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Stability of Dendriplexes Formed by Anti-HIV Genetic Material and Poly(propylene imine) Dendrimers in the Presence of Glucosaminoglycans

Michal Szewczyk,[†] Joanna Drzewinska,[†] Volha Dzmitruk,[‡] Dzmitry Shcharbin,*[,‡] Barbara Klajnert,[†] Dietmar Appelhans,[§] and Maria Bryszewska[†]

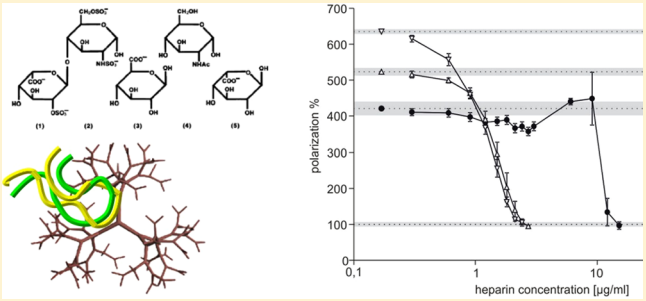
[†]Department of General Biophysics, University of Lodz, Pomorska 141/143, 90-237 Lodz, Poland

[‡]Institute of Biophysics and Cell Engineering of NASB, Akademicheskaja 27, 220072 Minsk, Belarus

[§]Leibniz Institute of Polymer Research Dresden, Hohe Strasse 6, D-01069, Dresden, Germany

Supporting Information

ABSTRACT: There are several barriers to the application of dendriplexes formed by poly(propylene imine) dendrimers and genetic material for gene therapy. One limitation is their interaction with extracellular matrix components such as glucosaminoglycans. These can displace the genetic material from the dendriplexes, affecting their transfection activity. In this study, we analyzed the interaction between dendriplexes and the four main glucosaminoglycans (heparin, heparan sulfate, chondroitin sulfate, and hyaluronic acid) by fluorescence polarization and gel electrophoresis. Dendriplexes were formed by combining three anti-HIV antisense oligodeoxynucleotides with three poly(propylene imine) dendrimers of the fourth generation: unmodified and partially modified with maltose and maltotriose (open shell glycodendrimers). The data showed that the effect of glucosaminoglycans on dendriplexes depends on the glucosaminoglycan type and the oligosaccharide serving as the surface group of the dendrimer. Heparin at physiological concentrations destroys dendriplexes formed by open shell glycodendrimers, but dendriplexes based on unmodified poly(propylene imine) dendrimers are stable in its presence. The other glucosaminoglycans at physiological concentrations cannot destroy dendriplexes formed by any of the dendrimers studied.



INTRODUCTION

Gene therapy is based on two conceptually different approaches. The first involves delivery of plasmid DNA or corresponding constructs for expression of the gene of interest under the control of an appropriate promoter. This results in an increase in the target activity, for example, in the production of a protein acting as a drug. The other involves the delivery of oligomeric genetic material such as antisense oligodeoxynucleotides or short interfering RNAs, which cause a decrease in the target activity, finally resulting in the inhibition of expression of harmful mRNA and/or synthesis of harmful proteins. Such key factors are considered in choosing an approach because of the number of genes involved in pathogenesis (monogenetic/polygenetic), duration of therapy (temporary or permanent), efficacy of approach, suitable targets, and methods for regulation of genetic drugs. None of the presently available viral vectors meets all these requirements. Viral vectors show high efficacy but have major limitations with regard to immunogenicity, risk of insertional mutagenesis, large-scale production, loading capacity, and often poor pharmacokinetics.[1−3] As an alternative, polymeric nonviral vectors have been developed with the aim of condensing DNA for protection against degradation, cellular uptake, and intracellular release. Dendrimers are promising materials for this purpose.[3,4] This class of polymers has structural advantages for gene transport. Dendrimers are monodisperse and stable. They are characterized by relatively low viscosity at high molecular mass and have numerous peripheral groups. Thus, peripheral cationic groups, being ionizable, can bind large amounts of genetic material efficiently.[4] Dendrimers are effective carriers for nonviral modification of stem cells ex vivo.[5−8] Dendrimer-driven modification of stem cells ex vivo and the subsequent in vivo implantation of these cells with overproduction of neurotrophic factors led to recovery of injured nerves in rats.[8] Among the polycationic dendrimers used for DNA delivery, poly(propylene imine) (PPI) dendrimers are prominent, owing to their relatively high gene transfer efficacy.[9,10] Since they show high cationic charge density at physiological pH because of their protonatable tertiary and primary amino groups, PPIs can form noncovalent complexes with all kinds of anionic nucleic acids. Once those complexes (dendriplexes) are internalized, their high gene transfer efficiency is governed by

