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# Interpolyelectrolyte Complexes Formed by DNA and Astramol Poly(propylene imine) Dendrimers

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Received April 19, 2000; Revised Manuscript Received October 2, 2000

**ABSTRACT:** Investigations have been carried out to clarify the binding interactions between two kinds of native DNA: one from salmon sperm (300–500 bp) and another from bacteriophage T4dC (166 kbp) and amine-terminated, diaminobutane core, poly(propylene imine) dendrimers (Astramol) of five generations (G1, G2, G3, G4, and G5). All dendrimers interacting with DNA at an equal concentration of amine and phosphate groups form electroneutral water-insoluble interpolyelectrolyte complexes (IPECs). However, G4 and G5 added to DNA solution in excess form positively charged water-soluble IPECs representing perfect objects to investigate the state of DNA molecules incorporated into IPEC. Using UV spectroscopy and CD spectroscopy combined with ultracentrifugation, it is shown that complexed DNA compacts, revealing a wound double-helical structure. Using fluorescence microscopy, we observed compaction of individual ultrahigh molecular mass DNA interacting with excess of G4 to form water-soluble positively charged IPECs “unimers”.

## Introduction

Native DNA, like other polyions in aqueous solutions, interacts with oppositely charged polyelectrolytes to form interpolyelectrolyte complexes (IPEC). DNA–polycation complexes are important to many issues related to basic and applied life science. In particular, gene delivery seems to be a novel rapidly growing area of their potential biomedical applications.<sup>1–3</sup> Another exciting field is the mimicking of nucleoprotein constructs such as nucleosomes, viruses, or phages.

Astramol poly(propylene imine) dendrimers, DAB-dendr-(NH<sub>2</sub>)<sub>x</sub>, are treelike molecules of well-defined three-dimensional structure built by starting with a diaminobutane core and adding propylene imine branches to it by an iterative procedure, which includes the Michael's addition reaction and reduction of nitrile end groups to amine groups, etc.<sup>4</sup> DAB-dendr-(NH<sub>2</sub>)<sub>x</sub> of five consecutive generations (G1, G2, G3, G4, and G5) with  $x = 4, 8, 16, 32$ , and 64 are presently available. All of them are highly water-soluble. The two senior generations exhibit a typical polyelectrolyte behavior when protonated and positively charged.<sup>5–7</sup> The structures of DAB-dendr-(NH<sub>2</sub>)<sub>4</sub> and DAB-dendr-(NH<sub>2</sub>)<sub>64</sub> are represented in Scheme 1. Primary and tertiary amine groups of DAB-dendr-(NH<sub>2</sub>)<sub>x</sub> molecules are partially ionized at physiological pH, providing electrostatic attraction to various polyanions. To date, there have been only few investigations of DNA–dendrimer interactions.<sup>6–9</sup> In particular, it was shown<sup>6</sup> that DAB-dendr-(NH<sub>2</sub>)<sub>x</sub> bound to DNA polyanions forms IPECs in water solution over a wide pH range below 10.5.

This paper describes some special features of complexation of this dendrimer family with DNA, characteristic of the DNA double helix and different from

ordinary flexible polyanions, such as poly(acrylate) or poly(styrenesulfonate) recently described.<sup>6</sup>

## Experimental Section

DAB-dendr-(NH<sub>2</sub>)<sub>x</sub> were produced at DSM. They are commercially available from DSM or Aldrich. Detailed information on the synthesis was published elsewhere.<sup>9,10</sup> The dendrimers of five different generations from DAB-dendr-(NH<sub>2</sub>)<sub>4</sub> to DAB-dendr-(NH<sub>2</sub>)<sub>64</sub>, i.e., G1, G2, G3, G4, and G5, were used.

Ultrahigh molecular mass native DNA (166 kbp) from bacteriophage T4dC was purchased from Nippon Gene and mainly used for single molecular observations by fluorescence microscopy. The fluorescent dye 4,6-diamidino-2-phenylindole (DAPI) and 2-mercaptoethanol (ME) were obtained from Wako Pure Chemical Industries Ltd. DAPI, able to intercollate into the DNA double helix, was used as a fluorescent label. ME was used as a free radical scavenger to reduce fluorescent fading and light-induced damage of DNA molecules.

The sample solutions, microscope slides, and coverslips were carefully prepared as in previous studies.<sup>11,12</sup> The concentration of the DAB-dendr-(NH<sub>2</sub>)<sub>x</sub> or DNA symbolized as [D<sub>x</sub>] or [DNA] represented the overall molar concentration of the dendrimer primary and tertiary amine groups or DNA phosphate groups, respectively.

The stock solution of DAB-dendr-(NH<sub>2</sub>)<sub>x</sub>, [D<sub>x</sub>] = 1 × 10<sup>3</sup> M, pH = 3 in 0.01 M NaCl, was added to a 10–20-fold excess by volume of 0.6 μM DNA solution in 0.01 M NaCl containing 0.3 μM of DAPI and 4% of ME to obtain the desired final concentration and dendrimer/DNA ratio. The mixture was gently shaken and kept for 15 min before observation. The sample solutions deposited on a slide were illuminated with 365 nm UV light to induce fluorescence of the DAPI label. The 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 by a high-sensitivity Hamamatsu SIT TV camera. The observations were carried out at 20 °C, and the pH was kept in the range 5–6. The apparent length of the long DNA axis assumed as the longest distance in the outline of the DNA image was estimated with an Argus 10 image processor (Hamamatsu Photonics).

