COMPUTATIONAL STUDY OF CYTOSINE INTERACTION WITH UV LIGHT

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Abstract. DNA interaction with UV radiation is an actual problem for medicine and biology, as prolonged sun exposure is the cause for the majority of skin cancers. This paper is focused on the computational modeling of UV light interaction with cytosine nucleotide by applying quantum chemical approach based on the semi-empirical method. The comparison of some energetic and structural parameters of cytosine nucleotide and its two derivatives resulted following UV radiation absorption has evidenced the linear dependence of dipole moment and total energy as provided by optimized structure analysis.

Key words: DNA nucleotide, UV radiation, quantum chemical method.

INTRODUCTION

There is a large range of physical, chemical and biological factors that can induce DNA damage, such as electromagnetic radiation [7, 23, 25], pesticides [17], tobacco [3]. One of the most common physical factors that causes DNA damage is ultraviolet light, due to constantly increasing UV radiation level at Earth's surface [23]. Numerous experimental studies have shown that the most important mechanisms underlying UV energy effect leading to DNA damage is formation of cyclobutane–pyrimidine dimers and 6–4 photoproducts [3, 4, 5, 9, 10, 19, 20, 24, 26]. These photoproducts are responsible for cell mortality, mutagenesis and induction of skin cancer in case of intensive UV light exposure [18, 21].

The understanding of the consequences of cyclobutane-pyrimidine dimer formation on the DNA structure could help in finding methods that will overcome the effects induced by UV exposure. Thus, numerous experimental and computational studies have been accomplished in order to obtain more information

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about the structural and energetic modifications UV induced in the structure of DNA [6].

When UV light interacts with cytosine, the photon breaks a double carbon bond and forms a single bond between these two carbons. The bonds are saturated by a radiolysed water molecule – one of the carbons attaches hydrogen and the other carbon attaches the hydroxyl group. This process is called cytosine hydration.

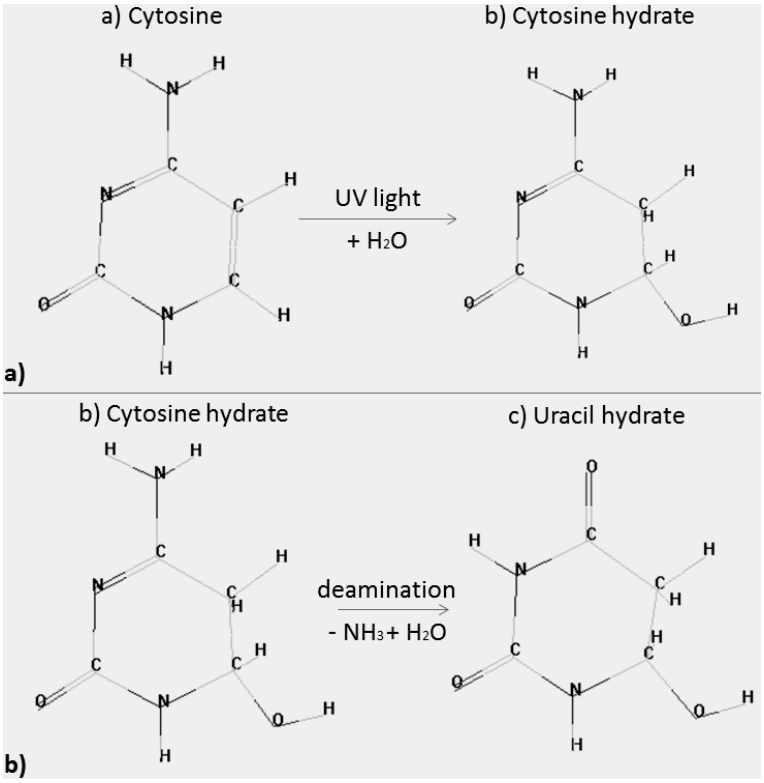

Fig. 1. Modifications in the structure of cytosine after hydration and deamination processes.

