Dextran-Spermine Conjugate: An Efficient Vector for Gene Delivery

*T. Azzam, H. Eliyahu, A. Makovitzki, A. J. Domb**

Department of Medicinal Chemistry and Natural Products, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel
Fax: +972-26758959
E-mail: adomb@md.huji.ac.il

Summary: Cationic Polysaccharides based on oligoamine-dextran conjugates were synthesized and tested as vectors for gene transfection. Dextran with 40 kDa in average molecular weight was oxidized under mild conditions by potassium periodate to obtain the respective polyaldehydes in relatively high yields (~90%). The oxidized dextran was reacted by reductive amination with various oligoamines of 2 to 4 amino groups to obtain the corresponding imine-conjugates. These water-soluble polymers were then reduced by excess of sodium borohydride to obtain the corresponding amine-conjuagtes in 30-40% overall yield. The electrostatic interactions of the representative polycations with plasmid DNA were evaluated as a function of charge ratio (+/-, polymer/DNA) and ionic strength of the medium applying the ethidium-bromide quenching assay. Although most synthetic polycations formed stable complexes with Plasmid DNAs, only the dextran-spermine conjugate of a defined amino content and molecular weight was able to transfect cells with high efficiency.

Keywords: dextran; polyamines; polyelectrolytes; reductive-amination; spermine

1. Introduction

Polycations are a leading class of nonviral gene-delivery systems in part because of their molecular diversity that can be modified to fine tune their physicochemical properties (1-2). These polycations are able to condense a large gene into smaller and compact structures and to mask the negative DNA charge, necessities for transfecting most types of cells (3-6). Polycations used for gene complexation are polyamines that become cationic at physiologic conditions. All polymers contain primary, secondary, tertiary, or quaternary amino groups capable of forming electrostatic complexes with DNA under physiologic conditions (7). The highest transfection activity is obtained usally at a 1-5 charge ratio of polycation to DNA, respectively (8). The most

 CCC 1022-1360/00/$ 17,50+.50/0

studied polycations used for gene complexation and delivery include linear and branched structures, block and graft copolymers (Figure 1). The linear polycation category include diethylamino-dextran (DEAE-dextran) (9), poly(vinyl pyridine) (10), linear poly(ethyleneimine) (11-12), chitosan (13) and poly(dimethyl aminoethyl methacrylate) (14-16). Branched backbone polycations include pAMAM dendrimer (17-18) and branched poly(ethyleneimine) (12). Block polycations include poly(ethyleneglycol)-poly(ethyleneimine) and poly(hydroxypropyl methacrylate)-poly(trimethyl aminoethyl methacrylate) copolymers (19). The last category (i.e., graft copolymers) include a copolymers of various molecular weights poly(L-lysines) and poly(ethylene glycol) (20), dextran (21), hyaluronic acid (22), poly(hydroxypropyl methacrylate) (20) or poly(dimethyl aminoethyl methacrylate) (23). All of the above are polycations with random distribution of the cationic sites along the polymer chains. This randomness is probably the reason for the fact that these polymers may work for some nucleotides and cell types and not for others. Most of these polymers are toxic to cells and nonbiodegradable, while the polymers based on amino acids such as poly(lysines) are immunogenic (24). More advanced polymeric gene delivery systems employ macromolecules with a very high cationic charge density that act as endosomal buffering systems, thus suppressing the endosomal enzymes activity and protecting the DNA from degradation. The high cationic charge mediates both DNA condensing and buffering capacity that diminish the requirement for the addition of endosomolytic agent (25-27).

In a recent publication (28), we reported on a new class of biodegradable polycations capable of complexing and administering various genes to many cell lines in relatively high yields. More than 300 different polycations were prepared starting from various polysaccharides and oligoamines having two to four amino groups. These polycations were prepared by reductive amination reaction between primary amines and periodate-oxidized polysaccharides. Transfection efficiencies of these synthetic polycations indicated that the oligoamine type has a crucial effect in the transfection activity of the synthetic polymers. Short oligoamines of 2 amino groups (i.e., aliphatic diamines) gave no transfection activity at all tested charge ratio. The most active oligoamine was the naturally occurring tetramine (spermine) conjugated to dextran with a defined aldehyde content and molecular weight. Other spermine analogues when conjugated to dextran resulted in drastic decrease in the transfection yield. The purpose of the present study was to evaluate the ability of these polymers to form complexes with plasmid DNAs and to evaluate the

transfection activity of the polymers in several cell lines as functions of conjugate type and charge ratio.