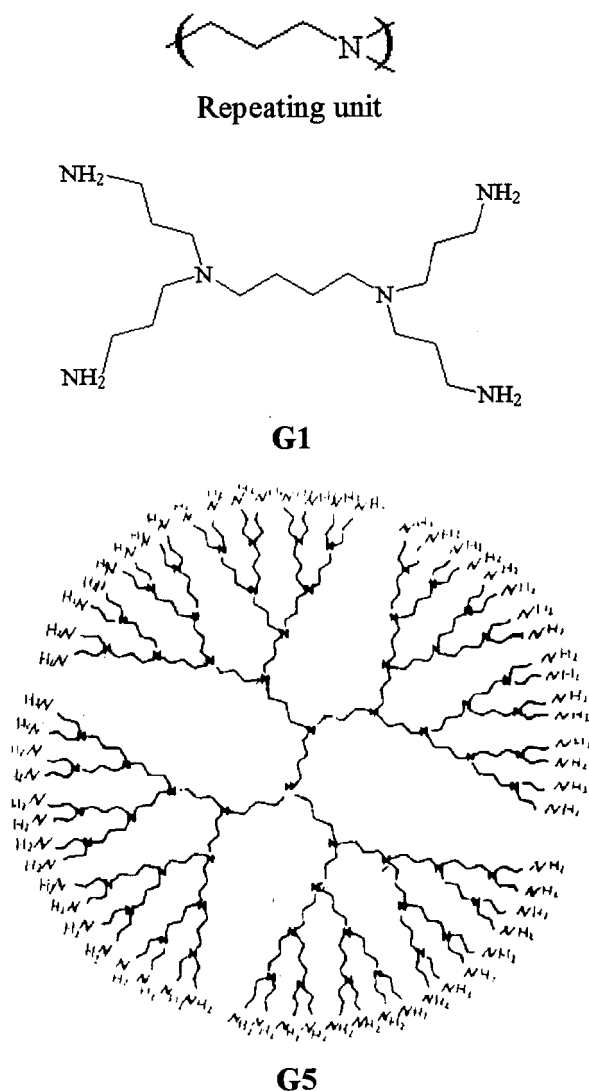
Salmon sperm native DNA (300–500 bp) purchased from GosNIIOKhT, Russia, for UV and CD spectroscopic studies as

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**Scheme 1. Schematic Representation of Dendrimer Repeating Unit, G1 and G5 Dendrimers**

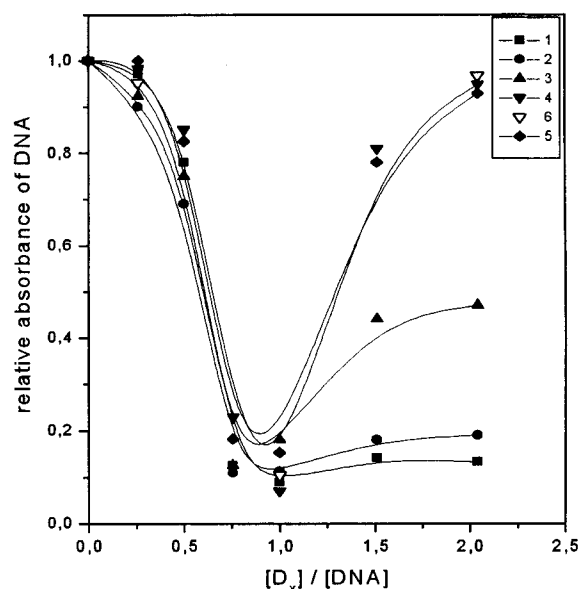
well as sedimentation measurements required sufficiently large samples.

UV absorption spectra recorded with a Specord M-40 spectrophotometer were used to measure DNA concentration in solution, assuming a molar extinction coefficient of  $6500 \text{ cm}^2/\text{mol}$  of the native DNA at its maximum of  $\lambda = 260 \text{ nm}$ .<sup>13</sup> In these experiments DAB-dendr- $(\text{NH}_2)_x$  solutions ( $[\text{D}_x] = 1 \times 10^{-3} \text{ M}$ ,  $\text{pH} = 3$  in  $0.01 \text{ M NaCl}$ ) were added in the required amounts to a series of Eppendorf microtubes containing a certain volume of native  $40\text{--}50 \mu\text{M}$  DNA in  $0.01 \text{ NaCl}$ . After incubation for 15 min the microtubes were centrifuged at  $15\,000 \text{ rpm}$  for 10 min, and the absorbance of the supernatant was measured. The CD spectra were recorded with a Jasco J-500C spectropolarimeter. Cells with an optical path of  $1 \text{ cm}$  were used for the UV and CD measurements. All measurements were carried out at  $25^\circ \text{C}$  with the pH in the range  $5\text{--}6$ .

