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Structure and properties of 2-hydroxy-adenine and 2-hydroxy-2′-deoxy-adenosine#

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Summary – *Ab initio* and PM3 semiempirical quantum chemistry calculations were carried out for 2-OH-adenine (2OH-A) and 2-OH-2′-deoxy-adenosine (2OH-dA) in different tautomeric forms. Among the six types of tautomers, the most stable is the enol-keto form with hydroxyl group in *syn*-to-N_3 atom conformation. The energy ordering remained the same for 2-OH-2′-deoxyadenoside. However, a significant dependence of the tautomerization of the stability of the *N*-glycosidic bond was observed. The most preferred enol-amino 2-OH-dA tautomer in the *syn* and *anti* conformations is characterized by a more stable *N*-glycosidic bond than the standard adenosine. In contrast the keto-amino form destabilizes this bond with respect of canonical adenosine. The stability of the Watson-Crick-like pairs with thymine was also analysed. The most stable dimer is formed by thymine with an enol-amino tautomer. This pair is stabilized by three hydrogen bonds.

2-OH-adenine / 2-hydroxy-2′-deoxy-adenosine / tautomerism / quantum chemistry calculations / N-glycosidic bond / Watson-Crick pairs

Introduction

The hydroxyl radical may cause various kinds of damage to DNA. Strand breaks and other lesions that block replications may be lethal. On the other hand, base damage that does not hinder replication may contribute significantly to mutagenesis. All nucleic acid bases that are targets of free radicals may form products with potential mutagenic properties. Dizdaroglou *et al* [1-3] have detected a broad spectrum of such base derivatives. Some were found to possess mutagenic potential. For example, the miscoding properties of 8-oxo-2′-deoxyguanosine, one of the major products of oxidative base damage, have been well characterized [4-6]. This derivative exists mainly as the 6,8-diketo tautomer and may form H-bonds with all four standard nucleosides [7, 8] mimicking the base-pairing properties of any DNA base. The biological consequences of hydroxyl radical thymine derivatives, thymine glycol and 5,6-dihydrothymine, have also been observed [9-11]. The promutagenic properties of two major products of 2′-deoxycytidine, 5-hydroxycytidine and 5-hydroxyuridine, have been investigated [12]. These modified bases may lead to C-T and C-G transversions. Adenine undergoes various modifications in native DNA leading to a variety of derivatives, including 2-OH-adenine (2-OH-A) and 8-OH-adenine (8-OH-A) are two major products [1-3]. Shibutani *et al* [13] used *in vitro* experiments to show that 8-OH-adenine has potential mutagenic properties. However, its miscoding abilities are less pronounced compared with the most popular hydroxyl radical nucleic acid derivative, 8-oxoguanine [14, 15]. The consequences of other DNA base derivatives on the properties and structure of DNA remain unknown. Before a detailed analysis of modified nucleic acid double strands can be made, an investigation of the fundamental characteristics of nucleic acid bases and nucleosides appears to be essential.

The aim of this paper is to characterize the relative stability of all possible tautomers of 2-OH-adenine and 2-OH 2′-deoxyadenosine. It is well known that the isolated standard nucleic acid bases, such as adenine and thymine, exist predominantly in one form (amino and dioxo, respectively) while guanine and cytosine form a mixture of at least two components. On the other hand, in polar environments such as liquid solution, solid state or inside DNA, all four canonical bases are found to exist as just one tautomer (amino or amino-oxo). The substitution of an additional hydroxyl group to the adenine molecule extends the possibilities of the tautomerization of these derivatives. In this paper, a quantum chemistry study of possible tautomeric forms of 2-OH-adenine and 2-OH-adenosine (2- OH-dA) is presented. We have only considered those tautomers of 2-OH-A in which a hydrogen is attached to the N_9 atom (*ie* those which may potentially occur in modified adeno-

