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Note

Dendriplexes and their characterisation

Chandrasekaran Ramaswamy [a,b], Thiagarajan Sakthivel [a], Andy F. Wilderspin [b], Alexander T. Florence [a,*]

[a] *Centre for Drug Delivery Research, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK*
[b] *Department of Pharmaceutical and Biological Chemistry, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK*

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Abstract

The interaction of DNA with partial dendrimers (dendritic polylysine containing seven lysines and eight terminal amino groups with or without a lipidic core) was studied. Compact complexes were formed which we term "dendriplexes". Agarose gel electrophoresis and exclusion of ethidium bromide confirmed the interaction. All the dendrons formed compact complexes above a 2:1 (+/−) charge ratio in water and HBSS. Photon correlation spectroscopy, electron microscopy and zeta potential measurements were used to determine, respectively, the particle size, shape and surface charge of the dendriplexes. The *z*-average diameter of the dendriplexes were found to be 60–70 nm irrespective of the dendron used and the zeta potential varied from 10 to 35 mV at a 3:1 (+/−) charge ratio depending on the dendron. The protection of the DNA component of these dendriplexes from nuclease degradation was confirmed by DNase protection assays.

Keywords: Dendrons; Partial dendrimers; Dendriplexes; Non-viral vectors

Gene transfer techniques are based on viral and non-viral vectors. Viral systems can be highly efficient but large-scale production, immunogenicity, regulatory constraints and recombination with wild type viruses restrict their usage and application. Plasmid DNA, in its naked form, is susceptible to degradation by plasma and serum proteins (Adami et al., 1998). Condensation of plasmid DNA provides protection against both chemical degradation and mechanical damage (Tsai et al., 1999) and forms the basis for targeting and improving its cellular uptake (Luo and Saltzman, 2000). The delivery performance of the vectors differs greatly depending on the physicochemical and colloidal properties of the complexes they form with DNA. Non-viral vectors include peptides, dendrimers, polymers, cationic lipids, and liposomes.

We synthesised cationic dendrons with which to condense DNA. Initial reports of transfection studies of some related dendrons produced in our laboratory have been published (Toth et al., 1999; Shah et al., 2000). In this study, we modified the lipid chains and systematically characterised the formation of complexes occurring spontaneously between dendrons and DNA. We refer to these complexes as "dendriplexes". The dendrons were synthesised by the divergent method adopting solid phase peptide synthesis following the procedures described earlier (Sakthivel et al., 1998). Dendriplexes of different charge ratios were prepared by fast addition of the dendron solution to DNA solution and equilibration for 30 min.

* Corresponding author. Tel.: +44-20-7753-5819; fax: +44-20-7837-5092.
E-mail address: a.t.florence@ulsop.ac.uk (A.T. Florence).

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Electrophoretic retardation of DNA and degradation of DNA by DNase in free form and in dendriplexes was observed by agarose gel electrophoresis.

The ability of dendrons to form tight complexes with DNA was determined by measuring changes in the ethidium bromide (EB)–DNA fluorescence (Perkins-Elmer spectrofluorometer) at excitation and emission wavelengths of 260 and 595 nm. The *z*-average particle size of the complexes was measured by dynamic light scattering (PCS4700 system, Malvern Instruments, Malvern, UK) at 25 °C, with a fixed angle of 90° and their zeta potentials by a Malvern Zetasizer. Transmission electron microscopy (TEM) was used to determine the shape of the dendriplexes after staining with 1% uranyl acetate (Philips CM 120 Bio Twin, Einhoven, The Netherlands; operating at a voltage of 120 kV).

In order to improve the transfection efficiency and understand how the chemical structure, and in particular, the lipid chains affect the properties of the dendriplexes, we systematically modified the lipid chain lengths, the number of lipid chains and terminal amino groups up to 32. We follow a simple nomenclature to differentiate these new compounds, e.g. $(C10)_3(L)_7(NH_2)_8$, where (C10) indicates the carbon chain length of the lipid chain, the suffix the number of chains; (L) indicates lysine, the suffix the number of branching units; and (NH_2) indicates the terminal amino groups with again the suffix denoting the number of terminal groups. The four dendrons used in this study have eight terminal amino groups, but differ in their core groups (Fig. 1).

