5-Bromodeoxyuridine Radiosensitization: Conformation-Dependent DNA Damage[†]

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Received October 10, 2006; Revised Manuscript Received April 13, 2007

ABSTRACT: DNA structure has recently emerged as one of the key factors governing the ability of 5-bromodeoxyuridine (BrdU) to radiosensitize DNA. Here, we report the dependence of the specific damage induced by BrdU for different DNA conformations. Strand breaks are specific for B-form DNA, whereas A-DNA only undergoes formation of piperidine-sensitive DNA lesions. Interstrand cross-links are only found in semi-complementary B-DNA. DNA conformation was altered by gradually rehydrating lyophilized DNA samples, which induces an A- to B-form transition. These results were also validated by irradiating DNA in solution, in the presence or absence of 80% ethanol to induce an A- or B-form, respectively. Alkali-labile DNA lesions were revealed using hot piperidine to transform both base and sugar lesions into strand breaks. We also analyzed the location of damage as a function of DNA structure: piperidine-sensitive lesions were observed exclusively at the site of BrdU substitution, whereas strand breaks were able to migrate along the DNA strand, with a clear preference for the adenine 5′ of the BrdU. Thus, not only the hybridization state but also the DNA conformation affect the degree of sensitization by BrdU by influencing the amount and type of damage produced. Although clinical trials using BrdU as a radiosensitizer have been disappointing up to this point, these new findings point to several key features of BrdU radiosensitization that may alter future radiotherapeutic studies.

5-Bromodeoxyuridine (BrdU[1]), an analogue of thymidine, radiosensitizes cells (*1*−*2*) leading to single and double strand breaks (*3*−*4*), chromosomal aberrations (*5*), and cell death. The mechanism for single strand break formation involves electron attachment to BrdU, followed by the departure of a bromide anion and the generation of a uracil-5-yl radical that further reacts to create strand breaks. Although the radiosensitizing activity of BrdU was discovered more than 40 years ago, clinical studies have given disappointing results (*6*−*10*), failing to show a survival advantage for patients with a range of tumor types. Nevertheless, the relatively low toxicity of BrdU as well as its rare ability to directly radiosensitize DNA suggest that further studies are warranted. We therefore decided to re-explore the molecular basis of BrdU radiosensitization in order to reach a better understanding of the conditions that favor BrdU-related damage and especially the structural requirements to maximize DNA sensitization by BrdU.

Changes in DNA structure with increasing levels of hydration have been thoroughly documented. Variations in structure (*11*−*13*), compaction (*14*−*15*), and reactivity to ionizing radiation (*16*−*21*) as a function of hydration have been extensively studied in the last decades. At low levels of hydration ($0 < \Gamma < 6$, where Γ represents the number of water molecules per nucleotide), Na-DNA adopts a B-like conformation, which changes to an A-form at higher levels ($6 < \Gamma < 20$). At these lower levels of hydration, radiation can ionize the water of hydration, but the initial ionization holes appear to be transferred to DNA in what is described as the quasi-direct effect. Thus, hydroxyl radicals are not observed below $\Gamma \approx 9$ (*22*−*23*), in what corresponds to the inner primary hydration shell. Hydroxyl radical formation has been identified in the outer primary hydration shell, which contains an additional 11−12 mol of water per mole of nucleotide. The secondary hydration shell is formed at higher levels of hydration ($\Gamma > 20$), and is indistinguishable from bulk water (*12*, *17*−*18*, *22*).

The importance of DNA structure for sensitization by BrdU has already been demonstrated by our group (*24*−*26*). It was shown that hybridization decreased sensitization by up to 20-fold compared to that of single stranded DNA. Moreover, a hybridized BrdU-substituted oligonucleotide with a 5-base mismatch produced an interstrand cross-link (ICL) that was dependent on the presence of a mismatch. Here, we examine other aspects of DNA conformation that affect BrdU sensitization. First, we increased the hydration level, thereby changing DNA conformation, and found that strand breaks for BrdU-substituted DNA are specific for B-form DNA and are not found in A-form DNA. We also investigated the effect of DNA conformation in solution by using ethanol to dehydrate DNA and produce an A-conformation. For both experimental approaches, irradiation

[†] This work was supported by the National Cancer Institute of Canada.

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[1] Abbreviations: A, Adenine; AB, bromodeoxyuridine-substituted oligonucleotide; AT, unsubstituted oligonucleotide; BrdU, 5-bromodeoxyuridine; CldC, 5-chlorodeoxycytidine; CldU, 5-chlorodeoxyuridine; dCMP, deoxycytidine monophosphate; DSBs, double strand breaks; DSc, complementary double stranded; DSsc, semi-complementary double stranded; $dU^{\cdot}$, deoxyuridinyl radical; e^{-}, electron; e_{aq}, solvated electron; e_{kin}, kinetic electron; EDTA, ethylenediaminetetraacetic acid; Γ, gamma (H_2O/nucleotide); ICL, interstrand cross-link; als, alkali-labile DNA lesion; sb, strand break; SS, single stranded; T, thymine; TA, complementary oligonucleotide; UHV, ultrahigh vacuum.