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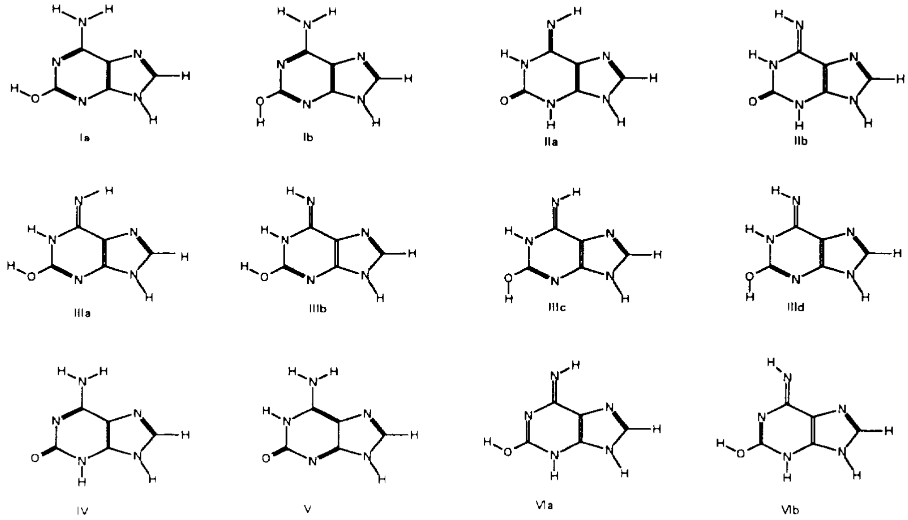

Fig 1. Tautomeric forms of 2-OH-adenine studied in this paper. Only three centers of hydrogen migration were taken into consideration : N_6-N_1, O_2-N_1 and O_2-N_3. These centers form six types of tautomeric isomers. The hydroxyl groups and imido substituent may be in *syn* or *anti* conformations with respect to the N_1-atom.

sine and oligonucleotides). The paper consists of the following sections. First, the quantum chemistry calculations of 2-OH-adenine tautomers are discussed. The formation of stable pairs with thymine is then considered. Next, 2OH-dA tautomers, in both *syn* and *anti* conformations, are characterized. Finally, the stabilization of *N*-glycosidic bond is analysed.

Calculation Details

The *ab initio* LCAO-MO method was used for the study of 2-OH-A and 2-OH-dA tautomerism. The quantum chemistry calculations were performed on both *ab initio* and semiempirical levels. First, the geometries of 2OH-A isomers were optimized on the basis of various semiempirical Hamiltonians, such as PM3, AM1, MNDO, MINDO/3 and CNDO. The full gradient optimization at the *ab initio* 6-31G level was then employed for four most stable tautomers. These calculations were performed with the aid of TurboMole program implemented in the BioSym molecular modelling software package [16]. Closed-shell restricted Hartree-Fock 6-31G calculations were applied to find the stationary points on the potential energy surface, which were supposed to be converged if the gradient norm was less than 0.001. The SCF convergence limit was chosen as 0.0001. The semiempirical quantum chemistry calculations were carried out on the basis of Insight [16] implemented Mopac 6.0 program [17]. In all calculations the keyword Precise was used to give sufficient accuracy.

Results and Discussion

Tautomerism of 2-hydroxyadenine

The 2-OH-adenine and the other *N*-heterocyclic compound may occur in variety of tautomeric forms. If we exclude atom N_9 from the analysis, there are three centers of hydrogen migration : N_6-N_1, O_2-N_1 and O_2-N_3. These centers form six types of tautomeric structures. Additionally some may have opposing conformations of imido and hydroxyl groups. All the tautomers of 2-OH-A studied in this paper are presented in figure 1. The first problem in the tautomerization of 2-OH-A is how to characterize the relative stability of each form. The most reliable results may be obtained on an *ab initio* quantum chemistry level using an extended basis set and corrections for electron correlation effects [18, 19]. However, because such calculations scale as N^5, where N is the number of basic functions, they are very demanding in terms of computer resources and calculation time. The typical time for one optimization cycle of an adenine-sized molecule exceeds 10 cpu hours of a Silicon Graphics workstation and about 1 GB of the disk space is necessary for integral storage in the direct mode. Thus, the temptation of using a much faster but less accurate semiempirical method seems to be justified. However, the problem of choosing the most suitable simplification arises. Some authors presented results proving the superiority of PM3 Hamiltonian over the NDDO one. On the other hand, some reports show that AM1 or MNDO parametrization are sometimes the most suitable approximation. For example, the PM3 method was assumed to be the most suitable for geometry optimization of rare adenine tautomers and adenine-thymine pairs [20], while the partial atomic charges were obtained on the basis of MNDO parametrization [21]. The tautomeric equilibrium of heterocyclic molecules were carried out on the basis of AM1 and PM3 methods [22]. Thus, before using any particular method, some preliminary calculations were performed on both *ab initio* and semiempirical levels. The comparison of these results enables us to choose the best semiempirical method for the further investigations.

