Biophysical and Structural Characterisation of Nucleic Acid Complexes with Modified Cyclodextrins Using Circular Dichroism

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ABSTRACT: Modified cyclodextrins (CDs) have shown great promise as non-viral gene and siRNA delivery vectors in a range of *in vitro* and *in vivo* studies. In the current study, structural and biophysical characterisation of selected CDs was carried out to enhance our understanding of their interaction with nucleic acids. The methods used for such characterisation were dynamic light scattering, zeta potential measurements and circular dichroism. Variations in the chemistries of individual CDs and in the type of formulation were shown to affect key properties of complexes such as size, surface charge and nucleic acid conformation. Furthermore, the effects of temperature and pH on the conformation of nucleic acids were investigated. pH studies were intended to mimic the conditions encountered by cationic complexes during endocytosis. Circular dichroism studies revealed that changes occurred in DNA and siRNA conformation upon complexation with CDs and when exposed to increasing temperature and decreasing pH. Overall, siRNA appeared to be more susceptible to conformational changes although complexation of siRNA with CDs tended to have a stabilising effect. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1346–1355, 2014

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INTRODUCTION

Gene delivery by non-viral vectors is the focus of extensive research to facilitate the development of novel therapies for neurological diseases and cancers.[1–3] A wide variety of non-viral vectors have been investigated, ranging from cationic lipids and polymers to dendrimers, peptides and antibodies.[4,5] We have developed a library of modified cyclodextrins (CDs) and have successfully delivered nucleic acids to a range of cell types *in vitro* and *in vivo*.[6] These CDs have been modified with cationic, lipophilic and stabilising [polyethyleneglycol (PEG)] moieties, as well as targetting ligands for prostate cancer and the liver and have been employed for delivery of genes and siRNA (small interfering RNA) to neurons, hepatocytes, intestinal epithelial cells and macrophages *in vitro* and to the striatum of the brain, prostate tumours and the colon *in vivo*.[6–12] Four different CDs were chosen for the current study, as examples of cationic, PEGylated and targeted CD gene delivery vectors. The CD structures are shown in Figure 1.

In addition, we investigated different types of formulation, including a formulation consisting of a single CD and formulations containing a blend of CDs, so-called co-formulation, which involves mixing two CDs before complexation of a nucleic acid. The co-formulation strategy has previously been reported for neuronal siRNA delivery[13] and hepatocyte pDNA delivery.[10]

Various analytical methods have been employed to characterise key properties of the complexes formed between nucleic acids and non-viral vectors. For example, dynamic light scattering is used to measure particle size, which impacts on the route and mechanism of intracellular uptake. The surface charge of particles is another important property; cationic complexes interact non-specifically with cell membranes for endocytic uptake, whereas neutral complexes are more desirable *in vivo* because of their prolonged circulation time and lack of interaction with plasma proteins. Surface charge of particles is usually measured by electrophoretic mobility. Additional methods used by us and other research groups to characterise nucleic acid complexes include gel electrophoresis (to assess complexation of nucleic acids by cationic vectors and complex stability), transmission electron microscopy (TEM) (to visualise complex morphology and for particle size measurement) and small-angle X-ray scattering (SAXS).[8,13–15] Previous analysis of cationic and co-formulated CD–siRNA complexes by TEM revealed irregular shaped particles, surrounded by a distinct 'shielding' layer in the case of the latter (PEGylated co-formulated complexes).[13] In-depth SAXS analysis of a range of modified CDs indicated that they may form multi-lamellar nano-assemblies or clusters when interacted with DNA, depending on the hydrophobic–hydrophilic balance within the CD molecule.[15]

Study of the conformation of nucleic acids within complexes has more recently become the focus of attention. Circular dichroism is a highly sensitive technique which can detect changes in the secondary structure of DNA and RNA molecules and has been used to monitor interaction of nucleic acids with non-viral gene delivery vectors.[16–20] It is an important analytical method which can be used to shed further light on the conformational requirements for transfection and on the relationship

