Formation of ssb, dsb, and Uracil in Monofilarly and Bifilarly Bromouracil-Substituted DNA Molecules

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DNA Strandbreaks, Bromouracil Substitution, 313 nm UV Irradiation

Bifilarly BU-substituted Col E 1 plasmid and monofilarly BU-substituted M 13 phage DNA were irradiated with UV light of 313 nm. Using agarose gel electrophoresis and "reversed phase" HPLC technique ssb, dsb induction and uracil formation, respectively, could be detected in the irradiated DNA in dependence on the UV fluence. The analysis of the strandbreaks in bifilar Col E 1 DNA shows a significant part of directly induced dsb.

Cross sections of ssb induction from 4.1 $m^2/J \times 10^7$ in 28%, 3.9 $m^2/J \times 10^7$ in 55% and 3.1 $m^2/J \times 10^7$ in 85–90% BU-substituted DNA were calculated. The cross section for dsb induction was found to be 0.04 $m^2/J \times 10^7$, estimated from the linear part of the fluence effect curve. In monofilar M 13 DNA a linear fluence effect curve for dsb induction was obtained. Excluding other than the direct production of dsb by using an *in vitro* approach for M 13 DNA, the results strongly support the hypothesis that dsb can be induced by one photochemical absorption event. The cross section for ssb was 3.8 $m^2/J \times 10^7$ and for dsb 0.05 $m^2/J \times 10^7$ in 41.5% monofilarly BU-substituted M 13 DNA.

The comparison of ssb, dsb, and uracil production in bifilar and monofilar DNA with similar BU substitution showed no significant difference between the two DNA systems (Col E 1, M 13), indicating that the location of BU molecules in one or in both DNA strands will not lead to a different number of lesions after UV_{313} exposure.

Introduction

The substitution of bromouracil (BU) for thymidine sensitizes DNA to UV light (reviews: Hutchinson [1]; Hutchinson and Köhnlein [2]).

Irradiation with long wave-length UV light leads to formation of uracil [3] by splitting the Br–C bond of bromouracil. The consequences of this chemical reaction (review: v. Sonntag [4]) are the induction of single strandbreaks (ssb) and double strandbreaks (dsb) (review: Hutchinson [1]).

In several earlier investigations the mechanism of ssb induction was examined [5–9] and dsb induction was shown [5, 9–12].

The hypothesis, that one photochemical absorption event leads to a dsb, as not yet been explained satisfactorily. Although the results of Krasin and Hutchinson [12] were really suggestive, these authors have worked with an *in vivo* system, in which they could not absolutely exclude the possibility of enzymatic repair reactions, which could lead to the second ssb opposite to an ssb induced by radiation at the BU molecule. In our present experiments we have used an *in vitro* system in which no enzymatic activity or repair processes are possible, we are able to confirm *in vivo* data of others (cited above) by an *in vitro* approach.

Since there is renewed interest in the halogenated pyrimidines as a class of non-hypoxic cell radiosensitizers (review: Mitchell *et al.* [13]) the basic sensitization mechanisms of halogenated DNA are also important again from the standpoint of medical applications [14, 15]. In this connection Ling and Ward [16] have recently shown, that induction of strandbreak after X-ray irradiation is independent of the location of BU in one or both DNA strands and only depends on the percent of BU substitution.

In the present study we have examined ssb and dsb induction after exposure to UV_{313} light of plasmid DNA (bifilar) homogeneously substituted with BU and M 13 phage DNA, in where only one strand was BU-substituted (monofilar). A linear dependence of the dsb induction on the fluence is evidence of dsb induction by one photochemical event in small closed circular DNA molecules. Furthermore, we have measured the fluence-de-

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pendent formation of uracil in both DNA systems. From comparison of results obtained in monofilar and bifilar DNA after UV exposure we obtain information on the importance of location of BU residues in one or both DNA strands.

