NOFIMA AS, AS (NORWAY)

UNIVERSIDAD COMPLUTENSE DE MADRID

Simulation of the three phosphorylation steps of several nucleoside reverse transcriptase inhibitors of HIV virus by MP2 ab initio method and M06-L and M06-2x DFT quantum chemical methods.

Experimental interpretation of these steps by infrared and Laser Raman vibrational spectroscopy

D. Kattan[a,b], M. Alcolea Palafox[a,b], N. Kristian Afseth[b] and U. Böcker[b]

[a]Departamento de Química-Física I, Facultad de Ciencias Químicas,Universidad Complutense, Madrid-28040, SPAIN

[b]Nofima AS the Norwegian Institute of Food, Fisheries and Aquaculture Research, PB 210, N-1431, Ås, Norway

NILS Science and Sustainability (ES07)
Coordinated Mobility of Researchers (ABEL-CM-2014A)
Supported by a grant from Iceland, Liechtenstein and Norway through the EEA Financial Mechanism.
Operated by Universidad Complutense de Madrid.

Abstract

• The conformational analysis and the hydration of two of the most used anti-HIV nucleoside analogues were carried out.

• The study was performed on ATP, Thymidine, AZT and D4T.

• ATP are molecules that cooperate in the transformation of nucleosides to nucleotides and incorporate them to our DNA.

• Thymidine (dT) is a natural nucleoside in our body.

• AZT and D4T are prodrugs that are used to stop the viral DNA of HIV.

Introduction

Human Immunodeficiency Virus (HIV) infects every year many people in the world. Unfortunately, the efficacy of the anti-virus used in clinic is very small. Our project presented is focused on anti-HIV drugs and in the three phosphorylation steps (fig. 1) by the corresponding Thymidine kinase enzymes. The main anti-HIV drug used in these days in the clinic is AZT but in fact it affects a lot the patients due to its secondary effects. The secondary effects come from the fact that this antidrug is used in a high concentration in order to pass the first phosphorylation step that is the crucial one to have a higher efficacy of the antidrug. In order to reduce secondary effects on patients and the concentration used of antidrug, we have developped the study to know why the low efficiency of this first phosphorylation step with the prodrugs.

D4T that is another anti-HIV drug that was used. Theoretical study is led by DFT methods and by ab initio. Experimental infrared and laser-Raman spectroscopy are used for the experimental study part.

Objectives

***I.** – The determination of the most stable conformer in the isolated state and in water solution of the anti-HIV nucleosides analogues AZT, D4T and Nikavir, as well as the natural nucleoside deoxythymidine (dT) and the adenosine 5´-triphosphate (ATP) molecule.

***II.** – The accurate characterization of the bands of whole experimental Raman spectra in water solution of all the molecules under study, as well as the IR bands using the special microhydration technique developed by us.

***III.** – The simulation of the crystal unit cell of all the molecules under study, and the further determination of their IR and Raman spectra. A detailed assignment of whole experimental IR and Raman bands of all the molecules under study in the solid state.

***IV.** – The resolution of the first phosphorylation step mechanism of the anti-HIV nucleosides under study, as well as on dT, through a simplified model which includes the interaction with the optimum structure of ATP, firstly in the isolated state, and after in water solution with different number of water molecules surrounding the system.

***V.** – The experimental interpretation of ATP interaction with the molecules under study in the first phosphorylation step through an accurate characterization of all the bands in the IR and FT-Raman spectra.

***VI.** - The determination of the second and third phosphorylation steps mechanisms of the mono- and diphosphate forms of the anti-HIV nucleosides under study, as well as on dT, through a simplified model which includes the interaction with the optimum structure of ATP, firstly in the isolated state, and after in water solution with different number of water molecules surrounding the system.

***VII.** – The detailed analysis of the last step through the bonding of the triphosphorylated anti-HIV nucleosides analogues in the DNA viral through the reverse transcriptase enzyme (RT) in a simplified model of the enzyme cavity with few residues.

Calculations

They were mainly carried out by using Density Functional methods (DFT), because they provide a very good overall description of medium-size molecules. Moreover, for the wavenumber calculations [1,2] they appear more accurate than HF and MP2, and at lower computational cost. Among the DFT methods, B3LYP is the most popular which uses a combination of the Becke's three-parameter exchange functional (B3) [3] and the correlation functional LYP (Lee, Yang, Parr) [4]. Because results obtained with both correlation functionals are similar, and also for simplicity, only the B3LYP results are given here. The default fine integration grid was employed. MP2 calculations were also carried out to confirm the geometry structure and atomic charges. These methods are implemented in the GAUSSIAN 09 [5] program package.
Basis set used was *6-31G****.