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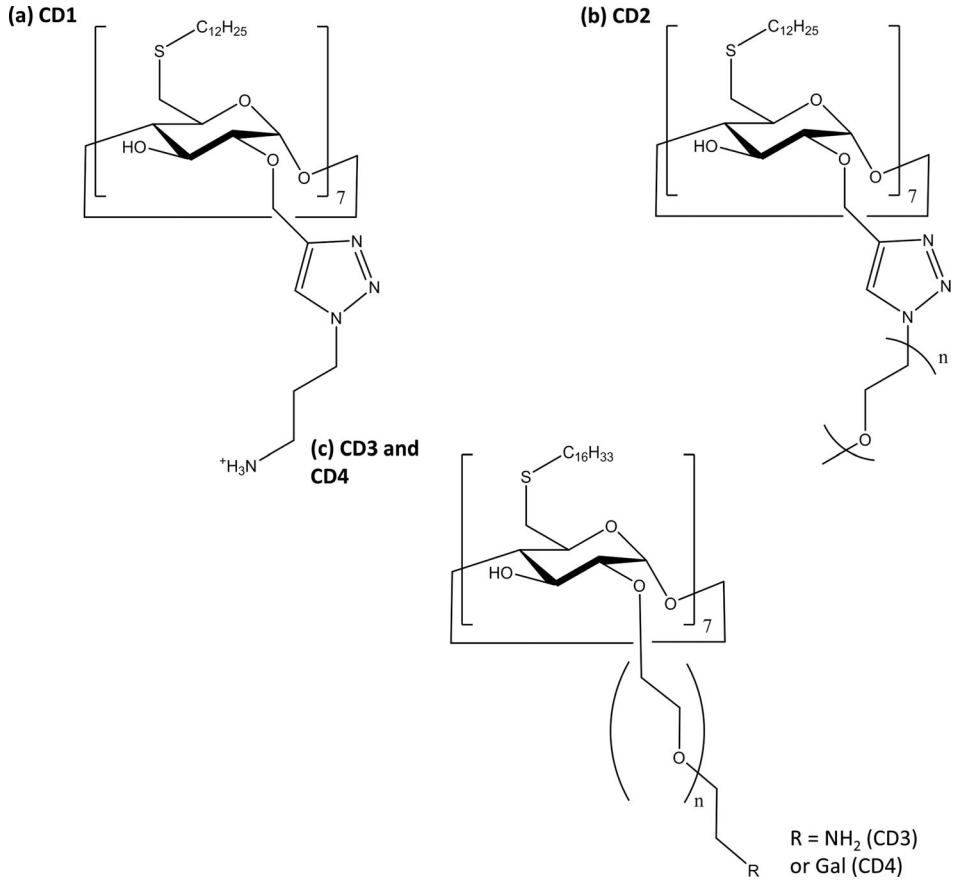

Figure 1. Chemical structures of the modified CDs used in the current study. (a) Amphiphilic cationic CD (CD1), (b) amphiphilic PEGylated CD (CD2) (n = 10–12) and (c) amphiphilic cationic (CD3) or galactosylated (CD4) CDs (R = -hydroxyl group or –galactose targeting ligand, n = 1–2).

between nucleic acid conformation and the ability to achieve gene expression or silencing. Circular dichroism has been used to investigate the changes in DNA secondary and tertiary conformation which occur after condensation by cationic vectors.[19] Transformation of DNA from the native B-form can be readily induced by a variety of factors including changes in temperature or interaction with ligands. Such changes in conformation could influence biological processes including transfection.[21] Circular dichroism has also been employed to investigate nonviral vector interaction with siRNA.[16,22] The native A-form secondary structure of siRNA is essential for its loading into the multi-protein RNA-induced silencing complex (RISC) complex for induction of gene silencing and, therefore, circular dichroism studies have been used to investigate whether chemical modification, interaction with cationic vectors and formulation and processing parameters will affect siRNA conformation.[16,23–26]

This paper aims to increase our insight into the conformation of nucleic acids in CD complexes using circular dichroism and also to assess the effects of other parameters such as the formulation components, temperature changes and pH changes.

MATERIALS AND METHODS

Preparation of CD–Nucleic Acid Complexes

siRNA was purchased from Qiagen (Manchester, UK). pCMV luciferase was purchased from Sigma (Wicklow, Ireland). All CDs used in this study were synthesised as previously described.[27–29] The CD structures are shown in Figure 1. For ease of reference, we have numbered the CDs from 1 to 4. CD1 (Fig. 1a) and CD2 (Fig. 1b) have the same lipophilic chain (C12) on their primary face but differ in their secondary face modification, with a primary amine on CD1 and a PEG group on CD2. CD3 and CD4 (Fig. 1c) are modified with a slightly longer lipophilic chain (C16) on the primary face and differ in their secondary face substitution. In CD3, the short PEG chain terminates in a primary amine, whereas in CD4, the PEG chain terminates in a galactose ligand, which targets the asialoglycoprotein receptor on liver hepatocytes.[10,30]

Three formulations were prepared as follows, according to previously published protocols.[10,13,28,31]

CD1 and pDNA: CD1 was dissolved in chloroform and a stream of nitrogen was applied to remove the solvent, leaving a CD film, which was rehydrated with water (CD concentration 1 mg/mL), followed by sonication at room temperature (RT) for 1 h for vesicle size reduction. A solution of plasmid DNA (pDNA) in nuclease free water was mixed with CD solution to give the required mass ratios (MRs; μg CD:μg nucleic acid) and allowed complex for 15–20 min at RT.

CD1/CD2 co-formulation and pDNA or siRNA: CD1 and CD2 were dissolved in chloroform and mixed together at a specified weight ratio (85% CD1 and 15% CD2), then formulated as described above.