Materials and Methods

Isolation of ColE1 plasmid DNA and M13 phage DNA

ColE1 plasmids were isolated from *E. coli* IC $411^{(thy-)}$ cells, as described elsewhere [17]. Single strand (ss) M13 phage DNA was used as a template for the *in vitro* polymerization of ds-BU-substituted M13 phage DNA. *E. coli* K1271-18 cells [18] were incubated in 100 ml YET medium (yeast 5 g, tryptone 10 g, NaCl 10 g in 1000 ml *aqua dest.*) at 30 °C. M13mp18 phage [19] were added to the growing cell culture (m.o.i. 10). The culture was then incubated with aeration to an OD_{550} of 0.5. M13 phage DNA was isolated by a procedure described in the Amersham handbook "Oligonucleotide-directed *in vitro* mutagenesis system" code RPN 2322.

In vitro polymerization

The *in vitro* reactions were performed essentially according to the procedures described by Maniatis *et al.* [20]. For the primer phosphorylation 10 ng of a 17-mere M13 primer (BRL) (10 µl), 20 units T4 kinase (20 µl) (BRL), kinase buffer (10 µl) (Tris/HCl 50 mM, pH 7.6, $MgCl_2$ 10 mM, DTT 5 mM, spermidine 0.1 mM, EDTA 0.1 mM) and 78 µl HPLC water (J. T. Baker) were incubated for 15 min at 37 °C and then for 10 min at 70 °C.

This phosphorylation batch was incubated with 12 µl ss-M13 phage DNA (50 mg/ml), 20 µl annealing buffer (NaCl 10 mM, Tris/HCl 1 mM, pH 7.8, EDTA 0.1 mM) and 68 µl HPLC water for 20 min at 15 °C. Subsequently, 56 µl of 1 mM dATP, dGTP, dTTP, dCTP (Boehringer, Mannheim) (14 µl each), d(5-^{3}H)CTP (1 µCi/ml) (Amersham), 18 units Klenow fragment of polymerase I (3 µl) (BRL), 30 ml nick translation buffer (Tris/HCl 50 mM, pH 7.2, $MgSO_4$ 10 mM, DTT 0.1 mM, BSA 50 µg/ml) and 10 units of T4 ligase (Boehringer, Mannheim) were added to the annealing batch. For BU substitution dTTP was replaced by bromodeoxyuridinetriphosphate (BrdUTP). Polymerization continued over night (20 h) at 15 °C and success was checked by agarose gel electrophoresis (Fig. 4).

Irradiation procedure and actinometry

The UV_{313} light used for the irradiation experiments was produced by a HBO 200 W superpressure mercury lamp (Osram) using a high intensity grating monochromator (Bausch and Lomb). The fluence rate was determined by the potassium ferrioxalate actinometry developed by Hatchard and Parker [21]. Each irradiation series was started with a fluence determination. The UV_{313} fluences were between 18 J/m^2 · sec and 26 J/m^2 · sec.

The DNA samples were irradiated in 10 mM phosphate buffer (pH 7.5) at room temperature in a 2 mm quartz cuvette. After predetermined fluences 25 µl aliquots of the irradiated DNA (0.1 µg M13 DNA and 1 µg ColE1 DNA, respectively) were withdrawn and stored in Eppendorf tubes at a dark, cool place until irradiation was finished. Subsequently, all samples were analyzed by agarose gel electrophoresis.

Agarose gel electrophoresis

Irradiated DNA samples were loaded onto a 1% agarose slap gel (100 × 100 × 2.5 mm thick) prepared with a buffer containing 50 mM Tris/HCl (pH 8.2), 20 mM sodium acetate, 2 mM EDTA and 18 mM NaCl. The electrophoresis was run in the same buffer for 4 h at 4 V/cm in a horizontal apparatus (Multiphor, LKB/Pharmacia) at 12 °C.

Gels were stained in the running buffer containing 0.5 µg/ml ethidium bromide (EtBr) for 2 h to visualize the well separated different forms of DNA molecules. For some experiments the DNA samples were analyzed in gels containing 5 µg/ml EtBr.

Quantification of DNA molecules in agarose gels

The 28%, 40%, and 55% BU-substituted ColE1 plasmid DNA were labeled with [^{3}H]thymidine and the M13 phage DNA was labeled with [5-^{3}H]-cytidine, while the 85–90% BU-substituted ColE1 plasmid DNA had no radioactive label.