The electrophoretic mobility of DNA was affected by all the dendrons used in this study. The mobility

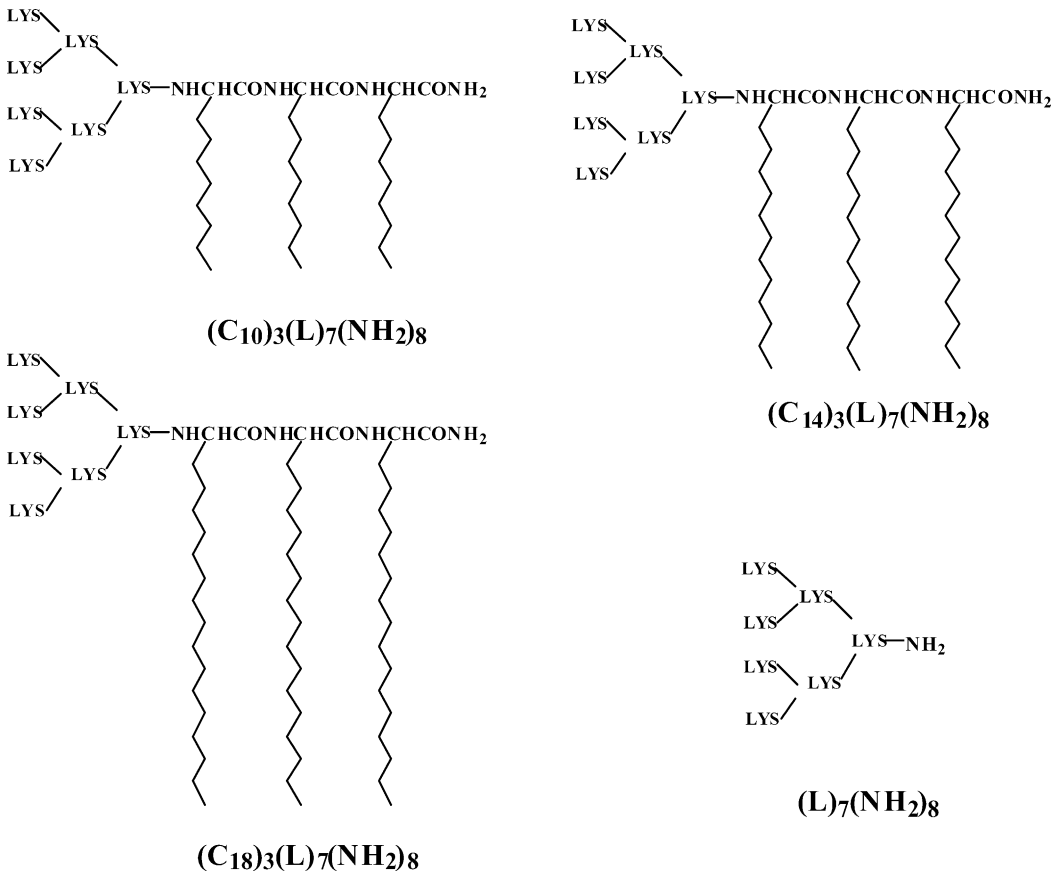

Fig. 1. Schematic structure of dendrons used in this study.

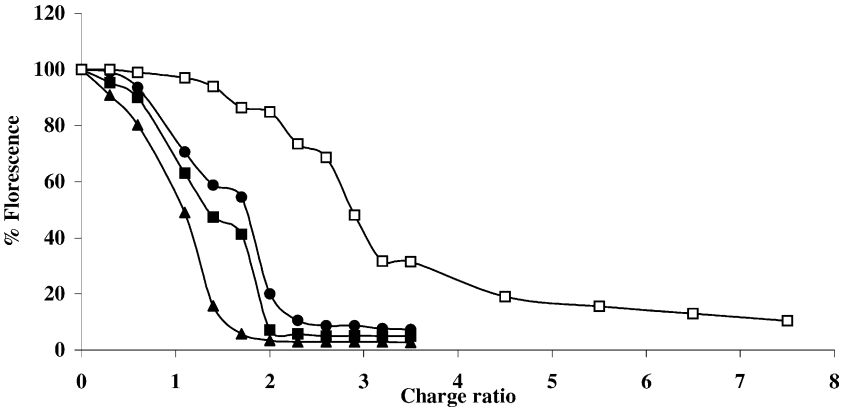

Fig. 2. Effect of dendron/polylysine concentration on fluorescence of EB with DNA. The fluorescence of DNA solutions (10 μg/ml) in water containing EB (500 ng/ml) was measured and set to 100%. Following stepwise addition of dendron/polylysine solutions in water, resulting fluorescence was measured after 2 min (●: $(L)_7(NH_2)_8$; ■: $(C10)_3(L)_7(NH_2)_8$; ▲: $(C18)_3(L)_7(NH_2)_8$; □: poly lysine).

