DNA Compaction by Divalent Cations: Structural Specificity Revealed by the Potentiality of Designed Quaternary Diammonium Salts

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DNA interaction with quaternary diammonium dications, $R(CH_3)_2N^+(CH_2)_nN^+(CH_3)_2R$, having various intercharge distances, lengths, and branching, and the chemical nature of the hydrophobic substituents were investigated by fluorescent microscopy and circular dichroism (CD) spectroscopy to reveal their structural specificity for binding to DNA. The conformational behavior of DNA was found to be highly sensitive to the structure of the dications with separated charges. The distance between two ammonium groups greatly influences the compaction activity of the dications. To explain this situation, we proposed a model that demonstrates that the charge density of the dication and the geometric fit between DNA phosphates and the ammonium groups in the dications play an important role in providing efficient DNA collapse. Elongation of the alkyl substituents (R) in the diammonium salts from ethyl to hexyl did not generate any significant alterations in the compaction activities, whereas the branching of substituents caused a drastic decrease in their compaction ability. Based on the results of CD spectroscopy, it was found that the ability of the dications to provoke a DNA transition from the B-form to A-form was also specific: it depended on their intercharge distances and was independent of the length of alkyl substituents.

Introduction

DNA compaction is of great interest because of possible biological applications for gene delivery into cells,[1, 2] and there have been numerous attempts to introduce DNA to different compaction agents in order to achieve controllable and practical DNA condensation.[3, 4] Compaction of DNA molecules can be caused by the action of various organic and inorganic multivalent cations.[5–10] According to Manning's condensation theory as modified by Wilson and Bloomfield,[11–14] charge neutralization of DNA, when the linear charge density of polymer is reduced by 89–90% by counterions, induces self-association and transition from an elongated coil state into a compact state. Under this theory, divalent cations cannot induce DNA compaction because the maximum charge neutralization of DNA does not exceed 88%. This theoretical state was later proved by Gosule and Shellemann,[15] who reported that DNA could not be collapsed by divalent Mg^{2+} ions in aqueous solutions. Divalent polyamines were also recognized as being generally ineffective compounds for DNA collapse in aqueous media.[16–18] However, at elevated temperatures[6] or in water/methanol,[14] the collapse of DNA can be induced even by divalent cations. Thus, the minimum charge of a molecule required to induce DNA condensation under normal conditions (aqueous solutions and ambient temperature) was established to be 3+,[19,20] and compaction agents such as spermidine (3+),[21] spermine (4+),[22] and hexamine cobalt(III) (3+),[7] have been commonly employed to study DNA condensation caused by multivalent cations.

So far, DNA interaction with multivalent cations with separated charges has been mainly studied in connection with DNA binding with charged polyamines and their different homologues in order to understand biological processes in vivo:[23–32] polyamine structure was found to influence the interaction with DNA in all such systems. The complexity of DNA–polyamine systems gave rise to numerous models and interpretations of DNA interactions with polyamines; however, the nature of the efficiency of such polycations with separated charges is still unclear. To explain the inconsistency of known binding models, it was suggested that DNA and polyamines may have a number of competing binding models, each of which correlates with a different polyamine function.[33]

DNA condensation by divalent cations attracted less interest than condensation by compounds with a higher number of

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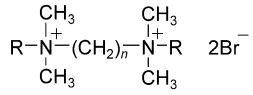
charged groups, probably due to the early reported results on ineffective DNA compaction by divalent ions in aqueous solutions.[34, 35] Despite several reports[26, 27] in which the possibility of DNA collapse by dications (diamines) was demonstrated and the structural effect was mentioned, currently available information about such systems is scant. However, understanding the structural effectivity of a dication as the simplest example of a multivalent cation with separated charges for DNA collapse can clarify more complex systems such as DNA with the natural polycations spermidine and spermine. Quaternary diammonium dications, $R(CH_3)_2N^+(CH_2)_nN^+(CH_3)_2R$, are suitable models for obtaining a wide spectrum of divalent condensing agents with molecular charges in various geometric locations, charge values on nitrogen atoms, and hydrophobicity, and for altering the molecular volume of the dication by appropriate changes in the number of methylene groups (*n*) between the N^+ atoms and in the chemical structure of substituents (R). From our recent studies it has become obvious that quaternary ammonium dications are able to induce DNA folding transition at the level of a single molecule. We have briefly reported that the structure and hydrophobicity of the dications (Scheme 1) influences the morphology of partly folded DNA products formed during compaction.[36]

In the present study, we focused on the relationship between the structure of the dicationic compaction agent and its activity to induce conformational transitions of DNA at the level of DNA large volume changes (the coil – globule transition), and at the level of internal changes in the DNA double helix (B- to A-family form transition).



EtMeEt HxPrHx

EtEtEt BzlPrBzl

EtPrEt (MePe)Pr(MePe)

EtBuEt (EtBu)Pr(EtBu)

BuPrBu

Scheme 1.

Results and Discussion

DNA compaction by quaternary diammonium salts

Fluorescence microscopy (FM) has been successfully applied to monitor the conformational changes of individual giant T4 DNA (166 kbp) molecules induced by various condensing agents in dilute aqueous solutions.[37–41] We used FM to visualize the conformational behavior of individual DNA molecules in the presence of different quaternary diammonium salts. Figure 1 A – D shows typical fluorescent images of T4 DNA molecules in the

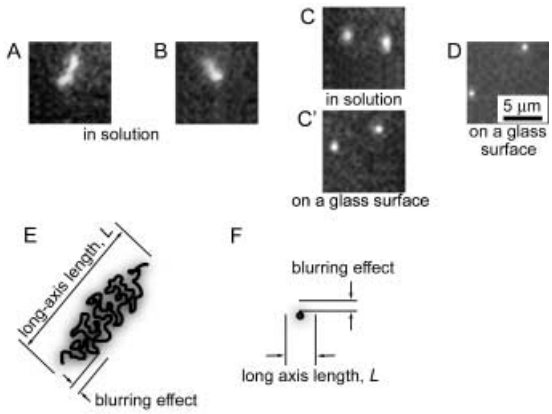


Figure 1. *A – D: Fluorescent images of T4 DNA in a 0.01 M Tris – HCl buffer solution (pH 7.8) at various concentrations of HxPrHx. A) Control, B) 2×10^{-4} M, C and C') 5×10^{-4} M, D) 5×10^{-3} M. E and F) Schematic illustrations of the fluorescent image of the DNA molecule and the definition of the characteristic parameter, DNA long-axis length* L, *for the coil (E) and the globule (F) states.*

presence of different amounts of the dication HxPrHx. As expected, at low concentrations T4 DNA adopts the coil conformation and moves randomly, exhibiting Brownian motion (Figure 1 A and B). An increase in the dication concentration results in the compaction of DNA into small particles, that is globules (Figure 1 D). At intermediate concentrations, coils and globules are observed simultaneously (Figure 1 C and C'). Thus, individual DNA molecules exhibit conformational change between the coil and globule states through interaction with diammonium salts.