For this non-radioactive DNA the sc-form was quantified by using a laser densitometer (Ultrascan XL, LKB/Pharmacia). The gels, stained with EtBr, were photographed by a C-58 Tectronix camera using 90 × 110 mm negative film (Kodak

T-Max 4053). Linear relation between DNA concentration in the sc-form and fluorescence by EtBr was shown up to 750 ng DNA in the sc-form. The concentration of the radioactive DNA was quantified after cutting and dissolving the gel pieces containing DNA in hot 2 M $NaClO_4$, by scintillation counting (Rackbeta, LKB/Pharmacia).

HPLC assay for uracil formation and BU substitution

M13 phage DNA and ColE1 plasmid DNA were treated with nuclease P1 (Serva) and alkali phosphatase (Serva) and the base composition was determined by reversed phase HPLC technique as described by Gehrke *et al.* [22].

Using a Supelcosil® LC 18 column with a high precision pump (300 C, Gynkotek), a LCC 500 operator (Pharmacia) and a variable wave-length monitor (2515, Pharmacia), separation was achieved with a 0.05 M KH_2PO_4 buffer + 5% MeOH at a flow rate of 1 ml/min. The column temperature was fixed at 35 °C and the signal of the UV detection at 270 nm was registered by the LCC 500 computer and a plotter (0.25 cm/min).

Using a known mixture of deoxynucleosides (dA, dG, dT, dC, dU, BrdU) as references, the dU-peak, which appeared in irradiated DNA, could be identified and its increase could be quantified from the peak areas. Similarly, the decrease of the BrdU peak with UV fluence was determined.

Results

Ssb and dsb induction in ColE1 plasmid DNA

The UV fluence-dependent conversion of the supercircular (sc) DNA form into the open circular (oc) and linear (lin) DNA form was quantified by agarose gel electrophoresis. A ssb converts the sc-form into the oc-form and a dsb converts either of these DNA forms into a linear plasmid molecule. Assuming a homogeneous distribution of the BU residues, a high number of absorption centers (BU molecules) as compared to the average number of strandbreaks, and a random distribution of strandbreaks in the DNA molecules, the number of ssb and dsb can be approximated by a Poisson distribution. The average number of dsb per DNA molecule (n_2) could be calculated from the increasing fraction of linear plasmid DNA form of full length [lin]. These molecules have exactly one dsb and can be represented by the first term of a Poisson distribution [23].

$$[\text{lin}] = n_2 \cdot \exp(-n_2). \tag{1}$$

The fraction [lin] was determined by (2):

$$[\text{lin}] = \frac{\text{lin}_{\text{after irradiation}} - \text{lin}_{\text{before irradiation}}}{(\text{sc} + \text{oc})_{\text{before irradiation}}}. \tag{2}$$

The average total number of ssb and dsb per plasmid molecule was obtained from the fraction of sc-form remaining after irradiation (sc) [17].

$$[\text{sc}] = \exp - (n_1 + n_2). \tag{3}$$

In the fluence range up to 8 kJ/m^2, dsb can be neglected so that strandbreaks are equal to ssb. The methods of determining strandbreaks in small supercircular DNA molecules by agarose gel electrophoresis have been described in more detail by Hempel and Mildenberger [24].

The phosphate buffer used in our experiments had no influence on the strandbreak rate within 10 mM to 200 mM. For the bifilarly BU-substituted ColE1 plasmid DNA with a BU substitution of 28%, 55%, and 85–90%, a linear fluence dependence of the ssb induction was found after UV_{313} irradiation in 10 mM phosphate buffer, while no ssb were noticed in normal DNA without any BU substitution up to 6 kJ/m^2 (Fig. 1).

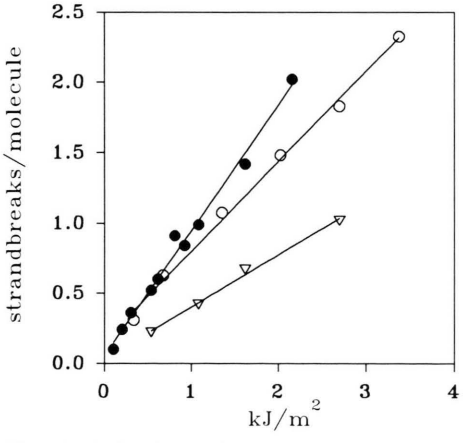

Fig. 1. Induction of strandbreaks in ColE1 plasmid DNA with different BU substitution in 10 mM phosphate buffer (pH 7.5) by UV_{313} light. ▽ = 28% BU substitution; ○ = 55% BU substitution; ● = 85–90% BU substitution.

