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Theoretical analysis of DNA intrastrand cross linking by formation of 8,5′-cyclodeoxyadenosine

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ABSTRACT

Formation of intramolecular cross links by addition of C(5′) deoxyribose radicals to the C(8)–N(7) double bond of an attached adenine base was analyzed by *ab initio* quantum-chemical methods. Conformational preferences that influence the stereospecificity of the reaction were investigated. A good correlation was found between the ratio of experimental yields of R and S stereoisomers of 8,5′-cyclodeoxyadenosine and the relative energy of conformations of the C(5′) radical that are precursors to these isomers. Molecular mechanics based on the AMBER force field was used to model the effect of 8,5′-cyclodeoxyadenosine on the conformation of the DNA dodecamer d(CGCGAATTCGCG)$_2$ with the lesion at the A6 position. The R and S stereoisomers of the intrastrand cross link cause comparable levels of DNA distortion with the major conformational changes occurring in backbone torsional angles at the site of the lesion.

INTRODUCTION

Highly reactive free radicals, such as the hydroxyl (OH) radical, generated by exposure to ionizing radiation interact with nucleic acids to produce a great variety of lesions (1) including intramolecular cross links between the base and deoxyribose moieties of DNA. 8,5′-cyclo(deoxy)nucleotides(sides) have been observed in both adenine- and guanine-containing monomers, in RNA and DNA polymers (2–7), and in DNA isolated from irradiated human cells (8,9). A three-step mechanism has been proposed (6) for induction of these lesions in polynucleotides and DNA by ionizing radiation: (i) abstraction of a hydrogen atom from the C(5′) position of sugar by an OH radical from water radiolysis, (ii) nucleophilic addition of this C(5′) radical to the C(8) position of the purine base to form the C(5′)–C(8) intramolecular bond, and (iii) one electron oxidation of the N(7)-centered radical on the purine base to yield the stable product.

The importance of OH radicals in formation of intrastrand cross links was confirmed by experiments showing that the yield of 8,5′-cycloAMP from irradiated solutions of 5′-AMP saturated with N_2O was twice that observed in N_2-saturated solutions (3) due to conversion of e_{aq-} to hydroxyl radicals (10). Addition of OH-radical scavengers, such as dimethyl sulfoxide or nitroaromatic radiosensitizers (11), to irradiated solutions of poly(A) reduced the yield of 8,5′-cycloadenosine in a manner consistent with OH-radical scavenging. The presence of oxygen (2,3,12) or nitroaromatic radiosensitizers (11) also reduced the yield of 8,5′-cyclo(deoxy)nucleotide(sides) due to reactions with the C(5′)-radical precursor that are more rapid than the nucleophilic addition to C(8).

The secondary structure of nucleic acids also affects intrastrand cross link formation. Yields of 8,5′-cyclodeoxypurines are higher in single-stranded DNA than they are in the double helix (7). Furthermore, the yields of these cross links at adenine and guanine bases are very similar in single-stranded DNA but the yield of 8,5′-cyclodeoxyadenosine is 50% higher than the yield of 8,5′-cyclodeoxyguanosine in duplex DNA. These observations lead Dirksen *et al.* (7) to speculate that strand separation in A–T rich DNA sequences provides a molecular environment that is favorable to intrastrand cross link formation.

Figure 1 shows the two stereoisomers of 8,5′-cyclodeoxypurines that result from different ligand configurations around C(5′). Both stereoisomers of 8,5′-cycloadenosine have been observed in irradiated solutions of adenosine, adenosine 5′-monophosphate (5′-AMP) and polyadenylic acid [poly(A)] (4,6) with R to S yield ratios of 1.80, 0.37 and 1.56, respectively. The relative yield of R and S isomers of 8,5′-cycloAMP was found to be strongly dependent on the pH of irradiated solutions of 5′-AMP with the S isomer predominating at neutral pH and the R isomer favored in acidic solutions (4). The R to S yield ratio of 1.8 observed for 8,5′-cyclodeoxyadenosine in single-stranded DNA (7) is close to that found for 8,5′-cycloadenosine in poly(A) (5); however, in duplex DNA, this yield ratio is reduced to 0.8 (7). An even greater shift in the stereospecificity of cross link formation is observed for 8,5′-cyclodeoxyguanosine where R/S ratios of 2.7 and 0.8 were found for single- and double-stranded DNA, respectively (7). These changes in stereospecificity may be linked to the reduced yield of intrastrand cross links in duplex DNA relative to single-stranded DNA.

