Analytical study of Linker Formation in Complexation of Two Dendrimers and one Polyelectrolyte

RESEARCH

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A B S T R A C T

The concept of gene therapy is to fix a genetic problem at its source by repairing defective genetic material. Dendrimers proved to be an effective carrier vectors for gene therapy that efficiently compact and protect DNA from degradation by serum nucleases in the blood when injected intravenously. The complexation between Poly(amido amine) (PAMAM) dendrimer and Deoxyribonucleic acid (DNA) has been studied using the penetrable sphere model describing the interaction between linear polyelectrolyte (LPE) chain and ion penetrable sphere (Porous sphere) representing the dendrimer. It was found that the wrapping degree of LPE around the dendrimer is increased by increasing the LPE length, and the Bjerrum length which represents the strength of the electrostatic interaction and is decreased by increasing the pH of the solution. As a result, the linker, represents the unwrapped part of the LPE chain, is decreased by increasing the Bjerrum length and increased by increasing the pH.

Keywords: Analytical model, Linear polyelectrolyte, Dendrimers, Complexes, Linker.

Introduction

Gene therapy is a medical technique that focuses on genetic modification to treat genetic disorder such as Parkenson's disease as well as an alternative procedure to conventional chemotherapy for curing cancer (Yang et al., 2007). Design efficient transporter vectors is needed for gene therapy to protect and condense DNA as it is rapidly degraded by serum nucleases in the blood when injected intravenously (Niven et al., 1998). Moreover, they must be transmitted into the cell and liberated the cargo DNA within the nucleus. viral vectors are found to be effective in transferring DNA for gene therapy, but the toxicity of these vectors promoted the seeking of other vectors for inserting the DNA into the targeted cell (Mintzer and Simanek, 2009; Gigante et al., 2019). A notable example of such nonviral vectors are dendrimers which have emerged as efficient and stable vectors for delivering DNA into cells. They are branched polymeric molecules having a definite size and shape and can be synthesized with a very narrow weight distribution. Because of the distinction of their structure, dendrimers are one of the most synthetic molecules with great potential as nonviral vectors for gene transfection (Gérard et al., 2013). Transfection efficacy and operation of dendrimer /DNA aggregate can be influenced and controlled by the structure, volume, and the charge density of the dendrimers (Qamhieh et al., 2009; Qamhieh et al., 2014; Qamhieh and Al-Shawwa, 2017).

The complexes of dendrimers/polyelectrolytes have been extensively studied, experimentally (Pi-Boleda et al., 2021; Forssell et al., 2007; Ainalem et al., 2009), theoretically (Nguyen and Shklovskii, 2001; Rosen et al., 2001; Schiessel et al., 2001) and using computer simulations (Stornes et al., 2021; Lyulin et

al., 2005; Larina et al., 2009; Lyulin et al., 2008). Here we use the term complex to refer to the entity formed by one dendrimer and the part of a polyelectrolyte molecule that wraps around it. DNA is symbolized as a polyelectrolyte, which can compact strongly with a cationic dendrimer to form a complex. The aim of this study is to provide further insights into the formation of the dendrimer/polyelectrolyte complexes and the linker formed between them, by applying a simple analytical model.

Analytical model

A penetrable sphere model (Khawla Qamhieh and Khaleel, 2014) was applied through our study, in which the dendrimer was considered as an ion penetrable (soft) sphere of radius R and charge Ze, and the linear polyelctrolyte as a rod of radius r = 1 nm, length L, persistence length l_p, and charge density $-e/b$, b is the axial spacing between charges. The sphere and the LPE chain are placed in a system characterized by Bjerrum length $l_B = e^2/\varepsilon k_B T$, and a Debye screening length, = $(8c_s \pi l_B)^{1/2}$ where ε is the dielectric constant of the solvent, c_s is the salt concentration, and $k_B T$ is the thermal energy. If the complexation is performed in 10 mM monovalent electrolyte, then = 3 nm and l_B = 0.7 nm using the dielectric constant of water at room temperature.