Table I. D_{37} values and cross sections for ssb induction in bifilarly BU-substituted ColE1 plasmid and monofilarly BU-substituted M13 phage DNA. The D_{37} values could be received directly from the linear regression drawn through the experimental points in Fig. 1 and 5, while cross sections were calculated using Eqn. (4).

	Bifilar ColE1 DNA			Monofilar M13 DNA
% BU substitution	28	55	85–90	41.5
D_{37} (ssb) [kJ/m^2]	2.55	1.37	1.08	1.48
σ (ssb) [m^2/J × 10^7]	4.1	3.9	3.1	3.8

As shown in Table I the D_{37} values for the induction of ssb decreases with BU substitution. The cross sections given in Table I were calculated according to Eqn. (4).

$$\sigma_{ssb} = \frac{\text{ssb/bp}_{(\text{plasmid molecule})}}{\text{fluence [J/m}^2\text{]} \cdot \text{BU molecules/bp}_{(\text{plasmid molecule})}} . \quad (4)$$

We found approximately the same cross sections for 28% and 55% BU substitution as expected, whereas the value for 85–90% BU substitution was smaller indicating less ssb per BU molecule at a given fluence.

In Fig. 2 we present the induction of dsb in bifilarly BU-substituted DNA as measured by the increasing amount of linear DNA form (1) *versus* UV fluences (F). The resulting curve shows a strong quadratic component suggesting dsb induction by coincidence of two ssb produced independently from each other. To analyze the data of Fig. 2 according to a linear quadratic relationship for dsb induction (n_2)

$$n_2 = aF + bF^2 \quad (5)$$

we replotted them in the form n_2/F *versus* fluence (F) (Fig. 3). From the general assumption of a linear quadratic fluence dependence on dsb induction, Eqn. (5) can be rearranged:

$$\frac{n_2}{F} = a + bF. \quad (6)$$

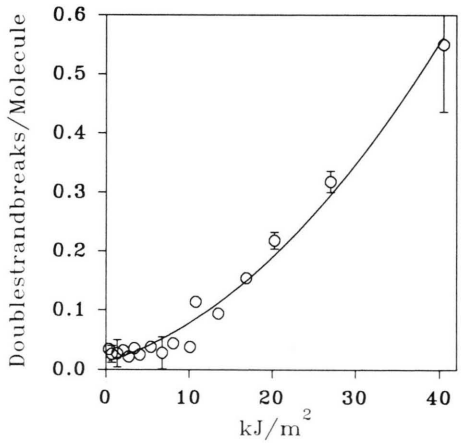

Fig. 2. Induction of dsb in ColE1 plasmid DNA bifilarly substituted with 55% BU by UV_{313} light. The data were obtained from four experimental series. The error bars result from 2–4 experimental points. The points without error bars indicate a single measurement. The curve is a second order regression.

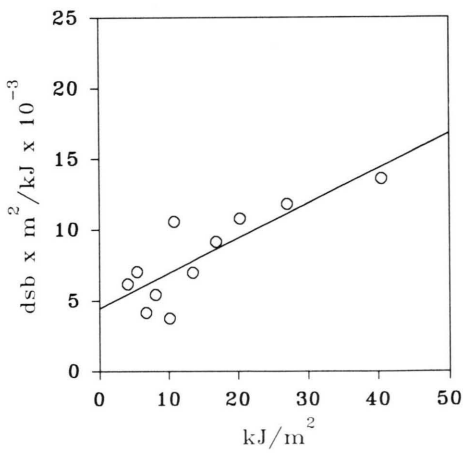

Fig. 3. Evaluation of the linear and quadratic term of the dsb induction by UV_{313} light obtained from the experimental points of Fig. 2. The data were calculated by Eqn. (6) assuming a linear quadratic relationship (5). For UV fluences below 5 kJ/m^2 small differences from the fit in Fig. 2 produced very large fluctuations of the resulting points in this graphic. Therefore, the data below 5 kJ/m^2 were excluded.

The intercept with the ordinate of the linear regression (Fig. 3), drawn through the experimental points, represent the linear component of the amount of dsb described by a. The quadratic component is given by the slope described by b.