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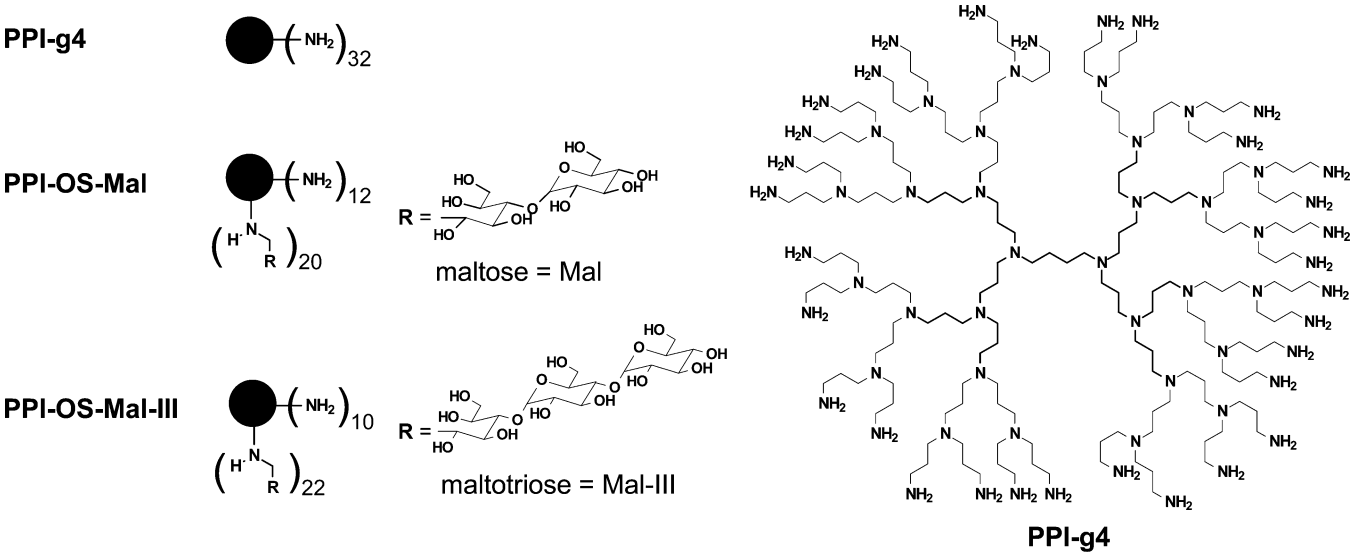

Figure 1. Chemical structure of dendrimers.

facilitated release from endosomes due to the so-called proton sponge effect.[11,12] However, the high cationic charge density of these dendrimers can lead to major drawbacks with regard to biocompatibility, toxicity, complex aggregation, and undesired nonspecific complex interactions with cellular and noncellular components, especially in vivo.[13,14] Their toxicity can be decreased by surface modification.[15,16] In the present article, we used three fourth generation PPI dendrimers (Figure 1): unmodified (PPI-g4), and maltose-(Mal)-modified, and maltotriose-(Mal-III)-modified. These oligosaccharide-modified dendrimers are called open shell glycodendrimers: PPI-OS-Mal and PPI-OS-Mal-III, respectively. PPI-OS-Mal and PPI-OS-Mal-III also have cationic surface charges,[17] as known from the pure PPI-g4, resulting from their residual peripheral amino groups (Figure 1). The oligosaccharide-shelled PPI glycodendrimers demonstrated high biocompatibility under in vitro[16−20] and in vivo conditions.[14] The key factor is the type of sugar shell (dense or open) attached to the dendritic scaffold.[16−22] This is responsible not only for the well-understood H-bond-driven interaction properties of dense shell PPI glycodendrimers in biological experiments[16,18] but also for the combination of H-bond and electrostatic interactions characteristic of open shell dendritic glycopolymers.[19,21,22] Another contribution of the maltose shell to dendrimeric scaffolds, not yet well understood, relates to the pathways for internalization, when polyplexes consisting of DNA and PEI decorated with open maltose shells simultaneously undergo clathrin-dependent and clathrin-independent endocytosis in SKOV-3 cells.[20] The main aim of our study was to investigate the stability of dendriplexes in the presence of glucosaminoglycans. Dendriplex stability in the extracellular matrix is a key factor for desirable transfection efficacy.

Dendriplexes were formed by the interaction between the above-mentioned dendrimers and anti-HIV antisense oligodeoxynucleotides: ANTITAR, GEM 91, and SREV. These oligodeoxynucleotides (ODNs) show enhanced inhibitory effects on HIV-1 replication and can be used in anti-HIV therapy:[23−25] ANTITAR is targeted against *Tat* and the TAR element (*Tat* encodes the protein Tat, transcriptional transactivator, which binds to the transactivation response element TAR of HIV-1); GEM 91 is targeted against *Gag* (*Gag* encodings the protein Gag, the major structural protein of HIV-1); SREV is targeted against *Rev* (*Rev* encodes the protein Rev, the regulatory protein of HIV-1). Previous analysis by our group indicated that PPI-g4 forms stable dendriplexes of 230−270 nm diameter with ANTITAR, GEM 91, and SREV at a molar ratio of 10:1 (dendrimer to ODN).[26] However, questions about the interaction of these dendriplexes with glucosaminoglycans remain unanswered. This problem is very important for successful application of dendriplexes in gene therapy, as in vivo gene transfer is usually less efficient than in vitro and is not easily predictable from in vitro results.[27] After local administration, dendriplexes can interact with plasma proteins, first of all with human serum albumin,[28,29] and then with extracellular matrix components such as proteoglycans. Proteoglycans consist of a core protein covalently linked to one or more sulfated or carboxylated glucosaminoglycans (e.g., hyaluronic acid, chondroitin sulfate, and heparan sulfate).[30] Extracellular polyanionic glucosaminoglycans might bind the dendriplexes and affect their mobility in the tissue[31] and their interaction with target cells.[32] Analysis of the interaction between dendriplexes formed by poly(amidoamine) dendrimers with DNA and glucosaminoglycans showed that the glucosaminoglycans could inhibit the transfection of such dendriplexes, correlating with their destruction.[30] However, the interaction of GAGs with dendriplexes had a complex character. It was found that hyaluronic acid did not release or relax DNA in any complex, but it inhibited transfection by some polyvalent systems (including dendrimers).[30] Gene transfer by other carriers was not affected by hyaluronic acid. Sulfated GAGs (heparan sulfate and chondroitin sulfates B and C) completely block dendrimer-mediated transfection.[30] Sometimes hyaluronic acid increases transfection by DNA complexes.[33,34] Transfection is inhibited by GAGs in many cases without disintegration of the complexes; that can be explained by different mechanisms of GAG action.[33,34] The significant differences in efficiency of transfection by different DNA carriers in the presence of glucosaminoglycans indicate that the study of dendriplex stability in the presence of glucosaminoglycans is a prerequisite for further transfection experiments.[30]

