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Toward a suitable structural analysis of gene delivery carrier based on polycationic carbohydrates by electron transfer dissociation tandem mass spectrometry

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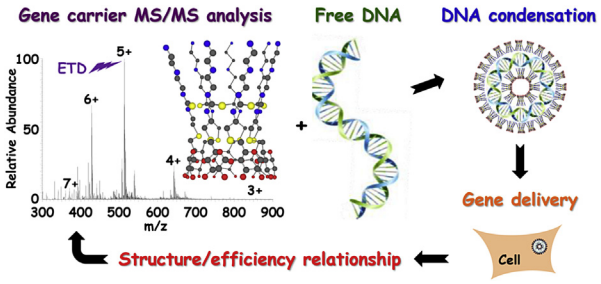
HIGHLIGHTS

- The first ETD-MS/MS characterization of polycationic carbohydrate based non-viral gene delivery agents.
- Suitable selection of charge states and fluoranthene reagent time improves ETD fragmentation efficiency.
- ETD with SA can complete structural deciphering of some building blocks which is not possible with CID only.
- ETD based fragmentation is more efficient with long grafted polycationic arms.
- MS/MS results can be used to correlate nitrogen/phosphorus ratio (N/P) in DNA compaction.

GRAPHICAL ABSTRACT



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ABSTRACT

Polycationic carbohydrates represent an attractive class of biomolecules for several applications and particularly as non viral gene delivery vectors. In this case, the establishment of structure-biological activity relationship requires sensitive and accurate characterization tools to both control and achieve fine structural deciphering. Electrospray-tandem mass spectrometry (ESI-MS/MS) appears as a suitable approach to address these questions. In the study herein, we have investigated the usefulness of electron transfer dissociation (ETD) to get structural data about five polycationic carbohydrates demonstrated as promising gene delivery agents. A particular attention was paid to determine the influence of charge states as well as both fluoranthene reaction time and supplementary activation (SA) on production of charge reduced species, fragmentation yield, varying from 2 to 62%, as well as to obtain the most higher both diversity and intensity of fragments, according to charge states and targeted compounds. ETD fragmentation appeared to be mainly directed toward pending group rather than carbohydrate cyclic scaffold leading to a partial sequencing for building blocks when amino groups are close to carbohydrate core, but allowing to complete structural deciphering of some of them, such as those including

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dithioureidocysteaminyl group which was not possible with CID only. Such findings clearly highlight the potential to help the rational choice of the suitable analytical conditions, according to the nature of the gene delivery molecules exhibiting polycationic features. Moreover, our ETD-MS/MS approach open the way to a fine sequencing/identification of grafted groups carried on various sets of oligo-/polysaccharides in various fields such as glycobiology or nanomaterials, even with unknown or questionable extraction, synthesis or modification steps.

1. Introduction

Polycationic polysaccharides can be encountered alone or conjugated to other biomolecules [1–3]. They can be obtained directly after extraction/purification steps from natural matrix as chitosan [4,5] or by chemical or enzymatic modification of neutral or anionic carbohydrates such as alginate, dextran, pullulan, cellulose, cycloamylose, carboxymethylcellulose or also hyaluronic acid [6–10]. Their applications are various ranging from agro-alimentary to medicine. A particular attractive field which has constantly gained interest is the capacity of some of them to efficiently compact polynucleic acids such as DNA/RNA acting as potential non-viral gene delivery agents [8,9,11–14]. Nevertheless, synthesis of such architectures requires several time consuming steps and the use of often heterogeneous starting materials. One relevant solution consists to use molecules with both well-defined structure and size to efficiently delineate a structure/activity relationship. In this sense, oligosaccharide based scaffolds such as cyclodextrins (CDs) after modification to enhance their basicity properties are attractive candidates for efficient gene delivery [15–20]. Nevertheless, even with such edifices, a straightforward structural control of the end products in term of size and number of grafted functions must be achieved. Nowadays, few analytical techniques are available to address those requirements.

