UV-induced strand break damage in single stranded bromodeoxyuridine-containing DNA oligonucleotides

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Aerobic UVB photolysis of single stranded bromodeoxyuridine (BrdU)-containing DNA oligonucleotides produces immediate and latent strand break products consistent with a specific BrdU base radical-mediated abstraction of the C1′ hydrogen atom from the 5′ neighbouring deoxyribose.

Substitution of genomic thymidine with bromodeoxyuridine (BrdU) renders aerobic cell cultures more sensitive towards the lethal effects of UV and γ-radiation.[1,2] One mechanism proposed to contribute to sensitisation is an increase in the yield of radiation-induced strand breaks caused by the presence of BrdU in DNA.[1,2] From examinations of the duplex structure it was initially proposed that the additional radiation-induced BrdU-mediated strand breakage occurs *via* the formation of reactive uracil(U)-5-yl base radicals, which in turn abstract hydrogen atoms from the 5′ neighbouring deoxyribose.[2,3] Subsequent molecular studies have indeed demonstrated that under anaerobic conditions UV photolysis of BrdU-containing DNA yields both immediate and latent (alkali labile) breaks at the nucleosides 5′ to the site of BrdU.[4–7] To date, however, there have been few definitive molecular studies of BrdU-mediated DNA strand breakage in the presence of oxygen[8] (conditions under which cellular sensitisation is observed) and none in single-stranded DNA.

Here we have examined immediate and alkali-induced strand break products from a 5′- or 3′-^{32}P-end labelled single stranded (*ss*) BrdU-containing DNA oligonucleotide upon UVB photolysis (*ca*. 0.41 mW cm^{-2}), using high resolution denaturing polyacrylamide gel electrophoresis (PAGE). The strand break products were assigned by reference to the products of Maxam and Gilbert reactions,[9] and by enzymatic end-group analysis. Aerobic UVB photolysis of 5′-GCTAGCTATT-BrdU-TTATC-GATCG-3′ **1** results in specific strand cleavage at the nucleoside situated immediately 5′ to BrdU and proceeds with the loss of this 5′-nucleoside. Thus, photolysis of **1** yields a strand break product **6** nine nucleotides in length [Fig. 1(*a*)] together with a strand break product **7** eleven nucleotides in length [Fig. 2(*a*)] (Scheme 1). Co-migration of the photo-induced fragments with products of Maxam and Gilbert reactions implies the presence of phosphate moieties at both termini of the induced breaks. This was confirmed by demonstrating that **6** was a substrate for the 3′-phosphatase activity (+3′P) of T4 polynucleotide kinase (T4PNK), yielding **8** [Fig. 1(*b*)], and that **7** was a substrate for the 5′-phosphatase activity of shrimp alkaline phosphatase (SAP), yielding **9** [Fig. 2(*b*)] (see also Scheme 1).

Piperidine treatment of photolysed **1** yields an approximate five-fold increase in the yield of **6** [Fig. 1(*c*)] suggesting that the majority of BrdU-mediated damage induced at the 5′-nucleotide are alkali labile lesions and not immediate strand breaks.[2,10] This observation of the majority of piperidine-induced breaks occurring at exactly the same location as immediate breaks, and yielding the same 3′-termini, implies a common mechanistic route for both types of strand break at this site. This is consistent with H-atom abstraction occurring from C1′ of the 5′-deoxyribose leading the formation of a deoxyribolactone lesion within the oligonucleotide (**5**)[11] and a small number of direct breaks possessing phosphate termini,[12,13] although the precise mechanism for the direct strand break pathway remains to be elucidated. Structure **5** is sensitive to the strand cleaving effects of alkali and subsequent piperidine treatment would yield further breaks possessing phosphate termini.[11] Interestingly, piperidine treatment of photolysed **1** also appears to produce minor fragmentation about the BrdU moiety possibly the consequence of an induced diffusible species giving rise to piperidine sensitive lesions.