Fig.9 IR instrument at NOFIMA AS.

Fig.10 Raman instrument at NOFIMA AS.

Experimental

ATP, Thymidine, AZT and D4T were obtained from M/s Aldrich Chemicals, Germany with a higher pourcentage of purity. Then the IR spectra at 4000-400 cm^{-1} range of ATP, Thymidine, AZT and D4T in powder form (Fig. 2) were recorded using KBr pellets (with 2 mg sample per 100 mg KBr) and by using ATR (Fig. 4), 32 scans were collected at 0,5 cm^{-1} resolution. Then we have performed the same study with microhydration technique developed by us on KBr pellets (Fig. 5) with the same resolution and the spectra on ATR (Fig. 3) were collected with the same conditions, both microhydrations were performed at different concentrations.
Then the Raman-Laser spectra in the 0-3500 cm^{-1} range of the same compounds were collected in powder form (Fig. 6 and Fig. 7) and after hydration. at different concentrations.
And in order to see interaction between ATP- anti-drugs, we have performed the same procedure in order to collect the Raman spectra at different concentrations (Fig. 8).

Summary and Conclusions

(1)-The molecular structure in water solution of three of the most important anti-HIV nucleosides used in clinic was determined.

(2)-The mechanism of the first phosphorylation step with D4T, AZT and dT nucleosides, including the energies involved in the interaction with ATP was described.

(3)-The first phosphorylation step through the experimental IR and Raman spectra in water solution was interpreted.

(4)-We determined the theoretical and experimental mechanisms of the second and third phosphorylation step of the mono- and diphosphate forms of the anti-HIV nucleosides under study, as well as on dT.

All the results obtained will help the scientific community in the design of new anti-HIV with high activity and could help for developing drugs with high anti-HIV activity and low toxicity. Thus, the expected impact of such results will be of great importance for the millions of the HIV infected people.

Below are some representative spectra recorded on IR and Raman instruments in NOFIMA AS:

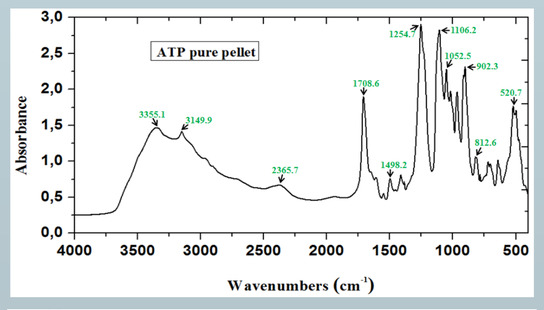

Fig.2 Experimental IR spectrum for ATP molecule.

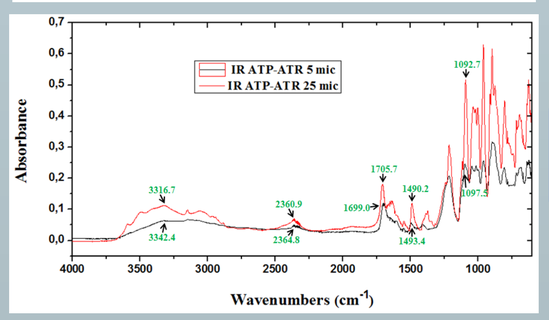

Fig.3 Experimental IR spectrum for microhydration of ATP molecule at different concentrations.

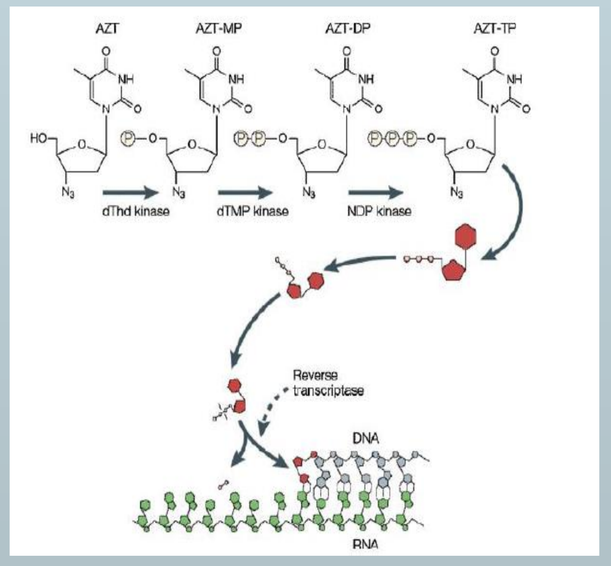

Fig.1 The 3 phosphorylation steps for AZT molecule. The same process as for D4T.