Complexation of a LPE chain with a single sphere

In Schiessel's model (Schiessel et al., 2001), the total free energy of a system consisting of one-sphere and one chain is given by:

$$F(l) = F_{compl}(l) + F_{chain}(L-l) + F_{compl-chain}(l) + F_{elastic}(l) \qquad (1)$$

Where l is the length of the part of DNA molecule wrapped around the sphere, and ($L-l$) is the length of the remaining chain. The first term is the electrostatic charging free energy of spherical complex of charge $Z(l)e$ where the sphere is considered as a soft sphere (ion penetrable sphere):

$$F_{compl}(l) = \frac{3}{8\pi} \frac{Z^2(l)k_B T}{(\kappa R)^2} \frac{exp(\kappa R)l_B}{R} \left[cosh(\kappa R) - \frac{sinh(kR)}{\kappa R} \right] \frac{e^{-\kappa R}}{R} \qquad (2)$$

The total charge of the complex can be given by,

$$Z(L) = Z - L/b$$

where the complex represents the sphere and the corresponding wrapped chain. The second term of the total entropic electrostatic free energy of the remaining chain ($L-l$) can be given by:

$$F_{chain}(L-l) \cong \frac{k_B T}{b} \Omega(a)(L-l)(1-\xi^{-1}) \qquad (3)$$

$$\Omega(a) \cong ln\left(\frac{4\xi\kappa^{-1}}{a}\right) \qquad (4)$$

Where $\Omega(a)$ is the entropic cost to 'confine' counterions close to the chain. and $\xi = L_B/b$ is so-called Manning parameter. The third term is the resulting free energy between the complexes (as soft sphere) and the unwrapped segment of the LPE chain and can be given as:

$$\frac{U_{compl-chain}(l)}{k_B T} \cong \frac{3Z(l)l_B}{4\pi(\kappa R)^2} \left[cosh(R) - \frac{sinh(\kappa R)}{\kappa R} \right]$$
$$\times \left[ln(r) - \sum_{n=0}^{\infty} \frac{(-1)^n}{(n+1)!(n+1)} (\kappa r)^{n+1} \right] \Big|_R^{L-Nl} \qquad (5)$$

The final term in Eq. (2.1), $F_{elastic}(l)$, is the elastic free energy required to bend l of the chain of radius of curvature around sphere of radius R, is the same as the one used in Scheissel's model:

$$F_{elastic}(l) \cong \frac{k_B T l_p}{2R^2} l \qquad (6)$$

Free energy calculation for a complexation between a LPE chain and multiple dendrimers

The total free energy for a system consisting of one LPE chain and N number of spheres is given by

$$F(N,l) = NF(l) + F_{int}(N,l) \qquad (7)$$

Where F_{int} is the repulsive electrostatic interaction between N ion-penetrable spheres that decorating the polymer chain., and can be obtained by summing over the electrostatic repulsion between all complexes within one chain, it is given as:

$$F_{int}(N,l) = \frac{9Z^2(l)k_B T l_B}{8\pi(\kappa R)^4} \left[cosh(\kappa R - \frac{sinh(\kappa R)}{\kappa R} \right]^2$$
$$\times \sum_{i=1}^{N-1} \left[\frac{N-i}{i} \right] \frac{e^{-\kappa D(N,l)}}{D(N,l)} \qquad (8)$$

Where $D(N,l) = (L-Nl+2NR)/N$ is the center-to-center distance between two neighboring complexes. The total free energy of the system can be written as:

$$\frac{F(N,l)}{k_B T} = \left\{ \frac{3}{4\pi} \frac{NZ(l)l_B A}{(\kappa R)^2} \left[\frac{Z(l)e^{-\kappa R}}{2R} \right. \right.$$
$$\left. + \frac{1}{b} \int_R^{L-Nl} \frac{e^{-\kappa r}}{r} dr + \frac{3Z(l)A}{2(\kappa R)^2} \sum_{i=1}^{N-1} X_i \left(\frac{N-i}{i} \right) \frac{e^{-\kappa D(N,l)}}{D(N,l)} \right]$$
$$\left. + \frac{1}{b} \Omega(a)(1-\xi^{-1})(L-Nl) + \frac{Nl_p}{2R^2} l \right\} \qquad (9)$$