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EXPERIMENTAL METHODS

Materials. Unmodified poly(propylene imine) dendrimer of the fourth generation (3514 g/mol) was purchased from SyMO-Chem (Eindhoven, The Netherlands). Open shell poly(propylene imine) dendrimers of the fourth generation modified with maltose or maltotriose groups were synthesized as described previously.[35,36] The data on the dendrimers used are presented in Table 1. The chemical structure of fourth generation PPI dendrimers is schematically presented in Figure 1.

Table 1. Molecular Weights (M_w) of Fourth Generation PPI Dendrimers and Number of Oligosaccharide Groups Attached on PPI Dendrimer Surface[a]

dendrimer	M_w g/mol	number of oligosaccharide
PPI-g4	3514	0
PPI-OS-Mal	10040	20
PPI-OS-Mal-III	14260	22

[a]Number of oligosaccharides attached on PPI dendrimer surface and M_w of PPI-OS-Mal and PPI-OS-Mal-III were determined by ^{1}H NMR approach.[24,25] Fourth generation PPI dendrimers have 32 peripheral amino groups.

Oligodeoxynucleotides (ODNs) labeled with fluorescein and unlabeled were purchased from Genomed (Poland): ANTI-TAR (AT) with the sequence 5′-GCT CCC GGG CTC GAC C-3′ (16 bases; M_w, 5192); GEM 91 (GEM) with the sequence 5′-CTC TCG CAC CCA TCT CTC TCC TTC T-3′ (25 bases; M_w, 8112.5); and SREV with the sequence 5′-TCG TCG CTG TCT CCG CTT CTT CCT GCC A-3′ (28 bases; M_w, 9086).

Glucosaminoglycans (GAGs) heparin (a mixture of polyanion chains with molecular weights ranging from 6 to 30 kDa, with most chains in the range 17−19 kDa), heparan sulfate (a polydisperse biomolecule with a broad molecular weight distribution 10−50 kDa with most chains in the range of ∼30 kDa), chondroitin sulfate (a polydisperse biomolecule with a broad molecular weight distribution 10−50 kDa, with a significant proportion of the chains in the range of ∼17 kDa) and hyaluronic acid (molecular weight range 100−10 000 kDa)[37,38] were purchased from Sigma-Aldrich (Poland).

Other chemicals were of analytical grade and were purchased from Sigma-Aldrich (Poland). Double-distilled water was used to prepare all solutions.

Fluorescence Polarization. Dendriplexes were prepared in phosphate buffered saline (0.01 M Na-phosphate buffer, 0.149 M NaCl, pH 7.4) by mixing the dendrimer with an ODN for 15 min in a Vortex mixer in the molar ratios determined previously.[26,39] The molar ratios are presented in Table 2.

Dendriplexes (ODN concentration 0.1 μM in each sample) were titrated against GAGs in phosphate-buffered saline, and the fluorescence polarization of fluorescein-labeled ODNs in the dendriplexes was measured. Dendrimers have no intrinsic fluorescence under these conditions. Fluorescence polarization was measured using a Perkin-Elmer LS 55 fluorescence spectrometer equipped with a thermostatted water bath. The excitation and emission wavelengths were 485 and 516 nm, respectively. The excitation and emission slits were 10 nm. Measurements were performed at a constant temperature of 37 °C. Fluorescence polarization was calculated using the formula $P_{ABS} = (I_{VV} - GI_{VH})/(I_{VV} + GI_{VH})$, where P_{ABS} = polarization value, I_{VV} = the intensity of vertically polarized fluorescence after excitation with vertically polarized light, I_{VH} = the intensity of horizontally polarized fluorescence after excitation with vertically polarized light, and G = G-factor value. The G-factor was calculated using the formula $G = I_{HV}/I_{HH}$, where I_{HV} = the intensity of vertically polarized fluorescence after excitation with horizontally polarized light, and I_{HH} = the intensity of horizontally polarized fluorescence after excitation with horizontally polarized light. The relative polarization (P, [%]) was calculated as $P = P_{ABS}/P_{ABS}^0 \times 100\%$, where P_{ABS} and P_{ABS}^0 are, respectively, the fluorescence polarization of the fluorescein-labeled ODN in the presence and absence of a dendrimer.