10.1021/bi062114e CCC: $37.00 © 2007 American Chemical Society
Published on Web 07/13/2007

Description	Sequence	Abbreviation
Non brominated single stranded oligonucleotide	5' C-G-A-G-T-A-C-T-G-C-A-A-T-A-A-C-G-T-G-T-A-C-A-G-C 3'	SS AT*
Non brominated complementary oligonucleotide	5' C-G-A-G-T-A-C-T-G-C-A-A-T-A-A-C-G-T-G-T-A-C-A-G-C 3' 3' G-C-T-C-A-T-G-A-C-G-T-T-A-T-T-G-C-A-C-A-T-G-T-C-G 5'	DSc AT*//TA
Non brominated semi-complementary oligonucleotide	5' C-G-A-G-T-A-C-T-G-C-A-A-T-A-A-C-G-T-G-T-A-C-A-G-C 3' 3' G-C-T-C-A-T-G-A-C-G-A-A-T-A-G-C-A-C-A-T-G-T-C-G 5'	DSsc AT*//AT
Brominated single stranded oligonucleotide	5' C-G-A-G-T-A-C-T-G-C-A-A-B-A-A-C-G-T-G-T-A-C-A-G-C 3'	SS AB*
Brominated complementary oligonucleotide	5' C-G-A-G-T-A-C-T-G-C-A-A-B-A-A-C-G-T-G-T-A-C-A-G-C 3' 3' G-C-T-C-A-T-G-A-C-G-T-T-A-T-T-G-C-A-C-A-T-G-T-C-G 5'	DSc AB*//TA
Bromnated semi-complementary oligonucleotide	5' C-G-A-G-T-A-C-T-G-C-A-A-B-A-A-C-G-T-G-T-A-C-A-G-C 3' 3' G-C-T-C-A-T-G-A-C-G-A-A-T-A-G-C-A-C-A-T-G-T-C-G 5'	DSsc AB*//AT

FIGURE 1: Sequences of the brominated (B) and non-brominated (T) oligonucleotides. The asterisk (*) indicates the labeled strand. The hybridization state is indicated as follows: single-stranded (SS), double stranded, complementary (DSc), and semi-complementary (DSsc).

of A-DNA produced BrdU-specific alkali-labile lesions, as revealed by treatment with hot piperidine, whereas BrdU-specific strand breaks were only found in B-DNA. ICL production also displayed the same specificity for the B-conformation.

MATERIALS AND METHODS

Oligonucleotides. 5-Bromodeoxyuridine modified and nonmodified oligonucleotides were purchased from the University Core DNA Services (University of Calgary, AB, Canada). Sequences are shown in Figure 1. Oligonucleotides were end-labeled with ^{32}P [γ-ATP] using T4 polynucleotide kinase (Amersham Pharmacia Biotech). Labeling was carried at an initial oligonucleotide concentration of 1 μM for 45 min at 37 °C with 10 U of kinase. The enzyme was inactivated by heating for 10 min at 75 °C. Oligonucleotides were diluted to 400 nM and purified on a G50 Sephadex microcolumn. Hybridization was carried out at a final concentration of 100 nM of the labeled strand and a 2-fold excess of the unlabeled strand. Samples were heated to 82 °C for 5 min, then cooled slowly for 3 h. Hybridization controls were carried out as described in Cecchini et al. (*25*). Deionized, sterile water was used in all experimental protocols.

Hydration of DNA Samples. Aliquots of the initial labeling reaction were diluted to a final concentration of 20 nM in phosphate buffer (10 mM, pH 7.5). DNA samples were pipetted into 0.5 mL Eppendorf tubes and dried using a Speedvad evaporator for 45 min, and then hydrated by placing the tubes in scintillation vials containing various saturated solutions in order to control the relative humidity. Scintillation vials contained roughly 0.5 g of crystals and 4 mL of the corresponding saturated solution. Samples were hydrated for 24 h at 4 °C before irradiation. Saturated solutions yielded levels of relative humidity similar to those reported by Stokes (*27*) and were measured using a VWR hygrometer: K_2CO_3 ($\approx$ 45%), NaCl ($\approx$ 76%), KCl ($\approx$ 84%), H_2O ($\approx$ 99%). The relationship between relative humidity and Γ, as determined by Huttermann (*14*, *16*), was used in the present study. In addition, this relationship between steady-state hydration levels and relative humidity was confirmed by our group using plasmid DNA (*28*). Under these conditions, control samples of dried, unirradiated samples showed less than 1% breaks. Control samples of deoxygenated DNA solutions were irradiated in the presence of 50 mM EDTA (pH 8.0) in order to prevent hydroxyl radical degradation, leaving only BrdU-specific strand breaks, as described in Cecchini et al. (*25*).

Ethanol Experiments. Single stranded (SS AB* and SS AT*), double stranded (DSc AB*//TA and DSc AT*//TA), and hybridized semi-complementary (DSsc AB*//AT and DSsc AT*//AT) oligonucleotides (20 nM final concentration, in 10 mM phosphate buffer at pH 7.5) were deoxygenated by bubbling for 1 min with N_2 and irradiated in water or 80% ethanol. Certain experiments were conducted in the presence of O_2 or N_2O to scavenge solvated electrons. All samples contained EDTA (25 mM, pH 8.0) to scavenge hydroxyl radicals, whether ethanol was present or absent.

Irradiation and Treatment of DNA Samples. DNA was irradiated in a Gammacell 220 (^{60}Co) with either 300 or 2400 Gy at a dose/rate of 3.06 Gy/min. After irradiation, hydrated samples were redissolved at their original concentration by adding 20 μL of water and gently pipetting the solution to resuspend the DNA. Samples from ethanol experiments were dried with a Speedvac and resuspended in 20 μL of water. For both experiments (hydration and ethanol), 10 μL was taken from each sample and treated with 10% hot piperidine (30 min at 90 °C) to reveal alkali-labile DNA lesions.