Sedimentation coefficients of DNA and its complexes with DAB-dendr- $(\text{NH}_2)_{32}$  were estimated using a Beckman E analytical ultracentrifuge in the scanning mode ( $\omega = 48\,000 \text{ rpm}$ ). Scanning was performed at  $\lambda = 260 \text{ nm}$ .

## Results and Discussion

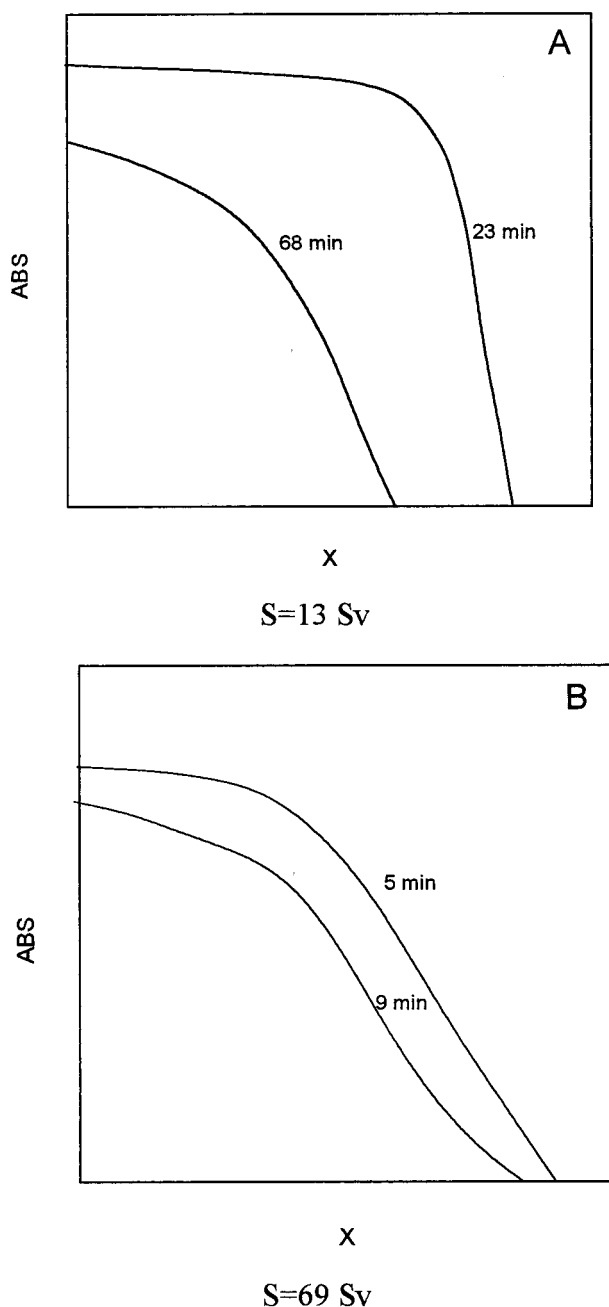
On addition of the aqueous solutions of DAB-dendr- $(\text{NH}_2)_x$  to the aqueous solutions of salmon sperm DNA, the mixtures remained transparent for the  $[\text{D}_x]/[\text{DNA}]$  ratio,  $Z$ , less than  $0.5$  but progressively turned turbid at  $Z > 0.5$  due to formation of insoluble IPEC as earlier



**Figure 1.** Residual optical density of DNA from salmon sperm in the supernatant after centrifugation ( $\omega = 11\,000 \text{ rpm}$ ) at various  $[\text{D}_x]/[\text{DNA}]$  ratios in  $0.01 \text{ M NaCl}$  solution,  $t = 20^\circ \text{C}$ , DNA-DAB-dendr- $(\text{NH}_2)_4$  (1), DNA-DAB-dendr- $(\text{NH}_2)_8$  (2), DNA-DAB-dendr- $(\text{NH}_2)_{16}$  (3), DNA-DAB-dendr- $(\text{NH}_2)_{32}$ , DNA-DAB-dendr- $(\text{NH}_2)_{64}$  (5), DNA-DAB-dendr- $(\text{NH}_2)_{32}$  reverse order of mixing (6).