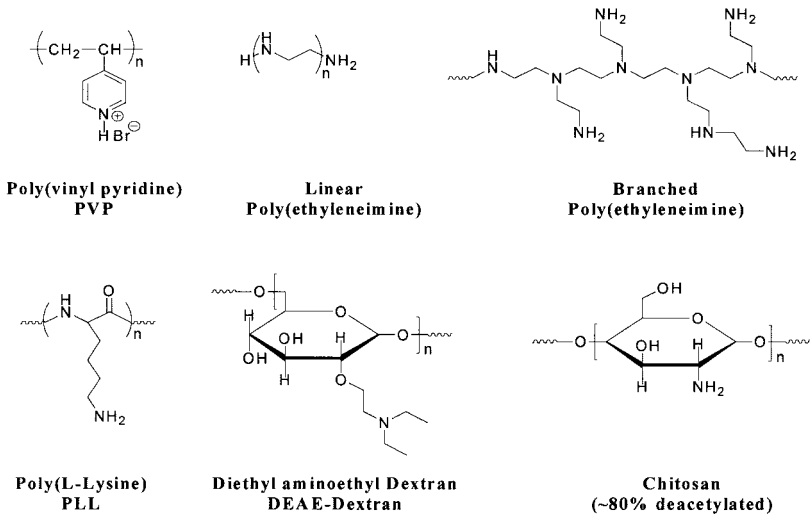


Figure 1: Structures of representative polycations commonly used in gene delivery.

2. Experimental

2.1 *Materials*

All solvents and reagents were of analytical grade and were used as received. Dextran of 40 kDa average molecular weight was obtained from Sigma Chemical Co. (St. Louis, MO). Spermine, Spermidine, N,N-dimethyl propaneamine, N,N-Bis(3-aminopropyl)-1,3-propanediamine { Tetramine [3:3:3] }, N,N-Bis(3-aminopropyl)-ethylenediamine { Tetramine [3:2:3] } , N,N-bis(2-aminoethyl)-1,3-propanediamine { Tetramine [2:3:2] }, potassium periodate and sodium borohydride were obtained from Fluka Chemie (Buchs, Switzerland). A sage-metering pump model-365 (Orion, NJ) was used for slow and reproducible addition of reactants. IR spectra were recorded on a Perkin Elmer System 2000 FT-IR. Molecular weights of starting polymers and conjugates were determined on GPC-Spectra Physics instrument (Darmstadth, Germany) containing a pump, column (Shodex KB-804 or KB-803) and refractive index (RI) detector. Molecular weights were estimated using pullulan standards (PSS, Mainz, Germany) of molecular

weights between 5,800 and 212,000. Eluents used were 0.05M $NaNO_3$ for the uncharged polymers and 3% acetonitrile in 5% Na_2HPO_4 (pH 4) for the cationic polymers. Elemental microanalysis (%N) of the polymers was performed on Perkin-Elmer 2400/II CHN analyzer. Primary amino contents of the synthetic polyamines were calculated by the TNBS method (29) using spermine as standard.

2.2 *Oxidation of Dextran*

Dextran (10 g, 62.5 mmole of glucose units) was dissolved in 200 ml of double deionized water (DDW) and to this solution was added potassium periodate at 1:1 mole ratio (IO_4^- / saccharide). The mixture was vigorously stirred in the dark at room temperature until a clear yellow solution was obtained (6-8 h). The resulting polyaldehyde derivative was purified from iodate (IO_3^-) and unreacted periodate (IO_4^-) by Dowex-1 (acetate-form) anion exchange chromatography, followed by extensive dialysis against DDW (12,000 cutoff cellulose tubing) for 2 days and at 4°C. Purified polyaldehyde derivative was freeze-dried to obtain a white powder in 90% average yield. FT-IR (KBr) = 1724 cm^{-1} (C=O).

The aldehyde content was determined by the hydroxylamine hydrochloride method (30).

2.3 *Synthesis of Dextran-Oligoamine Based Conjugates*

A solution of oxidized dextran (1 g) in 100 ml DDW (6.9 mmole aldehyde groups) was slowly added during 5 h (sage metering pump) to a basic solution containing 1.25 equimolar amount of oligoamine (to aldehyde) dissolved in 50 ml borate buffer (0.1 M, pH 11). The mixture was gently stirred at room temperature for 24 h and reduced by the addition of excess sodium borohydride (1 g) and stirring at room temperature for 48 h. The reduction was repeated with additional portion of $NaBH_4$ (1 g) and stirring for 24 h under the same conditions. The resulting light-yellow solution was dialyzed against DDW (6x6L) applying 3,500 cutoff cellulose tubing (Membrane Filtration Products Inc., San Antonio, TX, USA) followed by lyophilization to obtain a yellowish reduced amine-based conjugate in 30-40% overall yield.

2.4 *Ethidium-Bromide Quenching Assay (Condensation Studies)*

Polymer/DNA complexes were prepared in the appropriate medium according to literature (31). In brief, equal volumes (50 µl) of pLuc-DNA (0.6 µg/well) and ethidium bromide (4 µg/ml) were mixed on 96-well plates. Polymer solutions of 10 mg/ml (DDW) were diluted in the appropriate buffer and added in 50 µl to the wells to obtain different charge ratios between positive charges of

the polymers and the negative charge of the plasmid DNA. The range of +/- was chosen between 0.1 to 2 where the positive charge was determined according to primary amino content (TNBS) assuming 100% protonation and the negative charge according to the universal phosphate content of DNA (1μg = 3.08 nmole phosphate groups). Fluorescence was measured using excitation wavelength of 530 nm and emission at 590 nm. The extent of DNA condensation by polycation is inversely proportional to the percentage of remaining fluorescence after condensation. The maximum fluorescence (100%) is measured when ethidium-bromide is intercalated to DNA without addition of the polymer, but with addition of equal volume of blank buffer. Mediums used for the condensation studies were DDW, 150mM NaCl, 1.5M NaCl and 20mM HBS (hepes buffer saline).