During FM observations, it was noted that the numbers of globules on the bottom and cover glasses of the microscope cell were similar, and it was suggested that the appearance of DNA globules on both glasses was due not to the precipitation of neutralized DNA, but to the electrostatic interaction of the compact DNA with the negatively charged glass surface. In such a system, the positively charged divalent cations act as cationic "bridges" between the negatively charged DNA and the glass surface. Therefore, we carried out FM observations using diaminosilane-treated glass cells, the surfaces of which are positively charged in water solutions. In such cells, most DNA

globules were found to move freely in solution. In addition, centrifugation (12 000 rpm, 10 min) of the solution containing DNA globules did not remove the compact DNA, as detected by FM observations before and after centrifugation. These experiments proved that the final DNA – dication complexes are stable against precipitation in aqueous solutions. It is known that complexes of DNA with polyvalent cations (spermidine, spermine, surfactants, etc.) are insoluble in water and precipitate from aqueous solutions at the appropriate concentration of the compaction agent.[42, 43] In contrast, DNA complexes with divalent cations are soluble in aqueous media.

To examine the conformational transition of DNA molecules induced by diammonium salts quantitatively, a series of measurements was carried out at concentrations from 1 μM to 0.5 M. Figure 2 shows the evolution of the effective long-axis length *L* of a DNA molecule with the addition of dications, measured as shown in Figure 1 E and F. At the beginning of the interaction, all DNA molecules are in the coil state with the maximum *L* distribution close to 3.3 μM. Generally, with a further increase in the dication concentration, the values of *L* become smaller and globules (about 0.7 μM) appear as the final product of DNA collapse. The shaded areas in Figure 2 correspond to the regions where coils and globules coexist. Finally, only globules of DNA were observed. It becomes clear that all dications convert DNA conformation in a similar way from the coil state through gradual coil shrinking and coil/globule coexistence into the globule state. It should be noted that not all stages of DNA compaction are found for every dication due to variations in concentration, but the initial shrinking of DNA coils has been observed for all dications; this indicates that the DNA compaction routes are similar.

Shrinking of the DNA coil in solution is associated with strong intrachain segregation in the DNA molecule during interaction with the dications. The internal structure of the DNA coil in solution is "hidden" by the blurring effect of the dyed DNA molecules, but can be revealed by hydrodynamic stretching of DNA molecules, as was recently reported.[36] In addition to stretched DNA chains and compact globules as the beginning and final states, respectively, of DNA coil – globule transition, DNA molecules with intrachain segregation were also observed. Typical fluorescent images of segregated T4 DNA after hydrodynamic stretching are shown in Figure 3, here DNA molecules with various numbers of segregation centers on the unfolded chains like "beads on a string".[44] Thus, intrachain segregation is the intermediate morphology of T4 DNA, which undergoes a coil – globule conformational transformation.

To distinguish between different structural effects on DNA collapse, the synthesized compounds were divided into two groups. Compounds of the first group (EtMeEt, EtEtEt, EtPrEt, and EtBuEt Scheme 1, left) have the same substituents ($R = C_2H_5$), but a different number of methylene groups (*n*) between cationic nitrogen atoms. The second group (on the right side of Scheme 1) consists of compounds with the same trimethylene spacer ($n = 3$) and different substituents (R). This group consist of compounds with straight-chain alkyl substituents of different lengths: EtPrEt, BuPrBu, HxPrHx; different branched alkyl chains with the same carbon number: (MePe)Pr(MePe), (EtBu)Pr(EtBu);

