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Lipopolythiourea/DNA interaction: A biophysical study

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ABSTRACT

Lipopolythioureas (LPT) are original non cationic systems representing an alternative to cationic lipids. Their high transfection efficiency prompted us to investigate further their biophysical properties, and in particular how thiourea lipids interact with DNA. The interaction of lipopolythiourea with DNA was investigated by fluorescence correlation microscopy (FCS). Influence of the lipid length and nature of the thiourea head on the thiourea/DNA interaction were studied. FCS revealed a strong interaction between lipopolythiourea and DNA, occurring at 1 equivalent of a thiourea lipid by a DNA phosphate group, and leading to a condensed plasmid state. From previous *in vitro* experiments, we could conclude that the lipid leading to the more condensed state of DNA was also the more efficient to transfect cells.

1. Introduction

Non-viral gene therapy is a rapidly expanding field [1]. Many synthetic vectors have been commercialized and developed in order to improve transfection efficiency [2]. Of these carriers, the most widely used are cationic lipids: their efficiency has been demonstrated [3], they have been extensively characterized [4] and used in many clinical trials [5]. They are composed of a hydrophobic moiety, which provides self-association to form lipidic structures, a linker and a cationic head. A large panel of structures of this latter element has been investigated: it can be a quaternary ammonium head group, such as DOTMA [6], DOTAP [3], an amino acid [7], a guanidinium [8] or pyridinium moiety [9], or branched polyamines like DOGS [10]. In all these examples, the head group remains cationic, since this charge is responsible for the association with the negatively charged phosphate groups of DNA, and for cell-membrane interaction. This cationic character is also the source of several drawbacks, indeed unspecific interaction with blood proteins decreases strongly the *in vivo* efficiency [11] and induces non specific tissue accumulation [12]. Non cationic systems have been recently reported, like saccharidic clusters developed by Aoyama et al. [13], multilamellar neutral vesicles called spherulites [14] or nucleolipids [15].

We recently developed non cationic lipids based on a thiourea head group moiety (LPT) [16]. We investigated various lipid chain lengths, types of linker and structures of the head group in order to optimize the formulation and transfection properties [17]. Latest lipids, based on a serinol structure, demonstrated good formulation abilities and a high level of transfection efficiency *in vitro*. In particular, the compounds bearing 10 or 12 carbons in the lipid chain revealed the most efficient, reaching the transfection level of a cationic lipopolyamine [18]. Lipopolythiourea based on a lysine structure was also reported for its high transfection efficiency [19]. If their efficiency is now demonstrated, it appeared necessary to progress in the understanding of these systems. We aimed here at further studying these different structures in terms of lipopolythiourea/DNA interaction. Thiourea is known to create strong hydrogen bonds with anions and particularly phosphate groups

Abbreviations: LPT, Lipopolythiourea; FCS, Fluorescence correlation spectroscopy; NMR, Nuclear magnetic resonance; DOTMA, *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride; DOTAP, 1,2-dioleoyloxypropyl-3-(trimethylammonio)-propane; DOGS, Dioctadecylamidoglycylspermine 4trifluoroacetic acid, transfectam; DNA, Deoxyribonucleic acid; Na_2HPO_4, Sodium hydrogenophosphate; D_2O, Deuterated H_2O; Cn-TU4OH, Lipid containing a chain length of n carbons and a thiourea-tetraol head; Cn-TUP, Lipid identical to the Cn-TU4OH with the alcohol protected (P) by a isopropylydene moiety; Cn-TUMe, Lipid containing a chain length of n carbons and a thiourea-methyl head; TU/PO, Molar ratio of thiourea/DNA phosphate; LPT/PO, Molar ratio of lipopolythiourea/DNA phosphate.

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[20]. Variations of the LPT structure from a linear to a branched head group showed that interaction with DNA must actually occur thanks to the thiourea moiety [17]. Here, we compared the different lipopolythiourea lipids previously prepared (Fig. 1) to evaluate the chain length and the head group influences on the lipopolythiourea interaction with DNA, and gain comprehension into properties required for efficient non-viral gene transfection.

2. Experimental procedures

2.1. Materials

All solvents were purchased from Carlo Erba-SDS (Peypin France). Picogreen™ was purchased from Molecular Probes.

2.2. ^{31}P NMR

Na_2HPO_4 (3 mM) solution in acetone-d_6/D_2O (8:2) was added with different amounts of C10-TU4OH and the mixture was homogenized. The chemical shifts (δP) were measured by ^{31}P NMR on a BRUKER Avance DRX-300 spectrometer at 300.13 MHz. NMR spectra were processed using XwinNMR (Bruker) or Swan-NMR.

2.3. LPT formulation. Liposome preparation

LPT (5 mg, 6.4 μmol) was dissolved in 500 μL ethanol. This solution was added dropwise into 5 mL of stirred filtered water. The mixture was stirred overnight then evaporated under reduced pressure at RT to obtain a clear suspension of LPT (440 μL, 14.5 mM).

