Dicationic gemini surfactant gene delivery complexes contain cubic-lamellar mixed polymorphic phase

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ABSTRACT

The characterization of structural properties of novel dicationic (gemini) surfactant-based DNA complexes as micro/nano-scale self-assembling delivery systems for cutaneous gene therapy is described and discussed as related to measured transfection efficiencies. We have identified the Pn3m cubic phase in DNA-gemini-DOPE complexes with gemini surfactants having 12, 16 and 18:1 alkyl tail length. Increasing gemini/DNA charge ratios ($\rho_{+/-}$) from 0.5 to 10 resulted in increasingly mixed (Pn3m and H_{II} or Pn3m and L) polymorphic systems with lamellar (L) features becoming more predominant. DNA-gemini complexes exhibited very weak single scattering peaks representative of gemini- plasmid particles with no long range order and low transfection efficiency. In gemini – DOPE complexes the main complex geometry is inverted hexagonal (H_{II}) at low concentrations of gemini, with weakly ordered systems (generally lamellar) observed at increased concentrations. Overall, the presence of the newly identified cubic Pn3m phase in the DNA-gemini-DOPE complexes appears to be advantageous for increased transfection efficiency.

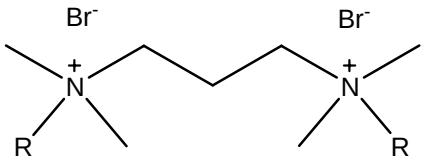
1 INTRODUCTION

An important focus of research in the medical and pharmaceutical sciences is the treatment of genetic disorders via gene therapy. A successful therapeutic mechanism requires the transfer and subsequent expression of exogenous DNA such that a defective gene can be replaced or a missing gene added. There are two basic methods for facilitating this transfer, through the use of viral and non-viral vectors. While an ideal choice from the point of view of nature where their function is precisely to facilitate DNA transfection, the use of viral vectors suffers from problems associated with potential immunological responses and a limited size for the plasmid DNA that can successfully be transfected. Non-viral vectors (typically cationic lipid or cationic polymers[1]) have the advantage of having, generally, low toxicity/immunogenicity, as well as having no limitation with regard to the size of DNA that can be delivered. From a manufacturing perspective non-viral vectors also benefit from simpler quality control, and more straightforward regulatory requirements.[2] The major problem facing the application of non-viral vectors to gene therapy is a low efficiency.[3]

The objective of this study was to characterize the structural and physicochemical properties of novel dicationic lipid-based DNA complexes by small-angle x-ray scattering (SAXS), zeta potential and particle size analysis in order to determine the optimum parameters required for cellular transfection.

2 MATERIALS AND METHODS

A series of cationic lipid-DNA complexes based on dicationic (gemini) surfactants and other lipids of various compositions were constructed. The synthesis of the gemini surfactant compounds used are this study is detailed elsewhere[4]; their structures are illustrated in scheme 1. Transfection mixtures consisting of plasmid – gemini surfactant complexes, (PGs) and plasmid – gemini surfactant – helper lipid vesicles (PGLs – with 1mM dioleoylphosphatidylethanolamine (DOPE) as helper lipid) were prepared, by first complexing the DNA (at a concentration of 0.075 mM; 50 μg/mL) with the cationic surfactant, followed by addition of DOPE.



Scheme 1: General structure of the m-3-m gemini surfactants; R = dodecyl, hexadecyl, or oleyl for the 12-3-12, 16-3-16, and 18:1-3-18:1 surfactants, respectively.

Murine keratinocytes (PAM212 cell line) at $5x10^4$ cells/well were grown to 60-70% confluency. The cells were transfected with PGs or PGLs containing 0.2 μg plasmid/well. The plates were incubated for 5 hours at 37°C in a CO_2 incubator. The supernatants were collected at 24 hours. The expressed protein (murine interferon γ) was determined by ELISA.

SAXS measurements were made using beamline X21 at the National Synchrotron Light Source at Brookhaven National Laboratory. The measurements were performed with 12KeV x-rays and the data covered a q-range from $0.008Å^{-1}$ to $0.5Å^{-1}$. Samples

were loaded into 1.5 mm capillaries and the scattering pattern was recorded using a 13cm Mar CCD detector (Mar USA, Evanston, IL), at 1.26m (calibrated with the scattering pattern of silver behenate) downstream of the sample. All spectra were processed to remove background contributions by subtracting the scattering profile obtained for a water-filled capillary.