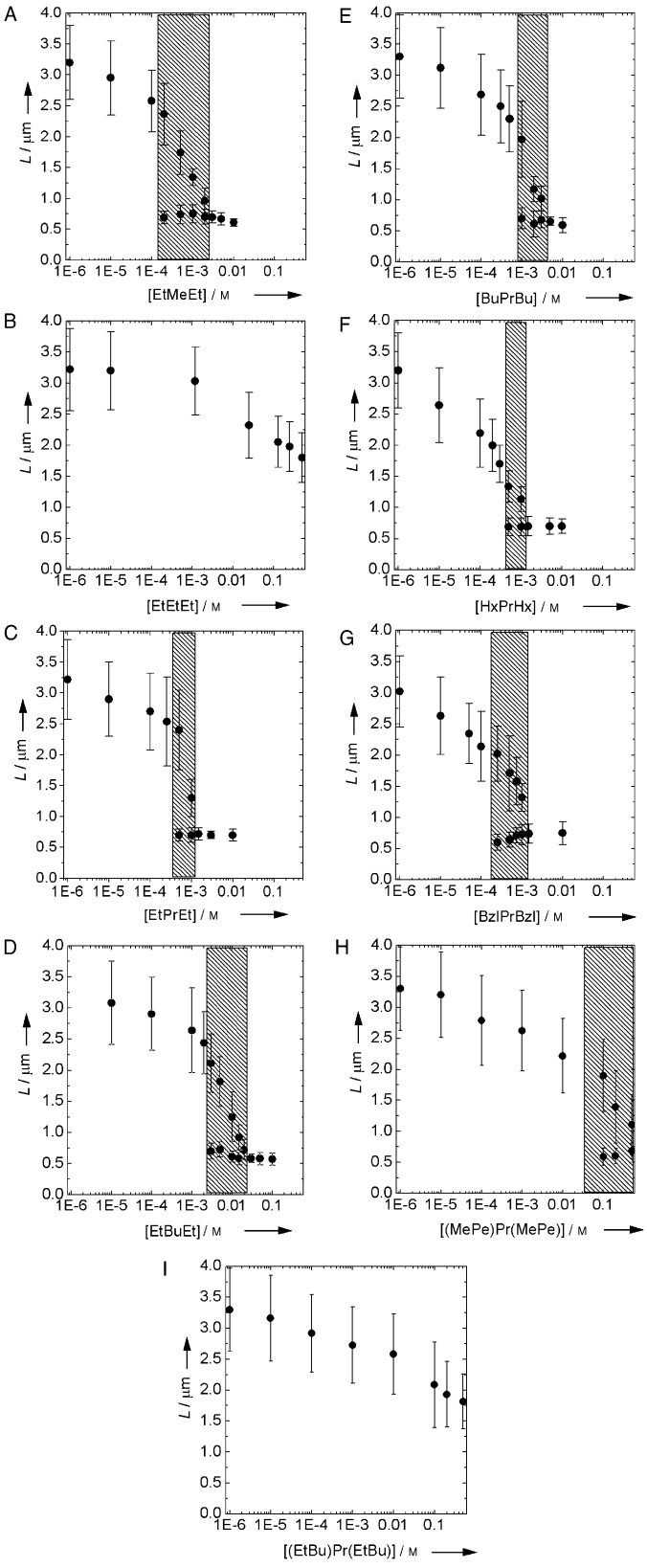


Figure 2. *Dependence of the long axis lengths (L) of T4 DNA molecules on the concentration of quaternary diammonium salts: A) EtMeEt, B) EtEtEt, C) EtPrEt, D) EtBuEt, E) BuPrBu, F) HxPrHx, G) BzlPrBzl, H) (MePe)Pr(MePe), I) (EtBu)Pr(EtBu). The circles on the plots indicate the maxima of the DNA length distributions; the statistical error of the distribution is given as a standard deviation. The striped areas represent the coil – globule coexistence region.*

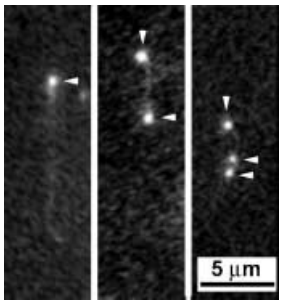


Figure 3. Typical examples of fluorescence images of T4 DNA molecules after interaction with EtPrEt and stretching on a microscopic glass surface by hydrodynamic flow, [EtPrEt] = 1×10^{-3} M. Pointers indicate segregation centers on the single DNA chains.

and a dication with aromatic substituents: BzlPrBzl. For both dication groups, correlation of dication activity with DNA compaction, obtained by FM, are summarized in Figure 4, where the bars indicate coil – globule coexistence regions.

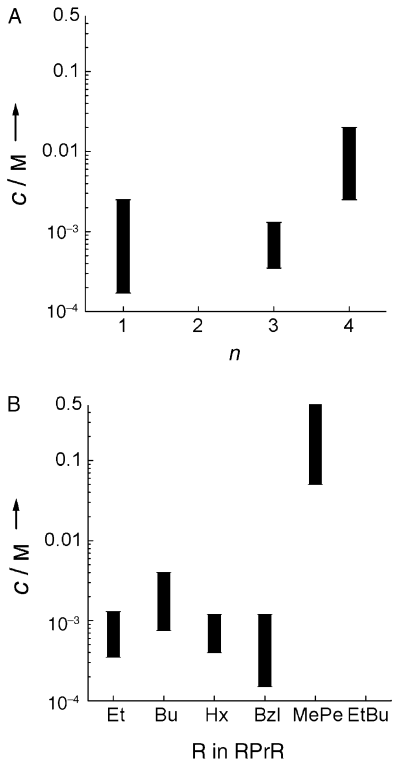


Figure 4. Summarized diagrams of DNA compaction activity of diammonium salts with various intercharge spacers n *(A) and various substituents R (B). Bars on the plots represent the coil – globule coexistence regions. For compounds EtEtEt (plot A,* n = *2) and (EtBu)Pr(EtBu) (plot B, R = (EtBu)), coexistence regions have not been achieved at the studied concentrations.*

The effect of the intercharge length on the compaction ability of dications

Figure 4 shows that EtMeEt induces collapse of DNA into globules at a concentration of about 10^{-4} M, and EtPrEt at a concentration twice as high, whereas a concentration of EtBuEt 10 times higher is necessary for complete DNA compaction. In contrast, DNA was not compacted into globules by EtEtEt even up to 0.5 M concentration. Thus, variations in the intercharge length result in a more than three orders of magnitude difference in the dication concentration required to achieve DNA collapse. The dependence of the diammonium cation activity on the number of methylene groups between charges is not monotonic and the experimentally found sequence of binding efficiency is as follows: EtEtEt $\ll$ EtBuEt $<$ EtPrEt $<$ EtMeEt.

Because DNA compaction is conducted by charge neutralization of the regularly spaced phosphates on the DNA double helix, cationic species with a higher positive charge density and with a structure affording better charge pairing are more effective for DNA compaction. Thus, we consider the two main factors responsible for the ability of the dications with separated charges to collapse DNA: the electrostatic factor and the geometric factor.

Electrostatics: To investigate the charge characteristics of the dications, the degree of dissociation of the diammonium salts of the first group was determined by using a bromide-selective

Table 1. *Degree of dissociation of diammonium cations with different intercharge distances.*

Diammonium salt	Degree of dissociation [%][a]		
	$c = 1.0 \times 10^{-5}$ M	$c = 1.0 \times 10^{-4}$ M	$c = 1.0 \times 10^{-3}$ M
EtMeEt	96	95	95
EtEtEt	94	93	93
EtPrEt	78	78	78
EtBuEt	88	88	88

[a] The degree of dissociation has been calculated as the ratio of the measured concentration of Br ions to the calculated concentration of Br ions in the range from 1×10^{-5} M to 1×10^{-3} M.

electrode. In Table 1 the dissociation degrees calculated as the ratios of experimentally measured bromide ion concentration in aqueous solutions of dications to the calculated bromide concentrations are shown. The obtained values are the same for each dication in the concentration range from 10^{-5} to 10^{-3} M. The degrees of dissociation found for dications with monomethylene and dimethylene spacers were nearly quantitative, whereas EtPrEt and EtBuEt dissociated to a lesser extent. These data might indicate that the chelate-like ion pairs of EtPrEt or EtBuEt are significant in attracting a bromide ion into the ammonium dications. In contrast, ion pairs of EtMeEt and EtEtEt with the same chelate structure are destabilized because of the strong internal stress in the dications. However, the 15% difference in the degree of dissociation of the different diammonium salts cannot play an essential role in the dication activity, since the concentration range over which DNA collapse by different dications occurs exceeds a few orders of magnitude.