Table 2. ODN/Dendrimer Molar Ratios for Dendriplexes Used in the Experiments

AT/PPI-g4 = 1/15	GEM/PPI-g4 = 1/20	SREV/PPI-g4 = 1/15
AT/PPI-OS-Mal = 1/5	GEM/PPI-OS-Mal = 1/4	SREV/PPI-OS-Mal = 1/2
AT/PPI-OS-Mal-III = 1/4	GEM/PPI-OS-Mal-III = 1/4	SREV/PPI-OS-Mal-III = 1/3

Agarose Gel Electrophoresis. For gel electrophoresis, a 4% agarose gel and TBE buffer (0.045 mM Trizma base, 0.044 mM boric acid, and 10 mM EDTA) were used. Ficoll 400 (15%) was applied as a loading buffer, which was prepared without dye to enable the fluorescence of fluorescein-labeled ODNs in the samples to be analyzed. Dendriplexes were prepared as described above in the molar ratios given in Table 2. To their solutions, various concentrations of GAGs were added, and the samples were incubated for 15 min at 37 °C. Electrophoresis was conducted at 80 V (8.8 V/cm) for two hours. After electrophoresis, the gel was visualized with an ultraviolet trans-illuminator and photographed.

Particle Size and Zeta Potential. Samples intended for light scattering analysis were prepared at 25 °C using phosphate-buffered saline, which was passed through a 0.22 μm filter to remove trace particulates. Complexes were prepared as described above. The particle size of the complexes was measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.). The light scattered from the incident light was fitted to an autocorrelation function using the method of cumulants. The particle size of a sample was determined from the average of four independent experiments (five replicates in each experiment) in a disposable Malvern plastic cuvette at 25 °C. Zeta potential experiments were carried out by phase analysis light scattering (PALS) using a Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.) at 25 °C. The electrophoretic mobility of the scattering (DLS) samples was determined from three independent experiments (five replicates for each experiment) with the electric field applied in a disposable Malvern plastic cuvette. The zeta potential of each complex was determined from the electrophoretic mobility using the Smoluchowski approximation.

Statistical Analysis. Results are presented as mean ± SEM (standard error of mean), n = 4. Data were analyzed using the Student-Fisher test.

RESULTS

Fluorescence Polarization of Dendriplexes in the Presence of GAGs. To study the stability of dendriplexes in the presence of GAGs by fluorescence polarization, the complexation ratios used between ODN and PPI dendrimers are presented in Table 2; these have been previously published

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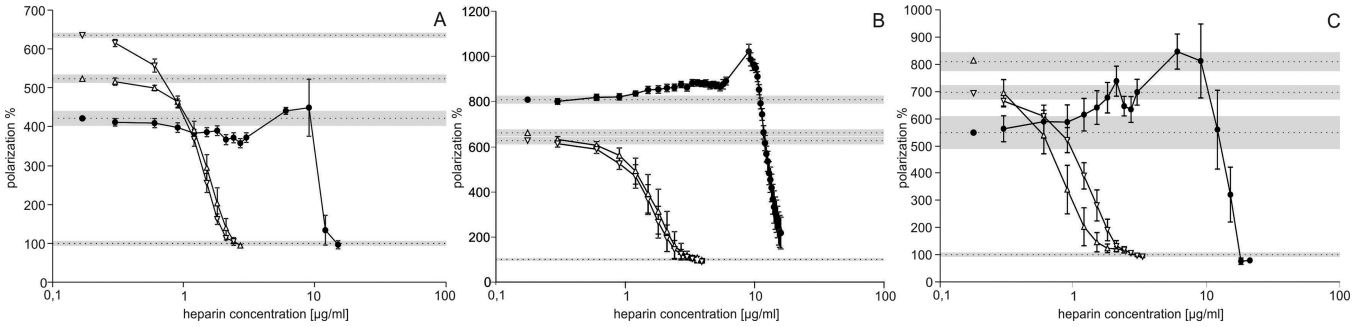

Figure 2. Fluorescence polarization of AT (A), GEM (B), and SREV (C) dendriplexes titrated with heparin. ●, PPI-g4; △, PPI-OS-Mal; ▽, PPI-OS-Mal-III.

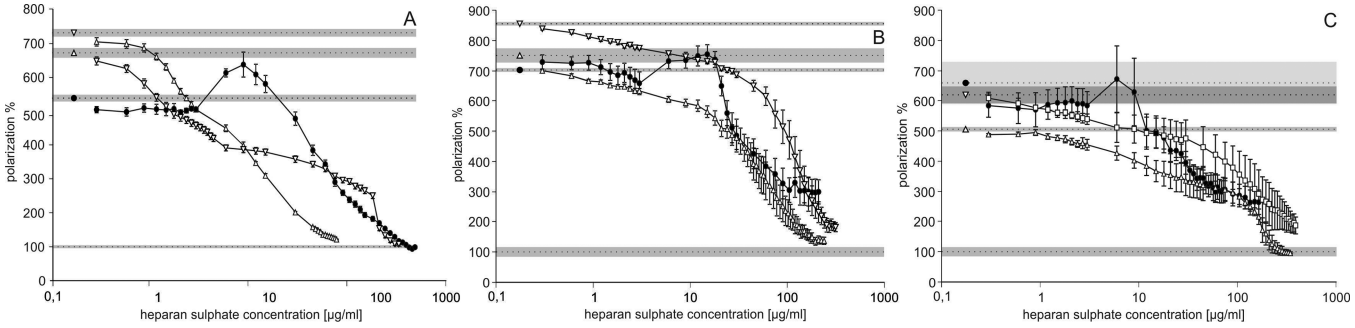

Figure 3. Fluorescence polarization of AT (A), GEM (B), and SREV (C) dendriplexes titrated with heparan sulfate. ●, PPI-g4; △, PPI-OS-Mal; ▽, PPI-OS-Mal-III.

