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5′,8-Cyclo-2′-deoxyadenosine (cdA) formation by γ-radiation. Theoretical quantum mechanics study

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Reactions of reactive oxygen species and more specifically — of hydroxyl radical (•OH) — with nucleosides may lead to the generation of radicals in the base and 2-deoxyribose moieties. In the present study emphasis was put on the possible reaction modes of 2'-deoxyadenosine (dA) radicals, leading to the formation of related 5′,8-cyclonucleosides. It appears that the prerequisite for the formation of 5′,8-cyclo-2′-deoxyadenosine (cdA) is the adoption of O4′-*exo* conformation by 2-deoxyribose; however, this is the least energetically favored conformer among the different puckered forms adopted by the furanose ring. The O4′-*exo* conformation was found to be present in each of the discussed mechanisms.

Keywords: 5′,8-cyclo-2′-deoxyadenosine, DNA damage, ionizing radiation, density functional theory (DFT)

INTRODUCTION

In the natural environment, the geometry of nucleic acids and nucleosides undergoes constant changes due to the flexibility of the sugar residues, ribose or 2-deoxyribose. These changes are described by the pseudorotation theory, according to which the pentagonal ring of the furanose moiety may adopt intermediate forms between two extreme conformers, namely C3′-*endo* and C2′-*endo* (Saenger, 1984). The position of the hydroxyl group at C5′ of 2′-deoxyribonucleosides, as well as the substituents at the C2′, C3′ positions of the sugar residue, have a significant influence on the sugar moiety geometry changes. In turn, the nucleobases have a rigid structure which can change only slightly; however, these small modifications may significantly influence the furanose geometry. Changes in the relative spatial positions of both nucleoside components may occur through the rotation around the N-glycosidic bond (O4′–C1′–N9–C4 (δ) dihedral angle). It is well documented that the *anti* form is usually predominant in the case of natural forms.

Nucleotides and nucleosides in the cellular environment are constantly exposed to several reactive oxygen species, the most reactive of them being •OH. The latter may be formed in cells as a result of initial generation of superoxide radical ($O_2^{-\bullet}$) through aerobic metabolism and subsequent Haber-Weiss reaction. Another possibility is γ-radiolysis of water molecules as a result of indirect effects of ionizing radiation (Cadet *et al*., 2003). The reactions of •OH with oligonucleotides and nucleosides may lead to base modifications and also sugar lesions that lead in most cases to the formation of strand breaks. Most of them can be removed by the base excision repair (BER) pathway (Sancar *et al*., 2004). However, modifications involving damage of both the sugar moiety and the base such as 5′,8-cyclo-2′-deoxyadenosine (**cdA**) — are not eliminated by the BER system, but by the more complex nucleotide excision repair (NER) pathway (Brooks *et al*., 2000). Moreover, such purine 5′,8-cyclonucleosides show different biological features depending on the *R* or *S* geometry of the C5′ (Kuraoka *et al*., 2001).

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Abbreviations: BER, base excitation repair; CPCM, conductor-like polarization continuum model; DFT, density functinal theory.

MATERIALS AND METHODS

Computation methodology of quantum mechanics study. The molecular geometries of 5′,8-cyclo-2′-deoxyadenosine (**cdA**) in gaseous phase were initially optimized by molecular mechanics using UFF (Universal Force Fields) (Rappe *et al*., 1992) implemented in Gaussian 03 software. All subsequent calculations were performed by the density functional theory (DFT) using the generalized gradient approximation (GGA) exchange-correction functional in which the B3LYP functional (Becke's three-parameter hybrid HF/DFT exchange functional (B3) (Becke, 1993), and the Lee-Yang-Parr correlation functional (LYP) were implemented (Lee *et al*., 1988). For all calculations the 6-31+G** (Hehre *et al*., 1986) basis set with polarization functions was used — yielding 397 basis functions per neutral **cdA**. The 6-31+G** basis set was represented as a number of atomic orbitals composed of $M_{C,N,O}/M_H$ (4s3p1d/2s1p), consisting of 19 basis functions per C, N, O atoms and five basis functions per H atoms (Šponer *et al*., 2006)**.** All the calculations were performed with convergence criteria of self-constructed fields equal to 10^{-6}. Moreover, using this strategy, the contribution of zero-point vibrational correction and the thermal contribution to the free energies were considered. For all energy calculations, the scale factor 0.96 for 6-31+G** basis set was used (Fridgen *et al*., 2006). For the characterization of the stationary point of all the investigated molecules, harmonic vibrations were calculated at the B3LYP/6-31+G** level. One may point out that for each structure being a minimum, no imaginary frequency was found; and for each structure being a transition state, one imaginary frequency was found.

