Cyclodextrin-Containing Polymers for Gene Delivery

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Abstract

Cyclodextrin-containing polymers are now being explored as vehicles for delivering nucleic acids into cells. The structures of the cyclodextrin-containing polycations affect the nucleic acid delivery efficiencies and their toxicities. Of interest is the fact that the cyclodextrin-containing polymers reveal lower toxicities than polymers that lack the cyclodextrins. The cyclodextrins endow the nucleic acid delivery vehicles with the ability to be modified by compounds that form inclusion complexes with the cyclodextrins, and these modifications can be performed without disruption of the polymer-nucleic acid interactions. Thus, cyclodextrin-containing polymers provide unique properties for gene delivery.

Introduction

The development of polyplexes (cationic polymer + nucleic acid) for gene delivery has grown at a rapid pace from initial work involving readily available polymers like poly-L-lysine (PLL) and polyethylenimine (PEI) to current studies that exploit polymers designed for this application. Cationic polymers are able to deliver DNA into cells by self-assembling with the anionic DNA via electrostatic interactions to subsequently form positively charged, small particles (sub-500 nm) that are taken up by cells.

While cationic polymers share a common mechanism of DNA delivery, their delivery efficiencies differ greatly from polymer to polymer. Additionally, significant variations in delivery efficiency and toxicity are observed by the use of various molecular weight fractions of the same polymer. Finally, little is known regarding the relationships between the molecular architecture of the polymer and the delivery properties of the polyplexes formed from that polymer.

Cyclodextrin-containing polymers have been known for quite some time. Examples of various classes of cyclodextrin-containing polymers are illustrated in Figure 1. In the mid-1990's, we began work on the synthesis of new cationic polymers for use as DNA delivery agents. We hypothesized that it may be possible to prepare low toxicity polycations from cyclodextrins [1] because numerous individual cyclodextrins (CD) were known to reveal low toxicity and to not elicit immune responses in animals. Additionally, cyclodextrins were exploited in drug formulation because of their ability to form inclusion complexes, and we planned on using this property in the assembly of fully formulated products that would be appropriate for systemic DNA de-
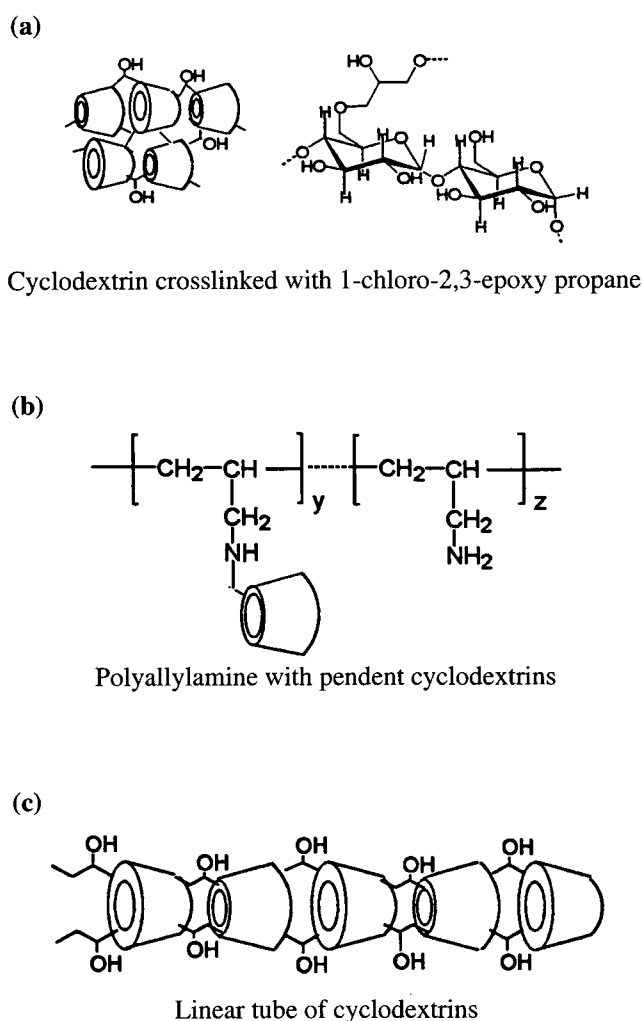
(a)

Cyclodextrin crosslinked with 1-chloro-2,3-epoxy propane

(b)

Polyallylamine with pendent cyclodextrins

(c)

Linear tube of cyclodextrins

Figure 1. Classes of cyclodextrin-containing polymers.

