Gas-Phase Ion/Ion Reactions of Transition Metal Complex Cations with Multiply Charged Oligodeoxynucleotide Anions

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Multiply deprotonated hexadeoxyadenylate anions, $(A_6-nH)^{n-}$, where n = 3–5, have been subjected to reaction with a range of divalent transition-metal complex cations in the gas phase. The cations studied included the bis- and tris-1,10-phenanthroline complexes of Cu^{II}, Fe^{II}, and Co^{II}, as well as the tris-1,10-phenanthroline complex of Ru^{II}. In addition, the hexadeoxyadenylate anions were subjected to reaction with the singly charged Fe^{III} and Co^{III} *N,N'*-ethylenebis(salicylideneiminato) complexes. The major competing reaction channels are electron-transfer from the oligodeoxynucleotide anion to the cation, the formation of a complex between the anion and cation, and the incorporation of the transition-metal into the oligodeoxynucleotide. The latter process proceeds via the anion/cation complex and involves displacement of the ligand(s) in the transition-metal complex by the oligodeoxynucleotide. Competition between the various reaction channels is governed by the identity of the transition-metal cation, the coordination environment of the metal complex, and the oligodeoxynucleotide charge state. In the case of the divalent metal phenanthroline complexes, competition between electron-transfer and metal ion incorporation is particularly sensitive to the coordination number of the reagent metal complexes. Both electron-transfer and metal ion incorporation occur to significant extents with the bis-phenanthroline ions, whereas the tris-phenanthroline ions react predominantly by metal ion incorporation. To our knowledge this work reports the first observations of the gas-phase incorporation of multivalent transition-metal cations into oligodeoxynucleotide anions and represents a means for the selective incorporation of transition-metal counter-ions into gaseous oligodeoxynucleotides. (J Am Soc Mass Spectrom 2008, 19, 281–293) © 2008 American Society for Mass Spectrometry

The development of gentle ionization methods such as matrix-assisted laser desorption ionization (MALDI) [1–5] and electrospray (ESI) [6–8] have allowed the formation of gas-phase ions of biopolymers, including oligodeoxynucleotides (ODNs), making these ions amenable to study by mass spectrometry. Like peptides and proteins, ODNs are amphoteric, [9] allowing formation of either positively or negatively charged ions. Formation of negatively charged oligonucleotides is quite facile using ESI and, as a result, negatively charged ODN ions have been the most widely studied [9]. Oligodeoxynucleotide ions with bound metals formed by MALDI or ESI, especially those containing transition metals, have shown fragmentation behavior, which is distinct and complementary to that of ions devoid of metals [6–8].

The study of the interaction of transition metals with DNA is of critical importance, given the variety of roles such interactions fulfill. Transition-metal complexes display nuclease activity and consequently complexes such as (methidium-propyl-EDTA)iron(II) have been utilized as footprinting agents [10]. Ruthenium(II) complexes have been extensively examined as spectroscopic probes of local DNA structure [11, 12]. The mechanism of action of the chemotherapeutic agent cisplatin involves binding to DNA to prevent replication [13–15]. Mass spectrometry has been used as a tool for the examination of ODN complexes with metal ions [16]. To date, in mass spectrometric studies, metal ion incorporation into the ODN occurs before, or in conjunction with, the ionization step, typically through the addition of metal salts to the sample matrix. This approach has the disadvantage of leading to a mixture of ions with different numbers of metal ion adducts, and in the case of ESI, different charge states.

Ion/ion reactions have been used to study the primary structure of biopolymers through processes such

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as electron-transfer dissociation (ETD), to manipulate charge states, and to form novel ion types which may not be available directly from ESI [17, 18]. Ion/ion reactions can be generally categorized into three types: charge-transfer (including proton and electron-transfer), complex formation, and metal cation exchange. Careful choice of reagents for ion/ion reactions can provide some control over the extent to which each of these reaction pathways contributes to the ion/ion reaction products observed [19, 20]. Factors that play important roles in determining the relative contributions of these reaction pathways are the reactant identities, the charges of the reactant ions, and the reaction exothermicity.