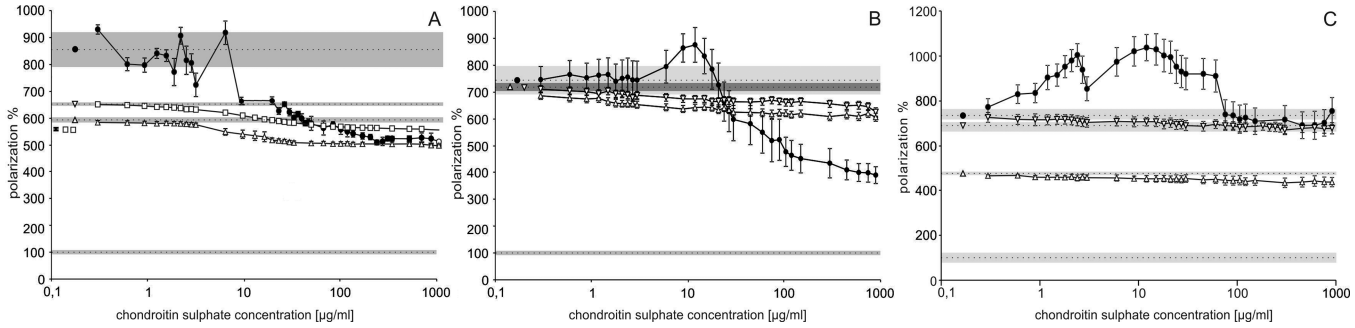

Figure 4. Fluorescence polarization of AT (A), GEM (B), and SREV (C) dendriplexes titrated with chondroitin sulfate. ●, PPI-g4; △, PPI-OS-Mal; ▽, PPI-OS-Mal-III.

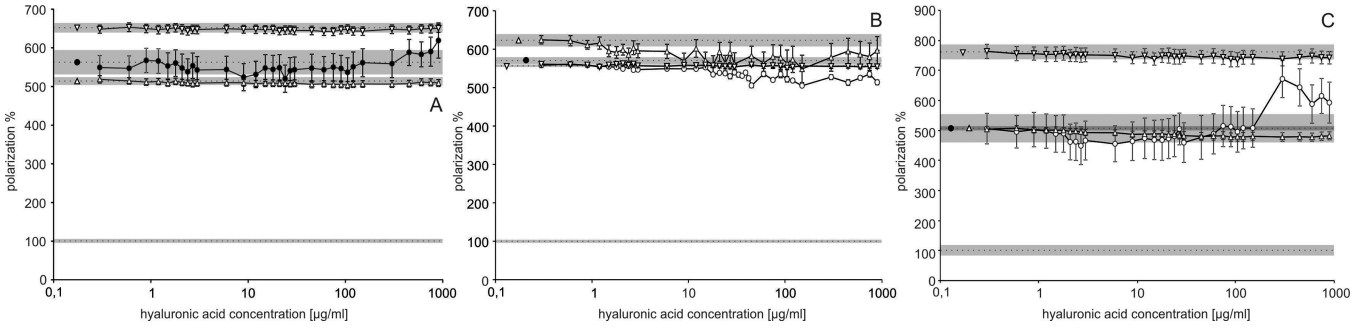

Figure 5. Fluorescence polarization of AT (A), GEM (B), and SREV (C) dendriplexes titrated with hyaluronic acid. ●, PPI-g4; △, PPI-OS-Mal; ▽, PPI-OS-Mal-III.

(for unmodified PPI dendrimers).[26] When a pure PPI dendrimer is used, it needs to be in high excess for dendriplexes to form (15 equivalents (equiv) of PPI for AT and SREV and 20 equiv for GEM). For oligosaccharide-modified PPI dendrimers, fewer molecules are necessary to form the desired dendriplexes (5 equiv of PPI-OS-Mal for AT, 4 equiv for GEM, 2 equiv for SREV; 4 equiv of PPI-OS-Mal-III for AT and GEM; and 3 equiv for SREV).[39] Such molar ratios were chosen on the basis of experiments on the interactions between unmodified and modified PPI dendrimers and ODNs.[39] They provide a

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balance between the biocompatibility of the dendrimers and their transfection efficiency.[18,39] The dendriplexes used in this study had slightly anionic surface charges under physiological conditions. With this in mind, we started to investigate their interaction properties upon the addition of different GAGs.

Figures 2−5 demonstrate the changes in fluorescence polarization of fluorescein-labeled ODNs in dendriplexes upon the addition of GAGs. The fluorescence polarization value of 100% in all the figures corresponds to the value for the pure ODN in solution. As follows from Figures 2−5, the fluorescence polarization of the ODNs in dendriplexes formed by all the dendrimers studied was 5−12 times greater than that of the pure ODNs in solution. These values did not depend significantly on the type of ODN or dendrimer used.

Heparin. The effect of heparin was strongly dependent on the dendrimer used to form the dendriplex. Dendriplexes formed from unmodified PPI-g4/ODNs showed constant or slightly increasing values of ODN fluorescence polarization at heparin concentrations up to 10 μg/mL. The addition of heparin to these dendriplexes at concentrations above 10 μg/mL led to a rapid decrease of the ODN fluorescence polarization, down to the value of the free ODN in solution at 20 μg/mL heparin. In contrast, dendriplexes consisting of PPI-OS-Mal and PPI-OS-Mal-III dendrimers showed gradually decreasing values of the ODN fluorescence polarization starting from 0.6 to 0.8 μg/mL of heparin. The polarization values fell to that of the free ODN at 2−3 μg/mL heparin.