Conductor-like polarization continuum model (CPCM) was used to describe the surrounding aqueous medium, with dielectric constant of water $\varepsilon = 78.39$ (Cancès *et al*., 1997). The standard free energy of the molecule in aqueous phase $\Delta G^0_{(AQU)}$ was described as the sum of the standard free energy of the molecule in gaseous phase $\Delta G^0_{(GAS)}$ and standard free energy of solvation $\Delta G^0_{(SOLV)}$ (Zhang & Eriksson, 2006). Suitable $\Delta G^0_{(SOLV)}$ values were obtained by single point calculation at the CPCM/B3LYP/6-31+G** level. Calculations of all the structures were performed using the Gaussian 03 Revision D.01 software (Frisch *et al*., 2003).

RESULTS AND DISCUSSION

Theoretically, three possible radical precursors (Fig. 1) of adenine 5′,8-cyclonucleosides can be formed as the result of the attack of a hydroxyl radical on the base and the sugar moiety of 2′-deoxyadenosine (**dA**). The nature of the radicals thus formed has a critical influence on the structure of the final reaction product. To gain insight into the formation of **cdA** we applied the DFT methodology with B3LYP (Becke's three-parameter exchange functional and the gradient-corrected functional of Lee-Yang-Parr) with the 6-31+G** basis set. The sequence of the discussed reactions is as follows:

- C8 radical of **dA** (**1**). Hydrogen atom abstraction from the C8 position of adenine followed by its transfer to C5′ of 2-deoxyribose.
- C5′ deoxygenated **dA** radical (**2**). The formation of this radical has been observed during the synthesis of **cdA** described as a photolysis product of the 5′-thiophenyl derivative of 2′-deoxyadenosine (Romieu *et al*., 1999). Moreover, this radical cannot be considered as a precursor of 5′,8-cyclo-2′-deoxyadenosines due to its conversion to the stable 5′,8-cyclo-2′,5′-dideoxyadenosine. Therefore, the chemical reactions of radical **2** are not further discussed.
- C5′ radical of **dA** (**3**) arising from abstraction of one of the hydrogen atoms from the 5′-hydroxymethyl function. The formation of C5′ radicals and the mechanism of **cdA** formation are discussed below. Moreover, attempts were made to rationalize the observed ratio between the *R* and *S* diastereomers (Fig. 2).

Experimental data show that the 5′*R* diastereomer of **cdA** is formed predominately over the 5′*S* one in free nucleoside and single-stranded DNA, while both diastereomers are produced in similar amounts in double-stranded DNA (Dickerson *et al*., 1988). An earlier study on the formation of 5′,8-cyclo-2′-deoxyadenosine was performed using quantum and semi-empirical methods, taking into account

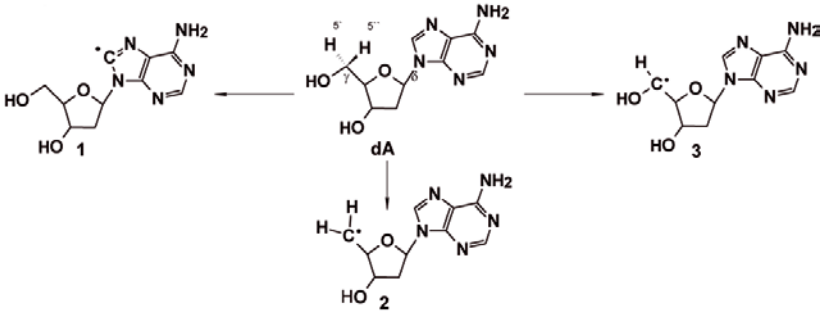


Figure 1. Three free-radical derivatives may be formed as a result of the attack of different free radicals on 2′-deoxyadenosine.