2.4. Preparation of LPT/DNA complexes

Plasmid DNA (pVax2luc [18], 100 μL, 0.02 g/L in H_2O) was added dropwise with constant vortexing to various amounts of LPT liposomes (in 100 μL H_2O) at RT. LPT/PO indicates the ratio in nmol of thiourea lipid versus DNA phosphates.

2.5. Agarose gel electrophoresis

Samples were prepared as described above (20 μL) and 5 μL of bromophenol blue was added. The mixtures were loaded into 0.8% agarose gel in TBE buffer (1 M Tris, 0.9 M boric acid, 0.01 M EDTA) at 80 V/cm. DNA was revealed with ethidium bromide and visualized under UV light.

2.6. Fluorescence correlation spectroscopy experiments

2.6.1. Instrumentation

The fluorescence correlation spectroscopy measurements were performed using an upgraded Confocor 1 [21] apparatus (Carl Zeiss GmbH, Jena; Evotec Biosystems GmbH, Hamburg; PicoQuant GmbH, Berlin, Germany) with a pulsed diode laser LDH-P-C-470 (470 nm, 40 MHz, FWHM of the overall IRF 500 ps) with a PDL800-B driver (PicoQuant GmbH, Berlin, Germany), a proper fluorescence filter set (HQ457/10, z470rdc, HQ515/50 — Chroma Technology, Rockingham, USA), water immersion objective C-Apochromat 40x/1,2 W and a detection system consisting of a single photon counting detector SPCM-AQR-13-FC (Perkin-Elmer). Laser power at the sample position was set to 1 μW for all FCS measurements.

2.6.2. Data acquisition and processing

Data acquisition was performed with the fast electronics (Picoharp 300, PicoQuant) in time-SymphoTime software (Picoquant, Berlin) and autocorrelation functions (ACF) were taken from the FCS data analysis using home built routines (DevC++, OriginPro70, OriginLab Corporation).

2.6.3. FCS data analysis

It has been shown that a confocal single focus FCS cannot be considered as a quantitative method for the determination of 3-dimensional diffusion coefficients [22]. Additionally, if the multiply labelled plasmid DNA exceeds like in the case reported herein a one and half the diameter of laser focus, the autocorrelation curve (ACF) for DNA molecules are difficult to model. For the freely diffusing plasmid DNA the segmental and the Brownian motions of the whole molecule are superimposed which usually causes an increase of the apparent number of particles in the focus and as well affects the apparent residence times. We therefore refrain from reporting absolute diffusion coefficients, and rather report residence times τ_{res} obtained from fits to those ACF [23].

$$G(\tau) = 1 + \frac{1}{PN(1-T)}\left(1-T\left(1-e^{-\frac{\tau}{\tau_0}}\right)\right)\cdot\left(\frac{1}{1+\left({}^{\tau}/_{\tau_{res}}\right)}\cdot\frac{1}{\left(1+\left({}^{\tau}/_{\tau_{res}}\right)\left({}^{\omega_0}/_{\omega_Z}\right)^2\right)^{\frac{1}{2}}}\right) \quad (1)$$

Where, (τ_{res}) — is residence time which we have assigned to lateral movement of the whole molecule, triplet-like term containing the "triplet" parameters T and τ_0 describe here the effect of segmental motion of the chain [24], PN is an apparent number of particles in the detection volume and ω_0 and ω_Z are half axes of that detection volume.

2.6.4. Sample preparation

Samples were prepared as follows: an appropriate amount of 1 nM solution of the PicoGreen labeled DNA in deionized water was placed into the measurement chamber; ($C_{PicoGreen}/C_{DNA\ phosphate}$ ratio of 0.02) and titrated with the lipopolythiourea lipids.

3. Results

3.1. Lipopolythiourea/phosphate interaction

A study was conducted by ^{31}P NMR to evaluate to what extent the attachment of the thiourea moiety to a lipid part affects its interaction with phosphates. This method had already been used to model interaction between DNA phosphates and nucleolipids [15]. It consists in a NMR titration of Na_2HPO_4 with a lipid, keeping the phosphate concentration constant and progressively increasing the lipid amount. The ^{31}P NMR signal, initially at 4.79 ppm, underwent an upfield shift to 1.75 ppm in the presence of C10-TU4OH (Fig. 2). A similar shift has been obtained with a cationic lipid taken as a reference. As can be seen in Fig. 2, we observed the presence of two phosphate species indicating an equilibrium between free and bonded anions at a ratio of LPT/P around 4. Total complexation was reached at 8 molar equivalents of lipid per phosphate (LPT/P) (Fig. 2).

