Formation of 5′,8-cyclo-2′-deoxyadenosine in single strand DNA. Theoretical quantum mechanics study†

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Reactions of reactive oxygen species, and more specifically, of hydroxyl radicals, with nucleotides, may lead to the generation of radicals in the base and the 2-deoxyribose moieties of DNA. In the present study, for the first time, emphasis was placed on the investigation of the possible reaction of 2′-deoxyadenosine-3′,5′-diphosphate radicals, leading to the formation of the related 5′,8-cyclo-2′-deoxynucleotide-3′,5′-diphosphate. It has been shown by several authors that 5′*R* and 5′*S* diastereomers of the discussed molecule are formed with different frequencies in DNA. The 5′*R* form of 5′,8-cyclo-2′-deoxyadenosine-3′,5′-diphosphate was found to be the most stable one. Moreover, the investigated reaction paths have shown that the formation of the 5′*R* isomer is energetically favourable in both the aqueous and gaseous phases. Therefore, the presented results are in good agreement with experimental data.

Introduction

DNA, which is the storehouse of genetic information, can exist in single- (ss-) or double-stranded (ds-) forms. The expression of ss-depends on the cell cycle, as well as other environmental conditions.[1] Damage that influences DNA strandedness and conformation is important for DNA expression in living organisms.[2] The constituents of the cell are continuously exposed to ionizing radiation, which can generate reactive oxygen species (ROS), like the hydroxyl radical.[3] Hydroxyl radicals can react with the sugar or base moieties of nucleotides/nucleosides, which may give rise to different kinds of DNA lesions.[4] Most of them can be removed from the genome under the base excision repair system (BER).[5] However, the tandem lesion, being the simultaneous injury of base and sugar units, for example 5′,8-cyclo-2′-deoxyadenosine (**cdA**), is dismissed from the genome by nucleotide excision repair machinery (NER).[6] Cadet *et al.* have shown that the presence of **(5′*S*)cdA** in matrix DNA strongly blocks the polymerization reaction.[7] It should be mentioned that the 5′*R* form of **cdA** can be bypassed by polymerases η, therefore it exhibits a less toxic effect.[7] The mechanism of cyclonucleoside formation has been theoretically investigated by Chatgilaloglu's, Miskiewicz's and Eriksson's groups.[8] It should be pointed out that the insertion of 5′,8-cyclo-2′-deoxyadenosine into DNA requires their conversion to 5′-triphosphate derivatives. Due to the rigid structure of both isomeric forms of **cdA**, their incorporation (from the outer source) to DNA occurs less frequently. Moreover, 5′*R* and 5′*S* diastereomers of **cdA** are produced in non-equivalent amounts. The 5′*R* isomer has been found to be the more propagating one under γ-radiation of an anaerobic water solution of 2′-deoxyadenosine or ss-DNA; however, in the case of ds-DNA, the **(5*S*)cdA** has been found to be the more abundant one.[9] In this article, for the first time, the formation of **cdA** in ss-DNA has been taken under theoretical consideration.

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Results and discussion

As mentioned in the introduction, the level of **cdA**s in mammalian cells depends on the conditions of the oxidative stress. The unusual influence of this tandem lesion on the oligonucleotides repair process means that the investigation of their formation mechanism in single stranded DNA is of primary importance. The 5′*R* and 5′*S* isomers of **cdA** can be effectively synthesized by known synthetic methods.[10,11] Both of them exploit the free radical intermediates of 2′-deoxyadenosine, ˙C8 or ˙C5′, formed under different radiation conditions. Moreover, the strategies mentioned above can be used for **cdA** formation in ss-DNA. However, the method described by Chatgilaloglu requires the introduction of a Br atom in the C8 position of adenine.[10a] The situation is less complicated in the case of the C5′ radical, which can be formed directly by the abstraction of one of the hydrogen atoms attached to the C5′ carbon of 2′-deoxyadenosine, as a result of the reaction with a hydroxyl radical in anaerobic conditions. The mechanism of 2′-deoxyadeno-5′-yl formation in the presence of oxygen, is still an open question. In 2006, Carrell *et al.* showed that the introduction of 8-bromo-2′-deoxyadenosine to hairpin DNA, containing the flavine electron injector in the loop structure, leads to the 5′C radical formation under radiation conditions.[12a] (The 5′C radical was recognised as a potential **cdA** precursor.[12b,c]) Even though the mechanism of 5′,8-cyclo-2′-deoxynucleoside formation has been theoretically investigated by different groups, a comprehensive study of **cdA** formation in ss-DNA is still required. To simplify the system of single strand oligonucleosides, the 2′-deoxyadenosine-3′,5′-diphosphate has been chosen as a model system.[13] Moreover, the 3′ and 5′ phosphodiester linkages have been terminated by methyl groups. The internucleotide bond, RO–PO_2^-–OR, moiety has been