As a result of the different charge separations in the dications, the charge density varies so that the compound with the

smallest methylene spacer (EtMeEt) has the largest charge density value, whereas the compound with the tetramethylene spacer (EtBuEt) has the smallest one. Positive charges in EtMeEt are separated by only one CH_2 group and it can be considered as a compact species with a condensed charge of 2+ (Figure 5).

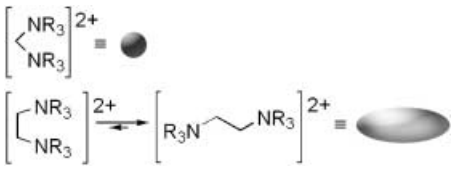


Figure 5. *Decrease in the dication charge density with increase in the intercharge spacer length.*

The compact (*syn*) conformations of EtEtEt and higher homologues are destabilized by the intercharge repulsion and the charge in these compounds becomes "diluted" as the length of the intercharge spacer increases (Figure 5). Dications with a higher charge density have a higher electrostatic binding to the negatively charged DNA chains, which leads to more effective compaction; this ability increases in the order EtBuEt < EtPrEt < EtEtEt < EtMeEt. This order of electrostatic efficacy reflects the actual experimental data (Figure 2) with the exception of EtEtEt, which is the weakest compacting agent within the diammonium homologues.

Geometric fitness: In order to understand the geometric correlations in the DNA – dication system, let us compare the intercharge distances in the diammonium salts obtained by molecular orbital calculations and the possible distances between the phosphate groups in DNA. The calculated distances between the nitrogen atoms of the fully extended zigzag conformation, which minimizes the electrostatic repulsion in dications, are 2.7, 3.9, 5.2, and 6.1 Å for EtMeEt, EtEtEt, EtPrEt, and EtBuEt, respectively. The distances between DNA phosphates on the opposite strands of the minor and major grooves are 13 and 17 Å, respectively, and the DNA intercharge distance between adjacent phosphates in the B-form is about 7.0 Å.[45] Binding across DNA grooves seems unlikely because the distances between such phosphates are significantly greater than the intercharge distances in the dications, and, in addition, incorporation into grooves is also unfavorable due to the bulky substituents of the diammonium salts. On the other hand, dication interactions with one or adjacent phosphates are most likely, and the specificity in compaction ability arises as a result of DNA and dication intercharge correlations, shown in Figure 6. The first homologue EtMeEt, which has the highest binding constant with DNA, has a 2.5 Å intercharge distance and can interact with only one DNA phosphate group. EtPrEt and EtBuEt, having maximum distances between nitrogen atoms of 5.2 and 6.3 Å, respectively, can interact through two ammonium groups with adjacent phosphates in DNA. Thus, the two ways of dication binding are suggested to be 1) two ammonium groups with two adjacent phosphates and 2) two close ammonium groups with one phosphate. The wider coexistence zone for EtMeEt (Figure 2) provides additional experimental evidence that EtMeEt and EtPrEt (or EtBuEt) bind with DNA in different ways. This may be related to the fact that the amount of EtMeEt, which binds with only one phosphate, needed to neutralize a certain amount of DNA phosphates is twice as high as is required for EtPrEt (or EtBuEt). Finally, taking into account these two mechanisms of DNA – dication interaction, the exclusive position of EtEtEt in this group of dications can be easily understood. The EtEtEt intercharge distance of 3.9 Å is too short to form two salt bonds with adjacent phosphates, and too long to realize two salt bonds with one DNA phosphate. Therefore, EtEtEt is able to form only one salt bond with DNA phosphate, similar to the monovalent counterion, and, as a result, the potential of EtEtEt to collapse DNA is very low. To summarize, the suggested model implies that the charge distribution on the dication and the geometric fit to DNA phosphates to form salt bonds through two ammonium groups play an important role in providing efficient DNA collapse by dication.

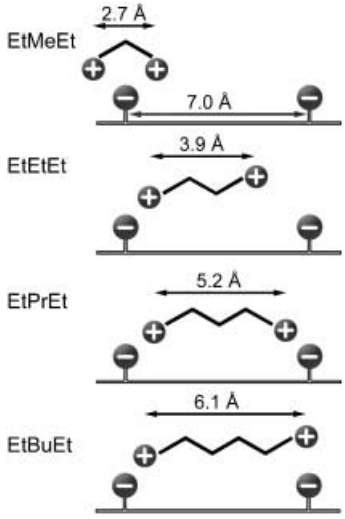


Figure 6. *Schematic representation of the correlation between intercharge distances in the diammonium salts and the distance between adjacent phosphates in DNA. The distances shown correspond to the calculated values of diammonium salts and literature data for the DNA intercharge distance.*[45]

The effect of hydrophobic substituents on the compaction ability of dications

Diammonium homologues having aliphatic substituents of different lengths (EtPrEt, BuPrBu, and HxPrHx) require similar concentration ranges to collapse DNA (Figure 2C, E, F and Figure 4B). EtPrEt and HxPrHx collapse DNA at a concentration of about 10^{-3} M, whereas the necessary concentration of BuPrBu is three times higher. The effectivity of the dication with different aliphatic substituents increases slightly in the sequence BuPrBu < HxPrHx < EtPrEt. Thus, the effectivity of these dications seemed to be mainly determined by the effectivity of the diammonium dication itself without alkyl groups, and their effectivity depends on the intercharge distance. Additional

factors that affect the effectivity of these compaction agents, are steric difficulties with increasing alkyl chain length, which have an unfavorable effect, and the hydrophobic interactions between alkyl chains, which have a favorable effect[18] on DNA compaction. Therefore, it is suggested that the lower compaction activity of BuPrBu compared with EtPrEt is the result of steric difficulties for the diammonium cation with longer alkyl substituents. Further "recovery of activity" in the HxPrHx case is due to strengthening of the hydrophobic interactions between alkyl chains, which stabilize the DNA – dication complex.