3.2. Lipopolythiourea/DNA interaction

3.2.1. Lipopolythiourea/DNA interaction by gel electrophoresis

As first reported, TU4OH LPT are able to form liposomes without any helper lipid [18]. Plasmid association was examined by gel electrophoresis and revealed by ethidium bromide (Table 1). According to this method, interaction could be detected from 10 TU/PO and total retention of DNA in the wells was observed at a ratio of 15 TU/PO for the TU4OH compounds. Higher amounts were necessary for full retention of DNA on an agarose for the other thiourea compounds.

3.2.2. Lipopolythiourea/DNA interaction by fluorescence correlation spectroscopy

3.2.2.1. Lipid length influence on DNA condensation. As we already reported [25] fluorescence correlation spectroscopy (FCS) allows to follow condensation of DNA molecule fluorescently tagged by

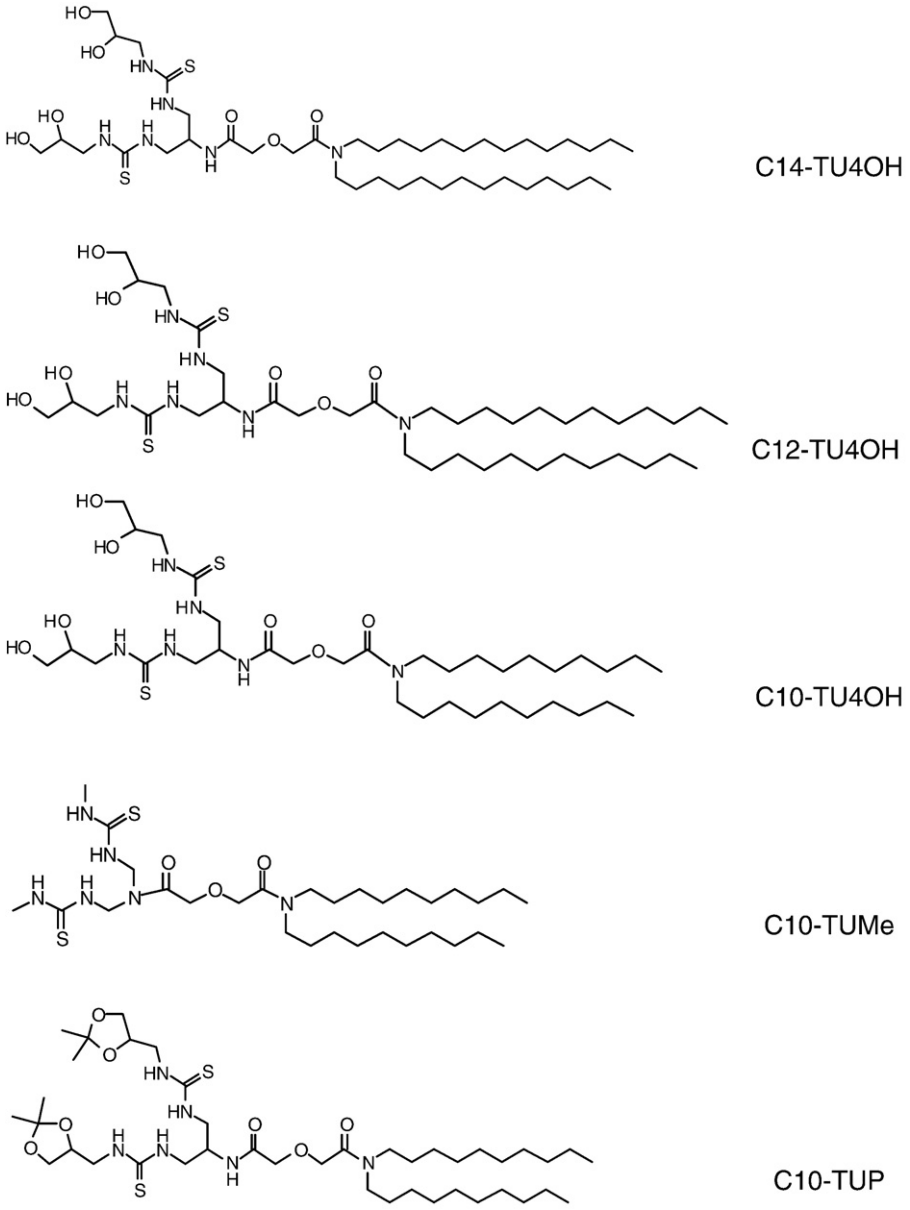

Fig. 1. Names and structures of the thiourea lipids evaluated for their interaction with plasmid DNA.