Table 1 The free energy and free energy of solvation of: substrates, transition states/products and final products of the C5′;C8 cyclization process obtained at the B3LYP 6-31++G** level in gaseous and aqueous phases

	Free energy in Hartree				
Molecule	G°_{gas}	G°_{gas} [a]	G°_{aq}	G°_{aq} [a]	ΔG°_{sol} [b]
1	-101.354379	-2101.002785	-2101.618398	-2101.2668	-165.6739914
2	-2101.357556	-2101.005801	-2101.622796	-2101.2710	-166.4409586
3	-2101.346868	-2100.995243	-2101.61812	-2101.2665	-170.2129622
4	-2100.697846	-2100.359499	-2100.96090	-2100.6226	-165.0688715
5	-2100.699635	-2100.361380	-2100.959848	-2100.6216	-163.2861295
6	-2100.700066	-2100.361898	-2100.961997	-2100.6238	-164.3641846
7	-2100.651361	-2100.312684	-2100.92362	-2100.5849	-170.8452094
8	-2100.659035	-2100.320038	-2100.926147	-2100.5871	-167.6151419
9	-2100.653076	-2100.314304	-2100.917061	-2100.5783	-165.6529448
10	-2100.651370	-2100.315705	-2100.916401	-2100.5807	-166.3091754
11	-2100.654755	-2100.318973	-2100.920662	-2100.5849	-166.8594510
12	-2100.680243	-2100.343071	-2100.941851	-2100.6047	-164.1615680
13	-2100.667229	-2100.330059	-2100.930700	-2100.5935	-165.3307186
14	-2100.687096	-2100.349877	-2100.943037	-2100.6058	-160.6055533
15	-2100.674724	-2100.337766	-2100.938804	-2100.6018	-165.7129158
16	-2100.705034	-2100.365834	-2100.974399	-2100.6352	-169.0294792
17	-2100.690183	-2100.351014	-2100.957812	-2100.6186	-167.9395831
18	-2100.712216	-2100.372968	-2100.971494	-2100.6322	-162.6991383
19	-2100.698114	-2100.359271	-2100.968544	-2100.6297	-169.6967791
(5′*S*)cdA	-2100.141433	-2099.812320	-2100.410313	-2100.0812	-168.7250367
(5′*R*)cdA	-2100.142869	-2099.813874	-2100.413926	-2100.0849	-170.0912881

[a] ZPE and thermal corrections are included. [b] Energy given in kcal mol^{-1}.

used in anionic form, due to the well known fact that phosphate groups are unprotonated in aqueous solution.[14] The initial conformations of the 5′-hydroxymethyl group of 2′-deoxynucleotide-3′,5′-diphosphate are critical for the final diastereomeric form of **cdA**. Each of the nucleosides/nucleotides exists in three rotameric forms: *gauche*(+), *gauche*(−) and *trans* (Fig. 1). The *gauche*(+) **2** has been found to be the most stable in gaseous and aqueous phases. The formation of •C8 leads to the appearance of radicals **7**, **8** and **9**; their thermodynamic stabilities are **8** > **9** > **7** in the aqueous phase, and **8** > **7** >**9** in the gaseous phase (Table 1, Fig. 2). (The pseudo-first-order rate constant of •C8 formation has been found as $k = 1.6 \pm 0.1 \times 10^{10}$ s^{-1}, for 8-Br-dA.[15])

It should be mentioned that the reaction energies of the •C8 radicals' formation are around 116 kcal mol^{-1}. On the other hand, the energy differences between **7**, **8** and **9**, and the corresponding C5′ radicals **4**, **5** and **6**, are approximately 26 kcal mol^{-1} in both aqueous and gaseous phases (Fig. 2). These results are in good agreement with the data obtained by Evangelista and Schaefer for different kinds of 2′-deoxyadenosine radicals.[16] Due to these facts, the formation of **4**, **5** and **6** from the corresponding nucleotides

