Effect of PEG-Polycation Chain Flexibility on siRNA loaded Polyion Complex Micelles Assembly

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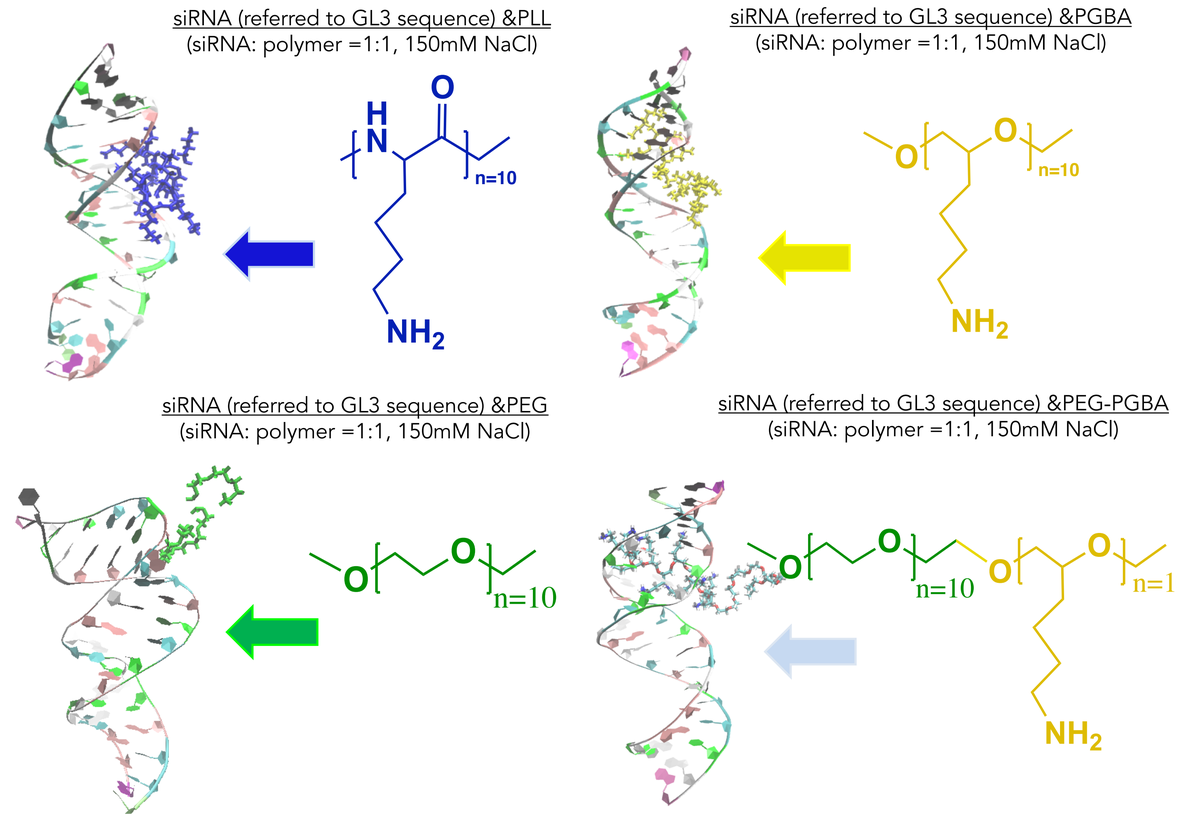
Introduction

RNA interference (RNAi) has emerged as a promising therapeutic approach for the treatment of a wide range of disorders. Small interfering RNAs (siRNAs), i.e. non-coding double-stranded RNA molecules, have been mainly used for RNAi. Because siRNA is susceptible for enzymatic degradation , the success of RNAi is strongly related to the design of efficient delivery technologies. And polymeric micelles self-assembled by polyion complexation have attracted much attention. We have demonstrated that the polycation flexibility influences the complexation with single stranded RNA molecules, affecting the delivery capability of the resulting micelles. On the other hand, the effects of the catiomer flexibility on micelles loading double stranded siRNA remains unknown. Thus, herein, we studied the effects of the polycation backbone flexibility on siRNA-loaded polyion complex (PIC) micelles by using complementary block copolymers poly(ethylene glycol)-poly(glycidylbutylamine) (PEG-PGBA). We found that PEG-PGBA effectively promoted self-assembly of PIC micelles. Computational studies of siRNA binding with polycations and PEG-polycations further supported the favorable binding process of flexible polycations with siRNA. Our results indicate the importance of polycation flexibility on the assembly of PIC micelles with siRNA, and its potential for developing innovative carrier systems.

