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Structure and Stability of Duplex DNA Containing (5'*S*)-5',8-Cyclo-2'-Deoxyadenosine: An Oxidatively-generated Lesion Repaired by NER

Tatiana Zaliznyak, Mark Lukin, and Carlos de los Santos*

Department of Pharmacological Sciences, School of Medicine. Stony Brook University, Stony Brook, NY, 11794-8651

Abstract

Cellular respiration and ionizing radiation generate 5',8-cyclo-2'-deoxyribonucleosides, an especial type of DNA damage that involves two modifications in the same nucleotide. These lesions evade the action of base excision glycosylases and their removal is a function of the nucleotide excision repair pathway. Diastereomeric 5',8-cyclo-2'-deoxyadenosine block mammalian DNA replication, diminish the levels of DNA transcription and induce transcriptional mutagenesis. Using solution state NMR spectroscopy and restrained molecular dynamics simulations, we have determined the structure of an undecameric DNA duplex having a centrally located (5'*S*)-5',8-cyclo-2'-deoxyadenosine residue paired to T. The damaged duplex structure is a right-handed helix having Watson-Crick base-pair alignments throughout and 2-deoxyribose puckers within the B-form conformation. Only small structural perturbations are observed at the lesion-containing and 5'-flanking base-pair. The 2-deoxyribose of the damaged nucleotide adopts the O4'-exo conformation and the *S*-cdA•T base-pair is propeller twisted. The 5'-lesion-flanking base is tilted forming a significantly buckled base-pair with its partner guanine. Analysis of UV-melting curves indicates mild thermal and thermodynamic destabilization on the damaged duplex. The *S*-cdA•T duplex structure shows many similarities and some intriguing differences with the recently reported structure of an *S*-cdG•dC duplex³¹ that suggest different lesion site dynamics.

INTRODUCTION

Oxidatively-generated damage produces a large variety of base and sugar lesions to cellular DNA, including 5',8-cyclo-2'-deoxyribonucleosides (cdPu).¹ Their formation follows the initial abstraction of a sugar H5-atom by the highly reactive hydroxyl radical² originating a carbon centered radical that attacks to the purine C8 position, generating an N7 radical. Ensuing oxidation of the N7 radical produces the 5',8-cyclic lesion whose glycosidic bond is more stable to acid conditions than that of the unmodified nucleoside.³ Since any of the sugar hydrogens at the 5-position is susceptible to radical abstraction, the cyclization reaction generates *R* and *S* diastereomers of the lesion in proportions that depend on the substrate identity, the reaction conditions and DNA conformation.^{4–15} The yields of the

Address all correspondence to cds@pharm.stonybrook.edu. Telephone: 631-444-3649. Fax: 631-444-3218.

SUPPORTING INFORMATION

Proton and phosphorus chemical shifts (Table S1) and J_{H1'-H2'} and J_{H1'-H2''} coupling constant values (Table S2) on the *S*-cdA duplex measured at 25 °C. One dimensional proton spectrum of the (5'*S*)-5',8-cdA duplex recorded in D₂O buffer at 25 °C (Figure S1), full 'finger print' region assignments on a 300 ms mixing time NOESY spectrum, recorded in 100% D₂O buffer at 25 °C for the damaged (Figure S2A) and undamaged (Figure S2B) strands of the duplex, one-dimensional phosphorus and [³¹P-¹H]-HMBC spectra recorded at a 161.85 MHz recorded in 100% D₂O buffer at 25 °C (Figure S3), three-dimensional model of an *R*-cdA•T duplex (Figure S4). This material is available at <http://pubs.acs.org>.

cyclization reaction diminishes in the presence of molecular oxygen,^{6,16} yet cdPu are formed under the oxygen concentrations existent in the cell.^{17–20}

CdPu are among the few oxidatively-generated lesions that are not substrates for base excision glycosylases. Their elimination from the cells is mediated, instead, by the Nucleotide Excision Repair (NER) pathway,^{21,22} which takes away these lesions with efficiency comparable to the removal of TT cyclobutane dimers and shows higher activity in excising the *R*- isomer.²¹ Accordingly, cdPu lesions are readily detected in keratinocytes from XPC and CSA patients exposed to low dose ionizing radiation^{23,24} as well as in tissues from knockout mice lacking CSB protein.²⁵ Furthermore, these lesions accumulate with ageing in mice tissues.²⁶

The presence of cdPu negatively impacts DNA replication and transcription. Both diastereomers of cdA block primer extension by T7 DNA polymerase and mammalian DNA pol- δ ,²¹ while pol- η can perform translesion synthesis on the *R*-isomer only.²⁷ The *S*-cdG lesion blocks DNA replication in *E. coli* and causes a large number of 5',8-cdG $\rightarrow$ dA transitions after the induction of the SOS response.²⁸ In addition to their effects on replication, 5',8-cdP lesions negatively affect DNA transcription. The presence of a single *S*-cdA lesion in the TATA box of the CMV promoter inhibits binding of the TATA box binding protein *in vitro* and strongly reduces gene expression *in vivo*.²⁹ Furthermore, bypass of *S*-cdA-containing DNA by human RNA polymerase II *in vivo* results in a majority of mutated transcripts showing single base changes or deletions of several lengths.³⁰