Figure 2a contains a comparison of the relative tautomer stability predicted by various methods. The re-

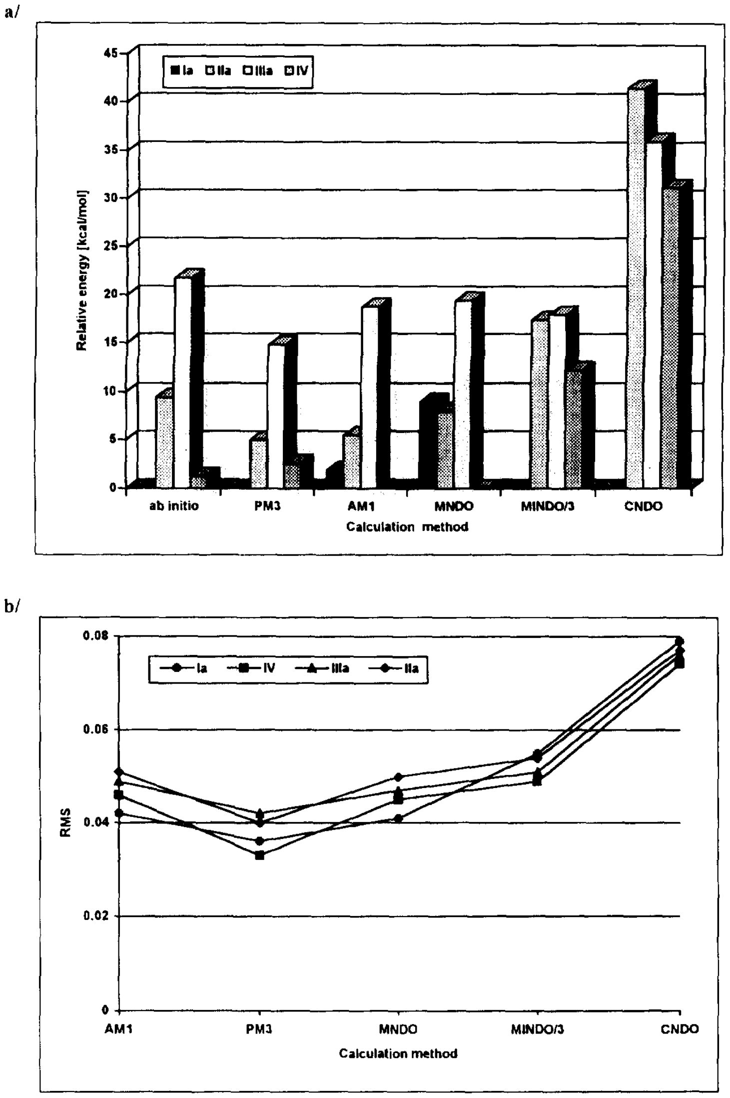

Fig 2. The comprehensive analysis of the results obtained on the basis of 6-31G *ab initio* and semiempirical quantum chemistry calculations.

a/ the total energy difference of four 2-OH-adenine tautomers; the tautomer predicted as most stable in given method was chosen as a zero level.

b/ mean square deviation (RMS) of the geometry optimized on the basis of different semiempirical methods with respect to *ab initio* ones.

sults for the four most favorable 2OH-A were presented. The first conclusion from these results is that only two semiempirical methods (PM3 and AM1) lead to relative tautomer stability in satisfactory agreement with *ab initio* results. With these methods, the energy ordering of studied species is correct but the semiempirical Hamiltonians overestimated the stability of enol-imido forms. All other semiempirical methods even failed in the description of the order of given tautomers. This corresponds to a practical rule that the MNDO method gives results in closer agreement with small basis expansions as STO-3G, while PM3 results are well-suited to much larger basis sets than 6-31G or better. Additionally, part b of figure 2 contains the root-mean-square deviation (RMS) of geometry optimized semiempirically and by *ab initio* methods. The best results were obtained for the PM3 and MNDO simplifications. The geometries optimized on the basis of the other semiempirical methods show much stronger deviations from those estimated by *ab initio* methods. As expected, the worst results correspond to CNDO calculations. It is a little surprising that MNDO led to slightly better results than AM1 method. The conclusion of this part of our work is that among the semiempirical methods available in Mopac 6.0, the PM3 Hamiltonian is most suitable for the 2-OH-A tautomer characteristics. The use of this method will be extended then to 2-OH-dA tautomers.