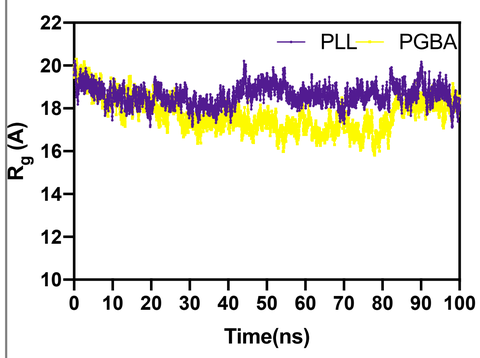
Results

Molecular dynamics simulation of siRNA binding with polycations and PEG-polycations



When inspecting the orientation of the cationic polymer on the siRNA, we found that in polycations systems, initial complexation will likely occur in the major groove of siRNA, where the maximum salt bridge may be formed. When monitoring the PEG-polycations RNA complex, it was found that polycation part was also apparent in the major groove, while PEG was positioned in near major groove, extended toward the external salt solution, which may be a result of strong hydrophilic of PEG.

Radii of Gyration of siRNA binding with PLL and PGBA



The radius of gyration (Rg), reports the average distance is the root mean square distance of the selected atoms from their center (optionally mass-weighted). This value provides some insight into the level of reorganization in the system. The Rg calculated for binding of PLL with siRNA is largely unchanged. While a lower Rg was observed in the second half of the simulation process, which indicate a more compact structure of PGBA siRNA complex than PLL siRNA complex.

Energy binding of polycations and PEG-polycations with siRNA

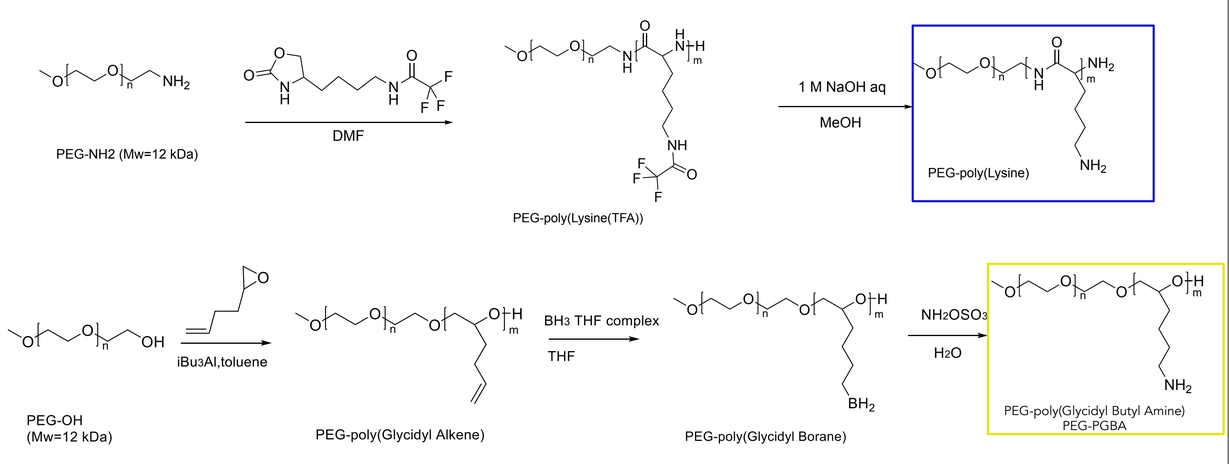
Complex	ΔE_{vdw}	ΔE_{ele}	ΔE_{GB}	ΔE_{SUFR}	ΔH_{bind}
PLL-siRNA	-2.317	-725.3	720.6	-0.804	-7.875
PGBA-siRNA	-3.800	-710.1	706.1	-0.902	-8.696
PEG-siRNA	-3.224	-1.3008	3.049	-0.346	-1.828
PEG-PGBA-siRNA	-4.183	-702.4	699.3	-0.925	-8.126

Energy of siRNA was normalized per charged amine, are expressed in kcal mol^{-1}

To get more details about the binding, the binding free energy components of all systems were calculated by using the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) method. The electrostatic energies (ΔE_{ele}), which supposedly originated from the interaction of positive charges on prime amine and negative charges on phosphate groups of RNA, are markedly favored for both two systems.

When considering polymer chain rigidity, the complexation of PGBA with siRNA resulted in lower binding enthalpy of -8.696 kcal mol^{-1} (-7.875 kcal mol^{-1} of PLL). PEG-PGBA siRNA system also showed favorable binding with binding enthalpy of -8.126 kcal mol^{-1}, the slight decrease in enthalpy is a result of influenced stretching of the polycations due to strong hydrophilic of PEG.