Results and discussions

In order to estimate the optimal wrapping length (l_{opt}) of the LPE chain around the dendrimer, Eq. (9) can be solved, by taking the first derivative of the free energy with respect to the wrapping length l and equal it to zero. All the systems in our study are at room temperature. Dendrimers can be thought of as soft and highly flexible sphere (Rosenfeldt et al., 2002; Likos et al.,2002). This means that the size of a dendrimer gets smaller when LPE chain wraps around it due to electrostatic interactions. For example, the complexation of semiflexible DNA that has a 50 nm persistence length l_p and a length $L = 680$ nm (2000 bp), with two G4 ethylenediamine cored dendrimers and two ammonia cord G4 dendrimers have been studied in aqueous solution containing 10 mM 1:1 salt by applying the penetrable sphere model, DNA is modeled as a semiflexible rod of radius r =1.0 nm, the axial spacing between adjacent charges of DNA is b = 0.17 nm, and the persistence length l_p is 50 nm. The results for the interaction between the DNA and two G4 ethylenediamine cored dendrimers are shown in table 1 as the radius of the dendrimer is decreased, the charge of the dendrimer $Z_{dend} = 64$, and the isoelectric length $L_{iso} = 10.88$. While Table 2 shows the results for the interaction between a

similar DNA and two G4 ammonia cored dendrimers of $Z_{dend} = 48$, and $L_{iso} = 8.16$. Figure 1a and Figure 1b show the results for l_{opt}, of the same systems, and number of turns around the dendrimer $l_{opt} / 2\pi R$, as a function of the radius of ethylenediamine cored dendrimer, and ammonia cord dendrimer respectively. Our results suggests that the optimal DNA wrapping length, l_{opt}, decreases with the dendrimer radius. The difference between the optimal wrapped DNA length and the length needed to neutralize the dendrimer charges $Dif = l_{opt} - l_{iso}$ decreases when the radius of the dendrimer decreases. The effective charge of the complex $Z*$ is negative for all values of the radius, that is, charge inversion is achieved for all considered dendrimer radii, means that the degree of dendrimer penetration does not affect the charge reversal. The $Z*/Z$ ratio is, in general, small, i.e., the electrostatic repulsion between complexes is low. l_{opt} and $Z*$ are larger for the complexes of ammonia cord dendrimers than ethylenediamine cored dendrimers. Number of turns around the dendrimers is increased as the radius is decreased. The linker D' = (D(N,l)-2R) nm between the two complexes, at specific radius, is longer with ethylenediamine cord dendrimers than it with ammonia cord dendrimers, and is increased by decreasing the radius of the dendrimer, where $D(N,l)$ is the center-to-center dendrimer spacing.

Effect of LPE chain length (L)

The total length of LPE chain is divided into two parts, the optimal wrapping length around the dendrimer, and the linker defined as the unwrapped part of the chain. Figure 2 shows the effect of LPE chain length on l_{opt} of the chain in complexes of G3 ethylenediamine cored dendrimers when two dendrimers are compacted with one flexible LPE chain of persistence length 3.0 nm, spacer of 0.7 nm, and a Debye screening length of

Table 1. Results for the compaction of two G4 ethylenediamine cored dendrimers and one DNA chain. R is the radius of the sphere where x defines the fraction of the noncompacted sphere of radius R = xR_0.

x (R=xR_0)	l_{opt}(nm)	Diff (nm)	$Z*_{complex}$	$Z*/Z$	D(N,l) (nm)	D'=D N,l)-2R (nm)	(D'+Dif)N/L	$l_{opt}/2\pi R$
1.0	16.4	5.52	-32.47	-0.51	7.53	3.03	0.44	1.16
0.9	16.2	5.32	-31.29	-0.49	7.29	3.23	0.44	1.27
0.8	15.8	4.92	-28.94	-0.45	7.23	3.63	0.44	1.40
0.7	15.5	4.62	-27.18	-0.42	7.09	3.93	0.44	1.56
0.6	15.1	4.22	-24.82	-0.39	7.03	4.33	0.44	1.78
0.5	14.6	3.72	-21.88	-0.34	7.09	4.83	0.44	2.06
0.4	13.5	2.62	-15.41	-0.24	7.37	5.93	0.44	2.39

Table 2. Results for the compaction of two G4 ethylenediamine cored dendrimers and one DNA chain. R is the radius of the sphere where x defines the fraction of the noncompacted sphere of radius $R = xR_0$.