This paper describes the use of quantum-chemical and molecular mechanics methods to investigate mechanisms of stereospecificity in 8,5′-cyclodeoxyadenosine formation. Our methods are briefly outlined in the first section. Results for both the energy and conformation of various species on the pathway from C(5′) radicals to stable intramolecular cross links are presented in the second section. The third section discusses our

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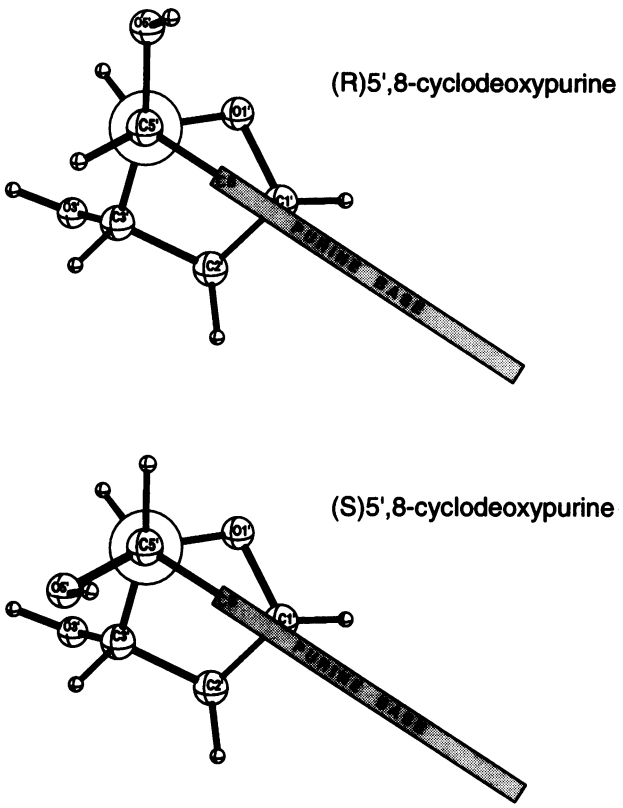

Figure 1. R and S stereoisomers of 8,5′-cyclodeoxypurine viewed along the C(5′)–C(4′) bond.

results in relation to the available experimental data and our conclusions are presented in the final section.

MATERIALS AND METHODS

Quantum chemical calculations

Quantum chemical calculations were performed with the GAUSSIAN92 package for *ab initio* calculations (13). Figure 2 shows the fragment consisting of deoxyribose and the five-membered ring of adenine that was used to model the formation of 8,5′-cyclodeoxypurine nucleotides. The notation used in this work for the molecules present at different stages of the reaction is also explained in Figure 2. C(5′)-centered radicals are labeled R or S depending on which stereoisomer of 8,5′-cyclodeoxypurines they lead to. Addition of the C(5′) radical to the C(8)–N(7) double bond gives a free-radical intermediate with asymmetric carbon centers at both C(5′) and C(8). We denote the four diastereisomers of this intermediate product as RR, RS, SR and SS, where the first and second letters refer to the stereoconfiguration at C(5′) and C(8), respectively. The final step of 8,5′-cyclodeoxypurine formation involves loss of a hydrogen atom from the paramagnetic intermediate to give the final diamagnetic product. This step was not specifically studied in this work apart from determining the energies and structures of the final products, which have only one asymmetric carbon, C(5′), resulting in two stereoisomers.

Geometries of all the molecular systems studied were optimized at the HF/6-31G level (UHF for open-shell systems). Single point calculations at MP2/6-31G* level were performed on the optimized structures. Zero point vibrational energies (ZPE) were calculated from the HF/6-31G frequencies. Projected MP2 energies (14) were used for all the open-shell systems due to the significant spin contamination in some of the systems studied. Solvation energies were calculated by the method of Rashin and Namboodiri (15) which has been successfully applied to small polar organic molecules (15,16). In this approximation, solvation energies are the sum of the enthalpy of electrostatic interaction between the molecule and the solvent (treated as a dielectric continuum) and the hydration enthalpy of a non-polar molecule forming the same cavity in the solvent. Standard cavity radii (15,16) were used in our calculations along with point charges derived from Mulliken population analysis of the 6-31G wavefunctions.

Molecular mechanics calculations

Molecular mechanics calculations were performed with the AMBER 4.0 program (17). The R and S stereoisomers of 8,5′-cyclodeoxyadenosine as well as two intermediate products in the configurations RR and SR were incorporated at the A6 position of the dodecamer duplex d(CGCGAATTCGCG)$_2$. The canonical right-handed B-DNA conformation was used as a starting structure. Atom types in the final products were the same as those in native adenosine; hence, C(5′) and C(8) were assigned the CT and CK AMBER atom types, respectively. Missing force field parameters for the C(5′)–C(8) intramolecular bond were derived from *ab initio* quantum calculations. For the intermediate products, a new carbon atom type C1 was created to describe C(8) since the intermediate structures have sp^3 carbon hybridization rather than the sp^2 hybridization of native adenosine. The template for this new atom type was the CT atom type for tetrahedral carbons with some modifications introduced according to the *ab initio* calculations on the intermediate products.

Distance constraints were appied to the C(5′)–C(8) bond to insure gradual formation of 8,5′-cyclodeoxyadenosines. Only the positions of water and counterions were optimized in the first step of energy minimization. Next, the energy of the whole system was minimized with the C(5′)–C(8) distance in A6 restrained at 3.0 Å; then this step was repeated with the C(5′)–C(8) distance decreased to 2.0 Å. Finally, the whole system was energy optimized without constraints. An additional constraint on the O(5′)–C(5′)–C(4′)–C(3′) torsion at A6 must be applied to form the R isomer of 8,5′-cyclodeoxyadenosine in B-DNA. This constraint was also removed in the last minimization. Minimized structures were analyzed using the Curves algorithm by Sklenar and Lavery (18,19).