2.5 *In Vitro Transfection*

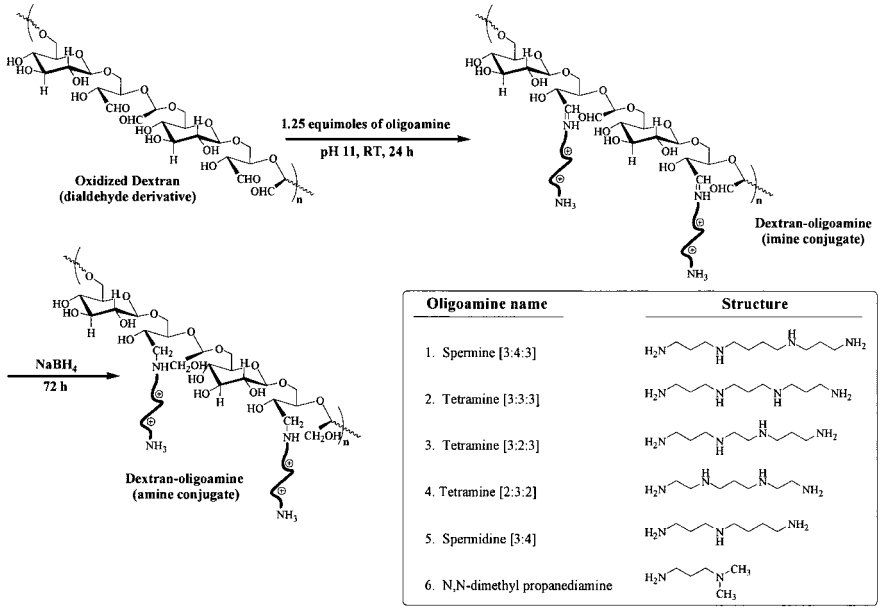
Transfections with polycations were performed and analyzed using expression plasmid pCMV-Luc containing luciferase gene under the control of cytomegalovirus long terminal repeat enhancer/promoters. 2 μg of purified plasmid (Qiagen kit) per well of transfected cells was mixed with dextran-oligoamine at a variety of weight ratios, from 1 to 20 +/- (polycation/DNA, respectively). The DNA/polycation complexes at a particular weight ratio were diluted to a final volume of 200 μl and allowed to stand at room temperature for 30 min. 24-well plates, seeded 24 h before the transfection with $1.5x10^5$ cells per well (NIH3T3), were washed with serum-free medium (SFM), and 200 μl of the complexes containing 2 μg of DNA in SFM were added to each well of cells, and incubated for 4 h at 37°C. The medium containing the complexes was then replaced with standard growth medium (10% FCS). The cells were incubated in culture for 48 h and the cells were lysed according to standard protocol. Lysed cells were removed from the wells and centrifuged (13,000 rpm, 5 min, 4°C). Samples of 20 μl were analyzed from supernatant by adding 100 μl of luciferene reagent and luminescence detected using luminometer TD-20/20 DLReady (Turner designs). Most transfection results were recorded relatively to the commercial transfecting reagent DOTAP/Chol 1/1 (Avanti Polar Lipids Inc., Alabaster, Alabama). In some experiments, the results were corrected for protein concentration using BSA as a standard (Microplate power wave-X, Bio-TEK instrument, Vermont, USA) and expressed as relative unit-light (RUL) per μg polymer.

DOTAP/Chol 1/1 lipid was used as control reference applying the manufacturer's instructions. In brief, 2.32 μl of 1mM DOTAP/Chol 1/1 solution in 10mM Hepes was added to 2 μg DNA solution and the mixture was diluted to 200 μl with the same buffer and allowed to stand at room temperature for 30 min. Then, it was added to cell well in SFM, incubated for 4 h and SFM was replaced with growing medium (10% FCS) and incubated for 48 h. the cells were lysed and the gene expression was evaluated as described above.

3. Results and Discussion

3.1 *Synthesis and Characterization of Dextran-Oligoamine Polycations*

Dextran of 40 kDa in average molecular weight was oxidized using 1 equimolar amount of potassium periodate (to saccharide units) in water and at room temperature for 7 h. The resulting polyaldehyde derivative was purified from iodate and unreacted periodate ions by DOWEX-1 anion-exchange chropmatography (acetate form) following extensive dialysis against DDW and finally lyophilization to obtain a white powder in 90% overall yields. The aldehyde content was determined according to the hydroxylamine hydrochloride method (30) and found to be 6.8-7.4 (mmole aldehyde per 1 g polymer, N=8). Polycations (dextran-oligoamine based conjugates) were prepared by reacting the desired oligoamine with oxidized dextran in aqueous medium by means of reductive-amination reaction. An aqueous solution of oxidized dextran at known average molecular weight and aldehdye content, was added dropwise during 5 h to a buffered aqueous solution (0.1M borate, pH 11) containing the desired oligoamine at 1.25 equimolar excess to aldehyde groups. The purpose of the slow addition was to minimize crosslinking and to facilitate grafting of oligoamine moieties onto the polymer backbone. After 24 h, the imine based conjugate was reduced by the addition of excess sodium borohdyride and allowed to react at room temperature for 48-72 h. In some cases, additional portion of borohydride was added and stirring was continued at the same condition for another 24 h. The resulting reduced polycation solution was purified from unreacted oligoamine by extensive dialysis (3,500 MWCO) against DDW at 4°C following lyophilization to dryness.