PicoGreen® intercalation [26] on a single molecule level. The residence time (τ_{res}) which we obtain from fitting the ACFs by Eq. (1) is related to the lateral movement of the labelled DNA molecule. In the experiments performed herein changes in τ_{res} mainly reflect changes in the size of the DNA molecule. Results for the τ_{res} in the presence of an increasing amount of TU4OH are plotted in Fig. 3. The three tested lipids exhibit a similar polyhydroxy polar head and 3 different lipid lengths C10, C12 and C14. Starting from free DNA, we observed a steep decrease in the residence time (τ_{res}) along with an increasing LPT/PO ratio until reached minimum values. Minimum residence times for DNA/C12-TU4OH and DNA/C14-TU4OH complexes amounted 15 ms and 10 ms respectively, mean 3- and 4.5-fold decreases of τ_{res} as compared to free DNA (45 ms). A substantially shorter minimum residence time (5 ms) was observed for the C10-TU4OH which then seems to be the most effective in tightening the supramolecular packing as the τ_{res} obtained at the plateau was 9-fold shorter in comparison to free DNA. Residence time decrease, i.e condensation of DNA, by polyhydroxy thioureas was dependent on the hydrocarbon chain length and also on the LPT/PO ratio. Thus, to reach a τ_{res} of 10 ms (4.5 fold decrease) about 5 molecules per phosphate group (LPT/PO) was required for C10-TU4OH, 10 for C12-TU4OH and as many as 20 for the C14-TU4OH (Fig. 3).

The simultaneous decrease of the residence time was followed by the drop of the apparent particle number (PN) values. The PN decrease reflects a size reduction of the DNA molecules till the DNA resembles a rigid point-like molecule much smaller than the size of the detection volume [24], where inner motions no longer contribute to the FCS signal. For the all TU4OH derivatives we have observed a significant (8–9 fold), continuous decrease of PN at the ratios LPT/PO in the range 4–5. This means that 4 to 5 molecules of TU4OH derivatives per 1 phosphate group were sufficient to decrease the size to coiled DNA molecules which meet the requirements of point-like molecule. For these three thiourea lipids, experimentally obtained PN (1.0) values for the condensation of DNA are similar to the theoretically calculated PN (0.6) from the use of plasmid concentration within the experimental error limit (+/−0.3).

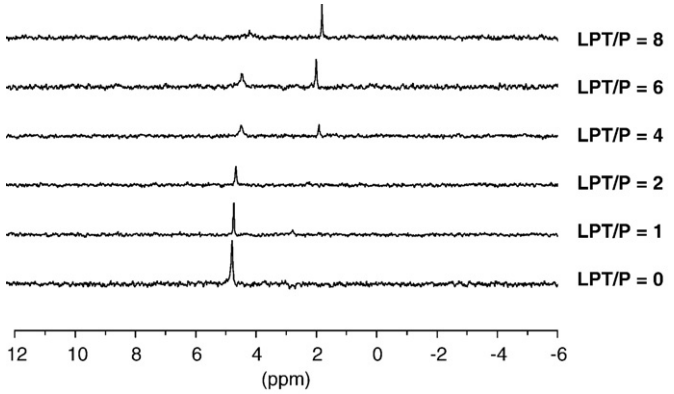

Fig. 2. ^{31}P NMR spectra of Na_2HPO_4 titration by C10-TU4OH (LPT/P = 0, 0.5, 1, 2, 3, and 4 from bottom to top) at 25 °C in acetone-d_6/D_2O (80/20). [Na_2HPO_4] = 3 mM. External reference H_3PO_4 in D_2O.

3.2.3. Polar head influence on DNA condensation

We have then studied three thiourea lipids exhibiting the same aliphatic chain length and different polar head (see in Fig. 1 C10-TU4OH, C10-TUMe and C10-TUP). The three polar heads are branched and bear two thiourea groups, however the polarity brought by these different heads are different. Keeping the protecting groups of the alcohol functions or replacing them by a methyl group highly reduced the hydrophilicity of the compound, the more hydrophilic being the C10-TU4OH as reflected by the log *P* calculated to be 3.3, 4.8 and 8.1 for C10-TU4OH, methyl derivative (C10-TUMe) and acetal protected diol (C10-TUP) respectively. From the tested compounds, C10-TU4OH was the most effective to condense DNA (Fig. 4). Residence times of the methyl derivative (C10-TUMe) have shown a 2-fold decrease whereas only a considerably smaller drop (from 40 ms to about 30 ms) was observed for the acetal protected thiourea along with increasing LPT/PO ratio up to a ratio of 20. This compared to the C10-TU4OH small decrease of τ_{res} for DNA–C10-TUMe and DNA–C10-TUP derivatives indicates that though a significant DNA conformation change occurred, the resulting hydrodynamic radius of the DNA/lipid complex is a magnitude of 4.0 larger than for C10-TU4OH but 1.5-fold smaller when compared to DNA. This conclusion is supported by the PN dependence. While a LPT/PO ratio up of about 5 is sufficient to form a nanoparticle much smaller than the size of the detection volume, for the other two derivatives the size of the complex remains close to the detection volume dimensions. However, for DNA–C10-TUMe a further increase to a LPT/PO ratio of 40 apparently further decreased the size to considerably smaller than the detection volume and thus PN (about 1.0) was close to the derived concentration, calculated theoretically (PN 0.6). In other words, the amount of C10-TUMe needed to complete the condensation is about LPT/PO = 40 which makes this compound 8-fold less effective when compared to C10-TU4OH. Considering the mixture DNA–acetal protected C10-TUP, an insignificant drop in the residence time has been observed along with a small drop of the PN (2-fold), which means that the nanoparticles were not formed.