The influence of the alkyl chain length in diammonium salts on DNA conformational behavior is clearly demonstrated by the degree of DNA shrinking at the critical concentration for globule formation. Figure 2 shows that DNA size gradually decreases with increasing dication concentration, and at the beginning of the coexistence region, the average size of DNA coils is between 1.5 and 2.5 μm. In Figure 7, DNA size *L* at the beginning of the coexistence region is plotted as a function of alkyl substituent length in the dication. The increase in the hydrocarbon chain length causes nearly linear shrinkage of DNA size. This effect can be explained as the local decrease in dielectric permittivity (ε) near the DNA chain. It was recently reported that the size of DNA in solvents with a low dielectric constant is smaller than in aqueous media.[46] In the same way, longer alkyl chain dications, interacting with DNA, create surroundings of lower polarities near DNA which cause DNA shrinking.

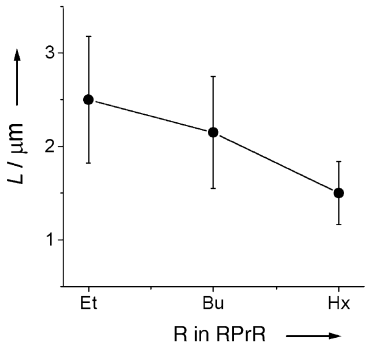


Figure 7. *Average long-axis lengths of T4 DNA molecules at the beginning of the coexistence region plotted as a function of the length of alkyl side chain in ammonium dication. The circles on the plots indicate the maxima of DNA length distributions; the statistical error of the distribution is given as a standard deviation.*

Dications with substituents with different branching (HxPrHx, (MePe)Pr(MePe), (EtBu)Pr(EtBu)) are very different in their compaction activity. As seen in Figure 2F, H, I and Figure 4B, the increase in branching of the alkyl substituent with six carbon atoms leads to rapid loss of dication efficiency for DNA compaction. Minor branching in the substituents of ((MePe)Pr-(MePe)) means that the concentration required to induce DNA collapse is 100 times higher than that needed with diammonium cation with n-hexyl groups (HxPrHx). Moreover, complete compaction of DNA into globules is not achieved until a 1 M concentration has been reached. A more branched dication, (EtBu)Pr(EtBu), has such a low potential for DNA collapse that even the coexistence region was not reached at molar concentrations of the compaction agent. It is unlikely that the main reason for the strong decrease in dication activity with increasing branching is the screening of charged ammonium groups, which might prevent electrostatic binding of the dication with DNA. Most probably, the main difficulties appear during filling the DNA chain with dications, which neutralize DNA negative charges. Branched dications bound with DNA significantly hinder adjacent DNA phosphates by their substituents. In extreme cases, both phosphates adjacent to the already occupied site on the DNA are blocked for further binding, and a maximum of only $^2/_3$ of phosphate groups can be neutralized by dications. This value of neutralization is much lower than that required for DNA collapse according to the counterion condensation theory (90%).

DNA compaction by dication with aromatic substituents (BzlPrBzl) is more effective than by diammonium salts with alkyl chains. Indeed, BzlPrBzl induced globule formation at a concentration of 1×10^{-4} M, and its effectivity is similar to that of EtMeEt in the first group. According to the calculated parameters of the diammonium salts, BzlPrBzl possesses a significantly higher charge on the nitrogen atoms (+0.73) than on other dications (+0.58 to +0.60). This increased charge appears to be due to the absence of β-hydrogen atoms in the molecule, which are able to effectively delocalize the charge on nitrogen. The positive-charge localization in BzlPrBzl is the obvious reason why the BzlPrBzl concentration required to induce DNA collapse is about five times lower than for dications with straight alkyl chains. Stacking interaction between aromatic rings of the diammonium molecules bound with DNA is an additional stabilization factor for DNA in a compact state.[47]

Transitions between DNA double-helix forms during interaction with dications

It is well known that the CD spectra of DNA are extremely sensitive even to small changes in the orientation of the polynucleotide bases in DNA, and provide important information about the geometric parameters of the DNA double helix.[44, 48] Interaction of DNA molecules with dications can cause conformational changes in the DNA secondary structure. To gain insight into such changes, we performed CD spectroscopic measurements of DNA in solutions of dications. Figure 8 shows the CD spectra of λ-DNA in a buffer solution at different concentrations of ammonium dications of the first group. The initial spectrum of DNA in a dication-free solution corresponds well with the spectrum of DNA in the B-form, which is the typical form for double helical DNA in aqueous media.[49] The original spectrum of DNA has nearly the same intensities of positive ($\lambda_{max} = 276$ nm) and negative ($\lambda_{min} = 245$ nm) bands and of the crossover ($\Delta\varepsilon = 0$) at the point corresponding to the adsorption maximum of DNA ($\lambda = 260$ nm). An increase in the concentration of EtPrEt or EtEtEt (Figure 8B and C) in the solution results in changes in the original DNA spectrum. The positive band intensity gradually increases and the intersection wavelength shifts to short wavelengths. In the second group of