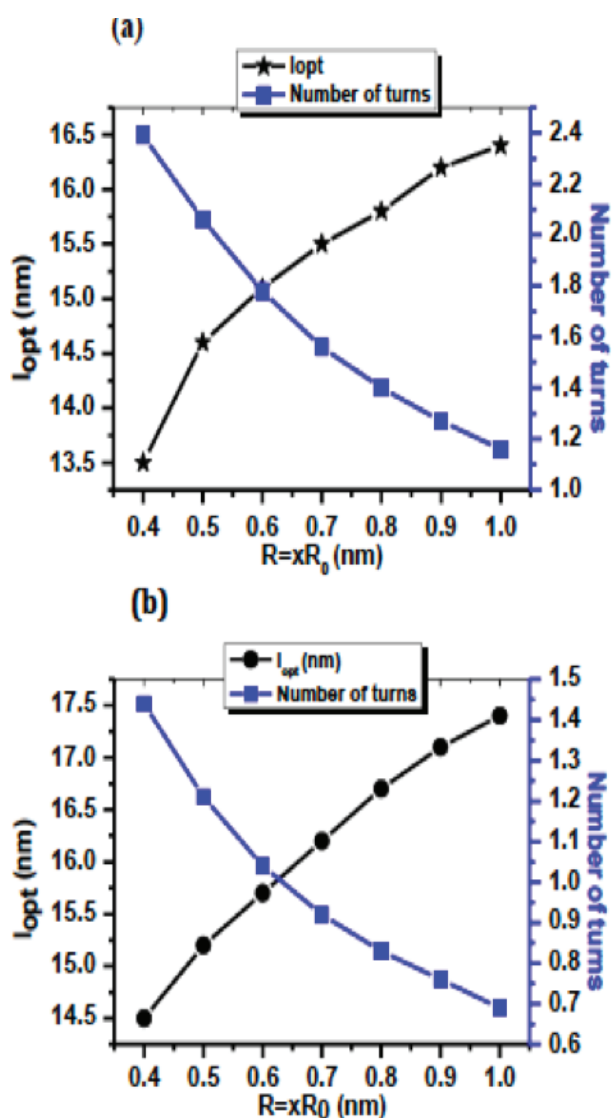| x ($R=xR_0$)	l_{opt}(nm)	Diff (nm)	$Z^*_{complex}$	Z^*/Z	$D(N,l)$ (nm)	$D'=D(N,l)-2R$ (nm)	$(D'+Dif)N/L$	$l_{opt}/2\pi R$
1.0	17.4	9.24	-54.35	-1.13	10.03	2.03	0.58	0.69
0.9	17.1	8.94	-52.59	-1.10	9.53	2.33	0.58	0.76
0.8	16.7	8.54	-50.24	-1.05	9.13	2.73	0.58	0.83
0.7	16.2	8.04	-47.29	-0.99	8.83	3.23	0.58	0.92
0.6	15.7	7.54	-44.35	-0.92	8.53	3.73	0.58	1.04
0.5	15.2	7.04	-41.41	-0.86	8.23	4.23	0.58	1.21
0.4	14.5	6.34	-37.29	-0.78	8.13	4.93	0.58	1.44

Figure 1. l_{opt} and number of turns around (a) dendrimer with ethylenediamine core, (b) dendrimer with ammonia core. Effect of different factors on the complexation have been studied, such as LPE chain length (L), Bjerrum length (l_B), and pH.
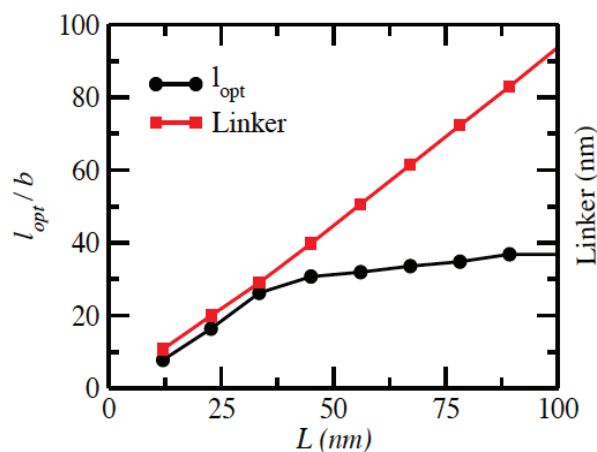

Figure 2. l_{opt} of LPE chain and the linker as a function of chain length.