Little is known about the structural and thermodynamics properties of DNA duplexes having 5',8-cyclo-2'-deoxyribonucleosides. Two recent manuscripts described the solution structures of three dodecameric duplexes containing an *S*-cdG lesion opposite dC, dA or T.^{31,32} The *S*-cdG•dC duplex is a right handed helix stabilized by formation of Watson-Crick alignments at all base-pairs, although the hydrogen bonds at the 3'-lesion-flanking pair are notably less stable. Some dihedral angles of the sugar-phosphate backbone of the damaged residue display significant deviation from canonical values that altered twist and stacking properties at the lesion site.³⁰ The structure of duplex DNA having cdA is presently known. Herein, we describe the NMR characterization, thermodynamic parameters and restrained molecular dynamics (rMD) structure of an undecameric DNA duplex having a centrally located *S*-cdA•T base-pair. The chemical structure of *S*-cdA and the duplex sequence under investigation are shown in Figure 1.

MATERIALS AND METHODS

Sample Preparation

We used standard solid-phase synthesis methods for the preparation of the modified and non-modified oligodeoxynucleotides,³³ using phosphoramidite precursors, including that of *S*-cdA, from Glen Research. For coupling of the *S*-cdA nucleoside, we used 5-fold excess of its phosphoramidite and 5-minute coupling time. For incorporation of the ensuing nucleoside we used 2 coupling reactions of 10-minute time, employing a fresh amidite-activator mixture on the second coupling. Purification of the DNA oligomers followed two rounds (DMT-on and DMT-off) of reverse-phase HPLC (C18 column), using as a mobile phase a 0.1 M triethylammonium acetate (TEAA) buffer (pH 6.8) and a linear 0 to 40 % acetonitrile gradient in 40 min. ESI-MS confirmed the composition of the oligodeoxynucleotides and assessed their purity. Percolation of the purified samples through a Sephadex G-25 column (2.5×80 cm) yielded the desalted oligomers. Based on ϵ_{260} values calculated with the Gene Runner (Hastings Software, Inc.) program, we mixed equal amount of each strand and annealed them by heating the mixture to 80 °C and slowly cooling the solution to room temperature. The NMR sample consisted of about 1.5 mM duplex

dissolved in 25 mM phosphate buffer, pH 6.5, containing 50mM NaCl and 1mM EDTA in '100 %' D₂O (Aldrich) or a 1:9 mixture of D₂O:MilliQ H₂O.

NMR Experiments

We recorded all proton spectra in a 600 MHz Varian Inova spectrometer, being the exception the imino proton temperature dependence study that was recorded at 700 MHz on a Bruker Avance instrument. We used sodium 3-(trimethylsilyl)propionate-2,2,3,3-d₄ at 0 ppm as chemical shift reference. We collected a one-dimensional ³¹P and a [³¹P-¹H]-HMBC spectra at 161.85 MHz using trimethyl phosphate (at 3.46 ppm) as reference. Proton NMR data set consisted of phase-sensitive NOESY (80, 150, and 300 ms mixing time), COSY, COSY45, and TOCSY (70 and 150 ms isotropic mixing time) spectra collected in '100%' D₂O buffer at 25 °C. A presaturation pulse applied during the relaxation delay of 1.5 s suppressed the residual solvent signal. We recorded the phase-sensitive proton NOESY (120 and 220 ms mixing time) spectra at 5 °C using a 'jump-return' pulse as the reading pulse. We used 'pulse sculpting' with a W5 pulse train for recording the temperature dependence of the imino proton signals. NMR data were processed and analyzed using NMRPipe³⁴ or Felix (Accelrys Inc., San Diego, CA, USA). Prior to the Fourier transformation, we multiplied the time-domain 2D data, generally consisting of 2048×300 complex points in the t₂ and t₁ dimensions, by shifted sine-bell window functions. For the COSY45 experiment we doubled the time domain data size to 4096×600 complex points. A polynomial baseline correction was applied to the frequency domain spectra as needed.