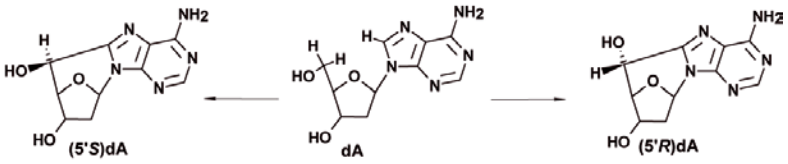


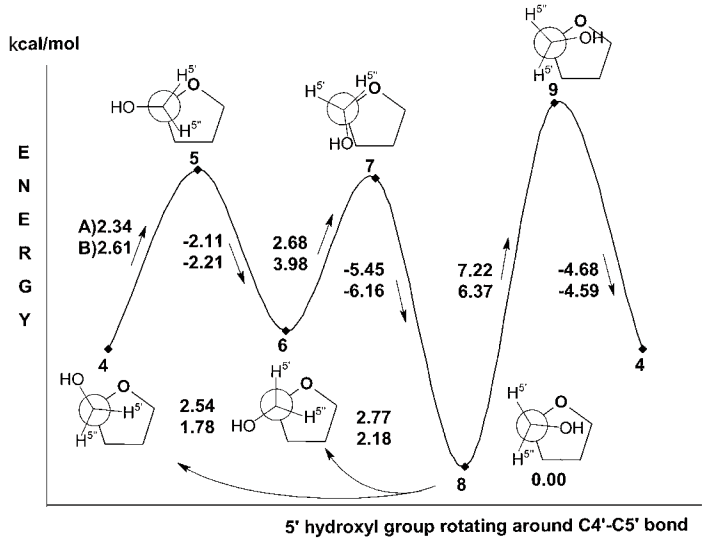
Figure 2. Graphical representation of two diastereomeric forms of cdA: 5′*R* and 5′*S*.

only the process of intramolecular cyclization (Miaskiewicz *et al*., 1995). It is important to mention that in that work only the potential energy was taken into account. Moreover, Zhang and Eriksson (2006) in their recent quantum mechanics study have reported data for the formation of the 5′*S* diastereomer of **cdA** only. In the present study, the mechanisms of the cyclization reaction, starting from free nucleoside, are discussed in terms of free energy values for both **(5′*S*)cdA** and **(5′*R*)cdA**.

The position of the 5′OH group in relation to the 3′OH function appears to have a crucial influence on the stereochemistry of the diastereomers formed in the free nucleoside. The hydroxyl group, rotating around the C4′–C5′ bond (C3'–C4'–C5'–O5' (γ) dihedral angle), adopts three positions corresponding to conformations of energy minimum, of which the form **8** exhibits the lowest energy (Scheme 1). These minima are separated by three conformers with higher energies, and the energetic barriers corresponding to the respective transformations are not equally valuable and fall between 2.11/2.21 and 7.22/6.37 kcal/mol in gaseous/aqueous phase, respectively (Scheme 1). As the *gauche* (+) (**8**), *trans* (**4**), *gauche* (–) (**6**) forms are the rotamers of the lowest energies, it may be assumed that they will be the precursors of the radical formed after hydrogen atom abstraction from adenine C8 position (Fig. 3).

Hydrogen atom abstraction from C8 of 2′-deoxyadenosine. Hydrogen atom abstraction by •OH from adenine C8 is not a favoured process. Reaction of •OH leading to the formation of 8-oxo-dihydro-2′-deoxyadenosine is a more likely one. However, taking into account the fact that one of the methods of **cdA** synthetic formation (Flyunt *et al*., 2000) postulates the generation of radical **1** as a result of γ-radiolysis of **8-Br-dA**, we assumed that this mechanism might be possible in extreme cases, despite its low probability in the case of **dA**.