References

1. (a) Alcolea Palafox M, *Recent Res. Devel. in Physical Chem*, 2 (1998) 213. (b) Alcolea Palafox M, *Int. J. Quantum Chem*., 77 (2000) 661.
2. Alcolea Palafox M; Rastogi VK, *Spectrochim Acta*, 58A (2002) 411.
3. Becke A D, *Phys Rev A*, 38 (1988) 3098.
4. Lee C, Yang W, Parr R G, *Phys Rev*, B37 (1988) 785.
5. Gaussian 09, Revision D.01, M.J. Frisch, et al., Gaussian, Inc., Wallingford CT, 2009.
6. Palafox M A, Rastogi V K, In *Perspectives in Modern Optics and Optical Instrumentation*, Sharma A, Joseph J, Rastogi, V K, Eds; Anita Publications, Delhi, Ghaziabad, India, 2002, p 91.
7. Rastogi V K, Palafox V K, *Spectrochim Acta*, A58 (2002) 411-440.

Publications

(under the present proyect)

With the NILS Programme appeared in Acknowledgements and with the Nofima AS address in Norway:

1. *Physical Chemistry Chemical Physics*, **16**, 24763-24783 (2014) **DOI:** 10.1039/c4cp03695f
2. *Asian J. of Physics*, **24** (1), 33-59 (2015). **ISSN** : 0971 - 3093
3. *Asian J. of Physics*, **24** (5) 751-769 (2015). **ISSN** : 0971 - 3093
4. *Chemical informatics, 1 (2:11) 1-13* (2015). **ISSN**: 2470-6973. Open access www.cheminformatics.imedpub.com/archive.php
5. *Journal of Molecular Structure*, **1106** 300-315 (2016). **DOI**: 10.1016/j.molstruc.2015.10.096
6. *Journal of Molecular Structure*, **1108,** 482-495 (2016). **DOI:** 10.1016/j.molstruc.2015.12.004
7. *Journal of Molecular Structure*, **1111,** 166-179 (2016). **DOI:** 10.1016/j.molstruc.2016.01.067
8. *Australian J. Chemistry,* in press (2016). **DOI:** 10.1071/ch15793
9. *Asian Chem Letts*, **20** (1-2) in press (2016). **ISSN** : 0971 - 9822
10. *Asian J. Physics*, **25** (2) in press (2016). **ISSN** : 0971 - 3093

With the Nofima AS address in Norway:

11. *Asian Chem Letts*, **19** (1), 1-25 (2015). **ISSN** : 0971 - 9822
12. *Journal of Applied Solution Chemistry and Modeling*, **5,** 30-47 (2016). **DOI:** 10.6000/1929-5030.2016.05.01.3

The following Congress:

1. *National Conference on Role of Optics and Philosophy in Environment Protection, ROPEP-2015*, pp. 37 . Tinsukia (India). 10-11 Marzo (2015)
2. *National Conference on Role of Optics and Philosophy in Environment Protection, ROPEP-2015*, pp. 38-39. Tinsukia (India). 10-11 Marzo (2015)
3. *16th European Conference on the Spectroscopy of Biological Molecules, ECSBM-2015* Topic: Biomolecular Simulations, ID: 71, P53. Ruhr, Bochum (Alemania). 6-10 Septiembre (2015)
4. *103rd Indian Scince Congress 2016, ISC-2016, accepted*. University of Mysore, Crawford Hall, Mysuru, Karnataka 570005 (India). 3-7 January (2016)

Future collaborations

We are under collaboration with Nils Kristian Afseth and Unrike Böcker, researchers in the biospectroscopy group, in NOFIMA AS. Also we are in the way to publish several pending works.

