An EPR Study of the Interactions between Starburst Dendrimers and Polynucleotides

M. F. Ottaviani* and B. Sacchi

Department of Chemistry, University of Florence, Via G. Capponi, 9, 50121 Firenze, Italy

N. J. Turro, W. Chen, and S. Jockusch

Department of Chemistry, Columbia University, New York, New York 10027

D. A. Tomalia

Michigan Molecular Institute, Midland, Michigan 48640

Received October 9, 1998; Revised Manuscript Received January 5, 1999

ABSTRACT: Interactions of nitroxide-labeled polyamidoamine dendrimers of generations 2 and 6 (2SBD-T and 6SBD-T, respectively) with double-stranded polynucleotides—Calf Thymus DNA (C.T.DNA), poly(deoxyadenylic−deoxythymidylic acid) (termed Poly(AT)), poly(deoxyguanylic−deoxycytidylic acid) (termed Poly(GC)), and a double-stranded oligonucleotide of 12 base pairs (DNA-12mer)—were investigated by EPR. Computer-aided analysis of the EPR spectra provided information on the mobility of the nitroxide labels and their partition in different environments, which, in turn, gave information on the interactions between dendrimers and polynucleotides. After complexes were formed between DNA and SBD, the labels retained fast mobility at room temperature. On the basis of EPR analysis at 258 K, interaction of oligo- or polynucleotides with SBDs decreased in the following order: DNA-12mer > C.T.DNA > Poly(GC) > Poly(AT). Small dendrimers (2SBD-T) at low pH (5.5) showed significant interaction with the polynucleotides, which decreased with an increase in concentration due to self-aggregation of dendrimer molecules. Conversely, interaction between large dendrimers (6SBD-T) and polynucleotides increased with an increase in SBD concentration until saturation of the interacting sites occurred. Comparison with previous studies on *n*SBD-T−vesicle systems indicated that interaction of dendrimers with vesicles is stronger than dendrimer−polynucleotide interaction. This study provides some insights into dendrimer−DNA interactions of particular interest in understanding the mechanism of gene transfer to mammalian cells by SBDs.

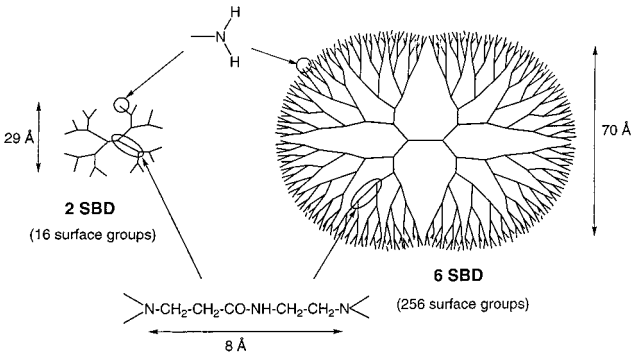
Introduction

Supramolecular assemblies of nucleic acids and polycationic macromolecules are important from the standpoint of molecular recognition, biological transport, and cell repair.[1,2] Examples can be found in host−guest interactions of DNA and histones, or DNA and spermidines, both of which serve a vital role in biological processes such as DNA stabilization or drug delivery. Synthetic polycationic macromolecules, such as dendrimers, serve as mimics of naturally occurring macromolecules, i.e., histones and spermidines.

Starburst dendrimers (SBDs) are a novel class of macromolecules possessing a well-defined molecular composition and constitution whose structure is created by stepwise attachment of layers, termed generations, onto a central core.[3,4] The SBDs used in our study, i.e., 2SBD and 6SBD (Scheme 1), are generated from amidoamine units emanating from an ethylenediamine ($NH_2-CH_2-CH_2-NH_2$) core, terminated with amino groups at the external surface.[4] These polyamidoamine dendrimers were therefore termed PAMAM-SBDs.

Recent reports[5−8] that SBDs act as gene transfer vectors to achieve highly efficient gene transfection in a wide range of mammalian cell lines provide a compelling motivation for basic scientific investigations in the pairwise and three-way interaction of SBD, DNA, and liposomes, the main components in this transfection process.

Scheme 1

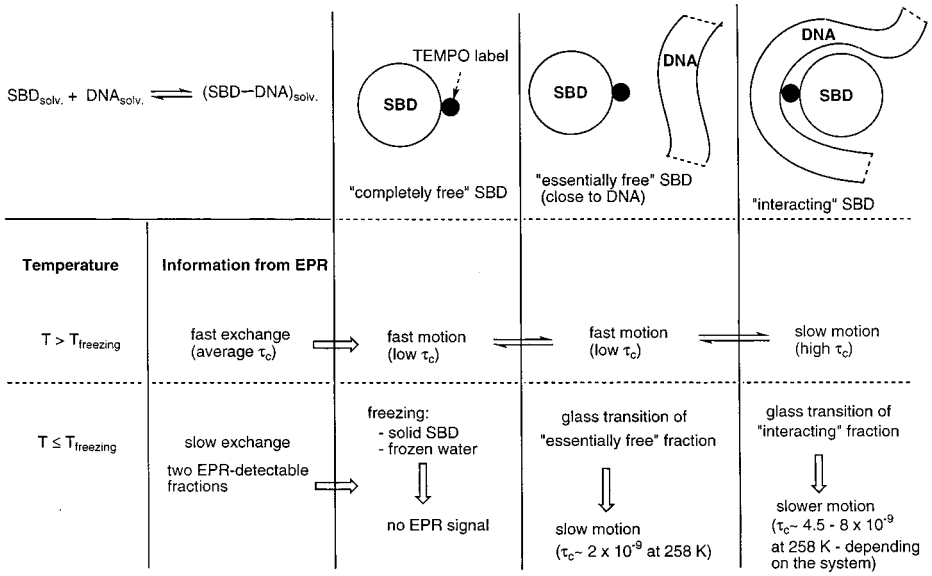


An understanding of a complex process such as gene transfection by means of the PAMAM-SBDs needs to be investigated and clarified from several points of view, by breaking the interacting system into simpler components starting from binary systems (SBD/liposome, SBD/DNA, or DNA/liposome, etc.) and by analyzing interactions of the dendrimers with each component of the models for the biosystem. Recently we have studied interactions of PAMAM-SBDs with liposomes, which mimic the structure of cell membranes.[9,10] Electrostatic interactions occur between the dendrimer surface (which is differently protonated) and negatively charged surface groups of vesicles. However, vesicle structure is not permanently perturbed by this interaction with dendrimers, which accounts for the biocompatibility of the SBDs.