4. Discussion

The lipopolythiourea were initially developed to provide non cationic DNA vectors. The purpose was to improve nucleic acid delivery using systemic administration in order to avoid vector

Table 1
Ratio thiourea/phosphate necessary to fully retain DNA in the wells of an agarose gel.

Compounds	C14-TU4OH	C12-TU4OH	C10-TU4OH	C10-TUMe	C10-TUP
TU/PO	15	15	15	40	40

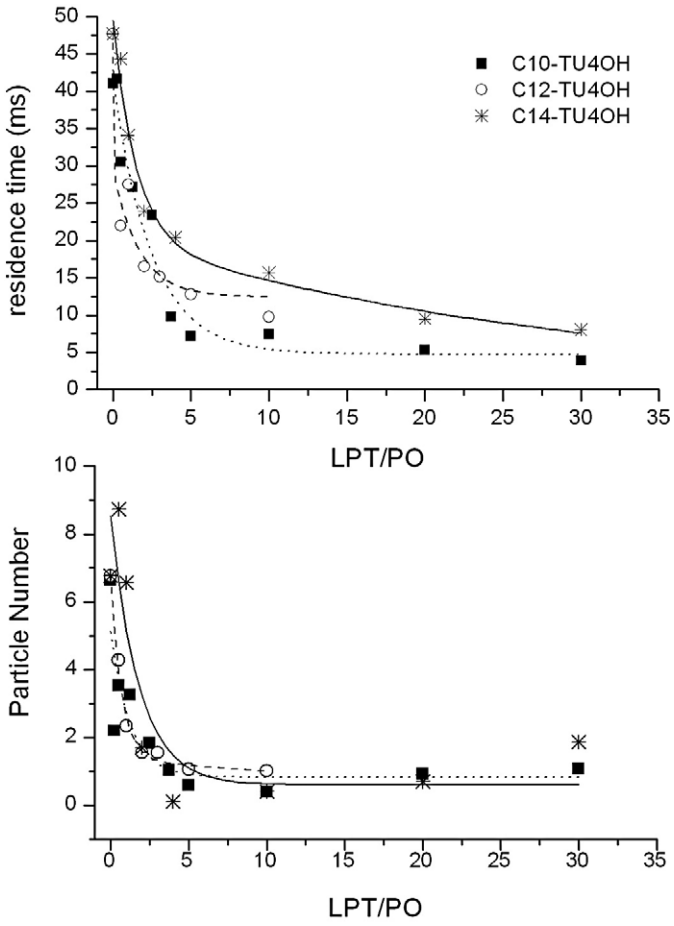

Fig. 3. Residence time and the particle number (PN) as a function of the LPT/PO ratio derived from FCS measurements during plasmid DNA condensation process with LPT serinol derivatives: C10-TU4OH (■),C12-TU4OH (○) and C14-TU4OH (*). PicoGreen was used at 0.02 dye/bp.

elimination of the bloodstream due to their cationic nature. The thiourea function is known to form strong hydrogen bonds with anions. Among them thiourea/phosphate complexes are the most stable [27]. Considering that previously described DNA carriers were mostly cationic, we assumed that the replacement of ionic interactions between the vector and DNA by hydrogen bond interactions would lead to less stable systems and would enhance DNA release. This hypothesis is consistent with the high lipid to DNA ratio needed to obtain a full DNA complexation on an agarose gel (10 to 20 LPT/PO according to the lipid studied). However, when evaluating the stability of these compounds in the presence of serum, we showed a high DNA stabilization indicating that the lipopolythiourea/DNA interaction was not that weak [18,19]. To provide more insight on the lipopolythiourea/DNA interaction, we performed NMR, gel electrophoresis and FCS experiments and evaluated the interaction of several lipopolythioureas with DNA.