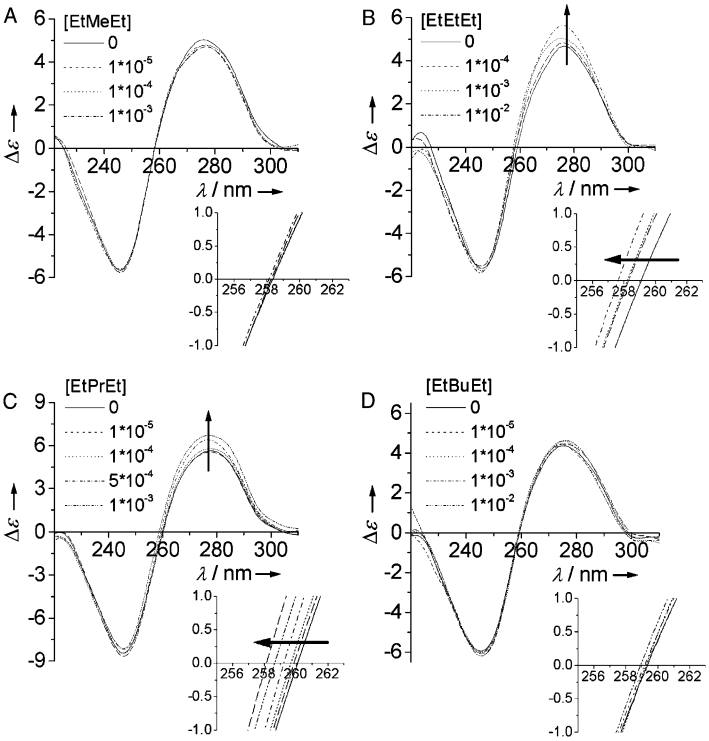


Figure 8. CD spectra of λ-DNA in 0.01 M Tris – HCl buffer solution (pH 7.8) at different concentrations of ammonium dication with various intercharge spacers: A) EtMeEt, B) EtEtEt, C) EtPrEt, D) EtBuEt; DNA concentration is about 1.5 × 10⁻⁴ M.

diammonium salts with the trimethylene intercharge spacer (EtPrEt, BuPrBu, HxPrHx), similar changes in CD spectra of DNA were observed (data not shown). We did not succeed in monitoring DNA spectral changes following the addition of BzlPrBzl due to the presence of BzlPrBzl in the sample solution, which strongly distorted the CD spectrum of DNA. In contrast to EtEtEt and EtPrEt, ammonium dications with methylene and tetramethylene "bridges" between two ammonium groups (EtMeEt and EtBuEt) have no effect on the initial CD spectrum of DNA (Figure 8 A and D). Even at high concentrations of these dications up to 1×10^{-2} M, the CD spectra of DNA retained their original shape. Thus, the ability of a dication with a different intercharge distance to change the DNA double helix is also specific. This specificity does not correlate with the dication activity for DNA compaction. However, the concentration ranges where CD changes have been detected for EtEtEt and EtPrEt are determined by the compaction activity of the dications, that is, the changes in CD spectra of DNA induced by EtPrEt appeared at much lower concentrations than those for EtEtEt.

According to published reports, changes in the CD spectrum of DNA induced by EtEtEt and EtPrEt correspond to the transition from the B-form family of DNA to the A-form family.[20] During the B – A transition, the geometric parameters of the DNA helix undergo structural changes. In particular, the distance between adjacent phosphate groups in DNA decreases from 7.0 (B-form) to 5.9 Å (A-form).[45] It is natural to expect that the specificity of the diammonium salts to induce this B – A transition is also based on the correlation between DNA and the dication intercharge distances in terms of the stabilization of B- or A-family forms of DNA. EtPrEt with an intercharge distance of 5.1 Å interacts with the adjacent phosphates on DNA in the B-form and tends to decrease the distance between phosphates in order to provide a better geometric fit between two phosphates and two ammonium groups as shown in Figure 9. We observed the same effect at high concentrations of EtEtEt (3.9 Å) and the nature of this

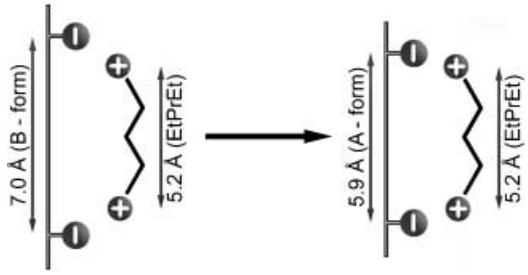


Figure 9. Schematic representation of the correlation between the intercharge spacer in EtPrEt and the distance between adjacent phosphates of DNA in the B-form (left) and the A-form (right). The distances shown correspond to the calculated values of diammonium salts and the literature data for DNA intercharge distances.[45]

effect was supposed to be the same as for EtPrEt. The intercharge distances in EtBuEt (6.1 Å) and in the B-form DNA (7.0 Å) are close to each other, therefore, the force needed to induce structural changes in DNA is weak or even absent, and thus DNA retains its initial geometric parameters of the double helix. As for EtMeEt, a molecule which was presumed to bind only with one phosphate group on DNA, it is evident that there is no preference to stabilize either the A-form or the B-form, and thus structural transitions in the DNA helix do not occur.

Compounds with the same trimethylene spacer changed CD spectra in a similar way and to a similar extent. Therefore, the intercharge distance of the dication is the main parameter responsible and influences the B – A transition in DNA, whereas small alkyl substituents have no significant effect.

It has already been established by Minyat et al. that polyamines stabilize the A- or B-form of DNA depending on their structure.[50] The authors suggested that the $-NH_2^+CH_2CH_2CH_2NH_2^+-$ moiety in polyamines is responsible for the B – A shift in DNA. Later, this suggestion was confirmed by the investigation of fibrous-oriented calf thymus DNA complexes with natural polyamines.[51] The contents of the A-form DNA in the complexes with 1,3-diaminopropane or spermidine ($NH_2(CH_2)_3NH_2(CH_2)_4NH_2$), which contain the trimethylenediamine unit, were significantly higher than the contents of the A-form DNA in complexes with putrescene (1,4-diaminobutane) or cadaverine (1,5-diaminopropane). According to our data, it is possible to generalize the conditions for the B – A shift in DNA induced by polycations. We can conclude that the B – A transition in DNA is dependent on the presence in the polycation of moieties with dimethylene or trimethylene spacers between the charged nitrogen atoms. The obtained results indicate that the transition from the DNA B-family form to the A-family form is caused by the adaptation of DNA helix parameters to the

geometric parameters of the polycation in order to provide the most effective electrostatic interactions between oppositely charged groups.