RESULTS

Energetics

Calculated energies relative to the most stable configuration in the absence of solvent are listed in Table 1 along with hydration energies. Absolute quantum-chemical energies are available by request. Inclusion of electron correlation at the MP2 level produces small quantitative differences but does not change the energetic preferences among stereoisomers observed in uncorrelated calculations. Hydration energies has a more dramatic effect because solvation strongly favors the R configuration at C(5′). Consequently, the R configuration at C(5′) is energetically preferred at all stages of the reaction, although this preference is small for the radical

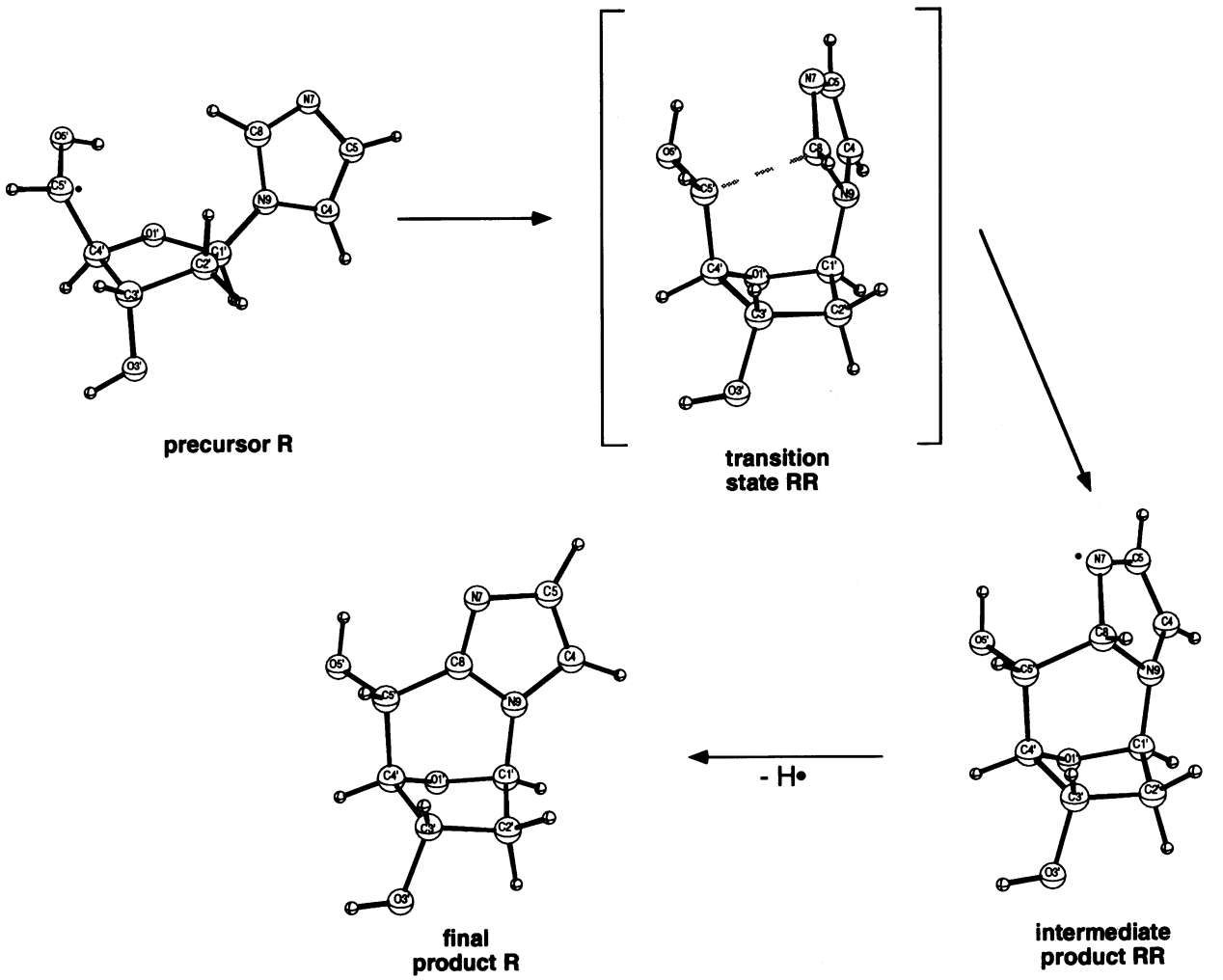

Figure 2. The fragment of purine nucleosides used in quantum chemical calculations and the stages of cyclodeoxypurine formation considered.

precursors. For each of the intermediate products with R or S symmetry at C(5′) there are two stereoisomers that differ in the orientation of substituents around the asymmetric C(8) position; however, the energetic preference for the R configuration at C(8) is large at all levels of approximation.

Calculations of the energy barriers for the addition reaction (Table 1) are highly dependent on the level of approximation. These barriers are very high at the Hartree–Fock level and even higher when correlation energy is included at the MP2 level. Removal of the spin contamination decreases the calculated energy barriers by about 14 kcal/mol. Sosa and co-workers (20,21) reported similar effects of the spin projection correction in calculations on radical addition to C–C multiple bonds. Hydration favors the transition state structure with the R configuration at C(5′), as was discussed above for products of the reaction. Including hydration energies, the barrier is lowest for the reaction leading to the RR isomer of the intermediate product.

