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Non-Electrostatic Complexes with DNA: Towards Novel Synthetic Gene Delivery Systems

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Abstract—We have developed new DNA complexing amphiphile based on Hoechst 33258 interaction with DNA grooves. The synthesis and physicochemical characterisation of lipid/DNA complexes, as compared to that of classical lipopolyamine for gene delivery, are described and discussed.

Cationic lipid vectors for gene delivery are based on electrostatic interaction between DNA (polyanion) and a polyammonium (cation) linked to a lipid moiety for self assembly formation. We and others[1] have described a variety of synthetic lipidic or polymeric gene delivery systems which, upon contact with DNA, form multilamellar bilayers[2] (lipopolyamines, lipopolyaminoguanidines), or toroids (polylysine,[3] polyethyleneimine[4]) suitable for in vitro and in vivo gene delivery.

Among the main issues are the fact that cationic DNA/vector complexes might be inactivated by interaction with anionic blood and extra-cellular fluid constituents. Moreover, cationic lipids and polymers have been described as displaying pro-inflammatory properties.[5]

The necessity of more efficient synthetic gene delivery methods prompted us to search for novel, non or less cationic, gene delivery systems. Such systems would thus not be based on electrostatic interactions with DNA. These non electrostatic complexes can be advantageous for in vitro and in vivo applications, since unlike cationic lipid/DNA complexes, the novel molecules could not lead to a compacted state of DNA, thus potentially leading to different kinetics of DNA release from complexes. Moreover, an advantage can be expected from the use of less cationic particles, as stated above.

Several compounds are able to bind to double stranded DNA along the grooves by formation of hydrogen bonds.[6] Groove binding typically exerts only subtle changes in DNA conformation, and DNA remains essentially in the native form.[7] Therefore, groove binding complexes will be essentially different from cationic lipid/DNA complexes, in which DNA is compacted within a multilamellar bilayer.

In order to set the prototype of an original DNA vector consisting of an amphiphile able to bind to DNA through hydrogen bond interactions, we have selected Hoechst 33258 (**1**), a well-known minor groove DNA binding agent.[8] This product is interesting for various reasons: (a) product (**1**) is known to give hydrogen bond interactions[9] along the minor groove of DNA through the benzimidazole moiety, both by hydrogen acceptor and donor; (b) its natural fluorescence (excitation: 350 nm; emission 450 nm) is increased when the compound interacts with DNA double strands, which allows a direct detection and easy characterisation of the complexes formed with DNA.[10]

However, the presence of one charge coming from the piperazine moiety in Hoechst 33258, necessitated a preliminary study to characterise the nature of the complexes. We have found that complexes formed between double stranded DNA and commercial Hoechst 33258, migrate normally in an electrophoretic field as compared to naked DNA, thus suggesting that the nature of the complex is hydrogen bonding rather than electrostatic, for which electrophoresis gel retardation of compacted DNA is commonly observed.[2,17] In the present

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work, we have synthesised alkyl derivatives of Hoechst 33258 (**1**). The complexes formed with DNA were characterised using physico-chemical methods, and included comparative studies using known cationic lipid/DNA complexes for gene delivery.

Results and Discussion

Chemistry

Several methods for direct derivatization of Hoechst 33258 were previously described, one of them[11] used high temperatures which induced benzimidazole oxidation and resulted in mixtures difficult to separate. We tried alkylation of the phenol under Williamson conditions,[12] but we obtained mixtures of polyalkylated bis-benzimidazole. Therefore, we chose to use alkylisocyanates to obtain the corresponding carbamate derivatives,[13] the phenolate group attacked the alkyl isocyanates under basic conditions, leaving intact the benzimidazole rings even under excess of isocyanate reagent. The synthesis of dodecyl (**2**) and octadecyl (**3**) carbamate derivatives of **1** are shown in Scheme 1.[14]