Conclusion

The results of this research demonstrate the influence of dication chemical structure on DNA conformational behavior and the form of the DNA double helix. Even among four homological diammonium salts with different intercharge spacers, every dication is unique because of its combination of efficiency for DNA collapse (effective: EtMeEt and EtPrEt; ineffective; EtEtEt and EtBuEt) and for realizing the shift from B- to A-form DNA (active: EtEtEt and EtPrEt: inactive: EtMeEt and EtBuEt). This flexibility in the effect of dications on DNA can be considered as the starting point for further research creating new chemicals for controlled DNA collapse.

Experimental Section

Materials and reagents: Bacteriophage T4dC DNA (166000 base pairs) was purchased from Nippon Gene (Japan) and was used for the fluorescence microscopy observations. λ-DNA (48502 base pairs), purchased from Takara Shuzo (Japan), was used for CD spectroscopic experiments. The fluorescent dye, 4,6′-diamidino-2-phenylindole (DAPI), and 2-mercaptoethanol (ME), which is a free-radical scavenger used to prevent fluorescence fading and light-induced damage of DNA molecules, were obtained from Wako Pure Chemical Industries (Japan) and were used without further purification. *N,N,N′,N′*-Tetramethyldiaminomethane, *N,N,N′,N′*-tetramethyl-1,2-diaminoethane, *N,N,N′,N′*-tetramethyl-1,3-diaminopropane, *N,N,N′,N′*-tetramethyl-1,4-diaminobutane, bromoethane, 1-bromobutane, 1-bromohexane, 1-bromo-4-methylpentane, 1-bromo-2-ethylbutane, benzyl bromide, and *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane were purchased from Tokyo Kasei Kogyo (Japan).

Instruments: NMR and ESI-TOF mass spectra were recorded on JEOL A-400 and MicroMass LC-T spectrometers, respectively. Elemental analyses were performed by the microanalytical center at the Faculty of Agriculture of Nagoya University. CD spectra were recorded on a Jasco J-790 spectropolarimeter in a $1.0 \times 1.0 \times 5.0$ cm quartz cell at room temperature. Bromide ion concentration in 1×10^{-3} M aqueous solutions of diammonium salts was measured using a Horiba ion meter (model F23-II) with a bromide-selective electrode at room temperature. Semiempirical (PM3) molecular orbital calculations were carried out using HyperChem® (Ver. 6) software.

Preparation of *N,N′*-diethyl-*N,N,N′,N′*-tetramethylmethanediammonium dibromide (EtMeEt): A general procedure for diammonium salts: A mixture of bromoethane (5.45 g, 0.05 mol) and *N,N,N′,N′*-tetramethylmethanediamine (1.02 g, 0.01 mol) in dry acetone (30 mL) was stirred for 5 h at room temperature. The reaction mixture was cooled to 0 °C, and the colorless precipitate was collected by decantation, washed twice with dry acetone, and dried under vacuum (0.1 kPa, 50 °C). The resulting product was obtained as colorless highly hygroscopic solids. ^{13}C NMR (100 MHz, CD_3OD) $\delta =$ 8.57, 50.30, 50.34, 50.38, 60.15, 60.18, 60.21, 88.07 ppm; elemental analysis calcd (%) for $C_9H_{24}N_2Br_2 \cdot 0.5\,H_2O$: C 32.84, H 7.66, N 8.51; found: C 32.98, H 8.21, N 8.50.

***N,N′*-Diethyl-*N,N,N′,N′*-tetramethyl-1,2-ethanediammonium dibromide (EtEtEt)**: Colorless powder, m.p. 235.5 – 236.5 °C; ^{1}H NMR (400 Hz, CD_3OD) $\delta = 1.49$ (t, $J = 7.3$ Hz, 6H), 3.28 (s, 12H), 3.61 (q, $J = 7.3$ Hz, 4H), 4.04 (s, 4H) ppm; ^{13}C NMR (100 Hz, CD_3OD) $\delta = 8.91$, 51.53, 56.65, 62.46 ppm; MS: *m/z*: 254 $[M - Br]^+$, 588 $[2M - Br]^+$; elemental analysis calcd (%) for $C_{10}H_{26}N_2Br_2$: C 35.95, H 7.84, N 8.38; found: C 35.94, H 7.92, N 8.20.

***N,N′*-Diethyl-*N,N,N′,N′*-tetramethyl-1,3-propanediammonium dibromide (EtPrEt)**: Colorless powder, m.p. 231 – 232 °C; ^{1}H NMR (400 MHz, CD_3OD), $\delta = 1.49$ (t, $J = 7.3$ Hz, 6H), 2.39 – 2.48 (m, 2H), 3.28 (s, 12H), 3.54 – 3.58 (m, 4H), 3.61 – 3.66 (m, 4H) ppm; ^{13}C NMR (100 Hz, CD_3OD) $\delta = 8.58$, 18.38, 51.04, 51.07, 51.11, 60.95, 61.77 ppm; MS: *m/z*: 268 $[M - Br]^+$; elemental analysis calcd (%) for $C_{11}H_{29}N_2Br_2$: C 37.95, H 8.11, N 8.05; found: C 37.95, H 8.39, N 8.00.

***N,N′*-Diethyl-*N,N,N′,N′*-tetramethyl-1,4-butanediammonium dibromide (EtBuEt)**: Colorless powder, m.p. > 250 °C; ^{1}H NMR (400 MHz, CD_3OD) $\delta = 1.43$ (tt, $J = 7.3$, 2.0 Hz, 6H), 1.90 (q, $J =$ 4.0 Hz, 4H), 3.13 (s, 12H), 3.44 – 3.50 (m, 8H) ppm; ^{13}C NMR (100 Hz, CD_3OD) $\delta = 8.63$, 20.70, 50.87, 50.90, 50.94, 61.14, 63.79 ppm; MS: *m/z*: 282 $[M - Br]^+$, 644 $[2M - Br]^+$; elemental analysis calcd (%) for $C_{12}H_{30}N_2Br_2$: C 39.79, H 8.35, N 7.73; found: C 39.79, H 8.62, N 7.69.