Gel Electrophoresis and Analysis. Samples were loaded on a 20% denaturing (7 M urea) polyacrylamide gel (35 $\times$ 43 cm). A molecular weight ladder was generated by a G+A Maxam & Gilbert sequencing treatment (*29*). Electrophoresis was carried out at 40 W for 2 h and 45 min, with a 30 min pre-run at 45 W. The gel was exposed overnight in a Phosphor Screen cassette (Molecular Dynamics, Inc.), and scanned with a fluorescence scanner (Storm, Molecular Dynamics Inc.). The bands were quantified using Im-

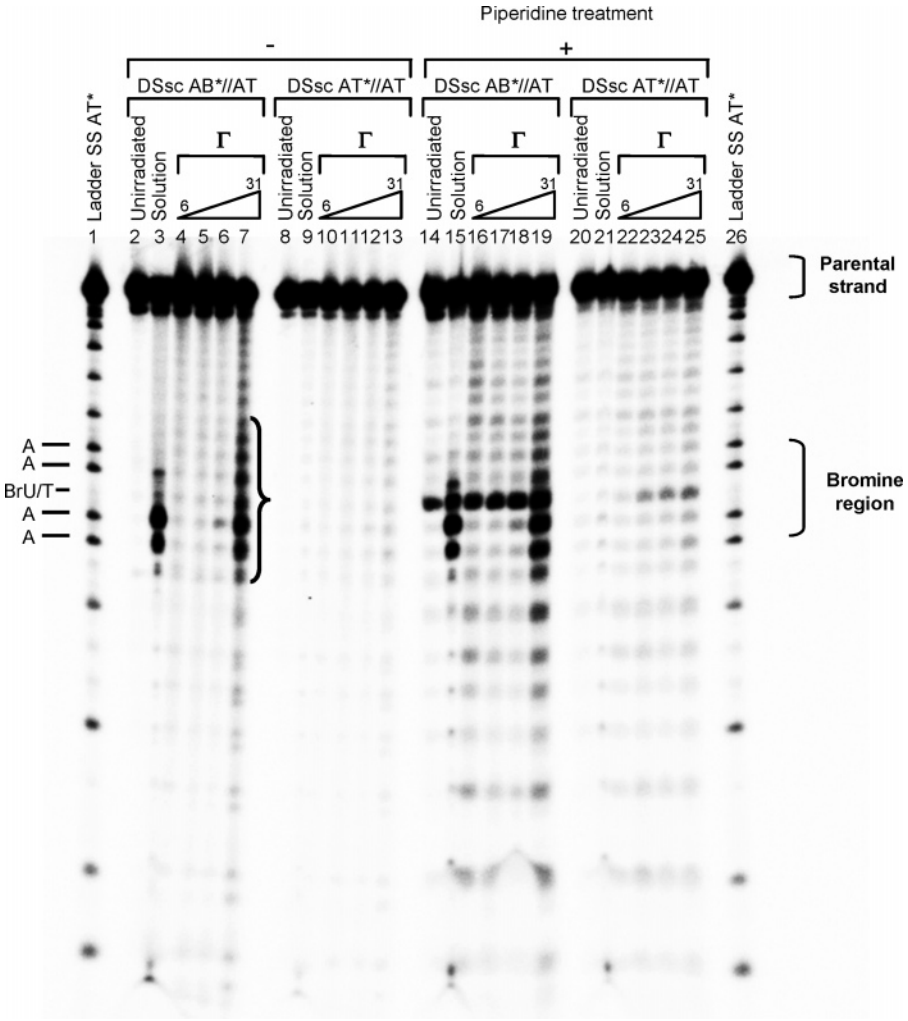

FIGURE 2: Strand breaks and base lesions as a function of hydration. Hybridized, semi-complementary DNA, unsubstituted (DSsc AT*//AT) or substituted (DSsc AB*//AT) with BrdU was irradiated with increasing levels of hydration (from $\Gamma \approx 6$ to $\Gamma \approx 31$). Lanes 2, 8, 14, and 20 are unirradiated controls, whereas lanes 3, 9, 15, and 21 are positive controls, irradiated in solution under a nitrogen atmosphere with EDTA in phosphate buffer. Lanes 14−25 were treated with hot piperidine to reveal alkali-labile DNA lesions. (Note: the portion of the gel between the wells and the parental oligonucleotide is not shown.)

ageQuant software (Molecular Dynamics) as described in Cecchini et al. (*24*).

RESULTS

Hydration and BrdU Sensitization. We first examined the role of DNA structure in BrdU sensitization by gradually increasing the DNA hydration level from $\Gamma \approx 6$ to $\Gamma \approx 31$. Although BrdU is a well-known radiosensitizer in solution (*30*−*32*) and in cells (*33*−*35*), we found no BrdU-specific strand breaks when a hybridized, semi-complementary oligonucleotide (DSsc AB*//AT, Figure 1) was hydrated between $\Gamma \approx 6$ and $\Gamma \approx 21$ (Figure 2, lanes 4−6 and 10−12 and Figure 3). Specific BrdU sensitization (strand breaks and ICLs) was only found when the hydration level reached $\Gamma \approx 31$ (Figure 2, lane 7), where there was a 5-fold increase in damage in DNA substituted with BrdU compared to that in unsubstituted DNA (Figure 3). BrdU-specific ICLs were only found at $\Gamma \approx 31$ (not shown). Because it has been reported that dehalogenation (*36*−*37*), uracil-5-yl radical production (*38*−*41*), and base fragmentation (*42*−*43*) occur in dehydrated BrdU-substituted DNA, we decided to test whether other types of BrdU-specific damage occurred at lower hydration levels. Thus, we treated irradiated DNA with hot piperidine to reveal alkali-labile DNA lesions.