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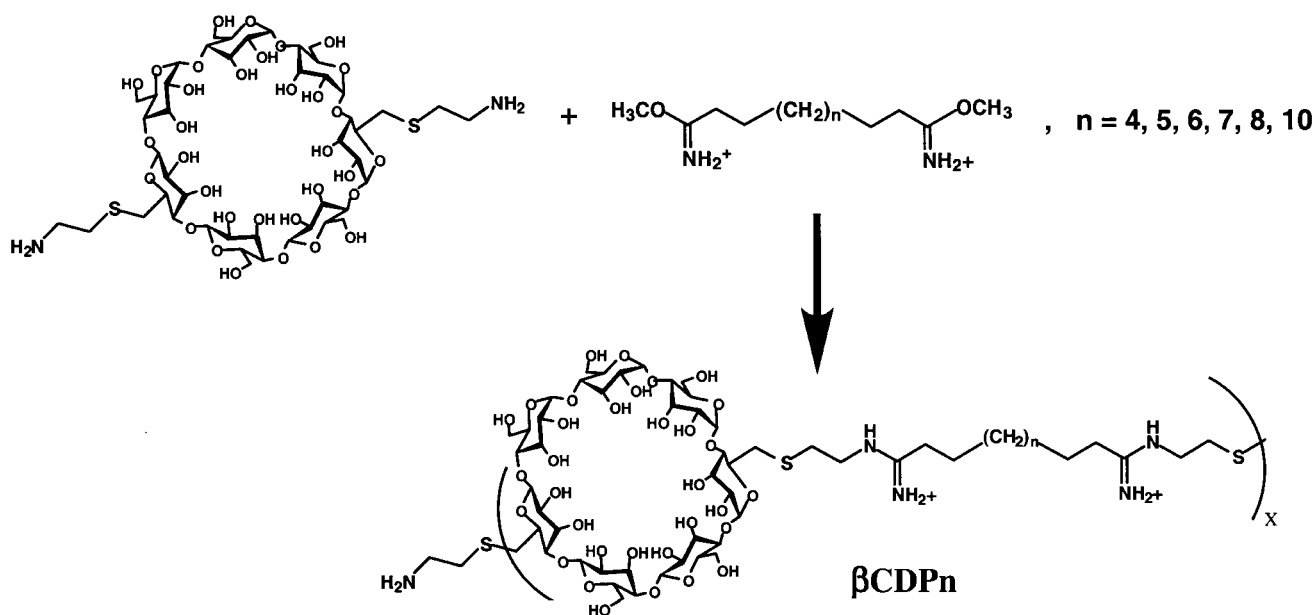
Figure 2. Polymerization scheme for β-cyclodextrin-containing polymers.

livery. In 1999, we reported on the synthesis of a new family of cyclodextrin-containing, cationic polymers (CDP) that were prepared by the condensation of difunctionalized CD monomers with other difunctionalized comonomers (see Figure 2) [1]. These linear polycations were able to provide effective DNA delivery to cultured cells with low toxicity [1, 2].

Numerous cyclodextrin-containing, cationic polymers currently exist. For example, within the class of cyclodextrin pendent polymers (see Table I), several are polycations, e.g. PEI, poly(allylamine), dendrimers. Although these materials have been known for some time, only recently has any of them been used to deliver genes to cultured cells. Arima *et al.* have delivered DNA to cells using α-, β- and γ-cyclodextrin-containing polyamidoamine dendrimers [3]. This work and ours with CDP are the only examples of cyclodextrin-containing polymers used for gene delivery.

Polyplex formulations optimized for *in vitro* delivery are typically not appropriate for *in vivo* use because successful systemic delivery requires different particle properties. After intravenous injection, cationic polyplexes interact with serum proteins and are quickly eliminated from the bloodstream by phagocytic cells. Additionally, the polyplexes rapidly aggregate at physiological ionic strength (150 mM salt concentration). Thus, cationic polyplexes require modification before they can be successfully applied for systemic gene delivery. A typical modification of polyplexes is to provide steric stabilization by PEGylating the particles (PEG: polyethylene glycol). Numerous methods are available for covalent attachment of PEG to create PEGylated particles.