* To whom correspondence should be addressed.

10.1021/ma9815859 CCC: $18.00
Published on Web 03/16/1999

Scheme 2



In this study we focus on an early, perhaps initial, step in the gene transfection process, i.e., the interaction of SBDs with polynucleotides. For most of our experiments we used Calf Thymus DNA (C.T.DNA), which is commonly employed as a representative and standard polynucleotide. To investigate interactions with the different base pairs constituting the DNA, we employed poly(deoxyadenylic−deoxythymidylic acid) (termed Poly-(AT)) and poly(deoxyguanylic−deoxycytidylic acid) [termed Poly(GC)], which contain only adenine−thymine base pairs or cytosine−guanine base pairs, respectively. Finally, to investigate the mechanism of binding of DNA with SBDs, a short sequence of 12 base pairs (termed DNA-12mer) was used.

The SBDs used in our studies (Scheme 1) are expected to interact electrostatically with the DNA bases. Thus, SBD−DNA interactions should depend on the degree of protonation of the external amino groups, which increases with decreasing pH.[11,12] Therefore, we investigated the pH dependence of SBD−DNA interactions.

SBD−DNA interactions were studied by CW EPR spectroscopy employing nitroxide radicals as spin-labels (covalently bound to dendrimers), a technique demonstrated previously to provide a powerful insight in investigating biosystems.[13,14] These labeled dendrimers, termed *n*SBD-T, where *n* indicates generation (either 2 or 6), have been used previously to investigate their interactions with liposomes.[9]

Computer-aided analysis of the EPR spectra of spin-labels[13,14] provides structural and mobility information on the label and its environment. Computation was carried out by means of the well-established procedure developed by Schneider and Freed.[14b] The following main parameters were included in the calculation (by means of a trial-and-error procedure, until the "best fitting" between the experimental and computed patterns was obtained). The principal components of the **g** tensor (for the Zeeman coupling between the electron spin and the magnetic field) were assumed constant: $g_{xx} = 2.009$, $g_{yy} = 2.006$, $g_{zz} = 2.003$; the hyperfine **A** tensor components (for the coupling between the unpaired electron spin and the nuclear nitrogen spin) of SBD-T in water solution were found to be $A_{xx} = 6$ G, $A_{yy} = 8$ G, $A_{zz} = 39$ G. A decrease in environmental polarity of the nitroxide usually induces a decrease in the A_{ii} components. $A_{zz} = 37.5$ G was used in the computation for the radicals interacting with polynucleotides, which indeed indicated a small decrease of polarity in vicinity of the DNA surface. Negligible variations were obtained for the various systems under study. Therefore, the A_{ii} components are not henceforth discussed in this paper. The diffusional rotational motion (assuming a Brownian model) was considered anisotropic, supposing an axial symmetry of the radicals. Therefore, parallel and perpendicular components of the correlation time for rotational motion of the nitroxide group ($\tau_{||}$ and $\tau_{\perp}$, respectively) may be calculated. However, it is known[14b] that, in the case of main rotation around the *Z* axis (the direction of the p orbital containing the unpaired electron), the perpendicular component is the main mobility parameter. Therefore, we report in the present study a $\tau_c = \tau_{\perp}$ as the mobility parameter. (The accuracy of τ, as obtained from spectral computation, is 5%.) The partition of the probes in different environments, when the exchange is slow in the EPR time scale, gave rise to the superposition of different signals to constitute the overall EPR line shape. A subtraction procedure of the computed components from the experimental signal, together with an addition procedure of the computed components to reproduce the experimental signal, allowed the extraction of parameters of each components. The different percentages (relative intensities) of the components were obtained by double integration of each computed component which were added to each other at the proper ratio to reproduce the experimental line shape. The precision in the parameters decreased for more than two components. Therefore, as an approximation, but supported by the model described in Scheme 2, the spectra analyzed in this study were assumed to be constituted by two components, as a maximum.

Experimental Section

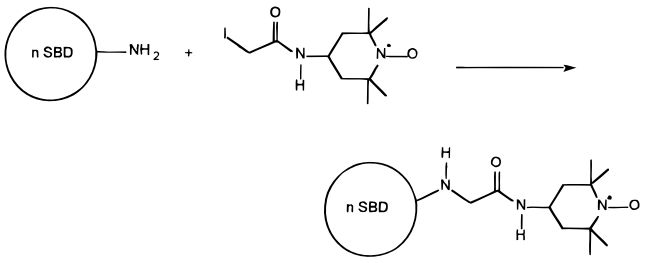
Calf thymus DNA, termed C.T.DNA (Sigma), was purified by standard methods.[15] Synthetic double-stranded poly(deoxyadenylic−deoxythymidylic acid) (termed Poly(AT), approximate average length in base pairs, 1620; MW = 1×10^6) (Pharmacia Biotech) and poly(deoxyguanylic−deoxycytidylic acid) (termed Poly(GC), approximate average length in base pairs, 1257; MW = 8.2×10^5) (Pharmacia Biotech) with alternating base pairs were used as received. The synthetic double-stranded oligonucleotide of 12 base pairs with a

sequence 5′-CGCGTTAACGCG-3′ (termed DNA-12mer) from Memorial Sloan-Kettering Cancer Center was used as received. The polynucleotides were suspended in a phosphate buffer solution (10 mM phosphate, 100 mM NaCl, pH = 7.5) and stirred for 1 h. Concentrations of polynucleotides were determined by UV−vis spectroscopy and given in terms of bases.