The existence of a linear part in the curve is indicative for the direct induction of a part of the measured dsb. These results strongly support our assumption of a dsb mechanism by one photochemical absorption event, but a conclusive proof for such a dsb induction required irradiation experiments with a definite monofilarly BU-substituted DNA.

Ssb and dsb induction in monofilar M 13 DNA

The success of the *in vitro* polymerization was shown by agarose gel electrophoresis. As shown in Fig. 4 nearly all of the used ss-M 13 DNA templates were polymerized to a relaxed circular (rc) ds-M 13 DNA form (Fig. 4).

The conversion of the rc-form to linear molecules takes place, if a dsb is induced directly or by coincidence of two ssb, while a ssb will not change the relaxed circular form of the M 13 molecule.

From the disappearance of the rc-form the number of dsb can be deduced with Eqn. (3) replacing (sc)-DNA by (rc)-DNA. In Fig. 5 the average dsb per M 13 molecule was plotted *versus* UV_{313} fluence. We found a well detectable number of dsb in the monofilar ds-DNA molecules, in direct proportion to fluence.

To estimate the probability for a dsb induction by coincidence of two ssb in the complementary DNA strands, the number of ssb in the thymine strand had to be determined before irradiation. Furthermore, the induction of a few ssb in normal thymine DNA at higher fluences could also influence the results of dsb induction. To obtain the number of the initial ssb in the *in vitro* synthesized DNA, an agarose gel-containing EtBr was run. Under these conditions the intact relaxed circular DNA is transformed to the sc-form by intercalation of the dye into the DNA. From the fraction of the DNA remaining in the rc-form, the number of ssb in the synthesized ds-M 13 DNA can be derived. In our experiments we found 0.87 ssb/molecule before irradiation or 0.44 ssb/single strand in a first approximation.

At the fluence which induces one dsb (D_{37} = 105.5 kJ/m^2) (Fig. 5) in M 13 phage DNA we found about 1.0 ssb/molecule in native DNA or

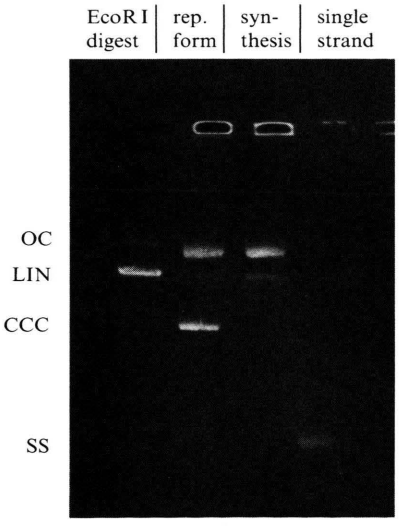

Fig. 4. *In vitro*-polymerized M 13 phage DNA and different M 13 DNA forms as a reference separated in an agarose gel. 1: EcoR I digest of isolated ds-M 13 phage DNA; 2: isolated M 13 phage DNA (replicative form); 3: *in vitro*-polymerized relaxed circular M 13 DNA; 4: isolated single-stranded M 13 phage DNA used as template for polymerization.

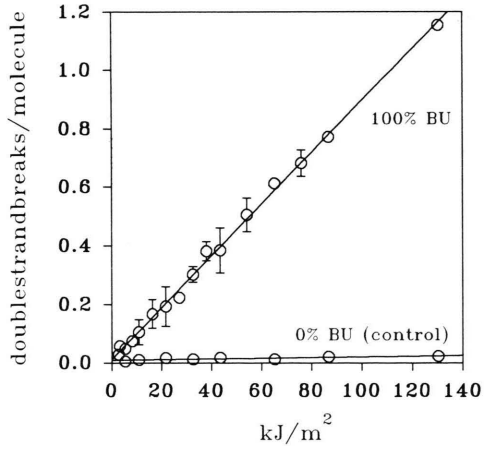

Fig. 5. Induction of dsb in monofilarly BU-substituted M 13 DNA in 10 mM phosphate buffer (pH 7.5) by UV_{313} light. The dsb per molecule could be calculated from the remaining relaxed circular (rc) DNA (Fig. 4) using Eqn. (3) after UV exposure. The quantification of the rc-form in the agarose gel was obtained from scintillation counting of the gel cut outs containing DNA.