First, we showed by NMR that, even part of supramolecular assemblies, the thiourea moiety conserved its ability to interact with phosphates. Thiourea functions were previously shown to interact with carboxylates and phosphates with increased affinity when part of a structural assembly such as a cage [28]. Here, we showed that thiourea functions interact with phosphates when covalently linked to a lipid entity, which obviously highly modifies their solubility and could have impaired the interaction of thiourea with hydrophilic molecules. The structure of the head moiety had a high importance though as the tetraol complexes did interact more efficiently with DNA as shown by FCS experiments. It cannot be excluded that a cage

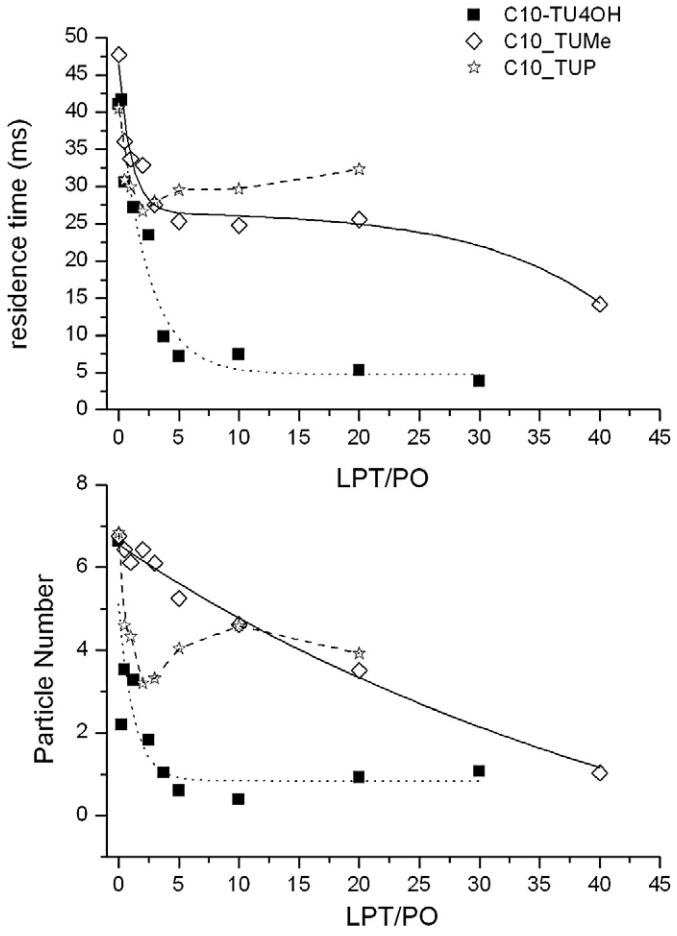

Fig. 4. Residence time and the particle number (PN) as a function of the LPT/PO ratio derived from FCS measurements during the plasmid DNA condensation process with C10-TU4OH, C10-TUMe and C10-TUP derivatives. PicoGreen was used at 0.02 dye/bp.

formed between the thiourea in the hydrophilic part of this particular lipid. Moreover, we had seen before that two thiourea functions were required for DNA interaction, as one thiourea function per lipid did not lead to efficient DNA condensation [29].

Second, we investigated the effect of the lipid length on DNA condensation. We and others had previously studied the effect of the lipid length on DNA transfection [30]. Here, we found that decreasing the length of the hydrophobic anchor led to a tightest supramolecular construct. As we previously reported that the transfecting properties of this lipopolythiourea family increased when the lipid chain length decreased [18], these data tend to indicate that the more tightest supramolecular constructs are the more efficient to transfect cells. These data appear consistent with another recent study on cationic lipids decrease which led to similar conclusions [31].

From a technical point of view, gel electrophoresis is the standard procedure to evaluate DNA condensation. We could by this method evidence an interaction that would delay DNA electrophoretic mobility, however a high amount of thiourea lipid appeared to be needed. This amount revealed to be far lower when using a more sensitive method, such as following the mobility of one single molecule of plasmid DNA. Complexation between DNA and LPT was shown by FCS to be an efficient process. Interaction could be detected as low as 1 LPT/PO reaching a plateau at 4 LPT/PO and led to a DNA condensed state. The fact that the TU4OH family was more efficient to interact with DNA than the other lipids has also been investigated by ^{13}C NMR, and tends to indicate that the thiourea moieties in these lipids are in a tautomeric thiourea/iminothiol form which might explain the strong difference of interaction obtained with these lipids [32]. The mixture of both ionic and hydrogen bonds would be a key to improve gene transfection as also indicated in a recent work where the authors added thiourea functions to their positively charged cyclodextrine based vectors [33,34].

Finally, as expected from our starting hypothesis FCS indicated that a LPT tetraol interacts strongly with phosphates. However, a larger amount of lipopolythiourea seems necessary to stabilize the complexes as no transfection levels are detectable below LPT/PO<10 [18]. As referred to DNA condensation via carbohydrate based hydrogen bonds [35], these data could support a multi-layer model in which a first LPT layer would interact rapidly with DNA through thiourea/phosphate interaction, then this primary construct would be stabilized by the addition of more LPT to enhance the hydrophobic forces probably via hydrogen bonds. This hypothesis is consistent with the disassembly of supramolecular constructs by the addition of salt or trifluoroethanol able to displace hydrogen bonds [32].