***N,N′*-Dibutyl-*N,N,N′,N′*-tetramethyl-1,3-propanediammonium dibromide (BuPrBu)**: Colorless powder, m.p. 159 – 167 °C; ^{1}H NMR (400 MHz, CD_3OD) $\delta = 1.09$ (t, $J = 7.3$ Hz, 6H), 1.45 – 1.55 (m, 4H), 1.81 – 1.89 (m, 4H), 2.34 – 2.43 (m, 2H), 3.23 (s, 12H), 3.45 – 3.52 (m, 8H) ppm; ^{13}C NMR (100 Hz, CD_3OD) $\delta = 13.98$, 18.64, 20.68, 25.60, 51.61, 61.57, 66.19 ppm; MS: *m/z*: 324 $[M - Br]^+$; elemental analysis calcd (%) for $C_{15}H_{37}N_2Br_2$: C 44.56, H 8.98, N 6.93; found: C 44.56, H 9.23, N 6.96.

***N,N′*-Dihexyl-*N,N,N′,N′*-tetramethyl-1,3-propanediammonium dibromide (HxPrHx)**: Colorless powder, m.p. 49 – 53 °C; ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 0.98 - 1.02$ (t, $J = 7.2$ Hz, 6H), 1.42 – 1.50 (m, 12H), 1.84 – 1.92 (m, 4H), 2.37 – 2.45 (m, 2H), 3.26 (s, 12H), 3.48 – 3.55 (m, 8H) ppm; ^{13}C NMR (100 Hz, $CDCl_3$) $\delta = 13.87$, 18.87, 22.36, 22.82, 25.89, 31.20, 51.24, 60.93, 66.47 ppm; MS: *m/z*: 380 $[M - Br]^+$; elemental analysis calcd (%) for $C_{19}H_{44}N_2Br_2$: C 49.57, H 9.63, N 6.08; found: C 49.48, H 9.70, N 6.11.

***N,N′*-Dibenzyl-*N,N,N′,N′*-tetramethyl-1,3-propanediammonium dibromide (BzlPrBzl)**: Colorless powder, m.p. 174 – 175 °C; ^{1}H NMR (400 MHz, CD_3OD) $\delta = 2.53 - 2.61$ (quint, $J = 7.3$ Hz, 2H), 3.25 (s, 12H), 3.59 – 3.63 (m, 4H), 4.80 (s, 4H), 7.59 – 7.72 (m, 10H) ppm; ^{13}C NMR (100 Hz, $CDCl_3$) $\delta = 18.91$, 50.71, 50.74, 50.78, 62.05, 69.72, 128.63, 130.45, 132.06, 134.32 ppm; MS: *m/z*: 392 $[M - Br]^+$; elemental analysis calcd (%) for $C_{21}H_{32}N_2Br_2$: C 53.40, H 6.83, N 5.93; found: C 53.39, H 6.87, N 6.01.

***N,N′*-Bis(4-methylpentyl)-*N,N,N′,N′*-tetramethyl-1,3-propanediammonium dibromide ((MePe)Pr(MePe))**: Colorless powder, m.p. 105 – 109 °C; ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 0.92$ (d, $J = 6.8$ Hz, 12H), 1.27 (q, $J = 7.8$ Hz, 4H), 1.58 – 1.68 (m, 2H), 1.78 – 1.76 (m, 4H), 2.71 – 2.81 (m, 2H), 3.41 (s, 12H), 3.52 (brt, $J = 7.8$, 4H), 3.91 (brt, 4H) ppm; ^{13}C NMR (100 Hz, $CDCl_3$) $\delta = 18.91$, 20.83, 22.34, 35.01, 51.27, 61.08, 66.84 ppm; elemental analysis calcd (%) for $C_{19}H_{44}N_2Br_2$: C 49.57, H 9.63, N 6.08; found: C 49.48, H 9.70, N 6.11.

***N,N′*-Bis(2-ethylbutyl)-*N,N,N′,N′*-tetramethyl-1,3-propanediammonium dibromide ((EtBu)Pr(EtBu))**: Colorless powder, m.p. 174 – 176 °C; ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 0.97$ (t, $J = 7.3$ Hz, 12H), 1.51 – 1.58 (m, 8H), 1.89 – 1.94 (m, 2H), 2.73 – 2.81 (m, 2H), 3.42 (s, 12H, t, 4H), 3.92 (brt, $J = 7.8$, 4H) ppm; ^{13}C NMR (100 Hz, $CDCl_3$) $\delta = 10.56$, 19.00, 25.88, 35.25, 51.13, 61.55, 71.23 ppm; elemental analysis calcd (%) for $C_{19}H_{44}N_2Br_2$: C 49.57, H 9.63, N 6.08; found: C 49.58, H 9.58, N 5.98.

Sample preparation: Diammonium salts were diluted with twice-distilled water (Milli-Q®) to the appropriate concentrations and

filtered through a hydrophobic PTFE syringe filter (pore size 0.5 μm; Toyo Roshi Kaisha (Japan)). The solutions for FM observations were prepared as follows: water (400 μL, Milli-Q®), Tris-HCl buffer solution (50 μL, 0.1 M, pH 7.8), 2-mercaptoethanol (20 μL), and DAPI solution (10 μL, 10 μM) were thoroughly mixed, and DNA solution (10 μL, 10 μM, in base pairs) was then added. The resulting DNA solution was gently mixed with a diammonium salt solution and kept for 1 h at room temperature to achieve equilibrium before observations. Samples for CD spectroscopic analyses were prepared by addition of the appropriate amounts of condensing agent solutions into a solution (0.15 mM) of λ-DNA in a Tris-HCl buffer (0.01 M, pH 7.8).

Fluorescent microscopy: The samples were illuminated with UV light (365 nm) from a high pressure Hg lamp, and fluorescence images of DNA molecules were observed using a Zeiss Axiovert® 135 TV microscope equipped with a 100× oil-immersed lens and recorded on S-VHS videotape through a Hamamatsu SIT TV camera. All observations were carried out at room temperature. The apparent long-axis length of DNA (L), which was defined as the longest distance in the outline of the DNA image, was calibrated with an Argus® 10 image processor (Hamamatsu Photonics (Japan)). Each value of L was obtained by measuring the effective size of at least 100 DNA molecules. Microscope slides and coverslips were carefully cleaned as in previous studies.[52] Modified microscope slides and coverslips were prepared by soaking in *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane for 3 h, repeated washing by distilled water and drying at 100 °C for 5 h.

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