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Revealing cooperative binding of polycationic cyclodextrins with DNA oligomers by capillary electrophoresis coupled to mass spectrometry

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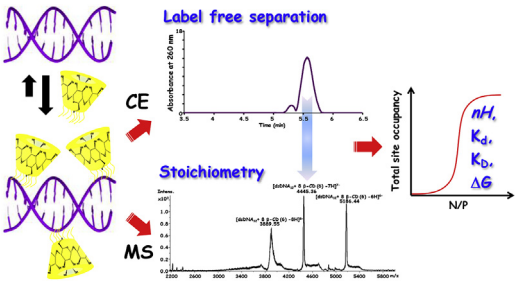
HIGHLIGHTS

- The first ACE-MS study of molecular association of polycationic cyclodextrins with DNA oligomers.
- The opportunity to screen a large set of nitrogen/phosphorus ratio (N/P) with reduced both sample consumption (nL-μL) and analysis time (<6 min/run).
- Access to both thermodynamics (K_d, K_D, and ΔG), and cooperativity (*nH*) key parameters of the supramolecular complexes.
- Unambiguous identification of ligand nature and complex stoichiometry.
- Faster but lower cooperative effect of polycationic cyclodextrins towards dsDNA as compare to ssDNA with the same bp composition.

GRAPHICAL ABSTRACT



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ABSTRACT

Gene delivery is critical for the development of nucleic acid-based therapies against a range of severe diseases. The conception of non-viral (semi)synthetic vectors with low cytotoxicity and virus-like efficiency is gathering a lot of efforts, but it represents a fantastic challenge still far from accomplishment. Carbohydrate-based scaffolds offer interesting features towards this end, such as easy availability, relatively cheap cost, tuning properties and a good biocompatibility. The lack of analytical methods providing quantitative and qualitative data on their binding properties with oligonucleotides (DNA/RNA), with a minimal time and sample consumption, represents a limitation for these channels. Here, we attempted to fill the gap by hyphenation of capillary electrophoresis with mass spectrometry (CE-MS). This coupling strategy allows discriminating free and complexed DNA oligomers with cationic cyclodextrins (CDs), determining the stoichiometry where the highest observed is always DNA_n: n/3(CD), and unambiguously

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Hill number
DNA
Gene delivery

assigning the partners through *m/z* detection. Very reliable data were obtained with migration time within 5.5 (standard deviation < 0.5%) and 25 min (standard deviation < 1.1%) for UV and MS detection, respectively. Furthermore, varying the nitrogen/phosphorus ratio (N/P), key parameters relating to the thermodynamics e.g. the micro and macroscopic dissociation constants K_d and K_D, respectively (both in low μM range) and the Gibbs free energy ΔG (−16.3 to −26.9 kJ mol^{-1}), and also the cooperativity as Hill number (*nH* between 0.98 and 15.75) of the supramolecular process can be delineated, providing a unique tool for the high throughput screening and selection of efficient gene delivery carriers.

1. Introduction

Gene therapy has a strong potential for the clinical treatment of a broad range of severe pathologies including cancer [1], Parkinson's disease [2], or Niemann−Pick type C disease [3]. The general approach requires that active nucleic acid drugs can be efficiently and safely delivered across biological membranes directly into the target cell [4]. Virions are widespread natural infectious particles able to perform transmission of their nucleic acid genome by exploiting the infected cell machinery. The possibility to achieve an oriented modification of their genetic content has represented up to now the most obvious and promising strategy for gene delivery [5]. Safety concerns about their immunogenicity and scaled up expression seriously limit their translation into the clinics. Indeed, some of them have revealed unsecure in advanced clinical trial stages [5−7]. This issue was soon recognized and fuelled an aggressive research in non-viral vectors [4,8]. However, despite the diverse systems developed along the years, the physicochemical properties of the carrier that endow efficacious DNA or RNA complexation and transport across the cellular barriers remain unclear [9].