PAMAM-SBDs were synthesized as described elsewhere.[4] Purification of the dendrimers was accomplished from subsequent precipitation, and their purity was determined by mass spectrometry.[16] The structural parameters of 2SBD and 6SBD are as follows:

dendrimer	mol wt (g/mol)	surface groups	spherical diameter (SEC in Å)
2SBD	3256	16	29
6SBD	58048	256	67

Labeling of the dendrimers (nSBD-T) was accomplished employing a modified method used by Pauly et al. for labeling DNA-nucleosides.[17] To a solution of 2 mmol (in surface groups) of *n*SBD in 20 mL of 0.01 M Na_3BO_3 (Aldrich) was added 0.05 mmol of 4-(2-iodoacetamide)-Tempo (Aldrich) in 1 mL of DMF and stirred for 6 h at room temperature. The mixture was then extracted six times with methylene chloride and hexane to remove the free Tempo. For further purification, dialysis in water was used employing Spectra/Por cellulose ester membranes from Spectrum Medical Industries, Inc. (MWCO 10 000 for 6SBD-T; MWCO 2000 for 2SBD-T). The labeling grade was evaluated as 1 label for approximately 50 surface amino groups of SBD. The reaction scheme is as follows:



Water solutions of 2SBD-T and 6SBD-T were prepared at a concentration of 0.1 M in surface amino groups. Unless otherwise specified, concentration of the SBD solutions is in surface groups. The solutions were stored under nitrogen to avoid oxidative degradation and kept in the refrigerator when not used. Different levels of protonation of the surface amino groups were accomplished by adding controlled amounts of diluted HCl (0.05 M, Merck). The final pH, after addition of polynucleotides, ranged between 8 and 5.5. The acid−base properties of the SBDs were determined in a previous study.[12] It has been found that at pH = 8 the external layer of amino groups is almost completely protonated, whereas at pH = 5.5, protonation of the subsequent (second layer) amino groups is accomplished. The more highly protonated-SBD-T (pH = 5.5) will henceforth be termed *n*SBD-T^+, to distinguish it from the less protonated form (pH = 8). In a series of measurements, the solutions were taken at the same pH; pure *n*SBD-T solutions were also prepared in the same buffer as in the presence of polynucleotides.

The dendrimer solutions were added to the polynucleotide solutions in order to obtain final concentrations between 0.01 and 0.05 M of *n*SBD-T and 1 mM of the polynucleotides.

EPR spectra were recorded on a Bruker 200D spectrometer operating at X band (9.5 GHz), interfaced with Stelar software to an IBM PC computer for data acquisition and handling. At least five scans of 2028 points each (sweep width 100 G) were accumulated. (The number of scans increased up to 30−50 for low-intensity spectra.) Field modulation and microwave power were settled at 0.8 G and at 20.9 mW, respectively. The temperature was controlled with the aid of a Bruker ST 100/700 variable-temperature assembly.

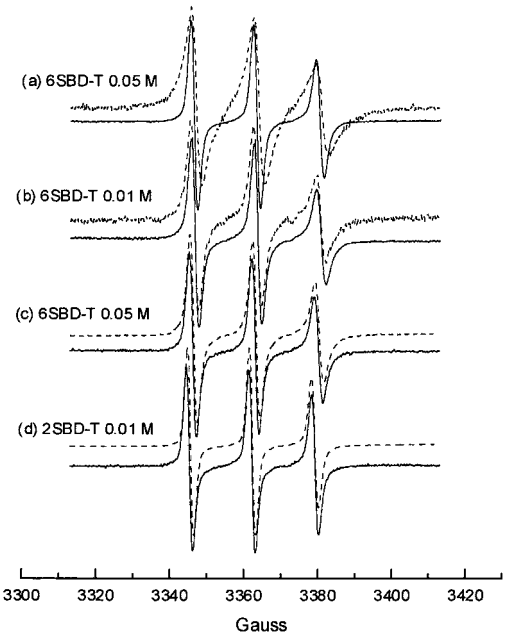


Figure 1. CW EPR spectra recorded at 298 K for (a) 6SBD-T^+ 0.05 M, (b) 6SBD-T 0.01 M, (c) 6SBD-T 0.05 M, and (d) 2SBD-T 0.01 M, respectively, in the absence (solid lines) and presence (dashed lines) of C.T.DNA. The spectra of each pair (with and without C.T.DNA) are normalized in the central hyperfine line. The pH of the solutions was 8 for *n*SBD-T and 5.5 for *n*SBD-T^+.

UV−vis measurements were performed on a Hewlett-Packard 8452A diode array spectrometer.

Results and Discussion

To explain the results, we refer to Scheme 2, which describes the conditions of the system under which the experimental analysis was carried out in the present study and clarifies the kind and extent of information obtained from EPR analysis:

The paradigm to be investigated is the equilibrium reaction giving rise to SBD−DNA adducts. To simplify, we describe the interaction with DNA which may be a polynucleotide or a oligonucleotide. We may identify three different physical conditions for the *n*SBD-Ts in water solutions in the presence of DNA in the experimental conditions of the present study: (a) "completely free" dendrimers far from the DNA surface, (b) "essentially free" (weakly bound and interacting) dendrimers in the vicinity of the DNA surface, and (c) dendrimers "significantly interacting" with DNA eventually wrapped up by DNA loops.

At room temperature, the EPR spectra are characterized by the usual three-line signals found for free nitroxides in solution in the "fast motion" limit. Examples are given in Figure 1, showing experimental EPR spectra for 2SBD-T (0.01 M), 6SBD-T (0.01 and 0.05 M), and 6SBD-T^+ (0.05 M) in the absence (solid lines) and in the presence (dashed lines) of C.T.DNA at 298 K. The small chain connecting the nitroxide to the dendrimer surface allows fast rotation with a correlation time for rotational motion τ_c about 10^{-10} s. By comparing spectra in the absence of DNA (solid lines), it is evident that the low generation dendrimer (2SBD-T, 0.01 M) showed faster mobility ($\tau_c = 1.2 \times 10^{-10}$ s) than the high-generation dendrimer (6SBD-T, 0.01 M, $\tau_c = 3.0 \times 10^{-10}$ s). The mobility increased slightly by increasing the 6SBD-T concentration from 0.01 to 0.05 M (τ_c from 3.0

$\times 10^{-10}$ to 2.6×10^{-10} s). Finally, the more protonated (pH = 5.5) high-generation dendrimer (6SBD-T$^+$, 0.05 M) showed faster mobility ($\tau_c = 2.0 \times 10^{-10}$ s) than the less protonated dendrimer at pH 8 (6SBD-T, 0.05 M, $\tau_c = 2.6 \times 10^{-10}$ s). In the adducts formed by adding DNA to the dendrimers, the labels are expected in slow motion conditions, but as also shown in Scheme 2, both fast exchange of the dendrimers among different localizations, at different distances from DNA surfaces, and a large fraction of "free" dendrimers gave rise to an average correlation time for motion, still in fast motion conditions. An increase of τ_c from 3.0×10^{-10} s for 6SBD-T (0.01 M) without DNA (solid line) to 4.4×10^{-10} s with C.T.DNA (dashed line) confirms at least a weak dendrimer−C.T.DNA interaction. Similar results were found for the interaction of *n*SBD-T with vesicles.[9]

The variation in concentration of both the dendrimer and the DNA in a large range of concentrations slightly modified the mobility (results not shown), indicating that the distribution of the dendrimers in the different regions depicted in Scheme 2 holds at both low and high concentrations.