3 RESULTS

Scattering curves for each of the gemini surfactant compounds were obtained for 4 different systems (all in aqueous solution); 1) gemini alone (G), 2) gemini with plasmid (PG), 3) gemini with DOPE (GL), 4) gemini with plasmid and DOPE (PGL). Aqueous solutions of surfactant gave generally weak scattering profiles, with a sharp peak at q = 0.156, 0.136, and 0.125 $Å^{-1}$ for the 12-3-12, 16-3-16, and 18:1-3-18:1 surfactants, respectively at concentrations of 3.75 mM.

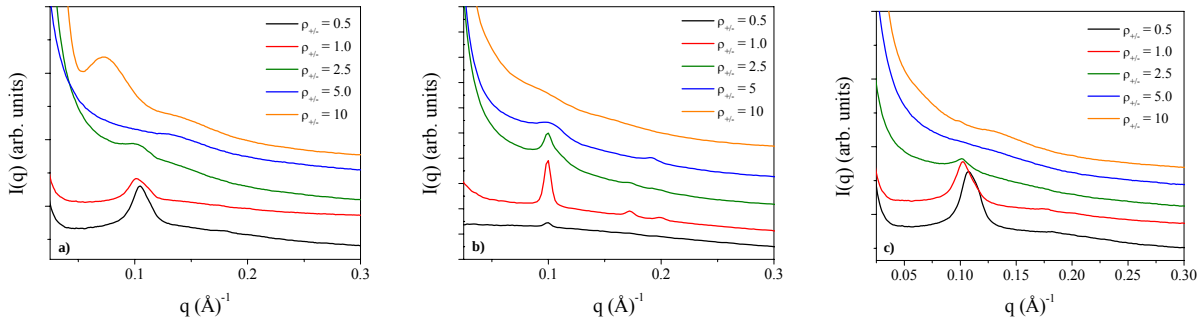


Figure 1: SAXS profiles for the gemini surfactant – DOPE (GL) systems at varying charge ratios; a) 12-3-12, b) 16-3-16, c) 18:1-3-18:1.

Table 1: SAXS Peak Positions (q) for the gemini surfactant – DOPE[a] systems at varying charge ratio

Concentration	q ($Å^{-1}$)	d or a (Å)	Phase	q ($Å^{-1}$)	d or a (Å)	Phase	q ($Å^{-1}$)	d or a (Å)	Phase
	12-3-12			16-3-16			18:1-3-18:1		
0[b]	0.106 0.184 0.214	68.4	H_{II}						
0.188	0.108 0.190	67.2	H_{II}	0.100 0.174 0.200 0.260	72.5	H_{II}	0.107 0.186 0.212	67.8	H_{II}
0.375	0.105 0.184	69.1	H_{II}	0.099 0.172 0.199	73.3	H_{II}	0.103 0.177 0.201	70.4	H_{II}
0.938	0.101	61.2		0.101 0.173 0.198	71.8	H_{II}	0.103	61.0	
1.875	-	-	-	0.102 0.191	62.2	L	0.100	62.8	
3.75	0.86 0.180	73.0		0.095 0.153	66.1 41.1		0.084 0.155	74.8	

[a] [DOPE] = 10 mM; [b]peak positions in absence of added gemini

This corresponds to average d-spacings (d = 2π/q) of 40.3, 46.2, and 50.3 Å and is consistent with the average diameter of gemini surfactant micelles as determined using dynamic light scattering.[5-7] Solutions containing both the gemini surfactant and plasmid DNA also gave, generally, weak scattering signals. At charge ratios ($\rho_{+/-}$) of 5 and 10 a single sharp peak is observed for both the 12-3-12 and 12-6-16 gemini surfactants with d-spacings of 45.5, and 49.1 Å, respectively, corresponding to the surfactant-complexed DNA. For the 18:1-3-18:1 surfactant, a scattering peak is observed at $\rho_{+/-}$ = 2.5, 5, and 10, with a d-spacing of 52.4 Å. Repeat patterns are not observed due to a lack of long range order.

