Comparing both polymers, PEI–DNA complexes interact to a higher extent compared to PDMAEMA–DNA complexes. Most likely, this can be ascribed to the higher number of positive charges on the surface of PEI–DNA complexes as derived from performed zeta-potential measurements (see Fig. 4).

The interaction of the polyelectrolyte complexes with hyaluronic acid does not lead to disruption of the complexes but to the formation of ternary complexes.

Conclusion

We proposed a SPR-based assay which can be used for studying the interaction between hyaluronic acid (HA) and different polymer–DNA complexes. The SPR assay indicated that the interaction between GAG and polymer–DNA complexes was both polymer and charge ratio dependent. This assay opens new perspectives in the field of non-viral polymer-based gene delivery, since the GAG–polyplex interaction is one of the barriers in the gene delivery process.

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Disulfide-containing poly(β-amino ester)s for gene delivery

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Summary

A group of new disulfide-containing poly(β-amino ester)s was synthesized and evaluated as non-viral gene delivery vectors. These linear polymers were obtained by Michael addition of a number of selected amines to bis(2-acryloyloxyethyl) disulfide. It was shown that the disulfide-containing poly(β-amino ester)s can condense plasmid DNA into nanoscaled and positively charged polyplexes, which could be rapidly destabilized in an intracellular reductive environment. Polyplexes of the polymer of histamine and bis(2-acryloyloxyethyl) disulfide show efficient transfection of COS-7 cells along with a relatively low cytotoxicity.

Introduction

One crucial aspect in the development of gene therapy is the availability of safe and efficient gene delivery vectors. Non-viral vectors have a potential advantage over viral vectors since they generally induce a low immune response and can be easily manufactured. However, non-viral vectors like polyethylenimine, polyamidoamine dendrimers and poly(2-(dimethylamino)ethyl methacrylate) (pDMAEMA) generally are associated with relatively low transfection activity and high cytotoxicity. The transfection activity of polyplexes of these cationic polymers is dependent on the properties of the polymers such as molecular weight, charge density, hydrophobicity and buffering capacity. The cytotoxicity could be due to their poor degradation profile.

Recently, degradable cationic polymers have received great interest for use in non-viral gene delivery systems because they revealed comparable *in vitro* transfection activity and, importantly, decreased cytotoxicity as compared to their non-degradable counterparts. Moreover, the polymeric degradation in the intracellular environment can be exploited to modulate the unpacking of polyplexes, yielding enhanced gene expression. Several linear [1] and branched [2] poly(β-amino ester)s with a variety of side groups have been studied as non-viral vectors, and a number of these polymers displayed relatively high transfection activity and low cytotoxicity. Their chemical degradation occurred via hydrolysis of ester bonds, and depending on polymeric structure and pH environment this process takes a few hours (pH 7) or a few days (pH 5). This suggests that hydrolytic degradation of poly(β-amino ester)s probably leads to inadequate DNA release inside the cytosol or nucleus yielding limited gene expression. An alternative approach to achieve biodegradable polymers is the introduction of disulfide linkages into the polymer main chains. By reductive cleavage of the disulfide0 linkages inside the cells, the polyplexes can be destabilized to release DNA for gene expression. In this paper, we synthesized a group of new cationic poly(β-amino ester)s containing disulfide linkages in polymeric backbone as gene delivery vectors. It was expected that the reductive cleavage of the disulfide

linkages in these polymers can promote the destabilization of the polyplexes inside the cell for DNA release, yielding enhanced gene expressions.

Experimental methods

Monomer synthesis

Bis(2-acryloyloxyethyl) disulfide (BAD) was synthesized by the esterification of bis(2-hydroxyethyl) disulfide with acryloyl chloride [3].