Addition of C.T.DNA to 6SBD-T$^+$ (0.05 M) caused a decrease in rotational motion of the label (τ_c about 5×10^{-10} s) and significant line broadening in the EPR spectrum. Such line broadening is usually caused by an increase in local concentration of the labels. We suggest that the interactions of the dendrimers with DNA facilitated the contact and collisions of the dendrimers themselves (spin−spin interactions producing line broadening).

By adding the dendrimers to the C.T.DNA solutions, a precipitate could be readily detected by visual inspection. However, this observation was not reflected by a significant change in the EPR spectra; i.e., the EPR spectra indicated that the labels remain mobile in the absence and presence of C.T.DNA.

To provide evidence of the SBD−DNA interactions, we adopted a "freezing" procedure as follows: the dendrimers that are far from the DNA surface and not affected by this surface itself behave as a salt in solution upon freezing. This means that this "free" dendrimers (the salt) separate from the ice-forming water at the characteristic freezing temperature of this salt solution (Scheme 2). In the dendrimer cluster formed upon freezing, the microcrystalline distribution of orientations of the magnetic tensors, together with strong spin−spin interactions, resulted in a lack of the correspondent EPR signal. The remaining dendrimers, whose labels contribute to the EPR signal, do not belong to the frozen solution. The "not freezing" solution shows a glass transition due to a modification of the water properties, which, in turn, are attributed to the DNA−dendrimer interactions. The "not interacting" dendrimers in the frozen solution were quantified by the decrease in signal intensity (evaluated from double integration of the spectra) upon freezing. For instance, only about 20% of the labels remained "visible" after the freezing transition of the 6SBD-T−C.T.DNA solution. This amount increased to about 40% for 2SBD-T−C.T.DNA at low dendrimer concentration (0.01 M), but it considerably decreased (down to 10%) at high 2SBD-T concentration (0.05 M), which presupposes self-aggregation of the dendrimer macromolecules. For 6SBD-T$^+$ (pH = 5.5) the frozen fraction was negligible; presumably, the structure of the water was modified by the positive charge of the dendrimer surface, and the solution with "free" dendrimers far from the DNA surface could not freeze (glass transition). For 2SBD-T$^+$, only 50−60% of the labels remained after freezing. The lower charge density of the dendrimer surface, due to the open structure of the 2SBD-T$^+$ dendrimers,[18] affected the hydration water properties much less than 6SBD-T$^+$.

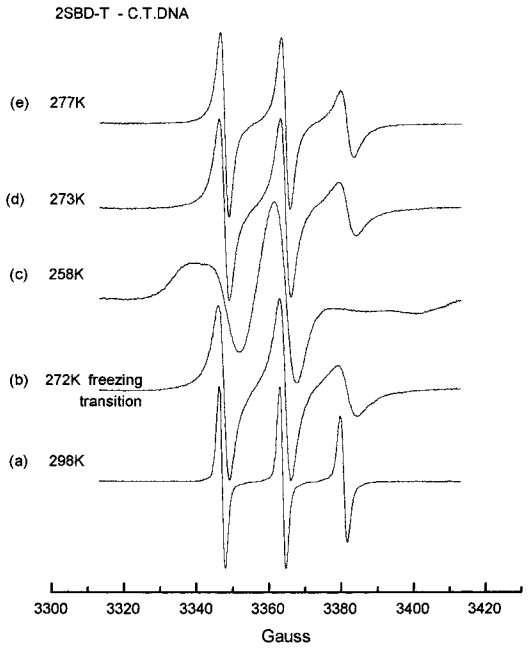


Figure 2. EPR spectra of a 2SBD-T (0.01 M)−C.T.DNA (1 mM) mixture recorded at various temperatures: 298 K (a), the temperature was decreased to 272 K (b), and 258 K (c). Afterward the temperature was increased to 273 K (d) and 277 K (e).

The validity of this "freezing" process presupposes that dendrimer−polynucleotide interaction and structures are not perturbed by freezing, and at least the local configuration of the interacting sites remains intact at low temperature. This has been already assumed for biostructures upon rapid freezing of their solutions.[19,20] Below the freezing transition, the spin-labels attached to "not frozen dendrimers" (corresponding to those of "free" SBDs close to DNA and "interacting" SBDs (Scheme 2)) showed the gradual immobilization with the decrease in temperature, which is usually found for glass transitions. As an example, Figure 2 shows the EPR spectra recorded at various temperatures for 2SBD-T (0.01 M) with C.T.DNA (1 mM). Starting from 298 K, the temperature was lowered to the transition (freezing) temperature; after a further decrease down to 258 K, the temperature was again increased, and spectra were recorded at higher temperatures. (Spectra at 273 and 277 K are reported as examples in Figure 2.) The spectral conditions found at the beginning of the process (room temperature) were restored by rising the temperature to the same initial temperature. The transition temperature was about 265−267 K for all samples (results not shown), with the exception of samples containing C.T.DNA (example given in Figure 2), for which the transition temperatures of diluted solutions were much higher, almost the same as for pure water (270−272 K). The heterogeneity of the dendrimer−C.T.DNA mixture accounts for the high transition temperature, i.e., freezing transition involves the free dendrimers which negligibly interact with C.T.DNA. These free dendrimers at low concentration are well separated from the dendrimers involved in the gel

formation with C.T.DNA. Therefore, the portion of the solution containing free dendrimers behaves as pure water.