Heparan Sulfate. The addition of heparan sulfate to all the dendriplexes studied led to the gradual decrease of ODN fluorescence polarization, down to the value of the free ODN in solution at a heparan sulfate concentration of about 500 μg/mL (except for the AT/PPI-OS-Mal dendriplex, which had a corresponding value at 100 μg/mL heparan sulfate). Unlike the effect of heparin, the effect of heparan sulfate did not depend significantly on the dendrimer type.

Chondroitin Sulfate. In contrast to heparin and heparan sulfate, chondroitin sulfate over a wide range of concentrations (0−1000 μg/mL) had no effect on the ODN fluorescence polarization of dendriplexes formed from OS glycodendrimers and ODNs. It had some perturbing effect on the fluorescence polarization of unmodified PPI-g4/ODNs dendriplexes, depending on the ODN used.

Hyaluronic Acid. Like chondroitin sulfate, hyaluronic acid over a concentration range of 0−1000 μg/mL did not affect the ODN fluorescence polarization of any of the dendriplexes.

Electrophoretic Mobility of ODNs in Dendriplexes in Presence of GAGs. Gel electrophoresis was used as an independent method for estimating dendroplex stability in the presence of GAGs. The result of a typical experiment (AT/dendrimers/heparin) is presented in Figure 6: the AT concentration in all wells was 0.02 μmol, and the dendrimer concentrations were those given in Table 2. Well 1 (A, B, C) contained pure AT. Wells 2−10 contained dendriplexes of AT with PPI-g4 (A), PPI-OS-Mal (B), and PPI-OS-Mal-III (C), without (well 2) and with (wells 3−10) heparin. The heparin concentrations (in μg/mL) used with PPI-g4 were 60 (well 3), 90 (4), 120 (5), 150 (6), 180 (7), 210 (8), 240 (9), and 270 (10); for PPI-OS-Mal and PPI-OS-Mal-III, they were 12 (well 3), 14 (4), 16 (5), 18 (6), 20 (7), 22 (8), 24 (9), and 26 (10). All other data on the stability of dendriplexes in the presence of GAGs are presented in the Supporting Information. As follows from Figure 6, AT bound to any of the dendrimers (arrows) showed no mobility in the gel, in contrast to the free form.

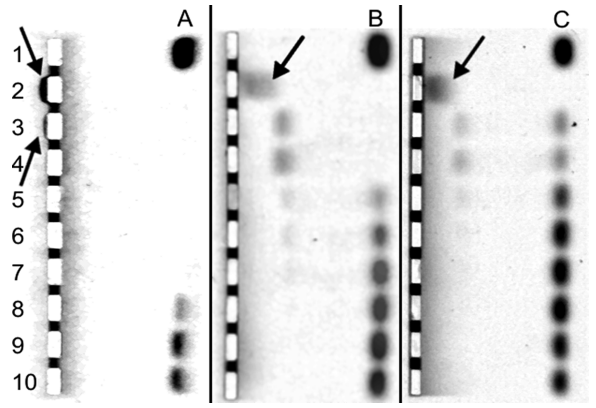

Figure 6. Gel electrophoresis of pure AT (well 1, A−C) and AT in dendriplexes with PPI-g4 (A), PPI-OS-Mal (B), and PPI-OS-Mal-III (C), without (well 2) and with (wells 3−10) heparin.

Increasing concentrations of heparin led to increased amounts of free AT, which moved in the gel. The results on the electrophoretic mobility of ODNs in all dendriplexes in the absence and presence of GAGs are in good agreement with the fluorescence polarization data. Some of the observed differences can be explained by differences in functioning of these two techniques.

Particle Size and Zeta Potential of Dendriplexes in the Absence and Presence of GAGs. The particle size and zeta potential of dendriplexes in the absence and presence of GAGs are presented in Table 3. The GAGs were added at maximal concentration when the dendriplexes were still stable. Only the data with polydispersity index (PDI) < 0.7 are presented (see the discussion section). These data represent the dendriplexes based on the unmodified PPI-g4 dendrimer. The addition of GAGs led to both an increase in the zeta-size of the complexes and a decrease of their zeta potential. The addition of hyaluronic acid to dendriplexes led to the formation

Table 3. Particle Size, Polidispersity Index (PDI), and Zeta Potential of Selected Dendriplexes in the Absence and in Presence of GAGs

dendriplex	zeta size (nm)	PDI	zeta potential (mV)
AT/PPI-g4	295 ± 9	0.18 ± 0.01	+6.4 ± 0.4
AT/PPI-g4 + heparin 1 μg/mL	405 ± 16	0.30 ± 0.02	+5.5 ± 0.3
AT/PPI-g4 + heparan sulfate 10 μg/mL	502 ± 46	0.60 ± 0.02	−5.0 ± 0.4
AT/PPI-g4 + chondroitin sulfate 10 μg/mL	356 ± 20	0.41 ± 0.02	+2.4 ± 0.2
GEM/PPI-g4	309 ± 16	0.19 ± 0.02	+11.4 ± 0.4
GEM/PPI-g4 + heparin 1 μg/mL	325 ± 14	0.19 ± 0.02	+8.7 ± 0.3
GEM/PPI-g4 + heparan sulfate 10 μg/mL	909 ± 99[a]	0.73 ± 0.03[a]	−1.8 ± 0.2[a]
GEM/PPI-g4 + chondroitin sulfate 10 μg/mL	536 ± 38	0.60 ± 0.04	+6.2 ± 0.3
SREV/PPI-g4	320 ± 11	0.23 ± 0.02	+12.6 ± 0.4
SREV/PPI-g4 + heparin 1 μg/mL	315 ± 9	0.21 ± 0.01	+6.8 ± 0.5
SREV/PPI-g4 + chondroitin sulfate 10 μg/mL	647 ± 55	0.64 ± 0.04	+2.3 ± 0.3

[a]The example of measurement in which PDI was higher than 0.7.