of free DNA decreased as the charge ratio (+/−) increased and was completely retarded above a charge ratio of 2:1 (+/−). The displacement of EB from DNA by cationic polymers has been reported as an indicator of the complex forming potential in DNA delivery systems (Tang and Szoka, 1997). We studied the EB exclusion from DNA–EB complex by dendrons to find the exact molar charge ratio at which they form a compact complex and compared to that of low molecular weight polylysine. As dendron solution was introduced to DNA the EB fluorescence was reduced and reached a minimum at 1.5:1 to 2:1 charge ratios for the lipidic dendrons and above a 2:1 for "non-lipidic" dendron. Further, linear polylysine (pLL; MW = 1000) was unable to form compact complexes even at a 5:1 charge ratio (Fig. 2). These results are in good agreement with the results obtained by Lucas et al. (1999), who reported that lower molecular weight pLL is required in higher molar quantities than higher molecular weight fractions.

The size of the vector and complex are important factors in transfection efficiency. The *z*-average

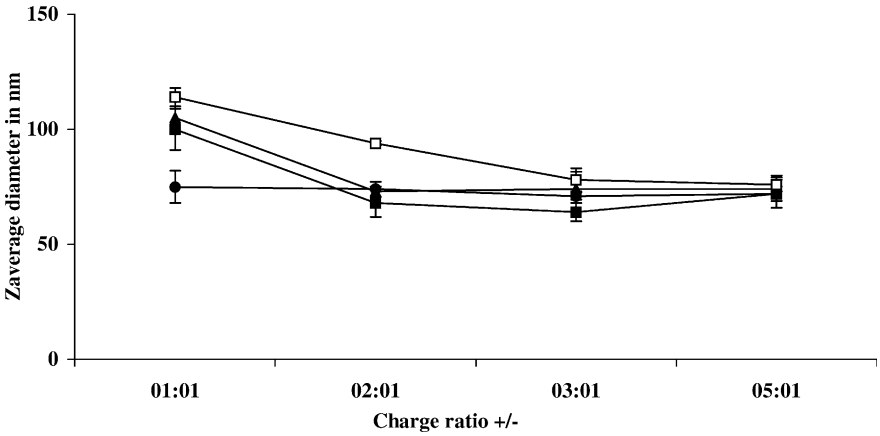

Fig. 3. The *z*-average diameter of the dendriplexes as determined by PCS at 25 °C: dendriplexes were prepared in water using 10 μg/ml of DNA and dendrons (●: $(L)_7(NH_2)_8$; ■: $(C10)_3(L)_7(NH_2)_8$; ▲: $(C14)_3(L)_7(NH_2)_8$; □: $(C18)_3(L)_7(NH_2)_8$) at different charge ratios after 30 min of equilibration.

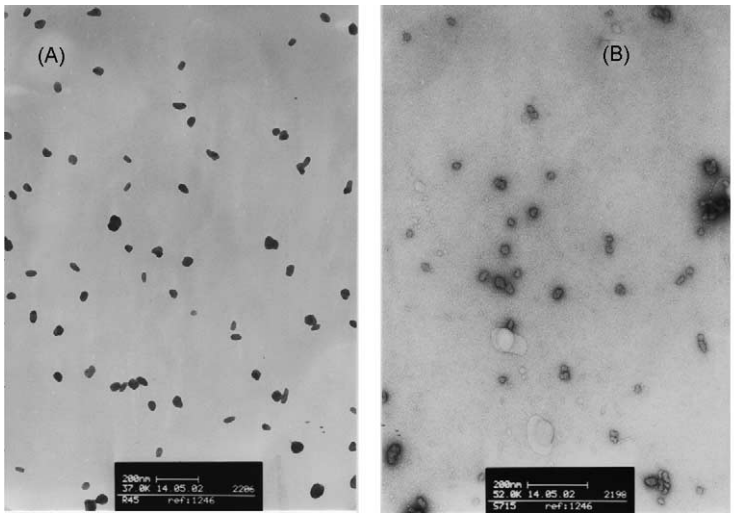

Fig. 4. TEMs of dendriplexes formed by utilising a dendron without lipid chains $(L)_7(NH_2)_8$ (A) and with lipid chains $(C14)_3(L)_7(NH_2)_8$ (B) in water.

