International Journal of Mass Spectrometry 316–318 (2012) 235–243

Contents lists available at SciVerse ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Ternary complexes of poly(ethylene imine), single-stranded oligodeoxynucleotides and glutamic acid moieties

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ARTICLE INFO

Article history:
Received 8 December 2011
Received in revised form 17 February 2012
Accepted 22 February 2012
Available online 3 March 2012

Keywords:
Polymer
Oligonucleotide
Non-covalent association
Terplex
Solution vs. intrinsic stability

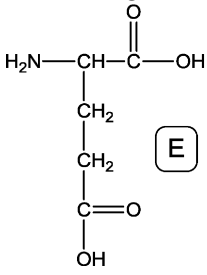
ABSTRACT

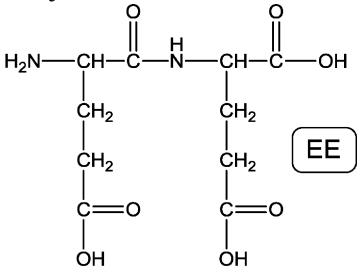
Ternary non-covalent complexes composed of poly(ethylene imine) (P), a pentadeoxynucleotide (N) and glutamic acid monomer or dipeptide (E or EE) were prepared by mixing aqueous P, N and E (or EE) solutions in various molar ratios. Five nucleotides were examined, viz., d(TTTTT), d(CCCCC), d(AAAAA), d(GGGGG) and d(GCGAT). The compositions, solution stabilities and intrinsic stabilities of the ternary complexes ("terplexes") were probed by electrospray ionization mass spectrometry (ESI-MS), tandem mass spectrometry (MS^2) and ion mobility mass spectrometry (IM-MS). ESI-MS experiments confirmed the formation of terplexes with four of the five N molecules tested, the favored stoichiometry being 1:1:1 P-to-N-to-E (or EE) in all cases; d(GGGGG) did not form any detectable ternary complexes. Other compositions, involving higher order terplexes with multiple units or P, N and/or E (or EE), as well as several binary combinations, could be identified by IM-MS. The solution stabilities of the ternary complexes, assessed from their relative intensities in ESI mass spectra, depend on the sequence of N for PNE terplexes, maximizing with thymine-rich oligonucleotides. This selectivity is lost in the more weakly bound PN(EE) terplexes, whose binding interactions are barely influenced by the oligonucleotide sequence. Gas-phase (intrinsic) stabilities, assessed by dissociation extents in MS^2 experiments, follow the same order as the corresponding solution stabilities, suggesting similar terplex structures in both media.

1. Introduction

Poly(ethylene imine) is a cationic polymer that has been extensively investigated for non-viral gene transfection *in vitro* and *in vivo* [1,2]. The complexes between poly(ethylene imine) and nucleic acids, termed polyplexes, have shown a high degree of trasfection efficiency in serum-free medium; however, a significantly lower efficiency has been observed in the presence of serum, which downgrades the *in vivo* application of these cationic vectors for gene delivery [3–5]. One of the reasons is that positively charged polyplexes bind non-specifically to negatively charged components in serum or blood, thus preventing successful gene therapy [6,7]. In order to protect the stability of DNA/poly(ethylene imine) complexes from non-specific interactions in the biological environment, these polymer-based systems can be chemically modified to improve their biocompatibility for *in vivo* applications [5,7,8]. One alternative way to modify these cationic systems is to electrostatically bind anionic polymers to their surface; indeed, ternary complexes generated by mixing polyplexes with polyglutamic acid (PGA), a non-toxic and biocompatible anionic polymer, showed significantly enhanced trasfection efficiency in the presence of serum [9,10].