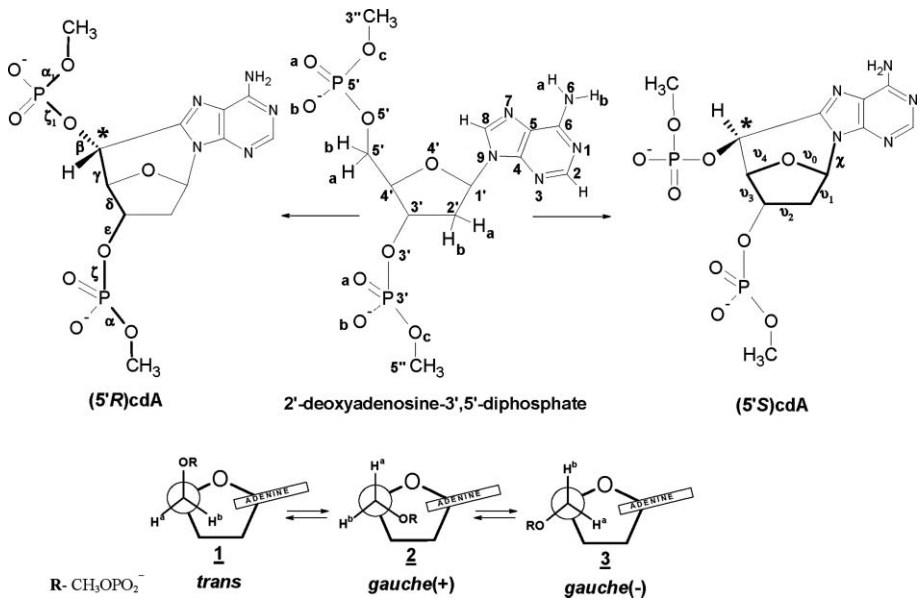

Fig. 1 Graphical representation of 2′-deoxyadenosine-3′,5′-diphosphate and the related 5′*R* and 5′*S* forms of 5′,8-cyclo-2′-deoxyadenosine-3′,5′-diphosphate. The atom numbers and dihedral angels are indicated.

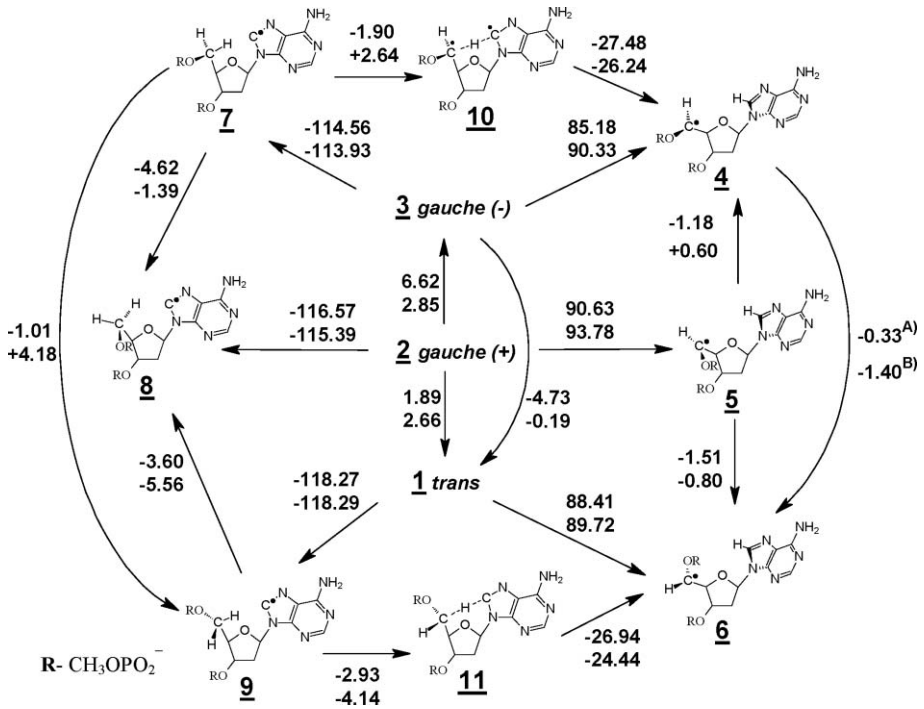

Fig. 2 Graphical representations of different types of radicals involved in **cdA** formation. Next to the arrows, the differences in energy between suitable compounds are given in kcal mol^{-1} (**A**-gaseous phase, **B**-aqueous phase).

1, **2** and **3**, by hydrogen atom abstraction from C5′, or by radical transfer from C8 (**7**, **8** and **9**) are privileged (Fig. 2). It should be pointed out that the transfer of the radical from the C8 carbon of **9** to 5′ of **11** leads to a negative value of $\Delta G = G^{\circ}_{11} - G^{\circ}_{9}$ in both discussed phases (Fig. 2). The same trend has been observed for the conversion of **7** → **10**. Therefore, this reaction should be exergonic in the gaseous phase. Conversely, the formation of **10** from radical **7** in the aqueous phase requires external energy to force the radical transfer.