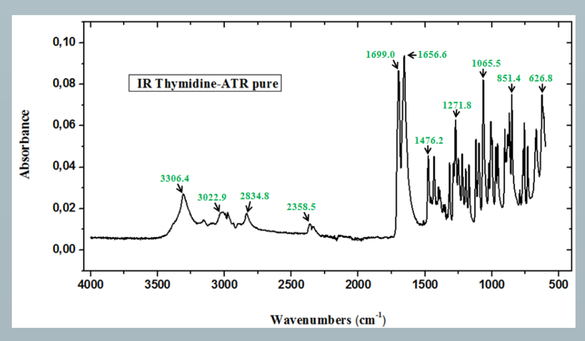

Fig.4 Experimental IR spectrum on ATR for Thymidine molecule.

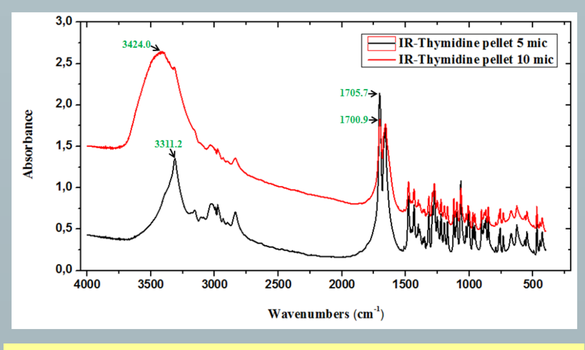

Fig.5 Experimental IR spectra for Thymidine molecule on pellet at different concentrations.

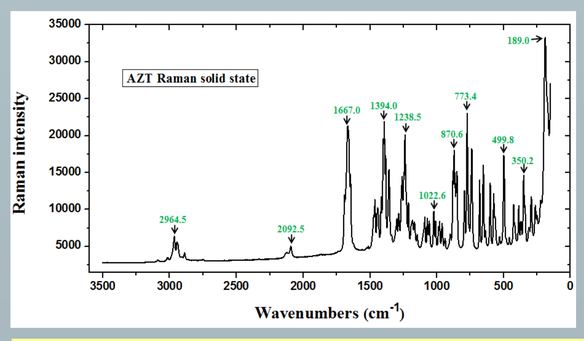

Fig.6 Experimental Raman spectrum for AZT molecule.

Organization of the Workshop

"Raman and Fluorescence spectroscopy applications in life sciences" from the NILS Science and Sustainability Programme

Nofima Research Institute, Ås (Norway)
September 23th (2015)
NILS Science and Sustainability Programme (ES07-EEA Grants). Convocatory code: 005-BBRR

Acknowledgements

We wish to thank to NILS Science and Sustainability Programme (ES07), 002-ABEL-CM-2014A Call for financial support. Supported by a grant from Iceland, Liechtenstein and Norway through the EEA Financial Mechanism. Operated by Universidad Complutense de Madrid.

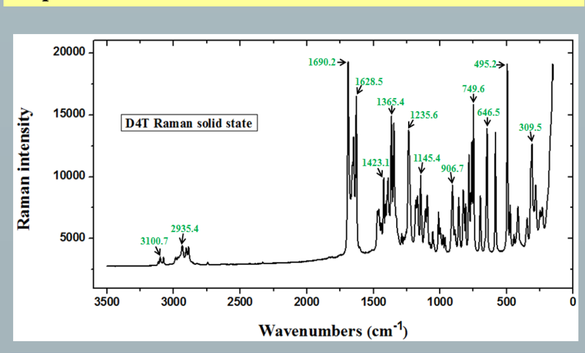

Fig.7 Experimental Raman spectrum for D4T molecule.

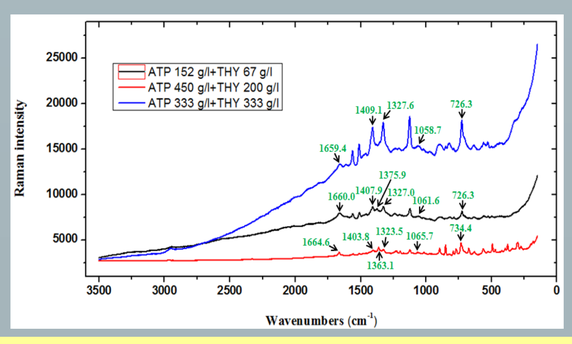

Fig.8 Experimental Raman spectrum for ATP-Thymidine interaction at different concentrations.