reported.<sup>6</sup> The insoluble DAB-dendr- $(\text{NH}_2)_x$ -DNA complexes were separated by ultracentrifugation. The concentration of DNA remaining in the supernatant was measured spectrophotometrically. The corresponding precipitation curves are shown in Figure 1. It is seen that a noticeable decrease of DNA concentration in the supernatant for dendrimers of all generations is observed for a  $Z \sim 0.5$ . Further addition of DAB-dendr- $(\text{NH}_2)_x$  causes a drastic decrease of DNA content in the supernatant. Finally, for  $Z$  ratio close to 1 the aqueous phase contains no measurable amount of DNA. Thus, DAB-dendr- $(\text{NH}_2)_x$ -DNA complexes of nearly equimolar composition apparently formed at  $0.5 < Z < 1$  are water-insoluble, just like stoichiometric IPECs formed by other oppositely charged polyelectrolytes. Indeed, formation of equimolar complexes of DNA with other polycations at  $Z < 1$  due to disproportionation was earlier observed.<sup>11</sup> However, as seen in Figure 1, by contrast to the G1 and G2 dendrimers, addition of the dendrimers of higher generations (G3, G4, and G5), namely DAB-dendr- $(\text{NH}_2)_x$ , where  $x = 16, 32$ , or  $64$ , above the equimolar ratio (i.e., at  $Z > 1$ ) results in an increase of residual DNA absorption in the supernatant. This probably indicates formation of the water-soluble DAB-dendr- $(\text{NH}_2)_x$ -DNA complexes incorporating an excess of the positively charged dendrimer amine groups, which provide positive charge and consequent solubility to the corresponding IPEC species. Nothing like that was observed on mixing the same dendrimers with ordinary flexible single-chain polyelectrolytes such as poly(acrylic) or poly(styrenesulfonic) acids. In these cases on addition of an excess of all generation dendrimers in neutral media, only water-insoluble 1:1 IPECs were formed while an overstoichiometric amount of the dendrimers remained in solution.

It is noteworthy that the above peculiar behavior of DAB-dendr- $(\text{NH}_2)_x$ -DNA mixtures at  $Z > 1$  is quantitatively reproduced when the components are mixed in the reverse order, suggesting that the system is at



**Figure 2.** Scan sedimentation profiles of DNA (A) and DNA-D<sub>32</sub> complex (B) in 0.01 M NaCl solution plotted against  $x$ , the distance from the bottom of the sedimentation cell. For details see Experimental Section.

equilibrium. It is significant that equilibration is achieved only in the presence of 0.01 M NaCl, which is known to accelerate considerably polyelectrolyte interchange reactions.<sup>14</sup> In salt-free solution the equilibrium distribution of the excess dendrimer molecules among the DNA chains is not achieved during the time of mixing. As a result, DNA separates in the form of insoluble nonequilibrium IPEC aggregates.

The typical sedimentation patterns of the original salmon sperm DNA and the corresponding DAB-dendr-(NH<sub>2</sub>)<sub>32</sub>-DNA complex in 0.01 M NaCl aqueous solutions recorded at different times are shown in Figure 2. Each profile has only a single step, indicating the constancy of the composition of the complex. Moreover, the value of sedimentation coefficient,  $S$ , for the DNA-D<sub>32</sub> complex formed at [D<sub>32</sub>]/[DNA] = 5 found about 5

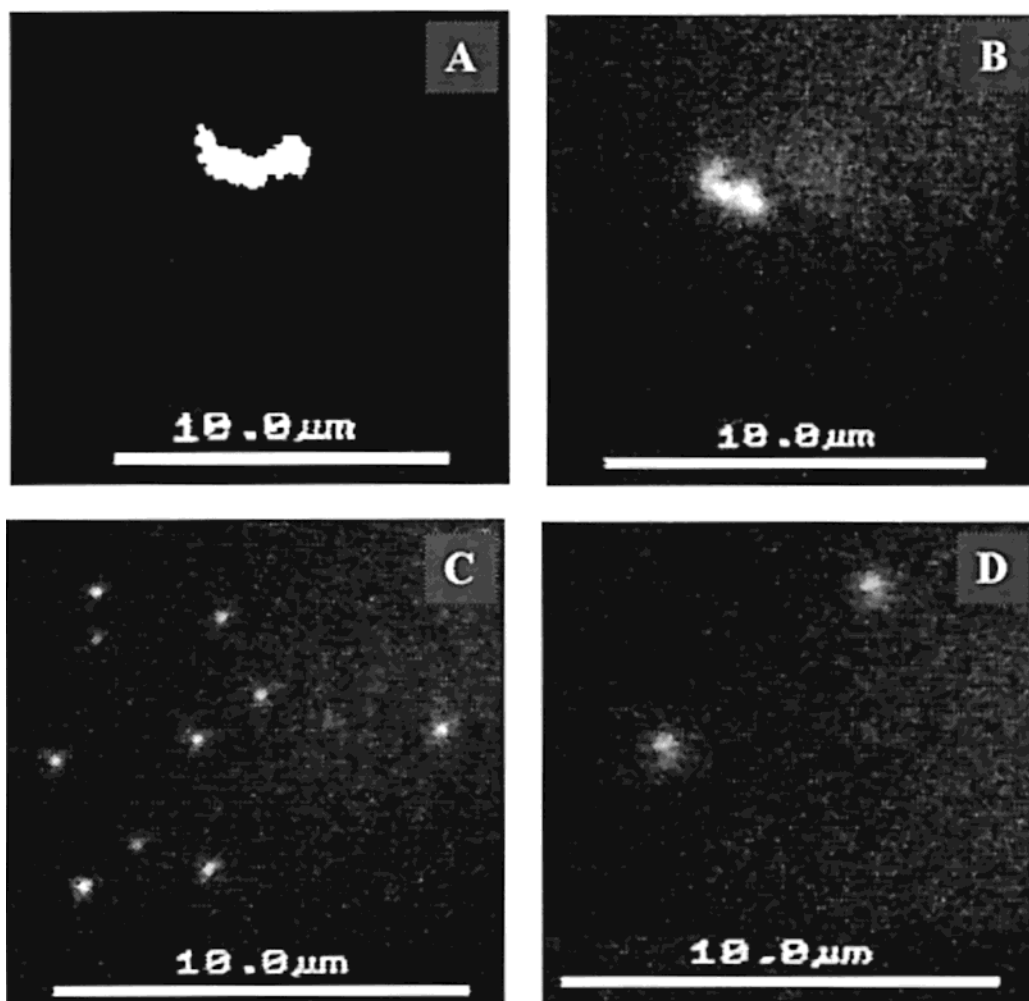
times larger than the original DNA at the same concentration. This difference can be attributed to either aggregation of DNA within complex species<sup>15</sup> or compaction of individual complexed DNA molecules.<sup>16–18</sup>