Polymer synthesis

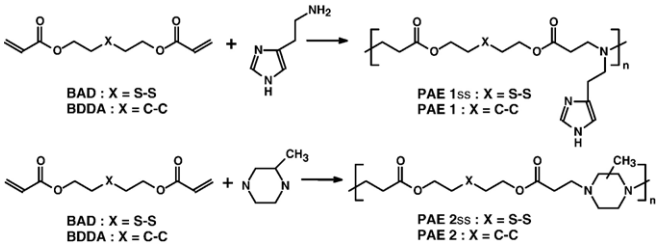
The disulfide-containing poly(β-amino ester)s (i.e., PAE1ss and PAE2ss) were prepared by Michael addition of histamine or methylpiperazine to BAD (Scheme 1) [1]. For comparison, 1,4-butanediol diacrylate (BDDA) instead of BAD was used to yield the corresponding poly(β-amino ester)s without the disulfide linkage (i.e., PAE1 and PAE2). The polymerisation reactions were performed at equal monomeric molar ratio in anhydrous chloroform or dimethyl sulfoxide at 45 °C under nitrogen atmosphere for several days. The reaction was terminated with excess of amine (10 mol% relative to starting amine) to block any unreacted acryl-groups. The polymer was precipitated in an acetone–HCl solution (pH 2) as the HCl-salt, and then dried for three days under vacuum at room temperature.

Biophysical characterization of the polymer/DNA polyplexes

The particle size of the polyplexes (in HEPES buffer pH 7.4 or pH 5.1, 5 wt.% glucose) was determined by dynamic laser scatting (DLS). The stability of the particle was followed by recording the particle size in time both in the absence and presence of 2.5 mM dithiothreitol (DTT). The surface charge of the polyplexes was determined by zeta potential measurements.

In vitro transfection efficiency and cytotoxicity assay

Transfection experiments were performed with COS-7 cells by using the plasmid pCMV-LacZ as reporter gene. Two parallel transfection series were carried out: one for the determination of reporter gene expression (β-galactosidase) and the other for the evaluation of cell viability by XTT assay.



Scheme 1. Synthesis of disulfide-containing poly(β-amino ester)s.

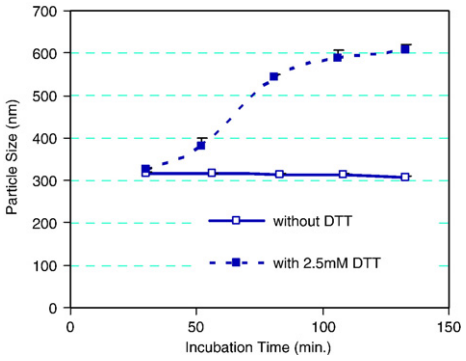


Fig. 1. Stability of PAE2ss based polyplexes at an N/P ratio of 32/1 in the absence (□) and presence (■) of 2.5 mM DTT at pH 7.4. The polyplexes were first incubated in HEPES buffer (20 mM, pH 7.4, with 5 wt.% glucose) at 37 °C for 30 minutes, and then the particle size of the polyplexes was determined by DLS at different time points.

Results and discussion

The synthesis of the poly(β-amino ester)s is depicted in Scheme 1. ^{1}H NMR spectra (D_2O, 300 MHz) indicated that these polymers have the expected composition.

Biophysical characterization of polyplexes

Dynamic light scattering (DLS) and zeta potential measurements showed that at pH 7.4 and a polymer/DNA weight ratio of 48/1 only PAE1ss was capable to condense plasmid DNA into nanosized (~400 nm) and positively charged (~+10 mV) polyplexes. The corresponding polymer without disulfide linkages, i.e. PAE1, condensed DNA into small (~200 nm) and positively charged (~+25 mV) polyplexes at a polymer/DNA weight ratio of 40/1. As expected, disulfide-containing poly(β-amino ester) based polyplexes show different stability in non-reductive and in reductive environment. As a typical example shown in Fig. 1, the particle size of PAE2ss based polyplexes were stable in time in the absence of dithiothreitol (DTT), but their size rapidly increased in the presence of 2.5 mM DTT. This