Comparison among the various *n*SBD-T−polynucleotide systems was accomplished at 258 K: the good resolution of the spectral features at this temperature allowed, by means of spectral computation, an easy differentiation among the various systems (dendrimers at different protonation extent, interacting with the various polynucleotides). Figure 3 shows the EPR experimental spectra (solid lines) at 258 K for 6SBD-T in the presence of the various polynucleotides, at two concentrations, 0.05 M (Figure 3a) and 0.01 M (Figure 3b), and for 6SBD-T^+ (0.05 M) in the presence of the various polynucleotides (Figure 3c). Figure 4 shows the EPR experimental spectra (solid lines) at 258 K for 2SBD-T (Figure 4a) and for 2SBD-T^+ (Figure 4b), both at a concentration of 0.05 M, in the presence of the various polynucleotides. The dashed lines, superimposed on the experimental spectra, are the spectra computed by means of the program of Schneider and Freed.[14b] The spectra of Poly(GC) and C.T.DNA in Figure 3b were computed with the same parameters (same dashed line between the two spectra). The sharp line which becomes evident in low-intensity spectra, such as the spectra of C.T.DNA and Poly(AT) in Figure 3b, at about 3370 G, arose from the reference (diphenylpicrylhydrazine = DPPH; $g = 2.0036$) introduced in the EPR cavity in several measurements to evaluate the magnetic parameters. In some cases the computation did not produce a good fitting with the experimental signal, even if obtained after accurate analysis and many calculation attempts. This is expected on the basis of the limits of the calculation procedure and, mainly, on the approximations assumed in the computation, such as the number of spectral components constituting the overall signal or the invariance of the magnetic parameters. As described in the Introduction, the main parameters extracted from the computation, which were chosen to provide information on the SBD−DNA interactions (mainly to differentiate the various systems from each other), were the correlation time for rotational motion and the relative percentages of the components contributing to the spectra. These parameters are listed in Table 1 for the "interacting component" of the spectra in Figures 3 and 4.

The occurrence of interactions between dendrimers and polynucleotides is confirmed by comparing the spectra for the various polynucleotides in Figures 3 and 4 and the corresponding parameters in Table 1. The following information was extracted from spectral analysis:

(1) The EPR signal consists of two components. This result is consistent with the working description in Scheme 2, which assumes that two fractions of SBDs underwent to glass transition by lowering the temperature, i.e., "essentially free" SBDs close to DNA and the SBDs "interacting" with DNA. Accordingly, the first component showed the same mobility conditions for all systems, that is, $\tau_c = 2.3 \times 10^{-9}$ s, with the exception of those systems containing 6SBD-T^+, for which the first component had $\tau_c = 1.4 \times 10^{-9}$ s. The difference in τ_c between 6SBD-T and 6SBD-T^+ reflects the difference already found for dendrimers in solution in the absence of DNA at room temperature. A high degree of protonation prevents the label from interacting with neighboring unprotonated amino groups at the dendrimer

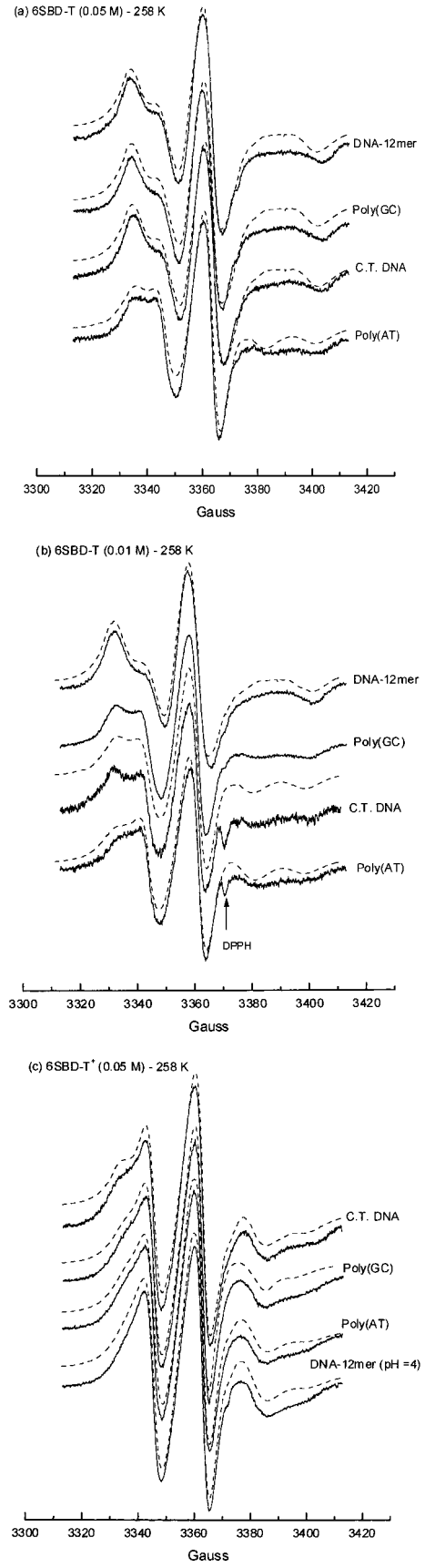


Figure 3. Experimental EPR spectra (solid lines) at 258 K of 6SBD-T and 6SBD-T^+ in the presence of the various polynucleotides: (a) 6SBD-T 0.05 M, (b) 6SBD-T 0.01 M, and (c) SBD-T^+ 0.05 M. The dashed lines, superimposed on the experimental spectra, are the simulated spectra.

