Supporting Information

Factors determining the superior performance of lipid/DNA/protammine nanoparticles over lipoplexes

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Lipid/DNA binding constants of lipid/DNA/protammine nanoparticles and lipoplexes

Lipid/DNA binding constants of lipid/DNA/protammine (LPD) nanoparticles and lipoplexes were determined by UV-Vis measurements. The absorption spectra were recorded on a Perkin Elmer Lambda 40 Spectrophotometer, with a slit of 2 nm and scan speed of 240 nm min^{-1}. Quartz cuvettes of 1 cm were used. The absorbance assessments were performed at pH 7.4 by keeping the concentration of DNA constant (4 mM), while varying the concentration of liposome (0.05–1 mM). To calculate the lipid–DNA binding constant, the data are treated according to the following equations (Marty et al., Nucleic Acids Res. **2009**, 37, 849–857):

$$\frac{1}{A - A_0} = \frac{1}{A_\infty - A_0} + \frac{1}{K(A_\infty - A_0)}\frac{1}{C}$$

where, A_0 is the absorbance of DNA at 260 nm in the absence of ligand, is the final absorbance of the ligated-DNA and A is the recorded absorbance at different ligand concentrations. The double reciprocal plot of $1/(A - A_0)$ versus $1/C$ is linear and the binding constant (K) is estimated from the ratio of the intercept to the slope. The initial absorbance of the free DNA at 260 nm (A_0=0.2218), was found to be slightly lower than that of protamine-condensed DNA (A_0=0.3065). This is consistent with reduction of base stacking interaction due to protein complexation (Marty et al., Nucleic Acids Res. **2009**, 37, 849–857). The recorded absorbance of DNA as a function of lipid concentration, C, is reported in Table 1. For both the systems, increasing lipid concentration resulted into an increase in UV light absorption. The double reciprocal plot of $1/(A-A_0)$ versus $1/(C)$ was found to be linear and the binding constant (K) can be estimated from the ratio of the intercept to the slope. The estimated binding constants ($K_{Lipoplex}$= (1.3±0.3) $\times 10^4$ M^{-1} and K_{LPD}= (2.1±0.5) $\times 10^4$ M^{-1}) are mainly due to the lipid–base binding. Thus, our findings indicating that more stable DNA adducts formed in the case of LPD nanoparticles is the result of a better DNA charge neutralization in the presence of cationic protammine.

Table 1. Recorded absorbance (260 nm) at different lipid concentrations, *C*.

	Absorbance (a.u.)	
***C* (mM)**	**Lipoplex**	**LPD**
47	0,31813	0,36236
95	0,34089	0,39002
190	0,36957	0,40012
382	0,37231	0,43193
763	0,49883	0,40651
1145	0,54636	0,41407

Kinetic stability of lipid/DNA/protammine nanoparticles and lipoplexes

Diameter, D, and zeta potential, ξ_P, of LPD nanoparticles (triangles) and lipoplexes (circles) over 24 hours. As evident, both the complexes were pretty stable in time.