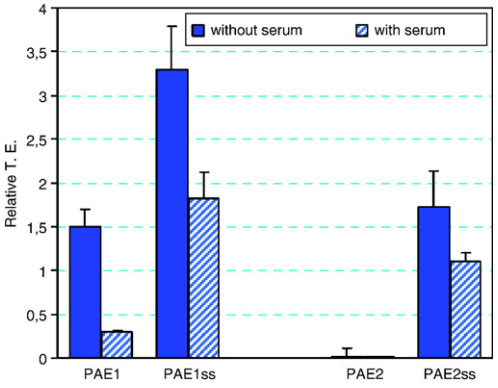


Fig. 2. Maximum transfection efficiency of polyplexes of the disulfide-containing poly(β-amino ester)s. Transfection efficiencies were optimized at polymer/DNA weight ratios (ranging from 6/1 to 48/1) in the absence and presence of serum. pDMAEMA/DNA polyplexes at a weight ratio of 3/1 were used as a reference. The data were expressed as mean values (standard deviations) of three experiments.

indicates that the PAE2ss polymer loses its DNA condensation ability in a reductive environment due to reductive degradation of the polymer.

Transfection efficiency and cell viability

As shown in Fig. 2, the optimum transfection was observed for PAE1ss based polyplexes at a polymer/DNA weight ratio of 48/1 in the absence of serum, with 3.3-fold higher efficiency than that of the reference (pDMAEMA polyplexes). In the presence of 5% serum, polyplexes of PAE1ss and PAE2ss showed a 1.7-fold and 1.1-fold higher efficiency than those of the reference polyplexes, respectively. In particular, the polyplexes of the disulfide-containing poly(β-amino ester)s showed a higher transfection efficiency than those of corresponding poly(β-amino ester)s without disulfide linkage. This indicates that the reductive cleavage of disulfide linkages in the cytosol may play an important role in the DNA dissociation from the polyplexes, resulting in facilitated gene release and enhanced gene expression. The XTT assays show that the cytotoxicity of all polyplexes is low (>85% cell viability) at the polymer/DNA ratios where the highest transfection activity is observed.

Conclusion

This study demonstrated that the introduction of disulfide linkages into poly(β-amino ester) polymers favorably contributes to the transfection activity of polyplexes based on these polymers. The disulfide-containing polymers are able to condense plasmid DNA into nanosized polyplexes, which display a high gene expression along with low cytotoxicity as compared to the polyplexes based on poly(β-amino ester)s without disulfide linkage.

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044

Water-soluble cationic poly(ferrocenylsilane): An efficient DNA condensation and transfection agent

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Summary

The DNA condensation and *in vitro* gene transfer properties of an organo-iron polymer, cationic poly(ferrocenylsilane), were investigated. Gel retardation assay, dynamic light scattering (DLS), atomic force microscopy (AFM) and zeta-potential measurements showed that cationic poly(ferrocenylsilane) is able to condense plasmid DNA into positively charged nano-sized particles at N/P ratios ≥2/1. These polyplexes displayed low cytotoxicity and were able to efficiently transfect COS-7 cells.

Keywords: poly(ferrocenylsilane), plasmid DNA, polyplexes, gene delivery

Introduction

In the past decade, there has been significant progress in the development of cationic polymer-based non-viral gene delivery systems. Several polymers such as polyethylenimine (PEI), poly(2-(dimethylamino)ethyl methacrylate) (pDMAEMA), and polyamidoamine (PAMAM) dendrimers have been evaluated *in vitro* as well as *in vivo* for DNA delivery. These current polymeric systems, however, are associated with certain cytotoxicity and show in general rather low transfection activity *in vivo* [1].

In this paper, we report a cationic organo-iron polymer, poly(ferrocenylsilane) (PFS, Chart 1), for DNA condensation and *in vitro* transfection. This is the first example of organometallic polymers for gene delivery applications. PFS polymers have attracted recent interest in nanotechnology due to their unique chemical and electrochemical properties. Tailor-made PFS can be obtained by anionic polymerization of ferrocenophanes [2].

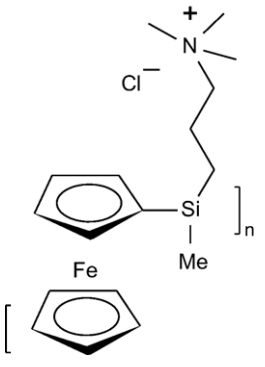

Chart 1. Chemical structure of water-soluble cationic poly(ferrocenylsilane) (PFS).