UV illumination under anaerobic conditions yields higher levels of strand breakage plus a markedly different pattern of 3′-termini damage, consisting of both phosphates and other unidentified moieties [Fig. 2(*c*)]. It is known from other studies that generation of a C1′ radical under both oxic and anoxic conditions leads to immediate and latent strand breaks possessing phosphate termini, with release of the undamaged base and the damaged sugar (probably as methylenefuranone **10**).[11–13] The present observation of different end groups following anaerobic photolysis of *ss* BrdU-containing oligonucleotides indicates that, in the absence of oxygen, H-atom abstraction must occur from a site other than (or in addition to) C1′, presumably C2′. These observations are similar to those of Cook and Greenberg[7] for duplexed BrdU-oligonucleotides. On the basis of kinetic isotope measurements these workers

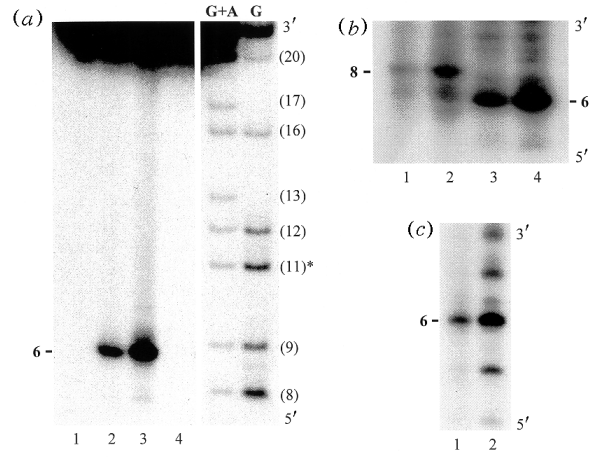


Fig. 1 Autoradiograms of the strand break products of 5′-^{32}P-labelled **1** upon aerobic photolysis and PAGE analysis. (*a*) Photolysis of **1** showing the specific formation of **6** (lane 1: no UV exposure; lane 2: 15 min UV; lane 3: 45 min UV; lane 4: nascent **1** (containing T in place of BrdU), 45 min UV); also shown are the fragment products of Maxam and Gilbert reactions specific for cleavage at G and G+A in 5′-GCTAGCTAGGTGGATCGATCG-3′; the bracketed numbers indicate fragment length with (*) indicating the location of BrdU in the BrdU-oligonucleotides. **(*b*)** Susceptibilty of **6** towards the 3′-phosphatase activity of T4 polynucleotide kinase (T4PNK) to give **8**. In these experiments the released strand break products were 5′-^{32}P-end labelled *after* photolysis using [γ-^{32}P]ATP plus T4PNK either possessing 3′-phosphatase activitity (+ 3′P) (from USB) or T4PNK lacking 3′-phosphatase activity (− 3′P) (from Boehringer Mannheim). {lane 1: 20 min UV + (+ 3′P); lane 2: 45 min UV + (+ 3′P); lane 3: 20 min UV + (− 3′P); lane 4: 45 min UV + (− 3′P)}. **(*c*)** Alkali lability of photolysed **1** revealing an enhanced formation of **6** upon piperidine treatment [lane 1: 15 min UV; lane 2: 15 min UV + piperidine (1 M, 90 °C, 30 min)].