The use of cyclodextrin-containing polycations for polyplex formation provides the means to create modified particles in an entirely new manner. Pun and Davis recently developed methodologies to modify the surface of polyplexes formed with cyclodextrin-containing polymers whether they be of the CDP-type [4, 5] or not [5]. This concept exploits the use of cyclodextrin/guest compound complexation to provide modified polyplexes appropriate for systemic application as gene delivery vehicles. As an example of this methodology, adamantane was conjugated to PEG and the resulting compound exposed to CDP-based polyplexes (see Figure 3) for self-assembly between the adamantane and the cyclodextrins. This methodology can provide CDP-based particles that are appropriate for systemic gene delivery [4].

In this paper, we discuss further issues: (i) associated with distributing charge centers along the CDP backbone, and (ii) modifying the surface of CDP-based polyplexes with adamantane-based compounds.

Materials and methods

All materials synthesis procedures and methods of characterization have been described previously, as have the cell transfection and toxicity protocols [1, 2, 4]. Further details on the surface modification compounds and their properties will be available shortly for cyclodextrin-containing polymers in general [5].

Results and discussion

Distribution of charge centers on CDP

Previously, Hwang *et al.* prepared a series of CDPs that varied the spacing between the charge centers by preparing polycations with spacer units containing 4–10 methylenes (see Figure 2) [2]. Table II shows the properties of these polymers and the polyplexes prepared from them. All the

Table 1. Examples of cyclodextrin pendent polymers

Type of polymer	Cyclodextrin	Preparation method
polyacrylic esters	α, β	polymerization of vinyl cyclodextrin derivatives
poly(allylamine)s	β	grafting of cyclodextrin to preformed polymer
acrylonitrile-methyl acrylate copolymer	β	grafting of cyclodextrin to preformed polymer
polymethacrylates	α, β, γ	polymerization of cyclodextrin methacrylate monomers
chitosan	β	grafting of cyclodextrin to preformed polymer
polyester	β	grafting of cyclodextrin to preformed polymer
polyethylenimine	β	grafting of cyclodextrin to preformed polymer
dendrimers	α, β, γ	grafting of cyclodextrin to preformed polymer

CDPs rapidly form polyplexes of approximately the same size. However, the gene delivery efficiencies as determined by luciferase gene expression assays and the cellular toxicities are strong functions of the spatial distribution of charge centers along the CDP backbone. The optimal gene delivery expression occurs with a CDP having 6 methylenes separating the charge centers (β-CDP6). As the distance between the charge centers is increased, the toxicity is diminished for spacings generated by 4–8 methylenes. The CDP with 10 methylenes spacing the charge centers becomes more toxic most likely because it reveals lower water solubility as compared to the other polycations [2]. Based on these results, two other CDPs have been prepared and they are schematically represented in Figure 4. β-CDP(NH) gives approximately the same gene delivery and toxicity properties as β-CDP6 while β-CD(NH)P6 shows significant cellular toxicity (see Figure 5). Thus, the spatial distribution of charge centers along the backbone of the CDP plays a significant role in the toxicity. We are currently attempting to understand the roles that charge distribution play in the cellular delivery of DNA by CDP-based polyplexes.

In addition to the charge distribution, the presence of the cyclodextrin has a significant effect on the toxicity of the linear polycations. For example, polyamidines (see Figure 6) prepared to mimic the charge distributions in CDPs reveal IC_{50}s of 0.005–0.034 mM at the conditions reported in Table II for the CDPs and a comparison of toxicities obtained from polyplexes prepared from these polycations is given in Figure 5. Thus, the cyclodextrin has a very large effect on the toxicity (or lack thereof) of the polycation.

Modification of polyplex surface

The concept of polyplex surface modification by entities that form inclusion complexes with the cyclodextrins of the cyclodextrin-containing polycations has recently been established [4, 5]. This method of polyplex modification does not involve the portions of the polycations that bind to the DNA so polyplex disruption is avoided. Although adamantane was initially used to form inclusion complexes with β-cyclodextrin-containing polycations [4], other combinations of guest species and cyclodextrin-types can be used [5]. The modifying agents contain PEG segments and can also contain anionic segments and targeting ligands at the
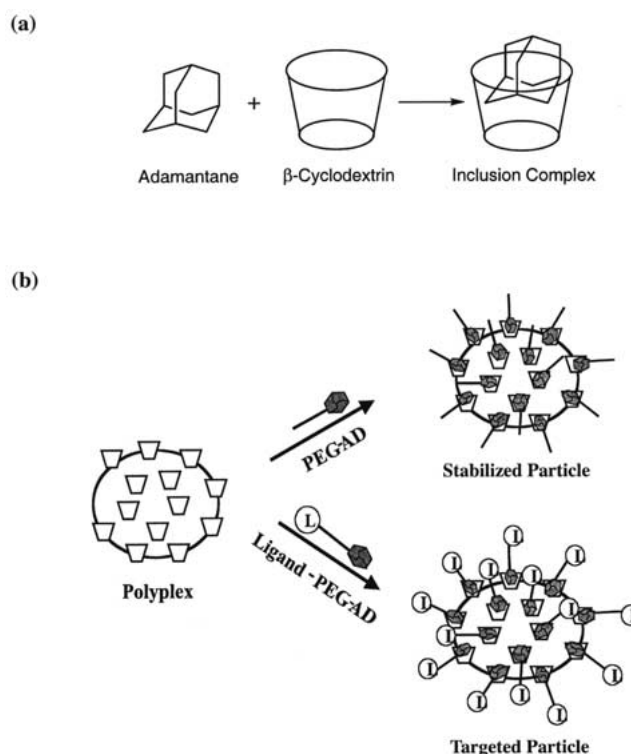