Scheme 1: Synthesis of dextran-oligoamine based conjugate. Names and structures of the oligoamines used for conjugation are summarized in the square above.

The oligoamines used for conjugation were spermine [3:4:3], spermidine [3:4], N,N-Bis(3-aminopropyl)-1,3-propanediamine [3:3:3], N,N-Bis(3-aminopropyl)-ethylenediamine [3:2:3], N,N-Bis(2-aminoethyl)-1,3-propanediamine [2:3:2] and N,N-dimethyl 1,3-propanediamine (scheme 1). The polycations were characterized by nitrogen elemental analysis (%N), primary amino content (TNBS) and average molecular weight (GPC) as shown in Table 1. Minor differences in %N and primary amino content (TNBS) were obtained using the various oligoamines indicating similar degree of amination. N,N-dimethyl propanediamine when conjugated to oxidized dextran gave as expected negative TNBS values indicating the absence of primary amino functionalities and the presence of tertiary amino groups (LS16, Table 1).

Table 1. Chemical Characterization of Dextran Derivatives Grafted with Various Oligoamines.[a]

Code	Oligoamine type	%N	TNBS (μmole/mg)	*Mw*	*Mn*	*P*
G7TA54	Spermine	10.84	1.25 ± 0.05	8,770	4,765	1.84
LS-5	Spermidine	10.05	1.48 ± 0.03	6,120	4,070	1.5
G7TA24	Tetramine [3:3:3]	9.58	1.07 ± 0.08	6,135	4,190	1.46
LS-15	Tetramine [3:2:3]	9.67	1.28 ± 0.05	6,950	5,190	1.34
G7TA23	Tetramine [2:3:2]	11.70	1.06 ± 0.03	5,480	4,530	1.21
LS-16	N,N-dimethyl propanediamine	6.36	0.05 ± 0.02	4,000	3,540	1.13

[a] Reaction conditions: oxidized dextran (6.9 mmole aldehyde/g) and the appropriate oligoamine (1:1.25 mole ratio, respectively) were allowed to react under similar conditions as described in the Experimental Section. %N was determined by elemental microanalysis and primary amino content was determined by the TNBS method (Experminetal) and expresses as μmole primary amine per 1 mg conjugate (n=3). *Mw*, *Mn* and polydispersity (*P=Mw/Mn*) were determined by GPC as described in Experimental Section for the polycationic materials.

3.2 *Condensation Studies Using Ethidium Bromide Quenching Assay*

Reduction in ethidium bromide-DNA fluorescence can be used to indicate condensation of DNA. Excited ethidium bromide fluoresces upon intercalating into DNA, while packing of DNA with a polycation results in ethidium bromide expulsion and fluorescence quenching (31). A series of polycations were tested for their ability to condense DNA as a function of charge ratio (+/-) and ionic strength of the medium. Complexes were allowed to stand at room temperature for at least 30 min prior the fluorescence reading (λ_{ex}=530 nm; λ_{em}=590 nm) to ensure complete condensation.

Figure 2 shows a typical condensation profile in 20 mM HBS medium (pH 7.4) of various cationic polymers complexed with pLuc-DNA at various charge ratios ranging from 0.1 to 2 (+/-). The charge ratios were expressed as primary amino content (TNBS) for the positive charge of the polymer, and phosphate groups for the negative charge of the DNA (1 μg = 3.08 nmole phosphate). Dextran-spermine based conjugate (Figure 2, •) efficiently condense DNA in respect to other grafted oligoamines. At 0.1 charge ratio (+/-) nearly 44% condensation of DNA helices was obtained with dextran-spermine, while conjugates grafted with spermidine and spermine

analogues resulted in low degree of condensation (~10%). Higher charge ratio (i.e., 0.25 +/-) resulted in maximum condensation in spermine and [3:2:3] tetramine (Figure 2 • and ▲, respectively). At 0.5 to 2 charge ratios (+/-) maximum condensations (~95%) were obtained in all tested polycations indicating a complete saturation of the negative charge of the DNA helices. The reason for the complete DNA condensation at low charge ratios (i.e. 0.25-0.5, +/-) is probably a result of a contribution of the electrostatic interactions between secondary amine functionalities and negative phosphate groups. In addition to primary amino group, each oligoamine moiety contains at least two secondary amino groups in the case of spermidine (triamine) and three secondary amino functionalities in the case of spermine and spermine analogues. These secondary amino groups are probably the reason for the ability of these polymers to mask the negative charge of the DNA in relatively low polymer concentrations.

