

# High Resolution LC/MS/MS Study of the Interactions of Inorganic Mercury (II) with Nucleic Acids Rich in Thymine



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## ABSTRACT

High resolution tandem mass spectrometry has proven to be a powerful tool for structural elucidations of DNA and oligonucleotides as well as their complexes with metal ions, drugs and small organic ligands. The interest in exploration of mercury ions interactions with nucleic acids stems from a wide variety of potential applications e.g. understanding of physiological functions of metal ions *in vivo* as well as molecular basis for their genotoxicity [1]; development of metal ion sensors [2] and metallo-DNA molecular magnets [3]. Here we report the results of high resolution LC/MS/MS study of the interactions of three oligonucleotides rich in thymine with mercury dications.

## INTRODUCTION

The unique binding mode of Hg(II) to thymine in T-Hg(II)-T base pair, determined using 15N NMR and Raman spectroscopy, involves bridging two thymine ligands accompanied by the displacement of two N3 protons by one Hg(II) [4] (Figure 1). However, structural information for the Hg(II)-DNA duplexes is still lacking. It is also unclear how the proximity of the mercury dications embedded in a DNA duplex will influence its overall stability. The computational studies suggest duplex-stabilizing metallophilic interactions between Hg(II) ions in the consecutive mercury-bridged thymine base pairs. [5] 3D modelling of mercury-bound DNA duplexes indicates that the T-Hg(II)-T pairs are well-accommodated without any significant distortion of the DNA duplex in a fashion similar to the canonical Watson-Crick base pairs.[5] Moreover, the theoretical modelling suggests that the well-stacked arrangement of mercuriated base pairs indicates that the interactions of the mercury atoms within DNA duplex is not repulsive. Earlier ESI-MS/MS studies demonstrated preservation of preferential interactions of Hg<sup>2+</sup> with thymine nucleobases from two separate ODN strands rather than with other binding sites available in the structure of thymine-rich oligonucleotides. [6] However, it is unclear whether the ESI process contributes in any way to the formation of mercuriated ODN adducts. In order to demonstrate the solution origin of Hg(II)-ODN complexes and gain insights into their structure we employed high resolution tandem mass spectrometry coupled with microLC separation approach to study interactions of inorganic Hg(II) with nucleic acids rich in thymine.

## MATERIALS AND METHODS

Three oligonucleotides with the sequences 5'-TTTT-3' (ODN1), 5'-GCTATTGC-3' (ODN2) and 5'-GCATTTGC-3' (ODN3) were acquired from ACGT Corp., Toronto, Canada. Mercury acetate (Sigma Aldrich) was used as a source of Hg(II) ions.

### Sample Preparation:

Working solutions (20 μM) of ODNs were prepared by dilution of the stock solutions with a mixed solvent of 90% water with 10% methanol. ODN2 and ODN3 oligonucleotides were annealed to form double-stranded species in 100 mM ammonium acetate buffer prior to microLC-ESI-MS/MS and direct infusion analyses. Annealing was performed by heating the solutions of ODNs to eighty degrees Celsius for 10 minutes and slowly cooling them down to room temperature to ensure the formation of the duplexes. The ratio of the concentration of Hg(II) to ODN was kept at 1 to 2.

### HPLC Conditions:

Three types of reverse phase micro-LC columns (Phenyl-Hexyl, C8, C18) and an Amide-based HILIC column were employed to achieve the best possible separation of bare and mercuriated single and double-stranded oligonucleotides. The results are shown for the C8 reverse phase column. A microLC 200 ultra HPLC system with an Inert Sustain C8, 100x0.3mm, 3μm column at 35° C with a gradient of eluent A, water + 2mM ammonium formate, and eluent B, methanol + 2mM ammonium formate, was used at a flow rate of 15μL/min. The injection volume was set to 7μL. 3-min pre-run flush at 100% starting condition was used to equilibrate the column.

### MS/MS Conditions:

An AB Sciex TripleTOF® 5600 system with Electrospray Ionization (ESI) probe was used in the negative ion mode. TOF MS scan was performed in the 100 to 2000 mass/charge range. Tandem mass spectrometric measurements (MS/MS) were performed in the product ion scan. MS/MS data were acquired at various fixed values of the laboratory frame collision energy.