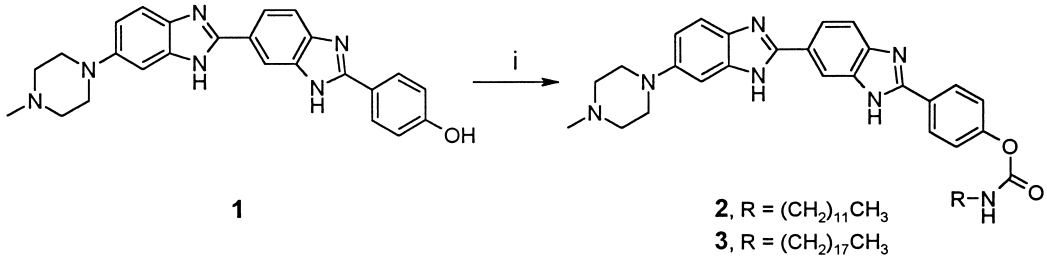
Physico chemical characterisation of complexes with DNA

The fluorescence properties of mixtures of DNA with increasing amounts of compound **2** or **3** were measured by excitation at 350 nm and detection at 450 nm. Results demonstrate the effective binding of the two compounds to DNA, with a DNA grooves saturation at 1 molecule of compound per bp DNA ratio (see Fig. 1(a) and (b)). Complexes formed with DNA by both compounds showed similar emission and excitation fluorescence spectra as compared to native Hoechst 33258 (data not shown).[6]

The complexes were also studied by gel electrophoresis[16] (Fig. 2(a)) and were compared to previously described cationic lipid complexes.[17] Direct observation of the gels could be done by taking advantage of the spectral absorption properties of compounds **2** and **3** when complexed (blue) or uncomplexed (yellow) with DNA. We have observed that DNA complexes of products **2** and **3** migrate on gel giving the typical blue absorption observed for native Hoechst/DNA complex (see Fig. 2(a); colours are not shown for technical reasons).

Increasing doses of **2** or **3** resulted in coloration increment of the DNA/compound complex band. At exceeding compound concentration, a yellow band characteristic of the aggregates formed by the excess of uncomplexed product **2** or **3**, was observed on the wells of the lanes.

After revealing the same gel with ethidium bromide (see Fig. 2(b))., the same amount of DNA was observed migrating on all lanes, and the electrophoretical mobility



Scheme 1. (i) Dodecyl or octadecyl isocyanate (1.5 eq), DIEA (excess), DMF, 50 °C, 18h, 41% (after purification by HPLC).

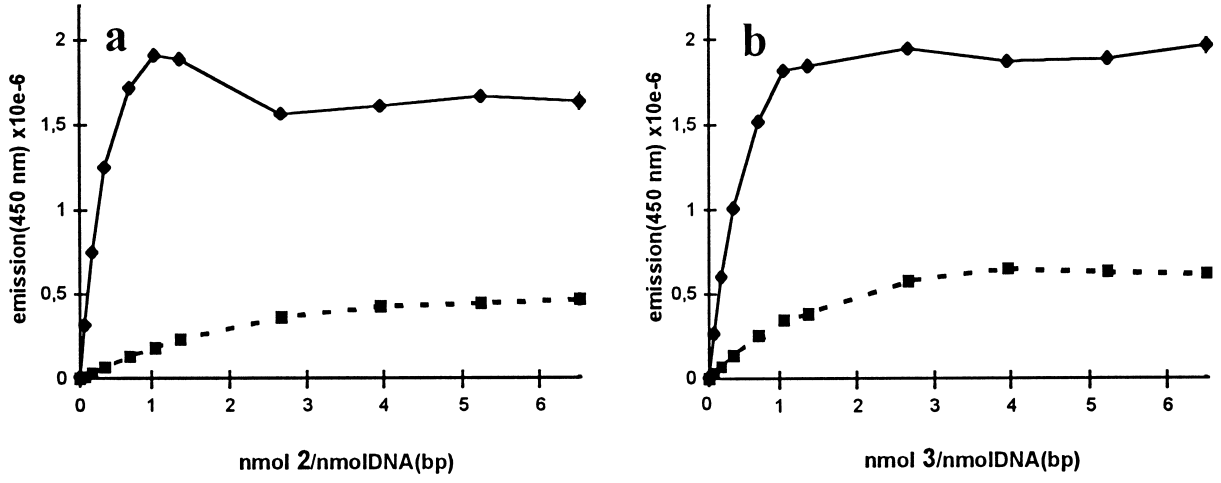


Figure 1. Fluorescence emission at 450 nm of complexes of DNA with compound **2** (panel a) or compound **3** (panel b) (—), and of compound alone (- - - - -). Ratios are indicated in nmol compound/nmol DNA(bp). Plasmid DNA contained 3671 base pairs, concentration was 50 μg/mL.[15]