Due to these facts, the conversion of **9** to **11** leads to the formation of **4** (precursor of **(5′*R*)cdA**); it can be postulated that **(5′*R*)cdA** should be formed as the predominant product during the radiation of single stranded oligonucleotides which contain the 8-bromo-2′-deoxyadenosine unit. These results are in good agreement with the experimental data, which show that during the discussed process, the formed diastereomers of 5′,8-cyclo-2′-deoxyadensine are produced in the ratio $5'R:5'S = 6:1$.[11] It should be pointed out that in the formed radicals **7**, **8** and **9**, the distance between the carbon atoms C5′ and C8 are similar to those found in the neutral compounds **1**, **2** and **3**. However, rather significant changes in the values of the dihedral angels around the *N*-glycosidic bond (C1′–N9) have been observed (Table 2). These data have been confirmed by the calculations of the pseudorotation parameters of the sugar unit, which show that the substrates and products of the discussed reactions (**1–9**) exhibit almost the same puckering, phase and amplitude (Table 2). Moreover, the changes in the dipole moment (DM) and charge distribution do not show significant fluctuation during the compound transformation in the discussed process (Table 3). Therefore, the abstraction of one of the hydrogen atoms from C5′ or the Br atom from the C8 position, leading to the corresponding free radical species' formation, has a low influence on the spatial geometry of the nucleotide/nucleoside.

As shown in Table 4, in compounds **7–9**, the spin density is mainly generated on the C8 carbon in all cases. The differences in the spin density have been observed in the C5′ radical series (however, it mainly accumulates on C5′). In compound **4**, the spin density has been diffused onto the C5′, C4′ and C1′ atoms (Table 3). Therefore, carbon 5′ in molecule **4** has demonstrated a decrease in its reactivity. For these reasons, **4** can be considered as a precursor of a different type of byproduct (other than the expected products **12** and **13**). It should be mentioned at this point that the 2′-deoxyadenosin-5′-yl radical attacks the double bond of adenine intramoleculary with the rate constant $k = 1.6 \times 10^5$ s^{-1}, to form the final product **cdA**[17]). Moreover, characteristic changes in the length of the bonds adjacent to the radical centres are observed, as presented in Table 2.

In the gaseous and aqueous environments, radical **6** (*trans*) has been found to be the most stable among all the 2′-deoxyadenosin-5′-yl forms (Fig. 3, Table 1). Surprisingly, in the gaseous phase, **4** (*gauche* (−)) is found as more stable than **5** (*gauche* (+)); however, in the aqueous phase the obtained results are opposite and in accordance with the stability order of the neutral species **1** > **3**. As a consequence, **4** and **6** can be recognized as precursors of 5′*S* and 5′*R* of 5′,8-cyclo-2′-deoxynucleotide-3′,5′-diphosphate, respectively, formed by direct hydrogen abstraction from the C5′ position of native 3′,5′-diphosphate **1** or **3**. In **4** and **6**, the initial distances between C5′ and C8 were 3.632 Å and 3.796 Å, respectively. These distances decreased by 1.4 Å in the transition states (**12–15**) and by another 0.7 Å in **16–19** (transition products). Additionally, significant changes in the sugar ring puckering have been observed (Table 2). It should be pointed out that in **12** and **14**, 2-deoxyribose adopts the unusual $_0T^1$ puckering,[18] the same as in the final products. Moreover, the new initially formed six-membered rings, adopt the boat or chair conformations (see

Table 2 Selected geometrical parameters of the substrates, transition states/products and final products obtained at the B3LYP 6-31++G** level