0.5 ssb/single strand. Together with the already existing 0.44 ssb/strand we have about 1 ssb in the thymine DNA strand, if the measured 1.87 (1.0 + 0.87) ssb were equally distributed in the thymine and the BU strand.

The probability of induction of the measured dsb (p_{dsb}) at 105.5 kJ/m² (Fig. 5) by coincidence of the induced ssb in the BU-containing DNA strand and the preexisting ssb in the thymine strand could be calculated by a Poisson distribution (7):

$$p_{dsb} = 1 - \exp\text{-}(q \cdot N_T \cdot R). \quad (7)$$

q = the quotient of the interaction distance over which two ssb in complementary strands can interact to form a dsb (we assumed 16 base pairs in 10 mM phosphate buffer according to Freifelder and Trumbo, 1969 [25], also higher and lower values have been reported in the literatur [24, 26]) and the number of base pairs per M 13 phage molecule (7249) ($q = 16/7249 = 2.21 \times 10^{-3}$);

N_T = number of ssb in the thymine strand at 105.5 kJ/m² (1 ssb);

R = number of ssb, induced by UV_{313} light irradiation in the BU-substituted strand (71 ssb) at 105.5 kJ/m² (obtained by analysis of EtBr-containing agarose gels with irradiated monofilarly BU-substituted M 13 phage DNA and linear extrapolation to a fluence of 105.5 kJ/m²).

From Eqn. (7) we obtained a probability of 14% that the measured dsb/molecule (Fig. 5) at 105.5 kJ/m² was induced by coincidence of two ssb in the complementary strands. Thus 86% of the measured dsb were induced directly.

When we compare the D_{37} values and the cross sections for ssb induction of bifilarly BU-substituted ColE1 plasmid DNA and the monofilarly substituted M 13 phage DNA (Table I) the data indicate that the location of the BU molecules has no crucial influence on induction of strandbreaks. We found similar D_{37} values for 55% bifilarly BU-substituted ColE1 DNA and 41.5% monofilarly BU-substituted M13 DNA. Because the ratio of the BU molecules to base pairs per molecule in these two DNA preparations is similar (M13 = 1783 BU/7249 bp {1:4}; ColE1 = 1884 BU/6646 bp {1:3.5} we conclude that the number of ssb at a given fluence depends on the percentage of substitution and not on its location in one or both DNA strands.

Uracil formation in bifilar ColE1 and monofilar M13 phage DNA

For the uracil formation, measured as deoxyuridine (dU) by HPLC technique, we found an increase of the dU peak with the UV fluence. In Fig. 6 we plot the increase of dU and the decrease of bromodeoxyuridine (BrdU) for 85–90% BU-substituted ColE1 DNA *versus* UV fluence. We found a linear decrease of BrdU and its disappearance was exactly balanced by the appearance of BU after irradiation with low fluences. At higher fluences hardly any further increase of the dU formation was found. To calculate the cross section for uracil formation (σ_U) Eqn. (8) was used.

$$\sigma_U = \frac{[dU]}{[BrdU] \cdot \text{fluence}\,[J/m^2]}. \quad (8)$$

Here [dU] denotes the uracil concentration after irradiation as determined by HPLC technique and [BrdU] the initial concentration. If the BrdU values after irradiation were used, for calculating cross sections, the resulting values would have been too high to explain the experimentally observed uracil production. In Fig. 6 the disappearance and production of BrdU and dU, respectively, are plotted *versus* UV_{313} fluence. As can be seen,

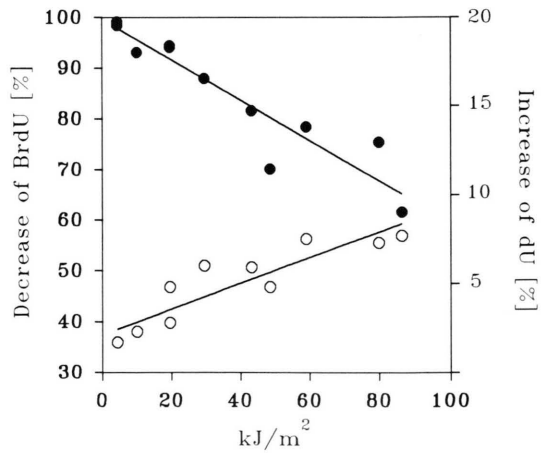

Fig. 6. Disappearance and appearance of BrdU and dU, respectively, in UV_{313}-irradiated ColE1 plasmid DNA substituted with 85–90% BU. The data were obtained using HPLC technique. ● = BrdU disappearance; ○ = dU appearance.