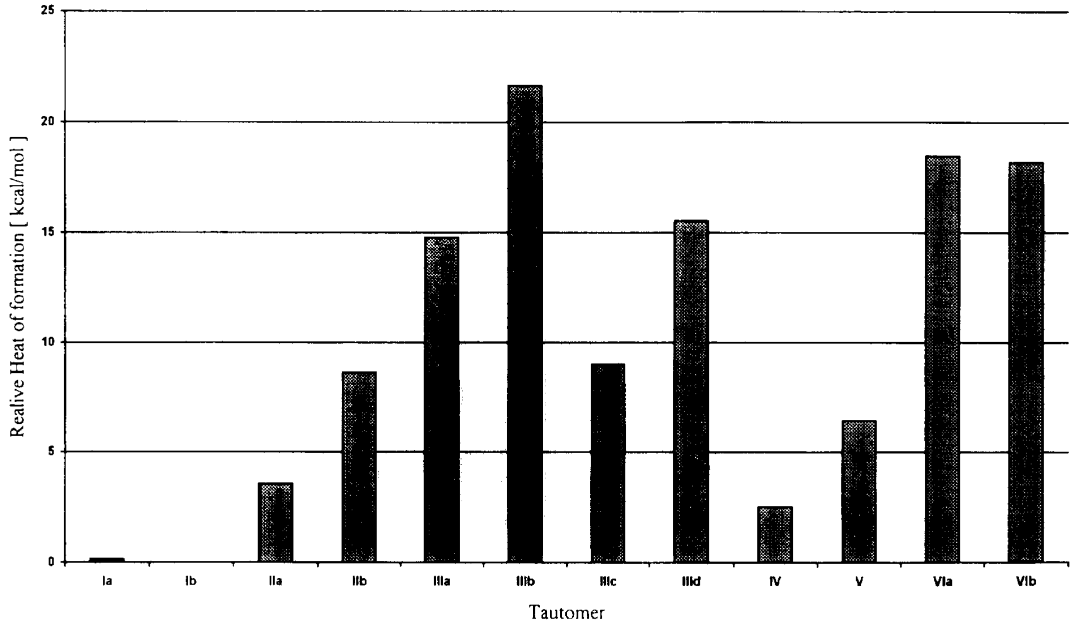

Fig 3. Relative heat of formation estimated in PM3 method. The most stable tautomer Ib was chosen as a reference point.

Figure 3 contains the PM3 heats of formation of all the studied 2-OH-A tautomers. The most stable in a vacuum is the enol-amino tautomer, Ib, with a *syn*-to-N_3-atom conformation of hydroxyl group. The rotation around C2-O2 bond leads to the slightly less stable rotamer, Ia. The problem of the conformation of hydroxyl group is also common to other tautomers. If the hydrogen atom is attached to the N_1 or N_3 centers the OH group is usually oriented to keep the hydrogen of hydroxyl group as far as possible from the H_1 or H_3 hydrogen atoms (*ie* those connected to N_1 or N_3 atoms, respectively). This is evident for all the III tautomers. This tendency is so strong that in the case of tautomer VI the *anti*-to-N_1-atom conformers are unstable since they do not form a deep enough minimum on the configuration hyperspace. Another problem is related to the stabilization effect of the amino-amino group. The presence of imino group always decreases significantly the stability of the structure. There are two possible conformations of the imino substituent but the rotation is hindered due to the double imino bond. As we would expect, the *anti*-to-N_1-atom conformations are now much more stable then the opposite conformations. The most surprising conclusion from the results presented in the figure 3 is that the keto-amino form is less stable then enol-amino one. This was also confirmed by *ab initio* calculations. The reason for this is the discrepancy in the polarities of these structures. The dipole moment of tautomer IV is equal to 6.364 D and is the highest for all tautomers of 2-OH-adenine. In contrast, the dipole moment of tautomer Ia is the lowest and equals 2.035 D, which justifies the good stability of this form in a non-polar environment.