The hydrogen atom removal from C8 of 2′-deoxyadenosine gives rise to three forms, depending on the position of the 5′OH methyl group (Fig. 3). Those compounds formed from the respective precursors are not energetically equivalent. As expected, **12** formed from **8** (Table 1, Fig. 3) is the most stable form. In order to allow the formation of **cdA**, radical translocation from C8 carbon to the C5′ function with a simultaneous transfer of one of the hydrogen atoms of the C5′ function onto C8 carbon atom is necessary. The H5′ and H5′′ protons in 2′-deoxyadenosine are not equally available for the C8 radical, and their availability is strictly dependent on the conformation adopted by the 5′-hydroxymethyl group. Analysis of these data shows that H5′ proton of compound **10** and H5′′of **11** are the most available — whereas the least available protons are found in the compound **12**. Table 1 reports distances between C8 and H5′ and H5′′. In the latter instance, the availability of the 5′-OH proton in 5′-hydroxymethyl group (3.75 Å) should be noted. It is well documented that radicals produced in aliphatic compounds are more stable than those located on heteroatoms or aromatic carbons (Evangelista & Schaefer, 2004). Additionally, reaching the suitable position enabling



Scheme 1. Graphical representation of 5′ hydroxyl group rotating around the C4′–C5′ bond with energetic different (kcal/mol) indication between suitable rotamers (A, gaseous phase; B, aqueous phase) **8** is indicated as the most stable rotamer.

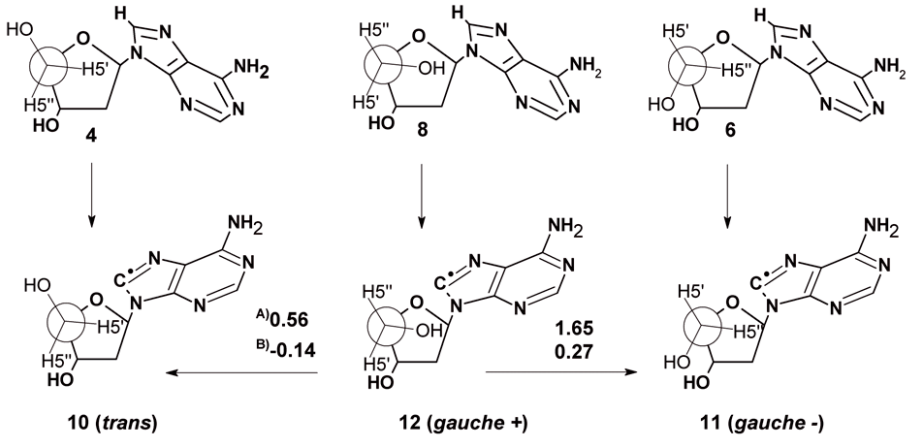


Figure 3. Hydrogen atom abstraction from C8 of 2′-deoxyadenosine leads to the creation of three radical forms, depending on the conformation of the 5′-hydroxymethyl group.
Differences in energy (kcal/mol) between possible C8 radicals are shown over the arrows. (A, gaseous phase; B, aqueous phase).

radical transfer by compound **12** requires surmounting the barrier of ΔG=5.45/6.16 kcal/mol for transformation **8**→**7**→**6** and of ΔG=7.22/6.37 kcal/mol for **8**→**9**→**4** in gaseous/aqueous phase, respectively.

It should be mentioned that transient **12** (*gauche* (+)) should be reorganized into form **11** (*gauche* (–)) or **10** (*trans*) to be involved in **cdA** formation (Fig. 3). This would lead to the preferential formation of **(5′*R*)cdA** in aqueous phase (ΔG(**10-12**)=–0.14 kcal/mol), which is consistent with experimental data. This is the reason why in the discussion transient **12** will not be further considered as a **cdA** precursor.