In this paper we present our computational approach on cytosine (4-amino-2-oxopyrimidine) transformation into hydrated uracil (2,4-dioxopyrimidine) aiming to compare the optimized structures of cytosine nucleotide derivatives with focus on some physical parameters that have changed by a UV light absorption mechanism.

In DNA molecule, cytosine is paired with guanine via two hydrogen bonds. But due to the fact that it is inherently unstable, cytosine hydrate can undergo a process called spontaneous deamination – through which it loses an ammonia group (NH_3) and again attaches one molecule of water. The resulting molecule is uracil hydrate. If this modification is not repaired by specialized enzymes, the deamination process can lead to a point mutation, also known as single base

substitution, which represents the replacement of a single DNA base with another one. According to base pairing rules, uracil is bounded to adenine through two hydrogen bonds.

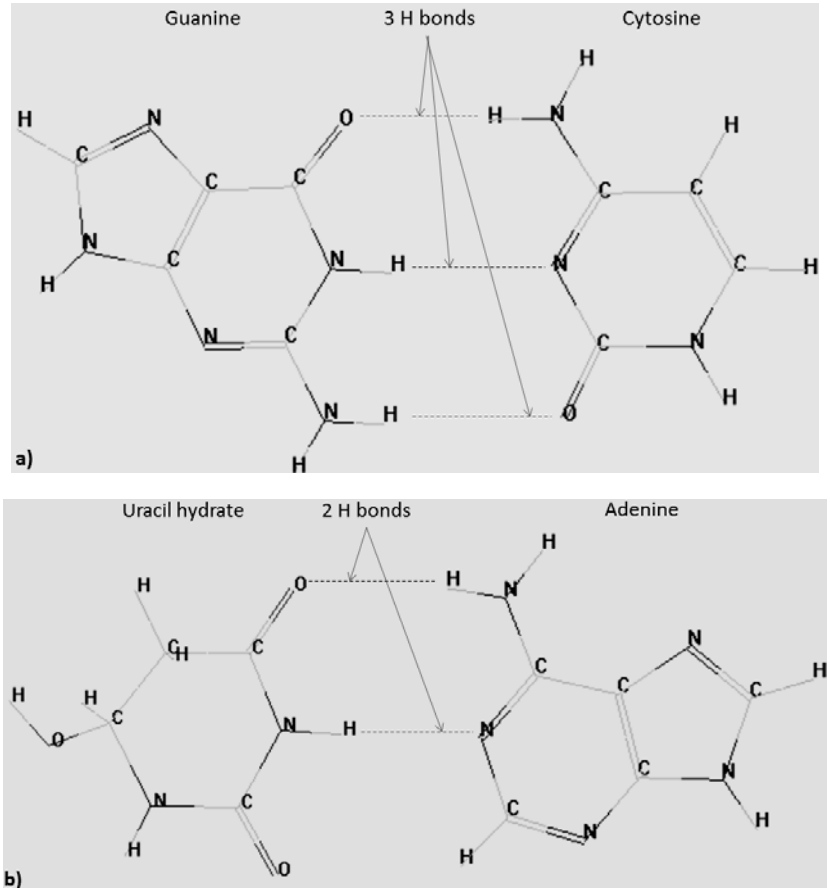

Fig. 2. Hydrogen bonds established between guanine and cytosine, respectively between uracil hydrate and adenine.