### Data Interpretation:

AB SCIEX PeakView® software was utilized for the data interrogation. The Formula Finder function of the software was used to independently assess the elemental composition of the peaks of interest. A prototype oligonucleotide fragmentation interpretation tool (OligoViewer 0.9) was used to gain insights into the CID pathways of the components of the reaction mixture.

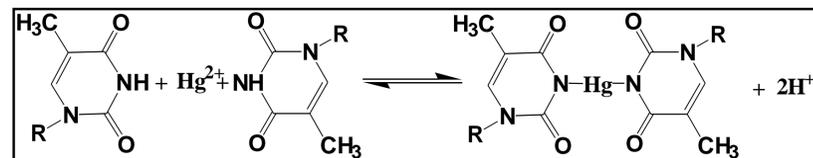


Figure 1. The reaction scheme of the T-Hg(II)-T base pair formation.

## RESULTS

Figure 2 provides the sum of the extracted ion chromatograms of the single-stranded ODN1 in different charge states (a). The added ion chromatograms were extracted from the TIC of a 20μM solution of ODN1. Utilization of a high resolution mass spectrometer enabled confirmation of the charge states of the observed ions as well as their fragments observed upon collision-induced dissociation of the ODNs. Examples c) and d) indicate a good correlation with theoretical monoisotopic masses calculated using OligoViewer 0.9 software. Upon incubation of ODN1 with 10 μM solution of mercury (II) acetate for at least 2 hours, a number of Hg(II)-containing species were observed as the result of LC separation.

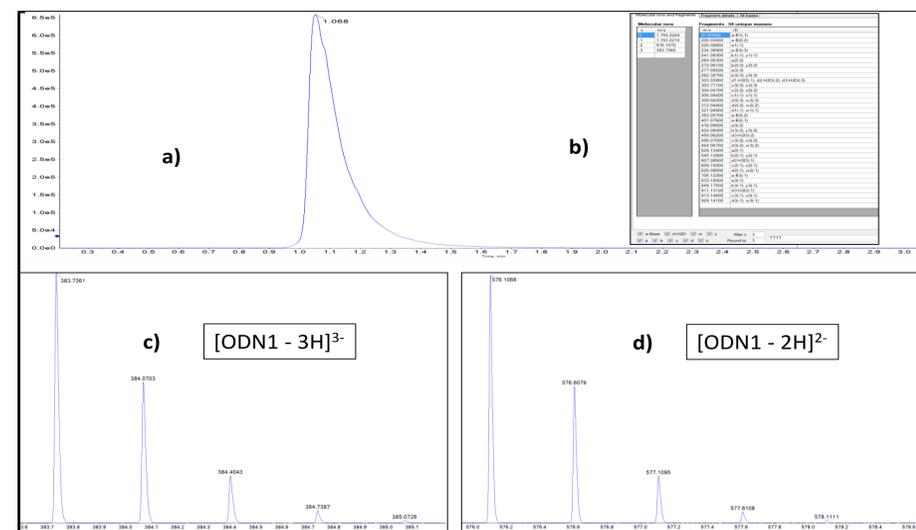


Figure 2. Sums of the extracted ion chromatograms of tetra-thymine oligonucleotide in three charge states - (a). Calculated monoisotopic *m/z* values for all possible ODN1 charge states - (b). Isotopic distribution of the single-stranded ODN1 in different charge states - (c) and (d).

It is essential to note that upon incubation of ODN1 with inorganic mercury, no significant presence of the single-stranded thymine adducts with Hg(II) was detected. Instead, multiply-mercuriated duplexes with the general formula [Hg<sub>n</sub>(ODN1)<sub>2</sub> - mH]<sup>l-</sup> with *l* = *m* - 2*n*, *n* = 3 and 4 always appear in the presence of mercury.

Among these species the predominantly observed species are of the general formula [Hg<sub>4</sub>(ODN1)<sub>2</sub> - mH]<sup>l-</sup>. Figure 3 provides the sum of the extracted ion chromatograms of the double-stranded tetra-mercuriated ions (a) as well as isotopic patterns for several observed charge states of the ion (b-d).