The postulated mechanism involving the formation of 5′,8-cyclo-2′-deoxyadenosine from the C8 radical derivative **dA** should proceed according to the model suggested by Miaskiewicz (Miaskiewicz *et al*., 1995*)* and independently by Chatgilialoglu *et al*. (2003) (starting from **8-Br-dA**). Following the C8 free radical formation the radical translocation to C5′ carbon atom takes place with the subsequent formation of the C5′–C8 covalent bond. The hydrogen atom is then eliminated from the intermediate compound formed with simultaneous reconstitution of adenine.

In order to challenge the above mechanism for **dA** we carried out semi-empirical scans of the possible reactions using molecular mechanics. Optimization with quantum mechanics using DFT was performed for the obtained critical points of the scanned reactions. Table 2 summarizes the obtained values for individual products or intermediate states (Fig. 4).

Table 1. ZPE corrected free energies and geometries of dA and C8 radical of dA conformers.

Obtained at the B3LYP/6–31+G** level.

Comp.	Energy [Hartree*]			Distances [Å]				Angle [°]	
	$\Delta G_{(GAS)}$	$\Delta G_{(AQU)}$	$^{A)}\Delta G_{(SOL)}$	$C_{5'}$–C_8	C_8–$H_{5'}$	C_8–$H_{5''}$	C_8–$OH_{5'}$	δ	γ
4	–888.12318	–888.16587	–0.04269	3.513	3.028	4.577	3.826	–174.28	175.73
5	–888.11945	–888.16171	–0.04226	3.847	2.969	4.429	5.209	171.79	–127.67
6	–888.12282	–888.16524	–0.04242	3.795	3.627	3.345	5.524	177.52	–68.05
7	–888.11854	–888.15890	–0.04035	3.860	4.607	2.973	4.997	173.51	–8.74
8	–888.12723	–888.16871	–0.04148	5.000	5.116	4.175	3.916	–118.25	50.17
9	–888.11573	–888.15856	–0.04283	4.096	4.478	5.042	3.859	–135.55	117.22
10	–887.44588	–887.48512	–0.03924	3.785	3.039	4.750	3.641	–97.85	174.40
11	–887.44415	–887.48447	–0.04032	3.667	3.873	2.854	5.211	–97.23	–66.65
12	–887.44678	–887.48490	–0.03812	4.836	5.810	5.172	3.545	–102.40	56.68