Most of the investigated non-viral gene delivery vectors are based on polycationic architectures that undergo self-assembling in the presence of polyanionic nucleic acids to form transfectious (nano)particles [4,5]. The tremendous interest garnered by polycations as designed carriers relies both in their capacity to protect the genetic payload from degradation during transport and their cell permeability enhancing effect [10]. In addition, they have demonstrated a low immunogenicity and can be obtained by efficient and versatile procedures, compatible with structural diversity strategies and scalable production schemes [5,11,12]. A myriad of cationic scaffolds can be tailored including cationic lipids [13], amine-containing synthetic polymers as for example the emblematic poly-L-lysine [14] and polyethylene imine (PEI) [15], or the polyamidoamine (PAMAM) dendrimers [16]. Unfortunately, most of these synthetic agents suffer from too low transfection efficiency and/or too high cytotoxicity [8]. To overcome such drawbacks, efforts have been paid to obtain biocompatible architectures, which can potentially serve as nucleic acid carriers along with presenting non-toxic and biodegradable features. In this sense, modified polysaccharides obtained from natural renewable resources [17−19] and glycopolymers [20,21] are particularly appealing. However, their polydisperse character represents a significant drawback, impairing the straightforward assessment of structure/activity relationships in search for optimal leads.

An alternative approach for gene vector design recently developed consists in the elaboration of discrete molecularly well-defined entities based on macrocyclic scaffolds [12], such as calixarenes [22], pillarenes [23], or cyclodextrins (CDs) [24,25]. The latter have been by far the most intensely investigated. CDs are cyclic carbohydrates composed of 6 (αCD), 7 (βCD) and 8 (γCD) α-(1 → 4)-linked D-glucopyranoside units. They are commercially available at relatively low cost and high purity, and exhibit no or low cytotoxicity [26−28]. The most accessible representative, namely βCD, has been elegantly exploited as molecular scaffold for the design of a large variety of polycationic derivatives to support gene delivery [26−33]. Five analytical methodologies were very frequently employed to study the successful formation of DNA complexes as a function of the ratio between the number of protonatable amino groups per molecule of the vector agent and the number of phosphate groups in the oligonucleotide (N/P ratio): (i) dynamic light scattering (DLS) [29,34], imparting an estimation of the hydrodynamic size of neo-formed particles; (ii) zeta potential measurements [29,34], which informs on the evolution of surface charges of the formed complexes; (iii-iv) transmission electron microscopy (TEM) [29,34] and atomic force microscopy (AFM) [33], which provide data on the size/shape and topography of the macromolecular assemblies; and (v) electrophoretic mobility shift assay (EMSA) [29,33,34], the forefront method to directly display the ability of complexation of a vector towards nucleic acids. In the latter case, an intercalating compound (ethidium bromide, GelRed®) is used to reveal the compaction state by fluorescence. This technique is qualitative and cannot be linked directly to the vector/DNA ratio. In spite of such analytical arsenal, none of these approaches can accurately address the molecular basis underpinning complex formation, i.e. the strength and type of interactions governing complexation between the vector and DNA. This information constitutes an essential primer to design more efficient cationic non-viral gene delivery vectors.

In the study herein, we have attempted to bridge this gap by developing for the first time a method based on online affinity capillary electrophoresis (ACE) hyphenated to mass spectrometry (MS). It has been previously demonstrated that CE used in a standalone mode allows the separation of single (ss) or double strand (ds) DNA either free [35,36] or complexed with small ligands [37−39], proteins [40,41] and even with a cationic dendritic tetrapod [42]. In addition to its inherent high resolution, suitability to study weak interactions and low sample requirements (nL and nM scale), it was recently demonstrated that kinetics and thermodynamics data can also be extracted [43−45]. In the other hand, MS is highly sensitive and allows not only to unambiguously ascribe free and complexed forms, but also to discriminate the various complexes which cannot be completely resolved by CE alone. As proof of concept, a library of cationic CDs (molecular weight range: 1000−3000 g mol^{-1}) previously characterized by MS and on other hand having demonstrated DNA complexing capabilities [34,46−49], was screened for the complexation abilities towards single stranded (ss) and double stranded (ds) DNA of various types and lengths (hexamers to 24-mers) as a function of the N/P ratio. The results obtained provide important information on the stoichiometries, dissociation constants and Gibbs free energies as well as on the existence of cooperativity, allowing a better understanding of the interaction mode at play and orientating the choice for efficient DNA complexation.

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