MATERIALS AND METHODS

In this paper we present the structural and energetic results obtained for cytosine, hydrated cytosine and hydrated uracil nucleotides using a quantum mechanical method – PM3 based on semi-empirical calculations that was carried out in the molecular modeling program – HyperChem^{TM} 8.0.10. Cytosine molecule used for this computational method was R-Cytosine (a cytosine nucleotide) from HyperChem databases. All further modifications (hydration and deamination) were

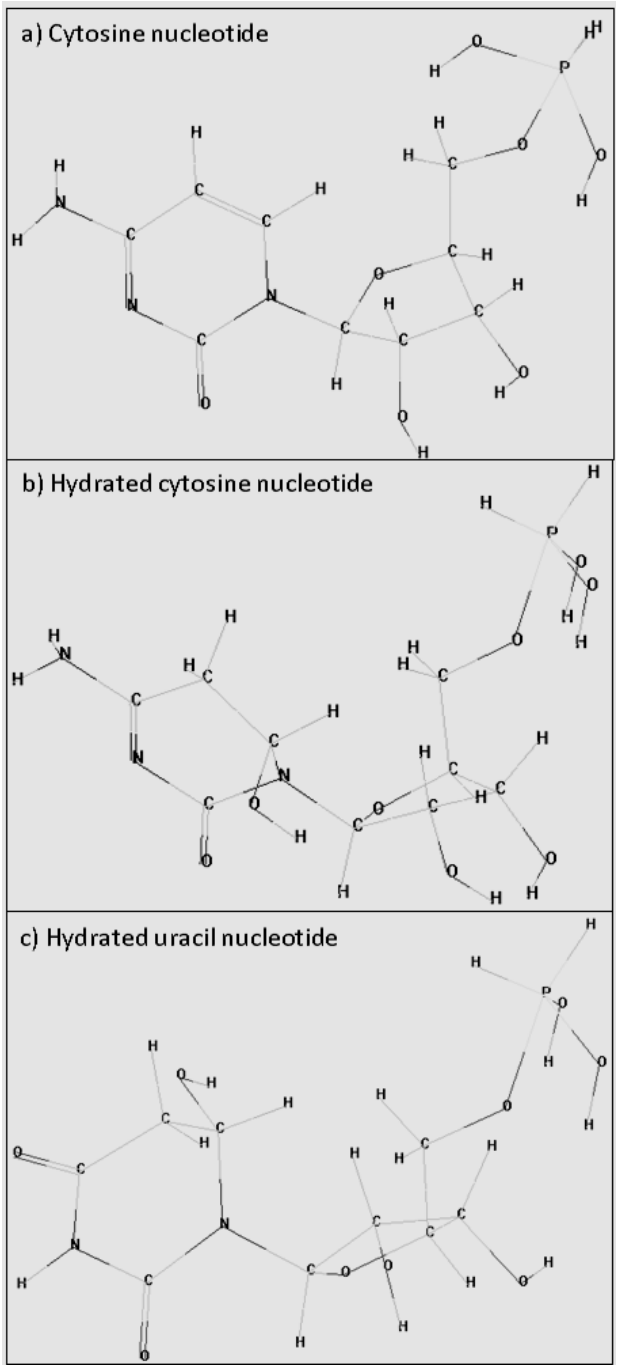

Fig. 3. Optimized structures resulted from quantum chemical modeling for a) cytosine, b) hydrated cytosine, respectively c) hydrated uracil.

simulated on this nucleotide. Polak-Ribiere optimization algorithm was used, including Restricted Hartree-Fock wavefunction with the convergence limit of 0.0001 and criterion of RMS gradient of 0.0001 kcal/(Å·mol). *Ab initio* Hartree–Fock calculations were conducted by settings similar to those used by Hutter [11].

RESULTS AND DISCUSSION

Optimized structures of R-cytosine are presented in the diagrams of Figure 3. As one can see from Fig. 3a, initially, cytosine molecule ($C_4H_5N_3O$) has a planar geometry, but when the double carbon bond is broken and a radiolysed water molecule is attached, forming cytosine hydrate (Fig. 3b), geometrical structure has a 3D (non planar) configuration (due to C–OH and C–H bonds, that come out of cytosine's plane). In case of uracil hydrate (Fig. 3c), geometry seems not to differ much from that of the cytosine hydrate.