The energy barriers shown in Table 1 are substantially higher than those usually calculated for radical addition to double bonds (22). This is due in part to an additional energetic contribution from the furanose ring which must adopt a high-energy puckering state to achieve the distance between C(5′) and C(8) in transition state structures. The pseudorotation phase angle (271°) and amplitude (42.4°) of this puckering state correspond to the maximum energy on the deoxyribose pseudorotation path (23,24). An estimate of the contribution from distortion of the deoxyribose ring to the reaction barrier can be obtained from a model of the deoxyribose molecule (R)-2-amino-(S)-4-hydroxy-(S)-5-methyltetrahydrofuran used in a previous study on deoxyribose and its radicals (25). For the present application, the furanose ring was frozen at the geometry observed in the transition state for the intramolecular cross link and only the parameters of ring substituents were optimized. At the MP2/6-31G*//HF/6-31G level, the structure optimized in this way had an energy 6.64 kcal/mol higher than the ^{2}E (C2′-endo) structure corresponding to the most stable conformation of deoxyribose. This result is close to the 5.8 kcal/mole barrier for deoxyribose puckering at P = 270° calculated by the molecular mechanics approach (23). The contribution to the energy barrier due to unfavorable deoxyribose ring puckering in the transition state structures (6.6 kcal/mol) is more than 60% of the whole barrier for RR addition at the MP2 level.

Table 1. Energetics of the 8,5′-cycloaddition reaction[a]

	UHF/6-31G	MP2/6-31G*	PMP2/6-31G*	PMP2/6-31G* +E_{hydr}	E_{hydr}
RELATIVE ENERGIES:					
Precursors:					
S(C5′)	0.00	0.00	0.00	0.00	–31.92
R(C5′)	0.93	2.31	2.29	–0.15	–34.36
Intermediate products:					
S(C5′)R(C8)	0.00	0.00	0.00	0.00	–26.42
S(C5′)S(C8)	5.63	5.10	5.55	4.84	–27.13
R(C5′)R(C8)	5.25	2.23	2.57	–6.77	–35.76
R(C5′)S(C8)	7.32	6.43	6.65	2.22	–30.85
Final products:					
S(C5′)	0.00	0.00		0.00[b]	–27.28
R(C5′)	4.13	2.34		–3.56[b]	–33.72
TRANSITION STATE BARRIERS:[c]					
S(C5′)	16.35	24.36	10.48	15.19	–27.21
R(C5′)	16.59	23.87	10.02	9.97	–34.41

[a]kcal/mole with ZPE included.
[b]MP2/6-31G* + E_{hydr}
[c]R configuration at C(8) assumed in transition-state structures due to its strong preference in intermediate products.

Structures

Conformational parameters calculated for 8,5′-cyclodeoxypurines and intermediates in the cyclization reaction are listed in Table 2. As was discussed above for the transition state, deoxyribose exhibits a very unusual conformation in the intermediate and final products of intrastrand cross linking. The phase angle (P) of the ring puckering is in the range of 270 to 290° and puckering amplitudes (τ_m) are between 46.5 and 48.8°. The puckering state is $_OE$ (O1′-exo) except for the SS intermediate and final S product where the puckering states are more like the adjacent ^1_OT. Similar puckering states were observed in the crystal structures of 8,5′-cycloAMP (26,27).

This unusual sugar puckering, which is required to bring C(5′) and C(8) close enough to form a new bond, forces the backbone dihedral angles γ and δ to assume values that are rarely observed in nucleic acids. The γ dihedral angles are in the ***-sc*** region in the SR intermediate and S final product, in the ***ap*** region in the RR intermediate and R final product, and in the ***ac*** region in intermediate products with an S configuration at C(8). The values of δ calculated for intermediate and final products are in the range of 140–156°.

The torsional angle about the glycosylic bond, χ, is found to have the common *anti* conformation in both R and S final products and the *high anti* (***-sc***) conformation in intermediate products with an R configuration at C(8). Only intermediate products with an S configuration at C(8) exhibit unusual values of χ in the ***ap*** conformational region.

Values of the Cremer and Pople general puckering parameters (28) are listed in Table 2 for the six-membered fused ring created by the intrastrand cross link. In both (R) and (S) 8,5′-cyclodeoxyadenosine, this ring has the half-boat (or envelope) conformation with O(1′) displaced from the plane (Fig. 3). The puckering amplitude (Q) is 0.683 Å for S isomer and 0.744 Å for R isomer. Large half-boat puckering was also found in the crystal structure of the S isomer of 8,5′-cycloadenosine-5′-monophosphate (26). In the crystal structure of the R isomer, the puckering was characterized as half-chair with C(4′) above and O(1′) below the plane (27). The half-boat conformation calculated for the final products, is not possible in the intermediate products due to the sp^3 configuration at C(8). Therefore, intermediate products with an R configuration at C(8) display a chair conformation with O(1′) above and C(8) below the plane, whereas intermediate products with an S configuration at C(8) prefer the boat conformation of the ring with C(8) and O(1′) above the plane. The imidazole ring that models the purine base in this work preserves its planarity in all of the calculated structures.