diameter of the particles formed by spontaneous interaction between DNA and dendrons was found to be 60–70 nm at a charge ratio of 3:1 irrespective of the dendron utilised. Fig. 3 shows the effect of charge ratio and lipid chain on the size of the dendriplexes. Although the *z*-average diameter of the complex determined by PCS was 60–70 nm, the TEM shows somewhat smaller sizes (Fig. 4). The majority of the population was less than 50 nm. Dendriplexes formed with dendrons $(C18)_3(L)_7(NH_2)_8$ and $(C14)_3(L)_7(NH_2)_8$ in HBSS medium do not aggregate, whereas $(C10)_3(L)_7(NH_2)_8$ and $(L)_7(NH_2)_8$ dendrons do. The zeta potential of the dendriplexes formed in water was further evidence that all the dendrons were incorporated into particles. The surface charge was positive above a 1:1 charge ratio, but the zeta potential of the dendriplexes does depend on the dendron component (Fig. 5). Dendriplexes formed

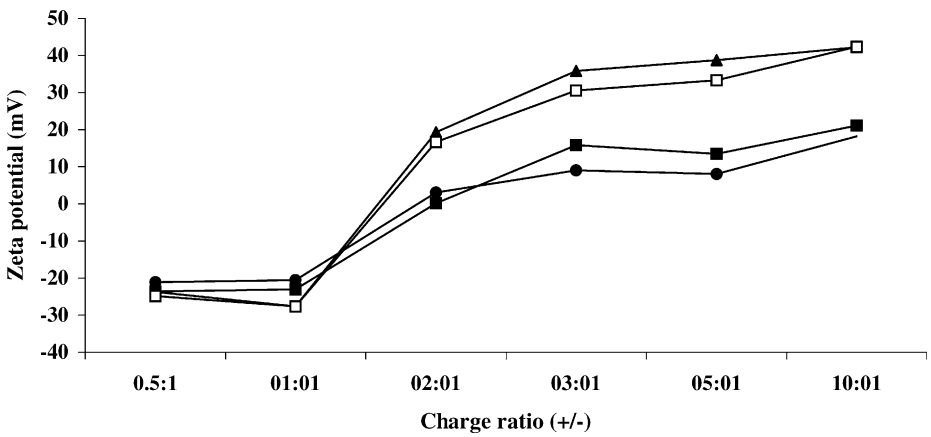

Fig. 5. Zeta potential of dendriplexes in water as a function of charge ratio using 10 μg/ml of DNA (●: $(L)_7(NH_2)_8$; ■: $(C10)_3(L)_7(NH_2)_8$; ▲: $(C14)_3(L)_7(NH_2)_8$; □: $(C18)_3(L)_7(NH_2)_8$).

with the non-lipidic dendron $(L)_7(NH_2)_8$ and with the $(C10)_3(L)_7(NH_2)_8$ system had zeta potentials up to 15 mV (at a 5:1 charge ratio) whereas the zeta potential of dendriplexes with $(C14)_3(L)_7(NH_2)_8$ and $(C18)_3(L)_7(NH_2)_8$ reached 40 mV. This may be due to shell formation of lipid chains around dendriplexes because of hydrophobic interactions of excess dendron leaving the terminal amino groups outside, but this requires further study.

Dendriplexes formed with low molecular weight dendrons seemed to protect the DNA from DNase I (10 times excess) even after 15 min of incubation at 37 °C as revealed by agarose gel electrophoresis (data not shown), while naked DNA was completely digested in 2 min. Dendriplexes formed with $(C10)_3(L)_7(NH_2)_8$ provided less protection when compared with dendriplexes formed with the other lipidic dendrons. This finding also requires further study.

Cationic dendrons based on lysine with or without amide linked lipid chains were able to form dendriplexes. The dendriplexes reported in this study suggest that these have suitable physicochemical and biological properties to make them useful as gene delivery carriers. The dendriplexes as revealed by light scattering and TEM studies indicate that their size is suitable for targeted biological application. They protect DNA from nuclease degradation, which may extend the lifetime of DNA in physiological conditions. However, transfection studies using these dendriplexes are required to confirm the differences observed here.

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