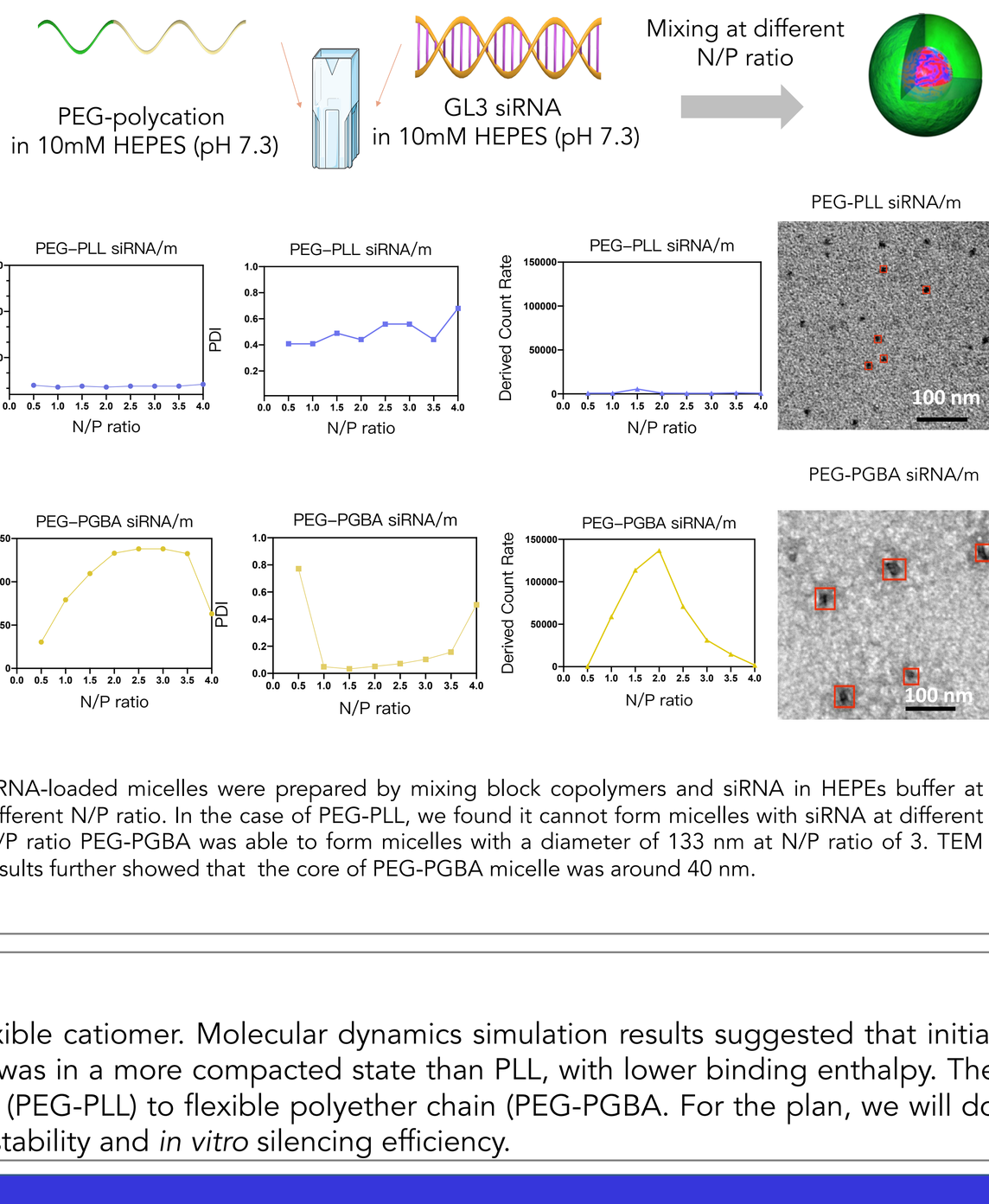
Block copolymers synthesis



Polymer	DP	M_w/M_n[2]	%Conversion
PEG-PLL	76	1.07	100
PEG-PGBA	90	1.05	100

The PEG-PLL block copolymer was synthesized by ring-opening polymerization (ROP) of lysine-TFA-NCA and followed by the deprotection of TFA groups. PEG-PGBA block copolymer was synthesized by ROPs followed by brown hydroboration-amination. The degree of polymerization was checked by ^{1}H-NMR, the DP of PEG-PLL and PEG-PGBA was calculated to be 76 and 90 respectively, and narrow molecular weight distribution were maintained.

siRNA loaded PIC micelle formation



siRNA-loaded micelles were prepared by mixing block copolymers and siRNA in HEPEs buffer at different N/P ratio. In the case of PEG-PLL, we found it cannot form micelles with siRNA at different N/P ratio PEG-PGBA was able to form micelles with a diameter of 133 nm at N/P ratio of 3. TEM results further showed that the core of PEG-PGBA micelle was around 40 nm.

Conclusion

In this study, we have successfully prepared siRNA-loaded micelles from flexible catiomer. Molecular dynamics simulation results suggested that initial complexation will likely occur in the major groove of siRNA. PGBA siRNA complex was in a more compacted state than PLL, with lower binding enthalpy. The DLS and TEM results showed that micelles were formed when changing rigid chain (PEG-PLL) to flexible polyether chain (PEG-PGBA. For the plan, we will do physiochemical characterization of PIC micelles structure, also evaluate PIC micelle stability and *in vitro* silencing efficiency.