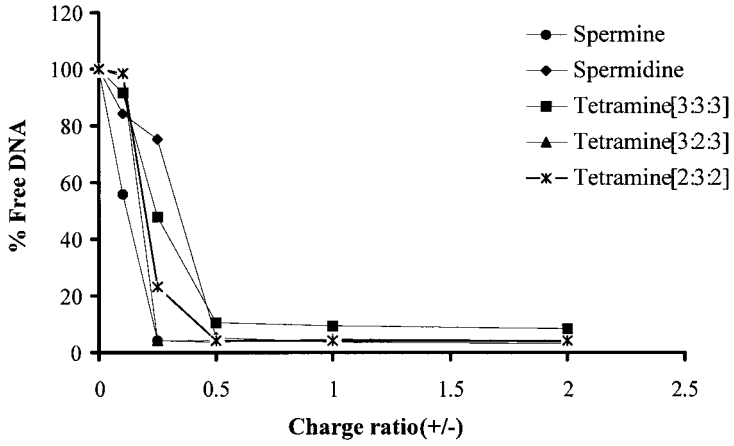


Figure 2: Condensation of pLuc-DNA with cationic dextran conjugated with spermine (•), spermidine (♦), N,N-Bis(3-aminopropyl)-1,3-propanediamine (■), N,N-Bis(3-aminopropyl)-ethylenediamine (▲) and N,N-Bis(2-aminoethyl)-1,3-propanediamine (*). 20 mM HBS (pH 7.4) was used as the condensation buffer (Experimental).

In a similar condensation experiment, dextran-spermine based conjugate was allowed to react with pLuc-DNA in various mediums and ionic strengths. Figure 3 summarizes the condensation profile of (dextran-spermine)-DNA applying the ethidium bromide quenching assay. When double-distilled water (DDW) was used as the condensation medium, maximum condensation

(~90%) was obtained at 0.25 charge ratio (primary amino groups / phosphate groups, respectively). Further addition of polycation (i.e. 0.5 to 2, +/-) did not increase the degree of condensation probably due to full saturation of the negative charge of the DNA. Mediums of low ionic strengths (20mM HBS and 150mM NaCl, Figure 3) resulted in large condensation disruption at low charge ratios (0.1 and 0.25, +/-) and minor disruption at 0.5 to 2 charge ratios where nearly ~90% condensation was obtained. High ionic strength medium (1.5M NaCl, Figure 3) resulted in drastic disruption in the polymer-DNA condensation at all tested charge ratios. At 0.1 to 0.5 charge ratio, slight and negligible condensation were obtained. The highest tested charge ratio (i.e. 2, +/-) resulted in only 45% condensation. The reason for the dependence between polymer-DNA condensation and the ionic strength of the medium is probably related to the nature of the electrostatic interaction between the negative phosphate groups and positive amines. High sodium and chloride ions deactivate the cationic and the anionic nature of the amino and phosphate groups, respectively.

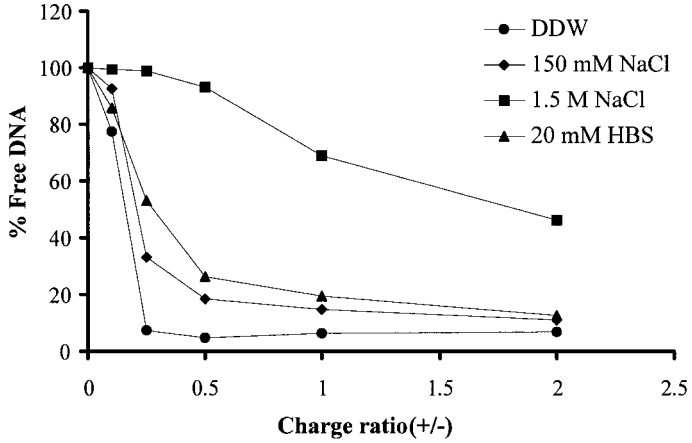


Figure 3: Condensation of pLuc-DNA with cationic dextran-spermine based conjugate in DDW (•), 150 mM NaCl (♦), 1.5 M NaCl (■) and 20 mM HBS pH 7.4 (▲).

The importance of the primary amino groups for condensation was tested in a cationic dextran lacking primary amino functionalities. N,N-dimethyl 1,3-propane diamine was conjugated to oxidized dextran under similar conditions (Scheme 1 and Table 1). The %N content of the polymer indicated almost a similar substitution of aldehyde groups in respect to other conjugated

oligoamines (spermine, spermidine and spermine analogues). The absence of primary amino groups was proven by the negative TNBS values obtained (LS-16, Table 1). This polycation was allowed to react with pLuc-DNA under similar conditions and the condensation profile (ethidium bromide assay) was recorded as functions of charge ratios and ionic strengths of the mediums (Figure 4).