100 nm. It is clear from the figure that as the length of the chain is increased the optimal wrapping length is increased linearly up to 40 nm chain length, then the change in the l_{opt} is decreased and starts to be almost constant for chain length greater than 40 nm. While the linker is increased linearly as the chain length is increased. The result agrees with what Larin et al. concluded in their simulation study. (Larin et al., 2010).

Effect of Bjerrum length

Figure 3 shows the optimal wrapping length and the linker formed between two ethylenediamine cored dendrimer 2G3 and oppositely charged flexible LPE as a function of Bjerrum length (l_B/b). The LPE chain has a persistence length of 3.0 nm, a bases spacer of 0.7 nm and 80 nm contour length, the dendrimer is considered as a penetrable sphere with radius R = 3.2 nm. When l_B is small the l_{opt} is small and the linker is large, but a small variation in l_B increases the l_{opt} very rabidly to reach a constant value at l_B b, and the linker decreases

significantly to reach a constant value at the same l_B, the positive charge of the complexes is reduced, as a result the repulsive electrostatic interaction between complexes is decreased.
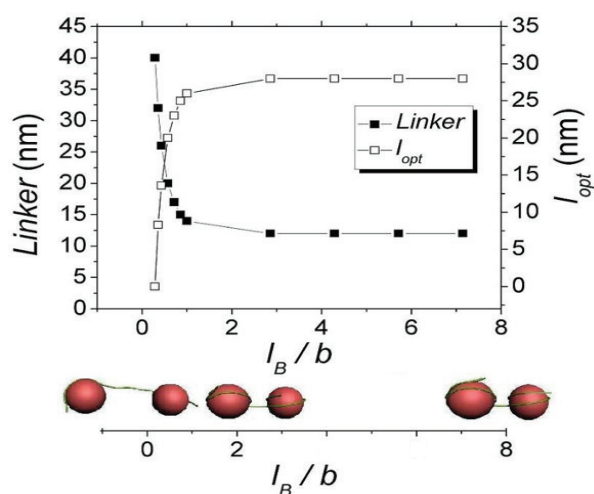

Figure 3: Linker formed between 2G3 complexes with LPE as a function of L_B/b.

Effect of pH

Figure 4 shows the effect of pH on linker formation between complexes when two G6 ethylenediamine cored dendrimers interact with a semiflexible DNA chain with 50 nm persistence length, 0.17 nm bases spacer, and a contour length L = 184 nm (541bps), the dendrimer is considered as a penetrable sphere with radius R = 3.5 nm. As pH increases l_{opt} decreases and the linker between the complexes increases. This can be explained by the reduction of attractive force between the negative DNA and the dendrimers, as the positive charge of the dendrimers decreases due to increasing the pH.
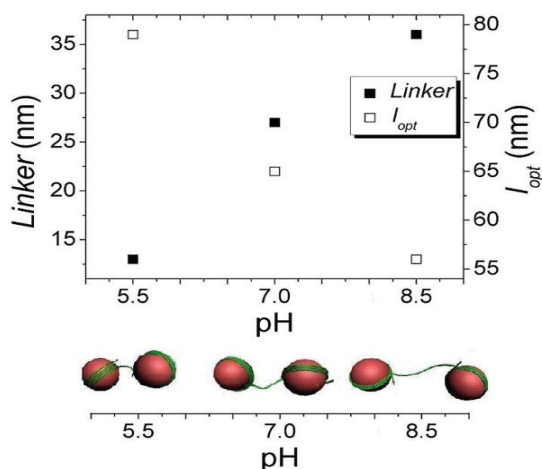

Figure 4. Linker formed between two G6 dendrimers and DNA as a function of pH.

Conclusions

The penetrable sphere model used in our study proved to be suitable to investigate the complexation between dendrimers as penetrable spheres, and the DNA modeled as Linear polyelectrolyte chain. We estimated the wrapping degree of the LPE chain around the dendrimer, and the linker formed between the complexes, which are found to be strongly affected by the medium environment as the Bjerum length, and the pH. Our results agree with the previous computer simulations (Lyulin et al., 2005; Larin et al., 2010); and theoretical studies (Netz & Joanny, 1999; Nguyen & Shklovskii, 2001; Boroudjerdi et al., 2011)

Competing interest

The author (s) declare (s) that they have no competing interests.

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