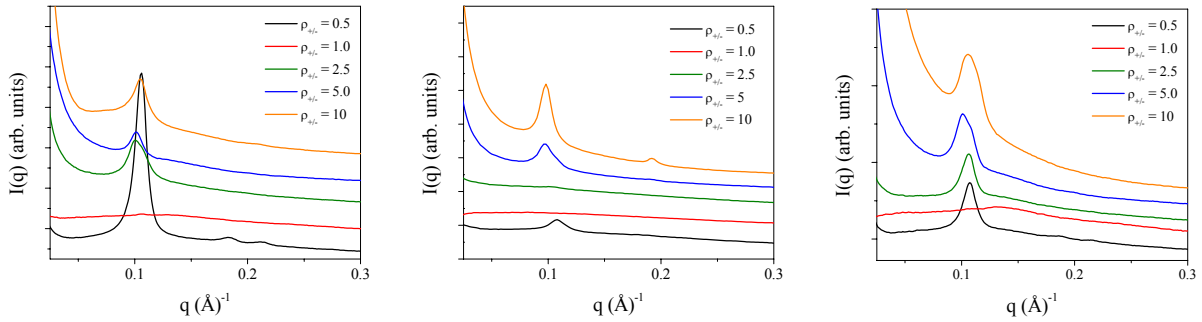


Figure 2: SAXS profiles for the plasmid – gemini surfactant – DOPE (PGL) systems at varying charge ratios; a) 12-3-12, b) 16-3-16, c) 18:1-3-18:1.

Table 2a: SAXS Peak Positions (q) for the 12-3-12 and 16-3-6 gemini surfactant – plasmid DNA – DOPE systems at varying charge ratio

$\rho_{+/-}$	q ($Å^{-1}$)	Phase	*hkl*	d or a (Å)	$\rho_{+/-}$	q ($Å^{-1}$)	Phase	*hkl*	d or a (Å)	$\rho_{+/-}$	q ($Å^{-1}$)	Phase	*hkl*	d or a (Å)
		12-3-12					16-3-16							
0.5	0.106	H_{II}	10	68.6	0.5	0.090	Pn3m	110	49.1	0.5	0.082	Pn3m	110	54.4
	0.115					0.108	H_{II}	10	67.3		0.106	Pn3m	111	
	0.183	H_{II}	20			0.109	Pn3m	111			0.107	L	100	67.5
	0.212	H_{II}	30			0.124	Pn3m	200			0.125	Pn3m	200	
						0.184	H_{II}	20			0.216	L	200	
						0.218	H_{II}	30						
2.5	0.082	Pn3m	110	54.1						2.5	0.097	Pn3m	110	45.6
	0.101	Pn3m	111								0.107	L	100	58.9
	0.105										0.121	Pn3m	111	
	0.129	Pn3m	200								0.199	L	200	
	0.162													
5	0.085	Pn3m	110	52.0	5	0.077	Pn3m	110	57.8	5	0.103	L	100	61.3
	0.101	Pn3m	111			0.097	Pn3m	111			0.132			
	0.102	L	100	61.8		0.099	L	100	63.6		0.166			
	0.136	Pn3m	200			0.127	Pn3m	200			0.198	L	200	
	0.201	L	200			0.189	L	200						
10	0.085	Pn3m	110	52.5	10	0.098	L	100	64.1					
	0.104	Pn3m	111			0.192	L	200						
	0.105	L	100	59.7										
	0.120	Pn3m	200											
	0.212	L	200											

At low surfactant concentrations (0.188 mM or less) the gemini surfactant/DOPE systems exhibit a hexagonal morphology, similar to that observed for DOPE alone. As the concentration of surfactant is increased, a transition is observed from the hexagonal phase to what appears to be a very weakly ordered lamellar phase. The results are consistent with the formation of mixed unilamellar vesicles, as observed from both dynamic light scattering measurements and electron microscopy[8], which do not show strong diffraction peaks due to a lack of long range order.[9]

