

Synthetic Intermediate Research Report

on project achievement in the whole execution period until present (September 2nd, 2013 – December 10th, 2014)

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Structural changes of LacDNA molecules [d(TAATGTGAGTTAGCTCACTCAT)-d(ATGAGTGAGCTAACTCACATTA)] induced by low pH treatment, in the absence of divalent metal ions are exemplified in detail by our experimental results. Large changes in the UV (275 nm) resonance Raman spectrum of LacDNA duplex were observed at acidic pH, in the absence of divalent metal ions, as compared with the corresponding spectrum at a reduced pH value. Main changes are: adenine protonation, GC base pair protonation, DNA bases unstacking and changes in the hydrogen bonding strength between the strands of LacDNA complex.

Because of the assumption, that Hoogsteen *syn* dG:dCH⁺ (guanine-cytosine) base pairs exist in DNA systems at acidic pH, numerical simulations of vibrational spectra (Raman and IR) of GC base pairs in DNA have been performed, in Watson-Crick (nonprotonated) and Hoogsteen (protonated) configurations, by including the anharmonic and solvent effects, respectively, in the theoretical calculations.

Using the DFT method with M06-2X meta-hybrid GGA exchange-correlation functional, including the anharmonic corrections and solvent effects, the theoretical IR and Raman spectra of the guanine-cytosine DNA base pairs in Watson-Crick and Hoogsteen configurations were computed. Our previously calculated values obtained with the B3PW91 hybrid GGA functional were compared with the results for harmonic frequencies and their anharmonic corrections. For the anharmonic corrections calculated with the two different DFT functionals, especially for the stretching modes, significant differences were obtained, while the corresponding harmonic frequencies did not differ considerable. The H⁺ vibration within the Hoogsteen GC base pair can be characterized as an asymmetric Duffing oscillator. Therefore unrealistic anharmonic corrections for normal modes, where this proton vibration is involved, have been

obtained. We have also discussed the spectral modification due to the anharmonic corrections, solvent effects and the influence of sugar-phosphate group for the Watson-Crick and Hoogsteen base pair configurations, respectively. Besides, the influence of stacking interaction on the theoretical IR and Raman spectra was analyzed. If one wants to obtain correct assignments of the theoretical frequency values, as compared with the experimental spectra, including the anharmonic correction in the normal mode analysis is essential for Watson-Crick configuration.

Divalent metal ions (Mn^{2+} and Ca^{2+}) were found to inhibit LacDNA protonation even at low concentrations. In this regard, Mn^{2+} ions are much more effective as compared with Ca^{2+} ions. A small influence of the divalent metal ions on binding of proton to LacDNA has been found in the case of Ca^{2+} . Binding of proton to LacDNA, in the presence of Mn^{2+} ions, seems to take place especially to adenine, as observed from the analysis of the bands at 1312 cm^{-1} (dA), 1347 cm^{-1} (dA) and 1585 cm^{-1} (dA, dG), respectively. Also, proton binding to N7 atom of guanine is not significant, in the presence of Mn^{2+} ions, as judged from the UV (275 nm) resonance Raman band around 1491 cm^{-1} [dG(N7), dA].

Moreover, structural changes of LacDNA molecules, induced at a reduced pH value, in the presence of divalent metal ions Mn^{2+} and Ca^{2+} have been discussed. As far as this aspect is concerned, binding of Mn^{2+} ions to N7 of guanine and, possibly, in a lesser extent to adenine was observed, as judging from the UV resonance Raman bands near 1314 cm^{-1} (dA), 1348 cm^{-1} (dA) and 1491 cm^{-1} [dG(N7), dA]. Low affinity of Ca^{2+} ions for binding to LacDNA molecules, at a reduced pH value, has been found.

Also, in different physico-chemical conditions, changes in the hydrogen bonding strength between the strands of LacDNA molecules and changes of the stacking interaction in the double-helical complexes were observed.

Present researches include principal components analysis (PCA) and hierarchical clusters analysis (HCA), characteristic to the UV resonance Raman spectra of double helical LacDNA molecule [d(TAATGTGAGTTAGCTCACTCAT)-d(ATGAGTGAGCTA-ACTCACATTA)].