Table II. Cross section of dU formation in bifilarly BU-substituted ColE1 plasmid and monofilarly BU-substituted M13 phage DNA. The appearance of dU after UV_{313} exposure was measured by HPLC technique (2.6) and the cross sections for every fluence could be calculated using Eqn. (8).

	% BU subst.	[kJ/m²]	σ dU [m²/J × 10⁷]
	40	21.3	5.9
		54	7.8
		8.8	10.2
	55	34	16.5
		75.6	7.4
		4.3	27
		10	27.1
Bifilar		19.5	21.8
ColE1 DNA		29.5	23.9
	85–90	43.2	15.2
		48.6	11.6
		59	14.9
		80	10.7
		86	10.5
Monofilar M13 DNA	41.5	82	9.4

even in the low fluence range, there is a remarkable deficit in uracil formation. Normally one would expect one value for the cross section of the deoxyuridine formation. The obtained values, however, scattered considerably between various substitution rates and they vary within a given BU substitution for different UV fluences by a factor of about 2, indicating saturation effects or the existence of a different photoproduct (Table II).

The cross section for dU formation from monofilarly BU-substituted M13 phage DNA compares rather well with the corresponding cross section obtained with ColE1 DNA (Table II).

Discussion

The major aim of this paper was to present definite experimental evidence for the direct induction of dsb in BU-substituted DNA by one photochemical absorption event. Furthermore, it could be shown by comparing bifilarly and monofilarly BU-substituted DNA, that the production of photolesions was independent of the location of the BU residues, either in one or in both DNA strands, and that it depends only on the percentage of BU substitution.

In vitro synthesized ds-M13 phage DNA with one normal DNA strand and one BU-substituted strand and homogeneously BU-substituted ColE1 plasmid DNA, which is well comparable in size with the M13 DNA system, was used.

Strandbreak induction and uracil formation in bifilarly BU-substituted ColE1 plasmid DNA

Before discussing dsb induction by one photochemical event and the influence of BU location on photolesions a phenomenon, observed in very high (85–90%) bifilarly BU-substituted DNA should be mentioned first. As shown in Fig. 2 and 6 and summarized in Tables I and II, the cross sections for ssb induction and for uracil formation were significantly smaller in 85–90% BU-substituted ColE1 DNA than in ColE1 DNA with smaller BU substitution. The reason for this is not yet clear. The high BU substitution might be responsible for an increasing uracil–dinucleotid formation at higher UV fluences. Sasson *et al.* [27] could identify 5′,5-diuridine after UV_{313} irradiation of bromouracil-polynucleotides. Experiments by Ehrlich and Riley [28, 29] with polybromouracil, poly(deoxybromouracil)-(deoxyadenine) and poly(bromouracil)-(adenine) also gave rise to this presumption. They found less strandbreaks than expected from a comparison with BU-substituted DNA.

If the high density of the BU molecules in one DNA strand would be responsible for the formation of dinucleotides, leading to the reduction of ssb induction and uracil formation per BU molecule at a given fluence, similar behaviour should also be observable with monofilarly substituted M13 DNA. Here the density of BU molecules in the BU strand was similar to that in each of the ColE1 DNA strands substituted with 85–90% BU. But the cross sections for ssb induction and uracil formation (Tables I and II) in M13 DNA do not agree with the data for ColE1 DNA with 85–90% BU substitution.

At very high BU substitutions an interaction between BU molecules in the complementary strands may have an influence on the absorption processes responsible for uracil formation and ssb induction.

Dsb induction by one photochemical absorption event

Looking at the dsb induction in the bifilarly BU-substituted ColE1 DNA (Fig. 2) one would mainly expect indirect induced dsb by coincidence

of two separately induced ssb. Analysis of the fluence effect curve, shown in Fig. 2, assuming a linear quadratic dependence, however, according to Eqn. (5) indicates a significant contribution of directly induced dsb (coefficient "a" in Eqn. (5) (Fig. 3).