	Pseudorotation parameters			Bond length/Å					Dihedral angle (°)	
Comp.	Puc.[a]	P[b]	Am[c]	C4′–C5′	C5′–O5′	C5′–C8	H5′–C8	C1′–N9	γ	χ
1	$^{2}T_{3}$	171.9	34.2	1.529	1.421	4.131	3.568	1.473	−178.97	−138.16
2	$^{2}T_{3}$	171.7	36.1	1.526	1.425	4.203	4.083	1.471	40.69	−134.29
3	$_{3}T^{2}$	181.1	36.8	1.531	1.420	3.879	3.374	1.478	−78.96	−127.18
4	$_{3}T^{2}$	197.2	36.2	1.501	1.359	3.632	3.287	1.480	−70.97	−112.88
5	$_{3}T^{2}$	182.8	36.1	1.480	1.361	4.025	4.671	1.479	48.56	−129.09
6	$_{3}T^{2}$	193.3	35.9	1.484	1.357	3.796	3.554	1.482	−174.85	−135.64
7	$^{2}T_{3}$	177.7	37.9	1.531	1.422	4.151	3.544	1.472	−76.68	−96.42
8	$_{3}T^{2}$	180.4	34.5	1.524	1.422	4.222	4.469	1.469	53.05	−95.51
9	$_{3}T^{2}$	192.1	33.0	1.533	1.425	3.868	3.255	1.468	−170.10	−112.74
10	$^{4}T_{3}$	226.1	36.5	1.540	1.387	2.909	1.834 1.162[d]	1.478	−57.09	−114.95
11	$^{1}T_{4}$	294.6	30.3	1.528	1.393	2.914	1.846 1.154[d]	1.488	−176.31	−126.61
12	$_{0}T^{1}$	271.4	40.9	1.521	1.344	2.240		1.464	−61.6	−79.3
13	$^{4}T_{0}$	251.1	42.3	1.536	1.347	2.174		1.487	−18.15	161.51
14	$_{0}T^{1}$	273.6	42.1	1.515	1.355	2.238		1.468	−176.57	−71.21
15	$_{0}T^{4}$	268.3	41.5	1.526	1.351	2.159		1.486	−162.68	162.68
16	$_{0}T^{4}$	269.6	45.6	1.554	1.405	1.568		1.465	−67.133	−94.290
17	$_{0}T^{4}$	255.2	48.7	1.575	1.409	1.574		1.477	−18.798	172.758
18	$_{0}T^{1}$	273.2	48.3	1.546	1.416	1.578		1.460	−177.85	−85.10
19	$_{0}T^{4}$	261.5	48.1	1.565	1.408	1.567		1.476	−138.68	175.214
(5′*S*)cdA	$_{0}T^{1}$	272.6	45.3	1.555	1.407	1.517		1.482	−60.12	−150.99
(5′*R*)cdA	$_{0}T^{1}$	275.6	47.4	1.548	1.419	1.517		1.481	−172.31	−150.13

[a] 2-Deoxyribose ring puckering. [b] Phase, calculated by equation: $\tan(P) = [(\upsilon_4 + \upsilon_1) - (\upsilon_3 + \upsilon_0)] / [2\upsilon_2(\sin(36) + \sin(72))]$. [c] Amplitude calculated by equation: $\phi_{max} = \upsilon_2/\cos(P)$.[1] [d] Distance between C5′ and H5′ atoms.

Table 3 Sum of charges determined by natural population analysis and dipole moments (in Debye) on the sugar, phosphates and base moiety, of the substrates, transition states/products and final products of the C5′;C8 cyclization process obtained at the B3LYP 6-31++G** level

	Component				
Molecule	5′-Phosphate	2-Deoxyribose	Adenine	3′-Phosphate	Dipole moment/D
1	-0.37	-0.86	-0.32	-0.40	10.3
2	-0.39	-0.91	-0.32	-0.39	11.7
3	-0.40	-0.86	-0.32	-0.41	17.7
4	-0.35	-0.90	-0.34	-0.38	16.3
5	-0.35	-0.92	-0.35	-0.37	16.7
6	-0.36	-0.86	-0.29	-0.40	10.0
7	-0.41	-0.82	-0.35	-0.40	19.2
8	-0.38	-0.90	-0.30	-0.38	13.7
9	-0.26	-0.86	-0.49	-0.40	9.3
10	-0.36	-0.75	-0.51	-0.38	17.6
11	-0.37	-0.73	-0.47	-0.39	14.5
12	-0.33	-0.84	-0.53	-0.38	9.5
13	-0.33	-0.80	-0.51	-0.39	11.1
14	-0.34	-0.83	-0.46	-0.39	6.0
15	-0.35	-0.88	-0.49	-0.39	5.0
16	-0.38	-0.83	-0.38	-0.38	14.9
17	-0.36	-0.87	-0.37	-0.39	13.5
18	-0.37	-0.87	-0.36	-0.40	7.1
19	-0.38	-0.84	-0.36	-0.40	9.9
(5′*S*)cdA	-0.36	-0.83	-0.41	-0.38	14.7
(5′*R*)cdA	-0.36	-0.82	-0.40	-0.40	10.4

Fig. 3). The transition states **12** and **14** with chair geometries have been found to be thermodynamically favoured ones in both discussed phases. In the transition structures **12–14**, the spin density mainly diffuses between C5′ in the sugar moiety and N7 in the adenine unit; however, it is still mainly located in the 2-deoxyribose unit of the nucleotides (Table 4). It should be pointed that a lower energy barrier has been found for the conversion of **6** to **14** in the gaseous phase (Fig. 3). Surprisingly, this barrier is on the same energy level for the **4** → **12** and **6** → **14** transformations in the aqueous phase—a negligible difference is found (0.08 kcal mol^{-1}). Therefore, the reaction paths involving the transformations **4** → **12** → **16** and **6** → **14** → **18** are discussed as the most probable paths of 5′*S* and 5′*R* 5′,8-cyclo-2′-deoxyadenosine-3′,5′-diphosphate formation, respectively (Fig. 3).

