Structure of fluorescent metal clusters on a DNA template.

A A Vdovichev, T S Sych, Z V Reveguk, A A Smirnova, D A Maksimov, R R Ramazanov, A I Kononov

Department of Molecular Biophysics and Polymer Physics, St. Petersburg State University, St. Petersburg, Russia
E-mail: artvd.jr@gmail.com

Abstract. Luminescent metal clusters are a subject of growing interest in recent years due to their bright emission from visible to near infrared range. Detailed structure of the fluorescent complexes of Ag and other metal clusters with ligands still remains a challenging task. In this joint experimental and theoretical study we synthesized Ag-DNA complexes on a DNA oligonucleotide emitting in violet-green spectral range. The structure of DNA template was determined by means of various spectral measurements (CD, MS, XPS). Comparison of the experimental fluorescent excitation spectra and calculated absorption spectra for different QM/MM optimized structures allowed us to determine the detailed structure of the green cluster containing three silver atoms in the stem of the DNA hairpin structure stabilized by cytosine-Ag+ -cytosine bonds.

Introduction

A few-atomic metal clusters are a subject of increasing interest in the past decade due to their bright emission from visible to near infra-red range [1,2]. For example, DNA-stabilized Ag clusters, exhibiting excellent brightness and photostability, are considered as a new promising type of emitters for various applications in photonic devices, bioassays, chemical sensors, biosensors [3-10]. Detailed structure of the fluorescent complexes of Ag and other metal clusters with ligands still remains a challenging task. In this joint experimental and theoretical study we synthesized Ag-DNA complexes on a DNA oligonucleotide emitting in violet-green spectral range. The structure of DNA template was determined by means of various spectral measurements (CD, MS, XPS). Comparison of the experimental fluorescent excitation spectra and calculated absorption spectra for different QM/MM optimized structures allowed us to determine the detailed structure of the green-emitting cluster-DNA complex consisting of three silver atoms in the stem of the DNA hairpin structure stabilized by cytosine-Ag+ -cytosine bonds.

Methods.

Cluster synthesis. Oligonucleotide strand 5’-CCTCCTTCCTCC-3’ (BioBeagle ltd.) and AgNO₃ aqueous solutions were mixed and stored for 15 min at room temperature. After that, NaBH₄ aqueous solution was added followed by vigorous stirring. The final concentrations were C_DNA = 20 μM, C_AgNO₃ = 120 μM, and C_NaBH₄ = 30 μM. The sample was kept in the dark about 1 month at 21 °C to reach maximum fluorescence.

Theoretical methods. DNA-cluster complex equilibrium structures were obtained with use of CP2K[11] program by utilizing computational protocol comprising of several steps. Initially from a nucleotide sequence described above was obtained the equilibrium DNA-hairpin structure stabilized by 4 silver ions in explicit solvent environment (Figure 1A). For this purpose we performed geometry optimization and dynamic equilibration of suggested structure by use of QM/MM scheme. The QM part is comprised of orthogonal box 19.73x18.12x33.16 Å, all nitrogenous bases and 4 silver ions linking of 4 cytosine pairs,
while the MM part is comprised of orthogonal water box 3x3x4 nm and sugar-phosphate backbone of DNA. The full charge of the QM/MM system was set to zero by adding of corresponded number of counterions Na+. QM/MM calculations where performed by use of pseudopotential plane-wave density functional theory in reciprocal space, namely pseudopotentials of Goedecker, Teter and Hutter (GTH)[12] for core electrons, molecularly optimized DZVP-MOLOPT-GTH basis set for valence electrons and hybrid functional PBE[13]. The model of DNA in MM part utilized periodic boundary conditions, TIP3P water solvent and parmbsc0 forcefield[14]. Subsequent MD equilibration performed in the NPT ensemble using a Nosé-Hoover thermostat with a 1 fs integration step. The obtained hairpin structure further was used as a template for Ag cluster formation.

![Figure 1. A) DNA-Ag⁺ equilibrium hairpin model structure obtained by QM/MM optimization protocol; B) DNA-silver green-emitting model structure obtained on the hairpin template after adding of one metal silver atom.](image)

Based on the obtained hairpin structure we constructed a QM part comprising of 4 silver ions, 1 metal silver atom and all of the nucleobases (Figure 1B). After QM/MM optimization procedure and addition dynamic equilibration the equilibrium cluster-nucleotide complexes where extracted for gas phase electronic spectra calculations. Calculations of absorption spectra were performed by use of the second order algebraic-diagrammatic construction scheme (ADC(2)[15]) method and def2-SVP basis set with Turbomole program[17].

**Results**

A palindrome 12-mer DNA sequence 5’-CCTCCTTCCTCC-3’ was used as a template for Ag cluster formation and theoretical investigations. The corresponding fluorescent emission and excitation spectra are shown in Figure 2.

![Figure 2.](image)

The major fluorescent fraction of the Ag-DNA complex has a maximum at about 520 nm. As has been shown previously [17], CD spectrum of the complex of DNA with Ag cations indicates formation of C-C pairs mediated by cytosine-Ag⁺-cytosine bonds. Since MS spectra show single-stranded DNA-Ag⁺ adducts only, it means formation of hairpin rather than a self-dimer structure. XPS analysis gives about 0.5 Ag/P ratio, suggesting that all silver cations are indeed bound with DNA matrix.

Comparison of experimental luminescent excitation spectra and preliminary calculated excitation spectra of different QM optimized isolated clusters and cytosine-cluster complexes consisting up to 3 silver atoms showed that green fluorescent silver clusters included not more than 3 silver atoms. We obtained an equilibrium structure of the DNA-cluster complex consisting of 3 silver atoms in fluorescent site in the stem of the DNA hairpin structure stabilized by cytosine-Ag⁺-cytosine bonds. This structure obtained with QM/MM approach is shown in Figure 3.

![Figure 3.](image)
Figure 2. Experimental emission and excitation spectra of green-emitting Ag clusters-DNA complex.

A reasonable agreement between the electronic spectrum of the complex, calculated by the ADC(2), and the experimental fluorescence excitation spectrum supports the model of the fluorescent DNA-cluster complex. The proposed approach can be further used for determining the structure of other ligand-stabilized fluorescent metal clusters.

Figure 3. Equilibrium structure of DNA and Ag$_3^{3+}$ green-emitting cluster and corresponded experimental and calculated excitation spectra.

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