}A} + x\mathrm{SM} - \mathrm{B}$$

where (LM−SM) is an interchain salt bond and A and B are the ions of the external salt.

Equilibrium constant K can be expressed as

$$K[(\mathrm{LM{-}SM})_x]C^x = ([\mathrm{LM{-}A}][\mathrm{SM{-}B}])^x$$

This equation is then transformed into

$$u/x(\mathrm{DP}) = v - \ln C(\mathrm{DP})$$

where $u = \ln\{K[(\mathrm{LM{-}SM})_x]\}$ and $v = \ln\{[\mathrm{LM{-}A}][\mathrm{SM{-}B}]\}$.

Gradual dissociation of salt bonds between the polyions that is considered in the model is evidenced by dissolution of stoichiometric PEC particles at salt concentrations much lower than that ones required for the complete dissociation of the complex.[13] To distinguish between "salt-bonded" and "unbound" groups, the relation between the Coulombic interactions and thermal energy kT (k = Boltzmann constant, T = absolute temperature) can be considered. If the former dominates, the two groups can be counted as "bound", otherwise the groups can be considered "unbound". The dissociation of PEC proceeds when the number of the "bound" groups is not enough to keep the chains from separation. It needs to be emphasized that distance between the charged groups of the polyions is most probably variable, and the model does not imply the univocal categorization of the ionene and DNA charged groups into the "salt-bonded" and "unbound".

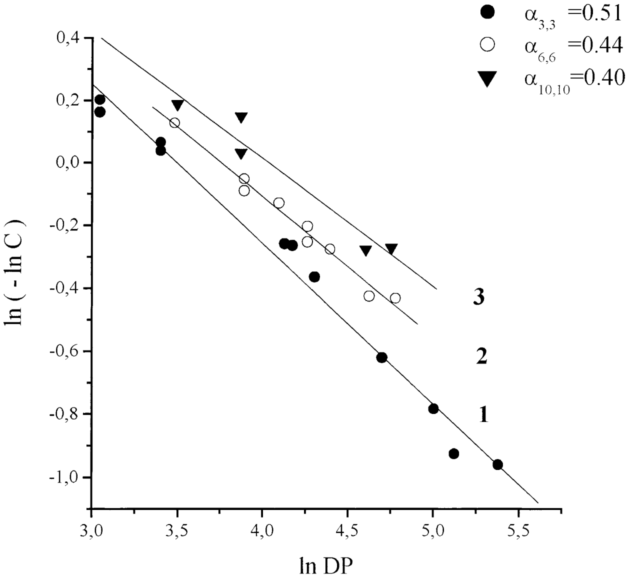


Figure 7. Dependence ln(−ln C) vs ln(DP) for DNA mixtures with X,X-ionenes: X = 3 (1); 6 (2) and 10 (3). The curves are constructed from the data presented in Figure 4.

As a first approximation, for PEC including SM with different DP at fixed initial concentrations of the polyions and temperature: (i) u and v are constant; (ii) the function x(DP) is the same.

In the system under surveillance LM is native rigid double stranded DNA. Sodium chloride has negligible effect on conformation of the double helix in the whole studied range of the ionic strength. Accordingly, the LM is a rod, at least on the length scale of a single ionene chain. If so, x will be proportional to the length of LM segment occupied by SM, i.e., to linear size of SM coil, which, in turn, is proportional to DP^{α}. Thus, the dependence of the "intrinsic" salt concentration on DP of SM should be linear in coordinates $-\ln C$ vs $\mathrm{DP}^{-\alpha}$, and the only varying parameter of the model, α, depends on the conformation of SM at the dissociation. Parameter α equals 1, $^3/_5$, $^1/_2$, and $^1/_3$ if at the dissociation SM is a rod, swollen coil (coil in athermic solution), θ-coil and globule, respectively.[27]