For further characteristics of studied adenine derivative tautomerism, the intramolecular proton migration was analysed. The following transformations were studied in detail : IV → Ia, V → Ib. and Ia → Ib. Plots of heats of formation versus reaction coordinates are presented in figure 4. In the first two cases the separation of the hydrogen atom from N_3 or N_1 atoms were chosen as tautomerization coordinate. The transition Ia → Ib was represented along the dihedral angle N_3-C_2-O_2-H_2. The semiempirical calculations were performed for modified molecule with fixed distance or torsion angle. The rest of the molecule was fully optimized for each value of a fixed parameter. In part a/ of figure 4, we present the transformation of keto tautomers into enol ones. The separation of mobile hydrogen atom was defined as the H-N_3 and H-N_1 distance for IV → Ia and V → Ib, respectively. On both plots we can see the same characteristic features : two minima separated by almost identical, very high barriers. These minima correspond to the stable tautomers. It is interesting to note that from the most preferred keto tautomer, IV, in the first step, we get a less stable enol tautomer, Ia. In contrast, we can observe the formation of the most stable tautomer of 2-OH-A, Ib, after proton migration from tautomer V. The transition between two enol forms, Ia → Ib, is presented in part b/ of figure 4. The modification of the dihedral angle containing the C_2-O_2 bond leads to two energy barriers located at ±90° corresponding to the orientation of the hydrogen atom above or below the molecule plane. Both maxima are characterized by almost equal heats of formation.

Figure 5 presents the *ab initio* optimization of the geometry of the most stable tautomers. All tautomers of 2-OH-adenine have symmetry *Cs* with hydroxyl, keto, amine and amine groups almost coplanar with the plane of the molecule. The coplanarity of keto and enol groups is with accuracy better than 0.6° for all of the studied 2-OH-A tautomers. Moreover, the improper torsion, H_{61}-N_6-H_{62}-C_6, whose value is a good indicator of the pyramidal character of the amino group, is equal to -179.106 and 179.441 for tautomers Ib and IV, respec-

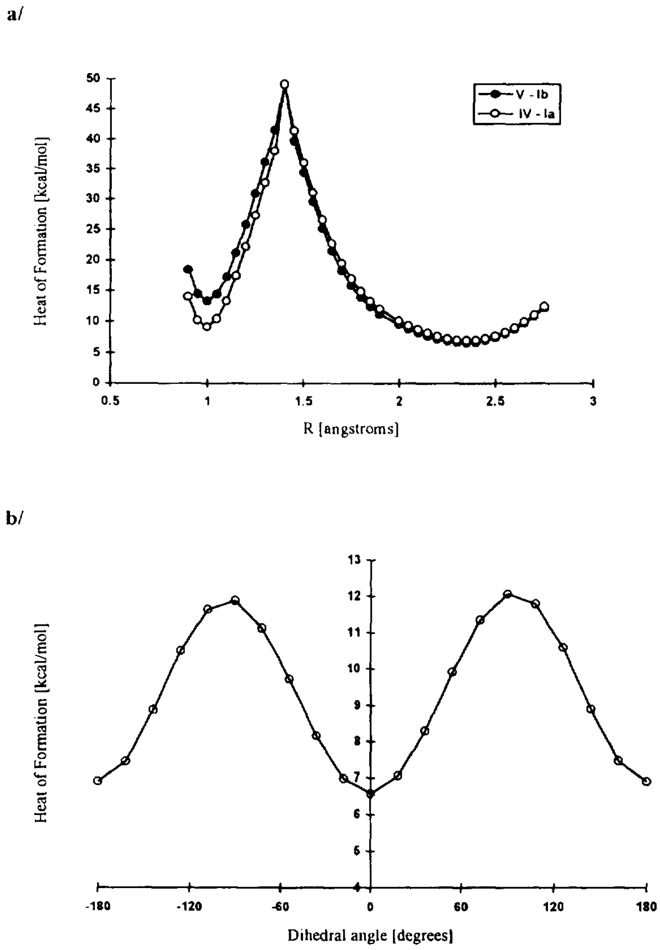

Fig 4. The tautomerization path calculated as the intramolecular migration of hydrogen atom between tautomerization centers.
a/ keto-enol transition : open circles correspond to IV → Ia while filled ones represent V → Ib
b/ transition between enol forms : Ia → Ib