The surfactant/DOPE/DNA systems also exhibit hexagonal and lamellar morphologies; however, a cubic phase can also be observed, which indexes to the Pn3m space group. This phase is one of several cubic phases that have been previously observed in lipid systems.[10]For $\rho_{+/-}$ = 0.5 the scattering profiles obtained for the 12-3-12 surfactant/DOPE/DNA system are again characteristic of a hexagonal morphology (see Figure 2 and Table 2); while those for the 16-3-16 and 18:1-3-18:1 systems suggest a mixture of both the Pn3m hexagonal structures. For surfactant concentrations corresponding to $\rho_{+/-}$ = 1.0 (i.e. the isoelectric point) no significant scattering profiles are observed. It should be noted that under these conditions, the complexes are observed to precipitate significantly. The scattering profiles obtained for the precipitates again show a hexagonal structure (Figure 3 a), with unit cell spacings of 67 – 68 Å, in excellent agreement with that obtained for the DOPE/DNA system in absences of added gemini surfactant. It is important to note that under these conditions transfection is not observed to occur for the gemini surfactant/DOPE/DNA systems.[8] Clearly the presence of a hexagonal morphology for a CLDC system does not necessarily indicate a system for which efficient transfection will occur (Table 3).

Table 3: Summary of transfection efficiencies for the plasmid (P) – gemini (G) – DOPE (L) systems

	Transfection Efficiency		
System	**12-3-12**	**16-3-16**	**18:1-3-18:1**
PL[a]	none	none	none
PG[b]	none	none	Low
PGL[c]	high	high	high

[a]plasmid – DOPE alone; [b]plasmid – gemini alone; [c]plasmid – gemini – DOPE complexes

As the concentration of surfactant is increased, different structures are observed, depending upon the identity (and therefore structure) of the gemini surfactant used to prepare the complexes. The Pn3m phase persists up to $\rho_{+/-}$ = 10 for the 12-3-12/DOPE/DNA systems; this phase is much less evident about charge ratios of 5 and 2.5 for the 16-3-16 and 18:1-3-18:1 systems, respectively. All 3 surfactants are observed to form complexes with a lamellar morphology (Figure 2 and Table 2), where complexes formed with the 16-3-16 surfactant being the most ordered. This is not unexpected, particularly if one considers the effect of the surfactant on a DOPE bilayer. The C_{12} tails are a poor match in terms of length to the oleyl tails of the DOPE molecules, allowing for a wider range of conformations. Similarly the oleyl tails of the 18:1-3-18:1 surfactants will have, due to the all-*cis* conformation, a disordering effect on the DOPE bilayers,[11, 12] while the C_{16} tails are expected to introduce the most order.

4 CONCLUSION

The incorporation of gemini surfactants into cationic lipid / DNA complexes results in a number of possible complex structures, depending upon the relative concentrations of surfactant and DOPE. Hexagonal structures exist at high mol ratios of DOPE, while a Pn3m phase is observed at high mol ratios of surfactant. Transfection has been previously observed only at high concentrations of surfactant indicating that it is not the presence of a hexagonal structure that promotes transfection, but rather the ability of the system as a whole to adopt various polymorphic structures.

REFERENCES

[1] M. A. Ilies, W. A. Seitz, and A. T. Balaban, Current Pharmaceutical Design **8**, 2441 (2002).

[2] A. D. Miller, Current Medicinal Chemistry **10**, 1195 (2003).

[3] K. Ewert, N. L. Slack, A. Ahmad, et al., Current Medicinal Chemistry **11**, 133 (2004).

[4] S. D. Wettig and R. E. Verrall, Journal of Colloid and Interface Science **235**, 310 (2001).

[5] T. Yoshimura, Y. Nagata, and K. Esumi, Journal of Colloid and Interface Science **275**, 618 (2004).

[6] S. D. Wettig, P. Nowak, and R. E. Verrall, Langmuir **18**, 5354 (2002).

[7] X. Li, S. D. Wettig, and R. E. Verrall, Journal of Colloid and Interface Science **282**, 466 (2005).

[8] I. Badea, R. E. Verrall, M. Baca-Estrada, et al., The Journal of Gene Medicine **7**, 1200 (2005).

[9] D. Uhrikova, M. Hanulova, S. S. Funari, et al., Biophysical Chemistry **111**, 197 (2004).

[10] R. Winter and R. Kohling, Journal of Physics-Condensed Matter **16**, S327 (2004).

[11] S. S. Feng, G. H. Zhu, and M. Y. Low, Langmuir **16**, 7401 (2000).

[12] T. Hianik, M. Haburcak, K. Lohner, et al., Colloids and Surfaces a-Physicochemical and Engineering Aspects **139**, 189 (1998).