A) ZPE and thermal correction are not included; *1 Hartree = 627.5095 kcal/mol

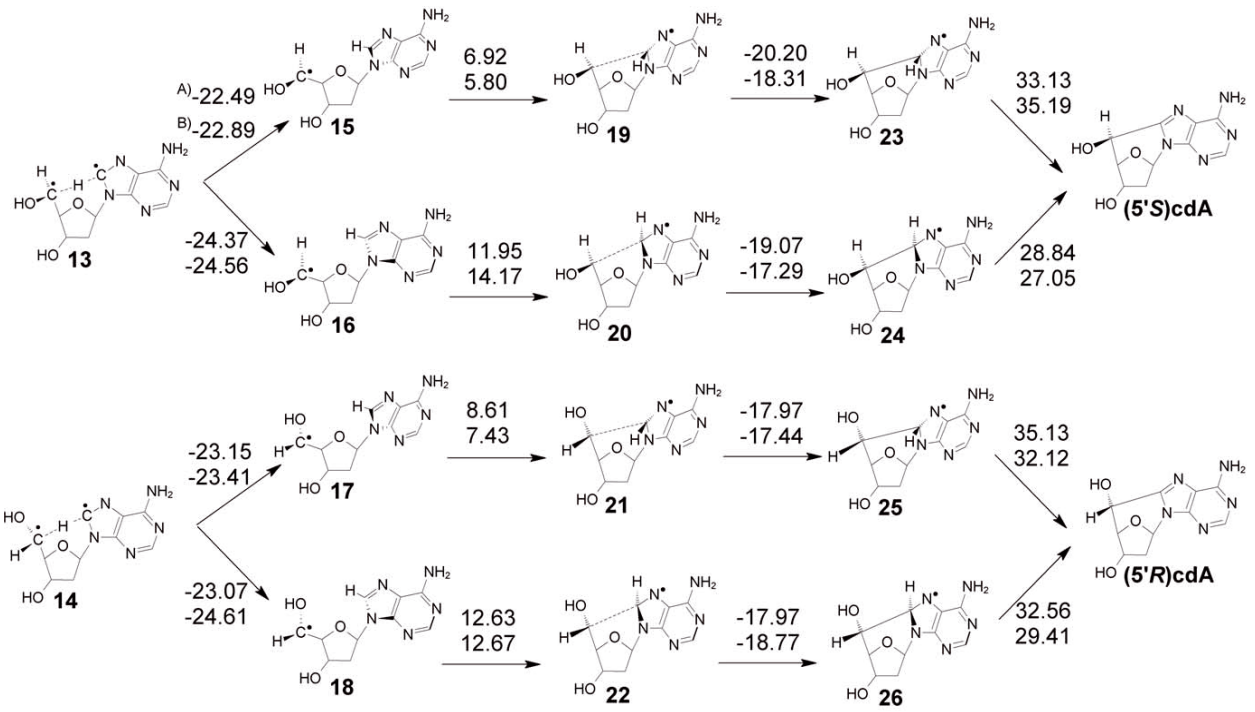


Figure 4. Possible reaction paths of 5′,8-cyclo-2′-deoxyadenosine formation.
Differences in energy (kcal/mol) between reaction steps are shown over the arrows. (A, gaseous phase; B, aqueous phase).

For intermediates **11** and **10,** the first stage of the reaction is the radical transfer from C8 to C5′. (It should be pointed out that in gaseous phase **12** was found as the most stable form, while surprisingly, in aqueous phase **10** exhibits the lowest energy (Fig. 3)). During this process, two intermediate states are formed: **13** and **14**, in which the radical is distributed between the C8 and C5′ carbons (Fig. 4, Table 2), whereas the remaining H5′ or H5′′ hydrogen atom is located between the two carbons. This stage requires a relatively small energy input, ΔG, of which 2.21/0.75 kcal/mol is for transition **10**→**14** and 2.43/0.39 kcal/mol for **11**→**13**, in gaseous/aqueous phase, respectively. The higher energetic barrier for this process results additionally from the necessity of a rotation of the 5′-hydroxymethyl group around the γ-bond, and was observed when compound **12** is the precursor of transient **13** with ΔG(**12**→**13**)=4.08 kcal/mol in gaseous phase, however, this value decrease to 0.66 kcal/mol in aqueous media. The situation is opposite when **12** is considered as the precursor of **14** in aqueous phase (ΔG(**12**→**14**)=2.77/–0.62 kcal/mol, in gaseous/aqueous phase, respectively). This leads to the formation of one of the intermediate products as a radical located at C5′ (**15; 16; 17; 18**) in which the C5′ carbon atom exhibits sp^2 configuration. The stability of the products thus generated plays a major role in the formation of the respective **cdA** diastereomers. The formation of **13** and **14** is more probable than the generation of compounds **10**, **11** or **12**. Moreover, the energetic difference between **13** and **15; 13** and **16** or **14** and **17; 14** and **18** is approximately –23/–24 kcal/mol, in gaseous/aqueous phase, respectively (Table 2, Fig. 4).

Since the intermediate product of the reaction is identical with the compound formed as the result of hydrogen atom abstraction from C5′, further reaction mechanism will be discussed as follows:

Hydrogen atom abstraction from C5′ of 2′-deoxyadenosine

The resulting C5′-yl radical in **15, 16, 17** and **18** is stabilized by its partial delocalization onto the oxygen atom of the hydroxyl group. It may be added that **17** (gaseous phase) and **18** (aqueous phase) are favoured thermodynamically. The process of intramolecular cyclization requires the proximity of C5′ and C8 atoms. In order to promote this process, the sugar moiety must adopt the O4′-*exo* conformation. Such a nucleoside conformer is characterized by high energy with a very low expected contribution of this conformation in the overall population of puckered forms. As a result of reaching a distance of about 2 Å between C5′ and C8 completion of a partial transfer of the radical onto the N7 nitrogen atom of adenine is possible and formation of intermediate states **19** and **20** as well as **21** and **22**, in which carbon atoms C5′ and C8 and N9 assume a

conformation close to sp^3. The energetic barriers for individual transformations are shown in Fig. 4.