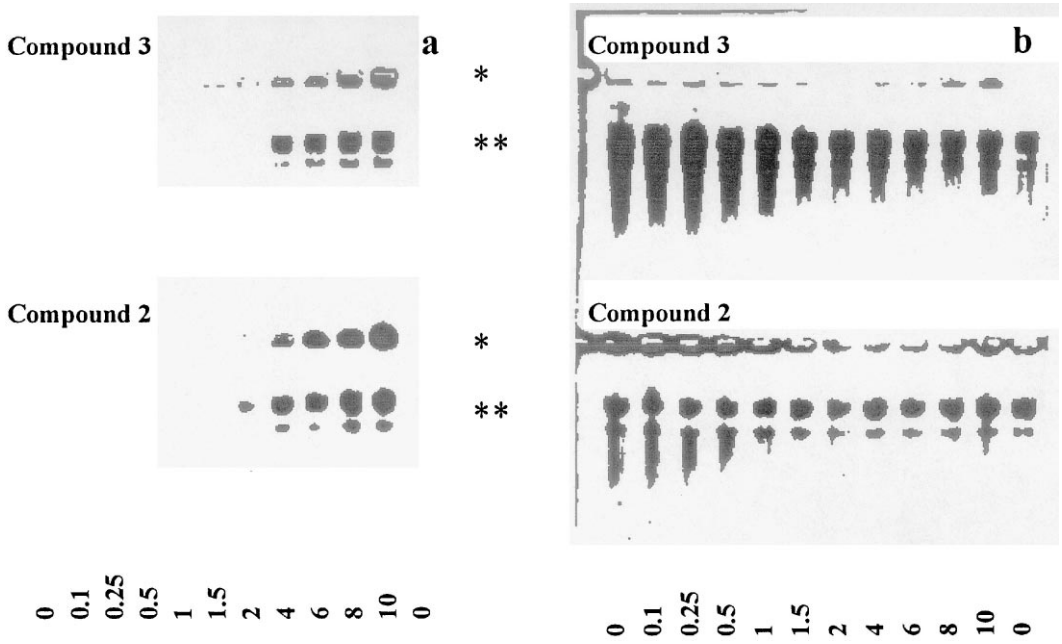


Figure 2. (a) 0.8% agarose gel of 1 μg plasmid DNA associated to an increasing concentration of product **2** (bottom) or **3** (top), indicated in nmol/μg DNA. Bands were revealed under UV light. *Yellow band: uncomplexed product on the lane wells. **Blue band: product complexed to DNA; (b) same as (a) with ethidium bromide staining.

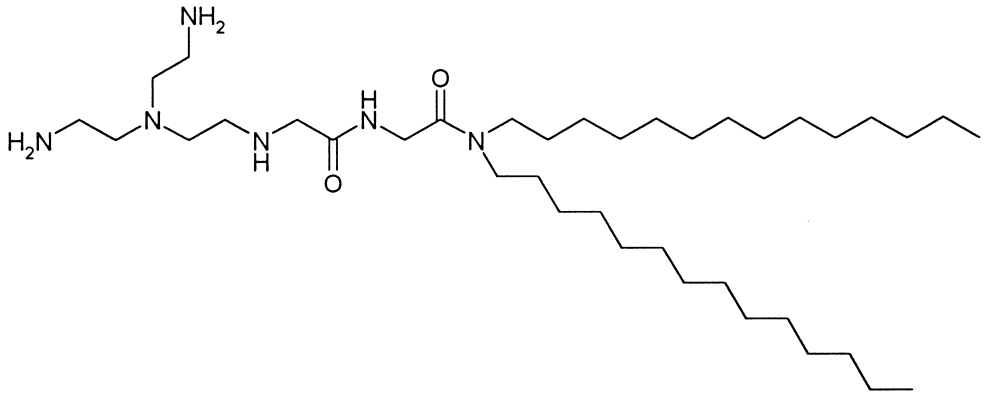


Figure 3. RPR203360, a typical cationic lipid with a polycation head and a double C-14 lipid chain.

of DNA complexes remained unperturbed as compared to naked DNA, suggesting that DNA was not in a compacted state and that complexes are mainly formed through hydrogen bonding rather than electrostatic forces.