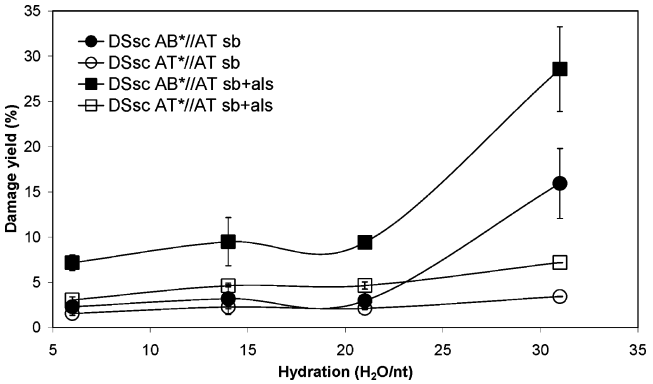

FIGURE 3: Damage yield as a function of DNA hydration. Strand breaks (sb; circles: ○, ●) and strand breaks + alkali-labile DNA lesions (sb + als); squares: □, ■) were measured for unsubstituted DNA (DSsc AT*//AT, open symbols: ○, □) and BrdU-substituted DNA (DSsc AB*//AT, filled symbols: ●, ■) in phosphate buffer.

Treatment with hot piperidine revealed DNA lesions that were created between $\Gamma \approx 6$ and $\Gamma \approx 31$ only in substituted DNA (lanes 14−19 of Figure 2 and Figure 3). Although

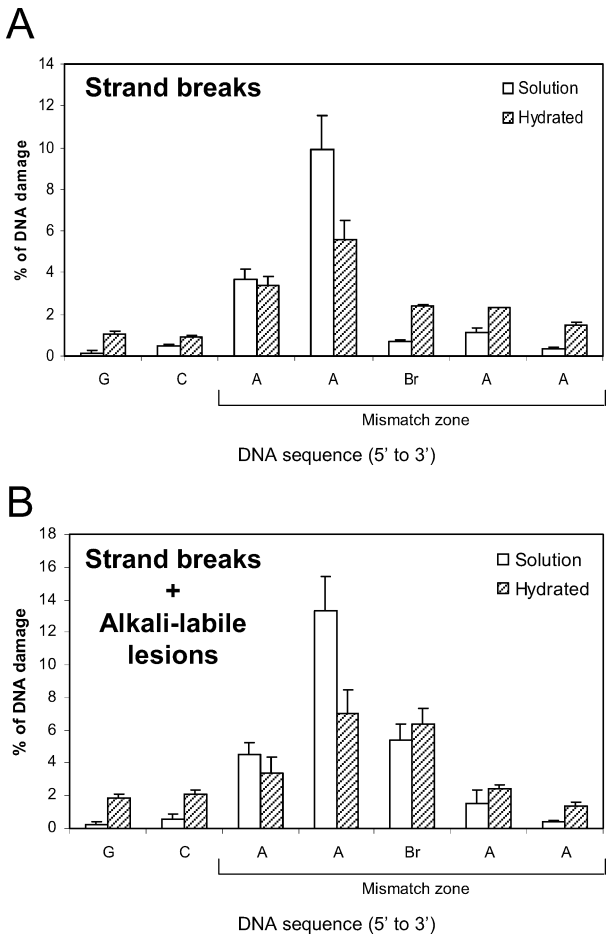

FIGURE 4: Damage as a function of DNA sequence. The relative yield of damage was measured for each base of the mismatch (AABrAA, see Figure 1 for the complete sequence) and for two bases 5′ of the mismatched, substituted oligonucleotide (DSsc AB*//AT). Strand breaks (panel A) and strand breaks + alkali-labile DNA lesions (panel B) were measured for both solution and hydrated DNA ($\Gamma \approx 31$, in phosphate buffer). Base lesions and sugar damage were revealed using hot piperidine. The background signal was removed by subtracting damage at each nucleotide of the unsubstituted oligonucleotide (DSsc AT*//AT).

treatment with piperidine revealed low levels of DNA lesions present in unirradiated, substituted oligonucleotides (Figure 2, lane 14), irradiation substantially increased the amount of DNA damage. Both strand breaks and alkali-labile lesions are quite specific for BrdU because irradiation of unsubstituted oligonucleotides induced strand breaks in fewer than 4% of the molecules under these conditions compared to 16% with substituted DNA (Figure 3). The only notable damage not specific to BrdU was the creation of alkali-labile DNA lesions at the central thymidine between $\Gamma \approx 14$ and $\Gamma \approx 31$ (Figure 2, lanes 23−25). Although BrdU-specific degradation at $\Gamma \approx 31$ is concentrated in the mismatch region (Figure 2, lane 7), leaving the double stranded portion of the oligonucleotide relatively unharmed, it extends farther on either side of the mismatch than in oligonucleotides irradiated in solution (lane 3). To further investigate this, we examined damage localization as a function of DNA sequence. Panel A of Figure 4 shows a clear bias for strand scission on the adenine 5′ of the BrdU, both in solution and in hydrated DNA, in accordance with previous studies (*24*). The second adenine 5′ of the BrdU (i.e., AABrU) is also affected, albeit to a lesser extent. Damage migration 3′ of the BrdU was also observed but occurred predominantly in hydrated DNA (Figure 4, panel A). Treatment with hot piperidine revealed that alkali-labile DNA lesions arise virtually exclusively at the BrdU site (Figure 4, panel B).