The highest ΔG values were observed for transformations **16→20** and **18→22**, being 11.95/14.17 kcal/mol and 12.63/12.67 kcal/mol in gaseous/aqueous phase, respectively. So high energetic barriers probably rule out the participation of precursors **16** and **18** in the formation of intermediate states **20** and **22** in **(5′*S*)cdA** and **(5′*R*)cdA** formation. It seems that the determining role in **cdA** diastereomer formation is played by radicals **15** and **17** for which ΔG of transition **15→19** and **17→21** is 6.92/5.80 kcal/mol and 8.61/7.43 kcal/mol in gaseous/aqueous phase, respectively, with the difference between them being 1.69/1.63 kcal/mol (gaseous/aqueous phase). Compound **17** is slightly thermodynamically more stable than **15** (ΔG(15–17)=1.97/0.60 kcal/mol) in both discussed phases.

The cyclization leads to the creation of a new chiral centre on the C8 carbon atom in the intermediate product. Due to the possibility of adenine rotation around the N-glycosidic bond in **15, 16, 17** and **18** the following diastereomeric arrangements at C5′ and C8 in the reaction intermediate products are possible: *SR*(**23**), *SS*(**24**), *RR*(**25**), *RS*(**26**) (Fig. 4). Selected geometrical parameters of these compounds are given in Table 2.

Their analysis using quantum mechanics calculations showed that products **23** and **25** are the most energetically favoured. Their formation have been characterized by the following energetic barriers: ΔG(**21→25**)=–17.97/–17.44 kcal/mol and ΔG(**19→23**)=–20.20/–18.31 kcal/mol, in gaseous/aqueous phase, respectively. Taking into account the obtained results one may assume that the formed intermediate products **25** and **23** are the most likely precursors of the 5′*R* and 5′*S* diastereomers of **cdA**, respectively, with corresponding energies –887.494163/–887.53719 and –887.49729/–887.54022 obtained in gaseous/aqueous phase, given in Hartree (1Hartree=627.5095 kcal/mol).

The final stage of the reaction is hydrogen atom elimination with simultaneous formation of the **cdA** molecule. Of the two diastereomers of **cdA** formed, the product with the 5′*S* configuration has been thermodynamically privileged in gaseous phase ΔG(**(5′*S*)cdA→(5′*R*)cdA**)=–3.96 kcal/mol, and a slightly higher one in aqueous phase ΔG(**(5′*S*)cdA →(5′*R*)cdA**)=1.18 kcal/mol.

Table 2. ZPE corrected free energies, geometries of intermediates, transition states and products of hydrogen atom abstraction reaction from adenine C8.

Obtained at the B3LYP/6–31+G** level.