For the ColE1 DNA it is difficult to calculate exact D_{37} values and cross sections for direct dsb induction because of the linear quadratic relationship of the fluence effect curve. For lower UV fluences (<9 kJ/m^2), however, where mainly direct dsb induction occurs, a cross section for direct induced dsb in ColE1 plasmid DNA of about 0.04 m^2/J × 10^7 could be estimated. From the results shown in Fig. 5a σ_{dsb} value for M13 DNA of 0.05 m^2/J × 10^7 was calculated. With the corresponding values for ssb (Table I), about 0.014 dsb per ssb were found in monofilar M13 DNA under neutral conditions. This value agrees well with those reported by other authors (Krasin and Hutchinson: 0.01 [12]; Beattie: 0.006 [30]; Köhnlein and Mönkehaus: 0.05 [31]; Lehmann: 0.01 [32]; Ley: 0.01 [33]).

The *in vitro* results presented here confirm the conclusions of earlier investigators. The conditions used here, however, unequivocally exclude the presence of BU in the parental DNA strand and the presence of repair reactions, which might have confounded the conclusions of earlier investigators (cited above).

The probability of dsb induction by coincidence of two ssb was estimated to be 14% for the monofilar-M13 phage DNA system under conditions where one dsb was observed. Thus, we present definite evidence for direct dsb induction by one photochemical absorption event.

Similar results were recently reported by Bothe *et al.* [34] who found a linear component in dsb induction in DNA by two step laser excitation.

Influence of the location of bromouracil in one or both DNA strands on strandbreak induction and uracil formation

The splitting of the Br–C bond of bromouracil leads *via* a uracilyl radical to the formation of uracil [6] which can be detected as dU by HPLC technique. From the results for dU formation, dsb, and ssb induction, respectively (Tables I and II) no indication was obtained that either ssb, dsb, or dU are produced at different rates in bifilarly or monofilarly BU-substituted DNA. Because of the almost identical D_{37} values for ssb induction in 55% BU-substituted ColE1 DNA and M13 DNA (41.5% BU substitution), where similar ratios of BU residues to basepairs per DNA molecule are found, the sensitivity of BU-substituted DNA apparently depends on the percentage of substitution and not on the distribution among the DNA strands in our *in vitro* approach.

For *in vivo* studies there is controversy about this problem in the literature. In Chinese hamster cells with more than 50% BU substitution, a higher sensitivity was found in cells substituted in both DNA strands than in cells substituted in one strand [35]; while Krasin and Hutchinson [12] noticed a very small difference between bifilarly and monofilarly substituted *E. coli* cells with substitution rates of 1–20% BU after UV_{313} exposure.

Studies which compare the uracil formation in bifilar and monofilar DNA have not been discussed in the literature. Krasin and Hutchinson [36] found an average cross section for uracil formation of 8 m^2/J × 10^7 in *E. coli* cells with less than 1% BU substitution and Lion (in Hutchinson and Köhnlein [2]) of 9 m^2/J × 10^7 in 6–100% BU-substituted DNA.

The cross sections of the uracil formation in 40% and 55% BU-substituted ColE1 DNA and 41.5% BU-substituted M13 DNA are similar (Table II), so that again no difference between monofilar and bifilar substitution was found. The values correspond very well to those found by others (cited above). The cross sections in 85–90% BU-substituted ColE1 plasmid DNA are higher and in addition a fluence dependency was observed indicating a possible saturation effect at fluences above 30 kJ/m^2 (Table II, Fig. 6).

Our results, together with those of Krasin and Hutchinson, agree with the findings of Ling and Ward [16], who also reported no difference in cell sensitivity of V79 hamster cells with one or two BU-substituted DNA strands after X-ray irradiation, indicating that the basic sensitization mechanisms in BU-substituted DNA are similar in the radiolytic and photolytic system. Consequently, the sensibilization properties of halogens incorporated into DNA against long wave-length UV light could also be used for explaining the sensitization mechanisms in DNA and cells in radiotherapy.

In this field selective experiments with X-rays would be difficult because of the broad spectrum of lesions appearing after irradiation (reviews: Hutchinson [37], v. Sonntag [4]).

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