Molecular Dynamics

We run rMD simulations with the program Xplor-NIH,³⁵ using an all-atom force field derived from CHARMM³⁶ and a dielectric constant set to 4.³⁷ We used the GAUSSIAN module of the HyperChem program package (HyperCube Inc.) in the minimal (3–21) basis set to calculate the partial electric charges of the cdA moiety. We built a starting B-form DNA model with the Biopolymer module of Insight 2000 (Accelrys Inc.), by deleting the pro S'H5' and H8 protons and bringing closer the C5'-C8 atoms of a central dA residue. Subsequently, we run 1000 steps of conjugate gradient energy minimization to optimize the geometry of S-cdA and remove unfavorable atomic interactions. As we have done previously,³⁸ we used a full relaxation-matrix approach³⁹ for computation of the experimental inter-proton distances. RMD simulations run enforcing 542 NOE-derived inter proton distances, 401 computed from resolved and 142 from overlapping NOE peaks. In addition, we use crystallographic distances between donor and acceptor heavy atoms to enforce W-C hydrogen bonds on the duplex. For the lesion containing and flanking base-pairs we increased the hydrogen bond distance bounds to 0.6 Å. No empirical restraints were used to enforce backbone dihedral angles at the lesion containing and flanking base-pairs. Distance restrained molecular dynamics simulations followed a standard protocol. Briefly, we started simulations at five different temperatures (100, 105, 110, 115, and 120 K) and heated the system to 500 K in 100 ps. During this time, we introduced a potential energy penalty function enforcing interproton distances with a penalty constant that increased from 10 to 300 kcal/(mol Å²) and stayed at this value until the end of the simulations. After completion of the high temperature step, we cooled the system down to 300 K in 100 ps, continuing the simulation at this temperature for additional 130 ps. We computed twenty five independently refined structures of the duplex by initiating the simulations at the five different temperatures mentioned above and running the high-temperature step for five different lengths (100, 105, 110, 115, and 120 ps). We averaged atom coordinates of the last 30 ps of each simulation and energy minimized them, obtaining a set of 25 refined models. Since these models belonged to a single structural family (see results) they were subsequently averaged and minimized generating the final refined structure presented here.

We used Curves⁴⁰ to compute helicoidal parameters and Chimera⁴¹ to visualize the refined duplex structure.

Melting Studies

We determined UV melting temperatures (T_m) of modified and unmodified duplexes in a CARY100 Bio UV-vis spectrophotometer (Varian, Inc.). Temperature readings were stable and accurate to within 1 °C. We allowed the Initial temperatures, 10 or 80 °C depending upon the experiment, to equilibrate for at least 10 minutes before starting data collection. Samples consisted of 0.2 to 2.2 OD₂₆₀ of duplex dissolved in 1 mL of 25 mM sodium phosphate buffer solution, pH 6.8, containing 150 mM NaCl and 0.5 mM EDTA. We registered UV absorption values every minute, heating (or cooling) the sample with a temperature change rate of 0.2 °C per minute. Using the first derivative of the melting curves, we computed three independent T_m values for each duplex concentration. We fit $(1/T_m)$ vs. $\ln(C_t)$ plots, where C_t is the duplex molar concentration, to a straight line and extracted ΔH° and ΔS° of duplex formation from the slope and the intercept, respectively.⁴² The Gibbs free energy (ΔG°) was derived from these values.

RESULTS

NMR Characterization

The one-dimensional proton spectrum of the *S*-cdA duplex recorded in D₂O buffer at 25 °C shows sharp and resolved signals indicating that the damaged duplex adopts a single conformation in solution, which is amenable to NMR determination (Figure S1, Supporting Information). Assignment of the non-exchangeable protons followed the analysis of a 300 ms mixing time NOESY spectrum recorded in '100%' D₂O buffer, at 25 °C. In right handed helices, each base proton (purine-H8/pyrimidine-H6) displays NOE peaks to the H1' sugar proton of the same and 5'-flanking nucleotide.⁴³ Figure 2 identifies these interactions on the internal d(A4–A8)•d(T15–T19) segment of the duplex, while Figures S2A and S2B (Supporting Information) do the same for whole sample. Indicative of a regular right-handed helix, we can trace the sequential interactions on the unmodified strand from G12 to G22 without any interruption (Figures 2 and S2B). On the modified strand the NOE trace proceeds normally from C1 to C5, breaks at the *S*-cdA nucleotide due to the lack of the H8 proton, and continues afterwards without any further interruption (Figures 2 and 2SA). We observe NOE cross-peaks between adenine-H2 and sugar-H1' protons (Figure 2, peaks A-T) and purine-H8 and 3'-flanking cytosine-H5 protons (Figure 2, peaks U-X), which additionally support the presence of a regular right-handed duplex in solution. We assigned the sugar H2', H2'', H3' and H4' protons of the *S*-cdA duplex following the examination of additional regions on the same NOESY spectrum and confirmed the assignments by analysis of COSY and TOCSY spectra (data not shown). Table S1 (Supporting Information) lists the proton chemical shifts on the *S*-cdA duplex at 25 °C.