The cyclization process leads to the creation of a new chiral centre on the C8 carbon atom in the intermediate product. Due

Table 4 Mulliken spin density of selected adenine or 2-deoxyribose atoms of the substrates (radicals) and the transition states/products of the C5′;C8 cyclization process obtained at the B3LYP 6-31++G** level

	Spin density									
Molecule	O5′	C5′	C4′	C1′	C8	N7	C6	C5	C2	H8
4	0.10	0.78	0.13	0.23	-0.01					
5	0.09	0.93	-0.01	-0.02	-0.01					
6	0.09	1.01	-0.03	0.05	-0.04					
7			-0.03	0.09	0.88			0.12		
8				0.07	0.87			0.14		
9			-0.02	0.03	0.86			0.06		
10	0.10	0.18	0.01	-0.01	0.67			0.13		
11	0.08	0.12	0.03	-0.06	0.78			0.12		
12	0.09	0.68	-0.18	0.05	-0.21	0.24	0.11	-0.07	0.07	0.01
13	0.10	0.58	-0.08	0.11	-0.20	0.24	0.12	-0.06	0.09	0.03
14	0.07	0.68	-0.18	0.15	-0.03	0.20	0.12	-0.05	0.07	0.03
15	0.09	0.58	-0.05	0.08	-0.21	0.25	0.11	-0.08	0.08	0.03
16	0.01	0.03	0.01	-0.01	-0.04	0.48	0.21	-0.14	0.20	0.05
17	0.01	0.04			-0.04	0.47	0.23	-0.14	0.20	0.05
18	0.01	0.08	-0.01	-0.01	-0.06	0.48	0.22	-0.13	0.20	0.04
19	0.01	-0.02	0.02	-0.01	-0.02	0.48	0.21	-0.11	0.20	0.06

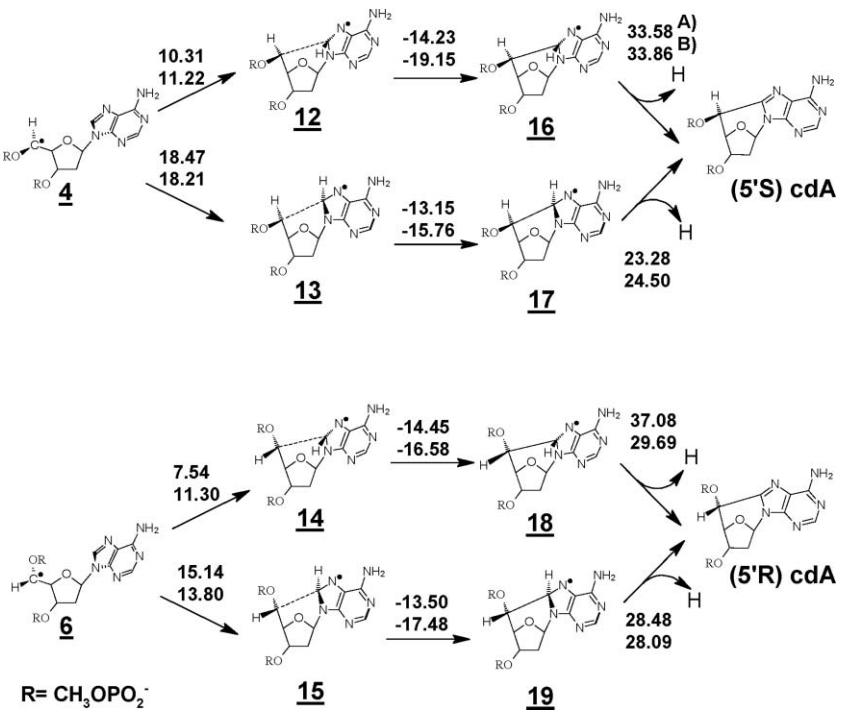

Fig. 3 Possible reaction paths of 5′,8-cyclo-2′-deoxynucleotide-3′,5′-diphosphate formation. Differences in energy (kcal mol^{-1}) between suitable molecules are given over the arrows. (**A**-gaseous phase, **B**-aqueous phase).

to the possibility of adenine rotation around the *N*-glycosidic bond in **16–18**, the following diastereomeric arrangements at C5′ and C8, in the reaction the following intermediate products are possible: *SR*(**16**), *SS*(**17**), *RR*(**18**), *RS*(**19**) (Fig. 3). The selected geometrical parameters of these molecules are given in Table 2. The results of quantum mechanics study have shown that products **16** and **18** are the most energetically favoured. Their formation is characterized by the following energetic barriers: $\Delta G(\mathbf{12} \rightarrow \mathbf{16}) = -14.23/-19.15$ kcal mol^{-1} and $\Delta G(\mathbf{14} \rightarrow \mathbf{18}) = -14.45/-16.58$ kcal mol^{-1} in the gaseous/aqueous phase, respectively. The thermodynamic stability of **16–19** is as follows: **18** > **16** > **19** > **17** and **16** > **18** > **19** > **17** in gaseous and aqueous phases, respectively (Table 1).