To conclude, first we have observed that increasing the LPT/PO ratio decreased the residence time together with decreased values of the particle number (PN), which is consistent with a condensation of DNA into thiourea nanoparticles. Next, the FCS experiments reported herein gave quantitative information on the packing density of DNA–LPT supramolecules and more detailed information on the LPT–DNA interactions as compared to the frequently used fluorescence intensity assays. Finally, we found that the more efficient lipid for intracellular gene transfer was also the tightest thiourea based supramolecular assembly.

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References

[1] T. Niidome, L. Huang, Gene therapy progress and prospects: nonviral vectors, Gene Ther. 9 (2002) 1647–1652.
[2] K. Kostarelos, A.D. Miller, What role can chemistry play in cationic liposome-based gene therapy research today? Adv. Genet. 53 (2005) 69–118.
[3] D. Simberg, S. Weisman, Y. Talmon, Y. Barenholz, DOTAP (and other cationic lipids): chemistry, biophysics, and transfection, Crit. Rev. Ther. Drug Carrier Syst. 21 (2004) 257–317.
[4] K. Ewert, N.L. Slack, A. Ahmad, H.M. Evans, A.J. Lin, C.E. Samuel, C.R. Safinya, Cationic lipid-DNA complexes for gene therapy: understanding the relationship between complex structure and gene delivery pathways at the molecular level, Curr. Med. Chem. 11 (2004) 133–149.
[5] M.L. Edelstein, M.R. Abedi, J. Wixon, Gene therapy clinical trials worldwide to 2007 — an update, J. Gene Med. 9 (2007) 833–842.
[6] P.L. Felgner, T.R. Gadek, M. Holm, R. Roman, H.W. Chan, M. Wenz, J.P. Northrop, G. M. Ringold, M. Danielsen, Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 7413–7417.
[7] J. Sen, A. Chaudhuri, Gene transfer efficacies of novel cationic amphiphiles with alanine, beta-alanine, and serine headgroups: a structure-activity investigation, Bioconjug. Chem. 16 (2005) 903–912.
[8] J. Sen, A. Chaudhuri, Design, syntheses, and transfection biology of novel non-cholesterol-based guanidinylated cationic lipids, J. Med. Chem. 48 (2005) 812–820.
[9] I. van der Woude, A. Wagenaar, A.A. Meekel, M.B. ter Beest, M.H. Ruiters, J.B. Engberts, D. Hoekstra, Novel pyridinium surfactants for efficient, nontoxic in vitro gene delivery, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 1160–1165.
[10] B. Schwartz, C. Benoist, B. Abdallah, D. Scherman, J.P. Behr, B.A. Demeneix, Lipospermine-based gene transfer into the newborn mouse brain is optimized by a low lipospermine/DNA charge ratio, Hum. Gene Ther. 6 (1995) 1515–1524.
[11] B.M. Tandia, M. Vandenbranden, R. Wattiez, Z. Lakhdar, J.M. Ruysschaert, A. Elouahabi, Identification of human plasma proteins that bind to cationic lipid/DNA complex and analysis of their effects on transfection efficiency: implications for intravenous gene transfer, Mol. Ther. 8 (2003) 264–273.
[12] H. Eliyahu, N. Servel, A.J. Domb, Y. Barenholz, Lipoplex-induced hemagglutination: potential involvement in intravenous gene delivery, Gene Ther. 9 (2002) 850–858.
[13] Y. Aoyama, T. Kanamori, T. Nakai, T. Sasaki, S. Horiuchi, S. Sando, T. Niidome, Artificial viruses and their application to gene delivery. Size-controlled gene coating with glycocluster nanoparticles, J. Am. Chem. Soc. 125 (2003) 3455–3457.