Figure S3 (Supporting Information) shows that the one-dimensional phosphorous spectrum of the *S*-cdA duplex recorded in '100%' D₂O buffer at 25 °C exhibits a poorly resolved group of signals appearing in the –3.7 to –4.4 ppm range along with a well-resolved peak at –5.7 ppm. Analysis of a HMBC allows the assignment of these resonances, revealing that the upfield signal belongs to phosphate group of the *S*-cdA residue. Table S1 (Supporting Information) lists the ³¹P chemical shifts on the *S*-cdA duplex at 25 °C.

The proton spectrum of the *S*-cdA duplex recorded in 10% D₂O buffer at 5 °C shows eight partially resolved exchangeable proton signals on the 12.0–13.6 ppm range (Figure 4, top spectrum) suggesting the formation of Watson-Crick (W-C) base-pair alignments. Assignment of the exchangeable protons follows the analysis of NOESY (120 and 220 ms

mixing time) spectra recorded at the same temperature. Each thymine imino proton displays a strong NOE interaction to the adenine H2 proton of its counter base, establishing the formation of W-C A•T base-pairs on the *S*-cdA duplex, including the lesion-containing *S*-cdA•T pair (Figure 3, peaks A-E). Similarly, each guanine imino proton exhibits strong NOE peaks to the hydrogen-bonded and non-hydrogen-bonded cytosine amino protons across all internal G•C pairs of the *S*-cdA duplex (Figure 3, peaks F/F'-I/I'). We observe NOE cross peaks between imino and adenine H2 protons of adjacent base-pairs (Figure 3, peaks J-P) and between imino protons of flanking base-pairs (Figure 3, peaks R-W) establishing base-pair stacking throughout the *S*-cdA duplex. Table S1 (Supporting Information) lists the chemical shift of the exchangeable protons on the *S*-cdA duplex at 5 °C.

It is worth noting that NOE cross peaks involving the imino proton of the lesion partner T17, specifically T17(H3)-*S*-cdA(H2) and T13(H3)-G16(H1), are weaker than similar interactions showed by other imino protons of the duplex, indicating faster solvent exchange at the lesion-containing base-pair. The temperature dependence of the imino proton signals provides additional evidence of decreased stability for the *S*-cdA•T base-pair. As shown in Figure 4, only the terminal imino protons of the duplex, which are fully exchanged and absent in the spectrum at 20 °C, show faster water exchange rate than T17. At 35 °C, the T17(H3) signal is not longer visible, whereas the imino protons of the lesion-flanking nucleotides, G16 and G18, appear minimally broadened by solvent exchange. Indicative of duplex melting, most imino proton resonances have disappear at 45 °C and only a broad hump is still visible at the position of the G16(H1) and G18(H1) signals.

Sugar Conformations

In order to estimate qualitatively 2-deoxyribose conformations on the *S*-cdA duplex, we recorded a COSY45 spectrum at 25 °C and compared the values of the $J_{H1'H2'}$ and $J_{H1'H2''}$ coupling constants that were measured from the analysis of the COSY cross-peak pattern. As Figure 5 shows, the majority of the nucleotides have $J_{H1'H2'} > J_{H1'H2''}$ establishing sugar conformations on the C2'-endo/C1'-exo range, which is characteristic of the B-form DNA conformation. For the damaged *S*-cdA residue, we only detect its $J_{H1'H2''}$ cross peak (Figure 5, peak A), indicating a small $J_{H1'H2'}$ coupling constant, observation that is consistent with a 2-deoxyribose conformation within the O4'-exo/C1'-endo/C2'-exo range (pseudorotation angle 270°–360°). Table S2 lists $J_{H1'H2'}$ and $J_{H1'H2''}$ coupling constants values on the *S*-cdA duplex.

S-cdA Duplex Structure

The 25 distance-rMD models comprise an ensemble of close related structures with a largest pair-wise Root Mean Square Deviations (RMSD) of 0.86 Å (Figure 4S, Supporting Information), suggesting that the duplex adopts a defined unique conformation in solution. Figures 6 and 7 show the averaged minimized model depicting the impact of the *S*-cdA lesion on the structure of duplex DNA. The presence of the lesion causes small structural perturbations that are localized to the lesion containing and flanking C5•G18 base-pairs. The *S*-cdA duplex is a mostly regular B-form helix, stabilized by formation of W-C base-pair alignments and proper base stacking. It displays a slight bent (26°) on the helical axis at the C5•G18/*S*-cdA•T17 step. The 2-deoxyribose of the damaged nucleotide adopts an O4'-exo conformation and bulges out of the sugar-phosphate backbone, appearing at the edge of the minor groove (Figure 6). The glycosidic torsion angle of *S*-cdA is in the *anti* range (–150°) and the *S*-cdA•T17 base-pair is propeller twisted to a small extent (28°). The C5•G18 base-pair is buckled (30°) and over twisted with respect to *S*-cdA•T17 ($\Omega = 48^\circ$), resulting in poor stacking at the C5•G18/*S*-cdA•T17 step. In contrast, twist and roll values at the *S*-cdA•T17/C7•G16 step are normal (Figure 7). Figure 8 displays the conformation of the fused 2-