The overall results show a different behaviour of the new lipid/DNA complexes, as compared to previously described cationic lipids, which classically compact DNA and retard DNA in gel electrophoresis. For example, the compound RPR 203360 (Fig. 3), a cationic lipid synthesised in our laboratory,[18] shows this typical effect of plasmid/DNA retardation in agarose gel electrophoresis, when used at increasing concentrations (Fig. 4).

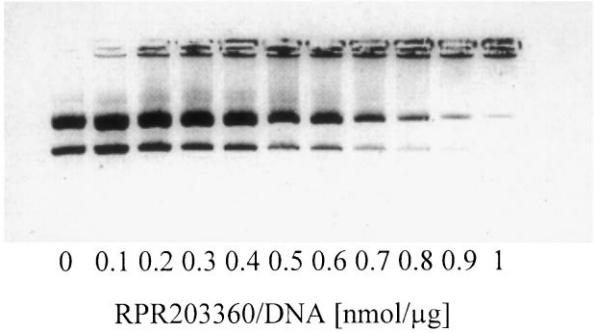


Figure 4. 0.8% agarose gel of 1 μg plasmid DNA associated to an increasing concentration of RPR 203660. DNA was revealed with ethidium bromide staining.

Conclusions

The present results demonstrate the effective binding of compounds **2** and **3** with DNA. The complexes have been characterised using fluorescence methods and gel electrophoresis. The data suggest that, unlike complexes with cationic lipids, DNA complexes with compounds **2** or **3** are not found in a compacted state and migrate normally on gel electrophoresis, as compared to naked DNA.

The different nature of the presented complexes, as compared to cationic lipid/DNA complexes, and specially the unperturbed electrophoretical mobility of the complexes, place the new complexes as potential self assembling systems for gene delivery. Evaluation of these compounds for their ability to increase gene expression is in progress, and will be reported separately.

Acknowledgements

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14. Two new compounds were characterised by NMR and MS. Analytical data for **3**: ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 0.86 (t, $J=7$ Hz, 3H); 1.15–1.40 and 1.51 (2 mt, 32H); 2.92 (s, 3H); 3.00–3.15 (mt, 2H); 3.10 (mt, 2H); 3.26 (mt, 2H); 3.61 (d, $J=10$ Hz, 2H); 3.91 (d, $J=10$ Hz, 2H); 7.20 (bs, 1H); 7.25 (d, $J=9$ Hz, 1H); 7.36 (d, $J=9$ Hz, 2H); 7.68 (d, $J=9$ Hz, 1H); 7.80–7.90 (mt, 2H); 8.06 (dd, $J=9$ and 1.5 Hz, 1H); 8.25 (d, $J=9$ Hz, 2H); 8.45 (bs, 1H); 9.70–9.90 (bb, 1H). LSIMS *m/z* (MBA): 720 [M + H]. Analytical data for **2**: ^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ 0.89 (t, $J=7$ Hz, 3H); 1.25–1.45 and 1.56 (2 mt, 20H); 2.93 (s, 3H); 3.15 (mt, 2H); 3.30–4.20 (mt, 8H:2H); 7.17 (dd, $J=9$ and 2 Hz, 1H); 7.22 (d, $J=2$ Hz, 1H); 7.34 (d, $J=8.5$ Hz, 2H); 7.45–7.60 (m, 1H); 7.63 (d, $J=9$ Hz, 1H); 7.81 (d, $J=8$ Hz, 1H); 8.06 (bd, $J=9$ Hz, 1H); 8.24 (d, $J=8.5$ Hz, 2H); 8.43 (bs, 1H). LSIMS *m/z* (MBA): 636 [M + H].
15. Fluorescence measurements were carried out on a Jobin-Yvon Spex fluoromax-2 spectrofluorometer (Longjumeau, France). Sample readings were conducted at an excitation wavelength of 350 nm and an emission wavelength of 450 nm. Complexes were prepared by mixing equal volumes of the product (**2** or **3**) solutions at different concentrations, strongly sonicated, and the plasmid DNA solution at the desired concentration. All solutions were in 1 mM Tris, pH 7.4, and 45 mM NaCl. Plasmid DNA used was pXL 3031; 1 μg of DNA corresponds to 0.4 pmol.
16. The vector (**2** or **3**)/DNA samples containing 1 μg of DNA were loaded on an agarose gel, and were run over 115 v during 40 min and direct detection of the complex grade under UV.
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