Many of the structural features discussed above for intermediate products of the cross linking reaction are also present in the transition-state structures (Fig. 4). Puckering of the deoxyribose ring is already close to that observed in the products and the conformation of backbone torsional angles is also similar. The transition states are about 0.1 Å later than those usually observed in the calculations for addition of carbon-centered radicals to C=C double bonds (22). The attack angle, C(5′)–C(8)–N(7), is similar to those found for other carbon-radical additions to double bonds (22) and indicates that the molecular plane of the base must be nearly perpendicular to the direction of attack by the C(5′) radical precursor. Values of the improper dihedral N(9)–C(8)–H(8)–N(7) in the transition states (143.3 and 144.8° for RR and SR, respectively) indicate significant pyramidalization at C(8).

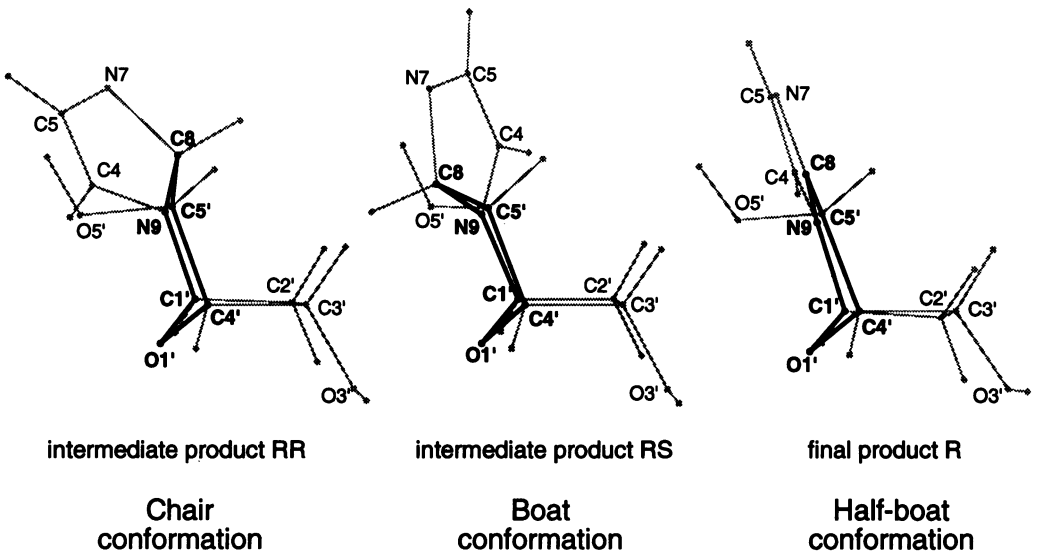

Figure 3. Conformation of fused six-membered ring (heavy lines) in intermediate and final products with R configuration at C(5').

Formation of 8,5'-cyclodeoxyadenosines inside DNA

Minimum-energy structures of the DNA dodecamer d(CGCGA ATTCGCG)$_2$ with A6 replaced by (R) and (S) 8,5'-cyclodeoxyadenosine or the RR and SR intermediate products indicate that intrastrand cross-link formation in duplex DNA requires large changes in backbone torsion angles near the lesion (Fig. 5). These perturbations include a shift in the ß torsion on the 5' side of (S) 8,5'-cyclodeoxyadenosine and shifts in both α and ß on the 5' side of the R isomer. Changes in the γ and δ torsion angles are a direct result of the unusual sugar puckering required to form the C(5')–C(8) bond of the intrastrand cross links.

All of the Watson–Crick hydrogen bonds were preserved in the minimun-energy structure; however, the length of the hydrogen bonds between the modified A6 residue and complementary T19 increased to about 2.4 Å and the bond angle decreased to about 160°, which indicates a weakening of these hydrogen bonds. Changes in structural parameters that affect base-stacking interactions are illustrated in Figure 6 for the strand containing the lesion. Results shown for tilt, roll, shift and slide indicate that base-stacking is highly distorted on both sides of 8,5'-cyclodeoxyadenosine. Changes in backbone torsion angles required to form the intrastrand cross link in DNA cause substantial perturbations of helical twist at the lesion site for both the R and S isomers (Fig. 6).