deoxyribose and 5',8-cyclo rings of the lesion. The 2-deoxyribose conformation is that of an almost perfect envelope, with C1', C2', C3' and C4' in a plane and O4' protruding toward the side of the O3' oxygen, exhibiting an angle of pseudorotation of 275°,⁴⁴ in the O4'-exo range. The conformation of the 5',8 cyclo ring is that of a half-boat, with only the O4' atom out of the 5',8-cyclo-dA moiety plane, which, in turn, is almost perpendicular to the 2-deoxyribose plane (Figure 8). The O4'-O3' distance is 3.1 Å, a value slightly shorter than the one usually observed for the unmodified sugars of the duplex. Table 1 lists statistics of the refinement and relevant parameters of the *S*-cdA duplex structure.

S-cdA duplex stability

The top panel of Figure 9 shows typical UV melting profiles of the lesion containing and the undamaged control duplexes under similar concentration and experimental conditions. The presence of the damaged nucleotide diminishes the melting temperature of the duplex by 7.5 °C, indicating that *S*-cdA has a significant destabilizing effect. The $(1/T_m)$ vs. $\ln(C)$ plot of the *S*-cdA duplex is a straight line without evidence of curvature (Figure 9, bottom panels), establishing a one-step duplex dissociation process that can be analyzed by van't Hoff formalism. The presence of the *S*-cdA lesion increases (less negative) the enthalpy of duplex formation, a destabilizing effect that is partially compensated for by an increase of entropy. The net result is a 3.1 kcal/mol decrease of the Gibbs free energy of duplex formation at 37 °C. Table 2 lists the thermodynamic parameters on the *S*-cdA and control duplexes.

DISCUSSION

NMR spectra

The presence and intensity of right-handed sequential interactions between the base (purine-H8/pyrimidine-H6) and sugar (H1', H2', H2'', H3') protons, as well as adenine-H2 and sugar (H1') protons conclusively establish the presence of a regular helical conformation in solution (Figures 2 and S2). The most significant deviation from the standard NMR properties of the non-exchangeable proton spectra of unmodified duplexes is the value of some proton chemical shifts, notably that of *S*-cdA(H2''), which appears up field from the *S*-cdA(H2') signal, and C5(H2'') that is significantly down field from the usual C(H2'') range (Figure 5 and Table S1). In addition, the conformation of the *S*-cdA 2-deoxyribose is on the very unusual O4'-exo range. On the exchangeable proton spectra, the most important perturbation is the weak intensity of T17(H3)–*S*-cdA(H2) and T17(H3) –G16(H1) NOE cross peaks, both involving the imino proton signal of the lesion containing base-pair (Figure 3). Furthermore, the temperature dependence of the exchangeable protons reveals a faster solvent exchange for the lesion-partner T17(H3) proton than that of G16(H1) and G18(H1), the imino protons of the lesion-flanking base-pairs. In fact, G16(H1) and G18(H1) are the most stable imino proton signals of the *S*-cdA duplex at elevated temperatures (Figure 4). All these observations indicate that the *S*-cdA duplex adopts a regular right handed helical conformation, minimally perturbed at the lesion containing and 5'-flanking base-pairs.

Solution structure

The rMD simulations defined a single conformation for the *S*-cdA duplex that is in excellent agreement with its NMR properties. Specifically, the average structure is a regular right-handed B-form helix that preserves W-C base-pair alignments throughout the duplex, and satisfies all the NOE-derived distances at the central three base-pair segment (Figure 6 and Table 1). The refined *S*-cdA duplex structure nicely explains some key NMR observations made at the lesion site. The 2-deoxyribose of the *S*-cdA nucleotide changes its conformation from the C2'-endo/C1'-exo range (characteristic of B-form helices) to the very unusual O4'-exo range. In this conformation, the $J_{H1'H2'}$ coupling constant is very small,^{43,44} thus