tively. In this case there are only slight deviations from a perfectly flat molecule. However, the PM3 methods predicted significant pyramidal structure for amino groups. This non-planarity was substantially decreased by *ab initio* 6-31G gradient optimization. The imino groups are coplanar with the rest of the molecule even with higher accuracy. The keto forms are characterized by an increase in the C_2-N_3 and C_2-N_1 bond lengths and a decrease in the N_1-C_2-N_3 bond angle with respect of corresponding enol forms. Similarly, the presence of the imino group increases the C_6-N_1 and C_6-C_5 bond lengths and decreases the N_1-C_6-C_5 bond angle compared with amino-forms of 2-OH-A. Generally, for all the tautomers studied here, the most significant differences in the geometry were observed for six-membered rings rather five-membered ones. Thus, the transformation of one tautomer into another is accompanied by significant geometry modification, especially around the tautomerization center.

Miscoding properties of 2-OH-adenine in the most stable tautomeric forms

The next problem related to the tautomerization of 2-OH-adenine is the coding potential of different tautomeric forms. The enol tautomers, Ia and Ib, and the most stable keto tautomer, IV, were coupled with thymine (T) to mimic a Watson-Crick (WC) pair. The full geometry optimization in an unrestricted Hartree-Fock method was performed. The semiempirical PM3 approximation was used. The results are collected in the table I. The most stable dimer is formed by thymine and tautomer Ia. Although the form Ia is itself slightly less stable than Ib, the T-Ia WC-like pair is more favorable than T-Ib due to the formation of three strong hydrogen bonds. The shortest H-bond is observed for nitrogen of Ia and hydrogen of T (see table I). The geometry of all pairs predicted by PM3 method are characterized by non-planar structures. The highest deviations from the

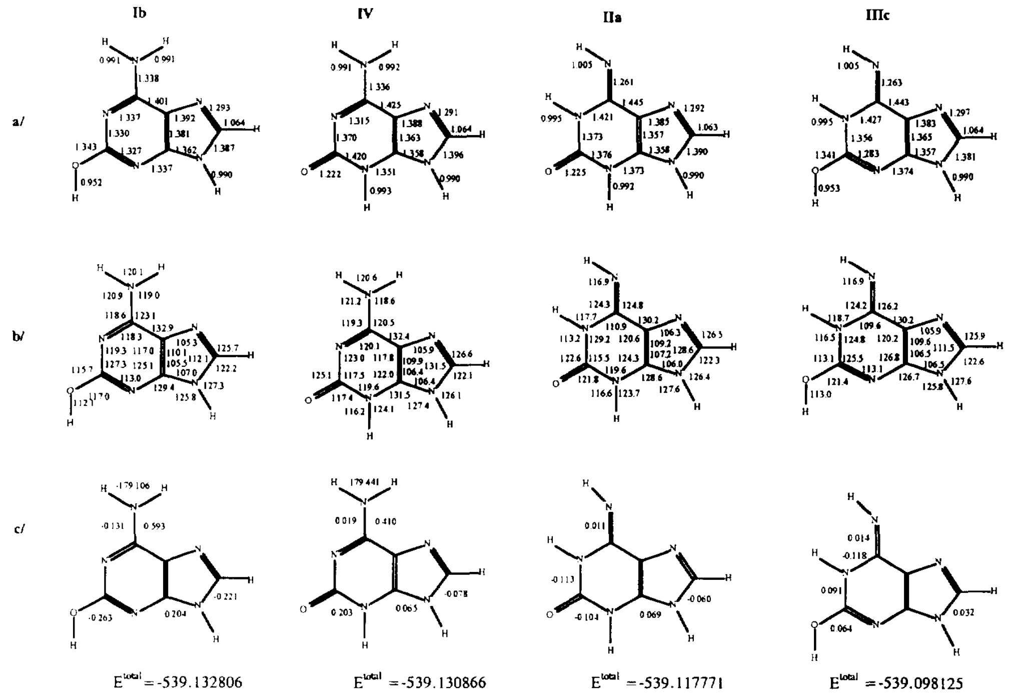

Fig 5. The geometry and total energies of four most stable tautomers of 2-OH-adenine calculated in 6-31G *ab initio* gradient optimization. The bond lengths are expressed in angstroms, the bond angles and dihedral angles are in degrees and energies in electron volts per mole.