Since two decades, mass spectrometry (MS) with its emblematic ionization mode, electrospray (ESI) has emerged as a forefront suitable technique to efficiently portray the solution content. Due to its gentle process and sensitivity features, ESI-MS allows the study of a wide variety of compounds even at trace levels and/or carrying labile moieties. These two essential criteria allowing a low sample consumption and access to a structural deciphering *via* multistep fragmentation, make to ESI-MS^n a major tool for oligo-/polysaccharide characterization. Nevertheless, CDs as other neutral oligosaccharides present an intrinsic preference for alkali metals than protons [21,22]. Consequently, the widely used collision induced dissociation (CID) fragmentation mode using multiprotonated molecules can be limited to finely characterize some moieties grafted on such oligosaccharides [23]. More recently, an efficient MS fragmentation mode named electron transfer dissociation (ETD), has been introduced by Hunt, Coon, Syka and coworkers [24–26]. ETD induces fragmentation of multiply charged molecules subsequently to electron transfer from an anionic reagent to cations. Initially, ETD has been developed to preserve labile post translational modifications of proteins and furthermore to increase fragmentation efficiency of multiply charged whole protein during ESI-MS^n analysis [25,26]. Nevertheless, ETD potential has been sporadically applied to other molecules such as synthetic polymers [27], combinatorial chemistry end products [28], pyridinium-based amino acid analogs (desmosine and isodesmosine) [29], crosslinking elastin [29], or also glycerophosphocholine lipids [30]. Few studies report the use of ETD for carbohydrate analysis and concerning exclusively neutral structures like milk oligosaccharides [31], maltodextrin pentamer [32] or lacto-*N*-fucopentaose/difucohexaose [33]. To obtain dicharged species, their ionization was assisted by coordination with ammonium or more specifically with cations of group IA (Na^+, Li^+, K^+ Rb^+, Cs^+), group IIA (Ca^{2+}, Mg^{2+}, Be^{2+}, Sr^{2+}, Ba^{2+}) and group IIB (Zn^{2+}, Hg^{2+}) [31–34]. Parameters affecting ion/ion reactions leading to optimal fragmentation efficiency of peptide such as the identity of the charge-bearing, cation charge state, the choice of the reagent, the reagent reaction time, the precursor/reagent ratio, and the Mathieu Q parameter have been thoroughly investigated [35–37]. All these studies have unambiguously demonstrated their influence on the ETD efficiency. Among them, it clearly appeared that the reaction time is the most important factor which directly correlates to the charge states as well as the location of charge sites i.e. the fine structure.

In this paper, we investigated the behaviour of a library of cyclic polycationic carbohydrates upon ETD-MS/MS. A particular attention was paid to delineate the effect of reagent reaction time both for various charge states of a given compound or for the same charge state of different compounds. Moreover, both the fragments and the charges of the reduced species content resulting from ETD, as well as the usefulness of supplementary activation were estimated. Considering an identical scaffold (cyclodextrins; CD), carrying various structural elements with protonation sites, the study herein aimed to establish particular reaction time/charge states/side modifications relationship occurred. ETD fragmentation efficiency was finally compared with CID only for the structural deciphering of the various molecules.

2. Materials and methods

2.1. Reagents

Methanol (MeOH) used for sample preparation was of HPLC grade and was purchased from VWR (West Chester, PA, USA). Water was of ultrapure quality, obtained from a MilliQ apparatus (Millipore, Milford, USA).

2.2. Samples

Synthesis of per-6-modified-β-CD (Fig. 1, Compounds **1**–**5**) were realized as previously described [38,39]. Samples were prepared at 1 mg/mL in water/methanol 1/1 (v/v).

2.3. Mass spectrometry

ESI-MS experiments were carried out using a LTQ-Orbitrap XL from Thermo Scientific (San Jose, CA, USA) and operated in positive ionization mode, with a spray voltage at 3.7 kV. A water/methanol 1/1 (v/v) mixture was continuously infused using a 500 μL syringe at 3 μL/min flow. Applied voltages were 31 and 115 V for the ion transfer capillary and the tube lens, respectively. The ion transfer capillary was held at 275 °C. Resolution was set to 60 000 (at m/z 400) for all studies, and the m/z ranges were set to 200–2000