explaining the absence of a COSY cross peak between these protons (Figure 4 and Table S2 (Supporting Information)). The O4'-exo conformation of the *S*-cdA lesion in double stranded DNA is identical to the one observed on the crystal structures of 5',8-cyclo ribonucleosides^{45,46} and suggested by the NMR spectrum of the (5'S) 5',8-cyclo-2'-deoxyadenosine⁴⁷ and the NMR/DFT data obtained for an *R*-cdA-containing dinucleotide,⁴⁸ indicating that any structural restraints imposed by the duplex formation do not affect the 2-deoxyribose conformation already present on the nucleoside. Furthermore, the recent NMR structure of an *S*-cdG-containing duplex shows that the damaged nucleotide also adopts the O4'-exo pucker,³¹ indicating that is the formation of the 5'-8 covalent bond that drives the unusual 2-deoxyribose conformation. The perturbations induced by *S*-cdA at the lesion site tilts the C5 residue bringing its H2'' proton to the plane of the *S*-cdA purine moiety with only 2.1 Å separation from the N7 atom (Figure 7). This perturbation is consistent with the unusual chemical shift of the C5(H2'') signal (Figure 5 and Table S1 (Supporting Information)), which resonates significantly down field from the normal H2'/H2'' range. Additional variations involve the ζ (O3'-P) and α (P-O5') torsion angles of phosphate groups linking A4/C5 and C5/*S*-cdA nucleotides, which change from a *gauche*, *gauche* conformation, typical of internal phosphate in B-form duplexes, to *trans*, *gauche* values more common on terminal residues. This change, however, does not explain the ³¹P up-field shift experienced by the phosphate of the *S*-cdA residue (Figure S3, Supporting Information), which would be expected to move down field with the observed ζ (*trans*) and α (*gauche*) torsion values.⁴⁹ Thus, the most likely explanation for the up-field value of the *S*-cdA phosphate is the electronegativity increase of C5' that results from the formation of the 5',8 covalent bond.

Our UV melting study indicates that the presence of *S*-cdA reduces the thermal stability of the duplex and increases its enthalpy (less negative) of formation (Table 2). The full preservation, however, of canonical W-C base-pair alignments on the refined structure and the absence of large perturbations of the B-form conformation indicates that the energetic destabilization is the result of several small structural adjustments. One of the most clearly visible conformational changes that can contribute to the duplex destabilization is the decrease of the C7(PO)/*S*-cdA(O5') distance that shortens by about 1 Å (Figure 7). In addition, the O3'-O4' distance on the damaged nucleotide diminishes by 0.3 Å, although this effect is compensated by the increase of the *S*-cdA(O5'-O4') distance. Buckling of the C5•G18 base-pair, propeller twist across the *S*-cdA•T17 pair and poor stacking at the C5•G18/*S*-cdA•T17 step (Figure 7) are additional perturbations that are likely to contribute to the decrease on the *S*-cdA duplex stability.

Comparison with the *S*-cdG lesion

Many spectroscopic and structural properties of the *S*-cdA duplex in solution are also present on a DNA duplex having a single *S*-cdG•dC pair in the 5'(C-X-T) sequence context.³¹ Both duplexes are right-handed B-form helices, having 2-deoxyribose conformation in the C2'-endo/C1'-exo range for all the undamaged nucleotides, W-C base-pair alignments at all steps of the sequence, the unusual O4'-exo pucker for the cyclo purine 2-deoxyribose and similar values for the backbone angles of the damaged nucleotide. Furthermore, shared NMR characteristics include the narrow non-exchangeable proton signals, the analogous chemical shift changes for the *S*-cdPu sugar protons and the significant down field shift of the 5'-lesion-flanking H2'' proton. A remarkable difference, however, is the behavior of the lesion-site imino protons that is an indication of the local duplex mobility. In the case of the *S*-cdG duplex, the imino proton signal at the lesion containing base-pair is clearly observed even at high temperatures³¹, whereas that of the *S*-cdA•T pair is visible at low temperature and almost disappears at 25 °C (Figure 4). We posit that this difference results from the intrinsic dynamics of the damaged purine residues and

the use of different DNA sequences. The presence of the 5',8-covalent bond severely restraints the rotation around the glycosyl bond of the damaged purine, which in the *S*-cdG•C pair diminishes the solvent exposure and exchange of the *S*-cdG(H1) proton, explaining its NMR observation at high temperatures. In the *S*-cdA duplex case, the lesion partner residue is unrestrained and, due to the decreased stability of the damaged base-pair, exhibits fastest solvent exchange among the internal imino protons. Hence, its observation on NMR spectrum is possible at low and up to room temperatures (Figure 4). More difficult to rationalize is the observation of G18(H1), the imino proton signal at the 5'-side of the lesion, that is present at all the examined temperatures (Figure 4), while the analogous imino proton signal in the *S*-cdG duplex is absent even at 5 °C.³¹ The higher stability of C•G over T•A pairs can help to explain this discrepancy, although, since other thymine imino protons are readily detected in the *S*-cdG duplex, some additional yet unidentified effects, induced by the *S*-cdG in that sequence context, must be at play.