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of polydisperse complexes with PDI > 0.7, probably because of the extremely high molecular weight (and length) of hyaluronic acid in comparison with the other GAGs. All dendriplexes based on glycodendrimers had PDI > 0.7.

DISCUSSION

Glucosaminoglycans, having a negative charge, can compete with the ODN for dendrimer binding. This causes release of the ODN from the dendriplex, resulting in a decrease of fluorescence polarization. Gel electrophoresis offers an independent technique for studying dendroplex stability in the presence of GAGs. Under the experimental conditions used, the naked ODNs are electrophoretically mobile and reach the end of a well. The formation of a dendriplex prevents their mobility in the gel.[28,29,40,41] GAGs competing with an ODN for dendrimer binding cause the release of the ODN and increase its mobility in the gel.

Heparin and Heparan Sulfate. Dendriplexes formed by PPI-g4 dendrimers were stable at concentrations of heparin up to 10 μg/mL. The addition of heparin to these dendriplexes in concentrations higher than 10 μg/mL led to their rapid destruction (the release of ODN from the dendriplexes was complete at a heparin concentration of ∼20 μg/mL). In contrast, dendriplexes formed by OS glycodendrimers showed a 10-fold reduction in stability in the presence of heparin. Since heparin has the highest negative charge density of the GAGs and PPI-g4 has the maximum number of terminal amino groups (positive charge density), one should expect a relatively strong electrostatic interaction between unmodified PPI-4 and heparin, which can result in the destabilization of the dendriplexes and release of the ODNs even at low heparin concentrations. However, it should be noted that the ODNs interact more strongly with the unmodified PPI than with the glycodendrimers.[26,39] The main reason for this difference is the lower cationic charge density and surface charge due to the attachment of oligosaccharide units on the fourth generation PPI scaffold.[16,17] Furthermore, the attached oligosaccharide units can also hamper a stronger interaction with the cationic dendritic PPI scaffold in PPI glycodendrimers. Finally, the lower molar ratio between glycodendrimer and ODN will also contribute to weakening the stability of dendriplexes formed from PPI glycodendrimers in comparison with unmodified PPI-g4.[26,39] Despite the lower stability of dendriplexes based on PPI glycodendrimers under in vitro conditions, one recent study[19] showed that cationic polyplexes based on siRNA and cationic maltose- and maltotriose-modified poly(ethylene imine)s (PEI) can undergo different biodistribution effects in organs from those of unmodified PEI, administered intravenously. This example for a cationic dendritic glycopolymer shows that different types of such polymers could have potential applications via bloodstream administration.[19]

It is interesting that the addition of heparan sulfate to all dendriplexes led to their gradual destruction and release of the ODN in concentrations ∼100 times higher than that of heparin. Five dendriplexes showed similar stabilities (concentration of heparan sulfate for total dendriplex destruction, ∼500 μg/mL), while the AT/PPI-OS-Mal dendriplex was destroyed at 100 μg/mL heparan sulfate. The heparin concentration causing the destruction of dendriplexes with OS glycodendrimers was comparable to or lower than that in the plasma of healthy human donors (1−2.4 μg/mL).[42] However, heparin in physiological (1−2.4 μg/mL)[42] or therapeutic (2.5−6.25 μg/mL) concentrations[43] in human plasma did not destroy the dendriplexes based on the unmodified PPI-g4 dendrimer. Unlike heparin, heparan sulfate at physiological concentrations (∼0.04−0.1 μg/mL)[44] in human plasma did not destroy any of the dendriplexes studied. The difference between the effects of heparin and heparan sulfate can be explained by their differences in chain length and percentage sulfation. Heparin has the highest negative charge density of any known biological molecule.[38] Heparan sulfate is structurally very similar to heparin, but its disaccharide units are organized into distinct sulfated and nonsulfated domains.[45]

Hyaluronic Acid and Chondroitin Sulfate. In contrast to heparin and heparan sulfate, these GAGs had little or no effect on dendriplex stability at any of the concentrations studied (0−1000 μg/mL). The concentration of hyaluronic acid in human plasma ranges from 0.01 to 0.1 μg/g (∼0.01−0.1 μg/mL),[36] in cerebrospinal fluid 0.02−0.32 μg/g (∼0.02−0.32 μg/mL),[46] and in joint fluid 2−4 mg/mL in healthy humans and ≤0.5 mg/mL in rheumatoid infusions.[47] Chondroitin sulfate is the main GAG in human plasma.[38] Its plasma concentration in healthy humans ranges from 3.69 to 6.65 μg/mL and in patients with arthrosclerosis from 4.0 to 8.56 μg/mL.[48] It is also present at a concentration of 0.55 mg/mL in the synovium and ∼0.04 mg/mL in synovial fluid.[47] We can conclude that hyaluronic acid and chondroitin sulfate at physiological concentrations in human plasma have no effect on dendriplex stability.