Metal cation exchange is a type of ion/ion reaction that may be used to synthesize new species in the gas phase. Such reactions have largely been used to substitute metal ions for protons in peptide ions. For example, metal containing anions of the form MetalX_{2-} where Metal is a singly charged alkali or transition-metal and X is a common counter ion such as a halide, acetate, or nitrate have been used as reagents [21–24]. Gas-phase metal containing peptide ions formed in this way have exhibited dissociation behavior identical to those formed in solution by mixing the metal salt with the peptide. The advantage of forming metal containing biopolymer ions via the ion/ion reaction approach is that it provides greater control over the selection of the reactant species and allows for independent optimization of ionization conditions for the analyte ion and the reagent ion.

Cationic metal complexes provide an opportunity for the gas-phase synthesis of new transition-metal-ODN complex ions via metal cation exchange reactions. These novel transition-metal-ODN ions can also provide a method to study the various factors influencing the relative contributions of electron-transfer, proton transfer, complex formation, and cation exchange in gas-phase ion/ion reactions with ODN anions. In addition, these transition-metal containing ions may provide new and complementary fragmentation information when subjected to collision induced dissociation (CID) or other dissociation techniques. Previous studies have shown that ESI can be used to readily form gaseous complex ions composed of a transition-metal and one or more multi-dentate ligands [25–28]. Such metal complex ions have been the subject of extensive study including CID [29–33] and ion/molecule reactions [34–36]. These studies have clearly shown the importance of coordination geometry and ligand identity on the reactivities of these ions and are consistent with the much larger body of work devoted to the chemistry of gaseous transition-metal ions [37–43]. They strongly suggest that a degree of control over ion/ion reactivity might be afforded by selection of metal, metal oxidation state, ligand, and ligand number.

Here, multiply charged hexadeoxyadenylate (hexa-dA) anions, $(\mathrm{A}_6{-}n\mathrm{H})^{n-}$, where $n = 3$–5, have been used as models for ODN anions in a study of the ion/ion reaction behavior of multiply charged anions with multiply charged divalent transition-metal multi-dentate ligand complex cations. The ion/ion reaction behavior of late-first row divalent transition metals Cu(II), Co(II), and Fe(II) in complexes with 1,10-phenanthroline (denoted herein as "phen") is emphasized. Results obtained with complexes of trivalent cobalt or iron and *N,N′*-ethylenebis(salicylideneiminato) (denoted herein as "salen") complexes as well as those for $[\mathrm{Ru^{II}(phen)_3}]^{2+}$ are also presented to illustrate the range of ion/ion reaction phenomenology observed.

Experimental

Mass Spectrometry

Nanospray emitters were pulled from borosilicate capillaries (1.55 mm o.d., 0.86 mm i.d.) using a P-87 Flaming/Brown micropipet puller (Sutter Instruments, Novato, CA). The electrospray solutions were loaded into these nanospray emitter tips, and a wire was inserted into the tip to make electrical contact with the analyte solution. For hexa-dA, a stainless steel wire was used, while a platinum wire was used for metal complex solutions.

Ionization was performed using a home-built pulsed dual nanoelectrospray ionization (nano-ESI) source that has been described previously [44]. Briefly, this pulsed dual source was comprised of two nano-ESI emitters, which were pulsed alternately on or off in each scan by the software. High voltages applied to the nano-ESI emitters were optimized separately and were independently generated by two high-voltage power supplies from the instrument and a fast high voltage pulser (GRX-1.5K-E; Directed Energy Inc., Fort Collins, CO). Ions of either polarity could be independently optimized and directed into the mass spectrometer using this method.

All experiments were performed with a quadrupole/time-of-flight tandem mass spectrometer (QSTAR XL; Applied Biosystems/MDS SCIEX, Concord, ON, Canada) that has been modified for ion/ion reactions [45]. Molecular nitrogen was used as the collision gas. The instrument was controlled by Daetalyst software, a research version of software developed by MDS SCIEX, which provided full control for initiating each ion source and for applying the appropriate potentials and their timing to the ion path.

In a typical experiment, hexa-dA anions were produced via the negative nano-ESI source and the ions of interest were isolated as they passed through Q1. The isolated anions were subsequently directed into the Q2 linear ion trap (LIT) (50 ms), where they were trapped. Reagent cations of interest were then similarly generated in the gas phase by positive nano-ESI of the corresponding metal solution, and similarly the reagent ion of interest was selected isolated in Q1, and introduced into the Q2 LIT (30–100 ms), where ions of both polarities were allowed to react in the LIT over a variable reaction period (20–200 ms). Note that due to significant variations in numbers of ions from one