Earlier it has been demonstrated that fluorescence microscopy is a useful tool to monitor the conformational change in individual ultrahigh molecular mass DNA molecules induced by various condensation agents in dilute aqueous solutions.<sup>19–22</sup>

We used fluorescence microscopy to characterize the overall conformational state of bacteriophage T4dC DNA in DAB-dendr-(NH<sub>2</sub>) <sub>$x$</sub> -DNA complexes. The typical examples of the fluorescence images of native T4DNA and its complexes with the dendrimers are represented in Figure 3. As expected, the original DNA molecules have an extended coil conformation (Figure 3A) in 0.01 M NaCl solution. The coil image observed under the microscope continuously fluctuates and moves randomly in the field of vision, exhibiting the classical thermal motion behavior. On addition of DAB-dendr-(NH<sub>2</sub>)<sub>32</sub> at  $Z < 0.5$  DNA molecules change to the partially folded state but still exhibit random intramolecular fluctuation (Figure 3B). Further increase of the dendrimers concentration to  $Z \geq 1$  induces the collapse of individual T4 dC DNA molecules to small compact particles (Figure 3C,D). The above observations are consistent with the results of earlier solution studies<sup>23–26</sup> and electron microscopy investigations.<sup>27</sup> However, in the present work we observed directly the whole dynamics of the condensation process for a single DNA molecule in the field of vision of the microscope. The qualitative characteristics of the fluorescence images observed in the sample solutions of various dendrimers at the different concentrations are represented in Table 1.

The data in the table show that the dendrimers of all studied generations added in sufficient amounts induce a transition of the individual DNA molecules from an extended coil to a compact conformation. However, the dynamic behavior of the compact complex species formed by an excess of the dendrimers of higher generations (G4 and G5) added to the DNA differs drastically from the lower generations (G1 and G2). The compact species formed by DNA and DAB-dendr-(NH<sub>2</sub>)<sub>4</sub> or DAB-dendr-(NH<sub>2</sub>)<sub>8</sub> at  $Z \geq 1$  always stick to the microscope slides and then remain motionless. The same holds for DAB-dendr-(NH<sub>2</sub>)<sub>32</sub> and DAB-dendr-(NH<sub>2</sub>)<sub>64</sub> at  $Z = 1$  (Figure 3C). By contrast, similarly looking but slightly swollen compact complex species observed with the excess of the dendrimers of higher generations, (i.e., in the  $Z$  region of solubility of the corresponding IPECs, see Figure 1) do not attach to the glass surface but exhibit translational thermal motion in the bulk solution disposed between the microscope slide and the coverslip (Figure 3D). The conceivable reason for such a different behavior may be the following. Referring to the data represented in Figure 1, one can conclude that the water-insoluble G1 and G2 dendrimer/salmon sperm DNA complex aggregates formed at  $Z \geq 1$  are electroneutral. In other words, DNA is not able to bind overstoichiometric amounts of G1 and G2 dendrimers. Correspondingly, the complex species formed by G1 and G2 dendrimers with individual T4 DNA molecules at the above  $Z$  ratios should also be neutral and therefore not restricted to adsorb on the microscope slide or coverslip surface apparently positively charged due to adsorption of the excess of the added dendrimer. Such compact complex





**Figure 3.** Fluorescence microscopy images of T4 (A), T4-DAB-dendr-(NH<sub>2</sub>)<sub>32</sub> (B) complex ([D<sub>32</sub>]/[DNA] = 0.5), T4-DAB-dendr-(NH<sub>2</sub>)<sub>32</sub> (C) complex ([D<sub>32</sub>]/[DNA] = 1), and T4-DAB-dendr-(NH<sub>2</sub>)<sub>32</sub> (D) complex ([D<sub>32</sub>]/[DNA] = 2) in 0.01 M NaCl solution. For details see Experimental Section.