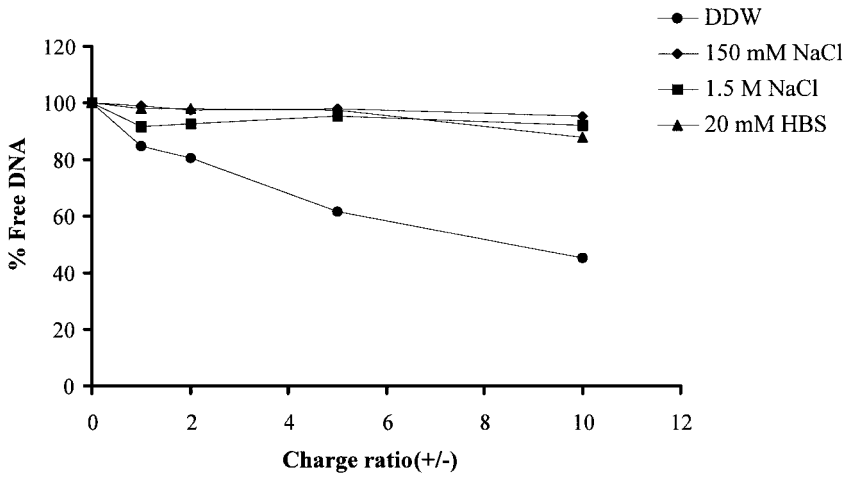


Figure 4: Condensation of pLuc-DNA with dextran-(N,N-dimethyl 1,3-propanediamine) based conjugate in DDW (•), 150 mM NaCl (♦), 1.5 M NaCl (■) and 20 mM HBS pH 7.4 (▲). The charge ratio in this case was expressed as nitrogen content (%N, elemental analysis) divided by the calculated amount of phosphate groups.

It could be shown clearly from Figure 4 that the absence of primary amino groups in this type of polymer reduces the cationic nature of the polymer and hence to a low capability of DNA-condensation. In DDW, only 40% condensation was obtained with 10 charge ratio (+/-). Higher charge ratios (up to 20, +/-) did not significantly improve condensation (data not shown). To the contrary, when condensation was conducted in low and high ionic strength mediums (i.e. 20 mM HBS, 150mM and 1.5M NaCl) low and negligible condensation were recorded at all tested charge ratios.

3.3 *In Vitro Transfection*

3.3.1 Type of Oligoamine

The transfection efficiencies of the synthetic polycations were evaluated in NIH3T3 cells applying pLuc-DNA as the marker gene. Solutions of the polycations and the plasmid DNA were separately mixed at increasing weight ratios ranging from 2 to 15 (polymer/DNA). The polymer-DNA complexes were allowed to stand at room temperature for at least 30 min prior to the transfection to insure complete condensation of the DNA. 48 h post transfection, cells were lysed and the protein content (luciferase) was determined according to standard protocol (Experimental). Figure 5 summarizes the relative transfection yields of pLuc-DNA applying five different polycations at several weight ratios.

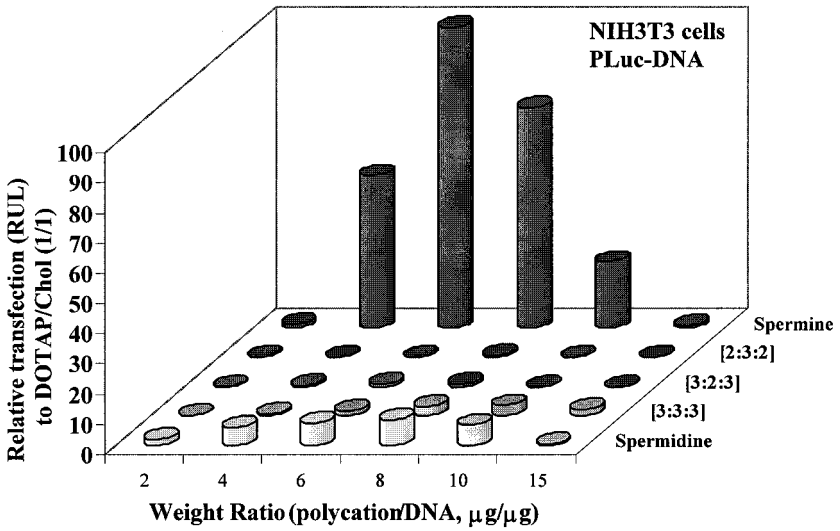


Figure 5: Relative transfection efficiencies (RUL) to DOTAP/Chol (1/1) of polycationic dextrans conjugated with spermidine, tetramine [3:3:3], tetramine [3:2:3], tetramine [2:3:2] and spermine. NIH3T3 cells and pLuc-DNA were used. Each polycation was tested at increasing weight ratios ranging from 2 to 15 (polymer/DNA, μg/μg) as described in Experimental section.

As shown in Figure 5, dextran conjugates of spermine analogues (i.e. tetramines of [3:3:3], [3:2:3] and [2:3:2]) resulted in no transfection at all tested charge ratios. Dextran-spermidine based conjugate when used as a vector resulted in low transfection yields (~8% in relative to

DOTAP/Chol 1/1) at 6 to 10 weight ratio (polymer/DNA). Other tested weight ratios resulted with no transfection. The only polymer which gave a satisfactory transfection results was dextran-spermine conjugate. Applying this polymer as a gene vector resulted in high transfection yields at 4 to 8 weight ratio. The optimal weight ratio of this polymer for cell-transfection was found to be 6 (polymer/DNA) which is equivalent to ~3.25 charge ratio (+/-, primary amines/phosphate groups). At this weight ratio, similar transfection yield was obtained in comparison to the control DOTAP/Chol 1/1. Low and high weight ratios (i.e. 2 and 15) resulted with low transfection. Dextran polycation lacking primary amino functionalities which was prepared by conjugation of dextran with N,N-dimethyl propanediamine (LS-16, Table 1) resulted as with no transfection at all tested charge ratios from 1 to 25 (data not shown).