The main changes resulted from computational modeling and presented in Fig. 4 were induced at the levels of carbon atoms initially related by double bond broken down by radiation impact, thus the electric charge of one carbon diminished about 3 times (from –0.288 to –0.093), while the other carbon charge increased from –0.043 to 0.114; also the charge of the nitrogen atom in the immediate vicinity of the last mentioned carbon atom has changed from 0.045 to –0.160 (Fig. 4a–b).

In case of uracil hydrate (Fig. 4c), the charge of the carbon connected by a double bond with a nitrogen atom increased more than 6 times (from 0.043 to 0.264), while the charge of the nitrogen atom decreased by a factor of almost 9 (from –0.247 to –0.028). Changes in the electrostatic potential map showed a remarkable difference between non-irradiated cytosine nucleotide (Fig. 5a) and the next intermediate product (Fig. 5b): the density of equipotential lines that was almost uniformly distributed on the three constitutive parts of the studied cytosine nucleotide (cytosine, deoxyribose and phosphate group) changed the configuration after the absorption of UV energy – equipotential lines are concentrated now mostly on the cytosine and deoxyribose. Further elimination of NH_3 group resulted in new electrostatic potential distribution, covering partially the cytosine, phosphate and deoxyribose groups, which suggested the formation of a new stable structure (Fig. 5c).

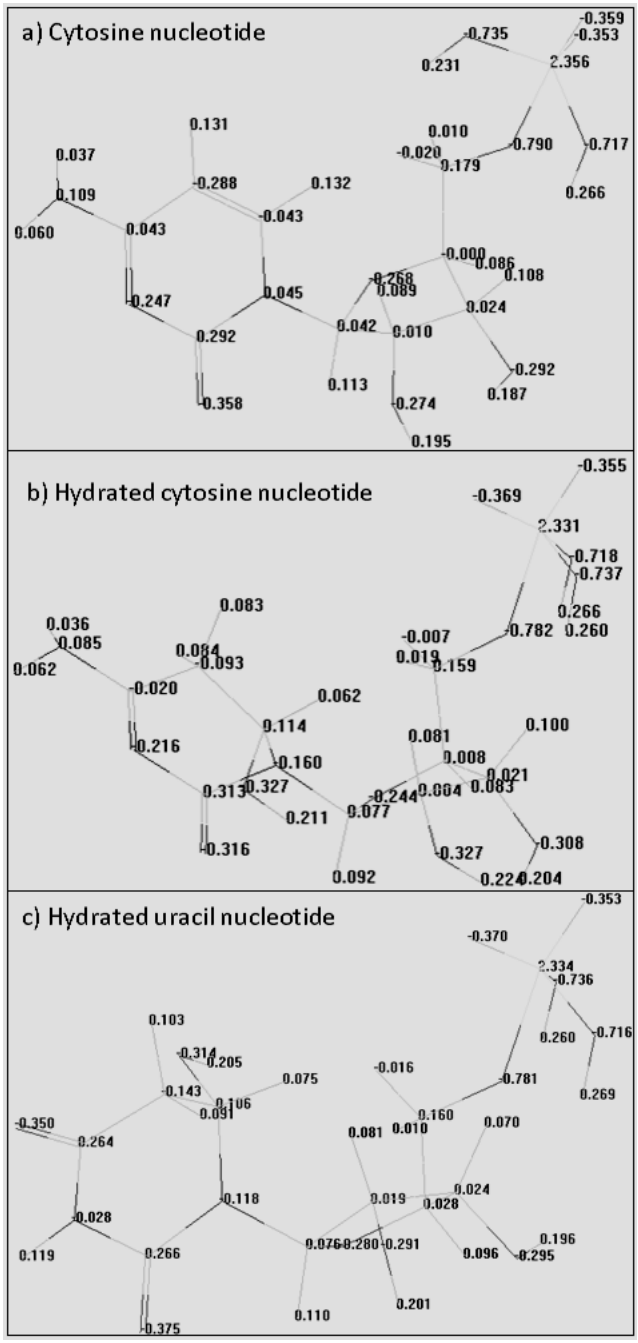

Fig. 4. Charge values for each atom of the three related molecular complexes as resulted from computational modeling.