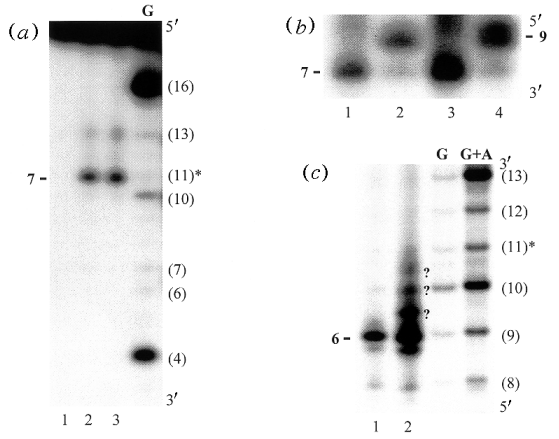
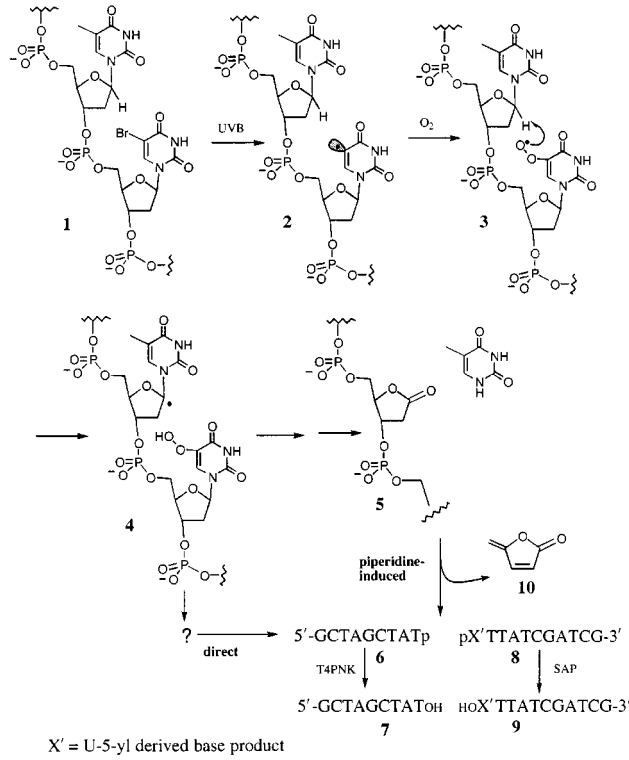


Fig. 2 (*a*),(*b*) Autoradiograms of the strand break products of 3′-^{32}P-ddA-labelled **1** upon aerobic photolysis and PAGE analysis. (*a*) Photolysis of **1** showing the specific formation of **7** (lane 1: no UV exposure; lane 2: 30 min UV; lane 3: 60 min UV); also shown are the fragment products of Maxam and Gilbert reactions specific for cleavage at G in **1**; the bracketed numbers indicate fragment nucleotide length (excluding 3′-ddA) with (*) indicating the location of BrdU. **(*b*)** Susceptibilty of **7** towards the phosphatase activity of SAP to give **9** (lane 1: 30 min UV; lane 2: 30 min UV + SAP; lane 3: 60 min UV; lane 4: 60 min + SAP). (*c*). Autoradiogram of the strand break products of 5′-^{32}P-labelled **1** upon aerobic or anaerobic photolysis and PAGE analysis. Photolysis of **1** showing the specific formation of **6** plus other unidentified products (denoted as **?**) (lane 1: 45 min UV, aerobic; lane 2: 45 min UV, anaerobic); also shown are the fragment products of Maxam and Gilbert reactions specific for cleavage at G and G+A in **1**; the bracketed numbers indicate fragment nucleotide length with (*) indicating the location of BrdU.



Scheme 1

conclude that under anaerobic conditions U-5-yl radicals abstract the C2′ hydrogen atom of the 5′-deoxyribose, ultimately yielding cleavage products containing 3′-phosphate and a labile 2′-deoxy-3′-ketonucleotide.[7] Clearly, if the anaerobic U-5-yl-mediated abstraction of C2′–H is occuring in our system then specific C2′ abstraction in duplexed BrdU-DNA is not simply a result of conformational effects imposed by the double helix, as it persists in *ss* systems under anaerobic conditions.