planarity were observed for T-IV pair and the lowest for most stable dimer T-Ia. The spatial orientation of the bases is described by the dihedral angles in table I.

Table I. Results of the optimization of WC-like pairs formed by thymine and tautomers of 2-OH-adenine. The H-bond lengths are expressed in angstroms, angles in degrees and heats of formation in kcal/mol.

Parameter	Ib-T	Ia-T	IV-T
O_4^*-H_{61}	1.809	1.859	1.811
N_1-H_3^*	1.808	1.742	1.802
O_2^*-H_2		1.812	
N_6^*-O_4^*-H_{61}	172.6	176.9	170.0
N_1-H_3^*-N_3^*	171.6	176.3	168.9
O_2-H_2-O_2^*		170.8	
C_6-N_1-N_3^*-C_4^*	−22.516	−4.675	40.877
N_1^*-N_3^*-N_1-N_3	−9.805	17.720	9.345
Heat of formation	−74.472	−77.872	−72.046

Stabilities of 2-hydroxy-2′-deoxyadenosine

Four tautomers of 2OH-dA were studied by means of a PM3 semiempirical method. First the restricted minimization was performed in order to preserve the C_2'-*endo* sugar puckering. A full gradient optimization was then carried out for both *syn* and *anti* conformations of adenine ring versus sugar backbone. The same four tautomers as above were considered. The resulting enthalpies of formation are presented on figure 6. The standard adenine results are also attached. The connection of modified adenosine to the deoxyribose backbone does not change the energy ordering of the tautomeric forms and the enol-amino isomer of 2-OH-dA is again the most stable tautomer in both the *syn* and *anti* conformations around the *N*-glycosidic bond. By the analogy to the standard adenosine, the *anti* form also predominates for C_2-modified adenosine, despite the fact that it form an internal H-bond between the N_3 and $H_{C5'}$ atoms by *syn* conformers. The most favorable enol-amino tautomer (Ib) of 2-OH-dA is more stable in the *anti* conformation and the energy difference between the *syn* and *anti* rotamers is equal to 0.611 kcal/mol. Analogous increases of the stability of the *anti* conformations is also common for other tautomers of 2-OH-dA.

The final subject of interest was the *N*-glycosidic bond stabilization/destabilization effect as a result of hydroxyl radical modification at C2 position. Thus, the following substitution reaction was considered :

$$\text{dA} + \text{2-OH-A} = \text{2-OH-dA} + \text{A}$$

The energetic effect of this reaction depends on the enthalpies of formation of standard and modified adenosine. This reaction is only *endo*-energetic if the *N*-glycosidic bond of standard adenosine is more stable than the modified one. In contrast, the *exo*-energetic reaction corresponds to a more stable *N*-glycosidic bond of 2-OH-dA compared with standard adenosine. The *N*-glycosidic bond stabilization was then estimated as the difference between standard enthalpies of formation

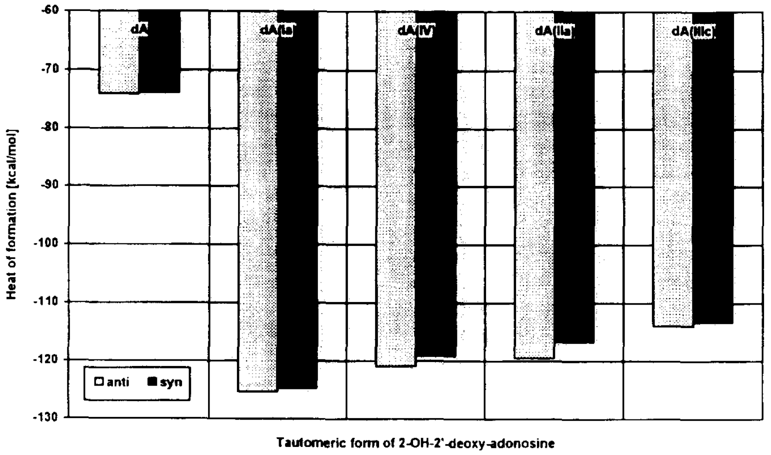

Fig 6. Heats of formation of standard and C-2 hydroxyl radical modified adenosine in *syn* and *anti* conformations estimated by the PM3 method.