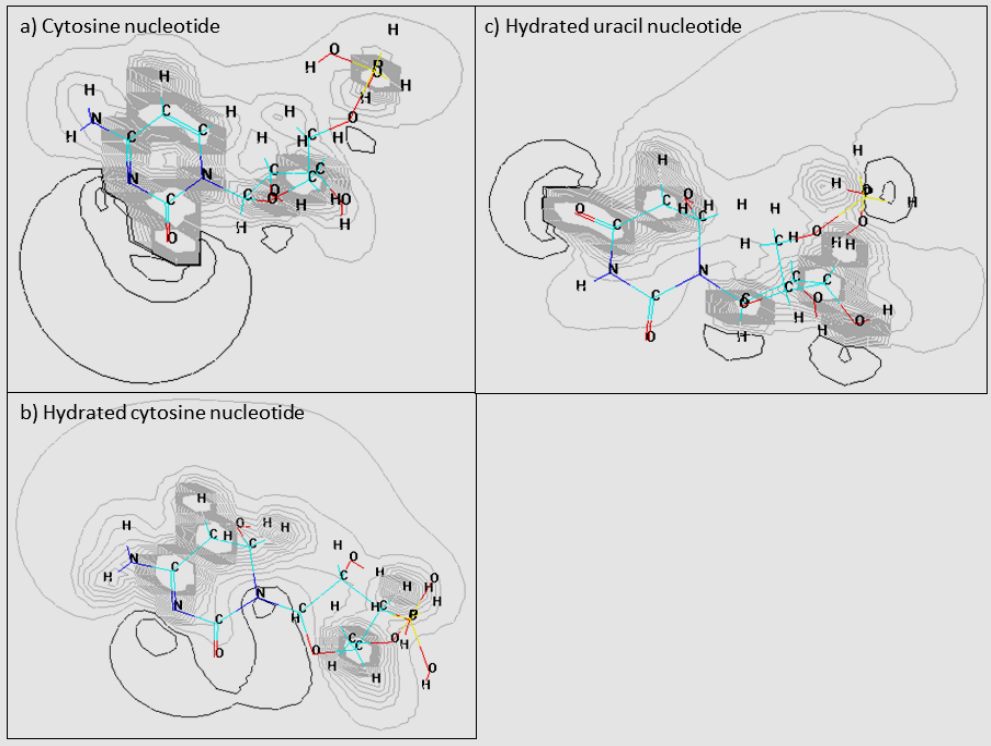

Fig. 5. Electrostatic potential maps for a) cytosine, b) hydrated cytosine, respectively c) hydrated uracil.

Charge density maps (Fig. 6a, b, and c) were simulated further. The isolated cytosine charge density map was also studied with a different computational algorithm by Mishra & Pal [15], and showed basically the same distribution of electronic density around molecular atoms. As one can see from Fig. 6 a–c, similar modifications were yielded as the ones presented above (Fig. 5 a–c), revealing the displacement of electronic charge from cytosine and phosphate groups to CO side groups of deoxyribose. After deamination, the electronic charge shifts back toward cytosine and phosphate group, shaping similar distribution like in original non-irradiated cytosine nucleotide (Fig. 6b–c). It seems that energetic stability is strongly related to this charge density configuration since the intermediate compound, the hydrated cytosine, is less stable. Also the hydrogen bond formation between complementary nucleotides from the two DNA strands requires significant electronic charge on side atoms like oxygen ones from cytosine.