Figure 3. Schematic of (a) inclusion complex formation and (b) surface modification of cyclodextrin-containing polyplexes. From [4].

opposite end of the adamantane [4, 5]. The targeting ligands used for binding to cell surface receptors can be small molecules, e.g. galactose, folate, and/or larger entities such as proteins [5].

The association between the adamantane-PEG (AD-PEG) molecules and the CDP-containing polyplexes was found to be quite strong and not what would be expected from the association of β-cyclodextrin and water-soluble adamantane analogues [6]. For example, CDP-containing polyplexes modified with AD-PEG$_{5000}$ are stable in PBS (see Figure 7) and to dilutions in PBS to concentrations in the microgram/milliliter range. The high association may arise because of the very high local concentration of cyclodextrins on the polyplexes, additional interactions between the PEG and the cyclodextrins, e.g. hydrogen bonding, and/or to other factors. We are currently attempting to un-
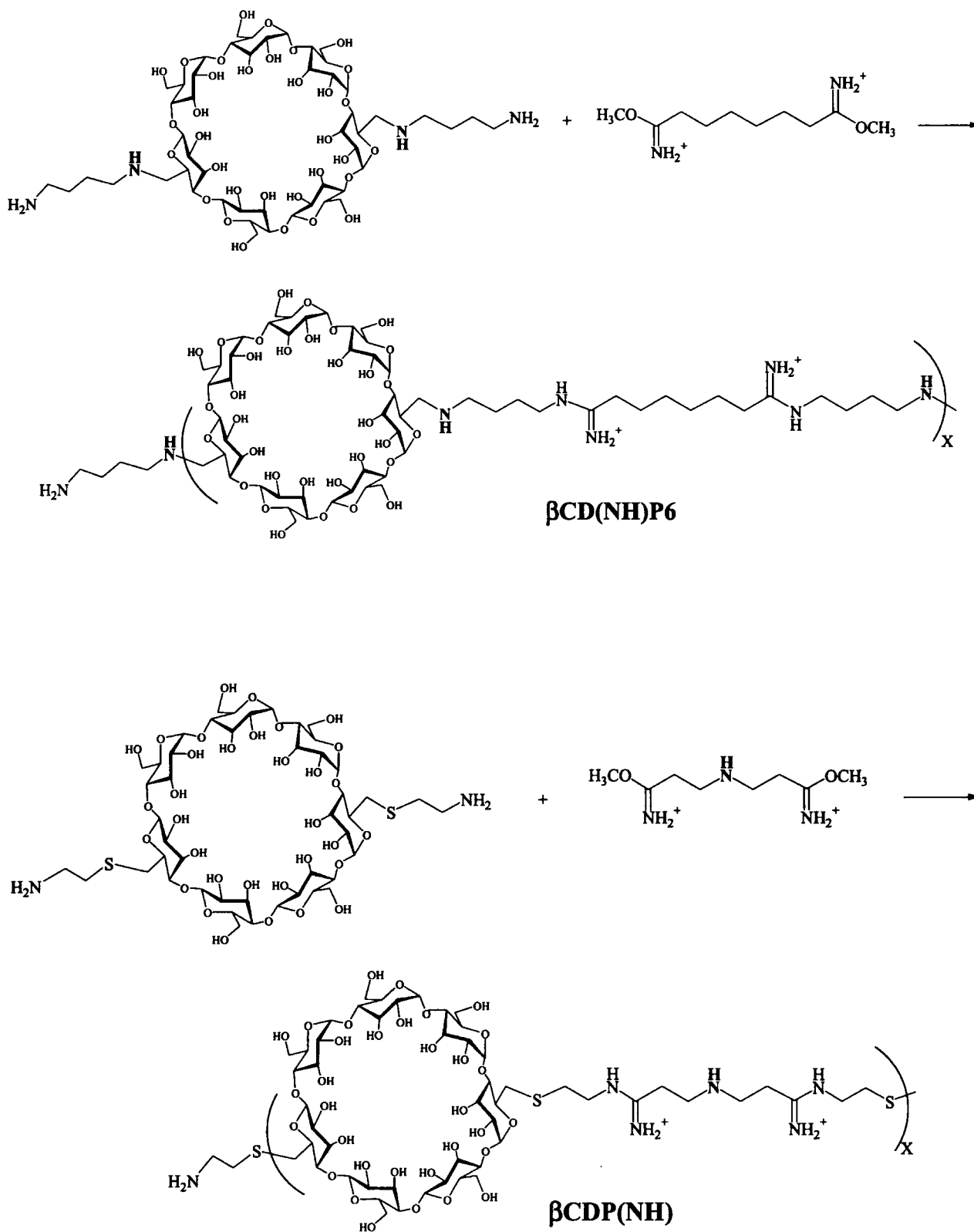
βCD(NH)P6