Recent studies have reported on the benefits of high transfection efficiency and low cytotoxicity of ternary DNA/poly(imine) complexes with anionic polymers and examined the secondary structures and sizes of these "terplexes" by circular dichroism spectroscopy and agarose gel electrophoresis [9,11]. Here, we describe the first molecular-level investigation of model terplexes composed of poly(ethylene imine), an oligodeoxynucleotide and either glutamic acid or glutamyl glutamic acid dipeptide by electrospray ionization mass spectrometry (ESI-MS), tandem mass spectrometry (MS^2) and ion mobility mass spectrometry (IM-MS). For brevity, these species will be symbolized by PNE and PN(EE), where P = polymer, N = oligodeoxynucleotide, E = glutamic acid and EE = glutamyl glutamic acid. The terplex stoichiometries, charge state distributions and stabilities in solution and the solvent-free environement are examined for systems prepared from P 400, five different single-stranded pentadeoxynucleotides and either E of EE.





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doi:10.1016/j.ijms.2012.02.016

2. Materials and methods

2.1. Materials

P 400, E and EE were acquired from Signa/Aldrich (Milwaukee, WI). HPLC-grade water, ammonium acetate, and the pentadeoxynucleotides 5′-d(TTTTT)-3′, 5′-d(CCCCC)-3′, 5′d(AAAAA)-3′, 5′-d(GGGGG)-3′ and 5′-d(GCGAT)-3′ were purchased from Fisher (Fair Lawn, NJ). All chemicals were used as received without further purification.

2.2. Sample preparation

E or EE, polymer and pentadeoxynucleotides were dissolved in 10 mM ammonium acetate buffer of pH = 7.4. Individual 0.5 mM solutions were prepared for poly(ethylene imine) (P) and the oligodeoxynucleotides (N), mixed in the ratio of 10:1 (v/v) and incubated for 10 min. Solutions with different concentrations of E or EE were then added to this mixture so that the final mixing ratio was 10:1:1 (v/v/v) P/N/E or P/N/EE. The resulting final mixtures were incubated for additional 10 min before introduction into the ESI source by direct infusion at a flow rate of 5 μL/min. The notation P/N/EE indicates the components of a ternary complex, whereas the notation PN(EE) designates a specific terplex with the P-to-N-to-EE stoichiometry of 1:1:1.

2.3. Mass spectrometry experiments

All experiments were carried out on a Synapt HDMS quadrupole/time-of-flight (Q/ToF) tandem mass spectrometer (Waters, Beverly, MA) equipped with ESI and traveling wave ion mobility mass spectrometry (TWIM-MS capabilites) [12]. The instrument contains a triwave device between the Q and ToF mass analyzers, consisting of three cells arranged in the order trap cell (closest to Q), ion mobility cell and transfer cell (closest to ToF). ESI mass spectra were aquired in positive mode utilizing the following settings: spray voltage 3.5 kV, sampling cone voltage 35 V, extraction cone voltage 3.2 V, source temperature 100 °C and desolvation temperature 150 °C. The desolvation gas flow rate was 400 L/h (N_2), the trap collision energy was set at 4.0 eV and the transfer cell collision energy at 6.0 eV. A further instrument parameter optimized was the pressure in the interface region between the ion source and the quadrupole mass filter (p_i), which was set at 6 mbar in order to maintain the non-covalent interactions. Experimental conditions were tuned to maximize the intensity of the doubly charged P–d(CCCCC)–E terplex; these conditions were then applied to all other terplexes. For the acquisition of MS^2 spectra, a specific terplex ion was isolated with the quadrupole using an isolation width of 4.5 and underwent collisions with Ar in the trap cell; the Ar gas flow was 1.5 mL/min. The collision energy in the MS^2 measurements was varied within 4–12 eV to induce fragmentation.

The normalized intensities of the P/N/E or P/N/EE ternary complexes in ESI mass spectra were used to determine the effect of solution concentrations on the yield and stoichiometry of the terplexes and to assess the relative solution stabilities of the terplexes [13–16]. Normalized intensities were obtained from peak heights by summing the intensities of the terplexes observed in a given stoichiometry and dividing this sum by the sum of intensities of uncomplexed species (N, P, E or EE) and binary complexes (PN, NE or N(EE), PE or P(EE)). All detectable charge states were used in this calculation. For the complexes containing poly(ethylene imine), the four most abundant oligomers were considered. Since uncomplexed N dissociated partly during ESI analysis, the N fragment intensities were added to those of intact N for the calculation of normalized intensities. The data provided in the text are the average values from two replicate ESI mass spectra.