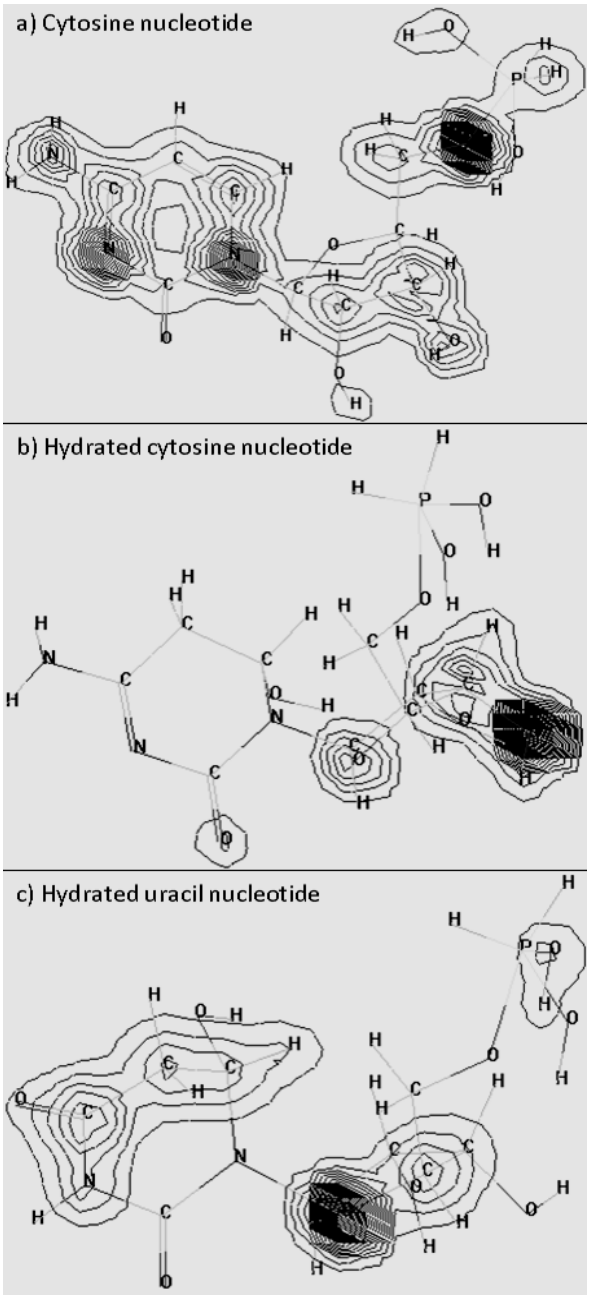

Fig. 6. Charge density maps for a) cytosine, b) hydrated cytosine, respectively c) hydrated uracil.

Indeed, according to data in Table 1, hydrated uracil resulted from irradiated cytosine is a more stable structure, with total energy smaller with more than 10% compared to non-irradiated cytosine. Also dipole moment diminished monotonously over the three consecutive structures directly proportional to total energy (Fig. 7) with a linear correlation coefficient higher than 0.94.

The value of cytosine dipole moment calculated by Kostko [13] using PM3 method was found to be 6.21 D for the biologically relevant cytosine tautomer, having a similar value as the dipole moment we found in our simulations (6.181 D). Govorun *et al*. [8] applied the semi-empirical quantum chemical AM1 method to calculate the dipole moment, and the result is analogous to the result obtained by Kostko [13].

Table 1

Properties of the three molecular complexes

Properties	**Cytosine nucleotide**	**Hydrate cytosine nucleotide**	**Hydrate uracil nucleotide**
Total energy (kcal/mol)	–91775.16	–99271.81 (8.17%)	–101963.06 (11.10%)
Dipole moment (*D*)	7.493	6.724 (10.26%)	6.181 (17.51%)
E_{HOMO} (eV)	–9.13	–9.51	–10.01
E_{LUMO} (eV)	–0.42	0.00073	0.41
$\Delta E = E_{HOMO} - E_{LUMO}$ (eV)	–8.71	–9.51	–10.42