[14] N. Mignet, A. Brun, C. Degert, B. Delord, D. Roux, C. Helene, R. Laversanne, J. Francois, The spherulites(TM): a promising carrier for oligonucleotide delivery, Nucleic Acids Res. 28 (2000) 3134–3142.
[15] J. Arigon, C.A. Prata, M.W. Grinstaff, P. Barthelemy, Nucleic acid complexing glycosyl nucleoside-based amphiphile, Bioconjug. Chem. 16 (2005) 864–872.
[16] I. Tranchant, N. Mignet, E. Crozat, J. Leblond, C. Girard, D. Scherman, J. Herscovici, DNA complexing polythiourea, Bioconjug. Chem. 15 (2004) 1342–1348.
[17] J. Leblond, N. Mignet, L. Leseurre, C. Largeau, M. Bessodes, D. Scherman, J. Herscovici, Design, synthesis and evaluation of enhanced DNA binding lipopolythiourea, Bioconjug. Chem. 17 (2006) 1200–1208.
[18] J. Leblond, N. Mignet, C. Largeau, M.V. Spanedda, J. Seguin, D. Scherman, J. Herscovici, Lipopolythiourea a new non cationic system for gene transfer, Bioconjug. Chem. 18 (2007) 484–493.
[19] J. Leblond, N. Mignet, C. Largeau, J. Seguin, D. Scherman, J. Herscovici, Lipopolythiourea transfecting agents: lysine thiourea derivatives, Bioconjug. Chem. 19 (2008) 306–314.
[20] Y. Takemoto, Recognition and activation by ureas and thioureas: stereoselective reactions using ureas and thioureas as hydrogen-bonding donors, Org. Biomol. Chem. 3 (2005) 299–4306.
[21] A. Benda, M. Hof, M. Wahl, M. Patting, R. Erdmann, P. Kapusta, TCSPC upgrade of a confocal FCS microscope, Rev. Sci. Instrum. 76 (2005) 76.
[22] J. Enderlein, I. Gregor, D. Patra, J. Fitter, Art and artefacts of fluorescence correlation spectroscopy, Curr. Pharm. Biotechnol. 5 (2004) 155–161.
[23] J. Humpolickova, A. Benda, J. Sykora, R. Machan, T. Kral, B. Gasinska, J. Enderlein, M. Hof, Equilibrium dynamics of spermine-induced plasmid DNA condensation revealed by fluorescence lifetime correlation spectroscopy, Biophys. J. 94 (2008) L17–L19.
[24] R.G. Winkler, S. Keller, J.O. Radler, Intramolecular dynamics of linear macromolecules by fluorescence correlation spectroscopy, Phys. Rev. E Stat. Nonlin. Soft Matter Phys. 73 (2006) 041919.
[25] T. Kral, K. Widerak, M. Langner, M. Hof, Propidium iodide and PicoGreen as dyes for the DNA fluorescence correlation spectroscopy measurements, J. Fluoresc. 15 (2005) 179–183.
[26] N. Adjimatera, T. Kral, M. Hof, I.S. Blagbrough, Lipopolyamine-mediated single nanoparticle formation of calf thymus DNA analyzed by fluorescence correlation spectroscopy, Pharm. Res. 23 (2006) 1564–1573.
[27] P. Buhlmann, M. Hayakawa, T. Ohshiro, S. Amemiya, Y. Umezawa, Influence of natural, electrically neutral lipids on the potentiometric responses of cation-selective polymeric membrane electrodes, Anal. Chem. 73 (2001) 3199–3205.
[28] M.P. Muley, J.J. Balsara, J.H. Jadhav, A.G. Chandorkar, Involvement of histaminergic mechanisms in the cataleptogenic effect of morphine in mice, J. Pharm. Pharmacol. 34 (1982) 34–37.
[29] E. Kabha, C. Jacquement, G. Pembouong, N. Mignet, D. Scherman, J. Herscovici, A single thiourea group is not enough to get stable thiourea lipoplexes, Bioorg. Med. Chem. 16 (2008) 4003–4008.
[30] G. Byk, C. Dubertret, V. Escriou, M. Frederic, G. Jaslin, R. Rangara, B. Pitard, J. Crouzet, P. Wils, B. Schwartz, D. Scherman, Synthesis, activity, and structure-activity relationship studies of novel cationic lipids for DNA transfer, J. Med. Chem. 41 (1998) 224–235.
[31] R. Koynova, B. Tenchov, L. Wang, R.C. Macdonald, Hydrophobic moiety of cationic lipids strongly modulates their transfection activity, Mol. Pharm. 6 (2009) 951–958.
[32] M. Breton, M. Bessodes, S. Bouaziz, J. Herscovici, D. Scherman, N. Mignet, Iminothiol/thiourea tautomeric equilibrium in thiourea lipids impacts DNA compaction by inducing a cationic nucleation for complex assembly, Biophys. Chem. 145 (2009) 7–16.
[33] A. Diaz-Moscoso, P. Balbuena, M. Gomez-Garcia, C.O. Mellet, J.M. Benito, L. Le Gourrierec, C. Di Giorgio, P. Vierling, A. Mazzaglia, N. Micali, J. Defaye, J.M.G. Fernandez, Rational design of cationic cyclooligosaccharides as efficient gene delivery systems, Chem. Commun. (2008) 2001–2003.
[34] A. Diaz-Moscoso, L. Le Gourrierec, M. Gomez-Garcia, J.M. Benito, P. Balbuena, F. Ortega-Caballero, N. Guilloteau, C. Di Giorgio, P. Vierling, J. Defaye, C.O. Mellet, J.M. G. Fernandez, Polycationic amphiphilic cyclodextrins for gene delivery: synthesis and effect of structural modifications on plasmid DNA complex stability, cytotoxicity, and gene expression, Chem. Eur. J. 15 (2009) 12871–12888.
[35] J.L. Blanco, P. Bootello, J.M. Benito, O. Mellet, G. Fernandez, Urea-, thiourea-, and guanidine-phosphate binders in water, J. Org. Chem. 71 (2006) 5136–5143.