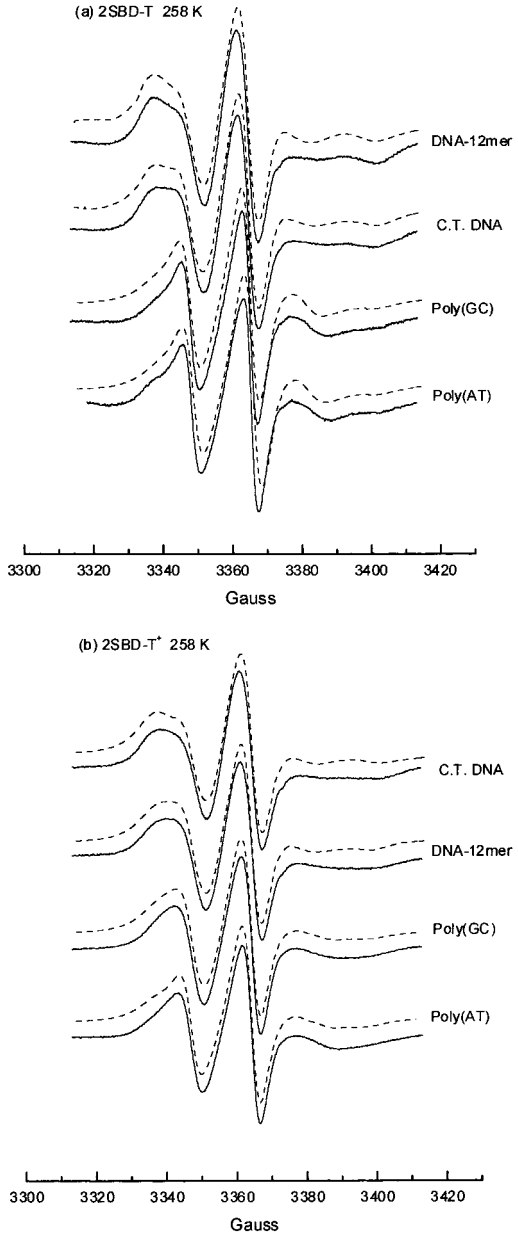


Figure 4. Experimental EPR spectra (solid lines) at 258 K of 2SBD-T (a) and 2SBD-T^+ (b), both at a concentration of 0.05 M, in the presence of various polynucleotides. The dashed lines, superimposed on the experimental spectra, are the simulated spectra.

surface, and therefore, the mobility of the probe increases. The second component showed a higher correlation time for motion, which changed from one system to another, together with its relative percentage. This second component is therefore called an "interacting component" and is diagnostic of the interacting ability (mainly electrostatic or dispersion force interactions) of the various polynucleotides toward the dendrimer surface.

(2) DNA-12mer interacted more strongly with *n*SBD-T than C.T.DNA and the other polynucleotides, on the basis of both the lower mobility and the higher fraction of radicals involved in the interaction. This can be explained by the short length of the DNA-12mer (approximately 42 Å) compared to the other polynucleotides. Both the length of the DNA-12mer and the diameter of the SBDs (see Scheme 1) are on the same order of magnitude. Especially in the case of 6SBD-T,

Table 1. Percentages and Correlation Times of the "Interacting Component" for the Various *n*SBD-T−Polynucleotide Systems at 258 K[a]

SBD	[SBD], M	polynucleotide	pH	%[b]	τ[c] ($\times 10^{-9}$ s)
6SBD-T	0.05	DNA 12-mer	8	93	8
6SBD-T	0.05	C.T. DNA	8	93	7.5
6SBD-T	0.05	Poly(GC)	8	93	7
6SBD-T	0.05	Poly(AT)	8	75	6.5
6SBD-T	0.01	DNA 12-mer	8	98	8
6SBD-T	0.01	C.T. DNA	8	75	6.5
6SBD-T	0.01	Poly(GC)	8	75	6.5
6SBD-T	0.01	Poly(AT)	8	65	6
6SBD-T^+	0.05	DNA 12-mer	4	50	4.5
6SBD-T^+	0.05	C.T. DNA	5.5	50	7
6SBD-T^+	0.05	Poly(GC)	5.5	50	5.5
6SBD-T^+	0.05	Poly(AT)	5.5	50	5
2SBD-T	0.05	DNA 12-mer	8	85	5.5
2SBD-T	0.05	C.T. DNA	8	75	5
2SBD-T	0.05	Poly(GC)	8	30	5
2SBD-T	0.05	Poly(AT)	8	30	5
2SBD-T^+	0.05	DNA 12-mer	5.5	77	5
2SBD-T^+	0.05	C.T. DNA	5.5	80	5.5
2SBD-T^+	0.05	Poly(GC)	5.5	70	4.5
2SBD-T^+	0.05	Poly(AT)	5.5	60	4.5

[a] See Schemes 2 and 3 for interpretation. [b] Percentage of "interacting component" which corresponds to the slower-motion component in the remaining EPR signal after freezing out of the "completely free" SBD; accuracy 5%. [c] Accuracy 5%.

it is likely that not more than one SBD molecule can interact with one molecule of DNA-12mer. But the situation should be different for the long polynucleotides, which possess more than 1000 base pairs. At the high SBD concentrations used for our studies, many SBD molecules will be involved in the interaction with one DNA molecule. The SBDs might hinder each other, causing a weaker interaction with the DNA backbone. This SBD concentration effect is currently under detailed investigation and will be the subject of an upcoming publication.

(3) Only a small DNA base component selectivity was observed. The interacting ability decreases slightly in the order of C.T.DNA, Poly(GC), and Poly(AT). It is known that alternating self-complementary Poly(AT) denatures locally on its double helix below the melting transition.[21,22] This locally denatured structure could reduce the interaction with the SBD.

(4) Both the correlation time for motion and the relative percentage of "interacting component" were lower in the case of 6SBD-T^+ (pH = 5.5) with respect to 6SBD-T (pH = 8) for all polynucleotides. The decrease in the correlation time for motion for the dendrimer−polynucleotide systems reflected the decrease found in the absence of polynucleotides. The relatively low percentage of "interacting" component for 6SBD-T^+ was expected, since some "free" dendrimers did not undergo freezing transition and still contributed to the EPR spectra at low temperatures.

(5) In contrast, for 2SBD-T, protonation gave rise to a larger fraction of "interacting" labels (for all polynucleotides studied, with the exception of DNA-12mer). In the latter case, freezing was more efficient in eliminating the fraction of free labels (free dendrimers). Furthermore, the small generation SBDs are known to form clusters.[23] Higher protonation (pH = 5.5) prevents the cluster formation and provides a higher concentration of the dendrimers to interact with the polynucleotides.