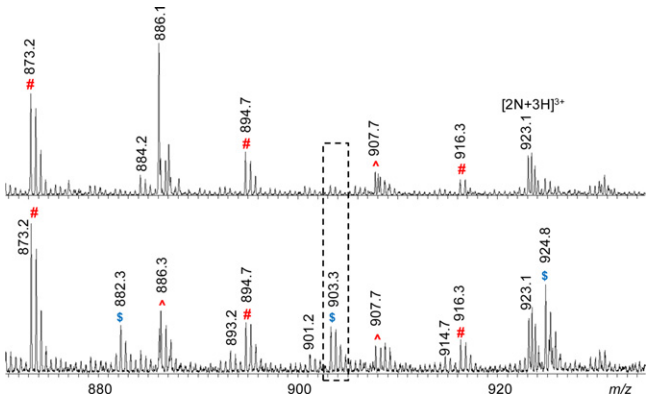

Fig. 1. Expanded view of the m/z 800–940 region of the ESI mass spectra of the P 400/5′-d(CCCCC)-3′/E terplex, acquired at a sample cone voltage of 35 V and backing pressure of (a) 2 mbar and (b) 6 mbar. The signs # and ∧ designate P–d(CCCCC) binary complexes containing linear (#) or cyclic (∧) P and having 1:1 stoichiometry. The sign $ designates P–d(CCCCC)–E terplexes containing linear P oligomers and having a stoichiometry of 1:1:1 P-to-N-to-E.

Fragmentation efficiency curves were constructed from MS^2 spectra of doubly charged terplexes by plotting the relative abundance of the selected terplex vs. the corresponding center-of-mass collision energy, E_{cm} [17]. E_{cm} values were calculated from the applied laboratory-frame collision energies, E_{lab}, via the equation $E_{cm} = E_{lab} \times [m_{Ar}/(m_{Ar} + m_{terplex})]$, where m_{Ar} and $m_{terplex}$ are the masses of the Ar atom and the selected terplex ion, respectively. Relative abundance was calculated by dividing the intensity of the terplex ion, I(terplex), by the sum I(terplex) + I(doubly charged fragments) + (1/2)I(singly charged fragments) [13–16]. The points were fitted into sigmoid curves using the Origin 8.1 graphing software [18]. The inflection point energies (E_{cm}) of the sigmoid curves provide an approximate measure of the gas-phase stabilities of the terplexes [19]; the values reported were derived from three replicate MS^2 spectra.

Two-dimensional (2D) ion mobility mass spectrometry (IM-MS) plots were acquired using a traveling wave velocity of 350 m/s and traveling wave height of 11 V. The IM gas (N_2) flow rate was 22.7 mL/min and the trap and transfer cell potentials were kept at 6.0 and 4.0 V, respectively.

3. Results and discussion

3.1. Terplexes with E

In an earlier study, we reported the characterization of polyplexes composed of P 400 or P 800 and oligodeoxynucleotides [16]. Here, we extend this work from binary to ternary cationic systems to gain insight about the structural interactions among the constituents of such ternary complexes and assess their solution and gas-phase (intrinsic) stabilities.

In order to preserve the non-covalent interactions within the ternary complexes during and after the ionization process, instrument parameters were selected that maximized ion intensities while minimizing dissociation. For the terplexes investigated, variations in the acceleration potential (sampling cone voltage) and source/desolvation temperatures were found to be less critical if the pressure in the first pumping stage of the mass spectrometer (backing pressure) is kept high [20]; thus, the key parameter optimized in this study was the backing pressure, which is the pressure in the extraction region between the ESI source and the quadrupole mass analyzer [21]. A number of studies have shown that this