In Table 1, the energy values of some representative molecular orbitals are given as resulted from mathematical modeling – namely E_{HOMO} and E_{LUMO} – as measure of the ionization energy (Koopmans' theorem [12]) and respectively an approximation of molecular electron affinity. According to Table 1, E_{HOMO} – the energy of the highest occupied molecular orbital – has lower values for hydrated cytosine nucleotide and hydrated uracil nucleotide compared to initial cytosine nucleotide. The lower the value of E_{HOMO} is, the more stable is the molecule, suggesting again that uracil hydrate nucleotide is a more favorable configuration from the point of view of ionization phenomena. The values of E_{LUMO} (energy of the lowest unoccupied molecular level) show an increasing tendency, having a negative value for cytosine nucleotide and a positive value for hydrated uracil nucleotide – which is in concordance with hydrogen bond formation favoring for the local stability of double DNA strand. Mishra & Pal [15] studied the hydrogen

bond formation between complementary bases guanine and cytosine belonging to neighboring DNA strands and evidenced the relatively high electron affinity of cytosine – that is concordant with our results for E_{LUMO} of cytosine. Overall, the difference between E_{HOMO} and E_{LUMO} called energy gap (ΔE, transition energy) is changed following the processes of hydration and deamination of the initial cytosine nucleotide.

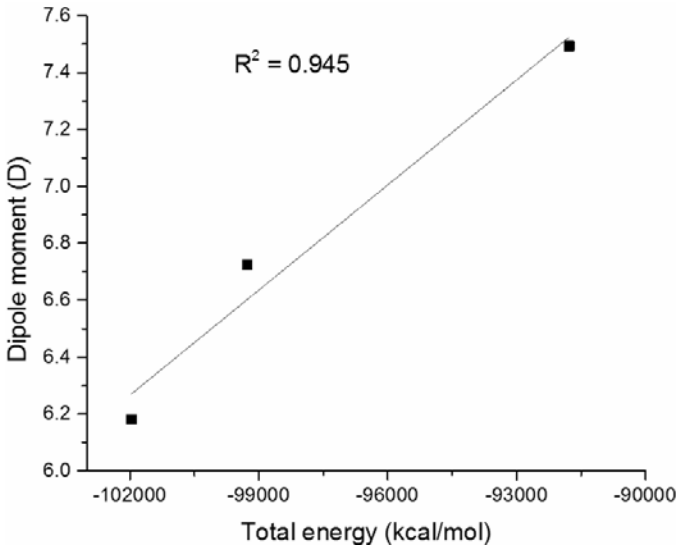

Fig. 7. Linear dependence between total energy of the molecules and their dipole moments.

Coexistence of cytosine tautomers [16] could complicate the interpretation of energy parameter estimation by different mathematical computational algorithms. Bazsó *et al*. [1] provided *ab initio* simulated spectra of isolated cytosine tautomers with transition energy bands between 5 and 7 eV approximatively. Lapinski *et al.* [14] found five cytosine photoisomers working with controlled UV laser irradiation. Sharonov *et al.* [22] reported an electronic spectral shift of cytosine nucleotide following cytosine side group changing; in that case it was studied the cytosine methylation effect that resulted in red shift of corresponding R-cytosine nucleotide. In the case studied by us, cytosine deamination effect was the blue shift of the corresponding R-cytosine electronic transition (from –8.71 eV to 10.42 eV).

CONCLUSION

In this report we presented some results obtained in the theoretical study of cytosine nucleotide interaction with UV photons, using PM3 quantum chemical approach. Radiation impact with the cytosine nucleotide influences both the structural and energetic parameters of the molecular complex (changes in relative

position and charge of the atoms, as well as changes in charge and electrostatic density maps). Thus, energetic parameters show that hydrate uracil nucleotide is a more stable configuration in comparison to cytosine and cytosine hydrate nucleotides. The study is planned to be extended with consideration of cytosine isomers and also of alternative simulation methods.

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