(6) As a consequence of cluster formation for 2SBD-T, and mainly for the similarity in size and shape

between 6SBD and histones, 6SBD-T resulted in more interaction with polynucleotides than did 2SBD-T. For more protonated dendrimers the trend was reversed; i.e., a higher percentage of labels in 2SBD-T^+ was involved in interactions with the polynucleotides when compared to 6SBD-T^+. This is consistent with the open structure of the small dendrimers, conferring a higher freedom of interaction between the protonated amino groups of the dendrimers, even from the internal layer, and the basic DNA groups. On the other hand, the protonated amino groups in the more densely packed surface structure of 6SBD-T^+ were poorly available to the interaction. Furthermore, higher protonation of the large dendrimers is expected to partially swell the dendrimers and thereby increase the mobility of the labels.

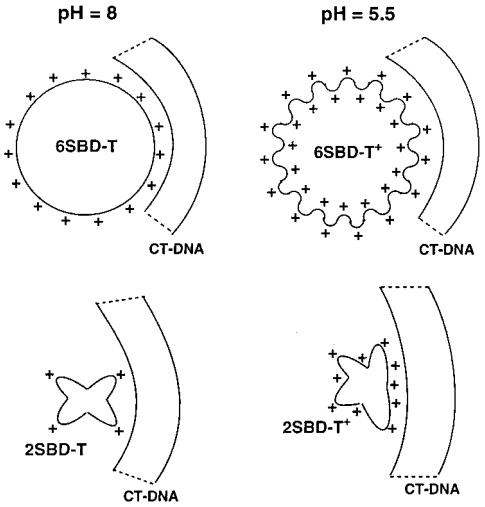
(7) The SBD−polynucleotide interaction strength and the percentage of interacting labels are expected to increase with increasing SBD concentration. This effect mainly holds for 6SBD-T, whereas for 2SBD-T the effect may be largely compensated by self-aggregation of the dendrimer macromolecules. Experimentally, it was found that the increase in τ_c and percentage of the interacting component reached a maximum at a certain concentration, which was different for the various systems and indicated a "saturation condition" of the interacting sites of the polynucleotides (results not shown).

In summary, we need to consider competitions among different kinds of interactions: dendrimer−polynucleotide, dendrimer−dendrimer (intramolecular interactions among the surface groups onto the same macromolecule), dendrimer−dendrimer (intermolecular interactions among different macromolecules), dendrimer−water and buffer components. The dendrimer−dendrimer interactions are responsible for the rather low mobility which was also found in the absence of the polynucleotides.

These results on Tempo-labeled dendrimers and polynucleotides provide some insight into these systems from the "dendrimer point of view". It is of interest that there is good agreement between these results and those obtained in a recent study[24] using a DNA intercalator, ethidium bromide, as a fluorescent probe to investigate DNA−dendrimer interaction from the "DNA point of view". Both studies have found that the interaction with polynucleotides favors high-generation rather than low-generation dendrimers, and interaction increases with concentration until saturation of the interacting sites. These results provide further support to the present conclusions.

It is noteworthy that the EPR line shapes (and, therefore, the mobility conditions) at 258 K for *n*SBD-T−polynucleotide systems are largely comparable to EPR line shapes found at 277 K (the temperature rises after the freezing transition) for *n*SBD-T−vesicle systems.[9] This means that at least the local direct interaction between the labels and surface groups of the biostructures is stronger for the vesicles than for the polynucleotides. Also, the spectra of the *n*SBD-T−vesicle systems in ref 9 show a variation of both the A_{ii} values, and the main rotation axis changed from one system to another. Conversely, the computation of the spectra for the various dendrimer−polynucleotide systems was accomplished by using the same A_{ii} values and main rotation axis for all systems. Therefore, labels interacting with the vesicles were more perturbed in their environment than those interacting with the polynucleotides.

Scheme 3



Conclusions

Aqueous solutions of nitroxide-labeled polyamino-amine dendrimers of generations 2 and 6 (2SBD-T and 6SBD-T, respectively) containing double-stranded polynucleotides, including Calf Thymus DNA, Poly(AT), and Poly(GC), and an oligonucleotide of 12 base pairs (DNA-12mer) were investigated to probe interactions between SBDs and DNA of interest in gene transfection in cell lines.

Computer-aided analysis of the EPR spectra of the nitroxide labels provided information on the mobility conditions of the labels and their partition in different environments, which, in turn, provides indirect information on the interacting abilities of dendrimers and polynucleotides.

As indicated in Scheme 2, room-temperature spectra showed a high mobility of the labels. Spectral analysis showed that, by lowering the temperature, a freezing transition excluded from EPR spectra the fraction of free dendrimers that are far from the DNA surface. The freezing temperature of this free dendrimer fraction, which separated from the SBD−C.T.DNA gel adducts, was almost equivalent to that of pure water. Comparison of the various systems at a selected temperature indicated that the interacting ability with SBDs was in the order DNA-12mer > C.T.DNA > Poly(GC) > Poly(AT). DNA-12mer was postulated to interact more strongly than polynucleotides because of its short length. However in the case of the long polynucleotides, the SBD molecules involved in the complex with one polynucleotide molecule could hinder each other.

Scheme 3 summarizes the conclusions on the basis of dendrimer size and level of protonation of the surface amino groups:

(1) DNA can "wrap" around 6SBD-T better than 2SBD-T, due to the differing size, distribution, and mobility of the interacting surface groups. Furthermore, the surface groups of small dendrimers are more mobile than those of large dendrimers, due to the open structure of the small SBDs (larger entropy).

(2) Higher protonation of the large dendrimers apparently decreased the interaction with DNA. Partial swelling of the dendrimer structure is expected upon electrostatic repulsion of the charged surface groups, which increased mobility. Nevertheless, despite this

destabilization at the local level (interacting sites), globally the interaction is expected to be stronger for more protonated dendrimers due to the electrostatic attraction with the DNA base pairs.

(3) On the other hand, higher protonation of small dendrimers increased the interaction with DNA, due to the large flexibility of the 2SBD-T^+ structure, which allows for greater electrostatic interaction with the DNA surface of the more internally protonated surface groups. Therefore, protonated small dendrimers showed significant interaction with the polynucleotides, which, however, decreased with an increase in SBD concentration, due to competing self-aggregation of the dendrimer molecules. Conversely, interaction between large dendrimers (which do not self-aggregate) and polynucleotides increased with an increase in SBD concentration until saturation of the interacting sites.