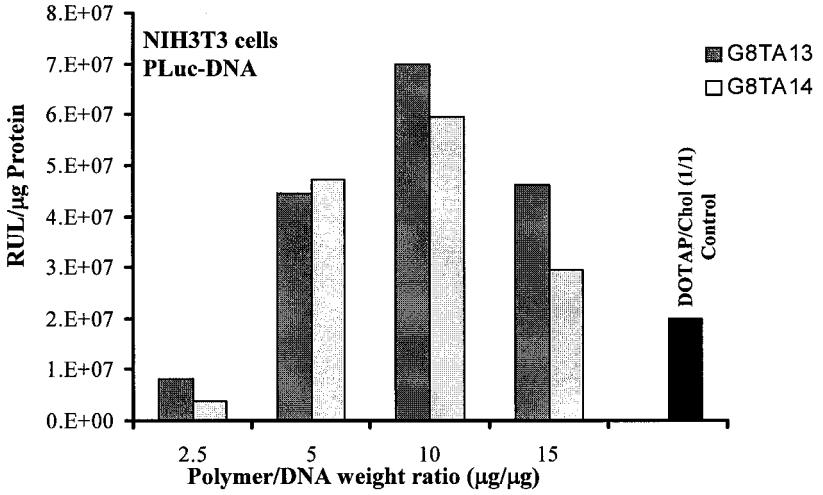


Figure 6: NIH3T3 cells transfected pLuc-DNA as the marker gene applying two identical dextran-spermine based conjugates (coded as G8TA13 and G8TA14) as the plasmid vectors. DOTAP/Chol 1/1 commercial transfecting reagent was used as reference and the transfection yields were corrected to the total concentration of proteins (Experimental).

In an additional experiment, two similar dextran-spermine based conjugates were synthesized and characterized as described earlier (Experimental). The two polymers (coded with G8TA13 and G8TA14) were tested for their transfection abilities as illustrated in Figure 6. The purpose of the experiment was to evaluate the reproducibility of the synthesis and activity of the polymers in

cell-transfection. The transfection yields were corrected to the total concentration of protein in each well. As shown in Figure 6 maximum transfections were obtained with the two polymers at 10 weight ratio (polymer/DNA) and the lucifirase content was determined and found to be in the region of 7.E+07 (RUL per μg total protein). At 5 and 15 weight ratio, the two polymers also gave a satisfactory transfection results in comparison to the control (3 to 4.5E+07 RUL/μg protein). At high weight ratios (up to 25, polymer/DNA) low and negligible transfection results were obtained (data not shown). The synthesis of dextran-spermine was found to be reproducible in terms of degree of conjugation and grafted spermine content. Transfection results on the other hand were also found to be reproducible but the weight ratio needed for optimal transfection varied from polymer to polymer. The optimal weight ratio needed for transfection in all tested dextran-spermine conjugates was found to be in the range of 6 to 10 (polymer/DNA). This variation could be explained by the slight changes in spermine content and degree of grafting, which in part could resulted with a major changes in DNA-condensation and therefore to a variation in complexation behaviors. Also, it was noticed that the commercial control (DOTAP/Chol 1/1) is non-reproducible transfecting reagent and the protein yields remarkably varied from experiment to experiment.

4. Conclusions

A series of synthetic polycations were prepared by reductive-amination reaction between oligoamines and oxidized dextran. The resulting imine conjugates were reduced to the stable amine conjugates by sodium borohydride. Nitrogen elemental analysis and primary amino content of the synthetic polymers indicated that the degree of substitution of the different oligoamines was almost similar in all conjugates. Although most synthetic polycations formed stable complexes with plasmid DNA as determined by the ethidium-bromide quenching assay, only the dextran-spermine conjugate was found to be active in transfecting cells in vitro. This work emphasize that the structure of the polycation has a significant role in the transfection activity.

Acknowledgments

Tony Azzam is grateful to the ministry of science, Israel, for the financial assistance. This work was supported in part by the AFIRST, French-Israeli Cooperation, and by the US-Israeli Bi-

National Fund (BSF). A. J. Domb is affiliated with the David R. Bloom Center for Pharmacy and with the Alex Grass Center for Drug Design and Synthesis at the Hebrew University of Jerusalem.