It is difficult to rationalise the aerobic abstraction of C1′–H and the anaerobic abstraction of C2′–H by simply evoking U-5-yl as being the sole abstracting species. We therefore propose that under anaerobic conditions it is the initial U-5-yl base radical that abstracts from a site other than C1′ (or a number of different sites), whilst under aerobic conditions the U-5-yl base peroxyl radical (U-5-yl-OO·), formed by the addition of oxygen to the 5-yl radical centre, mediates abstraction specifically from C1′ (Scheme 1).

Support for the above proposal, that in the presence of oxygen abstraction is from C1′, comes from the recent published observation of Greenberg *et al.* that direct strand breaks, mediated by the 5,6-dihydrothymid-5-yl radical in *ss* oligonucleotides in the presence of oxygen, proceed *via* the specific abstraction of the C1′ hydrogen atom by the corresponding base peroxyl radical species.[14] These authors also refer to their preliminary unpublished observations of C1′–H abstraction on photolysis of *ss* BrdU-oligonucleotides under aerobic conditions which directly supports our present observations.[14] Additional support for the specific abstraction of C1′–H by a peroxyl radical comes from calculations of enthalpies for H-atom abstraction in deoxyribose[15] which suggests that the C1′ may be the sole position from which a peroxyl radical could abstract.

Our observations are in broad agreement with those of Sugiyama *et al.*[8] who have demonstrated that under aerobic conditions it is the C1′ hydrogen atom which is predominatly abstracted in duplexed BrdU-containing oligonucleotides; however, they do not propose the base peroxyl radical intermediate as being formed and they propose some abstraction from C2′, both possibly resulting from constraints imposed by the more rigid duplex structure. Our present studies are continuing with examinations of duplexed BrdU-containing oligonucleotides, to investigate the effect of strandidness on BrdU-mediated radical reactions, and to allow for the examination of possible bi-strand lesions leading to double strand breaks and multiply damaged sites.[16]

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Notes and References

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1 C. F. Webb, G. D. D. Jones, J. F. Ward, D. J. Moyer, J. A. Aguilera and L. L. Ling, *Int. J. Radiat. Biol.*, 1993, **64**, 695.
2 F. Hutchinson, *Quart. Rev. Biophys.*, 1973, **6**, 201.
3 J. D. Zimbrick, J. F. Ward and L. S. Myers Jr., *Int. J. Radiat. Biol.*, 1969, **16**, 525.
4 J. Cadet and P. Vigney, in *Bioorganic Photochemistry*, ed. H. Morrisn, Wiley-Interscience, New York, 1990, vol 1, pp. 3–229.
5 I. Saito, *Pure Appl. Chem.*, 1992, **64**, 1305.
6 H. Sugiyama, Y. Tsutumi and I. Saito, *J. Am. Chem. Soc.*, 1990, **112**, 6720.
7 G. P. Cook and M. M. Greenberg, *J. Am. Chem. Soc.*, 1996, **118**, 10 025.
8 H. Suigiyama, K. Fujimoto and I. Saito, *Tetrahedron Lett.*, 1996, **37**, 1805.
9 A. M. Maxam and W. Gilbert, *Methods Enzymol.*, 1980, **65**, 499.
10 R. O. Rahn and H. G. Sellin, *Photochem. Photobiol.*, 1983, **37**, 661.
11 A. P. Breen and J. A. Murphy, *Free Radical Biol. Med.*, 1995, **18**, 1033.
12 B. K. Goodman and M. M. Greenberg, *J. Org. Chem.*, 1996, **61**, 2.
13 M. M. Meijler, O. Zelenko and D. S. Sigman, *J. Am. Chem. Soc.*, 1997, **119**, 1135.
14 M. M. Greenberg, M. R. Barvian, G. P. Cook, B. K. Goodman, T. J. Matray, C. Tronche and H. Venkatesan, *J. Am. Chem. Soc.*, 1997, **119**, 1828.
15 K. Miaskiewicz and R. Osman, *J. Am. Chem. Soc.*, 1994, **116**, 232.
16 J. F. Ward, *Int. J. Radiat. Biol.*, 1994, **66**, 427.

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