Table 2. Conformational parameters for the models of intermediate and final products of C5' to C8 cycloaddition

	Interm. prod. RR	Interm. prod. SR	Interm. prod. RS	Interm. prod. SS	Final prod. R	Final prod. S
C5'–C8 (Å)	1.550	1.539	1.531	1.546	1.496	1.498
Dihedral angles						
χ O1'–C1'–N9–C4 (°)	271.3	272.9	173.2	167.7	216.3	206.2
γ O5'–C5'–C4'–C3' (°)	182.3	293.2	221.7	220.9	194.2	302.9
δ C5'–C4'–C3'–O3' (°)	153.6	155.1	155.9	140.4	48.8	144.0
C5'–C8–N9–C1' (°)	32.0	38.2	–41.4	–25.2	4.2	13.0
Puckering of deoxyribose ring						
P (°)	271.7	273.7	271.6	289.6	277.5	286.6
τ_m (°)	47.8	48.8	47.7	47.6	47.0	46.5
Puckering of six-membered fused ring						
Q (Å)	0.781	0.748	0.879	0.879	0.744	0.683
θ (°)	28.3	26.6	83.5	75.4	60.1	49.1
φ (°)	54.9	59.9	62.7	61.0	58.4	60.1

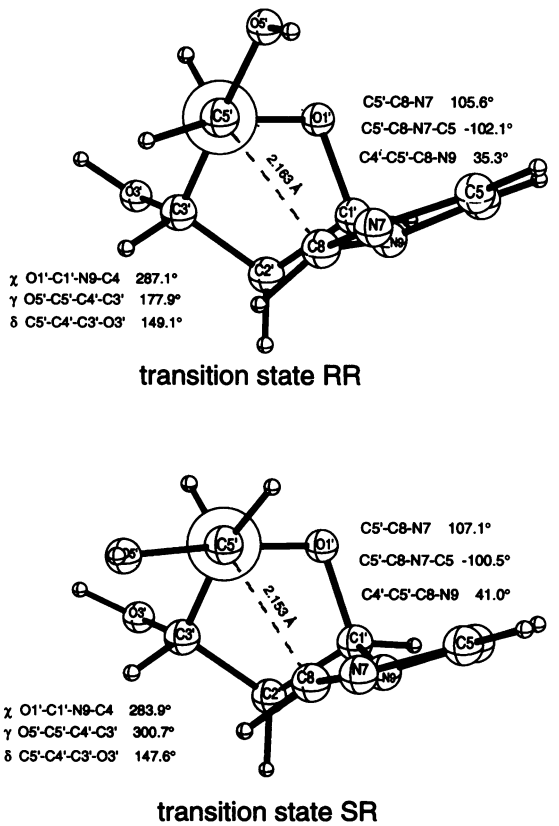

Figure 4. Transition state structures for cycloaddition calculated at the HF/6-31G level.

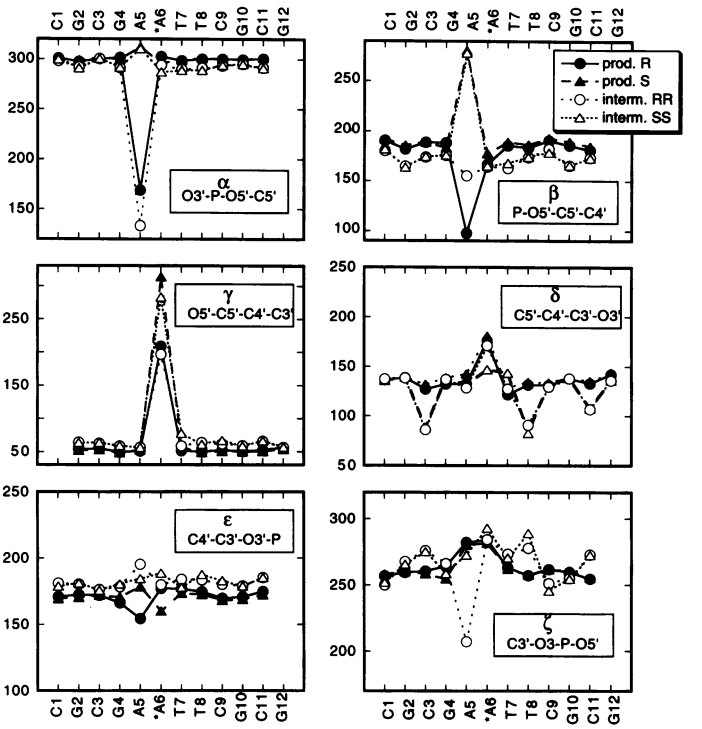

Figure 5. Backbone torsional angles in the energy-minimized structure of d(CGCGAATTCGCG)$_2$ with a cyclodeoxyadenosine lesion at A6.

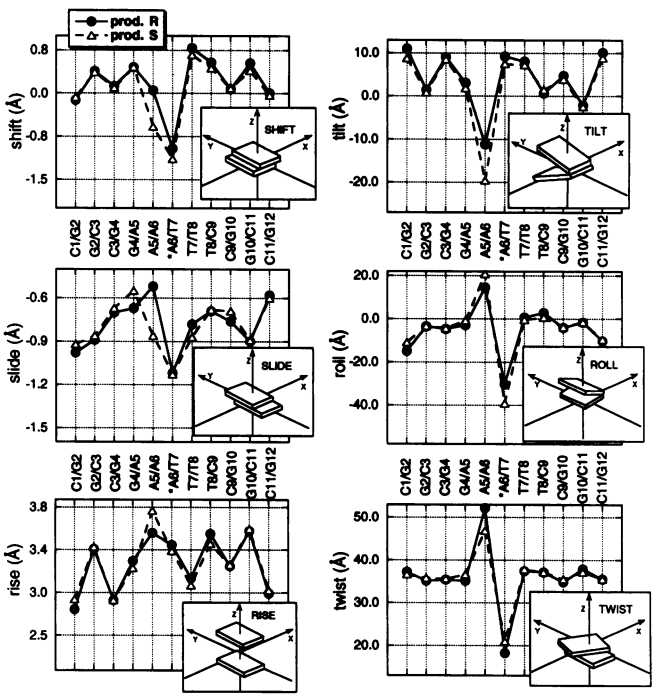

Figure 6. Geometrical parameters of base-stacking interactions in the energy-minimized structure of d(CGCGAATTCGCG)$_2$ with a cyclodeoxyadenosine lesion at A6.