The final stage of the reaction pathway is the hydrogen atom elimination with simultaneous formation of the 5′*R* and 5′*S* forms of 5′,8-cyclo-2′-deoxyadenosine-3′,5′-diphosphate. From the two formed diastereomers of 5′,8-cyclopurine-3′,5′-diphosphate, the product with the 5′*R* configuration is characterized by a lower energy compared to the 5′*S* one in both discussed phases (ΔG(**(5′*S*)cdA**–**(5′*R*)cdA**) = 0.98 and 2.34 kcal mol^{-1} in gaseous/aqueous phase, respectively) (Table 1, Fig. 1). Therefore, the presented results are in line with the experimental

data obtained by γ-radiation of aqueous solutions of short ss-DNA (tetramer d[ACGT]).[19] It has been assumed that precursors **6** and **14** of the **5′*R*** form of 5′,8-cyclo-2′-deoxyadenosine-3′,5′-diphosphate are the most stable ones of the discussed C5′ radical of 2′-deoxyadenosine-3′,5′-diphosphate in both calculation environments. Moreover, the lowest energetic barrier (7.54/11.3 kcal mol^{-1} gaseous/aqueous phase) of the **6** → **14** transition would lead to the preferential formation of the 5′*R* diastereomeric form in ss-DNA. The conductor-like polarisation continuum model (CPCM) single point calculations of previously optimised geometries in the gaseous phase, on the same level of theory, have shown a slight decrease of the reaction energy barrier for both diastereomers' formation process (Fig. 3). It should be pointed out that the solvation effect causes the significant decrease in energy of each of the discussed compounds (by approximately 170 kcal mol^{-1}) (Table 1). Due to the unusual structure of 5′,8-cyclo-2′-deoxyadenosine-3′,5′-diphosphate, the accumulation of the negative charge by the discussed substrates, transition states and products, are important for the above-mentioned reactions.

For this purpose, the full natural population analysis (NPA) was done for anionic and radical-anionic derivatives of 2′-deoxyadenosine-3′,5′-diphosphate and 5′,8-cyclo-2′-deoxyadenosine-3′,5′-diphosphate. Table 3 displays the charge distribution among the phosphate, sugar and adenine moieties of the discussed molecules. The NPA charge analyses have shown that the investigated molecules, independent of their anionic or radical-anionic forms, accumulate the negative charge almost evenly between nucleotide units. However, a twice higher accumulation of the negative charge is found at the 2-deoxyribose as compared to other units (Table 3).

Moreover, the charge, for all radical anions and anionic species, oscillates between –0.33 and –0.40, and from –0.37 to –0.40 for the phosphate groups attached to the 5′-end or the 3′-end, during the C5′;C8 cyclisation process (Table 3). As mentioned above, the fluctuation of the negative charge is negligible; however, it is possible to observe a strong correlation between the charge decreases on the part of molecule where the radical appears. (Fig. 3, and Table 3 and 4). The values of the dipole moments (DM) of the molecules involved in the cycloadenosine formation process have been presented in Table 3. (The DM is a valuable parameter for the description of the changes in the geometry of the molecule. It has been assumed that a lower value of DM provides a higher geometrical fluctuation of molecules and *vice versa*.[20]) The analysis of the DM has shown that in the privileged path of the 5′*S* diastereomer formation, the dipole moments of **3**, **4**, **12**, **16** and **(5′*S*)cdA** fluctuate between 9.5 and 17.7 Debyes. For the 5′*R* path (**1**, **6**, **14**, **18** and **(5′*R*)cdA**) the fluctuation of DM is between 7.1 and 10.4 Debyes. It has been postulated that for the 5′*S* and 5′*R* forms of **cdAs**, significant geometrical changes of molecules involved in the discussed process are necessary (Table 2 and 3). However, from the geometrical point of view, the formation of the 5′*R* diastereomer of **cdA** should be privileged. It has been found that after the initiation of the 5′C;C8 cyclisation process, the puckering of the sugar ring assumes the $_0T^1$ value exclusively. Moreover, the γ dihedral angle (C3′–C4′–C5′–O5′), responsible for the location of the 5′-end phosphate group in ss-DNA, requires only a slight rotation, around 6.7°, to force the conversion from **1** (*trans* rotamer of 2-deoxyribose) into **(5′*R*)cdA**. A more significant change, around 18.8°, of the position of the 5′ hydroxymethyl group, has been observed in the path of the transformation from **3** (*gauche*(–)) to **(5′*S*)cdA**. Finally, the DM values for the initial substrate **1** and the final product **(5′*R*)cdA** are found to be 10.3 and 10.4. (In the case of 5′*S* diastereomers DM had values of 17.7 and 14.7 for the starting molecule **3** and the terminal product **(5′*S*)cdA**, respectively.) Moreover, the previous results of structural analysis (DFT and NMR studies) of dT_{PO}(5′*R*)cdA and dT_{PO}(5′*S*)cdA have shown the significant difference in their geometries.[21] Therefore, it can be postulated that **(5′*R*)cdA** is "structurally similar" to natural 2′-deoxyadenosine-3′,5′-diphosphate as opposed to the 5′*S* diastereomer.