Comp.	Energy [Hartree]			Distance [Å]			Spin density			Angle [°]	
	$\Delta G_{(GAS)}$	$\Delta G_{(AQU)}$	[A)]$\Delta G_{(SOL)}$	$C_{5'}$–C_8	C_8–$H_{5'}$	C_8–$H_{5''}$	C5′	C8	O5′	δ	γ
13	–887.44028	–887.48385	–0.04357	2.706	3.079	1.517	0.074	0.596	0.318	–119.66	–49.65
14	–887.44236	–887.48393	–0.04158	2.721	1.557	3.392	0.075	0.676	0.308	–136.36	177.61
15	–887.47612	–887.52027	–0.04415	3.667	3.371		0.925	–0.022	0.102	–108.04	–72.16
16	–887.47912	–887.52299	–0.04387	3.741	4.067		0.927	–0.008	0.105	–179.08	41.34
17	–887.47925	–887.52123	–0.04198	3.461		4.098	1.063	–0.059	0.108	–97.88	159.20
18	–887.47912	–887.52315	–0.04403	3.485		3.414	0.924	–0.004	0.108	177.68	–170.32
									N7		
19	–887.46510	–887.51104	–0.04594	2.231	2.597		0.670	–0.143	0.230	–77.20	–57.28
20	–887.46007	–887.50041	–0.04035	2.193	2.576		0.640	–0.104	0.202	148.32	–23.71
21	–887.46553	–887.50940	–0.04386	2.222		2.663	0.627	–0.107	0.234	–77.66	168.95
22	–887.45899	–887.50297	–0.04398	2.177		2.646	0.610	–0.139	0.258	151.84	–164.01
23	–887.49729	–887.54022	–0.04293	1.558	2.164		0.046	–0.047	0.494	–92.59	–67.37
24	–887.49045	–887.52797	–0.03752	1.562	2.161		0.062	–0.083	0.463	171.22	–28.27
25	–887.49416	–887.53719	–0.04303	1.567		2.178	0.062	–0.083	0.463	–95.60	173.81
26	–887.49007	–887.53288	–0.04281	1.553		2.162	0.032	–0.054	0.481	171.82	–139.93
(5′*R*)cdA	–886.94450	–886.98414	–0.03964	1.508	2.126					–154.64	–56.12
(5′*S*)cdA	–886.93819	–886.98601	–0.04783	1.507		2.132				–153.47	–173.29

[A)]ZPE and thermal correction are not included

Additionally we assumed that precursors **17** and **18** of **(5′*R*)cdA** are the most stable of the discussed all C5′ radical of **dA**, in both calculated environments. Even though the energy barrier for **17**→**21** transition are slightly higher than for **15**→**19** one (Fig. 4), the lower stability of compound **15** discriminated this path of reaction as favourable one (Table 2). Additionally, CPCM single point calculations of previously optimized geometries in gaseous phase, on the same level of theory, show slightly lowed energy barriers of **(5′*S*)cdA** and **(5′*R*)cdA** formation (Fig. 4). It should be pointed out that solvation effects decrease significantly the energy of each discussed compound (by approx. 26.5 kcal/mol) (Tables 1 and 2).

Additionally, as mentioned above, in the cellular environment water molecules can stabilize the transient intermediates during the cyclization. It is important to mention that each nucleotide in dsDNA is coated by approximately 20 molecules of water. Moreover, the hydrogen bonds between nucleoside/nucleoside and water are well defined (Saenger, 1984). Due to that, in the case of the discussed mechanism, the location of the external H_2O molecule between the 5′OH group and O4′ atom of **dA** in the *trans* conformation, can force the cyclization in the **(5′*R*)cdA** direction, as the result of suitable hydrogen bond formation. Due to the lack of an electronegative atom in the 2-deoxyribose ring on the same side as the 5′OH function in the *gauche* (–) conformation of **dA**, the reaction path leading to the **(5′*S*)cdA** formation seems less favourable.

CONCLUSIONS

Reactions of reactive oxygen species and more specifically of $^{\bullet}$OH may lead to the generation of nucleoside radicals. In the present study, we analysed possible modes of **dA** radical reorganization, leading to the formation of 5′,8-cyclic adenine nucleosides.

Formation of radicals at C5′ and C8 atoms leads, through intramolecular cyclization, to **cdA** nucleosides. Using quantum chemistry methods the paths of the cyclization reaction were investigated in gaseous and aqueous phases. It was shown that the 5′,8-cyclonucleoside exhibiting a 5′*R* configuration might be predominant. Therefore, the results of our studies are consistent with the experimental data obtained from γ-irradiation of **dA** aqueous solution.

It seems that a prerequisite for adenine 5′,8-cycloinucleoside formation is the adoption of O4′-*exo* conformation by the 2-deoxyribose moiety; however, this is the least energetically favoured conformer among the puckered forms adopted by the furanose ring. This conformation was observed at each stage of the discussed mechanisms. Thus, in the natural environment, participation of nucleosides with this sugar conformation is expected to be marginal, so the levels of **(5′*S*)cdA** and **(5′*R*)cdA** should be equally small.

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