**Table 1. Characteristics of T4 DNA–Dendrimer Complex Images Observed in the Fluorescence Microscope at Various Dendrimer Concentrations in the Sample Solution**

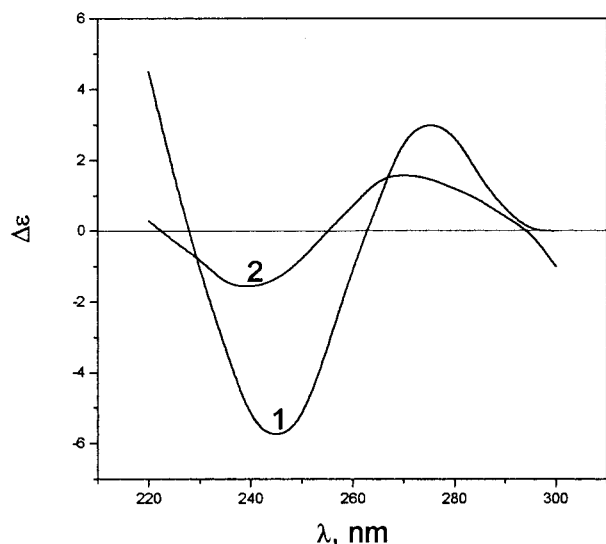
dendrimer	[D <sub>x</sub> ]		
	1 × 10 <sup>−6</sup> M	5 × 10 <sup>−6</sup> M	1 × 10 <sup>−5</sup> M
DAB-dendr-(NH <sub>2</sub> ) <sub>4</sub>	extended coil	extended coil	coil
DAB-dendr-(NH <sub>2</sub> ) <sub>8</sub>	extended coil	extended coil	fixed compact particle
DAB-dendr-(NH <sub>2</sub> ) <sub>16</sub>	extended coil	moving compact particle	
DAB-dendr-(NH <sub>2</sub> ) <sub>32</sub>	extended coil	moving compact particle	
DAB-dendr-(NH <sub>2</sub> ) <sub>64</sub>	extended coil	moving compact particle	

species approach the surface and fix there much readily than free DNA extended coils diffusing relatively slow. At the same time the G4 and G5 dendrimer/DNA compact water-soluble complex species positively charged by capturing an excess of the dendrimer, approaching the positive surface vicinity do not adsorb because of electrostatic repulsion.

Incorporation of DNA molecules in IPECs resulting in compaction of the double helix can also cause local conformational changes within the DNA secondary structure.<sup>3</sup> To gain an insight into such changes, we performed CD spectroscopic measurements of the water-soluble complexes of salmon sperm DNA with an excess of the dendrimers under investigation. Figure 4 shows the CD spectrum of the DAB-dendr-(NH<sub>2</sub>)<sub>32</sub>–DNA complex in comparison with the typical centrosymmetrical spectrum of the initial DNA characteristic of the B-form of the double helix in dilute aqueous solutions. The spectrum of the complexed DNA differs from free DNA

by a red shift of the crossover point as well as both absorption maxima and also the asymmetry due to higher intensity of the negative absorption band as compared to the positive one. Similar spectra were observed for soluble complexes of the DNA with and DAB-dendr-(NH<sub>2</sub>)<sub>64</sub>. It is significant that the above peculiarities were earlier observed in CD spectra of native DNA incorporated in medium-sized bacteriophage particles<sup>28</sup> and considered to be a characteristic of a highly wound DNA double helix.<sup>29,30</sup> Therefore, it is likely that such winding is also realized for the DNA molecules compacted within the soluble DNA–dendrimer complex species.

The reaction steps of DNA complexation with ionized primary and tertiary amine groups of the dendrimers can be represented as shown in Scheme 2. The driving force shifting the equilibrium from left to the right is mainly provided by an entropy increase due to the release of the low molecular counterions (Na<sup>+</sup>Cl<sup>−</sup>). It is