Role of DNA Structure in BrdU Sensitization in Solution. Single stranded (SS AB* and SS AT*, Figure 1), double stranded (DSc AB*//TA and DSc AT*//TA, Figure 1), and semi-complementary (DSsc AB*//AT and DSsc AT*//AT, Figure 1) oligonucleotides were irradiated in the presence or absence of 80% ethanol to induce an A- or B-form DNA, respectively. Single stranded oligonucleotides substituted with BrdU showed no evidence of conformation dependent strand break formation (Figure 5, panel A). However, for hybridized, semi-complementary DNA, an 8-fold increase in strand breaks in the BrdU region was observed when BrdU-DNA was in B-form rather than in A-form (Figure 5, panel C). Similar results were obtained for double stranded DNA (not shown). Treatment with hot piperidine exposed a similar tendency for alkali-labile DNA lesions. ICLs were also specific to B-form DNA, with a 4-fold increase (Figure 6) compared to A-DNA. Substitution with BrdU always produced substantially more damage than in unsubstituted oligonucleotides under all conditions (Figure 5, panels B and D). Irradiation in the presence of an electron scavenger (N_2O; $k_e = 9.1 \times 10^9$ L mol^{-1} s^{-1} (*44*)) reduced the percentage of damaged molecules from 9.7 ± 0.3 to 3.7 ± 0.1% (after correction for unirradiated DNA) following a dose of 2400 Gy. In the presence of ethanol, a hydroxyl radical scavenger ($k_e = 1.9 \times 10^9$ (*44*)), N_2O induced a further reduction in strand break formation from 1.2 ± 0.3% to 0.4 ± 0.2%. When DNA was irradiated with 300 Gy in the presence of another electron scavenger, O_2 ($k_e = 1.9 \times 10^{10}$ L mol^{-1} s^{-1} (*44*)), a similar reduction was observed from 3.5 ± 1.2% with N_2 to 0.7 ± 0.8% with O_2. Under these latter conditions, strand break levels were indistinguishable from unirradiated samples. At 300 Gy, the addition of ethanol had no effect on radioinduced damage (0.4 ± 0.5% and 0.4 ± 0.7% without or with O_2, respectively). Evidently, the presence of 25 mM EDTA in all samples efficiently reduced hydroxyl radical attack because total degradation of non-substituted DNA was less than 6% following a dose of 2400 Gy, compared to more than 60% in the absence of any hydroxyl radical scavenger (not shown).

DISCUSSION

Hydration and BrdU Sensitization. When a hybridized, semi-complementary oligonucleotide is irradiated at increasing levels of hydration, the frequency of strand breakage in BrdU-substituted DNA is indistinguishable from non-substituted DNA between $\Gamma \approx 6$ and $\Gamma \approx 21$. Specific BrdU sensitization (strand breaks + ICL) is only found at $\Gamma \approx 31$. In contrast, when irradiated DNA is treated with hot piperidine, alkali-labile DNA lesions that are specific for BrdU-substituted DNA are revealed over the entire hydration range (Figure 2, lanes 16−19, and Figure 3). Such evidence of DNA lesions under low hydration conditions is in agreement with previous results obtained under ultra high vacuum (UHV) (*42*−*43*). However, in these previous studies, no evidence of strand breaks was detected, most probably because irradiation under UHV precluded any hydration of DNA. Our experimental system indicates that a striking change in reactivity occurs between $\Gamma \approx 21$ and $\Gamma \approx 31$,