Implications for NER recognition

The NER system can excise cdA-containing duplexes with low efficiency, similar to that of cyclobutane TT dimers,²² and is more active in removing the *R* isomer of the lesion.²¹ Our characterization of the *S*-cdA duplex shows that the lesion induces minor structural perturbations in the canonical B-form DNA conformation and causes small decrease on duplex stability, which is of similar magnitude than the one observed for duplexes having a cyclobutane TT dimer.⁵⁰ The *S*-cdA-containing duplex thus remains slightly above the destabilization threshold for NER recognition, a value below which the XPC-Rad23B would not detect the lesion and bind the undamaged strand.⁵¹ Our preliminary MD simulations of an *R*-cdA duplex of identical sequence reveal that the *R* stereoisomer causes larger structural perturbations at the damaged site than the *S* counterpart (Figure S4, Supporting Information), giving some rationale for the more efficient NER recognition of the *R* isomer.²¹

Many similarities between the CPD and cdA lesions are worth noting. Both lesions lack a covalently-attached additional bulky moiety and minimally perturb the duplex conformation,⁵² thereby imposing only a small energetic penalty. Therefore, they may pose similar challenges for NER recognition apparatus. Mammalian cells have an auxiliary factor, the UV-DNA damaged binding protein (DDB) that is missing in XPE cells.⁵³ DDB is a heterodimer whose binding to CPD-damaged DNA enhances NER repair and suppresses UV-induced mutagenesis.^{54,55} The structure of DDB in complex with duplex DNA having a pyrimidine (6–4) pyrimidone photoproduct, or an abasic site analogue, shows that the DDB2 subunit binds damaged DNA by inserting a hairpin into the minor groove of the duplex and flipping the two residues of the photoproduct, or the AP site and its 3'-neighboring nucleotide, into a protein binding pocket (PDB entries 3EI1 and 3EI2).⁵⁶ Simple modeling of the *S*-cdA lesion into the DDB2 binding pocket (3EI2) reveals that this protein could readily accommodate the cdA and 3'-flanking nucleoside. Hence, we posit that 5',8-cdPu are likely targets for DDB2 binding and the level of these lesions increase in XPE cells. Investigations to verify these hypotheses are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

NER	Nucleotide Excision Repair
XPC	Xeroderma Pigmentosum Complementation Group C
CSA and CSB	Cockayne Syndrome Complementation Group A and B, respectively
CMV	cytomegalovirus
NOESY	Nuclear Overhauser Effect Spectroscopy Y
COSY	COrrrelation Spectroscopy Y
TOCSY	TOtal Correlation Spectroscopy Y
[³¹P-¹H]-HMBC	phosphorus-hydrogen Heteronuclear Multiple Bond Correlation

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C₁ G₂ T₃ A₄ C₅ X₆ C₇ A₈ T₉ G₁₀ C₁₁
G₂₂ C₂₁ A₂₀ T₁₉ G₁₈ T₁₇ G₁₆ T₁₅ A₁₄ C₁₃ G₁₂

S-cdA Duplex

(5'*S*)-5',8-Cyclo-2'-deoxyadenosine (*S*-cdA)

Figure 1. Sequence of the duplex used in this study and chemical structure of (5'*S*)-5',8-cyclo-2'-deoxyadenosine (*S*-cdA).

Figure 2.

Contour plot of the 'finger print' region on a 300 ms mixing time NOESY spectrum recorded with the sample dissolved in '100%' D₂O buffer at 25 °C. The figure depicts interactions between the base (7.1–8.5 ppm) and sugar H1' (5.3–6.4 ppm) protons, with red and blue traces following the sequential NOEs at the center of the duplex on the modified and unmodified strands, respectively. Nucleotide labels identify the intra residue base-H1' NOE cross peaks. Other labels are assigned as follows: A, A4(H2)-A4(H1'); B, A4(H2)-A20(H1'); C, A4(H2)-C5(H1'); D, A4(H2)-T19(H1'); E, *S*-cdA(H2)-*S*-cdA(H1'); F, *S*-cdA(H2)-G18(H1'); G, *S*-cdA(H2)-C7(H1'); H, *S*-cdA(H2)-T17(H1'); I, A8(H2)-A8(H1'), J, A8(H2)-G16(H1'); K, A8(H2)-T15(H1'); L, A8(H2)-T9(H1'); M, A14(H2)-A14(H1'); N, A14(H2)-G10(H1'); O, A14(H2)-T15(H1'); P, A14(H2)-T9(H1'); Q, A20(H2)-A4(H1'); R, A20(H2)-A20(H1'); S, A20(H2)-T3(H1'); T, A20(H2)-C21(H1'); U, A4(H8)-5(H5); V, G10(H8)-C11(H5); W, G12(H8)-C13(H5); X, A20(H8)-C21(H5); Z, *S*-cdA(H1')-C7(H6).

Figure 3.