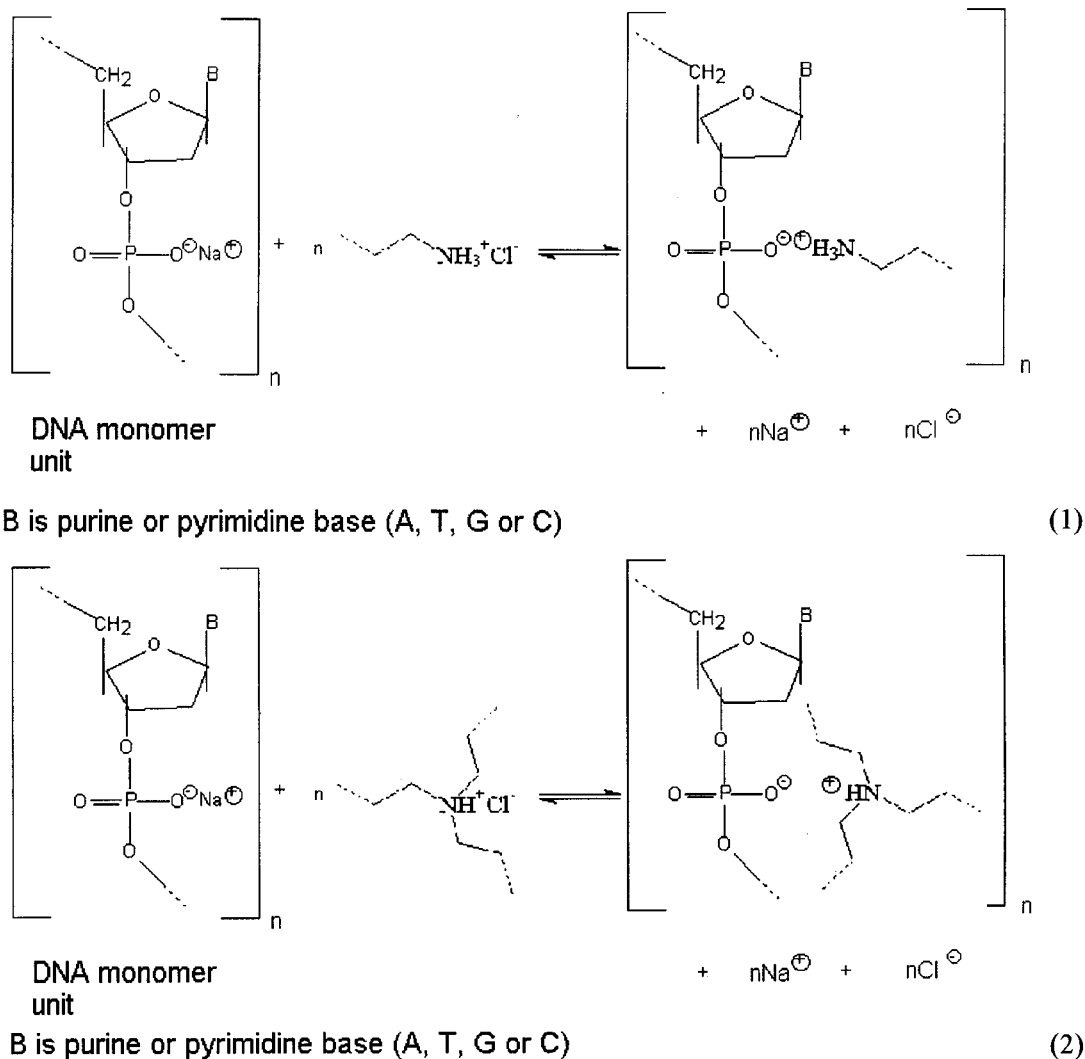
**Figure 4.** CD spectra of DNA (1) and DNA-DAB-dendr-(NH<sub>2</sub>)<sub>32</sub> complex (2) in 0.01 M NaCl solution. [D<sub>32</sub>]/[DNA] = 5, *t* = 25 °C.

obvious that the number of salt bonds in the formed IPEC is equal to the number of Na<sup>+</sup> or Cl<sup>-</sup> ions released. In our previous work,<sup>6</sup> it was shown that in the stoichiometric interaction of the same dendrimers with

oppositely charged flexible linear polyelectrolytes, i.e., poly(sodium acrylate) or poly(sodium styrenesulfonate), the precipitated IPEC contained practically neither free nor bound NaCl. All the simple salt was released and remained in the supernatant solution. This means that in these cases practically all primary and tertiary amine groups of DAB-dendr-(NH)<sub>x</sub> are available to form ion pairs with the polyanions. This indicates that dendrimers at least up to their fifth generation are fully penetrable for the rather flexible linear polyanions. In other words, such polyanions are able not only to wind around the dendrimer species but also to enter into its interior. This agrees with the conclusion in the recently published investigation of small-angle neutron scattering (SANS) by DAB-dendr-(NH<sub>2</sub>)<sub>32</sub> and DAB-dendr-(NH<sub>2</sub>)<sub>64</sub> dilute solutions in methanol.<sup>31</sup> In fact, it has been shown that the solvent occupies a large volume fraction of dendrimer; i.e., it is assessable also to other entities strongly interacting with the dendrimer functional groups.

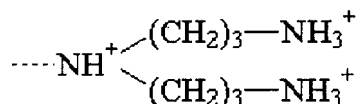
The above conclusion on DAB-dendr-(NH<sub>2</sub>)<sub>x</sub> full penetrability for flexible linear polyanions may not be true for rigid polyanionic chains. In fact, our earlier potentiometric study of interpolyelectrolyte complexation in equimolar mixture of DAB-dendr-(NH<sub>2</sub>)<sub>32</sub> with DNA as compared to equimolar DAB-dendr-(NH<sub>2</sub>)<sub>32</sub>-PAA mixture indicated that the extent of mutual availability of

**Scheme 2**



the oppositely charged polymer ionic groups as reflected by the corresponding titration curves was considerably lower in the case of rigid DNA double helices.<sup>6,32</sup>