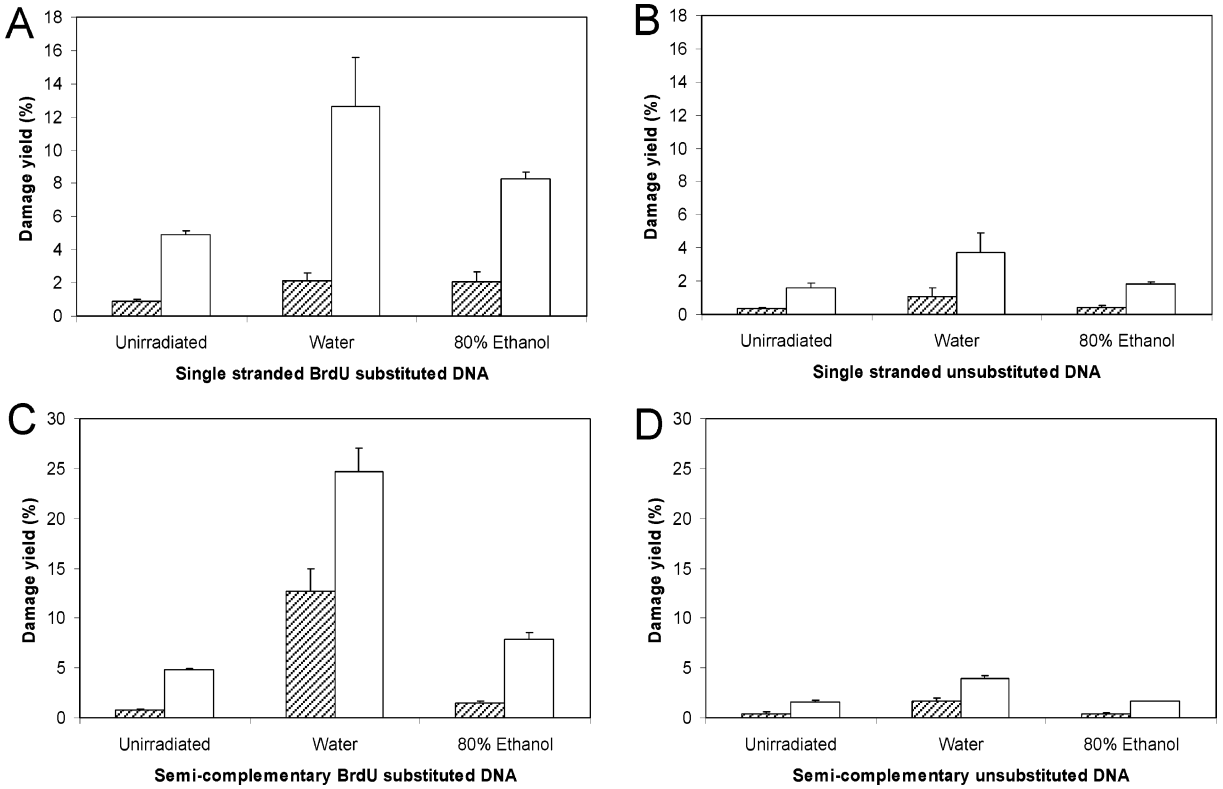

FIGURE 5: Damage yield in the mismatch zone as a function of DNA structure. Strand breaks (hatched) and strand breaks + alkali-labile DNA lesions (plain) were measured in the mismatch zone in the absence or presence of 80% ethanol for single stranded DNA with (SS AB*, panel A) or without (SS AT*, panel B) BrdU and hybridized, semi-complementary DNA with (DSsc AB*//AT, panel C) or without (DSsc AT*//AT, panel D) BrdU. DNA lesions (base lesions and sugar damage) were revealed using hot piperidine. Double stranded DNA adopts an A conformation in 80% ethanol and a B conformation in aqueous solution.

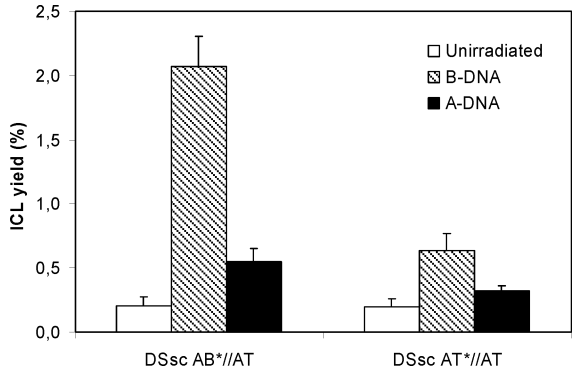

FIGURE 6: Interstrand cross-link yield as a function of DNA structure. Interstrand cross-links (ICLs) were measured in the absence (B-DNA) or presence (A-DNA) of 80% ethanol for mismatched DNA, brominated semi-complementary DNA (DSsc AB*//AT), or non-brominated semi-complementary DNA (DSsc AT*//AT), respectively.

leading to strand breaks as well as alkali-labile DNA lesions when DNA is in a higher hydration state.

DNA adopts an A-form between 45 ($\Gamma \approx 6$) and 90% ($\Gamma \approx 20$) of relative humidity, which shifts to a B-form when hydration is increased (*11*−*14*). Because BrdU sensitization has been shown to be extremely dependent on the hybridization state and on the presence of a mismatched region, we propose that the change in reactivity observed between $\Gamma \approx 21$ and $\Gamma \approx 31$ occurs because of a conversion from A-form to B-form DNA. However, it should be noted that at $\Gamma \approx 21$, the conversion to B-form is already relatively complete and thus one would expect to observe strand breaks at this level of hydration, according to the hypothesis stated above. However, the hydration levels reported here were not measured by us but were assumed on the basis of the measurement of relative humidity and the hydration levels published for these values. The use of synthetic oligonucleotides rather than plasmid DNA may produce slightly different levels of hydration. Plasmid or cellular DNA, even when every care is taken to purify it, often contains contaminants (e.g., proteins and Tris buffer) originating from the extraction protocol or the storage conditions. The presence of other molecules could affect the measurement of the hydration levels of plasmid DNA. Therefore, it is possible that the reason no strand breaks are observed at $\Gamma \approx 21$ is because the hydration level is slightly lower than $\Gamma \approx 20$, where DNA would mostly still be in A-form. For this reason, we proceeded to validate our hypothesis by inducing an A-form DNA in solution using ethanol to verify the effect of conformation on DNA radiosensitization by BrdU.