In the followings, the main results on the theoretical calculations of the effects of some divalent metal cations on the vibrational properties of GC and AT base pairs in DNA are given.

We present a DFT investigation of the vibrational properties of GC Watson-Crick and Hoogsteen base pairs, in the presence of Mg^{2+} , Ca^{2+} and Cu^{2+} ions. In this concern we have found, that if metals are bound at the N7 atom of guanine (in Watson-Crick structure) and at the N3 atom of guanine (in Hoogsteen structure), respectively, stable configurations are formed.

In order to take into account the effect of the molecular environment on the metal cation, the geometric model developed by us included the presence of five water molecules surrounding the metallic atom. The water molecules are distributed around the metal cation in an almost symmetric structure, as revealed by the analysis of the geometric properties. In the case of Watson-Crick configuration, the nitrogen-metal distances are slightly smaller. For the Watson Crick structure, the Ca and Mg are localized close to the GC plane. Also, for the Hoogsteen configuration Cu has the tendency to stay close to this plane. For all other configurations, a slight displacement of the metallic atom from the GC plane was noticed.

We have found, that the bands to be used as markers for the detection of the Ca^{2+} and Mg^{2+} ions are different from those allowing the detection of the Cu^{2+} ions, as suggested by the vibrational density of states (VbDOS), projected over the metallic atoms. Metal's vibrational amplitudes are important for the wavenumbers around 400 cm^{-1} , in the case of Ca^{2+} and Mg^{2+} . Also, around $100\text{-}150\text{ cm}^{-1}$, a second set of large peaks for the metal's vibrational amplitudes may be seen (depending on the configuration). Both configurations indicate an important peak around 260 cm^{-1} for Cu^{2+} ions, with relatively small differences between Watson-Crick and Hoogsteen structures.

A DFT investigation of the vibrational properties of AT base pairs, in the presence of Ca^{2+} and Mn^{2+} ions, is also presented in this work. We have found, that if metals are bound at the N7 atom of adenine, stable configurations are formed. The Mn^{2+} ions can also coordinate to the N3 atom of adenine, while for the Ca^{2+} we have found a strong deformation for this geometry.

In order to take into account the effect of local molecular environment on the metal cation, the geometric models used in our calculation, include the presence of five water molecules surrounding the metallic atom. The water molecules are distributed around the metal cation in an almost symmetric structure, as revealed by the analysis of the geometric properties.

The bands to be used as markers for the detection of Ca^{2+} and Mn^{2+} ions are located in the range 300-400 cm^{-1} and around 150-170 cm^{-1} , as suggested by the vibrational density of states (VbDOS), projected over the metallic atoms. Two vibrational bands are obtained also in the range 600-700 cm^{-1} , for the complex Mn^{2+} -AT with Mn atom coordinated to the N7 atom of adenine.

Detailed results of this synthetic intermediate research report can be found in the following disseminated papers.

1. A. Bende, C. M. Muntean (2014) "The influence of anharmonic and solvent effects on the theoretical vibrational spectra of the guanine-cytosine base pairs in Watson-Crick and Hoogsteen configurations", Journal of Molecular Modeling, Vol. 20, Nr. 3, Article Nr. 2113, 1-12.

<http://link.springer.com/article/10.1007/s00894-014-2113-z>

2. C. Morari, C. M. Muntean, C. Tripon, L. Buimaga-Iarinca, A. Calborean (2014) "DFT investigation of the vibrational properties of GC Watson-Crick and Hoogsteen base pairs in the presence of Mg^{2+} , Ca^{2+} and Cu^{2+} ions", Journal of Molecular Modeling, Vol. 20, Nr. 4, Article Nr. 2220, 1-7.

<http://link.springer.com/article/10.1007/s00894-014-2220-x>

In addition to the synthetic intermediate research report, full length research reports (63 pages) were submitted to the beneficiary.

Project manager,

A handwritten signature in blue ink, appearing to read 'Cristina M. Muntean', is centered below the text 'Project manager,'.

Dr. Cristina M. Muntean