Contour plot of expanded regions on a 220 ms mixing time NOESY spectrum recorded with the sample dissolved in 10% D₂O buffer at 5 °C, showing NOE interactions on the iminobase/ amino (top panel) and the symmetrical imino (bottom panel) proton regions. Labels are assigned as follows: A, T9(H3)-A14(H2); B, T15(H3)-A8(H2); C, T3(H3)-A20(H2); D, T19(H3)-A4(H2); E, T17(H3)-*S*-cdA(H2); F/F', G2(H1)-C21(N4H)_{hb/nhb}; G/G', G10(H1)-C13(N4H)_{hb/nhb}; H/H', G16(H1)-C7(N4H)_{hb/nhb}; I/I', G18(H1)-C5(N4H)_{hb/nhb}; J, T15(H3)-A14(H2); K, T9(H3)-A8(H2); L, G2(H1)-A2(H2); M, G10(H1)-A14(H2); N, G16(H1)-*S*-cdA6(H2); O, G16(H1)-A8(H2); P, G18(H1)-A4(H2); Q, T19(H3)-C5(N4H)_{hb}; R, T9(H3)-T15(H3); S, T9(H3)-G10(H1); T, T15(H3)-G16(H1); U,

T3(H3)-G2(H1); V, T19(H3)-G18(H1); W, T17(H3)-G16(H1). Hb/nhb refer to the hydrogen-bonded and non-hydrogen-bonded amino protons.

Figure 4. Temperature dependence of the imino proton signals of the *S*-cdA duplex. TER stands for terminal base-pairs.

Figure 5.

Contour plot of an expanded region of a COSY45 spectrum recorded with the sample dissolved in '100%' D₂O buffer at 25 °C. The figure shows J coupling interactions between the sugar H1' and H2'/H2'' protons (top panel) and the COSY peak between *S*-cdA(H5')-*S*-cdA(H4') that identifies the H5' proton of the lesion (bottom panel). Labeled peaks are assigned as follows: A, *S*-cdA(H1')-*S*-cdA(H2''); B, C5(H1')-C5(H2''); C, C5(H1')-C5(H2''); D, C7(H1')-C7(H2''); E, C7(H1')-C7(H2''); D, C7(H1')-C7(H2''); E, C7(H1')-C7(H2''); F, G16(H1')-G16(H2''); G, G16(H1')-G16(H2''); H, T17(H1')-T17(H2''); I, T17(H1')-T17(H2''); J, G18(H1')-G18(H2''); K, G18(H1')-G18(H2'). The circle is at the

position of the H1'-H2' COSY peak of the lesion, which is absent due to the small $J_{H1'H2'}$ constant value.

Figure 6.

Three-dimensional rMD structure of the *S*-cdA duplex (PDB ID: 2lsf) showing the nine internal base-pairs of the duplex with the major groove prominent (left panel) or the sugar-phosphate backbone of the damaged strand prominent (right panel). The figure displays only the heavy atoms (colored by type), the sugar H1' protons (white) and a ribbon tracing the sugar-phosphate backbone.

Figure 7.

Close up view of the lesion-site structure of the *S*-cdA duplex (PDB ID: 2lsf) depicting W-C base-pair alignments (left image) and stacking interactions (right images) with the atoms colored by type. Broken lines on the left image indicate anomalous short distances and the background base-pairs are filled on grey color on the right images.

Figure 8.
Close up view of the *S*-cdA conformation in the *S*-cdA duplex (PDB ID: 2lsf).

Figure 9.

Plots of UV_{260} absorption vs temperature (top) and $1/T_m$ vs $\ln(Ct)$ (bottom) for the *S*-cdA-containing ($\circ$) and unmodified ($\bullet$) duplexes. The inset on the top panel shows the melting curve derivatives.

Table 1***S*-cdA Duplex Structure**

Refinement Statistics ^a	
RMSD NOE Distances (Å) × 10 ⁻²	4.5
NOE distance violations ^b	6 – 4
Energy _{NOE} (kcal/mol Å ²)	8.7
RMSD bond distances (Å) × 10 ⁻³	5.1
RMSD bond angles (°)	2.3
RMSD dihedral angles (°)	0.9
Energy _{van der Waals} (kcal/mol)	–332.6
Structural Parameters ^c	
Helix bend (°)	21 – 30
<i>S</i> -cdA • T17 Propeller twist (°)	26 – 32
C5•G18 base-pair buckle (°)	28 – 32
<i>S</i> -cdA pseudorotation angle (°)	273 – 278

^aValues measured on the averaged minimized structure.

^bNo violations of distances involving the central three base-pairs. Six violations were on resolved and four on overlapped NOE peaks.

^cRange of values measured on the 25 rMD structures. On the average structure the *S*-cdA C1'-N9 torsion angle is –149° and the backbone dihedral angles are: α = –77°, β = 168°, γ = –56°, δ = 150°, ε = –154°, ζ = –52.

Table 2

Stability of the S-cdA Duplex

Duplex	T _m (°C)	ΔH ⁰ (kcal/mol)	ΔS ⁰ _{25 °C} (cal/mol°K)	ΔG ⁰ _{25 °C} (kcal/mol)	ΔG ⁰ _{37 °C} (kcal/mol)	ΔΔG ⁰ _{37 °C} (kcal/mol)
S-cdA	42.0	-71.7	-201.6	-11.6	-9.2	-3.1
Control	49.5	-101.0	-286.1	-15.7	-12.3	