To verify above model approach the experimental data obtained for X,X-ionenes (Figure 4) were transformed into dependences ln(−ln C) vs ln DP (Figure 7) and −ln C vs $\mathrm{DP}^{-\alpha}$ (Figure 8). Parameter C evidently coincides with C^*_{NaCl}. Indeed, the dependence −ln C vs $\mathrm{DP}^{-\alpha}$ for the 3,3-ionene (Figure 8, value of α determined from Figure 7) is linear (correlation coefficient R = 0.99), justifying the model approach. The values of the parameter α determined from the slopes of the linear graphs shown in Figure 7 for 3,3-, 6,6-, and 10,10-ionenes equals 0.51, 0.44, and 0.4, respectively. It suggests that at PEC dissociation 3,3-ionenes in the studied DP region are slightly more swollen than θ-coil,

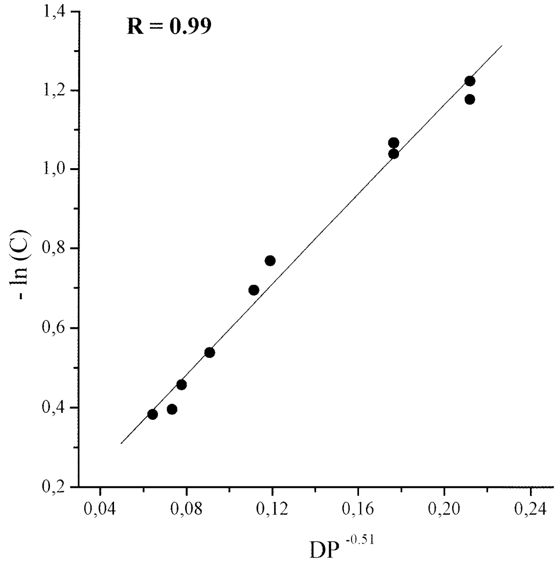


Figure 8. Dependence $-\ln(C)$ vs $DP^{-0.51}$ for DNA mixtures with 3,3-ionenes obtained from the data in Figure 4 (curve 1).

whereas the conformation of 6,6-ionenes is more compact than θ-coil and 10,10-ionenes are more compact than θ-coils approaching a globule.

This result is rather expected since the dissociation of PEC proceeds at $C_{\mathrm{NaCl}} > 0.3$ M. Rembaum et al. conducted an extensive research on viscosity of aqueous solutions of aliphatic ionenes with various distance between charges along the chains.[19] It was demonstrated that an increase in the external salt concentration leads to a pronounced decrease in the reduced viscosity of ionene solution with the most drastic changes at concentrations of potassium halides lower than 0.2 M. In 0.4 M KBr the parameter a in the Mark–Kuhn–Houwink equation was essentially constant for different ionenes and equaled 0.58–0.61, corresponding to the coiled conformation of the polyelectrolyte chain. The good accord in the conclusions based on the approach developed herein and the data of Rembaum et al.[19] suggests that, though the model is far from ideal, it nevertheless adequately describes the conformational state of the ionenes releasing to solution at PEC dissociation.

It needs to be emphasized that the thermodynamics of the polyions' interaction[15,16] as well as polyion–surface interaction[17,18] was studied by a number of researches with the key goal of the study on obtaining the values of the binding constant or its dependence on various parameters such as, temperature,[17,18] pH,[16] and ionic strength[15,16] of the solution, etc. In the model proposed, the explicit equation for the binding constant of the two polyions was left out of consideration, as the primary goal was set to elucidate the conformation of the interacting/dissociating polyelectrolyte chains. The evaluation of the binding constant of the utilized and other polyions is the subject of the ongoing research.

Thus, the effective charge density of the releasing ionenes is much higher than that calculated for the fully stretched chains. It explains the poorly pronounced dependence of PEC stability on the X and Y values of the ionenes in the region of their high DP. This also suggests that control over PEC stability by the choice of the ionene with a proper linear charge density can be realized only if the ionene is sufficiently short to provide PEC dissociation at moderate ionic strength when the releasing ionene is not too folded; otherwise, the control fails.

Acknowledgment. The financial support of this research by grants from INTAS (Project No. 00-113) and RFFI (Project No. 02-03-32263) is gratefully acknowledged.

References and Notes

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MA021361X