(1). Domb, A. J.; Levy, M. *Polymers in Gene Therapy: Frontiers in Biological Polymer Application*; Ottenbrite, R. M. ed.: Lancaster, PA, **1999**; 1-16.
(2). Putnam, D.; Gentry, C. A.; Pack, D. W.; Langer, R. *Proc. Natl. Acad. Sci. U. S. A.,* **98**, 1200 (2001).
(3). Haynes, M.; Garrett, R. A.; Gratzer, W. B. *Biochemistry, 9*, 4410 (1970).
(4). Plank, C.; Zatloukal, K.; Cotten, M.; Mechtler, K.; Wagner, E. *Bioconjugate Chem.,* **3**, 533 (1992).
(5). Plank, C.; Oberhauser, B.; Mechtler, K.; Koch, C.; Wagner, E. *J. Biol. Chem.,* **269**, 12918 (1994).
(6). Wagner, E.; Plank, C.; Zatloukal, K.; Cotten, M.; Birnstiel, M. L. *Proc. Natl. Acad. Sci. U. S. A.,* **89**, 7934 (1992).
(7). De Smedt, S. C.; Demeester, J.; Hennink, W. E. *Pharm. Res.,* **17**, 113 (2000).
(8). Abdallah, B.; Hassan, A.; Benoist, C.; Goula, D.; Behr, J. P.; Demeneix, B. A. *Hum. Gene Ther.,* **7**, 1947 (1996).
(9). Takai, T.; Ohmori, H. *Biochimica Et Biophysica Acta,* **1048**, 105 (1990).
(10). Yaroslavov, A. A.; Sukhishvili, S. A.; Obolsky, O. L.; Yaroslavova, E. G.; Kabanov, A. V.; Kabanov, V. A. *FEBS Lett.,* **384**, 177 (1996).
(11). Remy, J. S.; Abdallah, B.; Zanta, M. A.; Boussif, O.; Behr, J. P.; Demeneix, B. *Adv. Drug Deliv. Rev.,* **30**, 85 (1998).
(12). Ferrari, S.; Moro, E.; Pettenazzo, A.; Behr, J. P.; Zacchello, F.; Scarpa, M. *Gene Ther.,* **4**, 1100 (1997).
(13). MacLaughlin, F. C.; Mumper, R. J.; Wang, J. J.; Tagliaferri, J. M.; Gill, I.; Hinchcliffe, M.; Rolland, A. P. *J. Control. Rel.,* **56**, 259 (1998).
(14). Cherng, J. Y.; van de Wetering, P.; Talsma, H.; Crommelin, D. J. A.; Hennink, W. E. *Pharm. Res.,* **13**, 1038 (1996).
(15). van de Wetering, P.; Cherng, J. Y.; Talsma, H.; Hennink, W. E. *J. Control. Release,* **49**, 59 (1997).
(16). van de Wetering, P.; Cherng, J. Y.; Talsma, H.; Crommelin, D. J. A.; Hennink, W. E. *J. Control. Release,* **53**, 145 (1998).
(17). Haensler, J.; Szoka, F. C. *Bioconjugate Chem.,* **4**, 372 (1993).
(18). Tang, M. X.; Redemann, C. T.; Szoka, F. C. *Bioconjugate Chem.,* **7**, 703 (1996).
(19). Wolfert, M. A.; Schacht, E. H.; Toncheva, V.; Ulbrich, K.; Nazarova, O.; Seymour, L. W. *Hum. Gene Ther.,* **7**, 2123 (1996).
(20). Toncheva, V.; Wolfert, M. A.; Dash, P. R.; Oupicky, D.; Ulbrich, K.; Seymour, L. W.; Schacht, E. H. *Biochim. Biophys. Acta-Gen. Subj.,* **1380**, 354 (1998).
(21). Maruyama, A.; Katoh, M.; Ishihara, T.; Akaike, T. *Bioconjugate Chem.,* **8**, 3 (1997).
(22). Asayama, S.; Nogawa, M.; Takei, Y.; Akaike, T.; Maruyama, A. *Bioconjugate Chem.,* **9**, 476 (1998).
(23). Asayama, S.; Maruyama, A.; Cho, C. S.; Akaike, T. *Bioconjugate Chem.,* **8**, 833 (1997).
(24). Vanderkerken, S.; Vanheede, T.; Toncheva, V.; Schacht, E.; Wolfert, M. A.; Seymour, L.; Urtti, A. *J. Bioact. Compat. Polym.,* **15**, 115 (2000).
(25). Behr, J. P. *Chimia,* **51**, 34 (1997).
(26). Boussif, O.; Lezoualch, F.; Zanta, M. A.; Mergny, M. D.; Scherman, D.; Demeneix, B.; Behr, J. P. *Proc. Natl. Acad. Sci. U. S. A.,* **92**, 7297 (1995).
(27). Boussif, O.; Zanta, M. A.; Behr, J. P. *Gene Ther.,* **3**, 1074 (1996).
(28). Azzam, T.; Eliyahu, H.; Shapira, L.; Linial, M.; Barenholz, Y.; Domb, A. J. *J. Med. Chem.,* **45**, 1817 (2002).
(29). Snyder, S. L.; Sobocinski, P. Z. *Anal Biochem.,* **64**, 284 (1975).
(30). Zhao, H.; Heindel, N. D. *Pharm. Res.,* **8**, 400 (1991).
(31). Toncheva, V., Wolfert, M. A., Dash, P. R., Oupicky, D., Ulbrich, K., Seymour, L. W., Schacht, E. H., *Biochmi. et Biophys. Acta,* **1380**, 354 (1998).