Unfortunately, the great polydispersity of GAGs makes it very difficult to measure the sizes of the complexes they form with dendriplexes by dynamic light scattering (Table 3). The polydispersity index is a dimensionless measurement of the broadness of size distribution calculated from cumulant analysis.[49−52] If this index is high,[40] the mixture contains particles of widely different sizes and the hydrodynamic diameter will be an average of this mixture (for example, if the mixture comprises 80% of a 100 nm dendriplex and 20% of an approximately 1000 nm aggregate, the measured, average, hydrodynamic diameter will be 200−400 nm). The manufacturers' recommended range of PDI is below 0.7[49−52] since the distribution algorithms operate best over this range.[49−52] In our experiments, dendriplexes based on glycodendrimers had PDIs > 0.7. The zeta size and zeta potential results of dendriplexes (based on unmodified PPI-g4) in the absence and presence of GAGs showed that the addition of GAGs increased the zeta size and decreased the zeta potential of the complexes. This effect depended on the GAG used. It correlated with the slight increase of fluorescence polarization of dendriplexes in the presence of GAGs (except hyaluronic acid).

Analysis of the data allowed several steps in the interactions in an ODN−dendrimer−GAG system to be elucidated: (A) Interaction between ODNs and dendrimers and the formation of dendriplexes. The interaction between ODNs and dendrimers led to a 7−8-fold increase in the fluorescence polarization of fluorescein attached to the ends of the ODN and to changes in the zeta potential of a dendriplex from negative to positive values when increasing concentrations of dendrimers were added (data not presented). These interactions depended on both the type of ODN and the type of dendrimer. (B) Interaction between dendriplexes and GAGs. Such interactions led to an increase of zeta size, a slight increase of fluorescence polarization (the growth of a complex and the decrease of its molecular motions) and a slight decrease of the zeta potential of the complex up to the critical concentration of GAG. In contrast to other GAGs, hyaluronic acid differed in its interaction with dendriplexes (high PDI, no

change in fluorescence polarization). A possible reason is the extremely high molecular weight (and length) of hyaluronic acid in comparison with other GAGs. An increase of the GAG concentration over the critical value (which depended on GAG type) led to rapid destruction of a dendriplex. This process was similar to the interaction between dendriplexes and serum albumins.[28] This interaction is apparently driven by electrostatic forces.[28,30−34]

On the basis of previous studies,[30−34] we suppose that the interaction between GAGs and dendriplexes based on unmodified and modified PPI-g4 dendrimers can explain the decrease of transfection efficiency of the dendriplexes in the presence of GAGs in several ways.[30−34] First, binding to a GAG releases DNA from the complex. Second, this interaction changes the charge and size of the dendriplex, thereby affecting its cellular uptake or intracellular distribution.[30−34] Third, the complexes with GAGs might not behave as expected in the cells and endosomes: heparan sulfate may direct the complexes to a route unfavorable for transfection, while hyaluronic acid could lead to a more beneficial pathway.[30]

We also should notice that the fluorescence polarization and gel electrophoresis techniques proved much more useful for analyzing the destruction of dendriplexes by GAGs than zeta potential and zeta size measurements. This can be attributed to the selectivity of these techniques: both of them demonstrate the peculiarities of behavior of fluorescein-labeled ODNs in the system (complexation of ODNs with dendrimers and decomplexation under the action of GAGs). In contrast to zeta measurements, they are not sensitive to the presence of any complexes and aggregates that do not contain labeled ODNs. However, the information from zeta measurements is more helpful for understanding the nature of the interactions during dendriplex formation and destruction and for characterizing the dendriplexes.

CONCLUSIONS

Thus, heparin at physiological concentrations in human plasma can destroy dendriplexes formed by open shell glycodendrimers, while dendriplexes based on unmodified poly(propylene imine) dendrimers are stable in its presence. In contrast to heparin, heparan sulfate, chondroitin sulfate, and hyaluronic acid at physiological concentrations in human plasma cannot destroy dendriplexes formed by any of the dendrimers studied. Taking into account that heparin has the highest negative charge density of any known biological molecule,[42] we suppose that any other glucosaminoglycan (e.g., dermatan sulfate, keratan sulfate)[43] at physiological plasma concentrations will not be able to destroy dendriplexes formed by unmodified or modified PPI dendrimers. However, the analysis of transfection into wild-type and GAG-deficient CHO cells showed that cell surface GAGs (especially heparan sulfate) strongly inhibit transfection by lipoplexes and polyplexes.[30] Thus, our data indicate that the intravenous administration of dendriplexes based on OS glycodendrimers may result in a relatively rapid ODN release. This means that OS glycodendrimers could be used for the delivery of anticancer drugs or for disaggregating amyloids or scrapie proteins in the treatment of Alzheimer's or prion diseases.[16−22] Nevertheless, further studies will be directed to the application of dendriplexes for transfection of genetic material into cells, finally clarifying the question of using these nontoxic dendrimers for gene delivery.

ASSOCIATED CONTENT

Supporting Information

Data on stability of dendriplexes in the presence of glucosaminoglycans by gel-electrophoresis technique. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Fax: +375-17-2842359. E-mail: d.shcharbin@mail.ru.

Notes

The authors declare no competing financial interest.

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