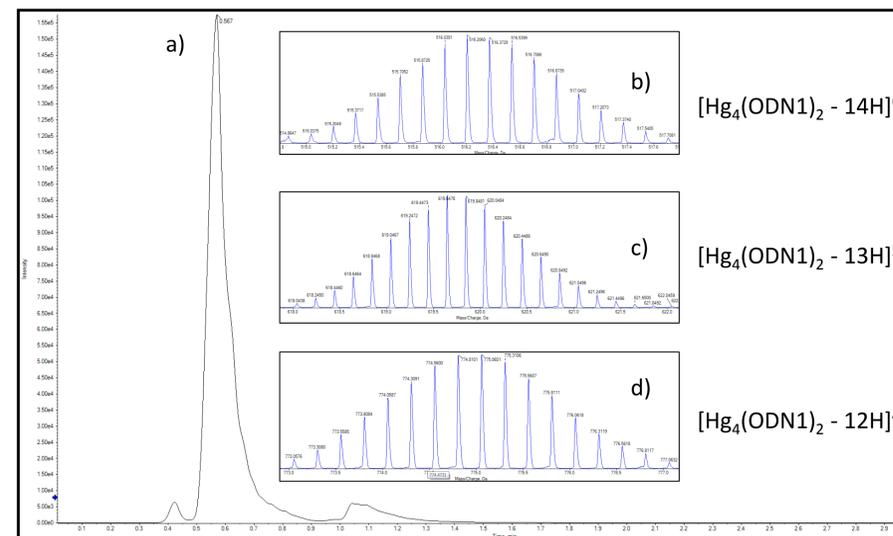


Figure 3. Sum of the XIC traces of the [Hg<sub>4</sub>(ODN1)<sub>2</sub> - mH]<sup>l-</sup> species in the observed charge states (a). Isotopic patterns of the mercury complexes in different charge states (b, c, d).

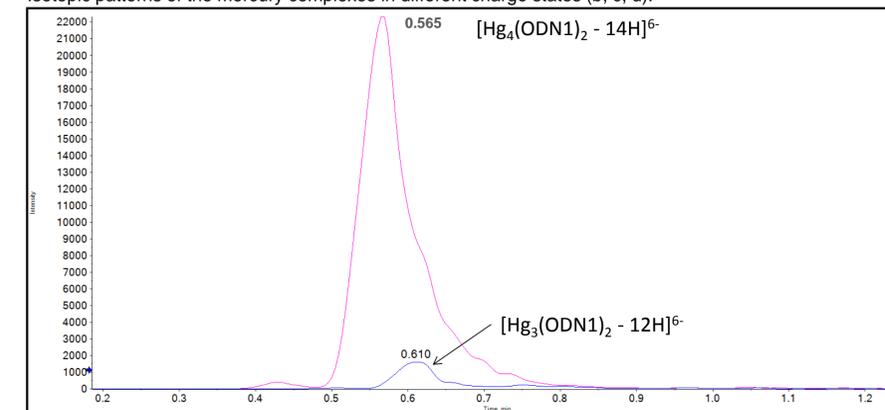


Figure 4. Extracted ion chromatograms of triply- and tetra-mercuriated duplex of ODN1 in the charge state of 6-.

No significant presence of singly and doubly-metallated duplexes was detected. Figure 4 provides a visual comparison of the XIC-s of triply- and quadruply-mercuriated duplexes of ODN1. Preferential formation of [Hg<sub>4</sub>(ODN1)<sub>2</sub> - mH]<sup>l-</sup> demonstrated in this study provides additional support of the hypothesis for the existence of the metallophilic interactions inside metallated DNA species theoretically predicted for Ag(I)-mediated imidazole-imidazole base pairs as well as thymine-Hg(II)-thymine pairs [7,8].

Complexation of ODN2 and ODN3 with Hg(II) seemed to occur with both single strands and duplexes. However, the formation of T-Hg(II)-T bridged structures was unambiguously detected via LC-MS/MS measurements (Figure 5).