βCDP(NH)

Figure 4. Further examples of linear, cyclodextrin-containing polymers.

Table 2. Effects of length between charge centers on CDPs [2]

No. of methylenes	M_w (kDa)	M_w/M_n	Polyplex size (nm)	Rel. gene Eff.[a]	IC$_{50}$ (mM) [b]
4	6.1	1.13	148	0.22	0.4
5	5.8	1.12	140	0.05	0.4
6	5.8	1.12	128	1.00	1.1
7	6.9	1.14	130	0.50	1.8
8	7.6	1.16	125	0.64	2.2
10	10.1	1.21	142	0.10	0.3

[a] Relative transfection efficiency (see [2] for details).
[b] IC50 of polycation alone with BHK cells (see [2] for details).
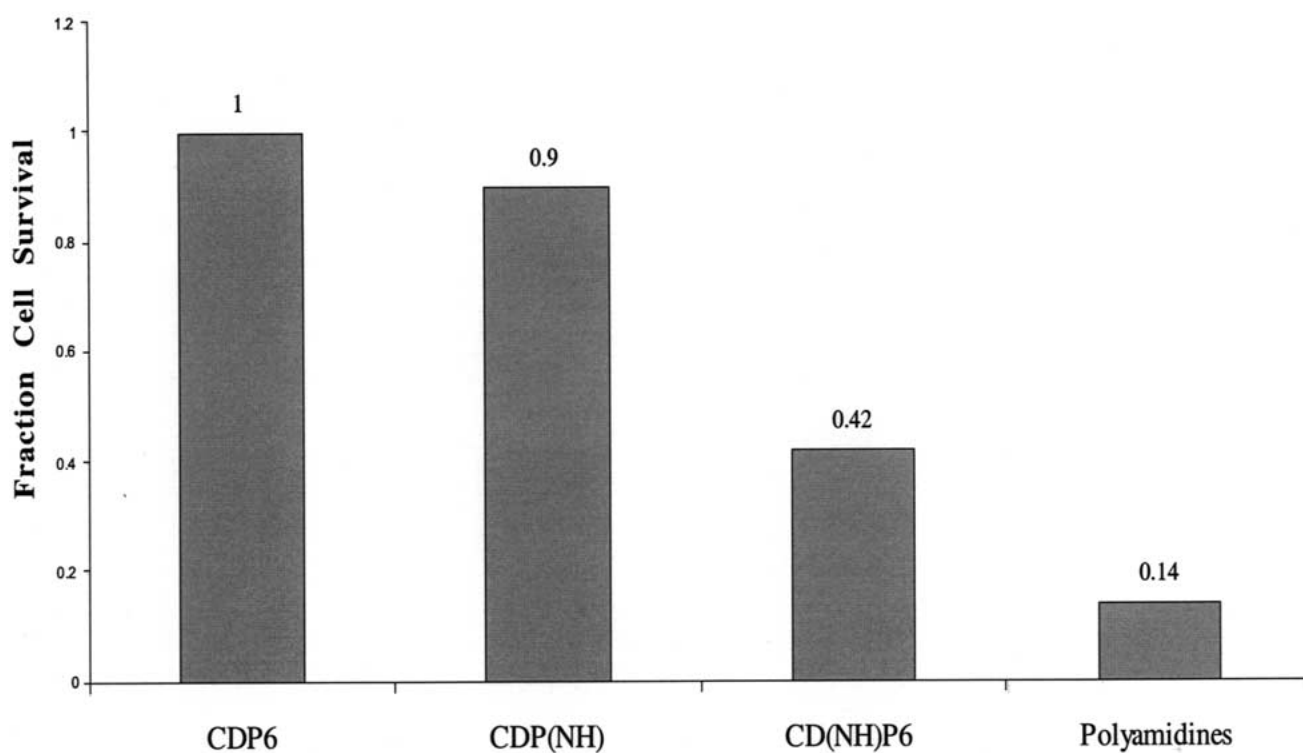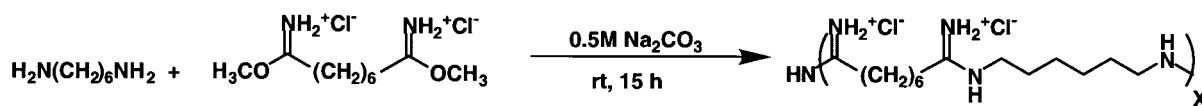