This is confirmed by measuring the content of residual  $\text{Cl}^-$  ions in the 1:1 IPEC precipitate formed on mixing of DAB-dendr-( $\text{NH}_2$ )<sub>32</sub> and salmon sperm DNA solutions at  $Z = 1$ . Elemental analysis showed that the thoroughly washed and dried IPEC sediment contains a certain amount of bound NaCl ( $3.0 \pm 0.2$  wt % Cl), indicating that about one-third (31%) of DNA phosphate groups has not reached the amine groups of the dendrimer to form interpolyelectrolyte salt bounds. This probably means that only protonated primary and tertiary amine groups, which belong to the outer branches of DAB-dendr-( $\text{NH}_2$ )<sub>32</sub>,



are sterically available for tight electrostatic binding to the DNA double helix in solution. The rest of protonated tertiary amine groups located in the interior of the dendritic polycation remains neutralized by  $\text{Cl}^-$  counterions. Also, one-third of sodium phosphate groups exposed on the surface of the DNA double helix in electroneutral 1:1 IPEC is still free to bind to protonated outer amine groups on the dendrimer polycations added in excess. The hydrodynamic radii of G4 and G5 dendrimers in water solutions<sup>33,34</sup> are about 15.6 and 19.8 Å, respectively, i.e., commensurable with the thickness of the DNA double helix (24 Å for B-form and 25.5 Å for A-form).<sup>35</sup> The ratio of the DNA persistent length ( $L = 450$  Å)<sup>3</sup> to the diameter of G4 and G5 dendrimers is 14.4 and 11.4. At the same time one can estimate that the electroneutral complex species at  $D_x/\text{DNA} = 1$  contain only 4.1 G4 or 2.06 G5 dendrimer molecules per L unit which apparently are uniformly distributed along the double helix. Therefore, it is not surprising that 1:1 IPECs have enough room to accept some excess of the dendrimer polycations and overcharge. The driving force for such extra bonding is provided by entropy increase due to the release of small counterions. Apparently this is how water-soluble positively charged IPECs are formed on mixing DNA with the dendrimers of higher generations at  $Z > 1$ . The above data are consistent with our potentiometric titration data<sup>6</sup> and recently published model for double-stranded DNA complexes with another polycationic dendrimer family, i.e., amine-terminated polyamidoamine starburst dendrimers studied using the ethidium bromide probe.<sup>8</sup>

## Conclusions

Investigations have been carried out to clarify the binding interactions between double-stranded DNA and DAB-dendr-( $\text{NH}_2$ )<sub>x</sub> dendrimers of five consecutive generations (G1–G5).

First it is shown that the dendrimers of all generations bind to the native DNA and form water-insoluble IPECs if the ratio  $Z = [D_x]/[\text{DNA}]$  is in the range 0.5–1. However, the behavior of G4 and G5 interacting with DNA differs entirely from G1 and G2 at  $Z > 1$ . G4 and G5 dendrimers added to DNA in excess form positively charged water-soluble IPECs in contrast with G1 and G2. The solubility of these complexes provides an opportunity to study by various techniques the state of complexed DNA. In particular, it is shown that the

native DNA complexed with an excess of the dendrimers is compacted exhibiting a wound double-helical structure. It is likely that soluble positively charged compact complex species of this kind but formed with plasmid DNA play an important part in DNA translocation into cells and transfection phenomena.<sup>2,36,37</sup>

In the case of ultrahigh molecular mass T4 DNA interacting with an excess of G4 dendrimer, we observed the compaction of individual DNA molecules forming water-soluble IPEC “unimers” apparently positively charged, which mimics to some extent DNA histone complex species.

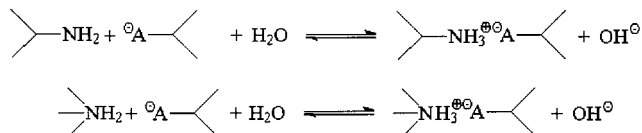
**Acknowledgment.** The authors gratefully acknowledge for Russian Foundation for Fundamental Research for the financial support of the above studies (Grant 99-03-33337). We also thank Associate Prof. N. Kumazawa, Faculty of Engineering, Ibaraki University, for technical assistance in fluorescence microscopy experiments and Prof. H. Morawetz for valuable comments and careful editing of the manuscript.

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- (32) It is known that polyamines interact with polyanions in water solution to form IPECs so that neutral amine groups protonate pairing with the anionic polymer units ( $^{-}A^{\ominus}$ ):



As a result, hydroxyl ions release in equivalent amount which can be detected by titration with the strong acid, e.g., HCl. Therefore, comparing the potentiometric titration curves for the free polyamine and polyamine/polyanion mixture, one can estimate the degree of polyion pairing, i.e., the extent of mutual availability of the polymer oppositely charged ionic groups (e.g., see: Kabanov, V. A. Basic Properties of Soluble Interpolyelectrolyte Complexes Applied to Bioengineering and Cell Transformation and the earlier references quoted in: *Macromolecular Complexes in Chemistry and Biology*; Dubin, P., Bock, J., Davis, R., Schulz, D. N., Eds.; Springer-Verlag: Berlin, 1994; p 151). Such estimation was done earlier with respect to the DAB-dendr-(NH<sub>2</sub>)<sub>32</sub>/PAA and DAB-dendr-(NH<sub>2</sub>)<sub>32</sub>/DNA systems.<sup>6</sup>

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MA000674U