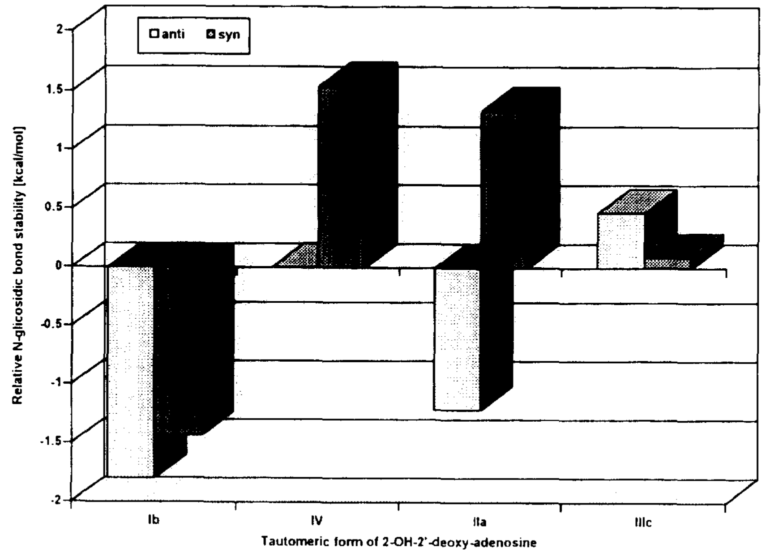

Fig 7. The relative stability of the *N*-glycosidic bond estimated by the PM3 method : heats of formation differences between standard and modified adenosine.

of the products and substrates. The results are shown in figure 7. All the *syn* conformations destabilize the *N*-glycosidic bond except the most favorable tautomer Ia. In contrast, all the *anti* conformers stabilize this bond except tautomer IIIc. The *anti*-tautomer IV has almost the same *N*-glycosidic bond stability as standard *anti*-adenosine. Thus, there is only one tautomer of modified adenine with a more stable *N*-glycosidic bond than standard adenosine in both *syn* and *anti* conformations. This conclusion is important if we consider the method of separation of modified nucleic acid bases from natural products. The typical route is to isolate pure DNA from biological material and to perform hydrolysis in formyl acid at high temperatures and low pH. If a significant increase of the *N*-glycosidic bond is observed the conditions of hydrolysis should be reconsidered and it should be checked whether all modified nucleic acid bases are separate from the polynucleotide chain. Our unpublished experimental results on the hydrolysis of 8-oxoguanine suggest that standard conditions are not sufficient for total hydrolysis of *N*-glycosidic bond due to the significant increase in the stability of the 6,8-diketo form. Similar remarks should be kept in mind when we consider the non-enzymatic hydrolysis of other modified nucleosides.

Conclusions

Although from the formal point of view the 2-hydroxyadenine may have six different types of tautomeric forms, most of them are insignificant due to energetic instabilities. The *ab initio* and PM3 calculation results demonstrated that only two isomers should be considered as probable : *ie* enol-amino and keto-amino tautomers. The same conclusion may be extended to modified 2-hydroxy-2′-deoxyadenosine. The *anti* conformations of modified adenosine is more stable than the *syn* conformation by the analogy to standard adenosine. However, significant changes were observed in the enthalpies of *N*-glycosidic bond formation. In both the *syn* and *anti* conformations the enol-amino form of 2-OH-dA increases, while keto-amino decreases the stability of the *N*-glycosidic bond with respect to non-modified adenosine.

In this paper, 2-hydroxyadenine was used as the name for the hydroxyl radical adenine modified at the C-2 position. This is justified by the energy ordering of enol and keto tautomers. This result is opposite to the common expectation that keto forms are predominant in the coupled heterocyclic systems.

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