By comparing the results from the present study with those obtained from SBD−vesicle systems, we obtained evidence of a preferential interaction of nSBD-Ts with vesicles vis-à-vis polynucleotides. Also, it is interesting to note the comparison between results obtained from this work and those obtained in a similar study on dendrimer−polynucleotide interactions by means of a fluorescent probe.[24] Further studies are in progress to clarify the gene transfection mechanism of SBDs.

Acknowledgment. M.F.O. and B.S. thank the Italian Ministero Università e Ricerca Scientifica (MURST). N.J.T. and S.J. thank the support of the National Science Foundation.

References and Notes

(1) Hayes, J. J. *Chem. Biol.* **1995**, *2*, 127.
(2) Behr, J.-P. *Acc. Chem. Res.* **1993**, *26*, 274.
(3) (a) *Advances in Dendritic Macromolecules*; Newkome, G. R., Ed.; JAI Press: Greenwich CT, 1993. (b) Newkome, G. R.; Moorefield, C. N.; Baker, G. R.; Johnson, A. L.; Behera, R. K. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1176. (c) Makelburger, H. B.; Jaworek, W.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1571. (d) Issberner, J.; Moors, R.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2413. (e) Alper, J. *Science* **1991**, *251*, 1562. (f) Krohn, K. *Org. Synth. Highlights* **1991,** 378. (g) Amato, L. *Sci. News* **1990**, *138*, 298. (h) Kim, Y. H.; Webster, O. W. *J. Am. Chem. Soc.* **1990**, *112*, 4592. (i) Hawker, C. J.; Wooley, K. L.; Fréchet, J. M. J. *J. Chem., Soc. Perkin Trans. 1* **1993**, 1287. (j) Fréchet, J. M. J. *Science* **1994**, *263*, 1710.
(4) (a) Tomalia, D. A.; Hall, M.; Hedstrand, D. M. *J. Am. Chem. Soc.* **1987**, *109*, 1601. (b) Tomalia, D. A.; Naylor, A. M.; Goddard, W. A. III *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 138. (c) Tomalia, D. A.; Durst, H. D. In *Topics in Current Chemistry*; Weber, E., Ed.; Springer-Verlag: Berlin, 1993; Vol. 165, p 193.
(5) Kukowska-Latallo, J. F.; Bielinska, A. U.; Johnson, J.; Spindler, R.; Tomalia, D. A.; Baker, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4897.
(6) Tang, M. X.; Redemann, C. T.; Szoka, F. C. J. *Bioconjugate Chem.* **1996**, *7*, 703.
(7) Bielinska, A.; Kukowska-Latallo, J. F.; Johnson, J.; Tomalia, D. A.; Baker, J. R. *Nucl. Acids Res.* **1996**, *24*, 2176.
(8) Haensler, J.; Szoka, F. C., Jr. *Bioconjugate Chem.* **1993**, *4*, 372−379.
(9) Ottaviani, M. F.; Matteini, P.; Brustolon, M.; Turro, N. J.; Jockusch, S.; Tomalia, D. A. *J. Phys. Chem. B* **1998**, *102*, 6029.
(10) Ottaviani, M. F.; Daddi, R.; Brustolon, M.; Turro, N. J.; Tomalia, D. A. *Appl. Magn. Reson.* **1997**, *13*, 347.
(11) Ottaviani, M. F.; Montalti, F.; Turro, N. J.; Tomalia, D. A. *J. Phys. Chem. B* **1997**, *101*, 158.
(12) Ottaviani, M. F.; Montalti, F.; Romanelli, M.; Turro, N. J.; Tomalia, D. A. *J. Phys. Chem.* **1996**, *100*, 11033.
(13) Berliner L. J., Ed. *Spin Labeling. Theory and Applications*; Academic Press: New York, 1976, Vol. 1; 1979, Vol. 2.
(14) (a) Berliner L. J., Reuben, J., Eds. *Biological Magnetic Resonance. Spin Labeling, Theory and Applications*; Plenum Press: New York, 1989; Vol. 8. (b) Schneider, D. J.; Freed, J. H. In *Biological Magnetic Resonance. Spin Labeling. Theory and Applications*; Berliner, L. J., Reuben, J., Eds.; Plenum Press: New York, 1989; Vol. 8, p 1.
(15) Chaires, J. B.; Dattagupta, N. C.; D. M. *Biochemistry* **1982**, *21*, 3933.
(16) Dvornic, P. R.; Tomalia, D. A. *Macromol. Symp.* **1994**, *88*, 123.
(17) Pauly, G. T.; Bobst, E. V.; Bruckmann, D.; Bobst, A. M. *Helv. Chim. Acta* **1989**, *72*, 110.
(18) Naylor, A. M.; Goddard, W. A. III; Kiefer, G. E.; Tomalia, D. A. *J. Am. Chem. Soc.* **1989**, *111*, 2341
(19) Hashimoto, S.; Thomas, J. K. *J. Am. Chem. Soc.* **1983**, *105*, 5230.
(20) (a) Hiff, T.; Kevan, L. *J. Phys. Chem.* **1989**, *93*, 1572. (b) Baglioni, P.; Kevan, L. *J. Phys. Chem.* **1987**, *91*, 1516.
(21) Millar, D. P.; Robbins, R. J.; Zewail, A. H. *J. Phys. Chem.* **1981**, *74*, 4200.
(22) Inman, R. B.; Baldwin, R. L. *J. Mol. Biol.* **1962**, *5*, 172.
(23) (a) Uppuluri, S.; Keinath, S. E.; Tomalia, D. A.; Dvornic, P. R. *Macromolecules* **1998**, *31*, 4498. (b) Ramzi, A.; Scherrenberg, R.; Brackman, J.; Joosten, J.; Mortensen, K. *Macromolecules* **1998**, *31*, 1621. (c) Briber, R. M.; Bauer, B. J.; Hammouda, B.; Tomalia, D. A. *Polym. Mater. Sci.* **1992**, *67*, 430.
(24) Chen, W.; Turro, N. J.; Tomalia, D. A. *Langmuir*, in press.

MA9815859