DISCUSSION

Our computational results provide insight into possible mechanisms for the conformational preferences observed in various experimental systems (4–7). A preference for either R or S isomers of 8,5′-cyclodeoxypurines can develop at several points along the reaction pathway. Since the rate constant for addition of C(5′) radicals to the C(8) position of purines is only 2.4×10^4 sec^{-1} (1), the relatively long lifetime of C(5′) radicals in the absence of oxygen should allow conformation equilibrium to be established with respect to rotation about the C(4′)–C(5′) bond. If this is the case, then the relative stability of conformations of the C(5′) radical that are direct precursors of R and S isomers of 8,5′-cyclodeoxypurines should be a contributing factor in the relative yields of stereoisomers, in addition to the energy barriers for cross link formation.

Our model calculations show that in the absence of solvent, radical precursors of the S isomer are 2.25 kcal/mol more stable than the R precursors but the energy barrier for addition of the R precursor to the C(8)–N(7) double bond is slightly lower than for the S precursor radical (Table 1). When solvation energies are included in the calculations, R-isomers are favored at every step of the reaction pathway (Table 1). The latter result agrees qualitatively with measured R to S yield ratios in adenosine, poly(A), 5′-AMP at low pH, and single-stranded DNA (4–7).

S isomers of 8,5′-cycloAMP become predominant as the pH in irradiated solutions of 5′-AMP increases (4). To investigate the mechanism for this effect, the 5′ hydrogens in the C(5′) radical precursors of the R and S isomers were replaced by HPO_4^{2-}

groups and the resulting structures were optimized at the HF/6-31G level. For both starting structures, a very strong interaction developed between the ionized 5′-phosphate group and the 3′-OH group that dramatically stabilized the S type conformation (*-sc*) around the C(4′)–C(5′) bond. This interaction is so strong that the R configuration (*ap*) at C(5′) is not stable and converges to *-sc* during geometry optimization. In a dielectric medium, *ap* conformations of the radical precursor of 8,5′-cyclo-AMP will undoubtedly be present; nevertheless, these calculations in vacuum strongly suggests that an ionized 5′-phosphate group will favor S isomers of 8,5′-cyclodeoxypurines if thermodynamic equilibrium is established among the conformations of the C(5′) radical precursor. Protonation of the 5′-phosphate group at low pH should weaken the interaction with the 3′-OH group and thus decrease the preference for S isomers.

The geometry of the transition state for C(5′) radical addition to the purine C(8)–N(7) double bond suggests that the base must rotate about the glycosylic bond to form the intrastrand cross link. Furthermore, this cross link appears to weaken Watson–Crick hydrogen bonds in duplex DNA at the site of the lesion. These results suggest that the structure of the double helix will inhibit the formation of 8,5′-cyclodeoxypurines, which corresponds to the experimentally observed reduction in the total yield of intrastrand cross links in duplex DNA relative to single-stranded DNA (7); however, we do not find any significant difference in the degree of DNA distortion induced by R and S isomers. The more rigid backbone of duplex DNA may retard equilibration of the *ap* and *-sc* conformations of C(5′) radicals so that kinetic factors like the relative accessibility of the two hydrogen atoms at C(5′) to OH-radical attack become important factors in determining the relative yield of R and S stereoisomers.

Paramagnetic intermediates in the production of C(5′) to C(8) intrastrand cross links can exist in four stereoisomeric forms. Our calculations on small model systems suggest that stereoisomers with an S configuration at C(8) have substantially higher energies than those with R symmetry at C(8). Furthermore, the orientation of the purine base calculated for isomers with S symmetry at C(8) ($\chi \sim 170°$) would be difficult to achieve inside a right-handed double helix without drastic perturbations in DNA conformation. Both of these factors suggests that paramagnetic intermediates on the pathway leading to 8,5′-cyclodeoxynucleotides in DNA will have R symmetry at C(8).

Formation of the C(5′)–C(8) bond causes extensive perturbations of the nucleoside structure; nevertheless, our molecular mechanics calculations show that the large number of torsional degrees of freedom in the DNA backbone allow either the R or the S isomer of 8,5′-cyclodeoxyadenosine to be accommodated by adjustment of dihedral angles in the immediate vicinity of the lesion. It should be noted that calculations of this type converge to the energy minimum closest to the starting structure. Hence, more global changes in DNA conformation may result from molecular dynamics calculations that sample a larger region of conformation space. Molecular dynamics simulations of the dodecamer d(CGCGAATTCGCG)$_2$ with an R or S isomer of 8,5′-cyclodeoxyadenosine at A6 are in progress.