Conclusions

Reactions of reactive oxygen species, and more specifically of ˙OH, may lead to the generation of nucleoside radicals in ss-DNA. In this article, for the first time, the analyses of the possible models of 2′-deoxyadenosine-3′,5′-diphosphate radical reorganization, leading to the formation of 5′,8-cyclo-2′-deoxyadenosine-3′,5′-diphosphate (**cdA**), were taken under consideration.

The formation of radicals at the C5′ and C8 atoms leads, through intramolecular cyclization, to the final product **(cdA)** formed in two diastereomeric forms, 5′*R* and 5′*S*. Quantum chemistry methods were employed to elucidate the various paths that result in cyclisation. The calculations were carried out in both gaseous and aqueous phases. It was shown that the 5′,8-cyclonucleoside, exhibiting the 5′*R* configuration, might be predominant, independent of the type of the initially formed radical (C5′ or C8 in ss-DNA). First of all, a lower energy barrier in the critical points (radical transfer from C8 to C5′ paths **9** → **11** → **6** and **6** → **14** → **18** in the cyclisation period) of the discussed process has been found in the case of 5′*R* diastereomer formation as compared to 5′*S*. Moreover, 5′*R* **cdA** has been found to be thermodynamically favoured in both discussed phases. Using the dipole moment analysis (almost the same values of DM have been found for **1** and **(5′*R*)cdA**), and considering the spatial geometry, it can be postulated that **(5′*R*)cdA** is "structurally similar" to natural 2′-deoxyadenosine-3′,5′-diphosphate instead of the 5′*S* diastereomer. Owing to the structural similarity, this lesion exhibited lower toxicity. Therefore, the results of these studies are consistent with the experimental data obtained during the γ-radiation of aqueous solutions of 2′-deoxyadenosine or 8-Br-2′-deoxynucleoside incorporated to ss-DNA.

Materials and methods

Computation methodology of quantum mechanics study

The molecular geometries of 5′,8-cyclo-2′-deoxyadenosine in the gaseous phase were initially optimized by molecular mechanics using UFF (Universal Force Fields)[22] 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),[23] and the Lee–Yang–Parr correlation functional (LYP)[24]) was implemented. For all calculations the 6-31++G**[25] basis set with polarisation functions was used, yielding

630 basis functions per neutral 5′,8-cyclo-2′-deoxyadenosine-3′,5′-diphosphate. The 6-31++G** basis set was represented as a number of atomic orbitals composed of $M_{C,N,O}/M_H$ (4s3p1d/3s1p), consisting of 19 basis functions per C, N, O atoms and 6 basis functions per H atom.[26] 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 thermal contribution to the free energies was considered. For all energy calculations, the scale factor 0.9854 for the 6-31++G** basis set was used.[27] For the characterization of the stationary point of all of 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.

The conductor-like polarisation continuum model (CPCM) was used to describe the surrounding aqueous medium, with dielectric constant of water $\varepsilon = 78.39$.[28] The standard free energy of the molecule in the aqueous phase, $\Delta G^\circ_{(AQU)}$, was described as the sum of the standard free energy of the molecule in the gaseous phase $\Delta G^\circ_{(GAS)}$ and the standard free energy of solvation, $\Delta G^\circ_{(SOLV)}$.[8c] Suitable $\Delta G^\circ_{(SOLV)}$ values were obtained by single point calculation at the CPCM/B3LYP/6-31++G** level. The calculations of all the structures were achieved with Gaussian 03 Revision D.01.[29]

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