Figure 5. Toxicity of CDP polyplexes to BHK-21 cells.

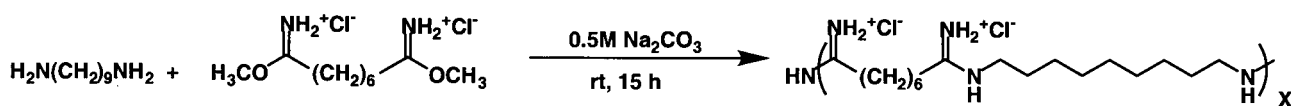**C6–C6 Polycation:**

C9–C6 Polycation:

Figure 6. Polymerization scheme for non-cyclodextrin-containing polymers.
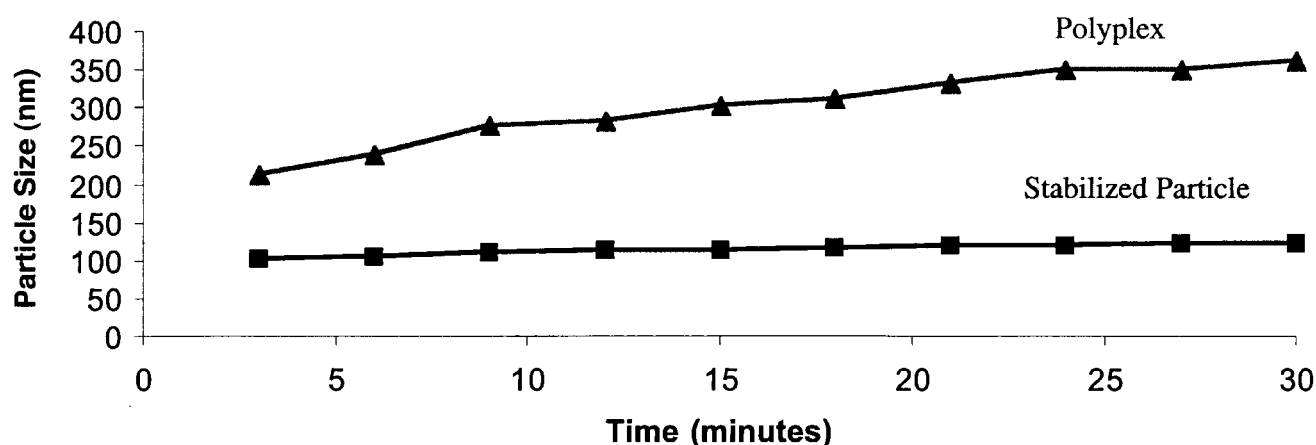
Figure 7. Particle size as a function of time in PBS.

ravel the underlying mechanisms for the stability of the AD-PEG modified CDP-containing polyplexes.

It is clear that the inclusion complex formation method of surface modification can be applied to cyclodextrin-containing polyplexes in general and this has been accomplished for several types of cyclodextrin-containing polyplexes [5]. Complete characterization of these stabilized gene delivery vehicles is forthcoming.

Conclusions

Cyclodextrin-containing polymers are revealing new and exciting properties when used as gene delivery vehicles. The cyclodextrins endow the gene delivery vehicles with low toxicity and can serve as hosts that can form inclusion complexes with appropriate guest species to decorate the surfaces of polyplexes.

References

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