CONCLUSIONS

Quantum-chemical calculations performed on model deoxypurine nucleosides indicate a preference for R stereoisomers at every step of the reaction leading to C(5′) to C(8) intramolecular cross links. This finding is consistent with experimental data on 8,5′-cyclodeoxypurine formation in nucleosides, nucleotides, poly(A) and single-stranded DNA. These calculations also suggest that an intramolecular interaction between ionized 5′ phosphate groups and 3′ OH groups causes the pH dependence of the relative yield of R and S isomers of 8,5′-cycloAMP in irradiated solutions of 5′-AMP.

Both stereoisomers of 8,5′-cyclodeoxyadenosine can be incorporated into the B-DNA conformation by changes in backbone dihedral angles that are near the site of the lesion. These molecular mechanics calculations did not reveal any substantial difference in the degree of DNA distortion caused by the two isomers.

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REFERENCES

1 von Sonntag, C. (1987) *The chemical basis of radiation biology,* Taylor & Francis: London–New York–Philadelphia.
2 Keck, K. (1968) *Z. Naturforsch.* **B23**, 1034–1043.
3 Raleigh, J. A., Kremers, W. and Whitehouse, R. (1976) *Radiat. Res.* **65**, 414–422.
4 Raleigh, J. A. and Fuciarelli, A. F. (1985) *Radiat. Res.* **102**, 165–175.
5 Fuciarelli, A. F., Shum, F. Y. and Raleigh, J. A. (1976) *Biochem. Biophys. Res. Commun.* **134**, 883–887.
6 Fuciarelli, A. F., Shum, F. Y. and Raleigh, J. A. (1987) *Radiat. Res.* **110**, 35–44.
7 Dirksen, M.-L., Blakely, W. F., Holwitt, E. and Dizdaroglu, M. (1988) *Int. J. Radiat. Biol.* **54**, 195–204.
8 Dizdaroglu, A. (1986) *Biochem. J.* **238**, 247–254.
9 Dizdaroglu, M., Dirksen, M.-L., Jiang, H. and Robbins, J. H. (1987) *Biochem. J.* **241**, 929–932.
10 Dainton, F. S, and Peterson, D.B. (1962) *Proc. R. Soc. A* **267**, 443–463.
11 Fuciarelli, A. F., Mele, F. G. and Raleigh, J. A. (1987) *Int. J. Radiat. Biol.* **51**, 629–639.
12 Fuciarelli, A. F., Koch, C. J. and Raleigh, J. A. (1988) *Radiat. Res.* **113**, 447–457.
13 Frish, M. J., Trucks, G. W., Head-Gordon, M., Gill, P. M. W., Wong, M. W., Foresman, J. B., Johnson, B. G., Schlegel, H. B., Robb, M. A., Replogle, E. S., Gomperts, R., Andres, J. L., Raghavachari, K., Binkley, J. S., Gonzales, C., Martin, R. L., Fox, D. J., Defrees, D. J., Baker, J., Stewart, J. J. P. and Pople, J. A. (1992) *Gaussian 92* (Gaussian, Inc. Pittsburgh, PA).
14 Schlegel, H. B. (1986) *J. Chem. Phys.* **84**, 4530–4534.
15 Rashin, A. A. and Namboodini, K. (1987) *J. Phys. Chem.* **91**, 6003–6012.
16 Rashin, A. A. (1990) *J. Phys. Chem.* **94**, 1725–1733.
17 Pearlman, D. A., Case, D. A., Caldwell, J. C., Seibel, G. L., Chandra Singh, U., Weiner, P. and Kollman, P. A. (1991) *AMBER4.0* (University of California, San Francisco).
18 Lavery, R. and Sklenar, H. (1988) *J. Biomol. Struct. Dynam.* **6**, 63–91.
19 Lavery, R. and Sklenar, H. (1989) *J. Biomol. Struct. Dynam.* **6**, 655–667.
20 Sosa, C. and Schlegel, H. B. (1987) *J. Am. Chem. Soc.* **109**, 4193–4198.
21 Sosa, C. and Schlegel, H. B. (1987) *J. Am. Chem. Soc.* **109**, 7007–7015.
22 Houk, K. N., Paddon-Row, N. M., Spellmeyer, D. C., Rondan, N. G. and Shigeru, N. (1986) *J. Org. Chem.* **51**, 2874–2879.
23 Levitt, M. and Warshel, A. (1978) *J. Am. Chem. Soc.* **100**, 2607–2613.
24 Olson, W. K. (1982) *J. Am. Chem. Soc.* **104**, 278–286.
25 Miaskiewicz, K. and Osman, R. (1994) *J. Am. Chem. Soc.* **116**, 232–238.
26 Haromy, T. P., Raleigh, J. and Sundaralingam, M. (1980) *Biochemistry* **19**, 1718–1722.
27 Birnbaum, G. I., Cygler, M., Dudycz, L., Stolarski, R. and Shugar, D. (1981) *Biochemistry* **20**, 3294–3301.
28 Cremer, D. and Pople, J. A. (1975) *J. Am. Chem. Soc.* **97**, 1354–1358.