Damage Localization and DNA Structure. It is well known that strand breaks occur predominantly at the nucleotide 5′ to the BrdU. This is because the pathway leading to strand breaks involves hydrogen abstraction from the 2′-deoxyribose moiety of the base 5′ to BrdU (*45*). When we examined strand break location as a function of DNA structure, we observed a similar tendency for both dissolved and hydrated DNA ($\Gamma \approx 31$, Figure 4, panel A), although damage spread farther in the hydrated sample (Figure 2, lane 7) than in solution (lane 2). In the former case, the limited availability of water molecules probably allows radicals to migrate farther along the DNA strand before being trapped by H_2O. Remarkably, piperidine-sensitive DNA lesions occurred exclusively at the site of BrdU substitution (Figure 4, panel

B). Several factors other than conformation may explain this observation; a higher hydration level may favor protonation of the uracil-5-yl radical and creation of a radical cation, thus allowing migration of the damage. However, no preference for the G's near the initial damage site was observed, as would be expected if this were the case. In addition, the greater mobility and wobbling afforded by a higher hydration level of DNA may facilitate charge transfer of radicals to distant bases (*46*). This could also explain why a Gaussian distribution of DNA damage is observed (Figure 4, panel B) when all types of damage (strand breaks + alkali-labile DNA lesions) are taken into account. The increase in mobility generated by the formation of a mismatch zone (*24*) has already been proposed by our group to be responsible for the increase in strand breaks and the production of interstrand cross-links observed in mismatched DNA. Indeed, the mobility of the DNA bases is likely to affect the ability of any radical created in DNA to react and migrate, and higher levels of hydration greatly increase the mobility of DNA compared to that of solid-state DNA. However, hydration also induces a change in conformation that affects the number of potential donors and acceptors near the radical created at the BrdU site. Therefore, it becomes extremely difficult to distinguish between the two factors. Because several factors can indeed affect the chemistry of solid-state DNA and for the reasons cited in the previous section, we proceeded to examine the effect of conformation on the radiosensitization of BrdU-substituted DNA in solution.

Role of DNA Structure in BrdU Sensitization in Solution. It is well established that ethanol will cause a conformational change in DNA from the canonical B-DNA to A-DNA (*47*). In light of our results with hydrated DNA, we examined the effect of conformation for BrdU-substituted DNA in solution. In this system, 25 mM EDTA ($k_{OH} = 4.0 \times 10^8$ L mol^{-1} s^{-1} (*48*)) was added to each sample to scavenge hydroxyl radicals and thus to reduce both random breakage of our DNA as well as site-specific strand breakage resulting from hydroxyl radical attack on BrdU. Therefore, the majority of strand breaks produced by irradiation of these samples were assumed to be due to the interaction of solvated electrons with BrdU. With this experimental system, we saw no effect of ethanol on the sensitization of single stranded DNA by BrdU (Figure 5, panels A and B), whereas mismatched DNA displayed a conformation dependency similar to what was observed with the hydration experiment, regardless of whether strand breaks (Figure 5, panels C and D) or ICLs (Figure 6) were considered. It is possible that the interaction of ethanol with the uracil-5-yl radical could prevent the attack of the latter on the sugar and the subsequent creation of a strand break. However, our observation that ethanol does not influence break formation in single stranded DNA argues against this hypothesis (Figure 5A). A slight reduction of alkali-labile DNA lesions was observed upon addition of ethanol and could be attributed to a low level of remaining hydroxyl radicals that are scavenged by ethanol. Although solvated electrons are thought to be the primary reactive species to interact with BrdU, hydroxyl radicals can also attack BrdU, leading to debromination and creation of a strand break (*37*). Because further scavenging of these hydroxyl radicals by ethanol could explain part of the reduction in strand breaks, we performed another experiment where DNA was again irradiated in the presence or absence of ethanol, but also in the presence of dinitrous oxide (N_2O), an efficient electron scavenger. Although we observed a diminution in strand breaks in both A-form and B-form DNA in the presence of N_2O, we were unable to eliminate all strand breaks produced at 2400 Gy. Because it is possible that a substantial amount of N_2O is consumed at this high dose, we lowered the dose to 300 Gy and used oxygen, another well-known electron scavenger. Strand breaks produced in B-form DNA were completely suppressed by irradiation in the presence of oxygen. However, the number of strand breaks produced in A-form DNA at 300 Gy was too low to observe an effect of the presence of oxygen. These results clearly indicate that although hydroxyl radicals may be responsible for a small portion of strand breaks generated in B-form DNA at 2400 Gy, the majority of damage to BrdU-substituted DNA indeed occurs through a reductive pathway and that the reduction in strand break formation observed upon addition of ethanol originates from a change in DNA conformation. Thus, the results obtained with ethanol support those from our hydration experiments: the type of DNA damage resulting from BrdU sensitization is dependent on DNA conformation. Irradiation of A-DNA will induce alkali-labile DNA lesions, whereas B-DNA will lead primarily to frank strand breaks and lower levels of alkali-labile DNA lesions.

DNA structure has an important influence on the production of strand breaks. Both simulations (*49*) and experimental results (*50*) suggest that hydroxyl radical attack is dependent on DNA structure, both on DNA form (A, B or Z) and on hybridization state (single or double stranded). Several papers also report that chromatin structure per se affects the extent of DNA damage by ionizing radiation (*51*−*55*). As for brominated DNA, recent evidence by Kimura et al. pointed to enhanced reactivity in Z-DNA compared to that in B-DNA in 8-bromo-2′-deoxyguanosine substituted oligonucleotides (*56*). Cecchini et al. (*25*) also presented evidence that hybridization lowered the total yield of single strand breaks induced by γ-rays in BrdU-substituted DNA by up to 20-fold and that substituted double stranded oligonucleotides containing a mismatch produced ICLs. Previous reports have already shown that DNA structure strongly influences the preference for hydrogen abstraction on C1′ or C2′ by a uracil-5-yl radical generated by photoirradiation of BrdU-substituted DNA (*57*−*58*; for a review, see ref *59*). Thus, it is likely that DNA conformation also affects the type of damage resulting from uracil-5-yl radical production. In our case, the A-form that exists between $\Gamma \approx 6-21$ probably prevents both the H-atom abstraction from the 2′-deoxyribose moiety and the migration of damage along the DNA strand, precluding the formation of frank strand breaks. Thus, the uracil-5-yl radical remains localized at the BrdU site, possibly allowing slower, competing pathways to create other types of lesions that can be revealed by piperidine treatment and are located exclusively at the site of BrdU substitution. However, the structural conditions necessary for the generation of strand breaks exist in B-form DNA at $\Gamma \approx 31$ and in solution. Although the nature of the alkali-labile lesions was not determined in these experiments, it is entirely possible that they coincide with the damage leading to the production of the base fragments observed under UHV (*42*−*43*). Electron attachment to BrdU leads to the formation of the

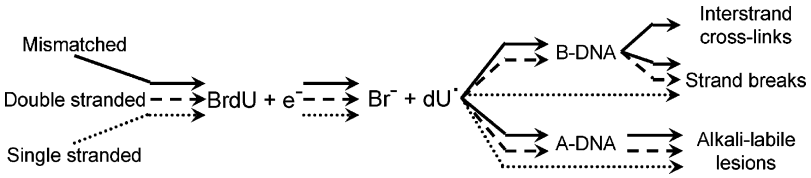

FIGURE 7: Major degradation pathway for BrdU-substituted DNA for single stranded, double stranded, and mismatched DNA for A- and B-DNA. (Note: only the most important lesions are indicated. Minor levels of alkali-labile lesions are generated in B-DNA.)

same uracil-5-yl radical, whether the electron is solvated or not (*38*−*41*), although it is also possible that the dissociative electron attachment mechanism that was proposed by Sanche's group (*60*) could also yield products different from those observed in solution. Because of the high dependence of BrdU sensitization on DNA structure, the experimental system presented here may be a sensitive probe for DNA conformation.

Figure 7 summarizes our conclusions. We propose that, during irradiation, electron attachment to DNA occurs in all oligonucleotides, independent of the hybridization state and structure. However, dehalogenation and creation of the uracil-5-yl radical, which are dependent on the probability of electron localization on BrdU, are strongly influenced by DNA structure (*25*). Furthermore, once the uracil-5-yl radical is formed, the type of damage will also depend on DNA conformation. We propose that in A-DNA, the uracil-5-yl radical is unable to abstract a hydrogen atom from the adjacent 2′-deoxyribose, thus localizing the radical at the site of substitution. Further reactions with adjoining donors will lead to the creation of alkali-labile lesions that possibly include the fragmented base that was observed by Sanche's group (*42*−*43*). In B-DNA, the structural requirements for hydrogen abstraction exist, and strand breaks will occur, although a certain amount of transient, unfavorable structure must also exist because a fraction of the uracil-5-yl radical population will generate alkali-labile DNA lesions and ICLs. B-DNA also allows the migration of the original radical to distant bases, thereby permitting delocalized induction of strand breaks. Therefore, A-DNA will produce piperidine-sensitive DNA lesions localized on BrdU, whereas B-DNA will also produce strand breaks that can spread on either side of the original radical. ICL production will be limited to mismatched DNA in B-form. Single stranded DNA will not be affected because of its less ordered configuration in solution. It is interesting to note that the reactivity of the mismatched nucleotides in semi-complementary DNA is affected by DNA conformation. This observation appears to favor the zipper-like structure for mismatches (*61*−*63*) rather than the open bubble model (*24*), which would possibly not be affected by DNA conformation.

BrdU and DNA Structure: Biological Implications. In light of the results presented in this article, we propose that the fate of BrdU upon irradiation is highly dependent on the regional conformation of DNA. BrdU-substituted DNA in A-form will only produce base lesions, as was observed in experiments studying dehydrated DNA. Strand breaks will occur at BrdU sites when DNA adopts a B-form because the DNA conformation is now favorable to the steps leading to sugar−phosphate backbone cleavage. How this hypothesis would translate into a cellular environment is still unknown; in cells, DNA is mostly in B-form but is wrapped tightly around nucleosomes to form chromatin. DNA packing and chromatin structure was shown to have an effect on radiation sensitivity (*64*), and some evidence points to a similar influence on BrdU sensitization (*34*, *65*).

Although clinical studies using BrdU as a radiosensitizer have been disappointing up to this point, a new class of halogenated pyrimidines has been emerging as a potent radiosensitizer (*66*−*67*). Sensitization by chlorodeoxycytidine (CldC) relies on the elevated levels of deoxycytidine kinase and dCMP deaminase in human tumors compared to normal tissues. CldC is incorporated in DNA as chlorodeoxyuridine (CldU). Because electron attachment to CldU will yield the same uracil-5-yl radical that is responsible for BrdU sensitization, it is extremely likely that similar sensitization mechanisms will also apply to CldC, including the production of ICLs. Therefore, it is crucial to pursue the study of the chemical processes leading to BrdU- and CldC-induced damage in order to develop and exploit tumor radiosensitizers.

ACKNOWLEDGMENT

We thank Drs. Jean Cadet, Tsvetan Gantchev, and François Bergeron for their helpful comments.

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BI062114E